2020

USP 43

THE UNITED STATES PHARMACOPEIA

NF 38

THE NATIONAL FORMULARY

Volume 1

By authority of the United States Pharmacopeial Convention.
Prepared by the Council of Experts and its Expert Committees

Official from May 1, 2020

The designation on the cover of this publication, "USP NF 2020," is for ease of identification only. The publication contains two separate compendia: The United States Pharmacopeia, Forty-Third Revision, and The National Formulary, Thirty-Eighth Edition.

THE UNITED STATES PHARMACOPEIAL CONVENTION
12601 Twinbrook Parkway, Rockville, MD 20852

www.webofpharma.com

SIX-MONTH IMPLEMENTATION GUIDELINE

The *United States Pharmacopeia–National Formulary* and its supplements become official **six months** after being released to the public. The *USP–NF,* which is released on November 1 of each year, becomes official on May 1 of the following year. This six-month implementation timing gives users more time to bring their methods and procedures into compliance with new and revised *USP–NF* requirements.

The table below describes the official dates of the *USP–NF* and its supplements. The 2019 *USP 42–NF 37,* and its supplements, *Interim Revision Announcements (IRAs)* and *Revision Bulletins* to that edition, will be official until May 1, 2020, at which time the *USP 43–NF 38* becomes official.

Publication	Release Date	Official Date	Official Until
USP 43–NF 38	November 1, 2019	May 1, 2020	May 1, 2021 (except as superseded by supplements, *IRAs,* and *Revision Bulletins*)
First Supplement to the USP 43–NF 38	February 1, 2020	August 1, 2020	May 1, 2021 (except as superseded by *Second Supplement, IRAs,* and *Revision Bulletins*)
Second Supplement to the USP 43–NF 38	June 1, 2020	December 1, 2020	May 1, 2021 (except as superseded by *IRAs* and *Revision Bulletins*)
USP 2021 Issue 1	November 1, 2020	May 1, 2021	May 1, 2022 (except as superseded by supplements, *IRAs,* and *Revision Bulletins*)

The table below gives the details of the *IRAs* that will apply to *USP 43–NF 38.*

IRA	PF Posting Date	Comment Due Date	IRA Posting Date	IRA Official Date
46(1)	January 2, 2020	March 31, 2020	May 29, 2020	July 1, 2020
46(2)	March 2, 2020	May 31, 2020	July 31, 2020	September 1, 2020
46(3)	May 1, 2020	July 31, 2020	September 25, 2020	November 1, 2020
46(4)	July 1, 2020	September 30, 2020	November 20, 2020	January 1, 2021
46(5)	September 1, 2020	November 30, 2020	January 31, 2021	March 1, 2021
46(6)	November 2, 2020	January 31, 2020	March 27, 2021	May 1, 2021

Revision Bulletins are published on the USP website and the *USP–NF Online* product and become official on the date specified in the *Revision Bulletin.*

NOTICE AND WARNING

Concerning U.S. Patent or Trademark Rights—Substances, products, or equipment referenced or described in the *USP–NF* may be associated with intellectual property rights, including but not limited to, registered trademarks, patents, and/or trade secrets. Nothing in the *USP–NF* should be construed as a representation as to such intellectual property rights. Furthermore, the inclusion in the *USP–NF* of a monograph, general chapter, or other reference addressing any substance, product, or equipment with respect to which intellectual property rights may exist shall not be deemed, and is not intended as, a grant of, or authority to exercise, any right or privilege protected by such patent, trademark, and/or trade secret. All such rights and privileges are vested in their respective owners, and no other person may exercise the same without express permission, authority, or license secured from such owner.

Concerning Use of USP–NF Text—Attention is called to the fact that *USP–NF* text is fully copyrighted. Authors and others wishing to use portions of the text should request permission to do so from the Legal Department of the USPC, legal@usp.org.

Copyright © 2019 The United States Pharmacopeial Convention 12601 Twinbrook Parkway, Rockville, MD 20852

All rights reserved.

ISSN 0195-7996 (print)

ISSN 1930-2932 (online)

ISBN 978-1-936424-95-5

Printed in the United States by United Book Press, Inc., Baltimore, MD

www.webofpharma.com

Contents

VOLUME 1

Mission Statement and Preface v
People 2015–2020 Revision Cycle ix
Officers ix
Board of Trustees ix
Council of Experts ix
Expert Committees x
In Memoriam xvii
Members and Delegates of the United States Pharmacopeial Convention, as of May 31, 2019 xviii
2018 Recognition of Monograph and Reference Material Donors xxv
Articles of Incorporation xxvii
USP Governance xxviii
Bylaws xxviii
Rules and Procedures xxviii
USP Policies xxviii
Admissions xxxi
Articles Admitted to USP 43 by Supplement xxxi
New Articles Appearing in *USP 43* That Were Not Included in *USP 42* Including Supplements xxxi
Articles Included in USP 42 But Not Included in USP 43 xxxi
Annotated List xxxiii
General Notices and Requirements 3
Guide to General Chapters 13

USP 43

USP 43, A-I Monographs 19
Index I-1

VOLUME 2

General Notices and Requirements 2485
Guide to General Chapters 2495
USP 43, J-Z Monographs 2501
Index I-1

VOLUME 3

General Notices and Requirements 4725
Guide to General Chapters 4735
Global Health Monographs 4741
Dietary Supplements Monographs 4745

NF 38

Admissions 5581
Articles Admitted to *NF 38* by Supplement 5581
New Articles Appearing in *NF 38* That Were Not Included in *NF 37* Including Supplements 5581
New Articles Appearing in *NF 38* 5581
Annotated List 5582
***USP* and *NF* Excipients** 5584
***NF 38* Monographs** 5597
Index I-1

VOLUME 4

General Notices and Requirements 6111
Guide to General Chapters 6121
Reagents, Indicators, and Solutions 6127
Reagent Specifications 6131
Indicators and Indicator Test Papers 6219
Buffer Solutions 6223
Colorimetric Solutions 6224
Test Solutions 6225
Volumetric Solutions 6237
Chromatographic Columns 6252
Reference Tables 6259
Containers for Dispensing Capsules and Tablets 6259
Description and Relative Solubility of *USP* and *NF* Articles 6273
Approximate Solubilities of *USP* and *NF* Articles 6334
Atomic Weights 6344
Half-Lives of Selected Radionuclides 6345
Alcoholometric Table 6346
Intrinsic Viscosity Table 6349
General Chapters
See page 6121 for detailed contents
General Tests and Assays 6405
General Requirements for Tests and Assays 6405
Apparatus for Tests and Assays 6445
Microbiological Tests 6449
Biological Tests and Assays 6488
Chemical Tests and Assays 6587
Physical Tests and Determinations 6819
Index I-1

www.webofpharma.com

VOLUME 5

General Notices and Requirements 7211
Guide to General Chapters 7221
General Information Chapters 7227
Dietary Supplement Chapters 8687
Index I-1

www.webofpharma.com

Mission and Preface *USP 43–NF 38* and *Supplements*

This section provides background information on the United States Pharmacopeial Convention (USP), as well as general information about the 43rd revision of the *United States Pharmacopeia* (*USP 43*) and the 38th edition of the *National Formulary* (*NF–38*) *Online* (*USP–NF Online* or *USP–NF*). Whether a document is official or not official will no longer be linked to when a specific publication such as the main *USP–NF* edition or one of the Supplements becomes official, but instead will be based on the individual document. USP will continue to publish the *USP–NF* standards on a set schedule, and each individual document will be associated with the indicated official date. Learn more about USP's transition to document-centric official dates on our website (https://www.uspnf.com/new-uspnf-online-faqs/end-user#irg1).

MISSION STATEMENT

USP–NF is published in continuing pursuit of the mission of USP: *To improve global health through public standards and related programs that help ensure the quality, safety, and benefit of medicines and foods.*

HISTORY

USP has a rich history, dating back to 1820, when 11 physicians met in the Senate Chamber of the U.S. Capitol building to establish a pharmacopeia for the United States. Learn more about USP's history and major milestones on the USP website (https://www.usp.org/about/usp-timeline).

CONTENT OF *USP–NF*

USP–NF contains official substance (ingredient) and product monographs for official articles recognized in *USP–NF* (see *General Notices 2.20 Official Articles*). *USP–NF* also includes monographs for compounded preparations. With few exceptions, such as articles covered by *Global Health* monographs, the intent is that all articles for which monographs are provided in *USP–NF* are legally marketed in the United States or are contained in legally marketed articles. *Global Health* monographs are provided for articles that are not approved or legally marketed in the United States, but that have been approved by a stringent regulatory authority [as defined by the World Health Organization (WHO)] and are used for essential purposes in other parts of the world.

A *USP–NF* monograph for an official substance, product, or preparation may consist of various components, including the article's name; definition; packaging, storage, and other requirements; and a specification. General chapters provide frequently cited procedures, sometimes with acceptance criteria, in order to compile into one location repetitive information that is applicable to many monographs. See *General Notices 3.10 Applicability of Standards* for more information about standards contained in *USP–NF* monographs and general chapters.

New and revised monographs and general chapters and omitted monographs from this edition are indicated in the *Admissions* section of the print publication and in the *USP Admissions List* section of the *USP–NF Online*.

***USP–NF* Organization**—The *USP–NF* is published online and printed as a five-volume set. To facilitate convenient use and reference, all five volumes include the combined index, as well as the *USP General Notices* and the *Guide to General Chapters*. *Volume 1* includes front matter (*Mission and Preface, People,* governance pages and websites, and *Admissions/Annotations*) and *USP* monographs A–I. *Volume 2* includes *USP* monographs J–Z. *Volume 3* includes *Global Health* monographs, *Dietary Supplements* monographs, *NF Admissions/Annotations*, *Excipients*, and *NF* monographs. *Volume 4* includes general chapters numbered below 1000 (*General Tests and Assays*—including chapter charts), *Reagents*, and *Reference Tables*. *Volume 5* includes general chapters numbered above 1000 (*General Information*) and *Dietary Supplements* general chapters. General chapters specific to dietary supplements are included in numerical order with the rest of the general chapters in *USP*. Excipient monographs are usually presented in *NF* but also may appear in *USP* with suitable cross-referencing when they are also drug substances. The *Excipients* section (*Volume 3*) presents a tabulation of excipients by functional category. [NOTE—*USP43–NF38* is the last publication that will be available in print as a five-volume set. Future publications will not be printed; only the online format will contain all current *USP–NF* content.]

Revisions to *USP–NF*—*USP–NF* is continuously revised by an exceptional process of public involvement and substantial interaction between USP and its stakeholders, both domestically and internationally. Revisions are presented in the *USP–NF* three times per year in November, February, and June. *Accelerated Revisions* [*Errata, Interim Revision Announcements* (*IRAs*), and *Revision Bulletins*] are published periodically and with greater frequency.

Standard Revisions—USP's standard revision process calls for publication of a proposed revision in the *Pharmacopeial Forum (PF)* for a 90-day notice and comment period and, after the revision is approved by the relevant USP Expert Committee, publication in the *USP–NF*.

Accelerated Revisions—The *Accelerated Revision* process is used to make revisions to *USP–NF* official more quickly than through USP's *Standard Revision* process. Learn more about *Revision Bulletins, Interim Revision Announcements (IRA), Errata*, and the criteria for and implementation of each on the USP website (http://www.uspnf.com/official-text).

Modification of Compendial References—USP and its Expert Committees periodically deem it necessary to modify general chapter titles or similar text that may be referenced in other standards throughout the *USP–NF*. When this occurs, USP staff undertake a rigorous process for identifying and updating such references. These updates may occur through a routine revision, or, in cases in which an update appears to

www.webofpharma.com

present no significant change in the affected standard, through a direct update of the reference in that standard without providing an opportunity for notice and comment. In all cases, USP will publish on its website a notice indicating the source change, any resulting references, and whether those references will be updated through a routine revision or a direct update.

Updating Chemical Information—Updates to the *Chemical Information* section at the beginning of monographs occur on an ongoing basis and are not identified with revision symbols. Chemical names and molecular weights are updated when a monograph undergoes revision to match the official source, *United States Adopted Names* (*USAN*). Chemical structures are updated on a continuous basis.

Chemical names typically reflect the naming conventions at the time of the monograph development or revision. If the nomenclature rules of CAS or IUPAC are significantly changed, the chemical names can be revised or added to implement those rules. Molecular weights are derived from the chemical formula and are based on the table of atomic weights. Atomic weights are recommended by the IUPAC and reflect the isotopic composition of normal terrestrial material. When the IUPAC recommended values are changed, it is understood that the changes in molecular weights will be made in due course.

Graphical representation of the chemical compound structures is intended as a visual aid to help establish chemical identity and is understood to represent one of many possible ways to depict the molecule. Addition of a graphical representation or changes in such representation, that result in the same chemical information, e.g., a flipped chiral molecule or adding a molecule structure, may be introduced outside of the revision process. It is also understood that in the case of tautomerism, the molecule depicted may be one of the tautomers, but it is intended to represent all isomers in equilibrium. Stereogenic centers depicted with plain bonds imply mixtures of pertinent stereomers—enantiomer, diastereomers, epimers (anomers), etc.
Depending on the timing of these updates, users may see a difference in a chemical structure between the publications in *PF* and *USP–NF*, and between the *USP–NF* and the *USP–NF Online*.

Shading and Symbols—Shading is used to identify text that has been modified, added, or deleted since it was last published. Symbols identify the beginning and end of each revision or nonharmonized text. The following table summarizes the types of symbols and the associated subscripts used in USP publications:

Revision Type	Revision Symbol and Text
In Process Revision (Book or Supplement)	▲▲ (USP 1-May-2020)
	▲▲ (NF 1-May-2020)
	▲▲ (GH 1-May-2020)
Interim Revision Announcement	▲▲(IRA 1-Jul-2020)
Revision Bulletin	▲▲(RB 1-Jul-2020)
Harmonization	◆◆
Errata	▲▲(ERR 1-Jul-2020)
Chapter References	▲▲(CN 1-Jul-2020)
	▲▲(Official 1-Jul-2020)
Pending	▲▲(TBD)

Commentary—For revisions that are published for public review and comment in *PF*, the proposal may advance to official status or be republished in *PF* for further notice and comment. If comments are received, they are considered and incorporated as appropriate by the Expert Committee(s). In cases where proposals advance to official status without republication in *PF*, a summary of comments received and the appropriate Expert Committee's responses are published in the *Commentary* section of the USP website at the time the revision is published.

The *Commentary* is not part of the official text and is not intended to be enforceable by regulatory authorities. Rather, it explains the basis of the Expert Committee's response to public comments. If there is a difference between the contents of the *Commentary* and the official text, the official text prevails. In case of a dispute or question of interpretation, the language of the official text, alone and independent of the *Commentary*, shall prevail.

Print and Electronic Presentations—See *General Notices 2.10 Official Text* for more information about *USP–NF* product formats. *USP43–NF38* is the last publication that will be available in print or on a flash drive. Future publications will not be printed or on flash drives; only the online format will contain all current *USP–NF* content.

USP–NF Translations—Translations of the *USP–NF* are available in Spanish, Russian, and Chinese. The Spanish translation is current; other translations are based on previous revisions of the *USP–NF*.

USP Reference Standards—The use of USP Reference Standards promotes uniform quality of drugs and supports reliability and consistency by those performing compliance testing and other users of *USP–NF*, including manufacturers, buyers, and regulatory authorities. USP Reference Standards are referenced in specific procedures in both monographs and general chapters. USP advances this material via careful characterization studies and collaborative testing, followed by review and approval of the compendial use of the reference material by Expert Committees of the Council of Experts. This program benefits from the widespread voluntary contribution of suitable materials and test data from pharmaceutical manufacturers. The *USP Catalog*, which lists the collection of USP Reference Standards, and more information about use and storage, can be accessed on USP's website (https://www.usp.org/reference-standards/reference-standards-catalog).

USP GOVERNING, STANDARDS-SETTING, AND ADVISORY BODIES

USP's governing, standards-setting, and advisory bodies include the USP Convention, the Board of Trustees, the Council of Experts and its Expert Committees, Expert Panels, Subcommittees, Joint Standard-Setting Subcommittees, and staff. Additional volunteer bodies include Stakeholder Forums and Project Teams, which offer stakeholders the opportunity to contribute, through advice and recommendations, to the advancement of USP's standards and processes. Learn about the composition and work of the USP Convention, Board of Trustees, Expert Committees, and Expert Panels on the USP website (https://www.usp.org/about/convention-membership). Learn more about Stakeholder Forums and Project Teams on the USP website (https://www.usp.org/get-involved/provide-input/stakeholder-forums). A listing of all current Voting Delegates of the USP Convention and members and Government Liaisons to the Council of Experts and its Expert Committees and Expert Panels is included in the *People* section.

Working with the Food and Drug Administration—USP works with the Secretary of the Department of Health and Human Services, and the principal agency in the Department for this work is the Food and Drug Administration (FDA). USP works in many ways with the agency, but the primary interaction is through the Government Liaison Program. The

www.webofpharma.com

Government Liaison Program allows representatives of FDA and other government agencies to participate in Expert Committee and Expert Panel meetings, enabling interactions between government staff and Expert Committees. Staff in the FDA Centers who are responsible for review of compendial activities provide specific links and opportunities for exchange of comments. The Office of Policy for Pharmaceutical Quality in the Center for Drug Evaluation and Research provides a primary compendial point of contact between FDA and USP.

RULES AND PROCEDURES

Governing Documents—*USP–NF* standards are recognized widely because they are authoritative and science-based and are established by a transparent and credible process. See the *Articles of Incorporation* section in this book; the Bylaws (https://www.usp.org/about/convention-membership/bylaws), and the *Rules and Procedures of the Council of Experts* (https://www.usp.org/about/leadership/policies-rules). Collectively, these documents serve USP volunteers and staff as the governing principles for USP's standards-setting activities.

LEGAL RECOGNITION

Recognition of *USP–NF*—*USP–NF* is recognized by law and custom in many countries throughout the world. In the United States, the Federal Food, Drug, and Cosmetic Act (FD&C Act) defines the term "official compendium" as the official *USP*, the official *NF*, the official *Homeopathic Pharmacopeia of the United States*, or any supplement to them. *USP–NF* standards play a role in the adulteration and misbranding provisions of the FD&C Act, which apply as well to biologics, a subset of drugs, under the Public Health Service Act (see *General Notices 2.30 Legal Recognition*).

FDA requires that names for articles that are not official must be clearly distinguished and differentiated from any name recognized in an official compendium. Drugs with a name recognized in *USP–NF* also will be considered misbranded unless they meet compendial standards for packaging and labeling (see *General Notices 3.10.10 Applicability of Standards to Drug Products, Drug Substances, and Excipients* for more information).

Drugs—USP's goal is to have substance and drug product monographs in *USP–NF* for FDA-approved drugs in the United States, including chemical and biologic medicines, and their ingredients. USP also provides monographs in *USP–NF* for legally marketed therapeutic products not approved by FDA, e.g., pre-1938 drugs, over-the-counter (OTC) drugs marketed under FDA's OTC Monograph system, dietary supplements, and compounded preparations. The *Global Health* section of *USP–NF* contains monographs for articles that are not approved or legally marketed in the United States, but that have been approved by a stringent regulatory authority (as defined by WHO) and are used for essential purposes in other parts of the world. Conformance with a *USP–NF* monograph, if applicable, is required at all times in the life of an article from production to expiration.

Biologics—In the United States, biologics are considered to be a subset of drugs, whether they were approved by FDA under the FD&C Act [and received a new drug application (NDA)] or under the Public Health Service Act [PHS Act, where they receive a biologics license application (BLA)]. As of March 23, 2020, applications for biological products approved under the FD&C Act will be deemed to be licenses for the biological products under the PHS Act. All PHS Act biologics are subject to the applicable drug regulatory requirements of the FD&C Act, which means they are required to comply with the adulteration and misbranding provisions of the FD&C Act, including *USP–NF* compendial requirements, to the extent that such requirements apply to a particular biologic product. This is equally so for biologics approved under the long-standing PHS Act "351(a)" pathway, as well as the new "351(k)" pathway for biosimilars added by the 2010 healthcare reform legislation (Biologics Price Competition and Innovation Act, Title VII, Subtitle A of the Patient Protection and Affordable Care Act, Public Law 111-148).

Dietary Supplements—The Dietary Supplement Health and Education Act of 1994 amendments to the FD&C Act provide that a dietary supplement may be deemed a misbranded food if it is covered by the specifications of an official compendium (e.g., *USP–NF*), is represented as conforming to the specifications of an official compendium, and fails to so conform. This contrasts with pharmaceutical products, wherein conformance to applicable compendial standards is mandatory, whether or not the product claims to conform.

Compounding—USP provides general chapters and monographs for compounded preparations, as well as monographs for bulk substances used in compounding. USP standards are recognized in various provisions of the FD&C Act and such provisions do not differentiate between manufactured and compounded medicines. Moreover, the FD&C Act and the 2013 Drug Quality and Security Act specifically reference USP standards for compounding. Learn more about compounding on the USP website (https://www.usp.org/compounding).

Medical Devices—Section 201(h) of the FD&C Act defines a device as an instrument, apparatus, similar article, or component thereof recognized in *USP–NF*. Section 502(e) of the FD&C Act defines the established name of a device in the absence of an FDA designation of the official name as the official title in an official compendium. Despite these statutory provisions, there is no comparable recognition of USP's role in establishing compendial standards for medical devices as exists for drugs and biologics. Under authority granted by the Food and Drug Administration Modernization Act of 1997, the Center for Devices and Radiological Health recognizes national and international standards, including some *USP* tests and assays, for medical devices.

Nomenclature—For information on the nomenclature development process, the Nomenclature and Labeling Expert Committee, and USP's work with United States Adopted Names (USAN), see the USP website (https://www.usp.org/expert-committees/nomenclature-and-labeling-expert-committee-work-plan).

Chemical Names and CAS Registry Numbers—Chemical subtitles given in the monographs are index names used by the Chemical Abstracts Service (CAS) of the American Chemical Society. They are provided only in monographs in which the titles specify substances that are definable chemical entities. The first subtitle is the inverted form of the systematic chemical name developed by CAS for the purpose of the Collective Index (CI). The second subtitle, given in uninverted form, is a preferred IUPAC name (PIN) sanctioned and used by the International Union of Pure and Applied Chemistry (IUPAC). Preferred IUPAC names also are used by the WHO. Occasionally a third subtitle is supplied for historical reasons or when the synonym uses an alternative, but equivalent, naming convention. Monographs with chemical subtitles also generally carry CAS registry numbers. These bracketed numbers function independently of nomenclature as invariant numerical designators of unique, unambiguous chemical substances in the CAS registry, and thus are convenient and widely used.

HARMONIZATION ACTIVITIES

USP participates in several collaborative activities with global pharmacopeias in both bilateral and multilateral

www.webofpharma.com

settings. Examples of USP's current activities include the following.

Pharmacopeial Discussion Group—USP harmonizes pharmacopeial excipient monographs and general chapters through the Pharmacopeial Discussion Group (PDG), which includes representatives from the European, Japanese, and United States pharmacopeias, and WHO (as an observer). According to the PDG definition, "a pharmacopeial general chapter or other pharmacopeial document is harmonized when a pharmaceutical substance or product tested by the document's harmonized procedure yields the same results, and the same accept/reject decision is reached." Information regarding PDG, including history, the PDG working procedure, a glossary, and lists of monographs and general chapters that have completed stages 1–4 of the pharmacopeial harmonization process, resulting in an approved USP Stage 4 Harmonization text, is available on USP's website (https://www.usp.org/harmonized-standards).

International Meeting of the World Pharmacopeias—USP works with WHO and global pharmacopeial partners on the strategy and establishment of Good Pharmacopoeial Practices (GPhP) as a set of guiding principles for the appropriate establishment of pharmacopeial standards.

Adopt/Adapt Agreements—USP grants the rights to copy and/or adapt USP standards for use in other pharmacopeias through this formal mechanism.

Bilateral Agreements—USP partners with pharmacopeias for the joint development of pharmacopeial standards using this informal process.

USP Exchange Programs—USP regularly welcomes the exchange of scientific personnel through this program, with the goal of sharing scientific knowledge among global organizations involved in standards setting and the effective use of standards.

OTHER USP COMPENDIA

USP Compounding Compendium—The *USP Compounding Compendium* is an online compendium that includes all compounding-related general chapters from the *USP–NF* as well as the supporting general chapters that are referenced in the compounding general chapters and in *GENERAL NOTICES AND REQUIREMENTS*. The purpose of the *USP Compounding Compendium* is to provide compounding practitioners with convenient access to associated general chapters.

USP Herbal Medicines Compendium—The *USP Herbal Medicines Compendium (HMC)* is an online compendium that helps ensure the quality of the herbal ingredients used in herbal medicines. *HMC* monographs provide quality specifications—tests, procedures, and acceptance criteria—with validated analytical procedures and allied reference materials that aid in conformity assessment. *HMC* can help ingredient manufacturers, herbal product manufacturers, regulatory agencies, and other stakeholders to assess conformance of herbal medicinal ingredients with independent public standards and to control the quality of articles moving in international commerce. The *HMC* is available at https://hmc.usp.org.

USP Dietary Supplements Compendium—The *Dietary Supplements Compendium* is an online compendium that combines *USP–NF* standards for dietary supplements, standards and information from the *Food Chemicals Codex*, regulatory and industry documents, and other tools and resources.

Food Chemicals Codex—The *Food Chemicals Codex* (*FCC*) is a compendium of internationally recognized monograph standards and tests for the purity and quality of food ingredients, e.g., preservatives, flavorings, colorings, and nutrients. *FCC* is published every two years, with supplements every six months, and is available in print and electronic formats. Proposed revisions to *FCC* are available for public viewing and comment through the *FCC Forum*. The *FCC Forum* can be accessed free of charge at http://www.foodchemicalscodex.org.

OTHER USP RESOURCES

Chromatographic Columns—This comprehensive reference, previously titled *Chromatographic Reagents*, provides detailed information needed to conduct chromatographic procedures found in *USP–NF*. *Chromatographic Columns* lists the brand names of the column reagents cited in every proposal for new or revised gas- or liquid-chromatographic analytical procedures that have been published in *PF* since 1980. *Chromatographic Columns* also helps to track which column reagents were used to validate analytical procedures that have become official. The branded column reagents list is updated bimonthly and maintained on USP's website.

USP Dictionary—The *USP Dictionary of USAN and International Drug Names* is an online resource containing the most up-to-date United States Adopted Names of drugs; official *USP–NF* names; nonproprietary, brand, and chemical names; graphic formulas; molecular formulas and weights; CAS registry numbers and code designations; drug manufacturers; and pharmacologic and therapeutic categories. The *Dictionary* helps to ensure the accuracy of the following: product labeling; reports, articles, and correspondence; FDA regulatory filings; and pharmaceutical package inserts. It is published annually. For more information about the *Dictionary* see the USP website (https://www.usp.org/products/usp-dictionary).

www.webofpharma.com

People

2015–2020 Revision Cycle

Officers of the USP Convention, Board of Trustees, and the Council of Experts, Expert Committees, Expert Panels, and Advisory Groups

Officers (2015–2020)

Jesse L. Goodman, M.D., M.P.H.
President
Washington, DC
Timothy R. Franson, B.S. Pharm., M.D.
Past President
Indianapolis, IN
John E. Courtney, Ph.D.
Treasurer
Bethesda, MD
Susan S. de Mars, J.D.
Secretary
Rockville, MD

Board of Trustees (2015–2020)

Thomas R. Temple, B.S. Pharm., M.S., F.A. Ph.A.
Chair
Trustee At-Large
Des Moines, IA
Gregory E. Amidon, Ph.D.
Trustee Representing the Pharmaceutical Sciences
Ann Arbor, MI
Laura Herman, M.B.A., M.A.
Trustee Representing the Public
New Canaan, CT
Robert J. Meyer, M.D.
Trustee Representing the Medical Sciences
Charlottesville, VA
Marilyn K. Speedie, Ph.D.
Trustee Representing the Pharmaceutical Sciences
Minneapolis, MN
Stephen P. Spielberg, M.D., Ph.D.
Trustee Representing the Medical Sciences
Upper Gwynedd, PA
Gail R. Wilensky, Ph.D.
Trustee At-Large
Bethesda, MD
Susan C. Winckler, R.Ph., J.D.
Trustee At-Large
Alexandria, VA
Ron T. Piervincenzi, Ph.D.
Chief Executive Officer, February 2014–present
(ex-officio)
Rockville, MD

Council of Experts (2015–2020)

Jaap Venema, Ph.D.
Chair, Council of Experts
Rockville, MD
Pascal Anger, Ph.D.
Chair, Biologics Monographs 4—Antibiotics
Verrières-le-Buisson, France
Richard A. Blessing, M.S.
Chair, Chemical Medicines Monographs 1
North Chicago, IL
Edward K. Chess, Ph.D.
Chair, Biologics Monographs 3—Complex Biologics
McHenry, IL
Stephanie Y. Crawford, Ph.D., M.P.H.
Chair, Nomenclature and Labeling
Chicago, IL
Gigi S. Davidson, B.S. Pharm., DICVP
Chair, Compounding
Pittsboro, NC
Michael R. De Felippis, Ph.D.
Chair, Biologics Monographs 1—Peptides and Insulins
Indianapolis, IN
James E. De Muth, Ph.D., R.Ph.
Chair, General Chapters—Dosage Forms
Madison, WI
Jonathan W. DeVries, Ph.D.
Chair, Food Ingredients
Coon Rapids, MN
Dennis E. Doherty, M.D., FCCP
Chair, Healthcare Quality and Safety

www.webofpharma.com

Lexington, KY
Mary G. Foster, Pharm.D., BFA
Chair, General Chapters—Packaging and Distribution
Fort Collins, CO
Dennis K.J. Gorecki, B.S.P., Ph.D.
Chair, Non-Botanical Dietary Supplements
Saskatoon, Saskatchewan, Canada
Xiaorong He, Ph.D.
Chair, General Chapters—Physical Analysis
Ridgefield, CT
Mary C. Houck, Ph.D.
Chair, Excipient Monographs 2
Fort Collins, CO
David Hussong, Ph.D.
Chair, General Chapters—Microbiology
Kensington, MD
Kim C. Huynh-Ba, M.S.
Chair, Chemical Medicines Monographs 4
Newark, DE
Amy J. Karren, B.S.
Chair, Chemical Medicines Monographs 5
Flagstaff, AZ
Nancy Lewen, B.S.
Chair, General Chapters—Chemical Analysis
Owasso, OK
Robin J. Marles, B.S., M.S., Ph.D.
Chair, Botanical Dietary Supplements and Herbal Medicines
Ottawa, Ontario, Canada
Michael G. Mulkerrin, Ph.D.
Chair, Biologics Monographs 2—Proteins
Hillsborough, CA
Eric J. Munson, Ph.D.
Chair, Excipient Monographs 1
West Lafayette, IN
Bernard A. Olsen, Ph.D.
Chair, Chemical Medicines Monographs 3
Wake Forest, NC
Ernest Parente, Ph.D.
Chair, Chemical Medicines Monographs 2
Overland Park, KS
Robert R. Singer, M.S.
Chair, General Chapters—Statistics
Union City, CA
Reinhard Walter, Ph.D.
Chair, Chemical Medicines Monographs 6
Whippany, NJ
Wesley E. Workman, Ph.D.
Chair, General Chapters—Biological Analysis
Saint Charles, MO

Expert Committees (2015–2020)

[NOTE—The following listing of Expert Committees includes the Expert Panels that serve in an advisory capacity to the specific Expert Committee. The listing of Expert Panels and their membership represents those that have been fully formed and approved as of July 2015. Expert Panels are continuously formed and concluded throughout the USP revision cycle, and other membership listings will appear in the future.]

Expert Panels for the Council of Experts Executive Committee

Spanish Translation Expert Panel
OSCAR QUATTROCCHI, M.S., *Chair*
Ofelia Espejo Gonzalez, Ph.D.; Monica I. Hirschhorn, M.S.; Francisco Kuribrena, Ph.D.; Pilar Pais, Ph.D.; José María Parisi, M.S.; Luisa Fernanda Ponce De León, Pharm.D.; Mauricio A. Seigelchifer, Ph.D.; Ofelia Villalva-Rojas, Pharm.D.; Caroline R. Weinstein-Oppenheimer, Pharm.D.

Expert Committees for the United States Pharmacopeia

Nomenclature and Labeling
STEPHANIE Y. CRAWFORD, PH.D., M.P.H., *Chair*
Mary B. Baker, Pharm.D.; Dawn M. Boothe, D.V.M., Ph.D.; Mike Cohen, M.S.; Elizabeth Igne Ferreira, Ph.D.; Karen Hauda, J.D., M.S.; Kent Johnson, M.Pharm.; Armen Melikian, Ph.D.; Ginette A. Pepper, R.N., Ph.D., FAAN; Thomas P. Reinders, Pharm.D.; Joanne G. Schwartzberg, M.D.; Maged Sharaf, Ph.D.; Eric Sheinin, Ph.D.; Thomas R. Tice, Ph.D.; Claudia Vincenzi, Ph.D., Pharm.D.; Gillian Woollett, Ph.D.

Pronunciation Expert Panel
WILLIAM M. HELLER, PH.D., *Chair*
Mary B. Baker, Pharm.D.; Stephanie Y. Crawford, Ph.D., M.P.H.; Doreen J. Elston, Pharm.D.; Kent Johnson, M.Pharm.; Joan C. May, Ph.D.; Anthony Palmieri, Ph.D.; Ginette A. Pepper, R.N., Ph.D., FAAN; Thomas P. Reinders, Pharm.D.

Healthcare Quality and Safety
DENNIS DOHERTY, M.D., FCCP, *Chair*
Timothy Albertson, Ph.D.; Phil Ayers, Pharm.D.; Danial E. Baker, Pharm.D.; Mark Decerbo, Pharm.D.; Peter Glassman, M.B.B.S.; Roy Guharoy, Pharm.D., M.B.A.; Raymond Hohl, M.D.; Duane Kirking, Ph.D.; Raymond Love, Pharm.D.; Melody Ryan, Pharm.D.; Joanne Schwartzberg, M.D.; Patricia Sokol, J.D.; J. Russell Teagarden, D.M.H.; Jeanne Tuttle, B.S. Pharm.; Terri Warholak, Ph.D.; Hsiang Shonna Yin, M.S.

Drug Allergy and Intolerance Classification Expert Panel
RAYMOND LOVE, PHARM.D., *Chair*
Dennis Doherty, M.D., FCCP; Gay Dolin, M.S.N.; Roy Guharoy, Pharm.D., M.B.A.; Robert Hausam, M.D.; Russell Leftwich, M.D.; Robert McClure, M.D.; Gerald McEvoy, Pharm.D.; Tejal Patel, Pharm.D.; Seth Powsner, Ph.D.; George Allen Robinson, B.S. Pharm.; Shelly Spiro, B.S. Pharm.

Exchange of Compounded Drug Preparation Information in Health IT System Expert Panel
ROY GUHAROY, PHARM.D., M.B.A., AND RICHARD PARRISH, PH.D., *Co-Chairs*
David Aguero, Pharm.D.; Sandra Benavides, Pharm.D.; Scott Louis Ciarkowski, Pharm.D.; Catherine Graeff, M.S.; John T. Kilbourne, M.D.; Leanne Murray, Pharm.D.; Tammy P. Powell, M.S.; Seth Strawbridge, Pharm.D.; Kathy Vieson, Pharm.D.

Health Literacy Expert Panel
JOANNE G. SCHWARTZBERG, M.D., *Chair*
Cindy Brach, MPP; Joseph Chebli, Pharm.D.; Terry Davis, Ph.D.; Radhika Devraj, Ph.D.; Dennis Doherty, M.D., FCCP; Gerald McEvoy, Pharm.D.; Juliet Nguyen, Pharm.D.; Ruth Parker, M.D.; Seth Powsner, Ph.D.; N. Lee Rucker, M.S.P.H.; Patricia Sokol, J.D.; J. Russell Teagarden, D.M.H.; Keith Trettin, B.S. Pharm.; Hsiang Shonna Yin, M.S.

Drug Classification Expert Panel
DUANE M. KIRKING, PH.D., *Chair*
Danial E. Baker, Pharm.D.; Dennis Doherty, M.D., FCCP; Lauren Hoffman, Pharm.D.; Gerald McEvoy, Pharm.D.; Seth Powsner, Ph.D.; N. Lee Rucker, M.S.P.H.; Brian Solow, M.D.; J. Russell Teagarden, D.M.H.; Jeanne Tuttle, B.S. Pharm.; Dennis West, Ph.D.

www.webofpharma.com

Parenteral Nutrition Safety Expert Panel
PHIL AYERS, PHARM.D., *Chair*
Mary B. Baker, Pharm.D.; Elizabeth Bobo, M.S.; Denise Bohrer, Ph.D.; Joseph Boullata, Pharm.D.; Mark Decerbo, Pharm.D.; Dennis Doherty, M.D., FCCP; Kathleen M. Gura, Pharm.D.; Deborah Houston, Pharm.D.; Matthew T. Jenkins, M.S.; Gordon S. Sacks, Pharm.D.; Maureen Schanck, Pharm.D.; David S. Seres, M.D.; Connie Rae Sullivan, B.S. Pharm.; Patricia Worthington, M.S.N.

Chemical Medicines Monographs 1
RICHARD A. BLESSING, M.S., *Chair*
Gennady Ananchenko, Ph.D.; Elizabeth (Betsy) Cariello, B.A.; Alain Duguet, Ph.D.; Rupa Iyer, M.S.; Monika Jain, Ph.D.; Greg Kaster, M.S.; Jasmina Novakovic, Ph.D.; Jeff Rohrer, Ph.D.; David Schuck, Ph.D.; Jan Srbek, Ph.D.; Giordano Trazzi, Ph.D.

Chemical Medicines Monographs 2
ERNEST PARENTE, PH.D., *Chair*
Mahmoud Al Omari, Ph.D.; Allan Bokser, Ph.D.; Matthew Borer, Ph.D.; Jama Elmi, Ph.D.; Michael Koberda, Ph.D.; Joan C. May, Ph.D.; Beth L. Minter, B.S.; Maria Ines Santoro, Ph.D.; Jeff Schwartzenhauer, M.S.; Dennis Stephens, Ph.D.; Luciano Virgili, Ph.D.; Zhenyu Wang, Ph.D.; Joseph Yakupkovic, Ph.D.; Patrick Yat, Ph.D.

Chemical Medicines Monographs 3
BERNARD A. OLSEN, PH.D., *Chair*
Samuel Akapo, Ph.D.; Bianca Avramovitch, Ph.D.; Amy Barker, Ph.D.; Lynn Blessing, M.S.; Thomas Broadbent, Ph.D.; Ian Chung, Ph.D.; Debashis Das, Ph.D.; Jeffrey Fleitman, Ph.D.; Yuri Goldberg, Ph.D.; Eric Kesslen, Ph.D.; Pauline Lacroix, M.S.; Donald Parsons, Ph.D.; David Reed, M.B.A.; Murugan Saravanan, M.S.; Joseph Stowell, Ph.D.

Chemical Medicines Monographs 4
KIM C. HUYNH-BA, M.S., *Chair*
Josep Maria de Ciurana Gay, M.S.; Simona Dragan, Ph.D.; Natalia Borisovna Epshtein, Pharm.D., M.S.N.; Quanyin Gao, Ph.D.; Jerome M. Lewis, Ph.D.; Oscar Liu, Ph.D.; Annarapu Malleswarareddy, Ph.D.; Mariann Neverovitch, M.S.; Patrick Noland, M.S.; James A. Ponto, M.S.; Hemant Kumar Sharma, Ph.D.; William Taraszewski, Ph.D.; Martin Williamson, Ph.D.; Min Xia, Ph.D.; Steve S. Zigler, Ph.D.

⟨821⟩ Identification and Assay of Radionuclides and ⟨1821⟩ Radioactivity Theory and Practice and ⟨1823⟩ Drugs for Positron Emission Tomography Expert Panel—CONCLUDED
SALLY W. SCHWARZ, M.S., *Chair*
Cathy Sue Cutler, Ph.D.; Jonathan M. Fitzsimmons, Ph.D.; Paula M. Jacobs, Ph.D.; Thijs Kroon, Pharm.D.; Jerome M. Lewis, Ph.D.; Roger Moroney, M.S.; James A. Ponto, M.S.; Duann V. Thistlethwaite, B.S.; Steven S. Zigler, Ph.D.

⟨825⟩ Radiopharmaceuticals Compounding Chapter
JAMES PONTO, M.S., *Chair*
David Barnes, B.S. Pharm.; Allegra DePietro, M.S.; Wendy Galbraith, Pharm.D.; Fred Patrick Gattas, Pharm.D.; Richard Lewis Green, B.S. Pharm.; Kim C. Huynh-Ba, M.S.; Brenda Sue Jensen, M.A.; Patricia C. Kienle, M.P.A.; Vivian Sue Loveless, Pharm.D.; Paul Barry Mahan, B.S. Pharm.; Rezaul H. Mannan, Ph.D.; Steve Zigler, Ph.D.

Non-Radioactive Imaging Agents Expert Panel—CONCLUDED
JEROME M. LEWIS, PH.D., *Chair*
Francisco Aguilar-Parrilla, Ph.D.; James Walter Brodack, Ph.D.; Dilip R. Choudhury, Ph.D.; Francette Delaloge, Ph.D.; Joseph Louis Glajch, Ph.D.; Ernest Victor Groman, Ph.D.; Gordon Craig Hill, Ph.D.; Kim C. Huynh-Ba, M.S.; Aurelie Mieze-Richard, Pharm.D.; Patrick Noland, M.S.; Scott Roberts

Radioactive Drugs Expert Panel
JAMES A. PONTO, M.S., *Chair*
Corinne Bensimon, Ph.D.; Jonathan M. Fitzsimmons, Ph.D.; Umesh Gangadharmath, Ph.D.; Kim C. Huynh-Ba, M.S.; Thijs Kroon, Pharm.D.; Adrian Nunn, Ph.D.; David Pipes, Ph.D.; Kara Weatherman, Pharm.D.; Martin Williamson, Ph.D.; Steve Zigler, Ph.D.

Chemical Medicines Monographs 5
AMY J. KARREN, B.S., *Chair*
D.J. Doan, M.S; Sushil Gangwal, Ph.D.; Min Li, Ph.D.; Judy Lin, M.S.; Pauline McGregor, Ph.D.; Marian Meyer, Ph.D., M.B.A.; Jonathan Parks, B.S.; Justin Pennington, Ph.D.; Vijaya Ramesh, B.Pharm.; Gurvinder Singh Rekhi, Ph.D.; Iffaaz Salahudeen, Ph.D.; Mary W. Seibel, B.S.; Hameraj Singh, Ph.D.; Michael Skibic, M.S.

Chemical Medicines Monographs 6
REINHARD WALTER, PH.D., *Chair*
Seamus Boland, B.S.; Robert Graham Buice, Ph.D., M.B.A.; Timothy Gilmor, Ph.D.; Carmen Gonzalez, Ph.D.; John Joseph Herries, Ph.D.; Todd Lewis, M.S.; William Long, Ph.D.; Phil Nethercote, Ph.D.; Raphael Ornaf, Ph.D.; David H. Rogers, Ph.D.; Thomas Rosanske, Ph.D.; Donna S. Seibert, Ph.D.; Christina Szabo, Ph.D.; Xiaoping Wang, Ph.D.; Kylen Whitaker, Ph.D.; Zeena Williams, Ph.D.

Acetaminophen OTC Expert Panel—CONCLUDED
KYLEN WHITAKER, PH.D., *Chair*
Tina M. Engel, Ph.D.; David A. Fay, Ph.D.; Saulius A. Gylys; Michael T. Rankin, M.S.; David H. Rogers, Ph.D.; Gregory K. Webster, Ph.D.; Jonathan Zeszotarski, Ph.D.

Biologics Monographs 1—Peptides and Insulins
MIKE DE FELIPPIS, PH.D., *Chair*
Wilfried Arz, Ph.D.; Chaim Eidelman, Ph.D.; Gyongyi Gratzl, Ph.D.; Gerhard Haas, Ph.D.; Morten Hach, M.S.; Marion King, Ph.D.; Peter Larsson; Jean-Marc Poudrel, Ph.D.; Harold Rode, Ph.D.; Raimon Rubires, Ph.D.; Ved Srivastava, Ph.D.; Michael Verlander, Ph.D.

Glatiramer Expert Panel
GYONGYI GRATZL, PH.D., *Chair*
Joseph Louis Glajch, Ph.D.; Satyanarayana Kota, Ph.D.; Barbara Mulloy, Ph.D.; Todd A. Osiek, Ph.D.; Marcia Cecilia Rusjan, Ph.D.; Rakesh Singh Shekhawat, Ph.D.; Rene Thuermer, Ph.D.; Patrick Vallano, Ph.D.; Michael Verlander, Ph.D; Vera Weinstein, Ph.D.

Glucagon Expert Panel—CONCLUDED
HAROLD RODE, PH.D., *Chair*
Jan Amstrup, Ph.D.; Matthew W. Borer, Ph.D.; Gerhard Manfred Haas, Ph.D.; Anne Munk Jespersen, Ph.D.; Elizabeth Kramer, Ph.D.

Insulin Expert Panel
HEATHER BOUX, PH.D., *Chair*
Jan Amstrup, Ph.D.; Wilfried Arz, Ph.D.; Chris Burns, Ph.D.; Jill Crouse-Zeineddini, Ph.D.; Morten Hach, M.S.; Elizabeth Kramer, Ph.D.; Ned Mozier, Ph.D.; Karthik Ramani, Ph.D.; Sarah Richer; Harold Rode, Ph.D.

Biologics Monographs 2—Proteins
MICHAEL MULKERRIN, PH.D., *Chair*
Gregory Beck, Ph.D.; Heather Boux, Ph.D.; Chris Burns, Ph.D.; Frédéric Carrière, Ph.D.; Jill Crouse-Zeineddini, Ph.D.; Michel Girard, Ph.D.; Anne Munk Jespersen, B.S.; Sridevi Khambhampaty, Ph.D.; Robert Mayer, Ph.D.; Ned Mozier, Ph.D.; Dhananjay Patankar, Ph.D.; Mauricio Seigelchifer, Ph.D.

www.webofpharma.com

Enzyme Expert Panel
FRÉDÉRIC CARRIÈRE, PH.D., *Chair*
Gregory Beck, Ph.D.; Francis Dwulet, Ph.D.; Olaf Friedrich, Ph.D.; Luigi G. Ghidorsi; Christopher Hosty, M.S.; Andreas Koerner, Ph.D.; Thomas K. Langdon, B.S.; Jeffrey Lessman

Fc Function Assays Expert Panel
JILL CROUSE-ZEINEDDINI, PH.D., *Chair*
Shan Chung, Ph.D.; Marina Feschenko, Ph.D.; Scott Kuhns, Ph.D.; LeeAnn Machiesky, M.S.; Bhavin Parekh, Ph.D.; Teresa Surowy, Ph.D.; Max Tejada, Ph.D.

Biologics Monographs 3—Complex Biologics
EDWARD K. CHESS, PH.D., *Chair*
Mehrshid Alai, Ph.D.; Pascal Anger, Ph.D.; Parastoo Azadi, Ph.D.; Svetlana Bergelson, Ph.D.; Barbara Blum, Ph.D., M.P.H.; Mirella Ezban, Ph.D.; Elaine Gray, Ph.D.; Donald MacLean, Ph.D.; Nicole Provost, Ph.D.; Elizabeth I. Read, M.D.; Peter Vandeberg, Ph.D.; Christian Viskov, Ph.D.; Darin Weber, Ph.D.

Bovine Unfractionated Heparin Expert Panel—CONCLUDED
WESLEY E. WORKMAN, PH.D., *Chair*
Tania Andrade, B.S. Pharm.; Irene Bartoli, B.S.; Maria Leticia Bertot, Pharm.D.; Elaine Gray, Ph.D.; Marco Guerrini, Pharm.D.; Kristian Johansen, Ph.D.; Robert Linhardt, Ph.D.; Barbara Mulloy, Ph.D.; Marilene Nuss Rangel; Zachary Shriver, Ph.D.; Pearle Torralba, Ph.D.; Christian Viskov, Ph.D.

CD-34 Positive Cells Expert Panel—CONCLUDED
NICOLE M. PROVOST, PH.D., *Chair*
Ruud Hulspas, Ph.D.; Elizabeth I. Read, M.D.; Michael D. Rosu-Myles, Ph.D.; Luisa Saraiva, Ph.D.; Richard J. Stebbings, Ph.D.; D. Robert Sutherland, M.S.; Lili Wang, Ph.D.; Albertus W. Wognum, Ph.D.

Low Molecular Weight Heparins Expert Panel
ELAINE GRAY, PH.D., AND EDWARD K. CHESS, PH.D., *Co-Chairs*
Christopher P. Bryant, Ph.D.; Ishan Capila, Ph.D.; Gyongyi Gratzl, Ph.D.; Kristian Johansen, Ph.D.; Annarapu Malleswarareddy, Ph.D.; Barbara Mulloy, Ph.D.; Anna K.Y. Nordin; Bruna Parma, M.S.; Christian Viskov, Ph.D.

Biologics Monographs 4—Antibiotics
PASCAL ANGER, PH.D., *Chair*
Elizabeth (Betsy) Bruce Cariello, B.A.; Sheila Deneau, B.S.; Colleen Guthrie, B.S.; Robert Klasson, M.S.; Mark G. Papich, M.S.; Cindy Reid, B.S.; Jennifer Jaye Schimmel, M.D.; Jonathan A. Stewart, B.S.; Yaozuo Yuan, Ph.D.

General Chapters—Biological Analysis
WESLEY E. WORKMAN, PH.D., *Chair*
Robert Bell, Ph.D.; Mahesh Bhalgat, Ph.D.; Christopher Jones, Ph.D.; Jeremy Kunkel, Ph.D.; Huijuan Li, Ph.D.; Kenneth Miller, Ph.D.; Anthony Mire-Sluis, Ph.D.; Anthony A.G. Ridgway, Ph.D.; Wendy Saffell-Clemmer, M.S.; Teruhide Yamaguchi, Ph.D.; Earl Zablackis, Ph.D.

Bioassay General Chapter
DAVID LANSKY, PH.D., *Chair*
Jan Amstrup, Ph.D.; Walter Hauck, Ph.D.; Bhavin Parekh, Ph.D.; Andrew Rugaiganisa, M.S.; Perceval Sondag, M.S.; Ralf Stegmann, Ph.D.; Ryan Yamagata, M.S.; Lingmin Zeng, Ph.D.

Biologics Stability Expert Panel
ANTHONY MIRE-SLUIS, PH.D., *Chair*
Kimberly Cheung, M.S.; Nila Das, Ph.D.; Stephanie Ferrari, M.S.; Jens Krogh Rasmussen, M.S.; Joseph Kutza, Ph.D.; Lori A. McCaig, Ph.D.; Nausheen Rahman, Ph.D.; Camilla Santos, Ph.D.; Michael Walsh, M.S.; Allison Wolf, M.S.

Cell Banking Expert Panel
ROBERT BELL, PH.D., *Chair*
Jeri Ann Boose, Ph.D.; Sunil Gairola, Ph.D.; Luhong He, Ph.D.; Ruud Hulspas, Ph.D.; Jette Dina Kreiberg, Ph.D.; Michael Laird, Ph.D.; Archie Lovatt, Ph.D.; Sam Yaghmour, M.S.; Earl Zablackis, Ph.D.

Residual DNA in Biotechnology-Derived Products Expert Panel
WESLEY E. WORKMAN, PH.D., *Chair*
Pascal Anger, Ph.D.; Jon R. Borman; Scott Kuhns, Ph.D.; Judith Shimoni, Ph.D.; Weihong Wang, Ph.D.

Vaccine Polysaccharide NMR Identity Testing Expert Panel
CHRISTOPHER JONES, PH.D., *Chair*
Yves Aubin, Ph.D.; Francesco Berti, Ph.D.; Cristiana Campa, Ph.D.; Thomas P. Jacques, Ph.D.; Michael T. Jones, Ph.D.; Jeremy P. Kunkel, Ph.D.; Neil Ravenscroft, Ph.D.; Philippe Talaga, Ph.D.; Earl Zablackis, Ph.D.

Validation of Commercial Test Kits Expert Panel—CONCLUDED
KENNETH MILLER, PH.D., *Chair*
Heather Boux, Ph.D.; David Good, M.S.; Wendy Saffell-Clemmer, M.S.

Viral Vaccines Expert Panel
EARL ZABLACKIS, PH.D., *Chair*
Luca Benetti, Ph.D.; Sunil Gairola, Ph.D.; Lucy Gisonni-Lex, M.S.; Keith Howard, Ph.D.; Christopher Jones, Ph.D.; Archie Lovatt, Ph.D.; Brij Patel, Ph.D.; Silke Schepelmann, Ph.D.; Vaneet Sharma, Ph.D.; Mark Van Ooij, Ph.D.

General Chapters—Chemical Analysis
NANCY LEWEN, B.S., *Chair*
Anthony Bevilacqua, Ph.D.; Christopher Burgess, Ph.D.; Robert T. Cambron, Ph.D.; Thomas DiFeo, Ph.D.; John Dolan, Ph.D.; Joseph Louis Glajch, Ph.D.; John P. Hammond, FRSC; John Hinshaw, Ph.D.; Brent Kleintop, Ph.D.; Steven Leinbach, M.S.; Gregory Martin, M.S.; Nuno Matos, B.S.; Oscar Quattrocchi, M.S.; Helmut Rockstroh, Ph.D.; Mark G. Schweitzer, Ph.D.; Timothy Shelbourn, M.S.; Rostyslaw O. Slabicky, B.S.; Teri Soli, Ph.D.; Kevin A. Swiss, Ph.D., Timothy J. Wozniak, Ph.D.

Analytical Methodologies Based on the Light Scattering Phenomena Expert Panel
KEVIN SWISS, PH.D., *Chair*
Emilia Byrne, M.S.; Nila Das, Ph.D.; Joseph Louis Glajch, Ph.D.; Thomas Gonyon, B.S.; John P. Hammond, FRSC; Stephen Hussey; Chris Jones, Ph.D.; Jonathan Kingsbury, Ph.D.; Richard Meury, B.S.; Helmut Rockstroh, Ph.D.; Jack Saad, B.S.; Zhigang Sun, Ph.D.; William F. Weiss, Ph.D.; Eloise Welfare, Ph.D.; Renliang Xu; Earl Zablackis, Ph.D.

Chemometrics Expert Panel—CONCLUDED
PEI CHEN, PH.D., AND NUNO MATOS, *Co-Chairs*
Chunsheng Cai, Ph.D.; Robert T. Cambron, Ph.D.; Peter de B. Harrington, Ph.D.; Mark J. Henson, Ph.D.; Yang (Angela) Liu, Ph.D.; Zhenqi (Pete) Shi, Ph.D.; Yvan C.D. Vander Heyden, Ph.D.; Stanislav O. Zakharkin, Ph.D.; Lin Zhang, Ph.D.

Elemental Impurities Expert Panel
NANCY LEWEN, B.S., *Chair*
Charles Barton, Ph.D., DABT; Courtney M. Callis, M.P.H., DABT; Steven J. Dentali, Ph.D.; Anna M. Fan, Ph.D., DABT; Edward J. Fletcher, B.S.; Bruce A. Fowler, Ph.D., A.T.S.; Roland Froetschl, Ph.D.; Richard Ko, Pharm.D., Ph.D.; Timothy L. Shelbourn, M.B.A., M.S.

www.webofpharma.com

Good Documentation Practices Expert Panel—CONCLUDED
KIM C. HUYNH-BA, M.S., *Chair*
Kathleen V. Brady, B.S.; Frank J. Diana, Ph.D.; Lisa Ann Fink, M.B.A.; Craig Hamilton, Ph.D.; Judy Lin, M.S.; Anjan K. Mittal, M.Pharm.; Kevin A. Swiss, Ph.D.

Modernization of Identification Tests Expert Panel
NANCY LEWEN, B.S., *Chair*
Anthony C. Bevilacqua, Ph.D.; Geoffrey P. R. Carr, Ph.D.; Pei Chen, Ph.D.; Jonathan W. DeVries, Ph.D.; Maryna Dmitriieva, Ph.D.; Michael Hornig, Ph.D.; Bernard A. Olsen, Ph.D.; Jeffrey S. Rohrer, Ph.D.

⟨467⟩ Residual Solvents Expert Panel
OSCAR A. QUATTROCCHI, M.S., *Chair*
Coleman C. Chasteen, M.S.; John Connelly, Ph.D.; Jeffrey Fleitman, Ph.D.; John V. Hinshaw, Ph.D.; Bruce P. Johnson, Ph.D.; Eric C. Kesslen, Ph.D.; Brent Kleintop, Ph.D.; Elizabeth Kovacs, B.S.; Paul W. Lockwood, M.S.; Gregory P. Martin, M.S.; Kevin A. Swiss, Ph.D.; Yuwen Wang, Ph.D.

Quality Standards for Pharmaceutical Continuous Manufacturing Expert Panel
NUNO MATOS, B.S., *Chair*
Shaukat Ali, Ph.D.; Ahmad Almaya; Brian Carlin, Ph.D.; Thomas P. Garcia, Ph.D.; Douglas Hausner, Ph.D.; Eric Jayjock, Ph.D.; Keith Jensen, Ph.D.; Johannes Khinast; Pramod Kotwal, Ph.D.; Marcus U. Krumme, Ph.D.; Kim Lamey, Ph.D.; Fernando J. Muzzio, Ph.D.; William G. Randolph, Ph.D.; Mark G. Schweitzer, Ph.D.; Raymond Skwierczynski, Ph.D.; Kelly Swinney, Ph.D.; Bernhardt Tout, Ph.D.; Amy Walia, M.A.

Validation and Verification Expert Panel—CONCLUDED
GREGORY P. MARTIN, M.S., *Chair*
Kimber L. Barnett, Ph.D.; Christopher Burgess, Ph.D.; Paul D. Curry, Ph.D.; Gyongyi Gratzl, Ph.D.; John P. Hammond, FRSC; Elizabeth Kovacs; David J. LeBlond, Ph.D.; Rosario LoBrutto, Ph.D.; Pauline L. McGregor, Ph.D.; Phil Nethercote, Ph.D.; Allen C. Templeton, Ph.D.; David P. Thomas, Ph.D.; M.L. Jane Weitzel

Water for Analytical and Pharmaceutical Purposes Expert Panel
ROSTYSLAW O. SLABICKY, B.S., *Chair*
Anthony C. Bevilacqua, Ph.D.; Lucia Clontz, D.H.S., M.S.; Max S. Lazar, B.A.; Nancy Lewen, B.S.; Bruno Rossi, M.D.; Teri C. Soli, Ph.D.

General Chapters—Physical Analysis
XIAORONG HE, PH.D., *Chair*
Shaukat Ali, Ph.D.; Lawrence H. Block, Ph.D.; Geoff P. R. Carr, Ph.D.; Martin J. Coffey, Ph.D.; Tim Freeman, B.A.; David J. Goldfarb, Ph.D.; Bruno Hancock, Ph.D.; Stephen Hoag, Ph.D.; Mario Hubert, Ph.D.; Richard Meury, B.S.; Matthew Mullarney, M.S.; Prabu Nambiar, Ph.D.; Myke Scoggins, Ph.D.; Changquan Sun, Ph.D.; Zhigang Sun, Ph.D.; Allen Templeton, Ph.D.; Eloise Welfare, Ph.D.; Dale Wurster, Ph.D.; Bing-Shiou Yang, Ph.D.; Geoff Zhang, Ph.D.

Impurities in Drug Substance and Drug Products Expert Panel
PRABU NAMBIAR, PH.D., M.B.A., *Chair*
Shaukat Ali, Ph.D.; Steven W. Baertschi, Ph.D.; Judy P. Boehlert, Ph.D.; Robert G. Buice, Ph.D.; Xiaorong He, Ph.D., M.B.A.; Kim C. Huynh-Ba, M.S.; Michael Koberda, Ph.D.; Robert E. Osterberg, R.Ph., Ph.D., Fellow-ATS; Ernest Parente, Ph.D.; Oscar Quattrocchi, M.S.; David H. Rogers, Ph.D.; Mark G. Schweitzer, Ph.D.; Mary W. Seibel; Rostyslaw O. Slabicky, B.S.; Teri C. Soli, Ph.D.; Kevin A. Swiss, Ph.D.

General Chapters—Dosage Forms
JAMES E. DE MUTH, PH.D., *Chair*
Emmanuel Akala, Ph.D.; Ilgaz Akseli, Ph.D.; Scott Aldrich, B.S.; Susan Cady, M.S.; Paul Curry, Ph.D.; Mario González, Ph.D.; Vivian A. Gray, B.S.; Ralph Heasley, Ph.D.; Anthony Hickey, Ph.D.; Munir A. Hussain, Ph.D.; Johannes Kraemer, Ph.D.; Stefan Leiner, Ph.D.; John Mauger, Ph.D.; Colin Minchom, Ph.D.; Jolyon Mitchell, Ph.D.; Pierre-Alain Muller, Ph.D.; Guirag Poochikian, Ph.D.; Chetan Pujara, Ph.D.; Shobhan Sabnis, Ph.D.; John Shabushnig, Ph.D.; Raymond D. Skwierczynski, Ph.D.; Jason Suggett, Ph.D., M.B.A.; Monica Tejwani, Ph.D.; Thomas R. Tice, Ph.D.; Kevin Warner, Ph.D.; Mehran Yazdanian, Ph.D.

⟨771⟩ Ophthalmic Preparation Expert Panel
ASHIM K. MITRA, PH.D., *Chair*
Dale S. Aldrich, Ph.D.; Martin Coffey, Ph.D.; Paul Curry, Ph.D.; Jeffrey S. Fleitman, Ph.D.; John Mauger, Ph.D.; Seshadri Neervannan, Ph.D.; Stacey M. Platzer; Chetan Pujara, Ph.D.; Satish K. Singh, Ph.D.; Monica Tejwani, Ph.D.; Thomas R. Tice, Ph.D.

⟨788⟩ Particulate Matter in Injections Expert Panel
DALE S. ALDRICH, PH.D., *Chair*
Dan Berdovich; Mary Lee Ciolkowski, Ph.D.; Kevin Dahl, Ph.D.; Linda Narhi, Ph.D.; Kent A. Peterson; Dean Ripple, Ph.D.

Liquid-Filled Capsules Expert Panel
VIVIAN A. GRAY, B.S., *Chair*
Joe Fotso, Ph.D.; Munir A. Hussain, Ph.D.; Stephen C. Tindal; Madhusudan Vudathala, M.Pharm., M.B.A.

Performance Test for Semisolid Dosage Forms Expert Panel
KAILAS THAKKER, PH.D., *Chair*
Bryan Crist; James E. De Muth, Ph.D.; Geoffrey N. Grove, Ph.D.; L. Thomas Hall, Ph.D.; John S. Heaney; Patricia L. Lee, M.S.; Patrick C. Mahn; William M. Rosenthal; Steve W. Shaw

Solubility Criteria for Veterinary Drugs Expert Panel
SUSAN CADY, M.S., *Chair*
Bryan Crist; Mario A. Gonzalez, Ph.D.; Mark G. Papich, M.S., D.V.M.; Alan F. Parr, Pharm.D., Ph.D.; Monica Tejwani, Ph.D.

Sutures Expert Panel
JAMES E. DE MUTH, PH.D., *Chair*
Edwin Anderson, M.S.; John C. Chen; Frank Corniello; Nomi Steen

Use of Enzymes in the Dissolution Testing of Gelatin Capsules Expert Panel
VIVIAN A. GRAY, B.S., *Chair*
Ewart Cole, Ph.D.; Luigi Ghidorsi; Jian-Hwa Guo, Ph.D.; Feixue Han, Ph.D.; Jian-Hwa Han, Ph.D.; Christopher T. Hosty; Johannes Kraemer, Ph.D.; Thomas Langdon; Steven R. Leinbach; Stefan Leiner, Ph.D.; Gregory P. Martin, M.S.; Steven M. Meyerhoffer, Ph.D.; Richard C. Moreton, Ph.D.; Edward Shneyvas, Ph.D.; Jason A. Suggett, Ph.D.; Stephen C. Tindal; Madhusudan Vudathala, M.Pharm., M.B.A.; Hu Wang, M.S.

Visual Inspection of Parenterals Expert Panel
RUSSELL E. MADSEN, M.S., *Chair*
Dale S. Aldrich, Ph.D.; John D. Ayres, M.D., J.D.; Roy Cherris; John G. Shabushnig, Ph.D.; Deborah Shnek, Ph.D.

General Chapters—Microbiology
DAVID HUSSONG, PH.D., *Chair*
James Agalloco, M.S.; James Akers, Ph.D.; Dilip Ashtekar, Ph.D.; Anthony M. Cundell, Ph.D.; Dennis Guilfoyle,

www.webofpharma.com

Ph.D.; Rajesh Gupta, Ph.D.; Russell Madsen, M.S.; Karen McCullough, M.S.; Robert Mello, Ph.D.; David Roesti, Ph.D.; Donald Singer, M.S.; Paul Stinavage, Ph.D.; Edward Tidswell, Ph.D.

Modern Microbiological Methods Expert Panel
ANTHONY M. CUNDELL, PH.D., AND EDWARD C. TIDSWELL, PH.D., *Co-Chairs*
Thierry Bonnevay, Ph.D.; Ralph Breton, B.S. Pharm.; Claudio Denoya, Ph.D.; Gary du Moulin, Ph.D.; John Duguid, B.S.; Rajesh K. Gupta, Ph.D.; David Hussong, Ph.D.; Matthew Jenkins, M.S.; Amy Lynn McDaniel, Ph.D.; Michael Miller, Ph.D.; Felix Montero Julian, Ph.D.; David Newton, Ph.D.; Kuldip Patel; Steven Richter, Ph.D.; David Roesti, Ph.D.; Yongqiang Zhang, Ph.D.; Steve S. Zigler, Ph.D.

General Chapters—Packaging and Distribution
MARY G. FOSTER, PHARM.D., BFA, *Chair*
Chris Anderson, M.A.; Bettine Boltres, Ph.D.; Glaucia K. Braga, Ph.D.; Jeffrey Carrico, Pharm.D.; Chris Chandler, Pharm.D.; Michael N. Eakins, Ph.D.; Dana Guazzo, Ph.D.; Renaud Janssen, Ph.D.; Dennis Jenke, Ph.D.; Wendy Mach, B.S.; Dan Malinowski; Daniel L. Norwood, Ph.D.; Diane Paskiet, M.S.; Robert Seevers, Ph.D.; Cheryl L. M. Stults, Ph.D.; Li Xiong, Ph.D.; Gao Yonghua, B.S. Pharm.

⟨381⟩ Elastomeric Closure for Injections Expert Panel
DIANE M. PASKIET, M.S., AND RENAUD JANSSEN, PH.D., *Co-Chairs*
Douglas J. Ball, M.S., DABT; Michael N. Eakins, Ph.D.; Dana Guazzo, Ph.D.; Dennis R. Jenke, Ph.D.; Douglas Kiehl, M.S., B.S.; Heinz Kirchmeyer, Ph.D.; Philippe LeGall, M.S.; Daniel L. Norwood, Ph.D.; Michael A. Ruberto, Ph.D.; Lisa M. Yoest, M.S.

⟨659⟩ Packaging and Storage Requirements Expert Panel—CONCLUDED
CHRIS CHANDLER, PHARM.D., *Chair*
Glaucia K. Braga, B.S.; Jeffrey Carrico, Pharm.D.; Mary G. Foster, Pharm.D., BFA; Eleanor Freeman, B.S.; Wendy Mach, B.S.; Devinder Pal, M.Pharm.; Robert H. Seevers, Ph.D.; Gao Yonghua, B.S. Pharm.; Li Xiong, Ph.D.

⟨660⟩ Containers—Glass Expert Panel
BETTINE BOLTRES, PH.D., AND MICHAEL EAKINS, PH.D., *Co-Chairs*
Michael E. Akers, B.A.; Alberto Biavati, Ph.D.; Juan Cerdan-Diaz; Carol Rea Flynn, M.S.; Emanuel Guadagnino, M.D.; Daniel Edward Haines, Ph.D.; Kevin McLean, B.S.; Kelly Murphy, Ph.D.; Volker Rekowski; Jennifer Martell Roark, B.S.; Holger Roehl, Ph.D.; Gao Yonghua, B.S. Pharm.

Biocompatibility of Materials Used in Packaging Systems, Medical Devices, and Implants Expert Panel
DANIEL L. NORWOOD, PH.D., *Chair*
Douglas J. Ball, M.S.; Stephen A. Barat, Ph.D.; William P. Beierschmitt, Ph.D.; Denise Bohrer, Ph.D.; Michael N. Eakins, Ph.D.; Jill A. Glosson, B.A.; John Iannone, B.S.; Renaud Janssen, Ph.D.; Douglas E. Kiehl, M.S.; Wendy Mach, B.S.; Robert Przygoda; Anita Y. Sawyer, M.S.; Cheryl L. M. Stults, Ph.D.

Clinical Trial Materials (GDP) Expert Panel—CONCLUDED
MARY G. FOSTER, PHARM.D., BFA, *Chair*
Christopher Anderson, M.A.; Rafik H. Bishara, Ph.D.; Glaucia K. Braga, Ph.D.; Jeffrey Carrico, Pharm.D.; Steven A. Jacobs, M.B.A.; Martin Jeiven, M.S.; Claude Jolicoeur, B.S.; Gao Yonghua, B.S. Pharm.

⟨661.3⟩ Plastic Systems Used for Manufacturing Pharmaceutical Products Expert Panel
DENNIS R. JENKE, PH.D., *Chair*
Weibing Ding, Ph.D.; Michael N. Eakins, Ph.D.; Mary G. Foster, Pharm.D., BFA; James Hathcock, Ph.D.; Jerold (Jerry) M. Martin, M.S.; Diane M. Paskiet, M.S.; Robert Steininger; Cheryl L. M. Stults, Ph.D.; Ken M. Wong, M.S.

⟨662⟩ Metal Packaging Components and Systems—CONCLUDED
CHERYL L. M. STULTS, PH.D., *Chair*
Peter Claessens; Benjamin Jeyaretnam, Ph.D.; Ralph Lessor, Ph.D.; Sara Miller, M.S.; Gaby Reckzuegel; John Willenbrock, B.S.

General Chapters—Statistics
ROBERT R. SINGER, M.S., *Chair*
Bruno Boulanger, Ph.D.; Richard Burdick, Ph.D.; David Christopher, M.S.; David Lansky, Ph.D.; Dave LeBlond, Ph.D.; Juris Meija, Ph.D.; Anthony Okinczyc, M.P.H.; Peter Rigsby, M.S.; Dennis Sandell, Ph.D.; Timothy Schofield, M.A.; Charles Tan, Ph.D.; Edwin van den Heuvel, Ph.D.; Jane Weitzel; Harry Yang, Ph.D.

Content Uniformity with Large Sample Sizes Expert Panel
DENNIS SANDELL, PH.D., *Chair*
Ilgaz Akseli, Ph.D.; James S. Bergum, Ph.D.; Paul Curry, Ph.D.; Walter Hauck, Ph.D.; Jeffrey Hofer, M.S.; Gregory L. Larner, M.S.; Raymond Skwierczynski, Ph.D.

Expert Committees for the *National Formulary*

Excipients Monographs 1
ERIC MUNSON, PH.D., *Chair*
Thiago Carvalho, Ph.D.; Brian Carlin, Ph.D.; Richard Cawthorne, Ph.D.; Richard Creekmore, Ph.D.; Vivek Dave, Ph.D.; Felicitas Guth; Otilia Koo, Ph.D.; Phil Merrell, Ph.D.; Dominic Moore; Chris Moreton, Ph.D.; Jasmine Musakhanian, M.S.; Charles Vesey, M.S.; Richard Wendt, Ph.D.; Jin Zhao, Ph.D.

⟨1059⟩ Chapter Excipient Performance Expert Panel
GREGORY AMIDON, PH.D., AND RICHARD MORETON, PH.D., *Co-Chairs*
Ilgaz Akseli; Shaukat Ali, Ph.D.; Lawrence Block, Ph.D.; Thiago Cardoso Carvalho, Ph.D.; Brian Carlin, Ph.D.; Richard Creekmore, Ph.D.; Stephen Hoag, Ph.D.; Johannes Khinast; Jasmine Musakhanian, M.S.; Natarajan Rajagopalan, Ph.D.; Hiroko Shibata, Ph.D.; Jiasheng Tu, Ph.D.; Katherine Ulman, B.S.; Maureen Taylor Vander Fliet, M.S.

⟨1059⟩ Excipient Performance Expert Panel—CONCLUDED
ERIC A. SCHMITT, PH.D., *Chair*
Abdullah M. Al-Mohizea, Ph.D.; Shaukat Ali, Ph.D.; Lawrence H. Block, Ph.D.; Patrick Deluca, Ph.D.; Carl Frey, M.S.; Xiaorong He, Ph.D., M.B.A.; Stephen W. Hoag, Ph.D.; Michelle A. Long, Ph.D.; Richard C. Moreton, Ph.D.; Prabu Nambiar, Ph.D., M.B.A.; James A. Ponto, M.S.; Kent Sternitzke, Ph.D.; Kevin A. Swiss, Ph.D.; Sean V. Taylor, Ph.D.

⟨1197⟩ Good Distribution Practices for Bulk Pharmaceutical Excipients Expert Panel—CONCLUDED
RICHARD C. MORETON, PH.D., *Chair*
Lawrence H. Block, Ph.D.; William Dale Carter, M.S.; Zak T. Chowhan, Ph.D.; Marc Fages; Elizabeth

www.webofpharma.com

Ferguson-Brown; Mary G. Foster, Pharm.D., BFA; Linda A. Herzog, M.B.A.; Ashok V. Katdare, Ph.D.; Zakiya Kurdi, Ph.D.; Edward G. Malawer, Ph.D., CQA; Frank Milek, Ph.D.; Becca Mitchell; Dwight Mutchler; Garnet E. Peck, Ph.D.; Mike Schultz, R.Ph.; Alexa Smith, M.S.; Glenn Sokoloski; Kelly Taylor; Jiasheng Tu, Ph.D.

Excipients Monographs 2
MARY C. HOUCK, PH.D., *Chair*
Lawrence H. Block, Ph.D.; Andrew Bluj; Tim Cabelka, Ph.D.; Arya Jayatilaka, Ph.D.; Russell Maus, Ph.D.; Robert E. Osterberg, R.Ph., Ph.D., Fellow-ATS; Julie Warner Pier, M.S.; Anisul Quadir, Ph.D.; Gwen Rucker; Barbara Serr, Ph.D.; Huimin Sun, Ph.D.; Jiasheng Tu, Ph.D.; Jacqueline Tordik; Fan Wu, Ph.D.; Timothy Yasika

Glycerin Expert Panel
TIM B. CABELKA, PH.D., *Chair*
Frances K. Byrne, M.S.; Ian A. Duncan, Ph.D.; Tanja Natterer; Marian J. Rinken, Ph.D.; Gwen E. Rucker, B.S.; David A. Sharknas, B.S.; Hong Zhou, Ph.D.

Povidones Expert Panel—CONCLUDED
BERNHARD D. FUSSNEGGER, PH.D., AND CARL PERINI, M.S., *Co-Chairs*
Feng Chen, Ph.D.; David J. Fillar, M.B.A.; Edward G. Malawer, Ph.D.; Syed A.A. Rizvi, Ph.D.; John W. Spink, Ph.D.; Fan Wu, Ph.D.

Talc Methods Expert Panel
JULIE WARNER PIER, M.S., AND MARTIN RUTSTEIN, PH.D., *Co-Chairs*
Daniel Crane; Sean M. Fitzgerald, B.S.; Mickey E. Gunter, Ph.D.; Don Halterman, M.S.; Mary C. Houck, Ph.D.; Lee Poye, B.S.; Matthew S. Sanchez, Ph.D.; Alan M. Segrave, B.S.; Gary P. Tomanino, B.S.; Drew R. Van Ordern, M.A.; James Webber, Ph.D.

Expert Committees for the *USP* and the *Dietary Supplements Compendium*

Botanical Dietary Supplements and Herbal Medicines
ROBIN J. MARLES, PH.D., *Chair*
Thomas Brendler, B.A.; Josef A. Brinckmann; Paula Naomi Brown, Ph.D.; Angela Calderon, Ph.D.; Steven Dentali, Ph.D.; Edward Fletcher, B.A.; Stefan Gafner, Ph.D.; Joerg Gruenwald, Ph.D.; De-an Guo, Ph.D.; Sukhdev Swami Handa, M.Pharm., Ph.D.; James Harnly, Ph.D.; Craig Hopp, Ph.D.; Scott A. Jordan, Ph.D.; Ikhlas Khan, Ph.D.; Richard Ko, Pharm.D., Ph.D.; Tieraona Low Dog, M.D.; Mirtha Navarro, Ph.D.; Pilar Pais, Ph.D.; Guido Pauli, Pharm.D., Ph.D.; Eike Reich, Ph.D.; Paul Schiff, Ph.D.; Shangmei Shi, B.S.

Green Tea Extract Hepatotoxicity Expert Panel
RICHARD KO, PHARM.D., AND AMY LYNNE ROE, PH.D., *Co-Chairs*
Joseph M. Betz, Ph.D.; Bill Gurley, Ph.D.; Kan He, Ph.D.; Scott Jordan, Ph.D.; Mahendra P. Kapoor, Ph.D.; Tieraona Low Dog, M.D.; Robin James Marles, Ph.D.; Victor Navarro, M.D.; Mary Paine, Ph.D.; Pradyumna Theertham Rao, Ph.D.; Cynthia Rider, Ph.D.

Herbal Medicines Compendium (HMC) East Asia Expert Panel
DE-AN GUO, PH.D., *Chair*
Yuan-shiun Chang, Ph.D.; ShiLin Chen, Ph.D.; Takashi Hakamatsuka, Ph.D.; Shen Ji, Ph.D.; Yeong Shik Kim, Ph.D.; Hwee-Ling Koh, Ph.D.; Clara Bik San Lau, Ph.D; Ping Li, Ph.D.; Qing Li, Ph.D.; Shuangcheng Ma, Ph.D.; Shangmei Shi; Viet Hung Tran, Ph.D.; Pengfei Tu, Ph.D.; Wanying Wu, Ph.D.; Zhongzhen Zhao, Ph.D.

Herbal Medicines Compendium (HMC) South Asia Expert Panel
SUKHDEV S. HANDA, PH.D., FNAIM, FNA.SC, *Chair*
Amit Agarwal, Ph.D.; Rasadah Mat Ali, Ph.D.; Mohamed Zahir Mohammed Farhad; C.K. Katiyar, Ph.D.; Bhawanishankar Madhira, Ph.D.; Ami Fazlin Syed Mohamed, Ph.D.; A. Nagarajan, Ph.D.; D.G. Naik, Ph.D.; Sankaran Natarajan, Ph.D.; M.K. Raina, Ph.D.; J.L.N. Sastry, Ph.D.; Rajeev Kr. Sharma, Ph.D.; R. Sundaram, Ph.D.; Neeraj Tandon, Ph.D.; Surapote Wongyai, Ph.D.

Dietary Supplements Safety Modeling—CONCLUDED
MARY L. HARDY, M.D., *Chair*
V.A. Shiva Ayyadurai, Ph.D.; Mary A. Fox, Ph.D., M.P.H.; Scott A. Jordan, Ph.D.; Mkaya Mwamburi, M.D., Ph.D., M.A. (Econ); Diane R. Mould, Ph.D.; Robert E. Osterberg, R.Ph., Ph.D., Fellow-ATS; Charlie Yoe, Ph.D.

Cannabis Expert Panel
IKHLAS A. KHAN, PH.D., *Chair*
Paula N. Brown, Ph.D.; Lawrence Deyton, M.D.; Mahmoud A. Elsohly, Ph.D.; Sytze Elzinga, M.S.; Christopher Hudalla, Ph.D.; Holly Johnson, Ph.D.; Robin Marles, Ph.D.; Jeremy Melanson, Ph.D.; Ethan Russo, M.D.; Gordon Vrdoljak, Ph.D.; Andrew Waye, Ph.D.; Joshua Wurzer, B.S.

Non-Botanical Dietary Supplements
DENNIS K.J. GORECKI, B.S.P., PH.D., *Chair*
Joseph M. Betz, Ph.D.; Michael S. Bradley, M.S.; James Brooks, Ph.D.; Bill Gurley, Ph.D.; Chung Hyun, M.S.; Joy A. Joseph, M.S.; Raimar Löbenberg, Ph.D.; Richard Myers, Ph.D.; James Neal-Kababick, B.S.; Peter Rice, Pharm.D., Ph.D.; Amy Roe, Ph.D.; Aniko Solyom, Ph.D.; Karunakar Sukuru, Ph.D.; Darryl Sullivan, Ph.D.

⟨2251⟩ Adulteration of Dietary Supplements with Drugs and Drug Analogs Expert Panel—CONCLUDED
DENNIS K.J. GORECKI, B.S.P., PH.D., *Chair*
Joseph M. Betz, Ph.D.; Pei Chen, Ph.D.; Hwee-Ling Koh, Ph.D.; Ikhlas A. Khan, Ph.D.; James Neal-Kababick, B.S.; John Spink, Ph.D.; Darryl Sullivan, Ph.D.

Extended-Release Dietary Supplements Expert Panel—CONCLUDED
JOY A. JOSEPH, M.S., *Chair*
Charles Barton, Ph.D., DABT; Joseph F. Borzelleca, Ph.D.; Michael S. Bradley, M.S.; James R. Brooks, Ph.D.; Marion Ehrich, Ph.D.; Vivian A. Gray, B.S.; Carol Johnston, Ph.D., R.D.; Raimar Löbenberg, Ph.D.; Alexander G. Schauss, Ph.D., FACN; Elizabeth A. Yetley, Ph.D.

Probiotics Expert Panel
MARY ELLEN SANDERS, *Chair*
Mike Bradley, M.S.; Marie-Eve Boyte; James Brooks, Ph.D.; Pierre Burguiere, Ph.D.; Scott Jackson, Ph.D.; David Keller; Marco Pane, M.S.; Amy Roe, Ph.D.; Jean Schoeni, Ph.D.; Buffy Stahl, B.S.; Christina Skovgaard Vegge, Ph.D.

www.webofpharma.com

Expert Committees for the *Food Chemicals Codex*

Food Ingredients
JONATHAN W. DEVRIES, PH.D., *Chair*
Richard C. Cantrill, Ph.D.; Junshi Chen, M.D.; Hwei-Fang Cheng, Ph.D.; Henry Chin, Ph.D.; Grady Chism, Ph.D.; Robin Churchill, Ph.D.; Roger Clemens, DrPH; John Clos, Ph.D.; Helen Darling, Ph.D.; Andrew Ebert, Ph.D.; Jaap Evers, Ph.D.; Carl Frey, M.S.; Einat Haleva, Ph.D.; Lori Klopf, Ph.D.; Hemant G. Koshia, Ph.D.; Dana Krueger, B.S.; Diane McColl, J.D.; Bert Popping, Ph.D.; Yoko Uematsu, Ph.D.; Yongning Wu, Ph.D.; Liangli Yu, Ph.D.

Dietary Proteins Expert Panel
SNEH D. BHANDARI, PH.D., *Chair*
Spencer Carter, Ph.D.; Jonathan W. DeVries, Ph.D.; Melanie Downs, Ph.D.; Jaap Evers, Ph.D.; Christophe Fuerer, Ph.D.; Boyan Gao, Ph.D.; Philip Haselberger, B.A.; Xiao Ping Huang, Ph.D.; Philip E. Johnson, Ph.D.; Steve Holroyd, Ph.D.; Andrew T. Mackey, Ph.D.; James Neal-Kababick, B.S.; Reto Portmann, Ph.D.; Haowei Song, Ph.D.; Chao Wu, Ph.D.; Jinchuan Yang, Ph.D.; We Zhu, Ph.D.

Food Adulteration Expert Panel
HENRY CHIN, PH.D., *Chair*
Grant Abernethy, Ph.D.; David Bolliet; Richard C. Cantrill, Ph.D.; Christophe Cavin, Ph.D.; Hwei-Fang Cheng, Ph.D.; Robin Churchill, Ph.D.; Helen Darling, Ph.D.; Jonathan W. DeVries, Ph.D.; Andrew Ebert, Ph.D.; Kim Huynh-Ba, M.S.; Shaun Kennedy; Dana Krueger, B.S.; Michele Lees, Ph.D.; Fernando Antunes Lopes; Bert Popping, Ph.D.; Lars Reimann, M.S.; Roman Romero, Ph.D.; John Spink, Ph.D.; Thomas Tarantelli, B.S.; Yoko Uematsu, Ph.D.; Saskia van Ruth, Ph.D.; Carl Winter, Ph.D.; Yongning Wu, Ph.D.

Food Adulterants Hazard Identification Expert Panel—CONCLUDED
HENRY CHIN, PH.D., *Chair*
Andrew Ebert, Ph.D.; Richard Lane, Ph.D.; Diane McColl, J.D.; Bert Popping, Ph.D.; Joseph Scimeca; Carl Winter, Ph.D.

Non-Targeted Methods for Milk Ingredients Expert Panel—CONCLUDED
ROBERT MAGALETTA, PH.D., *Chair*
Sned D. Bhandari, Ph.D.; Jonathan W. DeVries, Ph.D.; Gerard Downey, Ph.D.; Stephen Ellison, Ph.D.; James M. Harnly, Ph.D.; Elizabeth Hobbs; Steven Holroyd, Ph.D.; Gregory A. Israelson, B.S.; Joseph Katzenmeyer, Ph.D.; Andrew T. Mackey, Ph.D.; Benjamin B. Perston, Ph.D.; Jianwei Qin, Ph.D.; Roman Romero, Ph.D.; Paul Wehling; Thomas Wheat, Ph.D.; Steven Zbylut, Ph.D.

Olive Oil Authenticity and Quality Expert Panel
RICHARD C. CANTRILL, PH.D., *Chair*
Diego Luis Garcia-Gonzalez, Ph.D.; Claudia Guillaume, M.S.; Zohar Kerem, Ph.D.; Paul H. Miller, B.A.; Agusti Jordi Romero, Ph.D.; Selina Wang, Ph.D.

Expert Committees for the *USP* and *USP on Compounding*

Compounding
GIGI S. DAVIDSON, B.S. PHARM., DICVP, *Chair*
Lisa Ashworth, B.S. Pharm., R.Ph.; Gus Bassani, Pharm.D.; Edmund J. Elder, Jr., Ph.D.; Ryan Forrey, Pharm.D., M.S.; Deborah Houston, Pharm.D.; Brenda Jensen, M.A.; Patricia C. Kienle, M.P.A.; William A. Mixon, M.S.; John Musil, Pharm.D.; David Newton, Ph.D.; Alan Parr, Pharm.D., Ph.D.; Abby Roth; Robert Shrewsbury, Ph.D.; Connie Rae Sullivan, B.S. Pharm.; James T. Wagner; Brenda Yuzdepski, B.S. Pharm.

Compounding with Hazardous Drugs Expert Panel—CONCLUDED
PATRICIA C. KIENLE, M.P.A., *Chair*
Thomas H. Connor, Ph.D.; Eric Kastango, M.B.A., B.S. Pharm.; Melissa A. McDiarmid, M.D., M.P.H.; Kenneth R. Mead, Ph.D.; Martha Polovich, Ph.D.; Lucille A. Power; James T. Wagner

Government Liaisons to Expert Committees and Expert Panels

Jibril Abdus-Samad, Ph.D.; Rajiv Agarwal, Ph.D.; Mohammed Ahmed, M.S.; Om Anand, Ph.D.; Shalini Anand, Ph.D.; Kristen Anderson, Ph.D.; Matthew Barlow, RN, BSN; Julie N. Barrows, Ph.D.; Jacinta Batson, M.B.A.; Eden Bermingham, DVM, M.S., DACVCP; Ashwinkumar Bhirde, Ph.D.; Jonathan Bray, B.S.; Michael Brent; Michael Brewer, Ph.D.; Daniel Brown; Janice Brown, M.S.; Lana Bruney, Ph.D.; Teresa Cain, Ph.D.; Steven Casper, Ph.D.; Wiley Chambers, M.D.; Jane Chang, Ph.D.; Richard Chang, Ph.D.; Anissa Cheung, Ph.D.; Donna Christner, Ph.D.; John Cipollo, Ph.D.; David Claffey, Ph.D.; Maegen Colehour, M.S.; Celia Noemi Cruz, Ph.D.; Mike Darj, Ph.D.; Swapan De, Ph.D.; Ian DeVeau, Ph.D.; Julie Dohm, Ph.D.; Zedong Dong, Ph.D.; Jason Dreabit, M.A.; Sheila Dreher-Lesnick, Ph.D.; Stephanie Emory, Ph.D.; Okponanabofa Eradiri, Ph.D.; Erika E. Englund, Ph.D.; Cory Evans, Ph.D.; Raafat Fahmy, Ph.D.; Karen Farizo, Ph.D.; Adam Fisher, Ph.D.; Daniel Folmer, Ph.D.; Rick Friedman, M.S.; Michael Furness, M.S.; Christopher Galliford, Ph.D.; Zongming Gao, Ph.D.; Mary Papa Ghods, R.Ph.; Mohamed Ghorab, Ph.D.; Tapash Ghosh, Ph.D.; Devinder Gill, Ph.D.; Gurpreet Gill-Sangha, Ph.D.; Lillie Golson, Pharm.D.; Jennifer Goode, Ph.D.; Edisa Gozun, Pharm.D.; Yin Guo, Ph.D.; William Hallett, Ph.D.; Blake Hamann, Ph.D.; Bruce Harris, Ph.D.; Danielle Marie Harris, Pharm.D.; Joel Hathaway, Ph.D.; Lijuan He, Ph.D.; Mohammad Heidaran, Ph.D.; William Hess, B.S. Pharm.; Yong Hu, Ph.D.; Gloria Huang, Ph.D.; Laura Huffman, M.S.; Gregory Hunter, Ph.D.; Latiff Hussain, Ph.D.; Mai Huynh, Ph.D.; Karthik Iyer, Ph.D.; Edwin Jao, Ph.D.; Young Jhon; Jacquin L. Jones, MS, BSN, RN; Ravindra Kasliwal, Ph.D.; David Keire, Ph.D.; Michael Kennedy, Ph.D.; James Kenney, Ph.D.; Saeed Khan, Ph.D.; Erin Kim, Ph.D.; Kathryn E. King, Ph.D.; Anna Kooser, Ph.D.; Bogdan Kurtyka, Ph.D.; David Lau, M.A.; Hyoung S. Lee, Ph.D.; Sau Lee, Ph.D.; David Lewis, Ph.D.; Xihao Li; Jing Li, Ph.D.; Jennifer Liang, Ph.D.; June Liang, Ph.D.; Tsai-Lien Lin, Ph.D.; Heather Lombardi, Ph.D.; Ewa Marszal, Ph.D.; Marilyn Martinez, Ph.D.; Timothy McGovern, Ph.D.; Jeffrey Medwid, Ph.D.; Randa Melhem, Ph.D.; John Metcalfe, Ph.D.; Yana Mille, B.S. Pharm.; Adil Mohammad, Ph.D.; Magdi Mossoba, Ph.D.; Laura Moussa, Ph.D.; Karunakar Neelam, Ph.D.; Nina Ni, Ph.D.; Pallavi Nithyanandan, Ph.D.; Scott Edward Norris, Ph.D.; Sarai Obando, Ph.D.; Thomas O'Connor, Ph.D.; Steven Oh, Ph.D.; Andrea Ottesen, Ph.D.; Frank Perrella, Ph.D.; Erika Pfeiler, Ph.D.; Zhihao Peter Qiu, Ph.D.; Radhika Rajagopalan, Ph.D.; Muthukumar Ramaswamy, Ph.D.; Sam G. Raney, Ph.D.; Ashutosh Rao, Ph.D.; Shahnaz Read, Ph.D.; Bhagwant Rege, Ph.D.; James Rice, Ph.D.; Jason Rodriguez, Ph.D.; Sara Rothman; Allen Rudman, Ph.D.; Tracy Rupp, Ph.D.; R.D. Satzger, Ph.D.;

www.webofpharma.com

Zuben Erach Sauna, Ph.D.; Deborah Schmiel, Ph.D.; Suzanne Sechen, Ph.D.; Hamid Shafiei, Ph.D.; Rakhi Shah, Ph.D.; Millie Shah, Ph.D.; Balajee Shanmugam, Ph.D.; Meiyu Shen, Ph.D.; Akhtar Siddiqui, Ph.D.; Mark Skasko, Ph.D.; Fenhong Song, Ph.D.; Charudharshini Srinivasan, Ph.D.; Jannavi Srinivasan, Ph.D.; Marla Stevens-Riley, Ph.D.; Ann Stohlman, V.M.D.; Yichun Sun, Ph.D.; Jennifer Swisher, Ph.D.; Frank Switzer, Ph.D.; Neeru Takiar, M.S.; Carmen Tartera, Ph.D.; Jennifer Thomas, Ph.D.; Colleen Thomas, Ph.D.; Yiying Tsai, Pharm.D.; Saleh Turujman, Ph.D.; Katherine Tyner, Ph.D.; Ubrani Venkataram, Ph.D.; Steve Wolfgang, Ph.D.; Jeffrey Woodruff, Ph.D.; Bingyuan Wu, Ph.D.; Geoffrey Wu, Ph.D.; Larisa Wu, Ph.D.; Jo Wyeth, Pharm.D.; Jingyue Yang, Ph.D.; Yuda Zong, Ph.D.

Other Government Liaisons

Agency for Healthcare Research and Quality
Diane D. Cousins, R.Ph.; Deborah G. Perfetto, Pharm.D., M.S.

U.S. Centers for Disease Control and Prevention
Christopher A. Elkins, Ph.D.; Nadine Shehab, Pharm.D., M.P.H.; Melissa Schaefer, M.D.

Health Canada
Matthew Decan, Ph.D.; Jessica Priem, M.S.; Nana Bafi-Yeboa, Ph.D.

National Administration of Medicines, Foods, and Medical Technology (ANMAT)
Carolina Abba, Pharm.D.

National Institutes of Health
Rao Rapaka, Ph.D.; Cynthia Dyann Davis, Ph.D.

National Institute of Food and Drug Safety Evaluation
Kwangmoon Lee, Ph.D.

Saudi Food and Drug Authority
Ali Mohammed Alhomaidan, Ph.D.; Ali M. Alsamil, Ph.D.

In Memoriam

USP would like to acknowledge the following Expert Volunteers and Government Liaisons who have passed away during the 2015–2020 Cycle:
Stefan Christians, Ph.D. (Biologics Monographs 2—Proteins)
Scott V. W. Sutton, Ph.D. (General Chapters—Microbiology Expert Committee)

www.webofpharma.com

Members and Delegates of the United States Pharmacopeial Convention as of May 31, 2019

Academic Institutions and Associations Thereof—Academic Associations

American Association of Colleges of Nursing, Ginette A. Pepper, Ph.D.

American Association of Colleges of Osteopathic Medicine, Anthony J. Silvagni, D.O., Pharm.D., M.Sc., FACOFP, FAFPE

American Association of Colleges of Pharmacy, Lynette R. Bradley-Baker, R.Ph., Ph.D.

Association of American Medical Colleges, David W. Nierenberg, M.D.

Association of American Veterinary Medical Colleges, Dawn M. Boothe, D.V.M., Ph.D., M.S.

Association of Faculties of Pharmacy of Canada, Raimar Löebenberg, Ph.D.

Academic Institutions and Associations Thereof—Colleges and Schools of Medicine

Baylor College of Medicine, Lynn C. Yeoman, Ph.D.

Boston University School of Medicine, Carol T. Walsh, Ph.D.

Brody School of Medicine at East Carolina University, Abdel A. Abdel-Rahman, Ph.D., FAHA

Case Western Reserve University School of Medicine, Walter B. Geho, M.D., Ph.D.

Chicago Medical School at Rosalind Franklin University of Medicine and Science, Ann K. Snyder, Ph.D.

Columbia University College of Physicians and Surgeons, Rudina Odeh-Ramadan, Pharm.D.

Creighton University School of Medicine, Peter W. Abel, Ph.D.

Duke University School of Medicine, Sharon L. Ellison, Pharm.D.

East Tennessee State University James H. Quillen College of Medicine, Kenneth E. Ferslew, Ph.D.

Eastern Virginia Medical School, Arun Mehnaatamai Mohanram, M.D.

Florida State University College of Medicine, Graham A. Patrick, Ph.D.

Geisel School of Medicine at Dartmouth, Lionel D. Lewis, M.D.

Georgetown University School of Medicine, Thomas G. Sherman, Ph.D.

Howard University College of Medicine, Robert E. Taylor, M.D., Ph.D., FACP

Joan C. Edwards School of Medicine Marshall University, Gary O. Rankin, Ph.D.

Johns Hopkins University School of Medicine, Daniel M. Ashby, M.S., FASHP

Keck School of Medicine of University of Southern California, Paul D. Holtom, M.D.

Loma Linda University School of Medicine, John N. Buchholz, Ph.D.

Louisiana State University School of Medicine, Kurt J. Varner, Ph.D.

Loyola University Chicago Stritch School of Medicine, Jawed Fareed, Ph.D.

Mayo Medical School, Peter J. Post, Pharm.D., R.Ph.

Medical University of South Carolina College of Medicine, Kenneth D. Tew, Ph.D.

Meharry Medical College School of Medicine, Clivel G. Charlton, Ph.D.

Mercer University School of Medicine, Wayne Glasgow, Ph.D.

Morehouse School of Medicine, Ward Kirlin, Ph.D.

Mount Sinai School of Medicine, Joel S. Mindel, M.D., Ph.D.

New York Medical College, Mario A. Inchiosa, Ph.D.

New York University School of Medicine, Lewis S. Nelson, M.D.

Northeast Ohio Medical University College of Medicine, Moses O. Oyewumi, B.Pharm., Ph.D.

Northwestern University Feinberg School of Medicine, Steven M. Belknap, M.D.

Ohio State University College of Medicine, Robert J. Weber, Pharm.D., M.S.

Pennsylvania State University College of Medicine, Kelly D. Karpa, Ph.D., R.Ph.

Rutgers, the State University of New Jersey, New Jersey Medical School, Deborah Lazzarino, Ph.D.

Rutgers, the State University of New Jersey, Robert Wood Johnson Medical School, Susan Goodin, Pharm.D., FCCP, BCOP

Saint Louis University School of Medicine, Heather Macarthur, Ph.D., B.Sc.

San Juan Bautista School of Medicine, Marielis E. Rivera Ruiz, Ph.D.

Sidney Kimmel Medical College at Thomas Jefferson University, Walter Kraft, M.D., M.S., FACP

Southern Illinois University School of Medicine, Carl Faingold, Ph.D.

SUNY at Buffalo School of Medicine and Biomedical Sciences, Paul J. Kostyniak, Ph.D., DABT

SUNY Downstate Medical Center College of Medicine, Jacob V. Aranda, M.D., Ph.D., FRCPC

SUNY Upstate Medical University, David F. Lehmann, M.D., Pharm.D.

Temple University School of Medicine, Alan Cowan, Ph.D.

Texas A&M Health Science Center College of Medicine, D. Samba Reddy, Ph.D., R.Ph.

Texas Tech University Health Sciences Center School of Medicine, Michael P. Blanton, Ph.D.

The Warren Alpert Medical School of Brown University, Wayne D. Bowen, Ph.D.

www.webofpharma.com

Tufts University School of Medicine, Ross W. Thompson, M.S., R.Ph.

Tulane University School of Medicine, David W. Busija, Ph.D., M.D. (Hon.)

Universidad Central del Caribe School of Medicine, Harry Mercado, M.D.

University of Alabama at Birmingham School of Medicine, Richard J. Whitley, M.D.

University of Arizona College of Medicine, Hillary A. Franke, M.D., M.S.

University of Arkansas for Medical Sciences College of Medicine, Paul L. Prather, Ph.D., B.S.

University of California Davis School of Medicine, Timothy E. Albertson, M.D., M.P.H., Ph.D.

University of California San Francisco School of Medicine, Linda L. Liu, M.D.

University of Chicago Pritzker School of Medicine, Michael L. Maitland, M.D., Ph.D.

University of Cincinnati College of Medicine, Marianne F. Ivey, Pharm.D., M.P.H., FASHP

University of Connecticut Health Center School of Medicine, Kimberly J. Metcalf, Pharm.D.

University of Hawaii John A. Burns School of Medicine, Robert A. Nichols, Ph.D.

University of Iowa Carver College of Medicine, Donald E. Letendre, Pharm.D.

University of Kansas School of Medicine, Sam J. Enna, Ph.D.

University of Kentucky College of Medicine, Lisa A. Cassis, Ph.D.

University of Louisville School of Medicine, Demetra Antimisiaris, Pharm.D., CGP, FASCP

University of Maryland School of Medicine, Margaret M. McCarthy, Ph.D.

University of Massachusetts Medical School, Roy Guharoy, Pharm.D., M.B.A., FCP, FCC, FASHP

University of Michigan Medical School, Paul F. Hollenberg, Ph.D.

University of Mississippi School of Medicine, Deborah S. Minor, Pharm.D., FAHA

University of Missouri–Kansas City School of Medicine, James M. Wooten, Pharm.D.

University of Nevada School of Medicine, Iain L. Buxton, Pharm.D.

University of New Mexico Health Sciences Center School of Medicine, Arti Prasad, M.D.

University of North Dakota School of Medicine and Health Sciences, James E. Porter, Ph.D.

University of Oklahoma College of Medicine, Pramod K. Chetty, M.D.

University of Pennsylvania School of Medicine, Patrick J. Brennan, M.D.

University of Pittsburgh School of Medicine, Dennis P. Swanson, M.S.

University of Puerto Rico School of Medicine, Miguel A. Marrero, M.D.

University of South Alabama College of Medicine, Jack A. DiPalma, B.S., M.D.

University of South Carolina School of Medicine, Kenneth B. Walsh, Ph.D.

University of South Florida Morsani College of Medicine, Shufeng Zhou, M.D., Ph.D.

University of Tennessee, Memphis College of Medicine, Trevor W. Sweatman, Ph.D.

University of Texas Medical School at Houston, Gary C. Rosenfeld, Ph.D.

University of Utah School of Medicine, Richard J. Sperry, M.D., Ph.D.

University of Virginia School of Medicine, Matthew T. Jenkins, M.S., Pharm.D.

University of Washington School of Medicine, Suzanne M. Allen, M.D., M.P.H.

University of Wisconsin School of Medicine and Public Health, F. Michael Hoffmann, Ph.D.

Vanderbilt University School of Medicine, C. Michael Stein, M.D.

Virginia Commonwealth University School of Medicine, Dominic A. Sica, M.D.

Wake Forest University School of Medicine, Mary L. Bennett, B.S., Pharm.D.

Washington University School of Medicine in St. Louis, Evan D. Kharasch, M.D., Ph.D.

Wayne State University School of Medicine, Stephen A. Lerner, M.D.

Wright State University Boonshoft School of Medicine, Khalid M. Elased, Ph.D., R.Ph.

Yale University School of Medicine, Seth Powsner, M.D.

Academic Institutions and Associations Thereof—Colleges and Schools of Pharmacy

Albany College of Pharmacy and Health Sciences, Robert A. Hamilton, Pharm.D., M.P.H.

Auburn University Harrison School of Pharmacy, Jayachandra Ramapuram, Ph.D.

Butler University College of Pharmacy and Health Sciences, Sudip K. Das, Ph.D.

Campbell University School of Pharmacy, Jinsong Hao, Ph.D.

Chicago State University College of Pharmacy, Duc P. Do, Ph.D.

Creighton University School of Pharmacy and Health Professions, J. Christopher Bradberry, Pharm.D.

Drake University College of Pharmacy and Health Sciences, Abebe E. Mengesha, Ph.D.

Duquesne University School of Pharmacy, Ira S. Buckner, Ph.D.

Ernest Mario School of Pharmacy at Rutgers University, Longqin Hu, Ph.D.

Ferris State University College of Pharmacy, Kim E. Hancock, Ph.D.

Florida A & M University College of Pharmacy and Pharmaceutical Sciences, Mandip Sachdeva, Ph.D.

Hampton University School of Pharmacy, Chengan Du, Ph.D., M.P.H.

Howard University College of Pharmacy, Nursing and Allied Health Sciences, Emmanuel O. Akala, Ph.D.

Idaho State University College of Pharmacy, Kevin W. Cleveland, Pharm.D.

Lake Erie College of Osteopathic Medicine School of Pharmacy, Sachin S. Devi, B.Pharm., Ph.D.

Loma Linda University School of Pharmacy, Victoria Maskiewicz, Ph.D.

Long Island University Arnold and Marie Schwartz College of Pharmacy and Health Sciences, David R. Taft, B.S.Pharm., Ph.D.

Massachusetts College of Pharmacy and Health Sciences—Boston, David A. Williams, Ph.D.

Massachusetts College of Pharmacy and Health Sciences School of Pharmacy—Worcester, Robert Campbell, Ph.D., M.S.

Mercer University College of Pharmacy and Health Sciences, Ayman Akil, Ph.D.

Midwestern University Chicago College of Pharmacy, Anna Kabakov, Pharm.D.

Midwestern University College of Pharmacy—Glendale, Bill J. Bowman, Ph.D., R.Ph.

North Dakota State University College of Pharmacy, Nursing and Allied Sciences, Jagdish Singh, Ph.D.

Northeastern University Bouve College of Health Sciences School of Pharmacy, Mansoor M. Amiji, Ph.D.

NOVA Southeastern University College of Pharmacy, Hamid H. Omidian, Ph.D., M.Sc., B.Sc.

Ohio Northern University Raabe College of Pharmacy, Yousif B. Rojeab, B.Sc., Ph.D.

Oregon State University College of Pharmacy, J. Mark Christensen, Ph.D.

Pacific University College of Health Professions School of Pharmacy, Reza Karimi, Ph.D., R.Ph.

Palm Beach Atlantic University Gregory School of Pharmacy, Adwoa O. Nornoo, B.Pharm., M.S., Ph.D.

Purdue University School of Pharmacy and Pharmaceutical Sciences, Stephen R. Byrn, Ph.D.

www.webofpharma.com

Roseman University of Health Sciences College of Pharmacy, Mark C. Decerbo, Pharm.D., BCPS, BCNSP

Samford University McWhorter School of Pharmacy, John J. Arnold, Ph.D., B.S.

Shenandoah University Bernard J. Dunn School of Pharmacy, Nina Hengen, M.D., Ph.D.

South Carolina College of Pharmacy, James J. Sterrett, Pharm.D., BCPS

South Dakota State University College of Pharmacy, David L. Helgeland, B.S.Pharm., M.B.A., Ed.D.

South University School of Pharmacy, S. Craig Dyar, Ph.D.

Southern Illinois University Edwardsville School of Pharmacy, William M. Kolling, Ph.D.

Southwestern Oklahoma State University School of Pharmacy, Shelly Stockton, Ph.D.

St. John's University College of Pharmacy and Allied Health Professions, Abu Serajuddin, Ph.D.

St. Louis College of Pharmacy, John Pieper, Pharm.D.

SUNY at Buffalo School of Pharmacy and Pharmaceutical Sciences, Alfred T. Reiman, R.Ph.

Temple University School of Pharmacy, Michael Borenstein, Ph.D.

Texas Southern University College of Pharmacy and Health Sciences, Dong Liang, Ph.D.

Texas Tech University Health Sciences Center School of Pharmacy, Arthur A. Nelson, Ph.D., R.Ph.

The Ohio State University College of Pharmacy, Robert W. Brueggemeier, Ph.D.

The University of Arizona College of Pharmacy, Terri Warholak, Ph.D., R.Ph.

The University of Iowa College of Pharmacy, Mickey L. Wells, Ph.D.

The University of Louisiana at Monroe College of Pharmacy, Sami M. Nazzal, Ph.D.

The University of North Carolina at Chapel Hill Eshelman School of Pharmacy, Dennis M. Williams, Pharm.D.

The University of Texas at Austin College of Pharmacy, Janet C. Walkow, Ph.D.

The University of Toledo College of Pharmacy, Kenneth S. Alexander, R.Ph., Ph.D.

Touro University California College of Pharmacy, Alison McCormick, Ph.D.

University of Arkansas for Medical Sciences College of Pharmacy, Melanie Reinhardt, Pharm.D.

University of California San Diego Skaggs School of Pharmacy and Pharmaceutical Sciences, Grace M. Kuo, Pharm.D., Ph.D., M.P.H.

University of California San Francisco School of Pharmacy, Leslie Z. Benet, Ph.D.

University of Cincinnati James L. Winkle College of Pharmacy, Pankaj B. Desai, Ph.D.

University of Colorado Skaggs School of Pharmacy, Peter J. Rice, Pharm.D., Ph.D.

University of Florida College of Pharmacy, Rhonda Cooper-DeHoff, Pharm.D., M.S., FAHA

University of Georgia College of Pharmacy, Gurvinder Singh Rekhi, Ph.D., M.S.

University of Houston College of Pharmacy, F. Lamar Pritchard, Ph.D.

University of Illinois at Chicago College of Pharmacy, Stephanie Y. Crawford, Ph.D., M.P.H.

University of Kansas School of Pharmacy, Christian Schoneich, Ph.D.

University of Maryland School of Pharmacy, Natalie D. Eddington, Ph.D.

University of Michigan College of Pharmacy, Gregory E. Amidon, B.S., Ph.D.

University of Minnesota College of Pharmacy, Lynda Welage, Pharm.D., FCCP

University of Mississippi School of Pharmacy, Michael A. Repka, D.D.S., Ph.D.

University of Missouri–Kansas City School of Pharmacy, Cydney E. McQueen, Pharm.D.

University of Nebraska Medical Center College of Pharmacy, Michael F. Powell, B.S. Pharm., M.S.

University of Oklahoma College of Pharmacy, Vibhudutta Awasthi, Ph.D., M.Pharm.

University of Pittsburgh School of Pharmacy, Michael A. Zemaitis, Ph.D.

University of Rhode Island College of Pharmacy, David R. Worthen, Ph.D., J.D.

University of São Paulo College of Pharmacy, Prof. Terezinha de Jesus Andreoli Pinto

University of Southern California School of Pharmacy, Stan G. Louie, Pharm.D.

University of Tennessee Health Science Center College of Pharmacy, Hassan Almoazen, Ph.D.

University of the Pacific Thomas J. Long School of Pharmacy and Health Sciences, Xiaoling Li, Ph.D.

University of the Sciences in Philadelphia, Philadelphia College of Pharmacy, Edward F. Foote, Pharm.D., BCPS, FCCP

University of Utah College of Pharmacy, John W. Mauger, Ph.D.

University of Washington School of Pharmacy, Thomas K. Hazlet, Pharm.D., Dr.P.H.

University of Wisconsin–Madison School of Pharmacy, James E. DeMuth, Ph.D., R.Ph.

University of Wyoming School of Pharmacy, Kurt Dolence, Ph.D.

Virginia Commonwealth University/Medical College of Virginia School of Pharmacy, Barbara J. Exum, Pharm.D.

Washington State University College of Pharmacy, Danial E. Baker, Pharm.D., FASHP, FASCP

Wayne State University Eugene Applebaum College of Pharmacy and Health Sciences, Sheila M. Wilhelm, Pharm.D.

West Virginia University School of Pharmacy, Arthur I. Jacknowitz, Pharm.D.

Western University of Health Sciences College of Pharmacy, Sunil Prabhu, Pharm.D., R.Ph.

Wilkes University Nesbitt School of Pharmacy, Harvey A. Jacobs, Ph.D.

Xavier University of Louisiana College of Pharmacy, Tarun K. Mandal, Ph.D.

Academic Institutions and Associations Thereof—Colleges and Schools of Osteopathic Medicine

NOVA Southeastern University Dr. Kiran C. Patel College of Osteopathic Medicine, Lynne Lafferty, Pharm.D., N.D., M.B.A.

Consumer and Other Organizations Representing the Public Interest

AARP, Leigh Purvis

Alzheimer's Association, William Thies, Ph.D.

American Autoimmune Related Diseases Association, Virginia T. Ladd, R.T.

American Cancer Society, Inc., Leonard Lichtenfeld, M.D., MACP

American Heart Association, Robert Lee Page II, Pharm.D., M.S.P.H., FCCP, FAHA, FASHP, FASCP, BCPS, CGP

Arthritis Foundation, John H. Klippel, M.D.

Center for Science in the Public Interest, David G. Schardt, M.S.

Consumers Union, Lisa L. Gill, B.A.

ECRI Institute, Marcus Schabacker, M.D., Ph.D.

Institute for Safe Medication Practices, Michael R. Cohen, R.Ph., M.S., Sc.D., FASHP

National Organization for Rare Disorders, Ronald J. DeBellis, B.S., Pharm.D.

Governmental Bodies or Divisions or Associations Thereof

Agency for Healthcare Research and Quality, Scott R. Smith, Ph.D.

www.webofpharma.com

Argentine Pharmacopoeia, Héctor A. Giuliani, Pharm.D.

Association of Food and Drug Officials, Cynthia T. Culmo

Brazilian Pharmacopoeia Commission, Mônica da Luz Carvalho Soares

Brazil's National Institute of Quality Control in Health, Filipe Soares Quirino Silva, Ph.D.

British Pharmacopoeia Commission, James Pound

Centers for Disease Control and Prevention, Julian Jolly, Pharm.D., R.Ph.

Centers for Medicare & Medicaid Services, Jeffrey A. Kelman, M.D.

China National Center for Food Safety Risk Assessment, Junshi Chen, M.D.

Chinese Pharmacopoeia Commission, Zhang Wei

European Directorate for the Quality of Medicines and HealthCare, Susanne Keitel

FDA Center for Biologics Evaluation and Research, Ewa Marszal, Ph.D., M.S.

FDA Center for Devices and Radiological Health, Jose A. Centeno, Ph.D., FRSC

FDA Center for Drug Evaluation and Research, Pallavi Nithyanandan, Ph.D.

FDA Center for Food Safety and Applied Nutrition, Daniel E. Folmer, Ph.D.

FDA Center for Veterinary Medicine, Hilary Hoffman, M.S., Ph.D.

Federal Commission for Protection Against Sanitary Risks, Rocio del Carmen Alatorre Eden-Wynter, M.C.

Federal Service on Surveillance in Healthcare of Russian Federation, Valentina F. Kosenko, Ph.D., Pharm.

Food and Drugs Authority, Ghana, Eric Karikari-Boateng, M.S.

Food, Medicine and Health Care Administration and Control Authority of Ethiopia, Yehulu Denekew Alamneh, B.Sc., M.A.

General Directorate of Medicines, Supplies and Drugs, Laura Cerón, Pharm.D.

Health Canada, Karen Reynolds

Health Canada, Food Directorate, Barbara Lee

Indian Pharmacopoeia Commission, G.N. Singh, Ph.D., M.Pharm.

Indonesian Pharmacopoeia Commission, Augustine Zaini, M.S.

International Trade Administration—U.S. Department of Commerce, James D. Rice, B.A., M.A.

Japan's Ministry of Health Labour and Welfare, Pharmaceuticals and Medical Devices Agency, Nobuo Uemura

Jordan Food and Drug Administration, Dr. Lubna R. Qusous

Ministry of Food and Drug Safety, Dr. Jin-Ho Wang

Ministry of Health of the Republic of Belarus, Liudmila Reutskaya, Pharm.D.

Ministry of Health of the Republic of Uzbekistan, Mirzohidjon Kodirov, Ph.D.

Ministry of Health of Turkey, Filiz Bütev Koç, Ph.D.

Morocco's Directorate of Medicines and Pharmacy, Laila Hakkou

National Agency for Food and Drug Administration and Control, Dr. Monica H. Eimunjeze

National Association of Boards of Pharmacy, Elizabeth Scott Russell, B.S.Pharm.

National Center for Quality Control, Ofelia del Rosario Villalva Rojas, Q.F.

National Centre for Expertise of Drugs, Medical Products and Medical Equipment, Ardak U. Tulegenova, Ph.D.

National Drug Authority of Uganda, Gordon Katende Sematiko

National Health Surveillance Agency, Lais Santana Dantas, B.S.

National Institute for Biological Standards and Control, Adrian F. Bristow, Ph.D.

National Institute of Drug and Food Surveillance, Eduardo Vergel Bayona

National Institute of Metrology, Quality and Technology, Valnei Smarçaro da Cunha, Ph.D.

National Institute of Standards and Technology, Michael J. Tarlov, Ph.D.

National Institutes for Food and Drug Control, Bo Li, Ph.D.

National Institutes of Health, Robert DeChristoforo, R.Ph., M.S., FASHP

Permanent Commission of the Pharmacopoeia of the United Mexican States, Maria del Carmen Becerril-Martinez

Pharmacopoeia of Chinese Taipei, Jaw-Jou Kang, Ph.D.

Pharmacy Council of India, Bhojraj Suresh, M.Pharm., Ph.D., D.Sc.

Philippine Pharmacopeia, Maria Lourdes C. Santiago, B.S.Pharm., M.S.Pharm.

Saudi Food and Drug Authority, Abdullah S. Alhomoud, M.S.

State Administration of Ukraine on Medicinal Products, Andrii V. Shovkovyi

Tanzania Food and Drugs Authority, Yonah Hebron Mwalwisi, M.Sc., B.Pharm.

Thai Pharmacopoeia Committee, Kornvika Charupant, Ph.D.

The Pharmacy Board of Sierra Leone, Wiltshire C.N. Johnson, B.Pharm. (Hons.), M.Sc , MPSSL, FPCPharm

Therapeutic Goods Administration of Australia, Vivienne Christ, B.App.Sc., MASM

Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines, Oleksandr I. Gryzodub, Ph.D.

United States Agency for International Development, Anthony F. Boni

United States Air Force–Office of the Surgeon General, Deborah Myers, R.Ph., M.B.A.

United States Army–Office of the Surgeon General, John Spain, Pharm.D., BCPS

United States Department of Health and Human Services, Donald Wright, M.D., M.P.H.

United States Food and Drug Administration Office of the Commissioner, Hany W. Demian, M.S.

United States Navy Bureau of Medicine and Surgery–Office of the Surgeon General, Thinh V. Ha, Pharm.D., M.B.A., M.S.

United States Public Health Service–Office of the Surgeon General, David J. Skanchy, Ph.D., B.S.

Vietnamese Pharmacopoeia Commission, Luc Thi Thu Hang, Ph.D.

Other Health Practitioner, Professional and Scientific Associations

AACC International, Amy Hope

Academy of Managed Care Pharmacy, Afton Wagner, Pharm.D.

Academy of Nutrition and Dietetics, Ann Erickson, M.A., R.D.

American Academy of Neurology, Jack J. Chen, Pharm.D., FASCP, FCCP

American Academy of Nurse Practitioners, Jan Towers, Ph.D., NP-C

American Academy of Ophthalmology, Suber S. Huang, M.D., M.B.A.

American Academy of Physician Assistants, Marie-Michele Leger, M.P.H., PA-C

American Academy of Veterinary Pharmacology and Therapeutics, Carol A. Davis, Ph.D.

American Association of Pharmaceutical Scientists, Christopher McCurdy, Ph.D., R.Ph.

American Botanical Council, Mark Blumenthal

American Chemical Society, Mary M. Kirchhoff, Ph.D.

American College of Clinical Pharmacology, Peter Wiernik, M.D.

American College of Clinical Pharmacy, Michael S. Maddux, Pharm.D., FCCP

American College of Physicians, Douglas S. Paauw, M.D.

American Dental Association, Kathleen Ziegler, Pharm.D.

American Dental Education Association, Jeffrey Stewart, D.D.S., M.S.

American Geriatrics Society, Todd P. Semla, M.S., Pharm.D., BCPS, FCCP, AGSF

American Medical Association, Amy B. Cadwallader, Ph.D.

American Nurses Association, Rita Munley Gallagher, Ph.D., R.N.

American Optometric Association, Jimmy D. Bartlett, O.D.

www.webofpharma.com

American Pharmacists Association, Thomas E. Menighan, R.Ph., M.B.A., FAPhA

American Public Health Association, Deborah K. Walker, Ed.D.

American Society for Clinical Pharmacology and Therapeutics, Patricia W. Slattum, Pharm.D., Ph.D.

American Society for Microbiology, Amy L. Leber, Ph.D.

American Society for Nutrition, Robert M. Russell, M.D.

American Society for Pharmacology and Experimental Therapeutics, Mary Paine, Ph.D.

American Society for Quality, Donald C. Singer, M.S.

American Society of Anesthesiologists, Elizabeth Rebello, M.D.

American Society of Consultant Pharmacists, Arnold E. Clayman, Ph.D., FASHP

American Society of Health-System Pharmacists, Paul W. Abramowitz, Pharm.D., FASHP

American Veterinary Medical Association, Michael A. Strobel, D.V.M., M.S.

Argentine Association of Industrial Pharmacy and Biochemistry, Federico Montes de Oca, M.B.A.

Brazilian National Association of Compounding Pharmacists, Maria do Carmo Garcez

Calibration and Validation Group, Herman Lam, Ph.D.

Canadian Society for Pharmaceutical Sciences, Neal M. Davies, B.Sc. Pharm., Ph.D., R.Ph.

Drug Information Association, Katie N. Truong, B.A.

European Federation for Pharmaceutical Sciences, Hendrik J. de Jong, Ph.D.

Infusion Nurses Society, Mary C. Alexander, C.R.N.I., M.A., R.N., CAE, FAAN

Institute of Food Technologists, William Fisher, M.S.

International Academy of Compounding Pharmacists, Gary E. McCrory, B.S.Pharm.

International Council of Nurses, David C. Benton, RGN, RMN, BSC, MPhil, FFNF, FRCN

International Society for Cellular Therapy, Joseph C. Laning, Ph.D.

International Society for Pharmaceutical Engineering, Stephen M. Tyler, M.S.

Korea Biomedicine Industry Association, JungTae Park

National Alliance of State Pharmacy Associations, Rebecca P. Snead, R.Ph., B.S.Pharm.

National Community Pharmacists Association, Ronna B. Hauser, Pharm.D.

National Pharmaceutical Association, Barry A. Bleidt, Ph.D., Pharm.D.

National Pharmacy Technician Association, Michael J. Johnston, CPhT

New Jersey Pharmaceutical Quality Control Association, Barbara J. Ferguson, B.A.

Pan American Pharmaceutical Federation, Maria Elena Girard-Cuesy

Parenteral Drug Association, Inc., Tina S. Morris, Ph.D.

Regulatory Affairs Professional Society, Paul Brooks

Society of Critical Care Medicine, Timothy S. Yeh, M.D., FCCM

Society of Nuclear Medicine and Molecular Imaging, Jeffrey P. Norenberg, Pharm.D.

Western Compendial Discussion Group, Assad J. Kazeminy, Ph.D.

Health Practitioner, Professional and Scientific Associations—U.S. State Medical Societies

Connecticut State Medical Society, Henry Jacobs, M.D., J.D.

Hawaii Medical Association, Jone Geimer-Flanders, D.O.

Illinois State Medical Society, Maitreyi Janarthanan, M.D.

Indiana State Medical Association, Daria Schooler, M.D., R.Ph.

Iowa Medical Society, Harold W. Miller, M.D.

Kansas Medical Society, Tracie Collins, M.D., M.P.H.

Maine Medical Association, Stevan Gressitt, M.D.

Massachusetts Medical Society, Bruce G. Karlin, M.D.

MedChi–Maryland State Medical Society, Peter H. Rheinstein, M.D., J.D., M.S.

Medical Association of the State of Alabama, Allan R. Goldstein, M.D.

Medical Society of New Jersey, Joseph N. Micale, M.D.

Medical Society of the District of Columbia, Kim A. Bullock, M.D.

Medical Society of the State of New York, Richard S. Blum, M.D.

Missouri State Medical Association, Patrick J. Mills

Montana Medical Association, Jean Branscum

New Mexico Medical Society, Jerry D. McLaughlin, M.D.

North Dakota Medical Association, Robert (Rob) W. Beattie, M.D.

Pennsylvania Medical Society, Ralph Schmeltz, M.D., FACP, FACE

Puerto Rico Medical Association, Rolance G. Chavier-Roper, M.D.

Rhode Island Medical Society, Peter A. Hollmann, M.D.

South Dakota State Medical Association, E. Paul Amundson, M.D.

Tennessee Medical Association, John J. Ingram III, M.D.

Texas Medical Association, Kevin H. McKinney, M.D., FACE, FACP

Utah Medical Association, Michelle S. McOmber, B.S., M.B.A.

West Virginia State Medical Association, Kevin W. Yingling, M.D., R.Ph., FACP

Wisconsin Medical Society, Thomas M. Derrig, M.D.

Health Practitioner, Professional and Scientific Associations—U.S. State Pharmacy Associations

Alabama Pharmacy Association, Valerie A. Oakley, Pharm.D.

Alaska Pharmacists Association, Sara Doran-Atchison, Pharm.D., M.P.H., NCPS

Arizona Pharmacy Association, Kelly Fine, R.Ph.

Arkansas Pharmacists Association, Kristen G. Riddle, Pharm.D.

California Pharmacists Association, Jon R. Roth, CAE

Colegio de Farmaceuticos de Puerto Rico, Milagros Morales, R.Ph.

Colorado Pharmacists Society, Jennifer Davis, Pharm.D., M.B.A.

Connecticut Pharmacists Association, Peter J. Sposato, R.Ph., B.C.N.P.

Delaware Pharmacists Society, Kenneth (Kevin) Musto, R.Ph.

Florida Pharmacy Association, Michael A. Moné, J.D., R.Ph., FAPhA

Georgia Pharmacy Association, Larry L. Braden, B.S., R.Ph., D.Sc.

Hawaii Pharmacists Association, Ronald T. Taniguchi, Pharm.D.

Idaho State Pharmacy Association, Pam Eaton

Illinois Pharmacists Association, Norman A. Hoback, B.S.Pharm.

Indiana Pharmacists Alliance, Daniel D. Degnan III, Pharm.D., M.S.

Iowa Pharmacy Association, Thomas R. Temple, R.Ph., M.S.

Kansas Pharmacy Association, Alison Smith, Pharm.D.

Kentucky Pharmacists Association, Don B. Kupper, R.Ph., M.B.A.

Louisiana Pharmacists Association, Randall Brooks, B.S.

Maine Pharmacy Association, Laurier A. Lamie, R.Ph.

Maryland Pharmacists Association, Matthew G. Shimoda, Pharm.D.

Massachusetts Pharmacists Association, Steven D. Geoffroy, R.Ph.

Michigan Pharmacists Association, Eric D. Roath, Pharm.D.

Minnesota Pharmacists Association, Lowell J. Anderson, D.Sc., FAPhA, FFIP

Mississippi Pharmacists Association, Kimsey O'Neal, Pharm.D.

www.webofpharma.com

Missouri Pharmacy Association, Ron L. Fitzwater, CAE, M.B.A.

Montana Pharmacy Association, Lori Morin, M.B.A., Pharm.D.

Nebraska Pharmacists Association, Samuel C. Augustine, Pharm.D., FAPhA

New Hampshire Pharmacists Association, Elizabeth A. Robertson, R.Ph.

New Jersey Pharmacists Association, Steve H. Zlotnick, Pharm.D.

New Mexico Pharmacists Association, William G. Troutman, Pharm.D.

North Carolina Association of Pharmacists, Stephen F. Eckel, Pharm.D., M.H.A.

North Dakota Pharmacists Association, Michael D. Schwab

Ohio Pharmacists Association, Amelia S. Bennett, R.Ph.

Oklahoma Pharmacists Association, Wiley L. Williams, J.D.

Oregon State Pharmacy Association, Tiffany Pye, Pharm.D., BCPS

Pennsylvania Pharmacists Association, George R. Haynes, Pharm.D., Ph.D.

Pharmacy Society of Wisconsin, Susan M. Kleppin, R.Ph., FASHP

Rhode Island Pharmacists Association, John Grossomanides, Pharm.D.

South Carolina Pharmacy Association, Craig Burridge, M.S., CAE

South Dakota Pharmacists Association, Eric Grocott, Pharm.D., B.S.

Tennessee Pharmacists Association, Micah Cost, Pharm.D., M.S.

Texas Pharmacy Association, Debbie Garza, R.Ph.

Utah Pharmacists Association, Adam W. Jones

Virginia Pharmacists Association, William T. Lee, D.Ph, M.P.A, FASCP

Washington D.C. Pharmacy Association, Michael J. Kim, Pharm.D.

Washington State Pharmacy Association, Jeff Rochon, Pharm.D.

West Virginia Pharmacists Association, Patty Johnston, B.S.

Wyoming Pharmacy Association, William H. Rathburn, R.Ph.

Manufacturer, Trade and Affiliated Associations

American Chemistry Council, Michael P. Walls, Esq.

American Herbal Products Association, Holly E. Johnson, Ph.D.

American Hospital Association, John R. Combes, M.D.

Animal Health Institute, Richard A. Carnevale, V.M.D.

Association for Accessible Medicines, David R. Gaugh, R.Ph.

Association of the European Self-Medication Industry, Christelle Anquez-Traxler

Biotechnology Innovation Organization, Earl S. Dye, Ph.D.

Bulk Drug Manufacturers Association, Airra Krishna Reddy, B.Sc.

Compressed Gas Association, Michael B. Tiller, B.S., B.A.

Consumer Health Products Canada, Adam C. Kingsley, B.Sc., NP, CAE

Consumer Healthcare Products Association, John Punzi, Ph.D.

Council for Responsible Nutrition, James C. Griffiths, Ph.D.

Egyptian Chamber of Pharmaceutical Industry, Abdulla Molokhia, Ph.D.

European Generic Medicines Association, Julie Marechal-Jamal, M.S.

Flavor and Extract Manufacturers Association, John H. Cox, J.D.

Generic Animal Drug Alliance, Stephanie Batliner

Indian Drug Manufacturers' Association, Daara B. Patel, B.Com., D.I.M.M.

International Dairy Foods Association, John T. Allan III, B.S., M.S.

International Federation of Pharmaceutical Manufacturers and Associations, Caroline Mendy

International Food Additives Council, Robert Rankin

International Generic and Biosimilar Medicines, Nicholas Cappuccino, Ph.D.

International Pharmaceutical Excipients Council of the Americas, Priscilla Zawislak

Korea Pharmaceutical and Bio-Pharma Manufacturers Association, Hee-Mok Won, Pharm.D., M.S.

Mexico's National Chamber of the Pharmaceutical Industry, J. Rivelino Flores, M.Sc.

National Association of Chain Drug Stores, Alex J. Adams, Pharm.D.

Personal Care Products Council, Beth Anne Lange, Ph.D., B.S.

São Paulo State Pharmaceutical Manufacturers Association, Nelson dos Santos, Jr.

The Joint Commission, Jeannell M. Mansur, B.S. Pharm., Pharm.D.

The Jordanian Association of Manufacturers of Pharmaceuticals & Medical Appliances, Hanan J. Sboul, M.B.A., CAE

Nongovernmental Standards-Setting and Conformity Assessment Bodies

Accreditation Council for Pharmacy Education, Dimitra V. Travlos, Pharm.D., BCPS

American Type Culture Collection, Elizabeth J. Kerrigan, B.A.

Association for the Advancement of Medical Instrumentation, Joseph Carl Lewelling, B.A., M.A.

Chilean Pharmacopeia Foundation, Caroline R. Weinstein-Oppenheimer, Ph.D.

Clinical and Laboratory Standards Institute, Glen Fine, M.S., M.B.A., CAE

www.webofpharma.com

www.webofpharma.com

2018 Recognition of Monograph and Reference Material Donors

USP acknowledges and appreciates the support of the following companies that participated in the USP standards-setting process by providing information for the development of new monographs or by donating reference standard candidate materials. The companies listed are those that either provided monographs that were published as proposals in 2018 or donated reference material candidates that were released as USP Reference Standards in 2018.

Platinum Level Donors

AbbVIe
Ajanta
Amneal
Apotex
AstraZeneca
Aurobindo
Bayer
Boehringer Ingelheim
Cambrex
China Pharmaceutical University
Croda
DSM
DuPont
Evonik
FMC Corporation
Glaxosmith Kline
Glenmark
Himalaya
ICL Performance
Impax
Janssen
Lupin
Medichem
Medisca
Merck
Moehs Iberica
Mylan
Natural Remedies
Novartis
Par Pharma
Roche
Sanofi-Aventis
Shanghai Institute of Materia Medica
Shire
St. John Fisher College – School of Pharmacy
Sun Yat-Sen University
Teva
UCB Pharma
West-Ward
Zydus Cadila

Gold Level Donors

Ajinomoto
Albemarle
Bachem
Biogen
Chiesi
Cipla
Dow Chemical
Dr. Reddy's
Eastman
Fresenius Kabi
Gland Pharma
HRA Pharma
Indena
Jubilant
Koster Keunen
Lundbeck
Mallinckrodt
Merz
PCCA
Pfizer
Pharmavite
Scinopharm
Sicor
SMS Pharmaceuticals
Sun Pharma
Welch, Holme & Clark
Xavier University of Louisiana

Silver Level Donors

Aarti Industries
ACS Dofbar
Aizant
Alembic
Almirall
Alzo
Amano Enzyme

www.webofpharma.com

AMRI
AMSA
Apicore
Arbor
Arevipharma
Aspen
BASF
Baxter
Beijing Gingko
Beneo
Bergstrom Nutrition
Bimeda
BioPQQ
Biospectra
Blue California
Bristol-Myers Squibb
Budenheim Iberica
Cargill
CBC
Cellmark
Changzhou Pharmaceutical Factory
Chemische Fabrik
CKD Bio
Clariant
Corden
Daiichi Sankyo
Dipharma
Dishman
DMV-Fonterra
Dottikon
Eisai
Emcure
Enzyme Development
Eon Labs
Ethypharm
Excella
Excelvite
Fabbrica Italiana Sintetici
Ferrer
FineTech
Flamma
Flavor and Extract Manufacturers Association
Formosa
Fujian Fukang
Gattefosse
GE Healthcare
Helsinn
Herbalife
Hovione
Hunan Dongting
Icrom
Impossible Foods
Indivior
Indoco
Interquim
JB Chemicals
Jiaherb
K.A. Malle
K.D. Pharma
Kaneka
Kashiv Pharma
Katra Phyto Chem
Katwijk Chemie
Kongo Chemical
Kyowa Hakko
Lilly
L'Oreal
Lusochimica
Mangalam
Marksans
Menadiona
Micro Advanced Research Centre
Monteloeder
Mutchler
New Francisco (YunFu City) Biotechnology
Noramco
Novitium
Novo Nordisk
Olon
Orchid
Otsuka
Pfanstiehl
Piramal
Proto Chemicals
Purecircle
Quimica Sintetica
RPG Life Sciences
Sabinsa
Septodont
Sichuan Long March
Sichuan Xieli
Siegfried
Sintetica-Bioren
SPI Polyols
SST Corporation
T&R Chemical
Taiyo
Taro
Tennants
Thermo Fisher
Tianjin Tianyao
Trifarma
Unichem
Unique Pharmaceutical Labs
Upsher-Smith
Vanderbilt
Vertellus
Wilmar
Wockhardt
Yamasa
Zhejiang Apeloa Jianyuan
Zhejiang Hisoar
Zhejiang MedicineCo
Zhejiang University
Zoetis

www.webofpharma.com

Articles of Incorporation

In May of 1900, the USP Board of Trustees was directed by the Convention to incorporate the USP organization under the laws of the District of Columbia. Because the District of Columbia required that the majority of officers subscribing to the Certificate of Incorporation be residents, the filing was slightly delayed in order to appoint appropriate representatives. Nevertheless, the articles of incorporation were prepared, appropriately signed, and finally filed on July 11 of that year. The original certificate reads as follows:

Certification of Incorporation

This is to certify that we, whose names are hereunto subscribed, citizens of the United States, of full age, and a majority citizens of the District of Columbia, do associate ourselves together pursuant to the provisions of sections 545–552 inclusive of the Revised Statutes of the United States relating to the District of Columbia and of the Act of Congress to amend the same, approved the twenty-third day of April 1884, under the corporate name of The United States Pharmacopoeial Convention.

This Association is organized for a period of nine hundred and ninety-nine years. The particular objects and business of this Association are the encouragement and promotion of the science and art of medicine and pharmacy by selecting by research and experiment and other proper methods and by naming such materials as may be properly used as medicines and drugs with formulas for their preparation; by establishing one uniform standard and guide for the use of those engaged in the practice of medicine and pharmacy in the United States whereby the identity, strength, and purity of all such medicines and drugs may be accurately determined, and for other like and similar purposes; and by printing and distributing at suitable intervals such formulas and the results of such and similar selections, names and determinations among the members of this Association, pharmacists, and physicians generally in the United States and others interested in pharmacy and medicine.

The management and control of the affairs, funds, and property of this Association for the first year of its existence shall be vested in a Board of Trustees consisting of the seven following persons:

ALBERT E. EBERT
SAMUEL A.D. SHEPPARD
WILLIAM S. THOMPSON
CHARLES E. DOHME
GEORGE W. SLOAN
HORATIO C. WOOD
CHARLES RICE

In testimony whereof we have hereunto set our hands and affixed our seals this seventh day of July, 1900.

WILLIAM S. THOMPSON	[SEAL]	WILLIAM M. MEW	[SEAL]
G. LLOYD MAGRUDER	[SEAL]	FRANK M. CRISWELL	[SEAL]
JOHN T. WINTER	[SEAL]	MURRAY G. MOTTER	[SEAL]
THOMAS C. SMITH	[SEAL]		

www.webofpharma.com

USP Governance

Bylaws

The USP 2015–2020 Bylaws are available at *http://www.usp.org/about-usp/leadership/bylaws*.

Rules and Procedures

The Rules and Procedures of the 2015–2020 Council of Experts are available at *http://www.usp.org/about/leadership/policies-rules/council-experts*.

USP Policies

The USP policies are available at *http://www.usp.org/about-usp/leadership/policies-rules*.

www.webofpharma.com

USP 43

THE UNITED STATES PHARMACOPEIA

Official from May 1, 2020

FORTY-THIRD REVISION

www.webofpharma.com

Copyright ©2019 The United States Pharmacopeial Convention
12601 Twinbrook Parkway, Rockville, MD 20852
All rights reserved.

www.webofpharma.com

USP Admissions

Articles Admitted to *USP 43*

First Supplement (August 1, 2019)

GENERAL CHAPTERS
⟨64⟩ Probiotic Tests
⟨644⟩ Conductivity of Solutions

USP MONOGRAPHS
Azelastine Hydrochloride Ophthalmic Solution
Benzphetamine Hydrochloride
Benzphetamine Hydrochloride Tablets
Clindamycin Hydrochloride Compounded Oral Solution
Desvenlafaxine
Desvenlafaxine Fumarate
Desvenlafaxine Succinate
Diclofenac Sodium Topical Solution
Estriol Compounded Vaginal Cream
Famciclovir Tablets
Itraconazole Capsules
Leucovorin Calcium for Injection
Naproxen Compounded Oral Suspension
Phytonadione Compounded Oral Suspension
Tranexamic Acid Tablets

DIETARY SUPPLEMENTS
Cyanocobalamin Chewable Gels

Second Supplement (December 1, 2019)

GENERAL CHAPTERS
⟨60⟩ Microbiological Examination of Nonsterile Products—Tests for *Burkholderia Cepacia* Complex
⟨509⟩ Residual DNA Testing
⟨825⟩ Radiopharmaceuticals—Preparation, Compounding, Dispensing, and Repackaging
⟨1071⟩ Rapid Microbial Tests for Release of Sterile Short-Life Products: A Risk-Based Approach
⟨1085⟩ Guidelines on the Endotoxin Tests
⟨1229.16⟩ Prion Inactivation
⟨1236⟩ Solubility Measurements
⟨1430⟩ Analytical Methodologies Based on Scattering Phenomena—General
⟨1430.1⟩ Analytical Methodologies Based on Scattering Phenomena—Static Light Scattering
⟨1430.2⟩ Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size
⟨1430.4⟩ Analytical Methodologies Based on Scattering Phenomena—Electrophoretic Light Scattering (Determination of Zeta Potential)
⟨1430.5⟩ Analytical Methodologies Based on Scattering Phenomena—Small Angle X-Ray Scattering and Small Angle Neutron Scattering

USP MONOGRAPHS
Alosetron Hydrochloride
Alosetron Tablets
Amiodarone Hydrochloride Tablets
Amlodipine and Olmesartan Medoxomil Tablets
Rotigotine

DIETARY SUPPLEMENTS
Chinese Skullcap Root
Chinese Skullcap Root Dry Extract
Chinese Skullcap Root Powder
Cobamamide
L-alpha-Glycerylphosphorylcholine
Omega-3 Free Fatty Acids

New Articles Appearing in *USP 43* That Were Not Included in *USP 42* Including Supplements

[NOTE—The articles included in this list are noted in the book with the following symbols ▲▲USP 1-May-2020. This applies to new articles as well as sections of existing items that have been revised.]

GENERAL CHAPTERS
⟨856⟩ Near-Infrared Spectroscopy
⟨1850⟩ Evaluation of Screening Technologies for Assessing Medicine Quality

USP 43
Arginine Hydrochloride Compounded Oral Solution
Carboxymethylcellulose Sodium Compounded Intraperitoneal Solution, Veterinary
Chlorambucil Compounded Oral Suspension
Chloramphenicol Compounded Oral Suspension, Veterinary
Clonidine Hydrochloride Compounded Oral Suspension
Diclofenac Potassium for Oral Solution
Hydrochlorothiazide Compounded Oral Suspension
Phenytoin Compounded Topical Gel
Rotigotine Transdermal System
Sodium Benzoate Compounded Oral Solution
Torsemide Compounded Oral Suspension
Vancomycin Hydrochloride Compounded Oral Solution

DIETARY SUPPLEMENTS
Creatine

Articles Included in *USP 42* But Not Included in *USP 43*

USP MONOGRAPHS
Aminobenzoate Potassium for Oral Solution
Aminobenzoate Potassium Tablets
Aminoglutethimide
Aminoglutethimide Tablets

www.webofpharma.com

Aminosalicylate Sodium
Aminosalicylate Sodium Tablets
Aminosalicylic Acid Tablets
Astemizole
Astemizole Tablets
Atropine Sulfate Tablets
Azelastine Hydrochloride Ophthalmic Solution
Cefazolin Ophthalmic Solution
Chromium Cr 51 Edetate Injection
Cocaine and Tetracaine Hydrochlorides and Epinephrine Topical Solution
Dehydrocholic Acid Tablets
Demeclocycline Oral Suspension
Demeclocycline Hydrochloride Capsules
Dexamethasone Sodium Phosphate Cream
Dexamethasone Sodium Phosphate Ophthalmic Ointment
Dextroamphetamine Sulfate Capsules
Diazepam Capsules
Diazepam Extended-Release Capsules
Dicloxacillin Sodium for Oral Suspension
Diethylstilbestrol Injection
Diethylstilbestrol Tablets
Emetine Hydrochloride
Emetine Hydrochloride Injection
Ergoloid Mesylates Sublingual Tablets
Indium In 111 Ibritumomab Tiuxetan Injection
Indium In 111 Satumomab Pendetide Injection
Iobenguane I 131 Injection
Iodinated I 131 Albumin Aggregated Injection
Iodohippurate Sodium I 123 Injection
Iodohippurate Sodium I 131 Injection
Rose Bengal Sodium I 131 Injection
Krypton Kr 81m
Levodopa Capsules
Levodopa Tablets
Loracarbef
Loracarbef Capsules
Loracarbef for Oral Suspension
Magaldrate Oral Suspension
Magaldrate Tablets
Mazindol Tablets
Menadiol Sodium Diphosphate Injection
Menadiol Sodium Diphosphate Tablets
Menadione Injection
Manganese Sulfate Injection
Meclocycline Sulfosalicylate
Meclocycline Sulfosalicylate Cream
Mephenytoin
Mephenytoin Tablets
Meprednisone
Meprobamate Oral Suspension
Mesoridazine Besylate
Mesoridazine Besylate Injection
Mesoridazine Besylate Oral Solution
Mesoridazine Besylate Tablets
Metaproterenol Sulfate Inhalation Solution
Metaraminol Bitartrate
Methacycline Hydrochloride Capsules
Methdilazine Hydrochloride
Methdilazine Hydrochloride Oral Solution
Methdilazine Hydrochloride Tablets
Methenamine Oral Solution
Methenamine Mandelate for Oral Solution
Methenamine Mandelate Oral Suspension
Methenamine Mandelate Delayed-Release Tablets
Methylbenzethonium Chloride Ointment
Methylbenzethonium Chloride Topical Powder
Methyldopa Oral Suspension
Methyldopa and Chlorothiazide Tablets
Methylprednisolone Acetate Cream
Methysergide Maleate Tablets
Metyrapone Tablets
Mezlocillin Sodium
Mezlocillin for Injection
Miconazole Injection
Monobenzone Cream
Moricizine Hydrochloride Tablets
Morrhuate Sodium Injection
Nafcillin Sodium Capsules
Nafcillin Sodium for Oral Solution
Nafcillin Sodium Tablets
Nalidixic Acid
Nalidixic Acid Oral Suspension
Nalidixic Acid Tablets
Netilmicin Sulfate
Netilmicin Sulfate Injection
Norfloxacin
Norfloxacin Ophthalmic Solution
Norfloxacin Tablets
Omeprazole Oral Suspension
Oxacillin Sodium Capsules
Oxacillin Sodium for Oral Solution
Oxazepam Tablets
Oxprenolol Hydrochloride Tablets
Oxtriphylline Oral Solution
Oxtriphylline Tablets
Oxytetracycline and Nystatin Capsules
Oxytetracycline and Nystatin for Oral Suspension
Oxytetracycline Calcium
Oxytetracycline Calcium Oral Suspension
Pantoprazole Oral Suspension
Phenmetrazine Hydrochloride
Phenmetrazine Hydrochloride Tablets
Phensuximide
Phensuximide Capsules
Chromic Phosphate P 32 Suspension
Sodium Phosphate P 32 Solution
Potassium Perchlorate Capsules
Probucol
Probucol Tablets
Propyliodone Injectable Oil Suspension
Sisomicin Sulfate
Sisomicin Sulfate Injection
Suprofen
Suprofen Ophthalmic Solution
Technetium Tc 99m Albumin Injection
Technetium Tc 99m Albumin Colloid Injection
Technetium Tc 99m Apcitide Injection
Technetium Tc 99m Arcitumomab Injection
Technetium Tc 99m Depreotide Injection
Technetium Tc 99m Etidronate Injection
Technetium ^{99m}Tc Fanolesomab Injection
Technetium Tc 99m Gluceptate Injection
Technetium Tc 99m Lidofenin Injection
Technetium Tc 99m Nofetumomab Merpentan Injection
Thioridazine Oral Suspension
Xenon Xe 127
Xenon Xe 133 Injection

www.webofpharma.com

USP Annotated List

General Notices, Monographs, General Chapters, Reagents, and Tables Affected by Changes Appearing in USP 43

Page citations refer to the pages of USP 43. Note—In the lists below, if a section is new or if a subsection is added to or deleted from an existing section, it is labeled as such in parentheses after the section or subsection name. Items on this list that appear without the designation "new", "added", or "deleted" are items in which changes have been made to existing official text.

General Chapters

General Tests and Assays

General Requirements for Tests and Assays

⟨7⟩ Labeling (Revision Bulletin official May 1, 2019), 6435
- LABELS AND LABELING FOR DRUG PRODUCTS AND COMPOUNDED PREPARATIONS EXPRESSED AS ACTIVE MOIETY IN NAME AND STRENGTH
- LABELS AND LABELING FOR INJECTABLE PRODUCTS
- LABELS AND LABELING FOR PRODUCTS IN OTHER CATEGORIES
- LABELS AND LABELING FOR ANIMAL DRUG PRODUCTS

Biological Tests and Assays

⟨121⟩ Insulin Assays (Revision Bulletin official May 1, 2019), 6547
- ASSAY
  - *Rabbit Blood Sugar Method—Quantitative*
- ADDITIONAL REQUIREMENTS
  - *USP Reference Standards*

Chemical Tests and Assays

⟨203⟩ High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin, 6602
- INTRODUCTION
- EQUIPMENT
- PROCEDURE

⟨591⟩ Zinc Determination, 6817
- INTRODUCTION
- PROCEDURE

Physical Tests and Determinations

⟨701⟩ Disintegration (Stage 4 Harmonization official May 1, 2020), 6940

⟨800⟩ Hazardous Drugs—Handling in Healthcare Settings (Revision Bulletin official May 1, 2019), 7071

⟨856⟩ Near-Infrared Spectroscopy (new), 7161

General Information

⟨1010⟩ Analytical Data—Interpretation and Treatment, 7233

⟨1043⟩ Ancillary Materials for Cell, Gene, and Tissue-Engineered Products, 7381

⟨1046⟩ Cell-Based Advanced Therapies and Tissue-Based Products, 7400

⟨1047⟩ Gene Therapy Products, 7426
- INTRODUCTION
- MANUFACTURING OVERVIEW
- MANUFACTURING OF GENE THERAPY PRODUCTS
- ON-SITE PREPARATION AND ADMINISTRATION
- ANALYTICAL METHODS
- GLOSSARY
- APPENDIX

⟨1099⟩ Limit on Number of Large Deviations When Assessing Content Uniformity in Large Samples (Revision Bulletin official March 1, 2019), 7744

⟨1136⟩ Packaging and Repackaging—Single-Unit Containers (deleted), 7919

⟨1177⟩ Good Packaging Practices (deleted), 8003

⟨1850⟩ Evaluation of Screening Technologies for Assessing Medicine Quality (new), 8626

⟨1856⟩ Near-Infrared Spectroscopy—Theory and Practice, 8658

Reagents, Indicators, and Solutions

REAGENT SPECIFICATIONS

Glyceryl Monostearate (new), 6168
Lecithin (new), 6175
Lysolecithin (new), 6176
Manganese Chloride Tetrahydrate (new), 6177
Manganese Sulfate (new), 6176
Methyl Red (deleted), 6179
Polypeptone (new), 6192
Polysorbate 20 (new), 6192
Sodium Butyrate (new), 6202
Sodium Oleate (new), 6204
Sodium Propionate (new), 6205

www.webofpharma.com

Sodium Taurocholate (new), 6206
Sodium Taurodeoxycholate (new), 6206
INDICATORS
Methyl Red, 6221
Xylenol Orange, 6221
TEST SOLUTIONS
18 N Phosphoric Acid TS (new), 6233
50% Sodium Hydroxide TS (new), 6235
Xylenol Orange TS, 6237

Reference Tables

CONTAINER SPECIFICATIONS FOR CAPSULES AND TABLETS
Diazoxide Capsules (deleted), 6262
Oxprenolol Hydrochloride Tablets, Extended-Release (deleted),6268
Oxycodone and Acetaminophen Capsules (deleted), 6268
Piperazine Citrate Tablets (deleted), 6268
Pyrvinium Pamoate Tablets (deleted), 6269
Ritodrine Hydrochloride Tablets (deleted), 6269
Scopolamine Hydrobromide Tablets (deleted), 6270
DESCRIPTION AND RELATIVE SOLUBILITY OF USP AND NF ARTICLES
Anise Oil (new), 6276
Glyceryl Mono and Dicaprylate (new), 6297
Glyceryl Mono and Dicaprylocaprate (new), 6297
Medium-Chain Triglycerides, 6331
Neohesperidin Dihydrochalcone (new), 6309
Polypropylene Glycol 11 Stearyl Ether (new), 6317

Monographs (USP 43)

Allopurinol Compounded Oral Suspension, 133
DEFINITION
ASSAY (added)
SPECIFIC TESTS
ADDITIONAL REQUIREMENTS
Packaging and Storage, *Beyond-Use Date*, and *USP Reference Standards* (added)
Alprazolam Tablets (Revision Bulletin official March 1, 2019), 146
IMPURITIES (added)
Amitriptyline Hydrochloride, 261
IDENTIFICATION
Test A
IMPURITIES
Organic Impurities
Apomorphine Hydrochloride Tablets (deleted), 358
Arginine Hydrochloride Compounded Oral Solution (new), 372
Aurothioglucose Injectable Suspension (deleted), 434
Azatadine Maleate (deleted), 437
Azatadine Maleate Tablets (deleted), 438
Azathioprine Tablets, 441
IDENTIFICATION
ASSAY
PERFORMANCE TESTS
Dissolution
IMPURITIES (added)
ADDITIONAL REQUIREMENTS
Bendamustine Hydrochloride (Revision Bulletin official March 1, 2019), 493
IMPURITIES
Organic Impurities
Betaxolol Hydrochloride, 557
IDENTIFICATION
ASSAY
IMPURITIES
Organic Impurities
ADDITIONAL REQUIREMENTS
USP Reference Standards
Betaxolol Tablets, 559
IDENTIFICATION
Test B (added)
ASSAY
PERFORMANCE TESTS
Dissolution
IMPURITIES (added)
ADDITIONAL REQUIREMENTS
USP Reference Standards
Calcium Acetate Capsules (Revision Bulletin official May 1, 2019), 680
PERFORMANCE TESTS
Dissolution
ADDITIONAL REQUIREMENTS
Labeling (added)
Carboxymethylcellulose Sodium Compounded Intraperitoneal Solution, Veterinary (new), 775
Chlorambucil Compounded Oral Suspension (new), 925
Chloramphenicol Compounded Oral Suspension, Veterinary (new), 930
Cholecalciferol, 985
IDENTIFICATION
ASSAY
Cidofovir (Revision Bulletin official June 1, 2019), 999
IMPURITIES
Residue on Ignition (deleted)
Clonidine Hydrochloride, 1101
DEFINITION
IDENTIFICATION
Test A and *Test B*
ASSAY
IMPURITIES
Organic Impurities
Clonidine Hydrochloride Compounded Oral Suspension (new), 1103
Clonidine Transdermal System (Revision Bulletin official June 1, 2019), 1105
PERFORMANCE TESTS
Drug Release
Demeclocycline Hydrochloride, 1248
CHEMICAL INFORMATION
IDENTIFICATION
ASSAY
Demeclocycline Hydrochloride Tablets, 1249
IDENTIFICATION
Test B (added)
ASSAY
ADDITIONAL REQUIREMENTS
Packaging and Storage

www.webofpharma.com

Deslanoside (deleted), 1258
Deslanoside Injection (deleted), 1259
Diazoxide Capsules (deleted), 1333
Diazoxide Injection (deleted), 1334
Diclofenac Potassium for Oral Solution (new), 1344
Diflunisal Tablets, 1376
IDENTIFICATION
Test B
ASSAY
PERFORMANCE TESTS
IMPURITIES (added)
ADDITIONAL REQUIREMENTS
Packaging and Storage
Diltiazem Hydrochloride Extended-Release Capsules (Revision Bulletin official May 1, 2019), 1399
PERFORMANCE TESTS
Dissolution
IMPURITIES
Diphenhydramine Hydrochloride Oral Powder (new), 1428
Dobutamine Hydrochloride, 1466
IDENTIFICATION
ASSAY
IMPURITIES
Organic Impurities
SPECIFIC TESTS
Color of Solution (deleted)
ADDITIONAL REQUIREMENTS
USP Reference Standards
Donepezil Hydrochloride Tablets (Revision Bulletin official March 1, 2019), 1488
IDENTIFICATION
Ultraviolet Absorption, Test A
ASSAY
PERFORMANCE TESTS
Dissolution
IMPURITIES
Doxycycline Compounded Oral Suspension, Veterinary, 1530
DEFINITION
ASSAY
SPECIFIC TESTS
ADDITIONAL REQUIREMENTS
Packaging and Storage and *Beyond-Use Date*
Drospirenone and Ethinyl Estradiol Tablets (Revision Bulletin official April 1, 2019), 1556
IMPURITIES
Ergocalciferol, 1665
IDENTIFICATON
ASSAY
ADDITIONAL REQUIREMENTS
USP Reference Standards
Fenofibrate Capsules (Revision Bulletin official May 1, 2019), 1834
PERFORMANCE TESTS
Fentanyl, 1846
DEFINITION
IDENTIFICATION
Test A
ASSAY
IMPURITIES
Organic Impurities
ADDITIONAL REQUIREMENTS
USP Reference Standards
Fluticasone Propionate and Salmeterol Inhalation Powder (Revision Bulletin official February 8, 2019), 2010
ASSAY
PERFORMANCE TESTS
IMPURITIES
SPECIFIC TESTS
Foreign Particulate Matter
ADDITIONAL REQUIREMENTS
Labeling (added) and *USP Reference Standards*
Hydrochlorothiazide Compounded Oral Suspension (new), 2221
Hydrocortisone Gel, 2230
IDENTIFICATION
ASSAY
IMPURITIES (added)
SPECIFIC TESTS (added)
ADDITIONAL REQUIREMENTS
USP Reference Standards
Indomethacin Extended-Release Capsules, 2325
IDENTIFICATION
ASSAY
PERFORMANCE TESTS
IMPURITIES
ADDITIONAL REQUIREMENTS
Insulin (Revision Bulletin official May 1, 2019), 2335
CHEMICAL INFORMATION
DEFINITION
IDENTIFICATION
Test A
ASSAY
PRODUCT-RELATED SUBSTANCES AND IMPURITIES
Product-Related Substances
OTHER COMPONENTS
ADDITIONAL REQUIREMENTS
Labeling and *USP Reference Standards*
Insulin Injection (Revision Bulletin official May 1, 2019), 2338
IDENTIFICATION
ASSAY
OTHER COMPONENTS
ADDITIONAL REQUIREMENTS
Labeling and *USP Reference Standards*
Isophane Insulin Suspension (Revision Bulletin official May 1, 2019), 2351
IDENTIFICATION
ASSAY
ADDITIONAL REQUIREMENTS
Labeling and *USP Reference Standards*
Insulin Zinc Suspension (Revision Bulletin official May 1, 2019), 2356
IDENTIFICATION
ASSAY
ADDITIONAL REQUIREMENTS
Labeling and *USP Reference Standards*
Extended Insulin Zinc Suspension (Revision Bulletin official May 1, 2019), 2357
IDENTIFICATION
ASSAY

www.webofpharma.com

ADDITIONAL REQUIREMENTS
Labeling and *USP Reference Standards*
Prompt Insulin Zinc Suspension (Revision Bulletin official May 1, 2019), 2359
IDENTIFICATION
ASSAY
ADDITIONAL REQUIREMENTS
Labeling and *USP Reference Standards*
Itraconazole Capsules (Revision Bulletin official August 1, 2019), 2468
PERFORMANCE TESTS
Dissolution
IMPURITIES
ADDITIONAL REQUIREMENTS
Labeling (added)
Loperamide Hydrochloride, 2658
IDENTIFICATION
Test A
ASSAY
IMPURITIES
Organic Impurities
ADDITIONAL REQUIREMENTS
USP Reference Standards
Manganese Chloride for Oral Solution (deleted), 2732
Mazindol (deleted), 2742
Metformin Hydrochloride, 2821
DEFINITION
IDENTIFICATION
Test C (added)
ASSAY
IMPURITIES
Organic Impurities
ADDITIONAL REQUIREMENTS
USP Reference Standards
Metformin Hydrochloride Extended-Release Tablets (Revision Bulletin official March 1, 2019), 2824
PERFORMANCE TESTS
Dissolution
Methacholine Chloride, 2833
DEFINITION
IDENTIFICATION
Test A
ASSAY
IMPURITIES
Organic Impurities
SPECIFIC TESTS
ADDITIONAL REQUIREMENTS
USP Reference Standards
Methotrexate Injection (Revision Bulletin official May 1, 2019), 2857
IMPURITIES
Miconazole Nitrate (Revision Bulletin official March 1, 2019), 2947
SPECIFIC TESTS
Optical Rotation
Minocycline Hydrochloride Oral Suspension (deleted), 2966
Minoxidil, 2972
IDENTIFICATION
ASSAY
IMPURITIES
Organic Impurities
Naproxen Sodium and Pseudoephedrine Hydrochloride Extended-Release Tablets (Revision Bulletin official March 1, 2019), 3084
IMPURITIES
Nicardipine Hydrochloride Injection, 3149
DEFINITION
IDENTIFICATION
Test B (added)
ASSAY
IMPURITIES
OTHER COMPONENTS
SPECIFIC TESTS
Bacterial Endotoxins Test
ADDITIONAL REQUIREMENTS
Packaging and Storage and *USP Reference Standards*
Olmesartan Medoxomil Tablets (Revision Bulletin official March 19, 2019), 3244
PERFORMANCE TESTS
Dissolution
Oxprenolol Hydrochloride Extended-Release Tablets (deleted), 3324
Oxycodone and Acetaminophen Capsules (deleted), 3341
Pantoprazole Sodium Delayed-Release Tablets (Revision Bulletin official May 1, 2019), 3391
PERFORMANCE TESTS
Dissolution
Parachlorophenol (deleted), 3399
Phenytoin Compounded Topical Gel (new), 3525
Physostigmine Salicylate Ophthalmic Solution (deleted), 3536
Piperazine Citrate Tablets (deleted), 3575
Prednisolone Cream (deleted), 3660
Primidone Oral Suspension (deleted), 3684
Procaine and Tetracaine Hydrochlorides and Levonordefrin Injection (deleted), 3695
Promazine Hydrochloride Syrup (deleted), 3713
Propafenone Hydrochloride Extended-Release Capsules (Revision Bulletin official March 8, 2019), 3729
PERFORMANCE TESTS
Dissolution
IMPURITIES
Propoxycaine Hydrochloride (deleted), 3743
Propoxycaine and Procaine Hydrochlorides and Levonordefrin Injection (deleted), 3744
Propoxycaine and Procaine Hydrochlorides and Norepinephrine Bitartrate Injection (deleted), 3745
Propranolol Hydrochloride, 3746
DEFINITION
IDENTIFICATION
Tet A
ASSAY
IMPURITIES
Organic Impurities (added)
SPECIFIC TESTS
Melting Range or Temperature (deleted) and *Optical Rotation* (deleted)
ADDITIONAL REQUIREMENTS
USP Reference Standards

www.webofpharma.com

Propyliodone (deleted), 3754
Pseudoephedrine Hydrochloride Extended-Release Tablets (Revision Bulletin official March 1, 2019), 3764
PERFORMANCE TESTS
Dissolution
Pyrvinium Pamoate (deleted), 3789
Pyrvinium Pamoate Oral Suspension (deleted), 3789
Pyrvinium Pamoate Tablets (deleted), 3790
Quetiapine Extended-Release Tablets (Revision Bulletin official April 1, 2019), 3795
ASSAY
PERFORMANCE TESTS
Dissolution
IMPURITIES
Risedronate Sodium, 3900
IDENTIFICATION
Test C (added)
ASSAY
IMPURITIES
SPECIFIC TESTS
ADDITIONAL REQUIREMENTS
USP Reference Standards
Ritodrine Hydrochloride (deleted), 3910
Ritodrine Hydrochloride Injection (deleted), 3911
Ritodrine Hydrochloride Tablets (deleted), 3911
Rosuvastatin Tablets (Revision Bulletin official April 1, 2019), 3950
PERFORMANCE TESTS
Dissolution
Rotigotine Transdermal System (new), 3955
Saccharin Calcium
DEFINITION
IDENTIFICATION
Test A
ASSAY
SPECIFIC TESTS
Readily Carbonizable Substances Test and *Color of Solution*
ADDITIONAL REQUIREMENTS
USP Reference Standards
Scopolamine Hydrobromide Injection (deleted), 4012
Scopolamine Hydrobromide Ophthalmic Solution (deleted), 4013
Scopolamine Hydrobromide Tablets (deleted), 4013
Secobarbital (deleted), 4013
Sodium Benzoate Compounded Oral Solution (new), 4056
Sodium Fluoride Gel, 4071
IDENTIFICATION
ASSAY
ADDITIONAL REQUIREMENTS
Packaging and Storage and *USP Reference Standards*
Sodium Fluoride Tablets, 4073
IDENTIFICATION
Test B
ASSAY
ADDITIONAL REQUIREMENTS
Packaging and Storage and *USP Reference Standards*
Taurine (Revision Bulletin official March 1, 2019), 4223
ASSAY
Tetracycline Hydrochloride, 4303
IDENTIFICATION
Test A and *Test C*
IMPURITIES
SPECIFIC TESTS
Loss on Drying
Thioridazine Hydrochloride Oral Solution (deleted), 4354
Torsemide Compounded Oral Suspension (new), 4438
Vancomycin Hydrochloride Compounded Oral Solution (new), 4589
Vancomycin Injection (Revision Bulletin official March 1, 2019), 4585
DEFINITION
ASSAY
SPECIFIC TESTS
Composition of Vancomycin, *pH*, and *Particulate Matter in Injections*
ADDITIONAL REQUIREMENTS
Packaging and Storage and *Labeling*
Vitamin A Tablets, 4635
ASSAY
PERFORMANCE TESTS
Dissolution
ADDITIONAL REQUIREMENTS
Labeling

Monographs (Dietary Supplements)

Calcium with Vitamin D Tablets, 4843
IDENTIFICATION (added)
STRENGTH
Vitamin D (Cholecalciferol or Ergocalciferol), Method 1 and *Vitamin D (Cholecalciferol or Ergocalciferol), Method 2* (added)
Calcium and Vitamin D with Minerals Tablets, 4845
IDENTIFICATION (added)
STRENGTH
Vitamin D (Cholecalciferol or Ergocalciferol), Method 1 and *Vitamin D (Cholecalciferol or Ergocalciferol), Method 2* (added)
ADDITIONAL REQUIREMENTS
Labeling
Creatine (new), 4920
Oil-Soluble Vitamins Tablets, 5356
DEFINITION
IDENTIFICATION (added)
STRENGTH
Vitamin A, Method 1; *Vitamin A, Method 2*; *Vitamin A, Method 3*; *Vitamin D (Cholecalciferol or Ergocalciferol), Method 1*; *Vitamin D (Cholecalciferol or Ergocalciferol), Method 2*; *Vitamin D (Cholecalciferol or Ergocalciferol), Method 3*; *Vitamin E, Method 1*; *Vitamin E, Method 2*; *Vitamin E, Method 3* and *Vitamin A, Vitamin D, and Vitamin E, Method 4*; *Phytonadione, Method 3* (added)
ADDITIONAL REQUIREMENTS
Labeling and *USP Reference Standards*
Oil-Soluble Vitamins with Minerals Tablets, 5378
DEFINITION

www.webofpharma.com

IDENTIFICATION (added)
STRENGTH
Vitamin A, Method 1; Vitamin D (Cholecalciferol or Ergocalciferol), Method 1; Vitamin E, Method 1; Phytonadione, Method 1; Vitamin A, Vitamin D, Vitamin E, and Phytonadione, Method 2 (added) and *Beta Carotene*
ADDITIONAL REQUIREMENTS
Labeling and *USP Reference Standards*

Oil- and Water-Soluble Vitamins Tablets, 5419
DEFINITION
IDENTIFICATION (added)
STRENGTH
Vitamin A, Method 1; Vitamin A, Method 2; Vitamin A, Method 3; Vitamin D (Cholecalciferol or Ergocalciferol), Method 1; Vitamin D (Cholecalciferol or Ergocalciferol), Method 2; Vitamin D (Cholecalciferol or Ergocalciferol), Method 3; Vitamin E, Method 1; Vitamin E, Method 2; Vitamin E, Method 3; Vitamin A, Vitamin D (Cholecalciferol or Ergocalciferol), Vitamin E, Method 4; Phytonadione, Method 3 (added); *Ascorbic Acid, Calcium Ascorbate, and Sodium Ascorbate; Biotin, Method 2; Cyanocobalamin, Method 2; Folic Acid, Method 1; Folic Acid, Method 2; Calcium Pantothenate, Method 2; Niacin or Niacinamide, Pyridoxine Hydrochloride, Riboflavin, and Thiamine, Method 1; Niacin, Method 2; Niacinamide, Method 2; Riboflavin, Method 2; Thiamine, Method 2; Niacin or Niacinamide, Pyridoxine Hydrochloride, Riboflavin, and Thiamine, Method 3;* and *Folic Acid, Method 3; Ascorbic Acid, Niacin or Niacinamide, Pyridoxine Hydrochloride, Calcium Pantothenate, Riboflavin, and Thiamine, Method 4* (added)
ADDITIONAL REQUIREMENTS
Labeling and *USP Reference Standards*

Oil- and Water-Soluble Vitamins with Minerals Tablets, 5476
DEFINITION
IDENTIFICATION (added)
STRENGTH
Vitamin A, Method 1; Vitamin A, Method 2; Vitamin A, Method 3; Vitamin D (Cholecalciferol or Ergocalciferol), Method 1; Vitamin D (Cholecalciferol or Ergocalciferol), Method 2; Vitamin D (Cholecalciferol or Ergocalciferol), Method 3; Vitamin E, Method 1; Vitamin E, Method 2; Vitamin E, Method 3; Vitamin A, Vitamin D (Cholecalciferol or Ergocalciferol), and Vitamin E, Method 4; Phytonadione, Method 3 (added); *Ascorbic Acid, Calcium Ascorbate, and Sodium Ascorbate; Biotin, Method 2; Cyanocobalamin, Method 2; Folic Acid, Method 1; Folic Acid, Method 2; Calcium Pantothenate, Method 2; Niacin or Niacinamide, Pyridoxine Hydrochloride, Riboflavin, and Thiamine, Method 1; Niacin, Method 2; Niacinamide, Method 2; Riboflavin, Method 2; Thiamine, Method 2; Niacin or Niacinamide, Pyridoxine Hydrochloride, Riboflavin, and Thiamine, Method 3;* and *Folic Acid, Method 3; Ascorbic Acid, Niacin or Niacinamide, Pyridoxine Hydrochloride, Calcium Pantothenate, Riboflavin, and Thiamine, Method 4* (added)
ADDITIONAL REQUIREMENTS
Labeling and *USP Reference Standards*

Water-Soluble Vitamins Tablets, 5512
DEFINITION
IDENTIFICATION (added)
STRENGTH
Ascorbic Acid, Calcium Ascorbate, and Sodium Ascorbate; Cyanocobalamin, Method 2; Folic Acid, Method 1; Folic Acid, Method 2; Calcium Pantothenate, Method 2; Niacin or Niacinamide, Pyridoxine Hydrochloride, Riboflavin, and Thiamine, Method 1; Niacin, Method 2; Niacinamide, Method 2; Riboflavin, Method 2; Thiamine, Method 2; Niacin or Niacinamide, Pyridoxine Hydrochloride, Riboflavin, and Thiamine, Method 3 and *Folic Acid, Method 3; Ascorbic Acid, Niacin or Niacinamide, Pyridoxine Hydrochloride, Calcium Pantothenate, Riboflavin, and Thiamine, Method 4* (added)

Water-Soluble Vitamins with Minerals Tablets, 5552
DEFINITION
IDENTIFICATION (added)
STRENGTH
Ascorbic Acid, Calcium Ascorbate, and Sodium Ascorbate; Folic Acid, Method 1; Folic Acid, Method 2; Calcium Pantothenate, Method 2; Niacin or Niacinamide, Pyridoxine Hydrochloride, Riboflavin, and Thiamine, Method 1; Niacin, Method 2; Niacinamide, Method 2; Riboflavin, Method 2; Thiamine, Method 2; Niacin or Niacinamide, Pyridoxine Hydrochloride, Riboflavin, and Thiamine, Method 3 and *Folic Acid, Method 3; Ascorbic Acid, Niacin or Niacinamide, Pyridoxine Hydrochloride, Calcium Pantothenate, Riboflavin, and Thiamine, Method 4* (added)
ADDITIONAL REQUIREMENTS
USP Reference Standards

www.webofpharma.com

GENERAL NOTICES AND REQUIREMENTS

Applying to Standards, Tests, Assays, and Other Specifications of the United States Pharmacopeia

1. TITLE AND REVISION 3

2. OFFICIAL STATUS AND LEGAL RECOGNITION 3
2.10. Official Text 3
2.20. Official Articles 3
2.30. Legal Recognition 3

3. CONFORMANCE TO STANDARDS 4
3.10. Applicability of Standards 4
3.20. Indicating Conformance 5

4. MONOGRAPHS AND GENERAL CHAPTERS 5
4.10. Monographs 5
4.20. General Chapters 5

5. MONOGRAPH COMPONENTS 6
5.10. Molecular Formula 6
5.20. Added Substances 6
5.30. Description and Solubility 6
5.40. Identification 6
5.50. Assay 6
5.60. Impurities and Foreign Substances 7
5.70. Performance Tests 7
5.80. USP Reference Standards 7

6. TESTING PRACTICES AND PROCEDURES 7
6.10. Safe Laboratory Practices 7
6.20. Automated Procedures 7
6.30. Alternative and Harmonized Methods and Procedures 7
6.40. Dried, Anhydrous, Ignited, or Solvent-Free Basis 8
6.50. Preparation of Solutions 8
6.60. Units Necessary to Complete a Test 8
6.70. Reagents 8
6.80. Equipment 9

7. TEST RESULTS 9
7.10. Interpretation of Requirements 9
7.20. Rounding Rules 9

8. TERMS AND DEFINITIONS 9
8.10. Abbreviations 9
8.20. About 9
8.30. Alcohol Content 10
8.40. Atomic Weights 10
8.50. Blank Determinations 10
8.60. Concomitantly 10
8.70. Desiccator 10
8.80. Logarithms 10
8.90. Microbial Strain 10
8.100. Negligible 10
8.110. NLT/NMT 10
8.120. Odor 10
8.130. Percent 10
8.140. Percentage Concentrations 10
8.150. Pressure 10
8.160. Reaction Time 10
8.170. Specific Gravity 10
8.180. Temperatures 10
8.190. Time 10
8.200. Transfer 11
8.210. Vacuum 11
8.220. Vacuum Desiccator 11
8.230. Water 11
8.240. Weights and Measures 11

9. PRESCRIBING AND DISPENSING 12
9.10. Use of Metric Units 12

10. PRESERVATION, PACKAGING, STORAGE, AND LABELING 12
10.10. Packaging and Storage 12
10.20. Labeling 12

www.webofpharma.com

www.webofpharma.com

GENERAL NOTICES AND REQUIREMENTS

The *General Notices and Requirements* section (the *General Notices*) presents the basic assumptions, definitions, and default conditions for the interpretation and application of the *United States Pharmacopeia* (*USP*) and the *National Formulary* (*NF*).

Requirements stated in these *General Notices* apply to all articles recognized in the *USP* and *NF* (the "compendia") and to all general chapters unless specifically stated otherwise.

1. TITLE AND REVISION

The full title of this publication (consisting of five volumes and including its *Supplements*), is *The Pharmacopeia of the United States of America*, Forty-Third Revision and the *National Formulary*, Thirty-Eighth Edition. These titles may be abbreviated to *USP 43*, to *NF 38*, and to *USP 43–NF 38*. The *United States Pharmacopeia*, Forty-Third Revision, and the *National Formulary*, Thirty-Eighth Edition, supersede all earlier revisions. Where the terms "*USP*," "*NF*," or "*USP–NF*" are used without further qualification during the period in which these compendia are official, they refer only to *USP 43*, *NF 38*, and any *Supplement(s)* thereto. The same titles, with no further distinction, apply equally to print or electronic presentation of these contents. Although *USP* and *NF* are published under one cover and share these *General Notices*, they are separate compendia.

This revision is official beginning May 1, 2020 unless otherwise indicated in specific text.

Supplements to *USP* and *NF* are published periodically.

Accelerated Revisions, published periodically on the *Official Text* section of USP's website (http://www.usp.org/usp-nf/official-text), are designed to make revisions official more quickly than through the routine process for publishing standards in the *USP–NF*. *Interim Revision Announcements* are Accelerated Revisions to *USP* and *NF* that contain official revisions and their effective dates.

Revision Bulletins are Accelerated Revisions to official text or postponements that require expedited publication. They generally are official immediately unless otherwise specified in the *Revision Bulletin*.

Errata are Accelerated Revisions representing corrections to items erroneously published. Announcements of the availability of new USP Reference Standards and announcements of tests or procedures that are held in abeyance pending availability of required USP Reference Standards are also available on the "Official Text" tab of USP's website.

2. OFFICIAL STATUS AND LEGAL RECOGNITION

2.10. Official Text

Official text of the *USP* and *NF* is published in the *USP–NF Online* (www.uspnf.com) in the edition identified as "CURRENTLY OFFICIAL" and in Accelerated Revisions that supersede the *USP–NF Online* as described below.

Routine revisions are published in the *USP–NF Online* and become official on the date indicated, usually six months after publication. Accelerated Revisions supersede the *USP–NF Online* and become official on the date indicated. Links to Accelerated Revisions on the USP website can be found in any superseded monograph or general chapter in the *USP–NF Online*.

Print and USB flash drive versions of the *USP* and *NF* also are available. Routine revisions are provided with the same timing as the *USP–NF Online*. Official text published in *Supplements* supersedes that in the previously published print or USB flash drive versions of *USP–NF*. These versions also are superseded by Accelerated Revisions as described above.

In the event of any disparity between the print or USB flash drive versions and the *USP–NF Online*, the *USP–NF Online* will be deemed to apply.

2.20. Official Articles

An *official article* is an article that is recognized in *USP* or *NF*. An article is deemed to be recognized and included in a compendium when a monograph for the article is published in the compendium and an official date is generally or specifically assigned to the monograph.

The title specified in a monograph is the *official title* for such article. Other names considered to be synonyms of the *official titles* may not be used as substitutes for *official titles*. For drug products that incorporate a sensor to detect that the product has been administered, the *official title* shall be the title specified in the relevant drug product monograph plus the words "with sensor".

Official articles include both *official substances* and *official products*. An *official substance* is a drug substance, excipient, dietary ingredient, other ingredient, or component of a finished device for which the monograph title includes no indication of the nature of the finished form.

An *official product* is a drug product, dietary supplement, compounded preparation, or finished device for which a monograph is provided.

2.30. Legal Recognition

The *USP* and *NF* are recognized in the laws and regulations of many countries throughout the world. Regulatory authorities may enforce the standards presented in the *USP* and *NF*, but because recognition of the *USP* and *NF* may vary by country, users should understand applicable laws and regulations. In the United States under the Federal Food, Drug, and Cosmetic Act (FDCA), both *USP* and *NF* are recognized as official compendia. A drug with a name recognized in *USP–NF* must comply with compendial identity standards or be deemed adulterated, misbranded, or both. See, e.g., FDCA § 501(b) and 502(e)(3)(b); also FDA regulations, 21 CFR § 299.5(a&b). To avoid being deemed adulterated, such drugs must also comply with compendial standards for strength, quality, and purity, unless labeled to show all respects in which the drug differs. See, e.g., FDCA § 501(b) and 21 CFR § 299.5(c). In addition, to avoid being deemed misbranded, drugs recognized in *USP–NF* must also be packaged and

www.webofpharma.com

labeled in compliance with compendial standards. See FDCA § 502(g).

A dietary supplement represented as conforming to specifications in *USP* will be deemed a misbranded food if it fails to so conform. See FDCA § 403(s)(2)(D).

Enforcement of *USP* standards is the responsibility of FDA and other government authorities in the U.S. and elsewhere. USP has no role in enforcement.

3. CONFORMANCE TO STANDARDS

3.10. Applicability of Standards

Standards for an article recognized in the compendia (*USP–NF*) are expressed in the article's monograph, applicable general chapters, and *General Notices*. The identity, strength, quality, and purity of an article are determined by the official tests, procedures, and acceptance criteria, and other requirements incorporated in the monograph, in applicable general chapters, or in the *General Notices*. "Applicable general chapters" means general chapters numbered below 1000 or above 2000 that are made applicable to an article through reference in *General Notices*, a monograph, or another applicable general chapter numbered below 1000. Where the requirements of a monograph differ from the requirements specified in these *General Notices* or an applicable general chapter, the monograph requirements apply and supersede the requirements of the *General Notices* or applicable general chapters, whether or not the monograph explicitly states the difference.

General chapters numbered 1000 to 1999 are for informational purposes only. They contain no mandatory tests, assays, or other requirements applicable to any official article, regardless of citation in a general chapter numbered below 1000, a monograph, or these *General Notices*. General chapters numbered above 2000 apply only to articles that are intended for use as dietary ingredients and dietary supplements. General chapter citations in *NF* monographs refer to *USP* general chapters.

Early adoption of revised standards in advance of the official date is allowed by USP unless specified otherwise at the time of publication. Where revised standards for an existing article have been published as final approved "official text" (as approved in section *2.10. Official Text*) but have not yet reached the official date (6 months after publication, unless otherwise specified; see "official date", section *2.20. Official Articles*), compliance with the revised standard shall not preclude a finding or indication of conformance with compendial standards, unless USP specifies otherwise by prohibiting early adoption in a particular standard.

The standards in the relevant monograph, general chapter(s), and *General Notices* apply at all times in the life of the article from production to expiration. It is also noted that the manufacturer's specifications, and manufacturing practices (e.g., Quality by Design, Process Analytical Technology, and Real Time Release Testing initiatives), generally are followed to ensure that the article will comply with compendial standards until its expiration date, when stored as directed. Every compendial article in commerce shall be so constituted that when examined in accordance with these assays and test procedures, it meets all applicable pharmacopeial requirements (*General Notices*, monographs, and general chapters). Thus, any official article is expected to meet the compendial standards if tested, and any official article actually tested as directed in the relevant monograph must meet such standards to demonstrate compliance.

Some tests, such as those for *Dissolution* and *Uniformity of Dosage Units*, require multiple dosage units in conjunction with a decision scheme. These tests, albeit using a number of dosage units, are in fact one determination. These procedures should not be confused with statistical sampling plans. The similarity to statistical procedures may seem to suggest an intent to make inference to some larger group of units, but in all cases, statements about whether the compendial standard is met apply only to the units tested. Repeats, replicates, statistical rejection of outliers, or extrapolations of results to larger populations, as well as the necessity and appropriate frequency of batch testing, are neither specified nor proscribed by the compendia; such decisions are based on the objectives of the testing. Frequency of testing and sampling are left to the preferences or direction of those performing compliance testing, and other users of *USP–NF*, including manufacturers, buyers, or regulatory authorities.

Official products are prepared according to recognized principles of good manufacturing practice and from ingredients that meet *USP* or *NF* standards, where standards for such ingredients exist (for dietary supplements, see section *3.10.20. Applicability of Standards to Medical Devices, Dietary Supplements, and Their Components and Ingredients*).

Official substances are prepared according to recognized principles of good manufacturing practice and from ingredients complying with specifications designed to ensure that the resultant substances meet the requirements of the compendial monographs.

3.10.10. Applicability of Standards to Drug Products, Drug Substances, and Excipients

The applicable *USP* or *NF* standard applies to any article marketed in the United States that (1) is recognized in the compendium and (2) is intended or labeled for use as a drug or as an ingredient in a drug. Such articles (drug products, drug substances, and excipients) include both human drugs (whether dispensed by prescription, "over the counter," or otherwise), as well as animal drugs. The applicable standard applies to such articles whether or not the added designation "USP" or "NF" is used. The standards apply equally to articles bearing the official titles or names derived by transposition of the definitive words of official titles or transposition in the order of the names of two or more drug substances in official titles, or where there is use of synonyms with the intent or effect of suggesting a significant degree of identity with the official title or name.

3.10.20. Applicability of Standards to Medical Devices, Dietary Supplements, and Their Components and Ingredients

An article recognized in *USP* or *NF* shall comply with the compendial standards if the article is a medical device, component intended for a medical device, dietary supplement, dietary ingredient, or other ingredient that is intended for incorporation into a dietary supplement, and is labeled as conforming to the *USP* or *NF*.

Generally, dietary supplements are prepared from ingredients that meet *USP*, *NF*, or *Food Chemicals Codex* standards. Where such standards do not exist, substances may be used in dietary supplements if they have been shown to be of acceptable food grade quality using other suitable procedures.

3.10.30. Applicability of Standards to the Practice of Compounding

USP compounding practice standards, *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩ and *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩, as appropriate, apply to compounding practice or activity regardless of whether a monograph exists for the compounded preparation or these chapters are referenced in such a monograph. In the United States, ⟨795⟩ and ⟨797⟩ are not applicable to drugs compounded by entities registered with FDA as outsourcing facilities as defined by FDCA § 503B, because such facilities are required to comply with FDA's current good manufacturing practice requirements. Compounded preparations, including drug products compounded by outsourcing facilities, may also be

www.webofpharma.com

subject to applicable monographs; see section *2.20. Official Articles* and section *4.10. Monographs*.

3.20. Indicating Conformance

A drug product, drug substance, or excipient may use the designation "USP" or "NF" in conjunction with its official title or elsewhere on the label only when (1) a monograph is provided in the specified compendium and (2) the article complies with the identity prescribed in the specified compendium.

When a drug product, drug substance, compounded preparation, or excipient differs from the relevant *USP* or *NF* standard of strength, quality, or purity, as determined by the application of the tests, procedures, and acceptance criteria set forth in the relevant compendium, its difference shall be plainly stated on its label.

When a drug product, drug substance, compounded preparation, or excipient fails to comply with the identity prescribed in *USP* or *NF* or contains an added substance that interferes with the prescribed tests and procedures, the article shall be designated by a name that is clearly distinguishing and differentiating from any name recognized in *USP* or *NF*.

A medical device, dietary supplement, or ingredient or component of a medical device or dietary supplement may use the designation "USP" or "NF" in conjunction with its official title or elsewhere on the label only when (1) a monograph is provided in the specified compendium and (2) the article complies with the monograph standards and other applicable standards in that compendium.

The designation "USP" or "NF" on the label may not and does not constitute an endorsement by USP and does not represent assurance by USP that the article is known to comply with the relevant standards. USP may seek legal redress if an article purports to be or is represented as an official article in one of USP's compendia and such claim is determined by USP not to be made in good faith.

The designation "USP–NF" may be used on the label of an article provided that the label also bears a statement such as "Meets *NF* standards as published by USP," indicating the particular compendium to which the article purports to apply.

When the letters "USP," "NF," or "USP–NF" are used on the label of an article to indicate compliance with compendial standards, the letters shall appear in conjunction with the official title of the article. The letters are not to be enclosed in any symbol such as a circle, square, etc., and shall appear in capital letters.

If a dietary supplement does not comply with all applicable compendial requirements but contains one or more dietary ingredients or other ingredients that are recognized in *USP* or *NF*, the individual ingredient(s) may be designated as complying with *USP* or *NF* standards or being of *USP* or *NF* quality provided that the designation is limited to the individual ingredient(s) and does not suggest that the dietary supplement complies with *USP* standards.

4. MONOGRAPHS AND GENERAL CHAPTERS

4.10. Monographs

Monographs set forth the article's name, definition, specification, and other requirements related to packaging, storage, and labeling. The specification consists of tests, procedures, and acceptance criteria that help ensure the identity, strength, quality, and purity of the article. For general requirements relating to specific monograph sections, see section *5. MONOGRAPH COMPONENTS*.

Because monographs may not provide standards for all relevant characteristics, some official substances may conform to the *USP* or *NF* standard but differ with regard to nonstandardized properties that are relevant to their use in specific preparations. To assure substitutability in such instances, users may wish to ascertain functional equivalence or determine such characteristics before use.

4.10.10. Applicability of Test Procedures

A single monograph may include more than one test, procedure, and/or acceptance criterion for the same attribute. Unless otherwise specified in the monograph, all tests are requirements. In some cases, monograph instructions allow the selection of tests that reflect attributes of different manufacturers' articles, such as different polymorphic forms, impurities, hydrates, and dissolution. Monograph instructions indicate the tests, procedures, and/or acceptance criteria to be used and the required labeling.

The order in which the tests are listed in the monograph is based on the order in which they are approved by the relevant Expert Committee for inclusion in the monograph. Test 1 is not necessarily the test for the innovator or for the reference product. Depending on monograph instructions, a labeling statement is not typically required if Test 1 is used.

4.10.20. Acceptance Criteria

The acceptance criteria allow for analytical error, for unavoidable variations in manufacturing and compounding, and for deterioration to an extent considered acceptable under practical conditions. The existence of compendial acceptance criteria does not constitute a basis for a claim that an official substance that more nearly approaches 100% purity "exceeds" compendial quality. Similarly, the fact that an article has been prepared to tighter criteria than those specified in the monograph does not constitute a basis for a claim that the article "exceeds" the compendial requirements.

An official product shall be formulated with the intent to provide 100% of the quantity of each ingredient declared on the label. Where the minimum amount of a substance present in a dietary supplement is required by law to be higher than the lower acceptance criterion allowed for in the monograph, the upper acceptance criterion contained in the monograph may be increased by a corresponding amount.

The acceptance criteria specified in individual monographs and in the general chapters for compounded preparations are based on such attributes of quality as might be expected to characterize an article compounded from suitable bulk drug substances and ingredients, using the procedures provided or recognized principles of good compounding practice, as described in these compendia.

4.20. General Chapters

Each general chapter is assigned a number that appears in angle brackets adjacent to the chapter name (e.g., *Chromatography* ⟨621⟩). General chapters may contain the following:

- Descriptions of tests and procedures for application through individual monographs,
- Descriptions and specifications of conditions and practices for pharmaceutical compounding,
- General information for the interpretation of the compendial requirements,
- Descriptions of general pharmaceutical storage, dispensing, and packaging practices, or
- General guidance to manufacturers of official substances or official products.

When a general chapter is referenced in a monograph, acceptance criteria may be presented after a colon.

Some chapters may serve as introductory overviews of a test or of analytical techniques. They may reference other general chapters that contain techniques, details of the procedures, and, at times, acceptance criteria.

www.webofpharma.com

5. MONOGRAPH COMPONENTS

5.10. Molecular Formula

The use of the molecular formula for the official substance(s) named in defining the required strength of a compendial article is intended to designate the chemical entity or entities, as given in the complete chemical name of the article, having absolute (100%) purity.

5.20. Added Substances

Added substances are presumed to be unsuitable for inclusion in an official article and therefore prohibited, if their presence impairs the bioavailability, therapeutic efficacy, or safety of the official article; or they interfere with the assays and tests prescribed for determining compliance with the compendial standards (see section *3.20. Indicating Conformance*).

The air in a container of an official article may, where appropriate, be evacuated or be replaced by carbon dioxide, helium, argon, or nitrogen, or by a mixture of these gases. The use of such gas need not be declared in the labeling.

5.20.10. Added Substances in Official Substances

Official substances may contain only the specific added substances that are permitted by the individual monograph. Such added substances shall not exceed the quantity required for providing their intended effect. Where such addition is permitted, the label shall indicate the name(s) and amount(s) of any added substance(s).

5.20.20. Added Substances (Excipients and Ingredients) in Official Products

Suitable substances and excipients such as antimicrobial agents, pharmaceutical bases, carriers, coatings, flavors, preservatives, stabilizers, and vehicles may be added to an official product to enhance its stability, usefulness, or elegance, or to facilitate its preparation, unless otherwise specified in the individual monograph.

Added substances and excipients employed solely to impart color may be incorporated into official products other than those intended for parenteral or ophthalmic use, in accordance with the regulations pertaining to the use of colors issued by the FDA, provided such added substances or excipients are otherwise appropriate in all respects. (See also *Injections and Implanted Drugs Products* ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Vehicles and added substances, Added substances*.)

The proportions of the substances constituting the base in ointment and suppository products and preparations may be varied to maintain a suitable consistency under different climatic conditions, provided that the concentrations of drug substances are not varied and provided that the bioavailability, therapeutic efficacy, and safety of the preparation are not impaired.

5.20.20.1. In Compounded Preparations

Compounded preparations for which a complete composition is given shall contain only the ingredients named in the formulas unless specifically exempted herein or in the individual monograph. Deviation from the specified processes or methods of compounding, although not from the ingredients or proportions thereof, may occur provided that the finished preparation conforms to the relevant standards and to preparations produced by following the specified process.

Where a monograph for a compounded preparation calls for an ingredient in an amount expressed on the dried basis, the ingredient need not be dried before use if due allowance is made for the water or other volatile substances present in the quantity taken.

Specially denatured alcohol formulas are available for use in accordance with federal statutes and regulations of the Internal Revenue Service. A suitable formula of specially denatured alcohol may be substituted for Alcohol in the manufacture of official preparations intended for internal or topical use, provided that the denaturant is volatile and does not remain in the finished product. A preparation that is intended for topical application to the skin may contain specially denatured alcohol, provided that the denaturant is either a usual ingredient in the preparation or a permissible added substance; in either case the denaturant shall be identified on the label of the topical preparation. Where a process is given in the individual monograph, any preparation compounded using denatured alcohol shall be identical to that prepared by the monograph process.

5.20.20.2. In Dietary Supplements

Additional ingredients may be added to dietary supplement products provided that the additional ingredients (1) comply with applicable regulatory requirements, and (2) do not interfere with the assays and tests prescribed for determining compliance with compendial standards.

5.30. Description and Solubility

Only where a quantitative solubility test is given in a monograph and is designated as such is it a test for purity.

A monograph may include information regarding the article's description. Information about an article's "description and solubility" also is provided in the reference table *Description and Relative Solubility of USP and NF Articles*. The reference table merely denotes the properties of articles that comply with monograph standards. The reference table is intended primarily for those who use, prepare, and dispense drugs and/or related articles. Although the information provided in monographs and the information in the reference table may indirectly assist in the preliminary evaluation of an article, it is not intended to serve as a standard or test for purity.

The approximate solubility of a compendial substance is indicated by one of the following descriptive terms:

Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1,000
Very slightly soluble	From 1,000 to 10,000
Practically insoluble, or Insoluble	Greater than or equal to 10,000

5.40. Identification

A compendial test titled *Identification* is provided as an aid in verifying the identity of articles as they are purported to be, e.g., those taken from labeled containers, and to establish whether it is the article named in *USP–NF*. The *Identification* test for a particular article may consist of one or more procedures. When a compendial *Identification* test is undertaken, all requirements of all specified procedures in the test must be met to satisfy the requirements of the test. Failure of an article to meet all the requirements of a prescribed *Identification* test (i.e., failure to meet the requirements of all of the specified procedures that are components of that test) indicates that the article is mislabeled and/or adulterated.

5.50. Assay

Assay tests for compounded preparations are not intended for evaluating a compounded preparation before dispensing, but instead are intended to serve as the official test in the event of a question or dispute regarding the preparation's conformance to official standards.

www.webofpharma.com

5.50.10. Units of Potency (Biological)

For substances that cannot be completely characterized by chemical or physical means or that need confirmation of functionality or tertiary structure, it may be necessary to express quantities of biological activity in units of biological potency, each defined by an authoritative, designated reference standard. In cases where international reference materials have been discontinued, international units of potency may be defined in terms of molecular mass, such as in the cases of vitamins A, D, and E.

Where available, World Health Organization (WHO) international biological standards define the International Units (IU). *USP* monographs refer to the units assigned by USP Reference Standards either directly as International Units (IU) or as "USP Units." For some biological products, units of potency are value assigned against a corresponding U.S. Standard established by FDA, whether or not International Units or USP Units have been defined (see *Biologics* ⟨1041⟩). Note that product-related labeling, e.g., on containers, need not use the full phrase "USP [product name] Units" that appears in many *USP* monograph labeling sections. The term "USP Units" can be used on product labeling consistent with USP compendial requirements, provided it is clear from the context that the potency is stated in terms of USP [product name] Units. In such circumstances it should be clear that "USP Units" and "USP [product name] Units" share the same meaning.

5.60. Impurities and Foreign Substances

Tests for the presence of impurities and foreign substances are provided to limit such substances to amounts that are unobjectionable under conditions in which the article is customarily employed (see also *Impurities in Drug Substances and Drug Products* ⟨1086⟩).

Nonmonograph tests and acceptance criteria suitable for detecting and controlling impurities that may result from a change in the processing methods or that may be introduced from external sources should be employed in addition to the tests provided in the individual monograph, where the presence of the impurity is inconsistent with applicable good manufacturing practices or good pharmaceutical practices.

5.60.10. Other Impurities in USP and NF Articles

If a *USP* or *NF* monograph includes an assay or organic impurity test based on chromatography, other than a test for residual solvents, and that monograph procedure does not detect an impurity present in the substance, the amount and identity of the impurity, where both are known, shall be stated in the labeling (certificate of analysis) of the official substance, under the heading *Other Impurity(ies)*.

The presence of any unlabeled other impurity in an official substance is a variance from the standard if the content is 0.1% or greater. The sum of all *Other Impurities* combined with the monograph-detected impurities may not exceed 2.0% (see *Ordinary Impurities* ⟨466⟩), unless otherwise stated in the monograph.

The following categories of drug substances are excluded from *Other Impurities* requirements:

- Fermentation products and semi-synthetics derived therefrom,
- Radiopharmaceuticals,
- Biologics,
- Biotechnology-derived products,
- Peptides,
- Herbals, and
- Crude products of animal or plant origin.

Any substance known to be toxic shall not be listed under *Other Impurities*.

5.60.20. Residual Solvents in USP and NF Articles

All *USP* and *NF* articles are subject to relevant control of residual solvents, even when no test is specified in the individual monograph. If solvents are used during production, they must be of suitable quality. In addition, the toxicity and residual level of each solvent shall be taken into consideration, and the solvents limited according to the principles defined and the requirements specified in *Residual Solvents* ⟨467⟩, using the general methods presented therein or other suitable methods.

5.60.30. Elemental Impurities in USP Drug Products and Dietary Supplements

Elemental impurities in official drug products are controlled according to the principles defined and requirements specified in *Elemental Impurities—Limits* ⟨232⟩. Elemental contaminants in official dietary supplements are controlled according to the principles defined and requirements specified in *Elemental Contaminants in Dietary Supplements* ⟨2232⟩.

5.70. Performance Tests

Where content uniformity determinations have been made using the same analytical methodology specified in the *Assay*, with appropriate allowances made for differences in sample preparation, the average of all of the individual content uniformity determinations may be used as the *Assay* value.

5.80. USP Reference Standards

USP Reference Standards are authentic specimens that have been approved as suitable for use as comparison standards in *USP* or *NF* tests and assays. (See *USP Reference Standards* ⟨11⟩.) Where *USP* or *NF* tests or assays call for the use of a USP Reference Standard, only those results obtained using the specified USP Reference Standard are conclusive. Where a procedure calls for the use of a compendial article rather than for a USP Reference Standard as a material standard of reference, a substance meeting all of the compendial monograph requirements for that article shall be used. If any new *USP* or *NF* standard requires the use of a new USP Reference Standard that is not yet available, that portion of the standard containing the requirement shall not be official until the specified USP reference material is available.

Unless a Reference Standard label bears a specific potency or content, assume the Reference Standard is 100.0% pure in the official application. Unless otherwise directed in the procedure in the individual monograph or in a general chapter, USP Reference Standards are to be used in accordance with the instructions on the label of the Reference Standard.

6. TESTING PRACTICES AND PROCEDURES

6.10. Safe Laboratory Practices

In performing compendial procedures, safe laboratory practices shall be followed, including precautionary measures, protective equipment, and work practices consistent with the chemicals and procedures used. Before undertaking any procedure described in the compendia, the analyst should be aware of the hazards associated with the chemicals and the techniques and means of protecting against them. These compendia are not designed to describe such hazards or protective measures.

6.20. Automated Procedures

Automated and manual procedures employing the same basic chemistry are considered equivalent provided the automated system is properly qualified as being suitable to execute the compendial manual method and the analytical procedure is verified under the new equipment conditions.

6.30. Alternative and Harmonized Methods and Procedures

An alternative method or procedure is defined as any method or procedure other than the compendial method or

www.webofpharma.com

procedure for the article in question. The alternative method or procedure must be fully validated (see *Validation of Compendial Procedures* ⟨1225⟩) and must produce comparable results to the compendial method or procedure within allowable limits established on a case-by-case basis. Alternative methods or procedures can be developed for any one of a number of reasons not limited to simplification of sample preparation, enhanced precision and accuracy, improved (shortened) run time, or being better suited to automation than the compendial method or procedure. Only those results obtained by the methods and procedures given in the compendia are conclusive.

For evaluation as a potential replacement or addition to the standard, alternative methods and procedures should be submitted to USP (see section *4.10. Monographs*).

Certain general chapters contain a statement that the text in question is harmonized with the corresponding text of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia* and that these texts are interchangeable. Therefore, if a substance or preparation is found to comply with a requirement using an interchangeable method or procedure from one of these pharmacopeias, it should comply with the requirements of the *USP–NF*. When a difference appears, or in the event of dispute, only the result obtained by the method and/or procedure given in the *USP–NF* is conclusive.

6.40. Dried, Anhydrous, Ignited, or Solvent-Free Basis

All calculations in the compendia assume an "as-is" basis unless otherwise specified.

Test procedures may be performed on the undried or unignited substance and the results calculated on the dried, anhydrous, or ignited basis, provided a test for *Loss on Drying*, or *Water Determination*, or *Loss on Ignition*, respectively, is given in the monograph. Where the presence of moisture or other volatile material may interfere with the procedure, previous drying of the substance is specified in the individual monograph and is obligatory.

The term "solvent-free" signifies that the calculation shall be corrected for the presence of known solvents as determined using the methods described in ⟨467⟩ unless a test for limit of organic solvents is provided in the monograph.

The term "previously dried" without qualification signifies that the substance shall be dried as directed under *Loss on Drying* ⟨731⟩ or *Water Determination* ⟨921⟩ (gravimetric determination).

Where drying in vacuum over a desiccant is directed, a vacuum desiccator, a vacuum drying pistol, or other suitable vacuum drying apparatus shall be used.

6.40.10. Ignite to Constant Weight

"Ignite to constant weight" means that ignition shall be continued at 800 ± 25°, unless otherwise indicated, until two consecutive weighings, the second of which is taken after an additional period appropriate to the nature and quantity of the residue, do not differ by more than 0.50 mg per g of substance taken.

6.40.20. Dried to Constant Weight

"Dried to constant weight" means that drying shall be continued until two consecutive weighings, the second of which is taken after an additional drying period appropriate to the nature and quantity of the residue, do not differ by more than 0.50 mg per g of substance taken.

6.50. Preparation of Solutions

6.50.10. Filtration

Where a procedure gives direction to "filter" without further qualification, the liquid shall be passed through suitable filter paper or equivalent device until the filtrate is clear. Due to the possibility of filter effects, the initial volumes of a filtrate may be discarded.

6.50.20. Solutions

Unless otherwise specified, all solutions shall be prepared with Purified Water. Solutions for quantitative measures shall be prepared using accurately weighed or accurately measured analytes (see section *8.20. About*).

An expression such as "(1 in 10)" means that 1 part *by volume* of a liquid shall be diluted with, or 1 part *by weight* of a solid shall be dissolved in, a sufficient quantity of the diluent or solvent to make the volume of the finished solution 10 parts *by volume*. For example, a 1 in 10 solution is prepared by diluting 1 mL of a liquid or dissolving 1 g of a solid in sufficient solvent to make 10 mL of the solution. An expression such as "(20:5:2)" means that the respective numbers of parts, by volume, of the designated liquids shall be mixed, unless otherwise indicated.

6.50.20.1. Adjustments to Solutions

When a specified concentration is called for in a procedure, a solution of other normality or molarity may be used, provided that allowance is made for the difference in concentration and that the change does not increase the error of measurement.

Proportionately larger or smaller quantities than the specified weights and volumes of assay or test substances and Reference Standards may be taken, provided the measurement is made with at least equivalent accuracy.

Unless otherwise indicated, analyte concentrations shall be prepared to within ten percent (10%) of the indicated value. In the case in which a procedure is adapted to the working range of an instrument, solution concentrations may differ from the indicated value by more than ten percent (10%), with appropriate changes in associated calculations. Any changes shall fall within the validated range of the instrument.

When adjustment of pH is indicated with either an acid or base and the concentration is not indicated, appropriate concentrations of that acid or base may be used.

6.50.20.2. Test Solutions

Information on Test Solutions (TS) is provided in the *Test Solutions* portion of the *Reagents, Indicators, and Solutions* section of the *USP–NF*. Use of an alternative Test Solution or a change in the Test Solution used may require validation.

6.50.20.3. Indicator Solutions

Where a procedure specifies the use of an indicator TS, approximately 0.2 mL, or 3 drops, of the solution shall be added unless otherwise directed.

6.60. Units Necessary to Complete a Test

Unless otherwise specified, a sufficient number of units to ensure a suitable analytical result shall be taken.

6.60.10. Tablets

Where the procedure of a Tablet monograph directs to weigh and finely powder not fewer than a given number of Tablets, a counted number of Tablets shall be weighed and reduced to a powder. The portion of the powdered Tablets taken shall be representative of the whole Tablets and shall, in turn, be weighed accurately.

6.60.20. Capsules

Where the procedure of a Capsule monograph gives direction to remove, as completely as possible, the contents of not fewer than a given number of the Capsules, a counted number of Capsules shall be carefully opened and the contents quantitatively removed, combined, mixed, and weighed accurately. The portion of mixed Capsules contents taken shall be representative of the contents of the Capsules and shall, in turn, be weighed accurately.

6.70. Reagents

The proper conduct of the compendial procedures and the reliability of the results depend, in part, upon the quality of the reagents used in the performance of the procedures. Unless otherwise specified, reagents conforming to the specifications

www.webofpharma.com

set forth in the current edition of *Reagent Chemicals* published by the American Chemical Society (ACS) shall be used. Where such ACS reagent specifications are not available or where the required purity differs, compendial specifications for reagents of acceptable quality are provided (see the *Reagents, Indicators, and Solutions* section of the *USP–NF*). Reagents not covered by any of these specifications should be of a grade suitable to the proper performance of the method of assay or test involved.

Listing of these reagents, including the indicators and solutions employed as reagents, in no way implies that they have therapeutic utility; furthermore, any reference to *USP* or *NF* in their labeling shall include also the term "reagent" or "reagent grade." USP may supply reagents if they otherwise may not be generally commercially available.

6.80. Equipment

Unless otherwise specified, a specification for a definite size or type of container or apparatus in a procedure is given solely as a recommendation. Other dimensions or types may be used if they are suitable for the intended use.

6.80.10. Apparatus for Measurement

Where volumetric flasks or other exact measuring, weighing, or sorting devices are specified, this or other equipment of at least equivalent accuracy shall be employed.

6.80.10.1. Pipet/Pipette

Where a pipet/pipette is specified, a suitable buret may be substituted. Where a "to contain" pipet/pipette is specified, a suitable volumetric flask may be substituted.

6.80.10.2. Light Protection

Where low-actinic or light-resistant containers are specified, either containers specially treated to protect contents from light or clear containers that have been rendered opaque by application of a suitable coating or wrapping may be used.

6.80.20. Instrumental Apparatus

An instrument may be substituted for the specified instrument if the substitute uses the same fundamental principles of operation and is of equivalent or greater sensitivity and accuracy. These characteristics shall be qualified as appropriate. Where a particular brand or source of a material, instrument, or piece of equipment, or the name and address of a manufacturer or distributor, is mentioned (ordinarily in a footnote), this identification is furnished solely for informational purposes as a matter of convenience, without implication of approval, endorsement, or certification.

6.80.20.1. Chromatographic Tubes and Columns

The term "diameter" refers to internal diameter (ID).

6.80.20.2. Tubing

The term "diameter" refers to outside diameter (OD).

6.80.20.3. Steam Bath

Where use of a steam bath is directed, use actively flowing steam or another regulated heat source controlled at an equivalent temperature.

6.80.20.4. Water Bath

A water bath requires vigorously boiling water unless otherwise specified.

6.80.30. Temperature Reading Devices

Temperature reading devices suitable for pharmacopeial tests conform to specifications that are traceable to a National Institute of Standards and Technology (NIST) standard or equivalent. Temperature reading devices may be of the liquid-in-glass type or an analog or digital temperature indicator type, such as a resistance temperature device, thermistor, or thermocouple. Standardization of thermometers is performed on an established testing frequency with a temperature standard traceable to NIST. For example, refer to the current issue of American Society of Testing and Materials (ASTM) standards E1 for liquid-in-glass thermometers.

7. TEST RESULTS

7.10. Interpretation of Requirements

Analytical results observed in the laboratory (or calculated from experimental measurements) are compared with stated acceptance criteria to determine whether the article conforms to compendial requirements.

The reportable value, which often is a summary value for several individual determinations, is compared with the acceptance criteria. The reportable value is the end result of a completed measurement procedure, as documented.

Where acceptance criteria are expressed numerically herein through specification of an upper and/or lower limit, permitted values include the specified values themselves, but no values outside the limit(s). Acceptance criteria are considered significant to the last digit shown.

7.10.5. Nominal Concentrations in Equations

Where a "nominal concentration" is specified, calculate the concentration based on the label claim. In assay procedures, water correction is typically stated in the Definition and on the label of the USP Reference Standard. For other procedures, correction for assayed content, potency, or both is made prior to using the concentration in the equation provided in the monograph.

7.10.10. Equivalence Statements in Titrimetric Procedures

The directions for titrimetric procedures conclude with a statement of the weight of the analyte that is equivalent to each mL of the standardized titrant. In such an equivalence statement, the number of significant figures in the concentration of the titrant should be understood to correspond to the number of significant figures in the weight of the analyte. Corrections to calculations based on the blank determination are to be made for all titrimetric assays where appropriate (see *Titrimetry* ⟨541⟩).

7.20. Rounding Rules

The observed or calculated values shall be rounded off to the number of decimal places that is in agreement with the limit expression. Numbers should not be rounded until the final calculations for the reportable value have been completed. Intermediate calculations (e.g., slope for linearity) may be rounded for reporting purposes, but the original (not rounded) value should be used for any additional required calculations. Acceptance criteria are fixed numbers and are not rounded.

When rounding is required, consider only one digit in the decimal place to the right of the last place in the limit expression. If this digit is smaller than 5, it is eliminated and the preceding digit is unchanged. If this digit is equal to or greater than 5, it is eliminated and the preceding digit is increased by 1.

8. TERMS AND DEFINITIONS

8.10. Abbreviations

- RS refers to a USP Reference Standard.
- CS refers to a Colorimetric Solution.
- TS refers to a Test Solution.
- VS refers to a Volumetric Solution that is standardized in accordance with directions given in the individual monograph or in the *Reagents, Indicators, and Solutions* section of *USP–NF*.

8.20. About

"About" indicates a quantity within 10%.

If the measurement is stated to be "accurately measured" or "accurately weighed," follow the statements in *Volumetric Apparatus* ⟨31⟩ and *Balances* ⟨41⟩, respectively.

www.webofpharma.com

Illustration of Rounding Numerical Values for Comparison with Requirements			
Compendial Requirement	**Unrounded Value**	**Rounded Result**	**Conforms**
Assay limit ≥98.0%	97.96%	98.0%	Yes
	97.92%	97.9%	No
	97.95%	98.0%	Yes
Assay limit ≤101.5%	101.55%	101.6%	No
	101.46%	101.5%	Yes
	101.45%	101.5%	Yes
Limit test ≤0.02%	0.025%	0.03%	No
	0.015%	0.02%	Yes
	0.027%	0.03%	No
Limit test ≤3 ppm	3.5 ppm	4 ppm	No
	3.4 ppm	3 ppm	Yes
	2.5 ppm	3 ppm	Yes

8.30. Alcohol Content

Percentages of alcohol, such as those under the heading *Alcohol Content*, refer to percentage by volume of C_2H_5OH at 15.56°. Where a formula, test, or assay calls for alcohol, ethyl alcohol, or ethanol, the *USP* monograph article Alcohol shall be used. Where reference is made to "C_2H_5OH," absolute (100%) ethanol is intended. Where a procedure calls for dehydrated alcohol, alcohol absolute, or anhydrous alcohol, the *USP* monograph article Dehydrated Alcohol shall be used.

8.40. Atomic Weights

Atomic weights used in computing molecular weights and the factors in the assays and elsewhere are those established by the IUPAC Commission on Isotopic Abundances and Atomic Weights.

8.50. Blank Determinations

Where it is directed that "any necessary correction" be made by a blank determination, the determination shall be conducted using the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but with the substance itself omitted.

8.60. Concomitantly

"Concomitantly" denotes that the determinations or measurements are to be performed in immediate succession.

8.70. Desiccator

The instruction "in a desiccator" indicates use of a tightly closed container of suitable size and design that maintains an atmosphere of low moisture content by means of a suitable desiccant such as anhydrous calcium chloride, magnesium perchlorate, phosphorus pentoxide, or silica gel. See also section *8.220. Vacuum Desiccator*.

8.80. Logarithms

Logarithms are to the base 10.

8.90. Microbial Strain

A microbial strain cited and identified by its American Type Culture Collection (ATCC) catalog number shall be used directly or, if subcultured, shall be used not more than five passages removed from the original strain.

8.100. Negligible

"Negligible" indicates a quantity not exceeding 0.50 mg.

8.110. NLT/NMT

"NLT" means "not less than." "NMT" means "not more than."

8.120. Odor

"Odorless," "practically odorless," "a faint characteristic odor," and variations thereof indicate evaluation of a suitable quantity of freshly opened material after exposure to the air for 15 minutes. An odor designation is descriptive only and should not be regarded as a standard of purity for a particular lot of an article.

8.130. Percent

"Percent" used without qualification means:

- For mixtures of solids and semisolids, percent weight in weight;
- For solutions or suspensions of solids in liquids, percent weight in volume;
- For solutions of liquids in liquids, percent volume in volume;
- For solutions of gases in liquids, percent weight in volume.

For example, a 1 percent solution is prepared by dissolving 1 g of a solid or semisolid, or 1 mL of a liquid, in sufficient solvent to make 100 mL of the solution.

8.140. Percentage Concentrations

Percentage concentrations are expressed as follows:

- *Percent Weight in Weight* (w/w) is defined as the number of g of a solute in 100 g of solution.
- *Percent Weight in Volume* (w/v) is defined as the number of g of a solute in 100 mL of solution.
- *Percent Volume in Volume* (v/v) is defined as the number of mL of a solute in 100 mL of solution.

8.150. Pressure

Pressure is determined by use of a suitable manometer or barometer calibrated in terms of the pressure exerted by a column of mercury of the stated height.

8.160. Reaction Time

Reaction time is 5 minutes unless otherwise specified.

8.170. Specific Gravity

Specific gravity is the weight of a substance in air at 25° divided by the weight of an equal volume of water at the same temperature.

8.180. Temperatures

Temperatures are expressed in centigrade (Celsius) degrees, and all measurements are made at 25° unless otherwise indicated. Where moderate heat is specified, any temperature not higher than 45° (113° F) is indicated.

8.190. Time

Unless otherwise specified, rounding rules, as described in section *7.20. Rounding Rules*, apply to any time specified.

www.webofpharma.com

8.200. Transfer

"Transfer" indicates a quantitative manipulation.

8.210. Vacuum

"Vacuum" denotes exposure to a pressure of less than 20 mm of mercury (2.67 kPas), unless otherwise indicated.

8.220. Vacuum Desiccator

"Vacuum desiccator" indicates a desiccator that maintains a low-moisture atmosphere at a reduced pressure of not more than 20 mm of mercury (2.67 kPas) or at the pressure designated in the individual monograph.

8.230. Water

8.230.10. Water as an Ingredient in an Official Product

As an ingredient in an official product, water meets the requirements of the appropriate water monograph in *USP* or *NF*.

8.230.20. Water in the Manufacture of Official Substances

When used in the manufacture of official substances, water shall meet the requirements for drinking water as set forth in the U.S. Environmental Protection Agency National Primary Drinking Water Regulations or in the drinking water regulations of the European Union or of Japan, or in the World Health Organization's Guidelines for Drinking Water Quality. Additional specifications may be required in monographs.

8.230.30. Water in a Compendial Procedure

When water is called for in a compendial procedure, the *USP* monograph article Purified Water shall be used unless otherwise specified. Definitions for other types of water are provided in *Reagents, Indicators, and Solutions* and in *Water for Pharmaceutical Purposes* ⟨1231⟩.

8.240. Weights and Measures

In general, weights and measures are expressed in the International System of Units (SI) as established and revised by the *Conférence générale des poids et mesures*. For compendial purposes, the term "weight" is considered to be synonymous with "mass."

Molality is designated by the symbol *m* preceded by a number that represents the number of moles of the designated solute contained in 1 kilogram of the designated solvent.

Molarity is designated by the symbol M preceded by a number that represents the number of moles of the designated solute contained in an amount of the designated solvent that is sufficient to prepare 1 liter of solution.

Normality is designated by the symbol N preceded by a number that represents the number of equivalents of the designated solute contained in an amount of the designated solvent that is sufficient to prepare 1 liter of solution.

The symbol for degrees (°) without a qualifying unit of measure represents degrees Celsius.

Chart of Symbols and Prefixes commonly employed for SI metric units and other units:

	Units	Symbol	Notes
Length			
	meter	m	
	centimeter	cm	
	millimeter	mm	
	micrometer	µm	Previously referred to as a micron
	nanometer	nm	Previously the symbol mµ (for millimicron) was used
	Ångström	Å	Equal to 0.1 nm
Mass			
	kilogram	kg	
	gram	g	
	milligram	mg	
	microgram	µg	The symbol µg is used in the *USP* and *NF* to represent micrograms, but micrograms may be represented as "mcg" for labeling and prescribing purposes. The term "gamma," symbolized by γ, frequently is used to represent micrograms in biochemical literature.
	nanogram	ng	
	picogram	pg	
	dalton	Da	Also referred to as the unified atomic mass unit and is equal to 1/12 times the mass of the free carbon 12 atom.
	kilodalton	kDa	
Time			
	second	s	
	minute	min	
	hour	h	
Volume			
	liter	L	1 L is equal to 1000 cm^3 (cubic centimeters)
	deciliter	dL	
	milliliter	mL	1 mL is equal to 1 cm^3, sometimes referred to as cc
	microliter	µL	
Temperature			
	Celsius	°C	
Amount of Substance			
	mole	mol	Historically referred to as gram-molecular weight or gram-atomic weight
	millimole	mmol	
	micromole	µmol	
	femtomole	fmol	
	equivalent	Eq	Also referred to as gram-equivalent weight. It is used in the calculation of substance concentration in units of normality. This unit is no longer preferred for use in analytical chemistry or metrology.
	milli equivalent	mEq	
	osmole	Osmol	Osmotic pressure of a solution, related to substance concentration.
	milliosmole	mOsmol	
Pressure			
	pascal	Pa	
	kilopascal	kPa	

www.webofpharma.com

	Units	Symbol	Notes
	pounds per square inch	psi	
	millimeter of mercury	mmHg	Equal to 133.322 Pa
Electrical units			
	ampere	A	
	volt	V	
	millivolt	mV	
	hertz	Hz	Unit of frequency
	kilohertz	kHz	
	megahertz	MHz	
	electron volt	eV	
	kilo-electron volt	keV	
	mega-electron volt	MeV	
Radiation			
	becquerel	Bq	SI unit of activity for radionuclides
	kilobecquerel	kBq	
	megabecquerel	MBq	
	gigabecquerel	GBq	
	curie	Ci	Non-SI unit of activity for radionuclides
	millicurie	mCi	
	microcurie	μCi	
	nanocurie	nCi	
Other			
	acceleration due to gravity	g	Used to express rate of centrifugation
	revolutions per minute	rpm	Used to express rate of centrifugation

Selected SI Prefixes

Name	Symbol	Factor
giga	G	10^9
mega	M	10^6
kilo	k	10^3
deci	d	10^{-1}
centi	c	10^{-2}
milli	m	10^{-3}
micro	μ	10^{-6}
nano	n	10^{-9}
pico	p	10^{-12}
femto	f	10^{-15}

9. PRESCRIBING AND DISPENSING

9.10. Use of Metric Units

Prescriptions for compendial articles shall be written to state the quantity and/or strength desired in metric units unless otherwise indicated in the individual monograph [see also section *5.50.10. Units of Potency (Biological)* above]. If an amount is prescribed by any other system of measurement, only an amount that is the metric equivalent of the prescribed amount shall be dispensed. Abbreviations for the terms "Units" or "International Units" shall not be used for labeling or prescribing purposes. Apothecary unit designations on labels and labeling shall not be used.

9.20. Changes in Volume

In the dispensing of prescription medications, slight changes in volume owing to variations in room temperatures may be disregarded.

10. PRESERVATION, PACKAGING, STORAGE, AND LABELING

10.10. Packaging and Storage

All articles in *USP* or *NF* are subject to the packaging and storage requirements specified in *Packaging and Storage Requirements* ⟨659⟩, unless different requirements are provided in an individual monograph.

10.20. Labeling

All articles in *USP* or *NF* are subject to the labeling requirements specified in *Labeling* ⟨7⟩, unless different requirements are provided in an individual monograph.

www.webofpharma.com

Guide to General Chapters

(For complete alphabetical list of all general chapters in this Pharmacopeia, see under "General chapters" in the index.)

GENERAL TESTS AND ASSAYS

General Requirements for Tests and Assays

⟨1⟩ Injections and Implanted Drug Products (Parenterals)—Product Quality Tests 6405
⟨2⟩ Oral Drug Products—Product Quality Tests 6410
⟨3⟩ Topical and Transdermal Drug Products—Product Quality Tests 6415
⟨4⟩ Mucosal Drug Products—Product Quality Tests 6423
⟨5⟩ Inhalation and Nasal Drug Products—General Information and Product Quality Tests 6427
⟨7⟩ Labeling 6435
⟨11⟩ USP Reference Standards 6442

Apparatus for Tests and Assays

⟨17⟩ Prescription Container Labeling 6445
⟨31⟩ Volumetric Apparatus 6447
⟨41⟩ Balances 6448

Microbiological Tests

⟨51⟩ Antimicrobial Effectiveness Testing 6449
⟨55⟩ Biological Indicators—Resistance Performance Tests 6452
⟨60⟩ Microbiological Examination of Nonsterile Products—Tests For Burkholderia Cepacia Complex 6454
⟨61⟩ Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests 6457
⟨62⟩ Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms 6463
⟨63⟩ Mycoplasma Tests 6471
⟨64⟩ Probiotic Tests 6477
⟨71⟩ Sterility Tests 6480

Biological Tests and Assays

⟨81⟩ Antibiotics—Microbial Assays 6488
⟨85⟩ Bacterial Endotoxins Test 6508
⟨87⟩ Biological Reactivity Tests, in Vitro 6514
⟨88⟩ Biological Reactivity Tests, in Vivo 6516
⟨89⟩ Enzymes Used as Ancillary Materials in Pharmaceutical Manufacturing 6521
⟨89.1⟩ Collagenase I 6524
⟨89.2⟩ Collagenase II 6528
⟨90⟩ Fetal Bovine Serum—Quality Attributes and Functionality Tests 6533
⟨91⟩ Calcium Pantothenate Assay 6536

Biological Tests and Assays

⟨92⟩ Growth Factors and Cytokines Used in Cell Therapy Manufacturing 6539
⟨111⟩ Design and Analysis of Biological Assays 6543
⟨115⟩ Dexpanthenol Assay 6547
⟨121⟩ Insulin Assays 6547
⟨121.1⟩ Physicochemical Analytical Procedures for Insulins 6550
⟨123⟩ Glucagon Bioidentity Tests 6552
⟨124⟩ Erythropoietin Bioassays 6557
⟨126⟩ Somatropin Bioidentity Tests 6558
⟨127⟩ Flow Cytometric Enumeration of Cd34+ Cells 6560
⟨129⟩ Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies 6565
⟨130⟩ Protein A Quality Attributes 6570
⟨151⟩ Pyrogen Test 6577
⟨161⟩ Medical Devices—Bacterial Endotoxin and Pyrogen Tests 6579
⟨162⟩ Diphtheria Antitoxin Potency Testing for Human Immune Globulins 6582
⟨165⟩ Prekallikrein Activator 6583
⟨171⟩ Vitamin B12 Activity Assay 6584

Chemical Tests and Assays

IDENTIFICATION TESTS

⟨181⟩ Identification—Organic Nitrogenous Bases 6587
⟨191⟩ Identification Tests—General 6587
⟨193⟩ Identification—Tetracyclines 6592
⟨197⟩ Spectroscopic Identification Tests 6593
⟨198⟩ Nuclear Magnetic Resonance Spectroscopy Identity Testing of Bacterial Polysaccharides Used in Vaccine Manufacture 6596
⟨201⟩ Thin-Layer Chromatographic Identification Test 6600
⟨202⟩ Identification of Fixed Oils by Thin-Layer Chromatography 6601
⟨203⟩ High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin 6602

LIMIT TESTS

⟨206⟩ Aluminum 6605
⟨207⟩ Test for 1,6-Anhydro Derivative for Enoxaparin Sodium 6605
⟨208⟩ Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated and Low Molecular Weight Heparins 6611
⟨209⟩ Low Molecular Weight Heparin Molecular Weight Determinations 6614
⟨210⟩ Monosaccharide Analysis 6616
⟨211⟩ Arsenic 6621
⟨212⟩ Oligosaccharide Analysis 6623
⟨221⟩ Chloride and Sulfate 6636

www.webofpharma.com

Chemical Tests and Assays

⟨223⟩ Dimethylaniline..... 6636
⟨226⟩ 4-Epianhydrotetracycline..... 6636
⟨227⟩ 4-Aminophenol in Acetaminophen-Containing Drug Products..... 6637
⟨228⟩ Ethylene Oxide and Dioxane..... 6639
⟨232⟩ Elemental Impurities—Limits..... 6641
⟨233⟩ Elemental Impurities—Procedures..... 6645
⟨241⟩ Iron..... 6648
⟨251⟩ Lead..... 6649
⟨261⟩ Mercury..... 6650
⟨267⟩ Porosimetry by Mercury Intrusion..... 6653
⟨268⟩ Porosity by Nitrogen Adsorption—Desorption..... 6655
⟨271⟩ Readily Carbonizable Substances Test..... 6660
⟨281⟩ Residue on Ignition..... 6660
⟨291⟩ Selenium..... 6661

OTHER TESTS AND ASSAYS

⟨301⟩ Acid-Neutralizing Capacity..... 6661
⟨311⟩ Alginates Assay..... 6662
⟨341⟩ Antimicrobial Agents — Content..... 6664
⟨345⟩ Assay for Citric Acid/Citrate and Phosphate..... 6668
⟨351⟩ Assay for Steroids..... 6669
⟨381⟩ Elastomeric Closures for Injections..... 6669
⟨391⟩ Epinephrine Assay..... 6676
⟨401⟩ Fats and Fixed Oils..... 6676
⟨411⟩ Folic Acid Assay..... 6689
⟨413⟩ Impurities Testing in Medical Gases..... 6692
⟨415⟩ Medical Gases Assay..... 6693
⟨425⟩ Iodometric Assay - Antibiotics..... 6696
⟨429⟩ Light Diffraction Measurement of Particle Size..... 6697
⟨431⟩ Methoxy Determination..... 6702
⟨441⟩ Niacin or Niacinamide Assay..... 6704
⟨451⟩ Nitrite Titration..... 6709
⟨461⟩ Nitrogen Determination..... 6709
⟨466⟩ Ordinary Impurities..... 6710
⟨467⟩ Residual Solvents..... 6712
⟨469⟩ Ethylene Glycol, Diethylene Glycol, and Triethylene Glycol in Ethoxylated Substances..... 6733
⟨471⟩ Oxygen Flask Combustion..... 6735
⟨481⟩ Riboflavin Assay..... 6735
⟨501⟩ Salts of Organic Nitrogenous Bases..... 6741
⟨503⟩ Acetic Acid in Peptides..... 6741
⟨503.1⟩ Trifluoroacetic Acid (TFA) in Peptides..... 6743
⟨507⟩ Protein Determination Procedures..... 6744
⟨509⟩ Residual DNA Testing..... 6748
⟨511⟩ Single-Steroid Assay..... 6750
⟨525⟩ Sulfur Dioxide..... 6751
⟨531⟩ Thiamine Assay..... 6756
⟨541⟩ Titrimetry..... 6764

Chemical Tests and Assays

⟨551⟩ Vitamin E Assay..... 6767
⟨561⟩ Articles of Botanical Origin..... 6774
⟨563⟩ Identification of Articles of Botanical Origin..... 6788
⟨565⟩ Botanical Extracts..... 6798
⟨571⟩ Vitamin A Assay..... 6801
⟨580⟩ Vitamin C Assay..... 6805
⟨581⟩ Vitamin D Assay..... 6808
⟨591⟩ Zinc Determination..... 6817

Physical Tests and Determinations

⟨601⟩ Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests..... 6819
⟨602⟩ Propellants..... 6844
⟨603⟩ Topical Aerosols..... 6845
⟨604⟩ Leak Rate..... 6846
⟨610⟩ Alternative Microbiological Sampling Methods for Nonsterile Inhaled and Nasal Products..... 6846
⟨611⟩ Alcohol Determination..... 6848
⟨616⟩ Bulk Density and Tapped Density of Powders..... 6850
⟨621⟩ Chromatography..... 6853
⟨630⟩ Visual Comparison..... 6865
⟨631⟩ Color and Achromicity..... 6865
⟨641⟩ Completeness of Solution..... 6866
⟨643⟩ Total Organic Carbon..... 6867
⟨644⟩ Conductivity of Solutions..... 6868
⟨645⟩ Water Conductivity..... 6871
⟨651⟩ Congealing Temperature..... 6874
⟨659⟩ Packaging and Storage Requirements..... 6876
⟨660⟩ Containers—Glass..... 6881
⟨661⟩ Plastic Packaging Systems and Their Materials of Construction..... 6887
⟨661.1⟩ Plastic Materials of Construction..... 6893
⟨661.2⟩ Plastic Packaging Systems for Pharmaceutical Use..... 6912
⟨670⟩ Auxiliary Packaging Components..... 6916
⟨671⟩ Containers—Performance Testing..... 6922
⟨691⟩ Cotton..... 6929
⟨695⟩ Crystallinity..... 6931
⟨696⟩ Characterization of Crystalline Solids By Microcalorimetry and Solution Calorimetry..... 6931
⟨697⟩ Container Content for Injections..... 6934
⟨698⟩ Deliverable Volume..... 6935
⟨699⟩ Density of Solids..... 6938
⟨701⟩ Disintegration..... 6940
⟨705⟩ Quality Attributes of Tablets Labeled as Having a Functional Score..... 6944
⟨711⟩ Dissolution..... 6945
⟨721⟩ Distilling Range..... 6955
⟨724⟩ Drug Release..... 6957
⟨729⟩ Globule Size Distribution in Lipid Injectable Emulsions..... 6963
⟨730⟩ Plasma Spectrochemistry..... 6967

www.webofpharma.com

Physical Tests and Determinations

⟨731⟩ Loss on Drying 6970
⟨733⟩ Loss on Ignition 6970
⟨735⟩ X-Ray Fluorescence Spectrometry 6971
⟨736⟩ Mass Spectrometry 6975
⟨741⟩ Melting Range or Temperature 6980
⟨755⟩ Minimum Fill 6982
⟨761⟩ Nuclear Magnetic Resonance Spectroscopy 6984
⟨771⟩ Ophthalmic Products-Quality Tests 6993
⟨776⟩ Optical Microscopy 6998
⟨781⟩ Optical Rotation 7001
⟨782⟩ Vibrational Circular Dichroism Spectroscopy 7002
⟨785⟩ Osmolality and Osmolarity 7008
⟨786⟩ Particle Size Distribution Estimation by Analytical Sieving 7010
⟨787⟩ Subvisible Particulate Matter in Therapeutic Protein Injections 7014
⟨788⟩ Particulate Matter in Injections 7017
⟨789⟩ Particulate Matter in Ophthalmic Solutions 7020
⟨790⟩ Visible Particulates in Injections 7021
⟨791⟩ pH 7022
⟨795⟩ Pharmaceutical Compounding—Nonsterile Preparations 7025
⟨797⟩ Pharmaceutical Compounding—Sterile Preparations 7037
⟨800⟩ Hazardous Drugs—Handling in Healthcare Settings 7071
⟨801⟩ Polarography 7089
⟨811⟩ Powder Fineness 7093
⟨821⟩ Radioactivity 7094
⟨823⟩ Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses 7100
⟨825⟩ Radiopharmaceuticals—Preparation, Compounding, Dispensing, and Repackaging 7109
⟨831⟩ Refractive Index 7137
⟨841⟩ Specific Gravity 7137
⟨846⟩ Specific Surface Area 7139
⟨852⟩ Atomic Absorption Spectroscopy 7142
⟨853⟩ Fluorescence Spectroscopy 7146
⟨854⟩ Mid-Infrared Spectroscopy 7151
⟨855⟩ Nephelometry and Turbidimetry 7155
⟨856⟩ Near-Infrared Spectroscopy 7161
⟨857⟩ Ultraviolet-Visible Spectroscopy 7166
⟨861⟩ Sutures—Diameter 7175
⟨871⟩ Sutures—Needle Attachment 7178
⟨881⟩ Tensile Strength 7179
⟨891⟩ Thermal Analysis 7180
⟨905⟩ Uniformity of Dosage Units 7183
⟨911⟩ Viscosity—Capillary Methods 7187
⟨912⟩ Viscosity—Rotational Methods 7189
⟨913⟩ Viscosity—Rolling Ball Method 7193
⟨914⟩ Viscosity—Pressure Driven Methods 7195
⟨921⟩ Water Determination 7196

Physical Tests and Determinations

⟨941⟩ Characterization of Crystalline and Partially Crystalline Solids by X-Ray Powder Diffraction (XRPD) 7201

GENERAL INFORMATION

⟨1004⟩ Mucosal Drug Products—Performance Tests 7227
⟨1005⟩ Acoustic Emission 7230
⟨1010⟩ Analytical Data—Interpretation and Treatment 7233
⟨1024⟩ Bovine Serum 7260
⟨1025⟩ Pancreatin 7272
⟨1027⟩ Flow Cytometry 7281
⟨1029⟩ Good Documentation Guidelines 7296
⟨1030⟩ Biological Assay Chapters—Overview and Glossary 7300
⟨1031⟩ The Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants 7313
⟨1032⟩ Design and Development of Biological Assays 7320
⟨1033⟩ Biological Assay Validation 7337
⟨1034⟩ Analysis of Biological Assays 7351
⟨1039⟩ Chemometrics 7363
⟨1041⟩ Biologics 7379
⟨1043⟩ Ancillary Materials for Cell, Gene, and Tissue-Engineered Products 7381
⟨1044⟩ Cryopreservation of Cells 7389
⟨1046⟩ Cell-based Advanced Therapies and Tissue-based Products 7400
⟨1047⟩ Gene Therapy Products 7426
⟨1048⟩ Quality of Biotechnological Products: Analysis of The Expression Construct in Cells Used for Production of R-DNA Derived Protein Products 7452
⟨1049⟩ Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products 7454
⟨1050⟩ Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin 7459
⟨1050.1⟩ Design, Evaluation, and Characterization Of Viral Clearance Procedures 7472
⟨1051⟩ Cleaning Glass Apparatus 7481
⟨1052⟩ Biotechnology-Derived Articles—Amino Acid Analysis 7481
⟨1053⟩ Capillary Electrophoresis 7492
⟨1054⟩ Biotechnology-Derived Articles—Isoelectric Focusing 7499
⟨1055⟩ Biotechnology-Derived Articles—Peptide Mapping 7501
⟨1056⟩ Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis 7508
⟨1057⟩ Biotechnology-Derived Articles—Total Protein Assay 7515
⟨1058⟩ Analytical Instrument Qualification 7522
⟨1059⟩ Excipient Performance 7528
⟨1061⟩ Color—Instrumental Measurement 7553
⟨1062⟩ Tablet Compression Characterization 7556
⟨1063⟩ Shear Cell Methodology for Powder Flow Testing 7566
⟨1064⟩ Identification of Articles of Botanical Origin by High-Performance Thin-Layer Chromatography Procedure 7578
⟨1065⟩ Ion Chromatography 7588
⟨1066⟩ Physical Environments That Promote Safe Medication Use 7590
⟨1071⟩ Rapid Microbial Tests for Release of Sterile Short-Life Products: A Risk-Based Approach 7602
⟨1072⟩ Disinfectants and Antiseptics 7608

www.webofpharma.com

GENERAL INFORMATION *(continued)*

⟨1074⟩ Excipient Biological Safety Evaluation Guidelines..... 7613
⟨1078⟩ Good Manufacturing Practices for Bulk Pharmaceutical Excipients..... 7617
⟨1079⟩ Good Storage and Distribution Practices for Drug Products..... 7635
⟨1079.1⟩ Storage and Transportation of Investigational Drug Products..... 7644
⟨1080⟩ Bulk Pharmaceutical Excipients—Certificate of Analysis.... 7647
⟨1084⟩ Glycoprotein and Glycan Analysis—General Considerations..... 7655
⟨1085⟩ Guidelines on the Endotoxins Test..... 7665
⟨1086⟩ Impurities in Drug Substances and Drug Products..... 7678
⟨1087⟩ Apparent Intrinsic Dissolution—Dissolution Testing Procedures for Rotating Disk and Stationary Disk..... 7681
⟨1088⟩ In Vitro and in Vivo Evaluation of Dosage Forms..... 7684
⟨1090⟩ Assessment of Solid Oral Drug Product Performance and Interchangeability, Bioavailability, Bioequivalence, and Dissolution..... 7695
⟨1091⟩ Labeling of Inactive Ingredients..... 7706
⟨1092⟩ The Dissolution Procedure: Development and Validation. 7707
⟨1094⟩ Capsules—Dissolution Testing And Related Quality Attributes..... 7725
⟨1097⟩ Bulk Powder Sampling Procedures..... 7732
⟨1099⟩ Limit on Number of Large Deviations When Assessing Content Uniformity in Large Samples..... 7744
⟨1102⟩ Immunological Test Methods—General Considerations... 7746
⟨1103⟩ Immunological Test Methods—Enzyme-Linked Immunosorbent Assay (Elisa)..... 7753
⟨1104⟩ Immunological Test Methods—Immunoblot Analysis..... 7763
⟨1105⟩ Immunological Test Methods—Surface Plasmon Resonance..... 7773
⟨1106⟩ Immunogenicity Assays—Design And Validation of Immunoassays to Detect Anti-Drug Antibodies..... 7788
⟨1106.1⟩ Immunogenicity Assays—Design And Validation of Assays to Detect Anti-Drug Neutralizing Antibody..... 7802
⟨1111⟩ Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use..... 7819
⟨1112⟩ Application of Water Activity Determination to Nonsterile Pharmaceutical Products..... 7820
⟨1113⟩ Microbial Characterization, Identification, and Strain Typing..... 7823
⟨1115⟩ Bioburden Control of Nonsterile Drug Substances and Products..... 7827
⟨1116⟩ Microbiological Control and Monitoring of Aseptic Processing Environments..... 7833
⟨1117⟩ Microbiological Best Laboratory Practices..... 7845
⟨1118⟩ Monitoring Devices—Time, Temperature, and Humidity.. 7850
⟨1120⟩ Raman Spectroscopy..... 7856
⟨1121⟩ Nomenclature..... 7862
⟨1125⟩ Nucleic Acid-Based Techniques—General..... 7865
⟨1126⟩ Nucleic Acid-Based Techniques—Extraction, Detection and Sequencing..... 7870
⟨1127⟩ Nucleic Acid-Based Techniques—Amplification..... 7879
⟨1128⟩ Nucleic Acid-Based Techniques—Microarray..... 7887
⟨1129⟩ Nucleic Acid-Based Techniques—Genotyping..... 7893

GENERAL INFORMATION *(continued)*

⟨1130⟩ Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)..... 7897
⟨1132⟩ Residual Host Cell Protein Measurement in Biopharmaceuticals..... 7900
⟨1136⟩ Packaging and Repackaging—Single-Unit Containers..... 7919
⟨1151⟩ Pharmaceutical Dosage Forms..... 7929
⟨1152⟩ Animal Drugs for Use in Animal Feeds..... 7952
⟨1160⟩ Pharmaceutical Calculations in Pharmacy Practice..... 7953
⟨1163⟩ Quality Assurance in Pharmaceutical Compounding 7979
⟨1168⟩ Compounding for Phase I Investigational Studies..... 7985
⟨1174⟩ Powder Flow..... 7993
⟨1176⟩ Prescription Balances and Volumetric Apparatus Used in Compounding..... 7997
⟨1177⟩ Good Packaging Practices..... 8003
⟨1178⟩ Good Repackaging Practices..... 8006
⟨1180⟩ Human Plasma 8008
⟨1181⟩ Scanning Electron Microscopy..... 8025
⟨1184⟩ Sensitization Testing..... 8035
⟨1191⟩ Stability Considerations in Dispensing Practice..... 8044
⟨1195⟩ Significant Change Guide for Bulk Pharmaceutical Excipients..... 8049
⟨1197⟩ Good Distribution Practices for Bulk Pharmaceutical Excipients..... 8060
⟨1207⟩ Package Integrity Evaluation—Sterile Products 8079
⟨1207.1⟩ Package Integrity Testing in the Product Life Cycle-Test Method Selection and Validation..... 8086
⟨1207.2⟩ Package Integrity Leak Test Technologies..... 8097
⟨1207.3⟩ Package Seal Quality Test Technologies..... 8111
⟨1208⟩ Sterility Testing—Validation of Isolator Systems..... 8113
⟨1210⟩ Statistical Tools for Procedure Validation..... 8117
⟨1211⟩ Sterility Assurance..... 8129
⟨1216⟩ Tablet Friability..... 8137
⟨1217⟩ Tablet Breaking Force..... 8138
⟨1222⟩ Terminally Sterilized Pharmaceutical Products—Parametric Release..... 8142
⟨1223⟩ Validation of Alternative Microbiological Methods..... 8144
⟨1223.1⟩ Validation of Alternative Methods to Antibiotic Microbial Assays..... 8157
⟨1224⟩ Transfer of Analytical Procedures..... 8164
⟨1225⟩ Validation of Compendial Procedures..... 8166
⟨1226⟩ Verification of Compendial Procedures..... 8171
⟨1227⟩ Validation of Microbial Recovery From Pharmacopeial Articles..... 8172
⟨1228⟩ Depyrogenation..... 8176
⟨1228.1⟩ Dry Heat Depyrogenation..... 8181
⟨1228.3⟩ Depyrogenation by Filtration..... 8184
⟨1228.4⟩ Depyrogenation by Rinsing..... 8187
⟨1228.5⟩ Endotoxin Indicators for Depyrogenation..... 8190
⟨1229⟩ Sterilization of Compendial Articles..... 8194
⟨1229.1⟩ Steam Sterilization by Direct Contact..... 8199
⟨1229.2⟩ Moist Heat Sterilization of Aqueous Liquids..... 8202
⟨1229.3⟩ Monitoring of Bioburden..... 8206

www.webofpharma.com

GENERAL INFORMATION *(continued)*

⟨1229.4⟩ Sterilizing Filtration of Liquids..... 8209
⟨1229.5⟩ Biological Indicators for Sterilization..... 8216
⟨1229.6⟩ Liquid-Phase Sterilization..... 8219
⟨1229.7⟩ Gaseous Sterilization..... 8222
⟨1229.8⟩ Dry Heat Sterilization..... 8225
⟨1229.9⟩ Physicochemical Integrators and Indicators for Sterilization..... 8227
⟨1229.10⟩ Radiation Sterilization..... 8228
⟨1229.11⟩ Vapor Phase Sterilization..... 8231
⟨1229.12⟩ New Sterilization Methods..... 8233
⟨1229.13⟩ Sterilization-In-Place..... 8233
⟨1229.14⟩ Sterilization Cycle Development..... 8235
⟨1229.15⟩ Sterilizing Filtration of Gases..... 8238
⟨1229.16⟩ Prion Inactivation..... 8239
⟨1230⟩ Water for Hemodialysis Applications..... 8240
⟨1231⟩ Water for Pharmaceutical Purposes..... 8242
⟨1234⟩ Vaccines for Human Use-Polysaccharide and Glycoconjugate Vaccines..... 8276
⟨1235⟩ Vaccines for Human Use—General Considerations..... 8291
⟨1236⟩ Solubility Measurements..... 8306
⟨1237⟩ Virology Test Methods..... 8319
⟨1238⟩ Vaccines for Human Use—Bacterial VaccineS..... 8338
⟨1240⟩ Virus Testing of Human Plasma for Further Manufacture.. 8350
⟨1241⟩ Water-Solid Interactions in Pharmaceutical Systems..... 8359
⟨1251⟩ Weighing on an Analytical Balance..... 8363
⟨1265⟩ Written Prescription Drug Information—Guidelines..... 8368
⟨1285⟩ Preparation of Biological Specimens for Histologic and Immunohistochemical Analysis..... 8370
⟨1285.1⟩ Hematoxylin and Eosin Staining Of Sectioned Tissue for Microscopic Examination..... 8374
⟨1430⟩ Analytical Methodologies Based on Scattering Phenomena —General..... 8376
⟨1430.1⟩ Analytical Methodologies Based on Scattering Phenomena—Static Light Scattering..... 8379
⟨1430.2⟩ Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size..... 8386
⟨1403.4⟩ Analytical Methodologies Based on Scattering Phenomena—Electrophoretic Light Scattering (Determination of Zeta Potential)..... 8389
⟨1430.5⟩ Analytical Methodologies Based on Scattering Phenomena—Small Angle X-Ray Scattering and Small Angle Neutron Scattering..... 8392
⟨1467⟩ Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures..... 8404
⟨1601⟩ Products for Nebulization—Characterization Tests..... 8407
⟨1602⟩ Spacers and Valved Holding Chambers Used With Inhalation Aerosols—Characterization Tests..... 8411
⟨1644⟩ Theory and Practice of Electrical Conductivity Measurements of Solutions..... 8423
⟨1660⟩ Evaluation of The Inner Surface Durability Of Glass Containers..... 8429
⟨1661⟩ Evaluation of Plastic Packaging Systems and Their Materials of Construction With Respect to Their User Safety Impact..... 8434
⟨1663⟩ Assessment of Extractables Associated With Pharmaceutical Packaging/Delivery Systems..... 8442

GENERAL INFORMATION *(continued)*

⟨1664⟩ Assessment of Drug Product Leachables Associated With Pharmaceutical Packaging/Delivery Systems..... 8455
⟨1664.1⟩ Orally Inhaled and Nasal Drug Products..... 8467
⟨1724⟩ Semi-Solid Drug Products—Performance Tests..... 8473
⟨1730⟩ Plasma Spectrochemistry—Theory and Practice..... 8484
⟨1735⟩ X-Ray Fluorescence Spectrometry—Theory and Practice.. 8490
⟨1736⟩ Applications of Mass Spectrometry..... 8508
⟨1761⟩ Applications of Nuclear Magnetic Resonance Spectroscopy..... 8529
⟨1771⟩ Ophthalmic Products—Performance Tests..... 8549
⟨1782⟩ Vibrational Circular Dichroism Spectroscopy—Theory and Practice..... 8549
⟨1787⟩ Measurement of Subvisible Particulate Matter In Therapeutic Protein Injections..... 8562
⟨1788⟩ Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions..... 8575
⟨1790⟩ Visual Inspection of Injections..... 8587
⟨1821⟩ Radioactivity—Theory and Practice..... 8604
⟨1823⟩ Positron Emission Tomography Drugs-Information..... 8617
⟨1850⟩ Evaluation of Screening Technologies for Assessing Medicine Quality..... 8626
⟨1852⟩ Atomic Absorption Spectroscopy—Theory and Practice... 8632
⟨1853⟩ Fluorescence Spectroscopy—Theory and Practice..... 8641
⟨1854⟩ Mid-Infrared Spectroscopy—Theory and Practice..... 8650
⟨1856⟩ Near-Infrared Spectroscopy—Theory and Practice..... 8658
⟨1857⟩ Ultraviolet-Visible Spectroscopy—Theory and Practice..... 8670
⟨1911⟩ Rheometry..... 8679

DIETARY SUPPLEMENTS

⟨2021⟩ Microbial Enumeration Tests—Nutritional and Dietary Supplements..... 8687
⟨2022⟩ Microbiological Procedures for Absence of Specified Microorganisms—Nutritional and Dietary Supplements..... 8692
⟨2023⟩ Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements..... 8698
⟨2030⟩ Supplemental Information for Articles of Botanical Origin..... 8702
⟨2040⟩ Disintegration and Dissolution of Dietary Supplements... 8711
⟨2091⟩ Weight Variation of Dietary Supplements..... 8718
⟨2232⟩ Elemental Contaminants in Dietary Supplements..... 8719
⟨2250⟩ Detection of Irradiated Dietary Supplements..... 8722
⟨2251⟩ Screening for Undeclared Drugs and Drug Analogues..... 8725
⟨2750⟩ Manufacturing Practices for Dietary Supplements..... 8742

www.webofpharma.com

www.webofpharma.com

Official Monographs for USP 43

Abacavir Oral Solution

DEFINITION

Abacavir Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of abacavir ($C_{14}H_{18}N_6O$).

IDENTIFICATION

• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Trifluoroacetic acid and water (0.05: 99.95)

Solution B: Methanol and water (17:3)

Diluent: 1 mL of phosphoric acid diluted with water to 1000 mL

Mobile phase: See the gradient table below.

Time (min)	*Solution A* (%)	*Solution B* (%)
0	95	5
20	70	30
35	10	90
40	10	90
41	0	100
50	0	100
51	95	5
55	95	5

System suitability solution: 0.2 mg/mL of USP Abacavir System Suitability Mixture RS in *Diluent*

Standard solution: 0.46 mg/mL of USP Abacavir Sulfate RS in *Diluent*

Sample solution: Equivalent to 0.4 mg/mL of abacavir in *Diluent*, from Oral Solution. [NOTE—Sonicate, if necessary.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 0.8 mL/min

Injection size: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between abacavir and *trans*-abacavir, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{14}H_{18}N_6O$ in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of abacavir from the *Sample solution*

r_S = peak area of abacavir from the *Standard solution*

C_S = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of abacavir in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of abacavir mutiplied by 2, 572.66

M_{r2} = molecular weight of abacavir sulfate, 670.74

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Solution A, Solution B, Diluent, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Sensitivity solution: 0.2 µg/mL of USP Abacavir Sulfate RS in *Diluent*, from the *Standard solution*. [NOTE—The concentration of this solution is 0.05% of the nominal concentration of the *Sample solution*.]

Analysis

Samples: *Diluent, Standard solution, Sample solution,* and *Sensitivity solution*. [NOTE—In the *Sample solution* disregard any peaks corresponding to peaks identified in the *Diluent* and any peak with a peak area less than the abacavir peak area in the *Sensitivity solution*.]

Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of abacavir from the *Sample solution*

r_S = peak area of abacavir from the *Standard solution*

C_S = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of abacavir in the *Sample solution* (mg/mL)

F = relative response factor for each impurity from *Impurity Table 1*

M_{r1} = molecular weight of abacavir mutiplied by 2, 572.66

M_{r2} = molecular weight of abacavir sulfate, 670.74

www.webofpharma.com

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 2.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cyclopropyldiaminopurine abacavir[a]	0.57	1.4	0.3
Descyclopropyl abacavir[b]	0.68	1.0	0.8
Abacavir	1.00	—	—
trans-Abacavir[c]	1.04	1.0	—
Any individual unspecified impurity	—	1.0	0.2

[a] *N*^{6}-Cyclopropyl-9*H*-purine-2,6-diamine.
[b] [(1*S*,4*R*)-4-(2,6-Diamino-9*H*-purin-9-yl)cyclopent-2-enyl]methanol.
[c] {(1*R*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-cyclopent-2-enyl} methanol. It is a process impurity and monitored in the drug substance.

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 100 cfu/mL, and the total combined molds and yeast count does not exceed 10 cfu/mL. It also meets the requirement for absence of *Escherichia coli*.
- **pH** ⟨791⟩**:** 3.8–4.5

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Abacavir Sulfate RS
  USP Abacavir System Suitability Mixture RS
  A mixture containing abacavir sulfate and *trans*-abacavir

Abacavir Tablets

DEFINITION
Abacavir Tablets contain Abacavir Sulfate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of abacavir ($C_{14}H_{18}N_6O$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Diluent: 1.0 mL of phosphoric acid in 1 L of water
Solution A: Trifluoroacetic acid and water (0.05: 99.95)
Solution B: Methanol and water (85:15)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	70	30
35	10	90
40	10	90
41	95	5
50	95	5

System suitability solution: 0.2 mg/mL of USP Abacavir System Suitability Mixture RS in *Diluent*
Standard solution: 0.21 mg/mL of abacavir sulfate in *Diluent* (equivalent to 0.18 mg/mL of abacavir), from USP Abacavir Sulfate RS
Sample stock solution: Transfer the equivalent to 1500 mg of abacavir, from a portion of Tablets, into a 250-mL volumetric flask. Add 150 mL of *Diluent*. Shake mechanically for 45 min. Dilute with *Diluent* to volume. Pass a portion through a suitable filter of 0.45-µm or finer pore size. Discard the first 3 mL of the filtrate.
Sample solution: 0.18 mg/mL of abacavir in *Diluent* using the filtrate obtained in the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 15-cm; packing L1
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between abacavir and *trans*-abacavir, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of abacavir ($C_{14}H_{18}N_6O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of abacavir from the *Sample solution*
r_S = peak response of abacavir from the *Standard solution*
C_S = concentration of abacavir sulfate in the *Standard solution* (mg/mL)
C_U = nominal concentration of abacavir in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of abacavir multiplied by 2, 572.66
M_{r2} = molecular weight of abacavir sulfate, 670.74

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 15 min
Standard solution: 0.39 mg/mL of USP Abacavir Sulfate RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
Mode: UV
Analytical wavelength: 254 nm
Blank: *Medium*

www.webofpharma.com

Calculate the percentage of the labeled amount of abacavir ($C_{14}H_{18}N_6O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of abacavir multiplied by 2, 572.66
M_{r2} = molecular weight of abacavir sulfate, 670.74
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of abacavir ($C_{14}H_{18}N_6O$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Analysis

[NOTE—Record the chromatograms for 2.5 times the retention time of abacavir.]

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of abacavir from the *Standard solution*
C_S = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of abacavir in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Table 2*)
M_{r1} = molecular weight of abacavir multiplied by 2, 572.66
M_{r2} = molecular weight of abacavir sulfate, 670.74

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cyclopropyldiaminopurine abacavir[a]	0.57	1.4	0.2
Descyclopropyl abacavir[b]	0.68	1.0	0.2
Abacavir	1.0	—	—
trans-Abacavir[c, d]	1.04	—	—
O-Pyrimidine derivative abacavir[d, e]	1.24	—	—
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	1.0

[a] N^6-Cyclopropyl-9*H*-purine-2,6-diamine.
[b] [(1*S*,4*R*)-4-(2,6-Diamino-9*H*-purin-9-yl)-cyclopent-2-enyl]methanol.
[c] {(1*R*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-cyclopent-2-enyl} methanol.
[d] Process impurity monitored in the drug substance and not included in the total impurities.
[e] N^6-Cyclopropyl-9-{(1*R*,4*S*)-4-[(2,5-diamino-6-chloropyrimidin-4-yloxy) methyl]cyclopent-2-enyl}-9*H*-purine-2,6-diamine.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Abacavir Sulfate RS
USP Abacavir System Suitability Mixture RS
—A mixture of abacavir sulfate and *trans*-abacavir.

Abacavir and Lamivudine Tablets

DEFINITION

Abacavir and Lamivudine Tablets contain an amount of abacavir sulfate and lamivudine equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of abacavir ($C_{14}H_{18}N_6O$) and NLT 90.0% and NMT 110.0% of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$), respectively.

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: 0.1 N hydrochloric acid

Solution A: Water and trifluoroacetic acid (2000:1)

Solution B: Acetonitrile, methanol, and trifluoroacetic acid (1000:1000:1)

Mobile phase: See *Table 1*. [NOTE—Return to original conditions and re-equilibrate the system for about 7 min.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
12	70	30
12.1	40	60
13.1	40	60
13.2	100	0

System suitability solution: Dissolve the contents of one vial of USP Lamivudine Resolution Mixture C RS in 2.5 mL of *Diluent*. [NOTE—One vial of USP Lamivudine Resolution Mixture C RS contains 0.8 mg of USP Lamivudine Resolution Mixture C RS.]

Standard solution: 0.35 mg/mL of USP Abacavir Sulfate RS and 0.15 mg/mL of USP Lamivudine RS in *Diluent*. Sonicate to dissolve prior to final dilution.

www.webofpharma.com

Sample stock solution: Nominally 3 mg/mL of abacavir and 1.5 mg/mL of lamivudine in *Diluent* prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask. Add *Diluent* to about 50% of the final volume and shake for NMT 30 min to disperse the Tablets. Dilute with *Diluent* to volume. Pass through a suitable filter.

Sample solution: Nominally 0.3 mg/mL of abacavir and 0.15 mg/mL of lamivudine in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for lamivudine-*S*-oxide and lamivudine-*R*-oxide, in relation to the lamivudine peak, are 0.31 and 0.36, respectively; the relative retention times for lamivudine diastereomer and lamivudine are 0.88 and 1.0, respectively; *System suitability solution*.]

Suitability requirements

Resolution: NLT 1.0 between lamivudine-*S*-oxide and lamivudine-*R*-oxide; NLT 1.0 between lamivudine diastereomer and lamivudine, *System suitability solution*

Relative standard deviation: NMT 1.5% each for abacavir and lamivudine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of abacavir ($C_{14}H_{18}N_6O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of abacavir from the *Sample solution*

r_S = peak response of abacavir from the *Standard solution*

C_S = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of abacavir in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of abacavir multiplied by 2, 572.66

M_{r2} = molecular weight of abacavir sulfate, 670.74

Acceptance criteria: 90.0%–110.0% of the labeled amount of abacavir

Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lamivudine from the *Sample solution*

r_S = peak response of lamivudine from the *Standard solution*

C_S = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamivudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of lamivudine

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 75 rpm

Time: 30 min

Standard solution 1: 0.79 mg/mL of USP Abacavir Sulfate RS in *Medium*. Sonicate to dissolve prior to final dilution.

Standard solution 2: 0.33 mg/mL of USP Lamivudine RS in *Medium*. Sonicate to dissolve prior to final dilution.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

Mode: UV

Wavelength: 240–320 nm

Cell length: 0.2 mm

Blank: *Medium*

Analysis: The calculations of the percentages dissolved are performed using multi-component analysis software.

Tolerances: NLT 80% (*Q*) of the labeled amount of abacavir and lamivudine is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample stock solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual abacavir related impurity and each unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each abacavir related impurity or unspecified impurity

r_T = sum of the peak responses of abacavir, all abacavir related impurities, and all unspecified impurities

Calculate the percentage of each lamivudine related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each lamivudine related impurity

r_T = sum of the peak responses of lamivudine and all lamivudine related impurities

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cytosine[a]	0.12	0.2
Lamivudine-*S*-sulfoxide[b]	0.19	0.2
Lamivudine-*R*-sulfoxide[c]	0.21	0.2
Lamivudine-carboxylic acid[d]	0.49	—[e]
Lamivudine diastereomer (Lamivudine-*trans*)[f]	0.52	—[e]
Lamivudine	0.60	—
Lamivudine-uracil derivative[g]	0.78	0.2

www.webofpharma.com

Table 2 *(continued)*

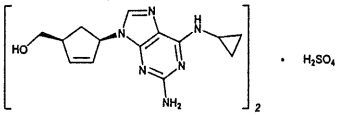
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cyclopropyldiaminopurine abacavir[h]	0.80	0.2
Descyclopropyl abacavir[i]	0.85	0.2
3-Hydroxyabacavir[i]	0.89	—[e]
Abacavir	1.0	—[e]
Any individual unspecified impurity	—	0.2
Total lamivudine related impurities[k]	—	0.6
Total abacavir related impurities[l]	—	1.0

[a] 4-Aminopyrimidin-2(1*H*)-one (lamivudine related impurity).
[b] 1-[(2*R*,3*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.
[c] 1-[(2*R*,3*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.
[d] (2*RS*,5*SR*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.
[e] Process impurity monitored in the drug substance.
[f] 1-[(2*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.
[g] 1-[(2*RS*,5*SR*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil.
[h] N^6-Cyclopropyl-9*H*-purine-2,6-diamine.
[i] [(1*S*,4*R*)-4-(2,6-Diamino-9*H*-purin-9-yl)cyclopent-2-enyl]methanol.
[j] (1*R*,2*R*,4*S*)-2-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-4-(hydroxymethyl)cyclopentan-1-ol.
[k] Includes all lamivudine related impurities.
[l] Includes all abacavir related and all individual unspecified impurities.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Abacavir Sulfate RS
  USP Lamivudine RS
  USP Lamivudine Resolution Mixture C RS
  This is a mixture of lamivudine and the following impurities (other impurities may also be present).
  Uracil: Pyrimidine-2,4(1*H*,3*H*)-dione.
  $C_4H_4N_2O_2$ 112.09
  Lamivudine-uracil derivative: 1-[(2*RS*,5*SR*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil.
  $C_8H_{10}N_2O_4S$ 230.24
  Cytosine: 4-Aminopyrimidin-2(1*H*)-one.
  $C_4H_5N_3O$ 111.10
  Lamivudine-*S*-sulfoxide: 1-[(2*R*,3*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.
  $C_8H_{11}N_3O_4S$ 245.26
  Lamivudine-*R*-sulfoxide: 1-[(2*R*,3*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.
  $C_8H_{11}N_3O_4S$ 245.26
  Lamivudine carboxylic acid: (2*RS*,5*SR*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.
  $C_8H_9N_3O_4S$ 243.24
  Lamivudine diastereomer: 1-[(2*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.
  $C_8H_{11}N_3O_3S$ 229.26
  Salicylic acid: 2-Hydroxybenzoic acid.
  $C_7H_6O_3$ 138.12

Abacavir Sulfate



$(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$ 670.74

2-Cyclopentene-1-methanol, 4-[2-amino-6-(cyclopropyl amino)-9*H*-purin-9-yl]-, (1*S*-*cis*)-, sulfate (salt) (2:1); (1*S*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol sulfate (salt) (2:1) [188062-50-2].

DEFINITION

Abacavir Sulfate contains NLT 97.0% and NMT 102.0% of $(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, obtained as directed in the test for *Organic Impurities, Procedure 2*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩
  Sample solution: 5 mg/mL

ASSAY

- **PROCEDURE**
  Mobile phase: Acetonitrile, phosphoric acid, and water (20:1:180)
  Standard solution: 0.04 mg/mL of USP Abacavir Sulfate RS in water
  Sample solution: 0.04 mg/mL of Abacavir Sulfate in water
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 5-cm; 5-µm packing L1
  Column temperature: 30°
  Flow rate: 1 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Relative standard deviation: NMT 1.5%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$ in the portion of Abacavir Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of abacavir from the *Sample solution*
r_S = peak area of abacavir from the *Standard solution*
C_S = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Abacavir Sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

USP Monographs

www.webofpharma.com

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.2%

ORGANIC IMPURITIES

• **Procedure 1: Related Compounds**

Solution A: Trifluoroacetic acid and water (0.05: 99.95)

Solution B: Methanol and water (17:3)

Mobile phase: See the gradient table below.

Time (min)	*Solution A* (%)	*Solution B* (%)
0	95	5
20	70	30
35	10	90
35.1	95	5
50	95	5

System suitability solution: 0.25 mg/mL of USP Abacavir Related Compounds Mixture RS in water

Sample solution: 0.25 mg/mL of Abacavir Sulfate in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between abacavir and *trans*-abacavir

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Abacavir Sulfate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_T = sum of the areas of all the peaks from the *Sample solution*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.8%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Descyclopropyl abacavir[a]	0.65	0.2
Abacavir	1.00	—
trans-Abacavir[b]	1.04	0.2
O-Pyrimidine derivative abacavir[c]	1.33	0.2
t-Butyl derivative abacavir[d]	1.67	0.2
Any unspecified impurity	—	0.1

[a] [(1*S*,4*R*)-4-(2,6-Diamino-9*H*-purin-9-yl)cyclopent-2-enyl]methanol.

[b] {(1*R*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-cyclopent-2-enyl} methanol.

[c] *N*⁶-Cyclopropyl-9-{(1*R*,4*S*)-4-[(2,5-diamino-6-chloropyrimidin-4-yloxy) methyl]cyclopent-2-enyl}-9*H*-purine-2,6-diamine.

[d] 9-[(1*R*,4*S*)-4-(*tert*-Butoxymethyl)cyclopent-2-enyl]-*N*⁶-cyclopropyl-9*H*-purine-2,6-diamine.

• **Procedure 2: Enantiomeric Purity**

Solution A: Heptane, 2-propanol, and diethylamine (850:150:1).

Solution B: Heptane and 2-propanol (1:1)

Mobile phase: See the gradient table below.

Time (min)	*Solution A* (%)	*Solution B* (%)	Flow Rate (mL/min)
0	100	0	1.0
25	100	0	1.0
27	0	100	0.8
37	0	100	0.8
39	100	0	1.0
55	100	0	1.0

Diluent: Methanol and trifluoroacetic acid (200:1)

System suitability solution: Transfer a quantity of USP Abacavir Stereoisomers Mixture RS to a suitable volumetric flask, add a volume of *Diluent* equivalent to 30% of the final volume, and sonicate until the solid is fully dissolved. Add a volume of 2-propanol equivalent to about 30% of the final volume, mix, and dilute with heptane to volume to obtain 0.4 mg/mL of USP Abacavir Stereoisomers Mixture RS.

Sample solution: Transfer 4 mg of Abacavir Sulfate to a 10-mL volumetric flask. Add 3 mL of *Diluent*, and sonicate until the solid is fully dissolved. Add 3 mL of 2-propanol, mix, and dilute with heptane to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 286 nm

Column: 4.6-mm × 25-cm; 10-µm packing L51

Column temperature: 30°

Injection size: 20 µL

System suitability

[NOTE—The relative retention times for *trans*-abacavir, abacavir enantiomer, and abacavir are 0.8, 0.9, and 1.0, respectively.]

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.0 between *trans*-abacavir and abacavir enantiomer; NLT 1.5 between abacavir enantiomer and abacavir

Analysis

Sample: *Sample solution*

Calculate the percentage of abacavir enantiomer in the portion of Abacavir Sulfate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of abacavir enantiomer from the *Sample solution*

www.webofpharma.com

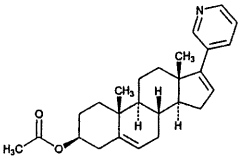
r_T = total peak areas of abacavir and abacavir enantiomer from the *Sample solution*

Acceptance criteria
Individual impurities: NMT 0.3% of abacavir enantiomer

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Abacavir Sulfate RS
  USP Abacavir Stereoisomers Mixture RS
  A mixture of abacavir sulfate, abacavir enantiomer, and *trans*-abacavir.
  USP Abacavir Related Compounds Mixture RS
  A mixture of abacavir glutarate, *O*-pyrimidine derivative abacavir, descyclopropyl abacavir, *trans*-abacavir, and *t*-butyl derivative abacavir.

Abiraterone Acetate



$C_{26}H_{33}NO_2$ 391.55
Androsta-5,16-dien-3-ol, 17-(3-pyridinyl)-, acetate (ester), (3β);
17-(Pyridin-3-yl)androsta-5,16-dien-3β-yl acetate [154229-18-2].

DEFINITION
Abiraterone Acetate contains NLT 98.0% and NMT 102.0% of abiraterone acetate ($C_{26}H_{33}NO_2$), calculated on the as-is basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 10 mM of ammonium acetate in water
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Acetonitrile (%)	Ethanol (%)
0	50	20	30
40	15	55	30
47	0	20	80
58	0	20	80
60	50	20	30

Table 1 *(continued)*

Time (min)	Solution A (%)	Acetonitrile (%)	Ethanol (%)
70	50	20	30

[NOTE—Protect solutions from light.]
System suitability solution: 0.625 mg/mL of USP Abiraterone System Suitability Mixture RS in acetonitrile. [NOTE—See *Table 2* for relative retention times of the main components of the mixture.]

Table 2

Name	Relative Retention Time
7-Ketoabiraterone acetate	0.42
α-Epoxyabiraterone acetate	0.62
β-Epoxyabiraterone acetate	0.66
Abiraterone	0.69
3-Deoxy-3-acetyl abiraterone-3-ene	0.85
Abiraterone acetate	1.0
Abiraterone ethyl ether	1.18
Abiraterone isopropyl ether	1.26
Anhydro abiraterone	1.29
3-Deoxy 3-chloroabiraterone	1.31
O-Chlorobutylabiraterone	1.33

Standard solution: 0.625 mg/mL of USP Abiraterone Acetate RS in acetonitrile
Sample solution: 0.625 mg/mL of Abiraterone Acetate in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3-mm × 15-cm; 3-µm packing L1
Column temperature: 15°
Flow rate: 0.45 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between anhydro abiraterone and 3-deoxy 3-chloroabiraterone peaks, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of abiraterone acetate ($C_{26}H_{33}NO_2$) in the portion of Abiraterone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Abiraterone Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Abiraterone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the as-is basis

www.webofpharma.com

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

[NOTE—Protect solutions from light.]

Solution A, Mobile phase, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity solution: 0.3 µg/mL of USP Abiraterone Acetate RS in acetonitrile, from *Standard solution*

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.0 between anhydro abiraterone and 3-deoxy 3-chloroabiraterone peaks, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Abiraterone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of abiraterone acetate from the *Standard solution*

C_S = concentration of USP Abiraterone Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Abiraterone Acetate in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any peak less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
α-Epoxyabiraterone acetate	0.62	0.26	0.25
β-Epoxyabiraterone acetate	0.66	0.26	0.25
Abiraterone	0.69	1.0	0.20
Abiraterone acetate	1.0	—	—
Abiraterone ethyl ether	1.18	1.0	0.20
Abiraterone isopropyl ether	1.26	1.0	0.20
Unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.80

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method Ic* ⟨921⟩: NMT 0.25%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature and protect from light.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Abiraterone Acetate RS

USP Abiraterone System Suitability Mixture RS

It contains Abiraterone Acetate and small amounts of the following:

Abiraterone
17-(Pyridin-3-yl)androsta-5,16-dien-3β-ol.
$C_{24}H_{31}NO$ 349.52

Abiraterone ethyl ether
3β-Ethoxy-17-(pyridin-3-yl)androsta-5,16-diene.
$C_{26}H_{35}NO$ 377.57

Abiraterone isopropyl ether
3β-Isopropoxy-17-(pyridin-3-yl)androsta-5,16-diene.
$C_{27}H_{37}NO$ 391.60

Anhydro abiraterone
17-(Pyridin-3-yl)androsta-3,5,16-triene.
$C_{24}H_{29}N$ 331.50

O-Chlorobutylabiraterone
3β-(4-Chlorobutoxy)-17-(pyridin-3-yl)androsta-5,16-diene.
$C_{28}H_{38}ClNO$ 440.07

3-Deoxy-3-acetyl abiraterone-3-ene
1-[17-(Pyridin-3-yl)androsta-3,5,16-trien-3-yl]ethanone.
$C_{26}H_{31}NO$ 373.53

3-Deoxy 3-chloroabiraterone
3β-Chloro-17-(pyridin-3-yl)androsta-5,16-diene.
$C_{24}H_{30}ClN$ 367.96

α-Epoxyabiraterone acetate
17-(Pyridin-3-yl)-16α,17α-epoxyandrost-5-en-3β-yl acetate.
$C_{26}H_{33}NO_3$ 407.55

β-Epoxyabiraterone acetate
17-(Pyridin-3-yl)-16β,17β-epoxyandrost-5-en-3β-yl acetate.
$C_{26}H_{33}NO_3$ 407.55

7-Ketoabiraterone acetate
7-Oxo-17-(pyridin-3-yl)androsta-5,16-dien-3β-yl acetate.
$C_{26}H_{31}NO_3$ 405.54

Abiraterone Acetate Tablets

DEFINITION

Abiraterone Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of abiraterone acetate ($C_{26}H_{33}NO_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 10 mM of ammonium acetate in water

Mobile phase: See *Table 1*.

www.webofpharma.com

Table 1

Time (min)	Solution A (%)	Acetonitrile (%)	Ethanol (%)
0	50	20	30
40	15	55	30
47	0	20	80
58	0	20	80
60	50	20	30
70	50	20	30

[NOTE—Protect solutions from light.]

System suitability solution: 0.625 mg/mL of USP Abiraterone System Suitability Mixture RS in acetonitrile. [NOTE—See *Table 2* for relative retention times of the main components of the mixture.]

Table 2

Name	Relative Retention Time
7-Ketoabiraterone acetate	0.42
α-Epoxyabiraterone acetate	0.62
β-Epoxyabiraterone acetate	0.66
Abiraterone	0.69
3-Deoxy-3-acetyl abiraterone-3-ene	0.85
Abiraterone acetate	1.0
Abiraterone ethyl ether	1.18
Abiraterone isopropyl ether	1.26
Anhydro abiraterone	1.29
3-Deoxy 3-chloroabiraterone	1.31
O-Chlorobutylabiraterone	1.33

Standard solution: 0.625 mg/mL of USP Abiraterone Acetate RS in acetonitrile

Sample solution: Nominally equivalent to 0.625 mg/mL of abiraterone acetate in acetonitrile, prepared from NLT 20 powdered Tablets as follows. Transfer the powder to a suitable volumetric flask. Add 50% of the flask volume of acetonitrile, shake by mechanical means for 30 min, and dilute with acetonitrile to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size, and use the clear solution for analysis.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm or diode array. [NOTE—Use diode array detector to perform *Identification* test *B*.]
Column: 3-mm × 15-cm; 3-µm packing L1
Column temperature: 15°
Flow rate: 0.45 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between anhydro abiraterone and 3-deoxy 3-chloroabiraterone peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of abiraterone acetate ($C_{26}H_{33}NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Abiraterone Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of abiraterone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
[NOTE—Protect solutions from light.]
Buffer: 56.5 mM of monobasic sodium phosphate in water. Adjust with 5 N sodium hydroxide or phosphoric acid to a pH of 4.5.
Medium: 0.25% of sodium lauryl sulfate in *Buffer*; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: 0.3 mg/mL of USP Abiraterone Acetate RS in *Medium* prepared as follows. Transfer USP Abiraterone Acetate RS into a suitable volumetric flask. Add 4% of the flask volume of acetonitrile to dissolve, and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter of 10-µm pore size. Use the filtrate.
Mobile phase: Acetonitrile, formic acid, and water (55: 0.05: 45)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 252 nm
Column: 4.6-mm × 3-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of abiraterone acetate ($C_{26}H_{33}NO_2$) dissolved:

$$(r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of abiraterone acetate ($C_{26}H_{33}NO_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
[NOTE—Protect solutions from light.]
Solution A, Mobile phase, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

USP Monographs

www.webofpharma.com

Sensitivity solution: 0.3 µg/mL of USP Abiraterone Acetate RS in acetonitrile from *Standard solution*

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.0 between anhydro abiraterone and 3-deoxy 3-chloroabiraterone peaks, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of abiraterone acetate from the *Standard solution*

C_S = concentration of USP Abiraterone Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of abiraterone acetate in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any peak less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
7-Ketoabiraterone acetate	0.42	1.4	0.50
α-Epoxyabiraterone acetate	0.62	0.26	0.80
β-Epoxyabiraterone acetate	0.66	0.26	0.80
Abiraterone	0.69	1.0	0.40
Abiraterone acetate	1.0	—	—
Abiraterone ethyl ether[a]	1.18	—	—
Abiraterone isopropyl ether[a]	1.26	—	—
Unspecified impurity	—	1.0	0.20
Total impurities	—	—	2.0

[a] This is a process impurity and is controlled in the drug substance monograph. It is included in the table for identification only, and it is not to be reported in the total impurities.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Abiraterone Acetate RS

USP Abiraterone System Suitability Mixture RS

It contains Abiraterone Acetate and small amounts of the following:

Abiraterone

17-(Pyridin-3-yl)androsta-5,16-dien-3β-ol.

$C_{24}H_{31}NO$ 349.52

Abiraterone ethyl ether

3β-Ethoxy-17-(pyridin-3-yl)androsta-5,16-diene.

$C_{26}H_{35}NO$ 377.57

Abiraterone isopropyl ether

3β-Isopropoxy-17-(pyridin-3-yl)androsta-5,16-diene.

$C_{27}H_{37}NO$ 391.60

Anhydro abiraterone

17-(Pyridin-3-yl)androsta-3,5,16-triene.

$C_{24}H_{29}N$ 331.50

O-Chlorobutylabiraterone

3β-(4-Chlorobutoxy)-17-(pyridin-3-yl)androsta-5,16-diene.

$C_{28}H_{38}ClNO$ 440.07

3-Deoxy-3-acetyl abiraterone-3-ene

1-[17-(Pyridin-3-yl)androsta-3,5,16-trien-3-yl]ethanone.

$C_{26}H_{31}NO$ 373.53

3-Deoxy 3-chloroabiraterone

3β-Chloro-17-(pyridin-3-yl)androsta-5,16-diene.

$C_{24}H_{30}ClN$ 367.96

α-Epoxyabiraterone acetate

17-(Pyridin-3-yl)-16α,17α-epoxyandrost-5-en-3β-yl acetate.

$C_{26}H_{33}NO_3$ 407.55

β-Epoxyabiraterone acetate

17-(Pyridin-3-yl)-16β,17β-epoxyandrost-5-en-3β-yl acetate.

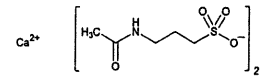
$C_{26}H_{33}NO_3$ 407.55

7-Ketoabiraterone acetate

7-Oxo-17-(pyridin-3-yl)androsta-5,16-dien-3β-yl acetate.

$C_{26}H_{31}NO_3$

Acamprosate Calcium



$C_{10}H_{20}CaN_2O_8S_2$ 400.48

1-Propanesulfonic acid, 3-(acetylamino)-, calcium salt (2:1); Calcium 3-(acetylamino)propane-1-sulfonate [77337-73-6].

DEFINITION

Acamprosate Calcium contains NLT 98.0% and NMT 102.0% of acamprosate calcium ($C_{10}H_{20}CaN_2O_8S_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Calcium*: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Add 5.0 mL of triethylamine per 1 L of water and adjust with phosphoric acid to a pH of 4.0.

System suitability solution: 10 mg/mL of USP Acamprosate Calcium RS and 0.005 mg/mL each of USP

USP Monographs

www.webofpharma.com

Acamprosate Related Compound B RS and glacial acetic acid in water. Sonication may be used to aid in dissolution.
Standard solution: 0.3 mg/mL of USP Acamprosate Calcium RS in water. Sonication may be used to aid in dissolution.
Sample solution: 0.3 mg/mL of Acamprosate Calcium in water. Sonication may be used to aid in dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 0.7 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of the acamprosate peak
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between acetic acid and acamprosate related compound B; NLT 1.3 between acamprosate related compound B and acamprosate, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acamprosate calcium ($C_{10}H_{20}CaN_2O_8S_2$) in the portion of Acamprosate Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Acamprosate Calcium RS in the *Standard solution* (mg/mL)
C_U = concentration of Acamprosate Calcium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Change to read:

• **LIMIT OF ACAMPROSATE RELATED COMPOUND A**
Solution A: 5 g/L of fluorescamine in acetonitrile. Use within 24 h of preparation.
Buffer: 13.8 g/L of monobasic sodium phosphate prepared as follows. Transfer a suitable amount of monobasic sodium phosphate to a volumetric flask. Dissolve in 90% of the final flask volume of water. Adjust with 10 N sodium hydroxide TS or phosphoric acid to a pH of 6.5. Dilute with water to volume.
Mobile phase: Acetonitrile, methanol, and *Buffer* (10:10:80)
Diluent: 24.6 g/L of boric acid prepared as follows. Transfer a suitable amount of boric acid to an appropriate volumetric flask. Dissolve in 90% of the final flask volume of water. Adjust with 10 N sodium hydroxide TS to a pH of 10.4. Dilute with water to volume.
Standard stock solution A: 250 µg/mL of USP Acamprosate Related Compound A RS in water
Standard stock solution B: 1 µg/mL of USP Acamprosate Related Compound A RS from *Standard stock solution A* in *Diluent*
Standard solution: Transfer 3.0 mL of *Standard stock solution B* to an appropriate container. Add 0.15 mL of *Solution A* and shake vigorously for 30 s. Heat in a water bath at 50° for 30 min. Cool under a stream of cold water, centrifuge, and pass the supernatant through a suitable membrane filter.
Sample stock solution A: 20 mg/mL of Acamprosate Calcium in water
Sample stock solution B: 2000 µg/mL of Acamprosate Calcium from *Sample stock solution A* in *Diluent*
Sample solution: Transfer 3.0 mL of *Sample stock solution B* to an appropriate container. Add 0.15 mL of *Solution A* and shake for 30 s. Heat in a water bath at 50° for 30 min. Cool under a stream of cold water, centrifuge, and pass the supernatant through a suitable membrane filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 261 nm
Column: 4.6-mm × 15-cm; 3- or 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of acamprosate related compound A
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for fluorescamine and acamprosate related compound A are about 0.5 and 1.0, respectively. Acamprosate calcium is not detected by this chromatographic system.]
Suitability requirements
Resolution: NLT 2.0 between fluorescamine and acamprosate related compound A
Relative standard deviation: NMT 5.0% for acamprosate related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acamprosate related compound A in the portion of Acamprosate Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of ▲USP Acamprosate Related Compound A RS▲ (ERR 1-Dec-2018) in the *Standard solution* (µg/mL)
C_U = concentration of Acamprosate Calcium in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.05%
• **ORGANIC IMPURITIES**
Mobile phase: Add 5.0 mL of triethylamine per 1 L of water and adjust with phosphoric acid to a pH of 4.0.
System suitability solution: 10 mg/mL of USP Acamprosate Calcium RS and 0.005 mg/mL each of USP Acamprosate Related Compound B RS and glacial acetic acid in water. Sonication may be used to aid in dissolution.
Standard solution: 0.005 mg/mL of USP Acamprosate Calcium RS in water. Sonication may be used to aid in dissolution.
Sample solution: 10 mg/mL of Acamprosate Calcium in water. Sonication may be used to aid in dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1

www.webofpharma.com

Flow rate: 0.7 mL/min
Injection volume: 20 µL
Run time: NLT 6 times the retention time of the acamprosate peak
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention time for acetic acid is 0.7; see *Table 1* for the other relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between acetic acid and acamprosate related compound B; NLT 1.3 between acamprosate related compound B and acamprosate, *System suitability solution*
Tailing factor: NMT 1.5 for acamprosate, *Standard solution*
Relative standard deviation: NMT 15.0% for acetic acid, *System suitability solution*; NMT 5% for acamprosate, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Acamprosate Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of acamprosate from the *Standard solution*
C_S = concentration of USP Acamprosate Calcium RS in the *Standard solution* (mg/mL)
C_U = concentration of Acamprosate Calcium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Calcium[a]	0.4	—
Acamprosate related compound B	0.8	0.05
Acamprosate	1.0	—
N-Methyl acamprosate[b]	1.9	0.05
Any individual unspecified impurity	—	0.05
Total impurities[c]	—	0.5

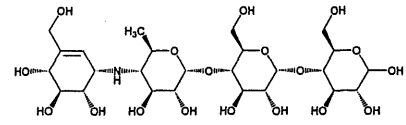
[a] Included for identification only. This peak is due to the calcium counterion and hence is not an impurity.
[b] 3-(*N*-Methylacetamido)propane-1-sulfonate.
[c] The sum of acamprosate related compound A from the *Limit of Acamprosate Related Compound A* test and all impurities from the test for *Organic Impurities*.

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 0.05 g/mL of Acamprosate Calcium in carbon dioxide-free water
Acceptance criteria: 5.5–7.0
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.4%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Store in tight containers.

• **USP Reference Standards** ⟨11⟩
USP Acamprosate Calcium RS
USP Acamprosate Related Compound A RS
3-Aminopropane-1-sulfonic acid.
$C_3H_9NO_3S$ 139.17
USP Acamprosate Related Compound B RS
Calcium 3-formamidopropane-1-sulfonate.
$C_8H_{16}CaN_2O_8S_2$ 372.42

Acarbose



$C_{25}H_{43}NO_{18}$ 645.60
D-Glucose, *O*-4,6-dideoxy-4-[[[1*S*-(1α,4α,5β,6α)]-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-; *O*-4,6-Dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino}-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose [56180-94-0].

DEFINITION
Acarbose is produced by certain strains of *Actinoplanes utahensis*. It contains NLT 95.0% and NMT 102.0% of acarbose ($C_{25}H_{43}NO_{18}$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Solution A: 0.6 mg/mL of monobasic potassium phosphate and 0.35 mg/mL of dibasic sodium phosphate in water
Mobile phase: Acetonitrile and *Solution A* (3:1)
System suitability solution: 20 mg/mL of USP Acarbose System Suitability Mixture RS in water
Standard solution: 20 mg/mL of USP Acarbose RS in water
Sample solution: 20 mg/mL of Acarbose in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4-mm × 25-cm; packing L8
Column temperature: 35°
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Identify the acarbose peak and the peaks due to the impurities listed in *Table 1*.
Suitability requirements
Peak-to-valley ratio: The ratio of the height of the impurity A peak to the height of the valley between the impurity A peak and the acarbose peak is NLT 1.2.
Chromatogram comparability: The chromatogram obtained is similar to the chromatogram provided with

www.webofpharma.com

USP Acarbose System Suitability Mixture RS for the known impurities found.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acarbose ($C_{25}H_{43}NO_{18}$) in the portion of Acarbose taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Acarbose RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩

Sample: 1.0 g

Acceptance criteria: NMT 0.2%

• **CHROMATOGRAPHIC PURITY**

Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Diluted sample solution: Dilute 1.0 mL of the *Sample solution* with water to 100.0 mL.

Analysis

Samples: *Sample solution* and *Diluted sample solution*

Calculate the percentage of each impurity in the portion of Acarbose taken:

$$\text{Result} = (r_U/r_A) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_A = peak response of the main acarbose peak from the *Diluted sample solution*

F = relative response factor for each impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity A[a]	0.9	1	0.6
Impurity B[b]	0.8	1.6	0.5
Impurity C[c]	1.2	1	1.5
Impurity D[d]	0.5	1.33	1.0
Impurity E[e]	1.7	0.8	0.2
Impurity F[f]	1.9	0.8	0.3
Impurity G[g]	2.2	0.8	0.3
Impurity H[h]	0.6	1	0.2
Any individual unknown impurity	—	—	0.2
Total impurities	—	—	3.0

[a] *O*-4,6-Dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino}-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-arabino-hex-2-ulopyranose.

[b] (1*R*,4*R*,5*S*,6*R*)-4,5,6-Trihydroxy-2-(hydroxymethyl)cyclohex-2-enyl 4-*O*-[4,6-dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino}-α-D-glucopyranosyl]-α-D-glucopyranoside.

[c] α-D-Glucopyranosyl 4-*O*-[4,6-dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino}-α-D-glucopyranosyl]-α-D-glucopyranoside.

[d] 4-*O*-[4,6-Dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino}-α-D-glucopyranosyl]-D-glucopyranose.

[e] *O*-4,6-Dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino}-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-arabino-hex-2-ulopyranose (4-*O*-α-acarbosyl-D-fructopyranose).

[f] *O*-4,6-Dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino}-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose (4-*O*-α-acarbosyl-D-glucopyranose).

[g] α-D-Glucopyranosyl *O*-4,6-dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino}-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranoside (α-D-glucopyranosyl α-acarboside).

[h] *O*-4,6-Dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino}-α-D-glucopyranosyl-(1→4)-*O*-6-deoxy-α-D-glucopyranosyl-(1→4)-D-glucopyranose.

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL in water

Acceptance criteria: +168° to +183°

• **pH** ⟨791⟩

Sample solution: 50 mg/mL

Acceptance criteria: 5.5–7.5

• **WATER DETERMINATION,** *Method Ic* ⟨921⟩ : NMT 4.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Acarbose RS

USP Acarbose System Suitability Mixture RS

Add the following:

▲Acarbose Tablets

DEFINITION

Acarbose Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acarbose ($C_{25}H_{43}NO_{18}$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020): The spectrum obtained from the *Sample solution* shows IR maxima in the regions of 3500–3200, 2950–2890, 1653–1633, and 1070–1000 cm^{-1}.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: 0.6 mg/mL of monobasic potassium phosphate and 0.35 mg/mL of dibasic sodium phosphate in water. Filter and degas.

Mobile phase: Acetonitrile and *Buffer* (75:25)

System suitability solution: 20 mg/mL of USP Acarbose System Suitability Mixture RS in water

Standard solution: 10 mg/mL of USP Acarbose RS

Sample solution: Nominally 10 mg/mL of acarbose in water, prepared as follows. Transfer a portion of the powder, from NLT 20 Tablets, equivalent to 100 mg of acarbose to a suitable volumetric flask and add water to 50%–70% of the flask volume. Sonicate to dissolve and dilute with water to volume. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.0-mm × 25-cm; 5-µm packing L8

Column temperature: 35°

Flow rate: 2 mL/min

Injection volume: 10 µL

Run time: NLT 2.5 times the retention time of acarbose

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Peak-to-valley ratio: The ratio of the height of the impurity A peak to the height of the valley between the impurity A peak and the acarbose peak is NLT 1.2, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acarbose ($C_{25}H_{43}NO_{18}$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acarbose from the *Sample solution*

r_S = peak response of acarbose from the *Standard solution*

C_S = concentration of USP Acarbose RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acarbose in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water, 900 mL, deaerated

Apparatus 2: 75 rpm

Time: 30 min

Determine the percentage of the labeled amount of acarbose ($C_{25}H_{43}NO_{18}$) dissolved by using the following procedure.

Buffer: 0.6 mg/mL of monobasic potassium phosphate and 0.35 mg/mL of dibasic sodium phosphate in water, adjusted to a pH of 6.8. Filter and degas.

Mobile phase: Acetonitrile and *Buffer* (5:95)

Standard stock solution: 10 mg/mL of USP Acarbose RS in *Medium*

Standard solution A (for Tablets labeled to contain 100 mg of acarbose): 0.1 mg/mL of USP Acarbose RS from the *Standard stock solution* in *Medium*

Standard solution B (for Tablets labeled to contain 50 mg of acarbose): 0.05 mg/mL of USP Acarbose RS from *Standard solution A* in *Medium*

Standard solution C (for Tablets labeled to contain 25 mg of acarbose): 0.025 mg/mL of USP Acarbose RS from *Standard solution A* in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*)

Mode: LC

Detector: UV 210 nm

Column: 4.0-mm × 12.5-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.8 mL/min

Injection volume: 100 µL

System suitability

Samples: *Standard solution A*, *Standard solution B*, or *Standard solution C*

Suitability requirements

Relative standard deviation: NMT 2.0% for the acarbose peak of *Standard solution A*, *Standard solution B*, or *Standard solution C*

Analysis

Samples: *Standard solution A*, *Standard solution B*, or *Standard solution C*; and *Sample solution*

Calculate the percentage of the labeled amount of acarbose ($C_{25}H_{43}NO_{18}$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (1/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from *Standard solution A*, *Standard solution B*, or *Standard solution C*

C_S = concentration of USP Acarbose RS in *Standard solution A*, *Standard solution B*, or *Standard solution C* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of acarbose ($C_{25}H_{43}NO_{18}$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution 1: Prepare a 0.2-mg/mL solution of USP Acarbose RS in water by pipetting 1.0 mL of the *Standard solution* from the *Assay* into a 50-mL volumetric flask. Dilute with water to volume.

Sensitivity solution: Prepare a 0.02-mg/mL solution of USP Acarbose RS in water by pipetting 10.0 mL of *Standard solution 1* into a 100-mL volumetric flask. Dilute with water to volume.

System suitability

Samples: *System suitability solution*, *Standard solution 1*, and *Sensitivity solution*

Suitability requirements

Peak-to-valley ratio: The ratio of the height of the impurity A peak to the height of the valley between the

www.webofpharma.com

impurity A peak and the acarbose peak is NLT 1.2, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution 1*
Relative standard deviation: NMT 2.0%, *Standard solution 1*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Sample solution* and *Standard solution 1*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of acarbose from *Standard solution 1*
C_S = concentration of USP Acarbose RS in *Standard solution 1* (mg/mL)
C_U = nominal concentration of acarbose in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

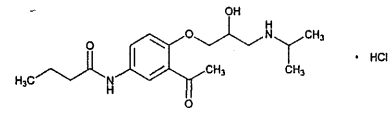
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity D[a]	0.5	1.3	1.2
Impurity B[b]	0.8	1.6	0.5
Impurity A[c]	0.9	1.0	1.6
Impurity C[d]	1.2	1.0	1.0
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	3.0

[a] 4-*O*-(4,6-Dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-en-1-yl]amino}-α-D-glucopyranosyl)-D-glucose.
[b] (1*R*,4*R*,5*S*,6*R*)-4,5,6-Trihydroxy-2-(hydroxymethyl)cyclohex-2-en-1-yl 4-*O*-(4,6-dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-en-1-yl]amino}-α-D-glucopyranosyl)-α-D-glucopyranoside.
[c] *O*-4,6-Dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-en-1-yl]amino}-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-arabino-2-hexulopyranose.
[d] α-D-Glucopyranosyl 4-*O*-(4,6-dideoxy-4-{[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-en-1-yl]amino}-α-D-glucopyranosyl)-α-D-glucopyranoside.

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total aerobic microbial count is NMT 10^3 cfu/g, and the total combined yeasts and molds count is NMT 10^2 cfu/g. It meets the requirements of the testing of products for the absence of *Escherichia coli* (per gram).

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Acarbose RS
USP Acarbose System Suitability Mixture RS▲ (USP 1-May-2019)

Acebutolol Hydrochloride



$C_{18}H_{28}N_2O_4 \cdot HCl$ 372.89
Butanamide, *N*-[3-acetyl-4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]-, monohydrochloride, (±)-;
(±)-3′-Acetyl-4′-[2-hydroxy-3-(isopropylamino)propoxy]-butyranilide monohydrochloride [34381-68-5].

DEFINITION
Acebutolol Hydrochloride contains NLT 98.0% and NMT 102.0% of acebutolol hydrochloride ($C_{18}H_{28}N_2O_4 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. Identification Tests—General** ⟨191⟩, *Chloride*: Meets the requirements of tests *A* and *B*

ASSAY
• **Procedure**
Mobile phase: Methanol, glacial acetic acid, and 0.3% aqueous solution of sodium dodecyl sulfate (675:20:325). Make adjustments if necessary to achieve a retention time for acebutolol of between 4 and 7 min.
Standard solution: 0.14 mg/mL of USP Acebutolol Hydrochloride RS in water
Sample solution: 0.14 mg/mL of Acebutolol Hydrochloride in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1500 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acebutolol hydrochloride ($C_{18}H_{28}N_2O_4 \cdot HCl$) in the portion of Acebutolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acebutolol from the *Sample solution*
r_S = peak response of acebutolol from the *Standard solution*
C_S = concentration of USP Acebutolol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Acebutolol Hydrochloride in the *Sample solution* (mg/mL)

USP Monographs

www.webofpharma.com

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A: Mix 2.0 mL of phosphoric acid and 3.0 mL of triethylamine, and dilute with water to 1 L.

Solution B: Acetonitrile and *Solution A* (1:1)

Standard stock solution 1: 0.2 mg/mL of USP Acebutolol Related Compound A RS prepared as follows. Dissolve a suitable amount of USP Acebutolol Related Compound A RS in a suitable volumetric flask, in 50% of the total volume of acetonitrile, and dilute with *Solution A* to volume.

Standard stock solution 2: 0.2 mg/mL of USP Acebutolol Related Compound B RS prepared as follows. Dissolve a suitable amount of USP Acebutolol Related Compound B RS in a suitable volumetric flask, in 50% of the total volume of acetonitrile, and dilute with *Solution A* to volume.

Standard solution A: 0.002 mg/mL of USP Acebutolol Hydrochloride RS in *Solution A*

Standard solution B: 0.004 mg/mL of USP Acebutolol Related Compound I RS in *Solution A*

Standard solution C: 0.002 mg/mL of USP Acebutolol Related Compound A RS in *Solution A* from *Standard stock solution 1*

Standard solution D: 0.004 mg/mL of USP Acebutolol Related Compound B RS in *Solution A* from *Standard stock solution 2*

System suitability solution: 0.4 µg/mL each of USP Acebutolol Hydrochloride RS and USP Acebutolol Related Compound I RS in *Solution A* from *Standard solution A* and *Standard solution B*

Sample solution: 2 mg/mL of Acebutolol Hydrochloride in *Solution A*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
2	98	2
30.5	10	90
41	10	90

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.0-mm × 12.5-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 25 µL

System suitability
Samples: *Standard solution A* and *System suitability solution*
Suitability requirements
Resolution: NLT 7.0 between acebutolol and acebutolol related compound I, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution A*

Analysis
Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, *Standard solution D*, and *Sample solution*
Calculate the percentage of acebutolol related compound A, acebutolol related compound B, and acebutolol related compound I in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acebutolol related compound A or acebutolol related compound B or acebutolol related compound I from the *Sample solution*
r_S = peak response of acebutolol related compound A or acebutolol related compound B or acebutolol related compound I from *Standard solution B*, *Standard solution C*, or *Standard solution D*
C_S = concentration of USP Acebutolol Related Compound A RS or USP Acebutolol Related Compound B RS or USP Acebutolol Related Compound I RS in *Standard solution B*, *Standard solution C*, or *Standard solution D* (mg/mL)
C_U = concentration of Acebutolol Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of acebutolol from *Standard solution A*
C_S = concentration of USP Acebutolol Hydrochloride RS in *Standard solution A* (mg/mL)
C_U = concentration of Acebutolol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acebutolol related compound B[a]	0.72	0.2
Acebutolol related compound I[b]	0.91	0.2
Acebutolol	1.00	—
Acebutolol related compound A[c]	1.48	0.1
Any unspecified impurity	—	0.10
Total impurities[d]	—	0.5

[a] *N*-{3-Acetyl-4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl}acetamide.
[b] *N*-{3-Acetyl-4-[3-(ethylamino)-2-hydroxypropoxy]phenyl}butyramide.
[c] *N*-(3-Acetyl-4-hydroxyphenyl)butyramide.
[d] Total impurities include specified and unspecified impurities.

SPECIFIC TESTS

- **pH** ⟨791⟩
Sample: 10 mg/mL of Acebutolol Hydrochloride in water
Acceptance criteria: 4.5–7.0
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Acebutolol Hydrochloride RS
USP Acebutolol Related Compound A RS
N-(3-Acetyl-4-hydroxyphenyl)butyramide.
$C_{12}H_{15}NO_3$ 221.25

www.webofpharma.com

USP Acebutolol Related Compound B RS
N-{3-Acetyl-4-[2-hydroxy-3-(isopropylamino)propoxy] phenyl}acetamide.
$C_{16}H_{24}N_2O_4$ 308.37
USP Acebutolol Related Compound I RS
N-{3-Acetyl-4-[3-(ethylamino)-2-hydroxypropoxy] phenyl}butyramide.
$C_{17}H_{26}N_2O_4$ 322.40

Acebutolol Hydrochloride Capsules

DEFINITION
Acebutolol Hydrochloride Capsules contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of acebutolol ($C_{18}H_{28}N_2O_4$).

IDENTIFICATION
- **A.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: Dissolve 2.4 g of sodium 1-decanesulfonate in 1000 mL of water. Adjust with glacial acetic acid to a pH of 3.5.
Mobile phase: Methanol and *Buffer* (60:40)
Standard solution: 0.22 mg/mL of USP Acebutolol Hydrochloride RS in methanol. [NOTE—This is equivalent to 0.2 mg/mL of acebutolol.]
Sample stock solution: Nominally 1 mg/mL of acebutolol prepared as follows. Transfer an equivalent to 200 mg of acebutolol, from the contents of NLT 20 Capsules, to a 200-mL volumetric flask. Add 180 mL of methanol, and stir by mechanical means for 30 min. Dilute with methanol to volume.
Sample solution: Nominally 0.2 mg/mL of acebutolol in methanol from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification A*, use a diode-array detector in the range of 200–400 nm.
Column: 3.9-mm × 15-cm; 4-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acebutolol ($C_{18}H_{28}N_2O_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of acebutolol from the *Sample solution*
r_S = peak response of acebutolol from the *Standard solution*
C_S = concentration of USP Acebutolol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acebutolol from the *Sample solution* (mg/mL)
M_{r1} = molecular weight of acebutolol, 336.43
M_{r2} = molecular weight of acebutolol hydrochloride, 372.89

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: A known concentration of USP Acebutolol Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 232 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acebutolol ($C_{18}H_{28}N_2O_4$) dissolved:

$$(A_U/A_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of acebutolol in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
M_{r1} = molecular weight of acebutolol, 336.43
M_{r2} = molecular weight of acebutolol hydrochloride, 372.89

Tolerances: NLT 80% (*Q*) of the labeled amount of acebutolol ($C_{18}H_{28}N_2O_4$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
- **ORGANIC IMPURITIES**

Test 1
Buffer: Prepare as directed in the *Assay*.
Mobile phase: Methanol and *Buffer* (44:56)
Diluent: Methanol and *Buffer* (50:50)
Standard stock solution: 0.6 mg/mL of USP Acebutolol Hydrochloride RS prepared as follows. To a suitable amount of USP Acebutolol Hydrochloride RS in a suitable volumetric flask, add methanol, about 24% of the flask volume, swirl to dissolve, and dilute with *Diluent* to volume.
Standard solution: 1.4 µg/mL of USP Acebutolol Hydrochloride RS in *Diluent* from *Standard stock solution*
Sample stock solution: Nominally 2.5 mg/mL of acebutolol prepared as follows. Transfer a portion of the contents of 20 opened Capsules, equivalent to 250 mg of acebutolol, to a 100-mL volumetric flask. Add 25 mL of methanol and shake by mechanical means for 15 min. Dilute with *Diluent* to volume. Centrifuge a portion of this solution and use the supernatant.
Sample solution: Nominally 250 µg/mL of acebutolol in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm

www.webofpharma.com

Column: 3.9-mm × 15-cm; 4-µm packing L1
Flow rate: 1 mL/min
Injection volume: 35 µL
Run time: NLT 2 times the retention time of acebutolol
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 6.0%
Analysis
Samples: *Diluent, Standard solution,* and *Sample solution*
Calculate the percentage of each impurity eluting before the acebutolol peak in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of acebutolol from the *Standard solution*
C_S = concentration of USP Acebutolol Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of acebutolol in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of acebutolol, 336.43
M_{r2} = molecular weight of acebutolol hydrochloride, 372.89

Acceptance criteria: NMT 0.5% of any individual impurity. Disregard any peaks from the *Diluent.*

Test 2
Buffer and **System suitability:** Proceed as directed in *Test 1.*
Mobile phase: Methanol and *Buffer* (50:50)
Standard stock solution: 0.6 mg/mL of USP Acebutolol Hydrochloride RS prepared as follows. To a suitable amount of USP Acebutolol Hydrochloride RS in a suitable volumetric flask, add methanol, about 24% of the flask volume, swirl to dissolve, and dilute with *Mobile phase* to volume.
Standard solution: 1.4 µg/mL of USP Acebutolol Hydrochloride RS in *Mobile phase* from *Standard stock solution*
Sample stock solution: Nominally 2.5 mg/mL of acebutolol prepared as follows. Transfer a portion of the contents of 20 opened Capsules, equivalent to 250 mg of acebutolol, to a 100-mL volumetric flask. Add 25 mL of methanol and shake by mechanical means for 15 min. Dilute with *Mobile phase* to volume.
Sample solution: Nominally 250 µg/mL of acebutolol in *Diluent* from *Sample stock solution* prepared as follows. Centrifuge a portion of *Sample stock solution,* and transfer 10.0 mL of the clear supernatant to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Proceed as directed in *Test 1* except for the *Injection volume* and *Run time.*
Injection volume: 70 µL
Run time: NLT 3 times the retention time of acebutolol
Analysis
Samples: *Mobile phase, Standard solution,* and *Sample solution*
Calculate the percentage of each impurity eluting after the acebutolol peak in the portion of Capsules taken:

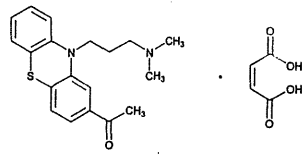
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of acebutolol from the *Standard solution*
C_S = concentration of USP Acebutolol Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of acebutolol from the *Sample solution* (µg/mL)
M_{r1} = molecular weight of acebutolol, 336.43
M_{r2} = molecular weight of acebutolol hydrochloride, 372.89

Acceptance criteria
Test 2: NMT 0.5% of any individual impurity. Disregard any peaks from the *Mobile phase.*
Sum of impurities from *Test 1* and *Test 2*: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Acebutolol Hydrochloride RS

Acepromazine Maleate



$C_{19}H_{22}N_2OS \cdot C_4H_4O_4$ 442.53
Ethanone, 1-[10-[3-(dimethylamino)propyl]-10*H*-phenothiazin-2-yl]-, (Z)-2-butenedioate (1:1);
10-[3-(Dimethylamino)propyl]phenothiazin-2-yl methyl ketone maleate (1:1) [3598-37-6].

DEFINITION
Acepromazine Maleate contains NLT 98.0% and NMT 101.0% of acepromazine maleate ($C_{19}H_{22}N_2OS \cdot C_4H_4O_4$), calculated on the anhydrous basis.
Throughout the following procedures, protect samples, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak for acepromazine of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **PROCEDURE**

Buffer: Add 6 mL of triethylamine to 700 mL of water, and adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile and *Buffer* (300:700)
Standard stock solution: 1 mg/mL of USP Acepromazine Maleate RS in 0.05 N hydrochloric acid
Standard solution: 0.1 mg/mL of USP Acepromazine Maleate RS in water from *Standard stock solution*
Sample stock solution: 1 mg/mL of Acepromazine Maleate in 0.05 N hydrochloric acid
Sample solution: 0.1 mg/mL of Acepromazine Maleate in water from *Sample stock solution*

Stimuli Article

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1500 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acepromazine maleate ($C_{19}H_{22}N_2OS \cdot C_4H_4O_4$) in the portion of Acepromazine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area response from the *Sample solution*
r_S = peak area response from the *Standard solution*
C_S = concentration of USP Acepromazine Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%
- **ORGANIC IMPURITIES**

Conduct this test without exposure to daylight, and with the minimum necessary exposure to artificial light.
Diluent: Methanol and diethylamine (19:1)
Sample solution: 20.0 mg/mL of Acepromazine Maleate in *Diluent*
Standard solution: 0.1 mg/mL of Acepromazine Maleate in *Diluent* from the *Sample solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: *n*-Heptane, isobutyl alcohol, and diethylamine (75:17:8)
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths the length of the plate. Remove the plate from the chamber and allow to air dry. Examine the plate under short-wavelength UV light.
Acceptance criteria: 0.5%; no spot, other than the principal acepromazine spot and any at the origin, observed in the chromatogram of the *Sample solution* is more intense than the principal spot observed in the chromatogram of the *Standard solution*.

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 136°–139°
- **pH** ⟨791⟩

Sample solution: 10 mg/mL of Acepromazine Maleate in water
Acceptance criteria: 4.0–5.5
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Acepromazine Maleate RS

Acepromazine Maleate Injection

DEFINITION
Acepromazine Maleate Injection is a sterile solution of Acepromazine Maleate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of acepromazine maleate ($C_{19}H_{22}N_2OS \cdot C_4H_4O_4$).
Throughout the following procedures, protect samples, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: To a volume of Injection, equivalent to 20 mg of acepromazine maleate, add 2 mL of water and 3 mL of 2 N sodium hydroxide, and extract with two 5-mL portions of cyclohexane. Combine the cyclohexane extracts, and evaporate to dryness under vacuum, using gentle heat if necessary.
Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: Add 6 mL of triethylamine to 700 mL of water, and adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile and *Buffer* (300:700)
Standard stock solution: 1 mg/mL of USP Acepromazine Maleate RS in 0.05 N hydrochloric acid
Standard solution: 0.1 mg/mL of USP Acepromazine Maleate RS in water from *Standard stock solution*
Sample stock solution: 1 mg/mL of Acepromazine Maleate in 0.05 N hydrochloric acid from an appropriately diluted volume of Injection
Sample solution: Nominally 0.1 mg/mL of Acepromazine Maleate in water from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1500 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of acepromazine maleate ($C_{19}H_{22}N_2OS \cdot C_4H_4O_4$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Acepromazine Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 4.5–5.8
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 4.5 USP Endotoxin Units/mg of acepromazine maleate
- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant, single-dose or multiple-dose containers as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Store at controlled room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Acepromazine Maleate RS

Acepromazine Maleate Tablets

DEFINITION
Acepromazine Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acepromazine maleate ($C_{19}H_{22}N_2OS \cdot C_4H_4O_4$).
Throughout the following procedures, protect samples, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: To a quantity of powdered Tablets, equivalent to 20 mg of acepromazine maleate, add 2 mL of water and 3 mL of 2 N sodium hydroxide, and extract with two 5-mL portions of cyclohexane. Combine the cyclohexane extracts, and evaporate to dryness under vacuum, using gentle heat if necessary.
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Buffer: Add 6 mL of triethylamine to 700 mL of water, and adjust with phosphoric acid to a pH of 2.5.
  Mobile phase: Acetonitrile and *Buffer* (300:700)
  Standard stock solution: 1 mg/mL of USP Acepromazine Maleate RS in 0.05 N hydrochloric acid
  Standard solution: 0.1 mg/mL of USP Acepromazine Maleate RS in water from *Standard stock solution*
  Sample stock solution: Transfer NLT 10 Tablets to a 200-mL volumetric flask, add 100 mL of 0.05 N hydrochloric acid, and sonicate for 10 min. Shake by mechanical means for 30 min, and dilute with 0.05 N hydrochloric acid to volume.
  Sample solution: Nominally 0.1 mg/mL of Acepromazine Maleate in water from *Sample stock solution*. Pass a portion of this solution through a filter of 0.5-µm or finer pore size.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 4-mm × 15-cm; 5-µm packing L7
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 1500 theoretical plates
  Tailing factor: NMT 2.5
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of acepromazine maleate ($C_{19}H_{22}N_2OS \cdot C_4H_4O_4$) in the portion of Tablets taken:

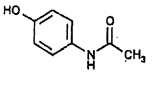
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Acepromazine Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** Label the Tablets to indicate that they are for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Acepromazine Maleate RS

Acetaminophen



$C_8H_9NO_2$ 151.16
Acetamide, *N*-(4-hydroxyphenyl)-;
4′-Hydroxyacetanilide [103-90-2].

DEFINITION
Acetaminophen contains NLT 98.0% and NMT 102.0% of acetaminophen ($C_8H_9NO_2$), calculated on the dried basis.

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Use low-actinic glassware for preparation of the *Sample solution*.

Solution A: 1.7 g/L of monobasic potassium phosphate and 1.8 g/L of dibasic sodium phosphate, anhydrous

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	99	1
3.0	99	1
7.0	19	81
7.1	99	1
10.0	99	1

Standard solution: 0.1 mg/mL of USP Acetaminophen RS in methanol

Sample solution: 0.1 mg/mL of Acetaminophen in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 10-cm; 3.5-µm packing L7

Column temperature: 35°

Flow rate: 1.0 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acetaminophen ($C_8H_9NO_2$) in the portion of Acetaminophen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = concentration of Acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **LIMIT OF FREE 4-AMINOPHENOL**

Solution A, Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 1.25 µg/mL of USP 4-Aminophenol RS in methanol

Sample solution: 25 mg/mL of Acetaminophen in methanol

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for 4-aminophenol and acetaminophen are 0.6 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 4-aminophenol in the portion of Acetaminophen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 4-aminophenol from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP 4-Aminophenol RS in the *Standard solution* (µg/mL)

C_U = concentration of Acetaminophen in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.005%

- **ORGANIC IMPURITIES**

Solution A: Methanol, water, glacial acetic acid (50:950:1)

Solution B: Methanol, water, glacial acetic acid (500:500:1)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	82	18
8	82	18
53	0	100
58	0	100
59	82	18
73	82	18

Diluent: Methanol

System suitability solution: 20 µg/mL of USP Acetaminophen RS and 80 µg/mL each of USP Acetaminophen Related Compound B RS and USP Acetaminophen Related Compound C RS in *Diluent*

Standard solution: 1.25 µg/mL of USP Acetaminophen Related Compound D RS and 0.25 µg/mL of USP Acetaminophen Related Compound J RS in *Diluent*

Sample solution: 25 mg/mL of Acetaminophen in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 0.9 mL/min

Column temperature: 40°

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 3* for relative retention time values.]

Suitability requirements

Tailing factor: NMT 2.0 for acetaminophen related compound D, *Standard solution*

www.webofpharma.com

Resolution: NLT 2.0 between acetaminophen and acetaminophen related compound B; NLT 1.5 between acetaminophen related compound B and acetaminophen related compound C, *System suitability solution*

Relative standard deviation: NMT 5.0% for acetaminophen related compound D, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acetaminophen related compound J in the portion of Acetaminophen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen related compound J from the *Sample solution*

r_S = peak response of acetaminophen related compound J from the *Standard solution*

C_S = concentration of USP Acetaminophen Related Compound J RS in the *Standard solution* (µg/mL)

C_U = concentration of Acetaminophen in the *Sample solution* (µg/mL)

Calculate the percentage of acetaminophen related compounds B, C, and D and any unspecified impurity in the portion of Acetaminophen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each specified or unspecified impurity from the *Sample solution*

r_S = peak response of acetaminophen related compound D from the *Standard solution*

C_S = concentration of USP Acetaminophen Related Compound D RS in the *Standard solution* (µg/mL)

C_U = concentration of Acetaminophen in the *Sample solution* (µg/mL)

F = relative response factor for each impurity shown in *Table 3*

Acceptance criteria: See *Table 3*. [NOTE—The relative retention times and relative response factors in *Table 3* (where applicable) are calculated relative to those of acetaminophen related compound D.]

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Acetaminophen	0.43	—	—
Acetaminophen related compound B[a]	0.67	1.2	0.05
Acetaminophen related compound C[b]	0.71	0.38	0.05
Acetaminophen related compound D[c]	1.0	1.0	0.05
Acetaminophen related compound J[d]	1.73	—	0.001
Individual unspecified impurity	—	1.0	0.05
Total impurities	—	—	0.1

[a] *N*-(4-Hydroxyphenyl)propanamide.
[b] *N*-(2-Hydroxyphenyl)acetamide.
[c] *N*-Phenylacetamide.
[d] *N*-(4-Chlorophenyl)acetamide (*p*-chloroacetanilide).

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° to constant weight.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature. Protect from moisture and heat.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Acetaminophen RS

USP Acetaminophen Related Compound B RS
N-(4-Hydroxyphenyl)propanamide.
$C_9H_{11}NO_2$ 165.19

USP Acetaminophen Related Compound C RS
N-(2-Hydroxyphenyl)acetamide.
$C_8H_9NO_2$ 151.16

USP Acetaminophen Related Compound D RS
N-Phenylacetamide.
C_8H_9NO 135.17

USP Acetaminophen Related Compound J RS
N-(4-Chlorophenyl)acetamide (*p*-chloroacetanilide).
C_8H_8ClNO 169.61

USP 4-Aminophenol RS
C_6H_7NO 109.13

Acetaminophen Capsules

DEFINITION

Acetaminophen Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Sample solution: 1 mg/mL of acetaminophen prepared as follows. Triturate from contents of the Capsules in methanol. Filter, and use the clear filtrate.

Chromatographic system

Developing solvent system: Methylene chloride and methanol (4:1)

Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Methanol and water (1:3)

Standard solution: 0.01 mg/mL of USP Acetaminophen RS in *Mobile phase*

Sample stock solution: Weigh the contents of NLT 20 Capsules, and calculate the average weight of the contents of each Capsule. Mix the combined contents of the Capsules, and transfer a portion, equivalent to 100 mg of acetaminophen, to a 200-mL volumetric flask. Add 100 mL of *Mobile phase*, shake by mechanical means for 10 min, and dilute with *Mobile phase* to volume. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass a portion of this solution through a filter of 0.5-µm or finer pore size, discarding the first 10 mL of the filtrate.

Sample solution: Nominally 0.01 mg/mL of acetaminophen from the *Sample stock solution* in *Mobile phase*. Pass a portion of this solution through a filter of

www.webofpharma.com

0.5-µm or finer pore size, discarding the first 10 mL of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 243 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: A known concentration of USP Acetaminophen RS in *Medium*
Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium* to obtain a concentration similar to that of the *Standard solution*
Instrumental conditions
Mode: UV
Analytical wavelength: 249 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES
• **4-Aminophenol in Acetaminophen-Containing Drug Products** ⟨227⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Acetaminophen RS

Acetaminophen Oral Solution

DEFINITION
Acetaminophen Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Sample solution: Nominally 1 mg/mL of acetaminophen in methanol from the Oral Solution
Chromatographic system
Developing solvent system: Methylene chloride and methanol (4:1)
Acceptance criteria: Meets the requirements

ASSAY
• **Procedure**
Mobile phase: Methanol and water (1:3)
Standard solution: 0.01 mg/mL of USP Acetaminophen RS in *Mobile phase*
Sample stock solution: Nominally 2 mg/mL in *Mobile phase*, prepared as follows. Transfer 500 mg of acetaminophen from a measured volume of Oral Solution to a 250-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: Nominally 0.01 mg/mL of acetaminophen in *Mobile phase* from the *Sample stock solution*. Pass a portion of this solution through a filter of 0.5-µm or finer pore size, discarding the first 10 mL of the filtrate. Use the clear filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 243 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Deliverable Volume** ⟨698⟩: Meets the requirements for oral solutions packaged in multiple-unit containers

USP Monographs

www.webofpharma.com

- **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements for oral solutions packaged in single-unit containers

IMPURITIES
- **4-Aminophenol in Acetaminophen-Containing Drug Products** ⟨227⟩: Meets the requirements

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.8–6.1
- **Alcohol Determination,** *Method II* ⟨611⟩ (if present)

Analysis: Determine by the gas–liquid chromatographic procedure, using acetone as the internal standard.
Acceptance criteria: 90.0%–115.0% of the labeled amount of alcohol (C_2H_5OH)

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Acetaminophen RS

Acetaminophen for Effervescent Oral Solution

DEFINITION
Acetaminophen for Effervescent Oral Solution contains, in each 100 g, NLT 5.63 g and NMT 6.88 g of acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION
- **A.** A 10-g portion dissolves, with effervescence, in water when dissolved as directed for the *Sample solution* in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Thin-Layer Chromatographic Identification Test** ⟨201⟩

Sample solution: Triturate 0.4 g of the powder with 25 mL of methanol, and filter.
Chromatographic system
Developing solvent system: Methylene chloride and methanol (4:1)
Acceptance criteria: Meets the requirements

ASSAY
- **Procedure**

Mobile phase: Methanol and water (1:3)
Standard solution: 0.01 mg/mL of USP Acetaminophen RS in *Mobile phase*
Sample stock solution: Dissolve 10 g of Acetaminophen for Effervescent Oral Solution in 200 mL of water in a 1000-mL volumetric flask, using gentle heat if necessary, until effervescence subsides, and then dilute with water to volume.
Sample solution: Nominally 0.16 mg/mL of acetaminophen in *Mobile phase* from the *Sample stock solution*. Pass a portion of this solution through a filter of 0.5-µm or finer pore size, discarding the first 10 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 243 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in g, of acetaminophen ($C_8H_9NO_2$) in 100 g of Acetaminophen for Effervescent Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F_1) \times L \times F_2$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)
F_1 = conversion factor, 1000 mg/g
L = label claim (mg/unit)
F_2 = conversion factor, 100, based on the label claim of g of acetaminophen per 100 g of sample

Acceptance criteria: 5.63–6.88 g

PERFORMANCE TESTS
- **Minimum Fill** ⟨755⟩: Meets the requirements for solids packaged in multiple-unit containers
- **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements for solids packaged in single-unit containers

IMPURITIES
- **4-Aminophenol in Acetaminophen-Containing Drug Products** ⟨227⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in air-tight containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Acetaminophen RS

Acetaminophen Oral Suspension

DEFINITION
Acetaminophen Oral Suspension is a suspension of Acetaminophen in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197K▲ (CN 1-May-2020)

Sample: Transfer a volume of Oral Suspension, equivalent to 240 mg of acetaminophen, to a separator. Add 50 mL of ethyl acetate, and shake. Filter the ethyl acetate extract through a funnel containing glass wool and 10 g of anhydrous sodium sulfate. Collect the filtrate in a beaker, and evaporate on a steam bath to dryness. Dry the residue under vacuum over silica gel.
Acceptance criteria: The crystals so obtained meet the requirements.

USP Monographs

www.webofpharma.com

• **B.** The retention time of the acetaminophen peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Add the following:

▲• **PROCEDURE**

Mobile phase: Methanol and water (1:3)

Standard solution: 0.01 mg/mL of USP Acetaminophen RS in *Mobile phase*

Sample stock solution: Nominally 0.5 mg/mL of acetaminophen prepared as follows. Transfer 100 mg of acetaminophen from a volume of Oral Suspension, previously well shaken, to a 200-mL volumetric flask. Add 100 mL of *Mobile phase*, and shake by mechanical means for 10 min. Dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.01 mg/mL of acetaminophen from the *Sample stock solution* in *Mobile phase*. Pass a portion of this solution through a filter of 0.5-µm pore size or finer, discarding the first 10 mL of the filtrate. Use the clear filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 243 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%▲▲ (RB 1-Aug-2018)▲ (ERR 1-Jul-2019)

Change to read:

• **PROCEDURE**

▲**Solution A:** Acetonitrile, trifluoroacetic acid, and water (14: 0.1: 86)

Solution B: Acetonitrile, trifluoroacetic acid, and water (90: 0.1: 10)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
4.0	100	0
5.0	0	100
6.0	100	0
10.0	100	0

Diluent: Methanol, phosphoric acid, and water (50: 0.1: 50)

Standard stock solution: 1.6 mg/mL of USP Acetaminophen RS in *Diluent*

Standard solution: 0.064 mg/mL of USP Acetaminophen RS in *Solution A*, from *Standard stock solution*

Sample stock solution: Nominally 1.6 mg/mL of acetaminophen prepared as follows. Transfer a quantity equivalent to about 160 mg of acetaminophen from a volume of Oral Suspension, previously well shaken, to a 100-mL volumetric flask. Add 60 mL of *Diluent*, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume. Mix well. Allow the sample to settle, or centrifuge.

Sample solution: Nominally 0.064 mg/mL of acetaminophen in *Solution A*, from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L11

Flow rate: 1 mL/min

Injection volume: 30 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*

r_S = peak response of acetaminophen from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)▲ (Postponed on 1-Aug-2018)

Acceptance criteria: 90.0%–110.0%

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
4.0	100	0
5.0	0	100
6.0	100	0
10.0	100	0

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For single-unit containers: Meets the requirements

• **DELIVERABLE VOLUME** ⟨698⟩

For multiple-unit containers: Meets the requirements

USP Monographs

www.webofpharma.com

IMPURITIES

Delete the following:

▲• **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩: Meets the requirements▲ (Postponed on 1-Aug-2018)

Add the following:

▲• **ORGANIC IMPURITIES**

Solution A: 0.2% trifluoroacetic acid in water

Solution B: 0.2% trifluoroacetic acid in acetonitrile

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	98	2
1.0	98	2
8.0	80	20
9.0	5	95
10.0	5	95
10.5	98	2
13.0	98	2

Buffer: 10 mM sodium citrate dihydrate, with a pH of 4.0, prepared by adding 1.1 g of sodium citrate dihydrate and 1.3 g of citric acid monohydrate to a 1-L volumetric flask, dissolving, and diluting with water to volume. Adjust with sodium citrate dihydrate to increase the pH or with citric acid monohydrate to decrease the pH, if necessary, to achieve a pH of 4.0.

Diluent: Acetonitrile and *Buffer* (10:90)

Sensitivity solution: 0.16 µg/mL of USP Acetaminophen RS and 0.08 µg/mL of USP 4-Aminophenol RS in *Diluent*

Standard solution: 1.6 µg/mL each of USP Acetaminophen RS and USP 4-Aminophenol RS in *Diluent*

Sample solution: Nominally 1.6 mg/mL of acetaminophen in *Diluent* prepared as follows. Transfer a quantity equivalent to about 160 mg of acetaminophen from a volume of Oral Suspension, previously well shaken, to a 100-mL volumetric flask. Add 60 mL of *Diluent*, and shake by mechanical means for 1 h. Dilute with *Diluent* to volume. Mix well. Pass a portion of this solution through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 272 nm

Column: 2.1-mm × 15-cm; 1.8-µm packing L1

Column temperature: 40°

Flow rate: 0.5 mL/min

Injection volume: 2.5 µL

System suitability

Samples: *Sensitivity solution* and *Standard solution*

[NOTE—See *Table 3* for relative retention times.]

Suitability requirements

Tailing factor: NMT 2.0 for acetaminophen and 4-aminophenol, *Standard solution*

Relative standard deviation: NMT 5.0% for acetaminophen and 4-aminophenol, *Standard solution*

Signal-to-noise ratio: NLT 10 for acetaminophen and 4-aminophenol, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 4-aminophenol in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 4-aminophenol from the *Sample solution*

r_S = peak response of 4-aminophenol from the *Standard solution*

C_S = concentration of USP 4-Aminophenol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Calculate the percentage of acetaminophen dimer or any unspecified impurity in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen dimer or any unspecified impurity from the *Sample solution*

r_S = peak response of acetaminophen from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. The reporting threshold is 0.05% for any impurities.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
4-Aminophenol	0.28	0.15
Acetaminophen	1.0	—
Acetaminophen dimer[a]	1.57	0.15
Any unspecified impurity	—	0.15
Total impurities	—	2.0

[a] *N,N'*-(6,6'-Dihydroxy-[1,1'-biphenyl]-3,3'-diyl)diacetamide.▲ (Postponed on 1-Aug-2018)

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.0–6.9

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Acetaminophen RS

USP 4-Aminophenol RS

Acetaminophen Suppositories

DEFINITION

Acetaminophen Suppositories contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

www.webofpharma.com

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Sample solution: Transfer the equivalent of 20 mg of acetaminophen from a portion of Suppositories to a beaker. Add 20 mL of methanol and heat on a steam bath until melted. Remove the beaker from the steam bath, allow to cool with occasional stirring, and filter. Use the clear filtrate.

Chromatographic system

Developing solvent system: Methylene chloride and methanol (4:1)

Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and water (1:3)

Standard solution: 0.01 mg/mL of USP Acetaminophen RS in *Mobile phase*

Sample stock solution: Nominally 0.5 mg/mL of acetaminophen prepared as follows. Tare a small dish and a glass rod, place NLT 5 Suppositories in the dish, heat gently on a steam bath until melted, stir, cool while stirring, and weigh. Transfer a weighed portion of the mass, equivalent to 100 mg of acetaminophen, to a separator, add 30 mL of solvent hexane, and dissolve. Add 30 mL of water, shake gently, and allow the phases to separate. If an emulsion forms, allow sufficient time for it to separate. Transfer the aqueous layer to a 200-mL volumetric flask, and wash the solvent hexane in the separator with three 30-mL portions of water, adding the washings to the volumetric flask. Dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.01 mg/mL of acetaminophen in *Mobile phase* from the *Sample stock solution*. Pass a portion of this solution through a filter of 0.5-µm or finer pore size, discarding the first 10 mL of the filtrate. Use the clear filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 243 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Suppositories taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩

Buffer: 4.0 g/L of sodium citrate dihydrate and 1.5 g/L of anhydrous citric acid, in water

Diluent: *Buffer* and acetonitrile (9:1)

Sample stock solution: Approximately 12–13 mg/mL of acetaminophen prepared as follows. Transfer an appropriate number of whole Suppositories to a suitable volumetric flask. Add *Diluent* until the flask is about half filled and sonicate for 1 h with frequent swirling. Allow to cool and then dilute with *Diluent* to volume.

Sample solution: Approximately 4.8–5.2 mg/mL of acetaminophen in *Diluent* from the *Sample stock solution* prepared as follows. Pipet 20.0 mL of the *Sample stock solution* into a 50-mL volumetric flask and dilute with *Diluent* to volume.

Standard stock solution: Prepare as indicated in the chapter.

Standard solution: Add 20.0 mL of the *Sample stock solution* and 15.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature or in a cool place.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Acetaminophen RS

Acetaminophen Tablets

DEFINITION

Acetaminophen Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION

• **A.** The retention time of the acetaminophen peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.** The UV spectrum of the acetaminophen peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 1% (v/v) glacial acetic acid in water

Solution B: Methanol

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for 4 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
4.0	90	10
4.1	20	80
6.0	20	80

Diluent: Methanol and water (10:90)

Standard solution: 0.01 mg/mL of USP Acetaminophen RS in *Diluent*

Sample stock solution: Nominally 0.1 mg/mL of acetaminophen in *Diluent* prepared as follows. Transfer an appropriate amount of acetaminophen from NLT 10

www.webofpharma.com

Tablets to a suitable volumetric flask and dilute with *Diluent* to volume. Centrifuge or pass a portion of this solution through a suitable filter. [NOTE—Sonication or shaking may be necessary.]

Sample solution: Nominally 0.01 mg/mL of acetaminophen in *Diluent* from the *Sample stock solution*. Pass a portion of this solution through a suitable filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 243 nm. For *Identification B*, use a diode array detector in the range of 220–400 nm.
Column: 3.0-mm × 10-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 0.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*
r_S = peak response of acetaminophen from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: pH 5.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: A known concentration of USP Acetaminophen RS in *Medium*
Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium* to obtain a concentration similar to that of the *Standard solution*
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 243 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$) is dissolved.

For Tablets labeled as chewable
Medium: pH 5.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL
Apparatus 2: 75 rpm
Time: 45 min
Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed above.
Tolerances: NLT 75% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
It is suggested to protect all solutions containing acetaminophen or 4-aminophenol from light.
Buffer: Dissolve 1.9 g of ammonium formate in 1 L of water. Add 1.0 mL of formic acid.
Solution A: Dissolve 3.1 g of ammonium acetate in 1 L of water. Add 1.0 mL of trifluoroacetic acid.
Solution B: Acetonitrile, methanol, and water (10:75:15)
Solution C: Dissolve 3.1 g of ammonium acetate in 1000 mL of *Solution B*. Add 1.0 mL of trifluoroacetic acid.
Mobile phase: See *Table 2*. Return to original conditions and re-equilibrate the system for 4 min.

Table 2

Time (min)	Solution A (%)	Solution C (%)
0	97	3
5	70	30
10	10	90
11	10	90

Diluent: Methanol and *Buffer* (5:95)
Sensitivity solution: 0.000175 mg/mL of USP 4-Aminophenol RS in *Diluent*. Sonicate to dissolve, if necessary.
Standard solution: 0.00175 mg/mL of USP 4-Aminophenol RS and 0.0035 mg/mL of USP Acetaminophen RS in *Diluent*. Sonicate to dissolve, if necessary.
Sample stock solution: Nominally 5 mg/mL of acetaminophen in *Diluent* from NLT 10 Tablets. [NOTE—It is recommended to shake on a flat bed at low speed (180 oscillations/min) to dissolve, if necessary.]
Sample solution: Nominally 3.5 mg/mL of acetaminophen in *Diluent* prepared as follows. Pass a portion of the *Sample stock solution* through a suitable filter of 0.2-µm pore size. Discard the first 2 mL of the filtrate. Dilute a suitable volume of the filtrate with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 272 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 0.9 mL/min
Injection volume: 25 µL
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0% for 4-aminophenol and acetaminophen, *Standard solution*
Signal-to-noise ratio: NLT 10 for 4-aminophenol, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 4-aminophenol in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 4-aminophenol from the *Sample solution*

www.webofpharma.com

r_S = peak response of 4-aminophenol from the *Standard solution*
C_S = concentration of USP 4-Aminophenol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of acetaminophen from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
4-Aminophenol	0.53	0.15
Acetaminophen	1.0	—
Any unspecified impurity	—	0.15
Total impurities	—	0.60

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label Tablets that must be chewed to indicate that they are to be chewed before swallowing.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Acetaminophen RS
  USP 4-Aminophenol RS
  4-Aminophenol.
  C_6H_7NO 109.13

Acetaminophen Extended-Release Tablets

DEFINITION

Acetaminophen Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: A portion of powdered Tablets
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Solution A: Phosphoric acid and water (1:9)
  Mobile phase: Methanol, *Solution A*, and water (300:1:700)
  Standard solution: 0.65 mg/mL of USP Acetaminophen RS in *Mobile phase*. Prepare by first dissolving in methanol, and then diluting with *Mobile phase* to volume.
  Sample stock solution: Transfer 10 Tablets to a 250-mL volumetric flask containing 50 mL of water and a magnetic stir bar. Stir for at least 30 min or until the coating has dissolved. Add 150 mL of methanol, and stir for 45 min. Tablet cores should be disintegrated at least 15 min before ending the stirring. Remove the magnetic stir bar, and rinse into the flask with methanol. Dilute with methanol to volume, and centrifuge. Use the clear supernatant.
  Sample solution: Dilute 5 mL of the *Sample stock solution* with *Mobile phase* to 200 mL.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 295 nm
  Column: 3.9-mm × 15-cm; packing L1
  Flow rate: 2.0 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 3.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium: Simulated gastric fluid TS (without enzyme); 900 mL
  Apparatus 2: 50 rpm
  Times: 15 min, 1 h, and 3 h
  Standard solution: A known concentration of USP Acetaminophen RS in *Medium*
  Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium* to obtain a concentration similar to that of the *Standard solution*
  Instrumental conditions
  Mode: UV
  Analytical wavelength: 280 nm
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) dissolved.
  Tolerances: See *Table 1*.

Table 1

Time	Amount Dissolved
15 min	45%–65%
1 h	60%–85%

www.webofpharma.com

Table 1 *(continued)*

Time	Amount Dissolved
3 h	NLT 85%

The percentages of the labeled amount of acetaminophen ($C_8H_9NO_2$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

For gelatin-coated Tablets

Medium, Apparatus, Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed in *Test 1*.

Times: 30 min, 90 min, and 4 h

Tolerances: See *Table 2*.

Table 2

Time	Amount Dissolved
30 min	40%–60%
90 min	55%–85%
4 h	NLT 80%

The percentages of the labeled amount of acetaminophen ($C_8H_9NO_2$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Apparatus, Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed in *Test 1*.

Times: 15 min, 1 h, and 3 h

Tolerances: See *Table 3*.

Table 3

Time	Amount Dissolved
15 min	40%–60%
1 h	55%–75%
3 h	NLT 80%

The percentages of the labeled amount of acetaminophen ($C_8H_9NO_2$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where the Tablets are gelatin-coated, the label so states. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Acetaminophen RS

Acetaminophen and Aspirin Tablets

DEFINITION

Acetaminophen and Aspirin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$) and aspirin ($C_9H_8O_4$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Use clean and dry glassware. Inject the *Standard solution* and the *Sample solution* promptly after preparation.]

Solution A: Chloroform, methanol, and glacial acetic acid (78:20:2)

Mobile phase: Transfer 225 mg of tetramethylammonium hydroxide pentahydrate to a 1000-mL flask. Add 750 mL of water, 125 mL of methanol, 125 mL of acetonitrile, and 1.0 mL of glacial acetic acid. Stir for 3 min, and pass through a membrane filter of 0.5-µm or finer pore size.

Internal standard solution: 20 mg/mL of benzoic acid in *Solution A*

Standard solution: 3.25 mg/mL each of USP Acetaminophen RS and USP Aspirin RS, and 2.0 mg/mL of benzoic acid, from *Internal standard solution*, in *Solution A*

Sample solution: Nominally 3.25 mg/mL of acetaminophen in *Solution A*, prepared as follows. Transfer an equivalent of 325 mg of acetaminophen, from NLT 20 finely powdered Tablets, to a 100-mL volumetric flask. Add 10.0 mL of *Internal standard solution* and 50 mL of *Solution A*, and sonicate for 3 min. Dilute with *Solution A* to volume. Pass a portion of this solution through a filter of 2.5-µm or finer pore size. Use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention times for acetaminophen, salicylic acid (if present), aspirin, and benzoic acid are about 2, 3, 5, and 8 min, respectively.]

Suitability requirements

Relative standard deviation: NMT 3.0% for acetaminophen or aspirin for four replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate individually the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) and aspirin ($C_9H_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of acetaminophen or aspirin to benzoic acid from the *Sample solution*

R_S = peak response ratio of acetaminophen or aspirin to benzoic acid from the *Standard solution*

C_S = concentration of USP Acetaminophen RS or USP Aspirin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen or aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$) and aspirin ($C_9H_8O_4$)

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Procedure for a pooled sample

Medium: Water; 900 mL

Apparatus 2: 50 rpm

USP Monographs

www.webofpharma.com

Time: 45 min
Solution A, Mobile phase, and **Chromatographic system** (except for ***Injection volume***): Proceed as directed in the *Assay*.
Injection volume: 20 µL
Internal standard solution: 1 mg/mL of benzoic acid in methanol
Standard stock solution A: 70 µg/mL of USP Salicylic Acid RS in *Solution A*
Standard solution A: Combine 4.0 mL of *Standard stock solution A* and 1.0 mL of *Internal standard solution*.
Standard stock solution B: 360 µg/mL each of USP Acetaminophen RS and USP Aspirin RS in *Solution A*
Standard solution B: Combine 4.0 mL of *Standard stock solution B* and 1.0 mL of *Internal standard solution*.
Sample stock solution: Filtered portions of sample suitably diluted with *Medium* to a concentration that is similar to that of *Standard stock solution A* or *Standard stock solution B*
Sample solution: Combine 4.0 mL of the *Sample stock solution* and 1.0 mL of *Internal standard solution*.
Analysis
Samples: *Standard solution A, Standard solution B,* and *Sample solution*
[NOTE—The relative retention times for acetaminophen, salicylic acid, aspirin, and benzoic acid are about 0.3, 0.4, 0.6, and 1.0, respectively.]
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) dissolved:

$$\text{Result} = (R_U/R_S) \times (C/W) \times 90$$

R_U = relative peak response ratio of acetaminophen to the internal standard from the *Sample solution*
R_S = relative peak response ratio of acetaminophen to the internal standard from *Standard solution B*
C = concentration of USP Acetaminophen RS in *Standard solution B* (µg/mL)
W = labeled amount of acetaminophen (mg)

Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) dissolved:

$$\text{Result} = \{[90C_1 \times (R_{U1}/R_{S1})] + [90C_2 \times (R_{U2}/R_{S2}) \times 1.3044]\}/W$$

C_1 = concentration of USP Aspirin RS in *Standard solution B* (µg/mL)
R_{U1} = relative peak response ratio of aspirin to the internal standard from the *Sample solution*
R_{S1} = relative peak response ratio of aspirin to the internal standard from *Standard solution B*
C_2 = concentration of USP Salicylic Acid RS in *Standard solution A* (µg/mL)
R_{U2} = relative peak response ratio of salicylic acid to the internal standard from the *Sample solution*
R_{S2} = relative peak response ratio of salicylic acid to the internal standard from *Standard solution A*
W = labeled amount of aspirin (mg)

Tolerances: NLT 75% (Q) of the labeled amount of acetaminophen ($C_8H_9NO_2$) and aspirin ($C_9H_8O_4$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*: Meet the requirements with respect to acetaminophen and to aspirin

IMPURITIES
• **LIMIT OF SALICYLIC ACID**
Solution A, Mobile phase, Internal standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard stock solution: 1.0 mg/mL of USP Salicylic Acid RS in *Solution A*
Standard solutions: Transfer 1.0-, 5.0-, and 10.0-mL portions of *Standard stock solution* to separate 100-mL volumetric flasks. Add 10.0 mL of *Internal standard solution* to each flask, and dilute with *Solution A* to volume.
Analysis
Samples: *Sample solution* and *Standard solutions*
Plot the ratios of the peak responses for salicylic acid and benzoic acid for each of the *Standard solutions* versus concentrations, in mg/mL, of salicylic acid, and draw the straight line best fitting the three plotted points. From the graph so obtained, and from the ratio of the peak responses for salicylic acid and benzoic acid from the *Sample solution* as obtained in the *Assay*, determine the concentration, in mg/mL, of salicylic acid ($C_7H_6O_3$) in the *Sample solution*.
Calculate the percentage of salicylic acid in relation to the concentration of aspirin in the *Sample solution*, as obtained in the *Assay*.
Acceptance criteria: NMT 3.0%
• **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetaminophen RS
USP Aspirin RS
USP Salicylic Acid RS

Acetaminophen, Aspirin, and Caffeine Tablets

DEFINITION
Acetaminophen, Aspirin, and Caffeine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$).

IDENTIFICATION
• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Inject the *Standard solution* and the *Sample solution* within 8 h of preparation.
Mobile phase: Methanol, glacial acetic acid, and water (28:3:69)
Diluent: Methanol and glacial acetic acid (95:5)
Internal standard solution: 6 mg/mL of benzoic acid in methanol
Standard stock solution: 0.25 mg/mL of USP Acetaminophen RS, 0.25J mg/mL of USP Aspirin RS, and 0.25J' mg/mL of USP Caffeine RS in *Diluent* (J being the ratio of the labeled amount, in milligrams, of aspirin to the labeled amount, in milligrams, of acetaminophen per Tablet; and J' being the ratio of the labeled amount, in

USP Monographs

www.webofpharma.com

milligrams, of caffeine to the labeled amount, in milligrams, of acetaminophen per Tablet)

Standard solution: 0.1 mg/mL of USP Acetaminophen RS, 0.1*J* mg/mL of USP Aspirin RS, and 0.1*J'* mg/mL of USP Caffeine RS in *Diluent*, prepared as follows. Transfer 20.0 mL of the *Standard stock solution* and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Sample stock solution: Nominally 2.5 mg/mL of acetaminophen in *Diluent* prepared as follows. Transfer an equivalent of 250 mg of acetaminophen, from NLT 20 finely powdered Tablets, to a 100-mL volumetric flask. Add 75 mL of *Diluent*, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume.

Sample solution: Nominally 0.1 mg/mL of acetaminophen in *Diluent* prepared as follows. Transfer 2.0 mL of the *Sample stock solution* and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Column temperature: 45 ± 1°
Flow rate: 2 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for acetaminophen, caffeine, aspirin, benzoic acid, and salicylic acid are about 0.3, 0.5, 0.8, 1.0, and 1.2, respectively.]
Suitability requirements
Resolution: NLT 1.4 between any of the analyte and internal standard peaks
Tailing factor: NMT 1.2 for each analyte peak
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate individually the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of acetaminophen, aspirin, or caffeine to the internal standard from the *Sample solution*
R_S = peak response ratio of acetaminophen, aspirin, or caffeine to the internal standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen, aspirin, or caffeine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of acetaminophen, aspirin, and caffeine

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 100 rpm
Time: 60 min
Mobile phase, Diluent, Internal standard solution, Standard stock solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: Transfer 20.0 mL of *Standard stock solution*, 3.0 mL of *Internal standard solution*, and 20 mL of water to a 50-mL volumetric flask, and allow to stand for 30 s. Dilute with *Diluent* to volume. Use within 8 h.
Sample solution: Transfer 20.0 mL of a filtered portion of the solution under test to a 50-mL volumetric flask. Add 3.0 mL of *Internal standard solution* and 20 mL of *Diluent*, mix, and allow to stand for 30 s. Dilute with *Diluent* to volume.
Analysis: Proceed as directed for *Analysis* in the *Assay*.
Calculate individually the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$) dissolved:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of acetaminophen, aspirin, or caffeine to the internal standard from the *Sample solution*
R_S = peak response ratio of acetaminophen, aspirin, or caffeine to the internal standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen, aspirin, or caffeine in the *Sample solution* (mg/mL)

Tolerances: NLT 75% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*: Meet the requirements with respect to acetaminophen, aspirin, and caffeine

IMPURITIES

• **LIMIT OF SALICYLIC ACID**
Mobile phase and **Diluent:** Prepare as directed in the *Assay*.
Standard solution: 0.02 mg/mL of USP Salicylic Acid RS in *Diluent*
Sample solution: Nominally 2.5 mg/mL of aspirin in *Diluent*, prepared as follows. Transfer an equivalent of 250 mg of aspirin, from NLT 20 finely powdered Tablets, to a 100-mL volumetric flask. Add 75 mL of *Diluent*, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 302 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Column temperature: 45 ± 1°
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.6
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid ($C_7H_6O_3$) relative to the labeled amount of aspirin in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*

www.webofpharma.com

r_S = peak response of salicylic acid from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0%
• **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetaminophen RS
USP Aspirin RS
USP Caffeine RS
USP Salicylic Acid RS

Acetaminophen and Caffeine Tablets

DEFINITION
Acetaminophen and Caffeine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of acetaminophen ($C_8H_9NO_2$) and caffeine ($C_8H_{10}N_4O_2$).

IDENTIFICATION
• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, relative to the internal standard, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: Methanol and glacial acetic acid (95:5)
Mobile phase: Methanol, glacial acetic acid, and water (28:3:69)
Internal standard solution: 6 mg/mL of benzoic acid in methanol
Standard stock solution: 0.25 mg/mL of USP Acetaminophen RS and 0.25*J* mg/mL of USP Caffeine RS in *Solution A*; *J* being the ratio of the labeled amount, in mg, of caffeine to the labeled amount, in mg, of acetaminophen per Tablet
Standard solution: 0.1 mg/mL of USP Acetaminophen RS and 0.1*J* mg/mL of USP Caffeine RS, prepared by transferring 20.0 mL of *Standard stock solution* and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and diluting with *Solution A* to volume
Sample stock solution: Nominally 2.5 mg/mL of acetaminophen in *Solution A*, prepared as follows. Transfer a portion of the powder equivalent to 250 mg of acetaminophen, from NLT 20 finely powdered Tablets, to a 100-mL volumetric flask. Add 75 mL of *Solution A*, and shake by mechanical means for 30 min. Dilute with *Solution A* to volume.
Sample solution: Transfer 2.0 mL of the *Sample stock solution* and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Solution A* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Column temperature: 45 ± 1°
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for acetaminophen, caffeine, and benzoic acid are about 0.3, 0.5, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.4 between any of the analyte and internal standard peaks
Tailing factor: NMT 1.2 for each analyte peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate individually the percentages of the labeled amounts of acetaminophen ($C_8H_9NO_2$) and caffeine ($C_8H_{10}N_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of acetaminophen or caffeine to the internal standard from the *Sample solution*
R_S = peak response ratio of acetaminophen or caffeine to the internal standard from the *Standard solution*
C_S = concentration of USP Acetaminophen RS or USP Caffeine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen or caffeine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of acetaminophen ($C_8H_9NO_2$) and caffeine ($C_8H_{10}N_4O_2$)

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 100 rpm
Time: 60 min
Solution A, Mobile phase, Internal standard solution, Standard stock solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: Transfer 20.0 mL of the *Standard stock solution*, 3.0 mL of *Internal standard solution*, and 20 mL of water to a 50-mL volumetric flask, and allow to stand for 30 s. Dilute with *Solution A* to volume. Use within 8 h.
Sample solution: Transfer an aliquot of a filtered portion of the solution under test to a 50-mL volumetric flask to obtain an expected concentration of 0.1 mg/mL of acetaminophen and 0.1*J* mg/mL of caffeine, where *J* is defined for the *Standard stock solution*. Add 3.0 mL of *Internal standard solution* and 20 mL of *Solution A*, and allow to stand for 30 s. Dilute with *Solution A* to volume.
Analysis: Proceed as directed in the *Assay*, using the *Standard solution* and *Sample solution* prepared within the *Dissolution* test.
Calculate the percentages of the labeled amounts of acetaminophen ($C_8H_9NO_2$) and caffeine ($C_8H_{10}N_4O_2$) dissolved:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of acetaminophen or caffeine to the internal standard from the *Sample solution*
R_S = peak response ratio of acetaminophen or caffeine to the internal standard from the *Standard solution*
C_S = concentration of USP Acetaminophen RS or USP Caffeine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen or caffeine in the *Sample solution* (mg/mL)

www.webofpharma.com

Tolerances: NLT 75% (*Q*) of the labeled amounts of acetaminophen ($C_8H_9NO_2$) and caffeine ($C_8H_{10}N_4O_2$) is dissolved.

- **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

- **4-Aminophenol in Acetaminophen-Containing Drug Products** ⟨227⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Acetaminophen RS
  USP Caffeine RS

Capsules Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine

» Capsules Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ($C_8H_9NO_2$), chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$), and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) or pseudoephedrine sulfate [$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$].
[NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.]

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Acetaminophen RS
USP Chlorpheniramine Maleate RS
USP Dextromethorphan Hydrobromide RS
USP Pseudoephedrine Hydrochloride RS
USP Pseudoephedrine Sulfate RS
Labeling—The label for each article encompassed by this monograph bears a name composed of the active ingredients contained in the article. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article.

Identification—
A: If pseudoephedrine hydrochloride or pseudoephedrine sulfate is purported to be present, the retention time of the major peak for pseudoephedrine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for pseudoephedrine hydrochloride* or the *Assay for pseudoephedrine sulfate.*
B: If acetaminophen is claimed in the labeling to be present, the retention time of the major peak for acetaminophen in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for acetaminophen.*
C: If chlorpheniramine maleate is claimed in the labeling to be present, the retention time of the major peak for chlorpheniramine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for chlorpheniramine maleate.*
D: If dextromethorphan hydrobromide is claimed in the labeling to be present, the retention time of the major peak for dextromethorphan in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for dextromethorphan hydrobromide.*

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Test preparation—Mix 9.0 mL of a filtered portion of the solution under test with 1.0 mL of 1% phosphoric acid solution.
Procedure—Determine the amounts of pseudoephedrine hydrochloride or pseudoephedrine sulfate (as appropriate), acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide dissolved, employing the procedures set forth in the *Assay for pseudoephedrine hydrochloride* or *Assay for pseudoephedrine sulfate, Assay for acetaminophen, Assay for chlorpheniramine maleate,* and *Assay for dextromethorphan hydrobromide,* respectively, making any necessary volumetric adjustments.
Tolerances—Not less than 75% *(Q)* of the labeled amounts of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) or pseudoephedrine sulfate [$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$], acetaminophen ($C_8H_9NO_2$), chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), and dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$) are dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay for pseudoephedrine hydrochloride (where pseudoephedrine hydrochloride is the salt form used, if present in the formulation)—
Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.*
Chlorpheniramine standard preparation—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate.*
Dextromethorphan standard preparation—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide.*
System suitability solution 1 (for Capsules that contain either all four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation.*
System suitability solution 2 (for Capsules that contain no chlorpheniramine)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation.*
Assay preparation—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about

USP Monographs

www.webofpharma.com

100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with water to obtain a solution having a concentration of about 0.12 mg of pseudoephedrine hydrochloride per mL.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the Capsules taken by the formula:

$$(CL/D)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation; L* is the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Capsule; *D* is the concentration, in mg per mL, of pseudoephedrine hydrochloride in the *Assay preparation,* based on the number of Capsules taken, the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Capsule and the extent of dilution; and r_U and r_S are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for pseudoephedrine sulfate (where pseudoephedrine sulfate is the salt form used, if present in the formulation)—

Mobile phase, System suitability solutions, and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.*

Chlorpheniramine standard preparation—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate.*

Dextromethorphan standard preparation—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide.*

Standard preparation—Dissolve an accurately weighed quantity of USP Pseudoephedrine Sulfate RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 2.5 mL of methanol, dilute with 0.1% phosphoric acid to volume, and mix.

Assay preparation—Proceed as directed for the *Assay preparation* in the *Assay for pseudoephedrine hydrochloride* to obtain a solution having a concentration of about 0.24 mg of pseudoephedrine sulfate per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay for pseudoephedrine hydrochloride.* Calculate the quantity, in mg, of pseudoephedrine sulfate $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$ in each Capsule taken by the formula:

$$(CL/D)(r_U/r_S)$$

in which the terms are as defined therein, pseudoephedrine sulfate being substituted for pseudoephedrine hydrochloride.

Assay for acetaminophen *(if present)*—

Mobile phase—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (79:20:1). Make adjustments, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 25 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 4 mL of methanol, and mix until solution is complete. Add 0.2 mL of phosphoric acid, dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.25 mg per mL.

Assay preparation—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.25 mg of acetaminophen per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for Procedure: the tailing factor for the acetaminophen peak is not greater than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ($C_8H_9NO_2$) in each Capsule taken by the formula:

$$(CL/D)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation; L* is the labeled quantity, in mg, of acetaminophen in each Capsule; *D* is the concentration, in mg per mL, of acetaminophen in each mL of the *Assay preparation,* based on the number of Capsules taken, the labeled quantity, in mg, of acetaminophen in each Capsule, and the extent of dilution; and r_U and r_S are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for chlorpheniramine maleate *(if present)*—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.*

Standard preparation—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 8 µg per mL.

Assay preparation—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 8 µg of chlorpheniramine maleate per mL.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in each Capsule taken by the formula:

$$(CL/D)(r_U/r_S)$$

www.webofpharma.com

in which *C* is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation; L* is the labeled quantity, in mg, of chlorpheniramine maleate in each Capsule; *D* is the concentration, in mg per mL, of chlorpheniramine maleate in each mL of the *Assay preparation,* based on the number of Capsules taken, the labeled quantity, in mg, of chlorpheniramine maleate in each Capsule, and the extent of dilution; and r_U and r_S are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for dextromethorphan hydrobromide *(if present)*—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.*

Standard preparation—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 0.4 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 0.04 mg per mL.

Assay preparation—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.04 mg of dextromethorphan hydrobromide per mL.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$) in each Capsule taken by the formula:

$$(370.33/352.32)(CL/D)(r_U/r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively; *C* is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation; L* is the labeled quantity, in mg, of dextromethorphan hydrobromide in each Capsule; *D* is the concentration, in mg per mL, of dextromethorphan hydrobromide in each mL of the *Assay preparation,* based on the number of Capsules taken, the labeled quantity, in mg, of dextromethorphan hydrobromide in each Capsule, and the extent of dilution; and r_U and r_S are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Oral Powder Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine

» Oral Powder Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ($C_8H_9NO_2$), chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$), and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) or pseudoephedrine sulfate [$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$].

[NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.]

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Acetaminophen RS
USP Chlorpheniramine Maleate RS
USP Dextromethorphan Hydrobromide RS
USP Pseudoephedrine Hydrochloride RS
USP Pseudoephedrine Sulfate RS

Labeling—The label for each article encompassed by this monograph bears a name composed of the active ingredients. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article.

Identification—

A: If pseudoephedrine hydrochloride or pseudoephedrine sulfate is claimed in the labeling to be present, the retention time of the major peak for pseudoephedrine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for pseudoephedrine hydrochloride* or the *Assay for pseudoephedrine sulfate.*

B: If acetaminophen is claimed in the labeling to be present, the retention time of the major peak for acetaminophen in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for acetaminophen.*

C: If chlorpheniramine maleate is claimed in the labeling to be present, the retention time of the major peak for chlorpheniramine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for chlorpheniramine maleate.*

D: If dextromethorphan hydrobromide is claimed in the labeling to be present, the retention time of the major peak for dextromethorphan in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for dextromethorphan hydrobromide.*

Minimum fill ⟨755⟩**:** meets the requirements.

Uniformity of dosage units ⟨905⟩—
FOR ORAL POWDER PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

www.webofpharma.com

Assay for pseudoephedrine hydrochloride (where pseudoephedrine hydrochloride is the salt form used, if present in the formulation)—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.*

Chlorpheniramine standard preparation—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate.*

Dextromethorphan standard preparation—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide.*

Standard preparation—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, dilute with 0.1% phosphoric acid to volume, and mix.

System suitability solution 1 (for Oral Powder that contains either all four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation.*

System suitability solution 2 (for Oral Powder that contains no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation.*

Assay preparation—Transfer the contents of 10 unit-dose containers of the Oral Powder to a 2000-mL volumetric flask. Add 1000 mL of water and 2.0 mL of phosphoric acid. Gently heat to about 60° until the powder is fully dispersed. Cool the flask to room temperature, add 40 mL of methanol, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.24 mg of pseudoephedrine hydrochloride per mL.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$), in each unit-dose container of Oral Powder taken by the formula:

$$(CL/D)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation; L* is the labeled quantity, in mg, of pseudoephedrine hydrochloride in each unit-dose container; *D* is the concentration, in mg per mL, of pseudoephedrine hydrochloride in each mL of the *Assay preparation,* based on the number of unit-dose containers taken, the labeled quantity, in mg, of pseudoephedrine hydrochloride in each unit-dose container, and the extent of dilution; and r_U and r_S are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for pseudoephedrine sulfate (where pseudoephedrine sulfate is the salt form used, if present in the formulation)—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.*

Chlorpheniramine standard preparation—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate.*

Dextromethorphan standard preparation—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide.*

Standard preparation—Dissolve an accurately weighed quantity of USP Pseudoephedrine Sulfate RS in water to obtain a solution having a known concentration of about 6.0 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, dilute with 0.1% phosphoric acid to volume, and mix.

System suitability solution 1 (for Oral Powder that contains either all four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation.*

System suitability solution 2 (for Oral Powder that contains no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation.*

Assay preparation—Proceed as directed for the *Assay preparation* in the *Assay for pseudoephedrine hydrochloride* to obtain a solution having a concentration of about 0.48 mg of pseudoephedrine sulfate per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay for pseudoephedrine hydrochloride.* Calculate the quantity, in mg, of pseudoephedrine sulfate $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$ in each unit-dose container of Oral Powder taken by the formula:

$$(CL/D)(r_U/r_S)$$

in which the terms are as defined therein, pseudoephedrine sulfate being substituted for pseudoephedrine hydrochloride.

Assay for acetaminophen *(if present)*—

Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.*

Assay preparation—Transfer the contents of 10 unit-dose containers of the Oral Powder to a 2000-mL volumetric flask. Add 1000 mL of water and 2 mL of phosphoric acid. Gently heat to about 60° until the powder is fully dispersed. Cool the flask to room temperature, add 40 mL of methanol, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.50 mg of acetaminophen per mL.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ($C_8H_9NO_2$) in each unit-dose container of Oral Powder taken by the formula:

$$(CL/D)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation; L* is the labeled quantity, in mg, of acetaminophen in each unit-dose container; *D* is the concentration, in mg per mL, of acetaminophen in the *Assay preparation,* based on the number of unit-dose containers taken, the labeled quantity, in mg, of acetaminophen in each unit-dose container, and the extent of dilution; and r_U and r_S are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for chlorpheniramine maleate *(if present)*—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—*

www.webofpharma.com

Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.

Standard preparation—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 8 µg per mL.

Assay preparation—Transfer the contents of 10 unit-dose containers of Oral Powder to a 2000-mL volumetric flask. Add 1000 mL of water and 2 mL of phosphoric acid. Gently heat to about 60° until the powder is fully dispersed. Cool the flask to room temperature, add 40 mL of methanol, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of 8 µg of chlorpheniramine maleate per mL.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in each unit-dose container of Oral Powder taken by the formula:

$$(CL/D)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; L is the labeled quantity, in mg, of chlorpheniramine maleate in each unit-dose container; D is the concentration, in mg per mL, of chlorpheniramine maleate in each mL of the *Assay preparation*, based on the number of unit-dose containers taken, the labeled quantity, in mg, of chlorpheniramine maleate in each unit-dose container, and the extent of dilution; and r_U and r_S are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for dextromethorphan hydrobromide *(if present)*—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.*

Standard preparation—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of 0.08 mg per mL.

Assay preparation—Transfer the contents of 10 unit-dose containers of Oral Powder to a 2000-mL volumetric flask. Add 1000 mL of water and 2 mL of phosphoric acid. Gently heat to about 60° until the powder is fully dispersed. Cool the flask to room temperature, add 40 mL of methanol, dilute with water to volume, and mix. If necessary, quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a concentration of 0.08 mg of dextromethorphan hydrobromide per mL.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$) in each unit-dose container of Oral Powder taken by the formula:

$$(370.33/352.32)(CL/D)(r_U/r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively; C is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*; L is the labeled quantity, in mg, of dextromethorphan hydrobromide in each unit-dose container; D is the concentration, in mg per mL, of dextromethorphan hydrobromide in each mL of the *Assay preparation*, based on the number of unit-dose containers taken, the labeled quantity, in mg, of dextromethorphan hydrobromide in each unit-dose container, and the extent of dilution; and r_U and r_S are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Oral Solution Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine

» Oral Solution Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ($C_8H_9NO_2$), chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$), and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) or pseudoephedrine sulfate [$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$].

[NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.]

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Acetaminophen RS
USP Chlorpheniramine Maleate RS
USP Dextromethorphan Hydrobromide RS
USP Pseudoephedrine Hydrochloride RS
USP Pseudoephedrine Sulfate RS

Labeling—The label for each article encompassed by this monograph bears a name composed of the active ingredients. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article.

Identification—

A: If pseudoephedrine hydrochloride or pseudoephedrine sulfate is claimed in the labeling to be present, the retention

USP Monographs

www.webofpharma.com

time of the major peak for pseudoephedrine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for pseudoephedrine hydrochloride* or the *Assay for pseudoephedrine sulfate.*

B: If acetaminophen is claimed in the labeling to be present, the retention time of the major peak for acetaminophen in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for acetaminophen.*

C: If chlorpheniramine maleate is claimed in the labeling to be present, the retention time of the major peak for chlorpheniramine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for chlorpheniramine maleate.*

D: If dextromethorphan hydrobromide is claimed in the labeling to be present, the retention time of the major peak for *dextromethorphan* in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for dextromethorphan hydrobromide.*

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

pH ⟨791⟩**:** between 3.7 and 7.5.

Alcohol Determination, *Method II* ⟨611⟩ ***(if present)*** **:** between 90.0% and 110.0% of the labeled amount of C_2H_5OH.

Microbial enumeration tests ⟨61⟩ **and** ***Absence of specified microorganisms*** ⟨62⟩—The total bacterial count does not exceed 100 cfu per g, the total combined molds and yeasts count does not exceed 10 cfu per g, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli.*

Assay for pseudoephedrine hydrochloride (where pseudoephedrine hydrochloride is the salt form used, if present in the formulation)—

Mobile phase—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.15 g of triethylamine hydrochloride, 0.25 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 1.5 mg per mL. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, add 8.0 mL of *Mobile phase,* dilute with water to volume, and mix.

Chlorpheniramine standard preparation—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate.*

Dextromethorphan standard preparation—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide.*

System suitability solution 1 (for Oral Solution that contains either all the four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation.*

System suitability solution 2 (for Oral Solution that contains no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation.*

Assay preparation—Transfer an accurately measured volume of the Oral Solution, equivalent to 15 mg of pseudoephedrine hydrochloride, to a 100-mL volumetric flask, add 80.0 mL of *Mobile phase,* dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor for the pseudoephedrine peak is not greater than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%. Separately inject about 10 µL of *System suitability solution 1* or *System suitability solution 2,* as appropriate. The resolution, *R,* between pseudoephedrine and chlorpheniramine or between pseudoephedrine and dextromethorphan is not less than 2.0.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in each mL of the Oral Solution taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation; V* is the volume, in mL, of the Oral Solution taken; and r_U and r_S are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for pseudoephedrine sulfate (where pseudoephedrine sulfate is the salt form used, if present in the formulation)—

Mobile phase, System suitability solutions, and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride.*

Chlorpheniramine standard preparation—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate.*

Dextromethorphan standard preparation—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide.*

Standard preparation—Dissolve an accurately weighed quantity of USP Pseudoephedrine Sulfate RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, add 4.0 mL of *Mobile phase,* dilute with water to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to 30 mg of pseudoephedrine sulfate, to a 100-mL volumetric flask, add 80.0 mL of *Mobile phase,* dilute with water to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay for pseudoephedrine hydrochloride.* Calculate the quantity, in mg, of pseudoephedrine sulfate [$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$] in each mL of the Oral Solution taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which the terms are as defined therein, pseudoephedrine sulfate being substituted for pseudoephedrine hydrochloride.

www.webofpharma.com

Assay for acetaminophen *(if present)*—
Mobile phase—Prepare a suitable degassed and filtered mixture of water, methanol, and glacial acetic acid (79:20:1). Make any necessary adjustments (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer about 16.5 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 2.5 mL of methanol, and mix until solution is complete. Dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.165 mg per mL.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 33 mg of acetaminophen, to a 200-mL volumetric flask, add 5 mL of methanol, and mix. Dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor for the acetaminophen peak is not greater than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ($C_8H_9NO_2$) in each mL of the Oral Solution taken by the formula:

$$200(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation; V* is the volume, in mL, of the Oral Solution taken; and r_U and r_S are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for chlorpheniramine maleate *(if present)*—
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride.*
Standard preparation—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 1 mg per mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, add 80 mL of *Mobile phase,* dilute with water to volume, and mix.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 1 mg of chlorpheniramine maleate, to a 100-mL volumetric flask. Add 80 mL of *Mobile phase,* dilute with water to volume, and mix.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in the Oral Solution taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation; V* is the volume, in mL, of the Oral Solution taken; and r_U and r_S are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for dextromethorphan hydrobromide *(if present)*—
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride.*
Standard preparation—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 1.5 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 80 mL of *Mobile phase,* dilute with water to volume, and mix.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 7.5 mg of dextromethorphan hydrobromide, to a 100-mL volumetric flask, add 80 mL of *Mobile phase,* dilute with water to volume, and mix.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$) in each mL of the Oral Solution taken by the formula:

$$(370.33/352.32)(100C/V)(r_U/r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively; C is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation; V* is the volume, in mL, of the Oral Solution taken; and r_U and r_S are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine

» Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ($C_8H_9NO_2$), chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$), and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) or pseudoephedrine sulfate [$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$].
[NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.]

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Acetaminophen RS

USP Monographs

www.webofpharma.com

USP Chlorpheniramine Maleate RS
USP Dextromethorphan Hydrobromide RS
USP Pseudoephedrine Hydrochloride RS
USP Pseudoephedrine Sulfate RS

Labeling—The label for each article encompassed by this monograph bears a name composed of the active ingredients. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

Identification—

A: If pseudoephedrine hydrochloride or pseudoephedrine sulfate is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for pseudoephedrine hydrochloride* or the *Assay for pseudoephedrine sulfate*, exhibits a major peak for pseudoephedrine, the retention time of which corresponds to that exhibited by the *Standard preparation*.

B: If acetaminophen is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for acetaminophen*, exhibits a major peak for acetaminophen, the retention time of which corresponds to that exhibited by the *Standard preparation*.

C: If chlorpheniramine maleate is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for chlorpheniramine maleate*, exhibits a major peak for chlorpheniramine, the retention time of which corresponds to that exhibited by the *Standard preparation*.

D: If dextromethorphan hydrobromide is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for dextromethorphan hydrobromide*, exhibits a major peak for dextromethorphan, the retention time of which corresponds to that exhibited by the *Standard preparation*.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—

TEST 1—

Medium: pH 5.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Test preparation—Mix 9.0 mL of a filtered portion of the solution under test with 1.0 mL of 1% phosphoric acid solution.

Procedure—Determine the amounts of pseudoephedrine hydrochloride or pseudoephedrine sulfate (as appropriate), acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide dissolved, employing the procedures set forth in the *Assay for pseudoephedrine hydrochloride* or *Assay for pseudoephedrine sulfate*, *Assay for acetaminophen*, *Assay for chlorpheniramine maleate*, and *Assay for dextromethorphan hydrobromide*, respectively, making any necessary volumetric adjustments.

Tolerances—Not less than 75% *(Q)* of the labeled amounts of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) or pseudoephedrine sulfate [$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$], acetaminophen ($C_8H_9NO_2$), chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), and dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$) are dissolved in 45 minutes.

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: water; 900 mL.

Apparatus, Time, Test preparation, Procedure, and *Tolerances*—Proceed as directed for *Test 1*.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay for pseudoephedrine hydrochloride (where pseudoephedrine hydrochloride is the salt form used, if present in the formulation)—

Mobile phase—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.3 g of triethylamine hydrochloride, 0.15 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 1.0 mL of this solution to a 25-mL volumetric flask, add 2.5 mL of methanol, dilute with 0.1% phosphoric acid to volume, and mix.

Chlorpheniramine standard preparation—Prepare as directed for the *Standard preparation* in the *Assay for chlorpheniramine maleate*.

Dextromethorphan standard preparation—Prepare as directed for the *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

System suitability solution 1 (for Tablets that contain either all the four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

System suitability solution 2 (for Tablets that contain no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 6 mg of pseudoephedrine hydrochloride, to a 50-mL volumetric flask. Add 5 mL of methanol, and sonicate to disperse the powder. Dilute with 0.1% phosphoric acid to volume, mix, and filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor for the pseudoephedrine peak is not greater than 2.5, and the relative standard deviation for replicate injections is not more than 2.0%. Separately inject about 10 µL of *System suitability solution 1* or *System suitability solution 2*, as appropriate. The resolution, *R,* between pseudoephedrine and chlorpheniramine or between pseudoephedrine and dextromethorphan is not less than 2.0.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Tablets taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation,* and r_U and r_S are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for pseudoephedrine sulfate (where pseudoephedrine sulfate is the salt form used, if present in the formulation)—

Mobile phase, Chlorpheniramine standard preparation, Dextromethorphan standard preparation, System suitability solutions, and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride*.

www.webofpharma.com

Standard preparation—Dissolve an accurately weighed quantity of USP Pseudoephedrine Sulfate RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 2.5 mL of methanol, dilute with 0.1% phosphoric acid to volume, and mix.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 12 mg of pseudoephedrine sulfate, to a 50-mL volumetric flask. Add 5 mL of methanol, and sonicate to disperse the powder. Dilute with 0.1% phosphoric acid to volume, mix, and filter.

Procedure—Proceed as directed for *Procedure* in the *Assay for pseudoephedrine hydrochloride.* Calculate the quantity, in mg, of pseudoephedrine sulfate $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$ in the portion of Tablets taken by the formula:

$$50C(r_U/r_S)$$

in which the terms are as defined therein, pseudoephedrine sulfate being substituted for pseudoephedrine hydrochloride.

Assay for acetaminophen *(if present)*—

Mobile phase—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (79:20:1). Make adjustments, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 50 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 4 mL of methanol, and mix until solution is complete. Dilute with 0.1% phosphoric acid to volume, and mix.

Assay preparation—Weigh and powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of acetaminophen, to a 50-mL volumetric flask. Add about 7.5 mL of methanol, and sonicate to disperse the powder. Add 0.5 mL of phosphoric acid, dilute with water to volume, mix, and filter. Transfer 25.0 mL of the filtered solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor for the acetaminophen peak is not greater than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken by the formula:

$$200C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation;* and r_U and r_S are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for chlorpheniramine maleate *(if present)*—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride.*

Standard preparation—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 8 µg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2 mg of chlorpheniramine maleate, to a 250-mL volumetric flask. Add 25 mL of methanol, and sonicate to disperse the powder. Add 1 mL of phosphoric acid, dilute with water to volume, mix, and filter.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in the portion of Tablets taken by the formula:

$$250C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation;* and r_U and r_S are the peak responses for chlorpheniramine obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for dextromethorphan hydrobromide *(if present)*—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride.*

Standard preparation—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 0.6 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 0.06 mg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 6 mg of dextromethorphan hydrobromide, to a 100-mL volumetric flask. Add 10 mL of methanol, and sonicate to disperse the powder. Add 0.4 mL of phosphoric acid, dilute with water to volume, mix, and filter.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$) in the portion of Tablets taken by the formula:

$$(370.33/352.32)(100C)(r_U/r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively; C is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation;* and r_U and r_S are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Acetaminophen, Chlorpheniramine Maleate, and Dextromethorphan Hydrobromide Tablets

DEFINITION

Acetaminophen, Chlorpheniramine Maleate, and Dextromethorphan Hydrobromide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of

USP Monographs

www.webofpharma.com

acetaminophen ($C_8H_9NO_2$), chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), and dextromethorphan hydrobromide monohydrate ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$).

IDENTIFICATION

- **A.** The retention time of the major peak for acetaminophen of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Acetaminophen*.
- **B.** The retention time of the major peak for chlorpheniramine of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Chlorpheniramine Maleate*.
- **C.** The retention time of the major peak for dextromethorphan of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Dextromethorphan Hydrobromide*.

ASSAY

- **ACETAMINOPHEN**

Mobile phase: Methanol, glacial acetic acid, and water (20:1:79)

Standard solution: 0.5 mg/mL of USP Acetaminophen RS prepared as follows. Transfer an appropriate amount of USP Acetaminophen RS to a suitable volumetric flask and add methanol using 4% of the final volume. Mix until solution is complete and dilute with 0.1% phosphoric acid to volume.

Sample stock solution: Nominally 2 mg/mL of acetaminophen prepared as follows. Transfer a portion of powdered Tablets (NLT 20), equivalent to 100 mg of acetaminophen, to a 50-mL volumetric flask. Add 7.5 mL of methanol, and sonicate to disperse the powder. Add 0.5 mL of phosphoric acid, dilute with water to volume, and filter.

Sample solution: Nominally 0.5 mg/mL of acetaminophen from *Sample stock solution* in water

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*
r_S = peak response of acetaminophen from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$)

- **CHLORPHENIRAMINE MALEATE**

Mobile phase: Methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.3 g of triethylamine hydrochloride, 0.15 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution

Standard stock solution: 0.8 mg/mL of USP Chlorpheniramine Maleate RS in water

Standard solution: 8 µg/mL of USP Chlorpheniramine Maleate RS in 0.1% phosphoric acid from *Standard stock solution*

Sample solution: Nominally 8 µg/mL of chlorpheniramine maleate prepared as follows. Transfer a portion of powdered Tablets (NLT 20), equivalent to 2 mg of chlorpheniramine maleate, to a 250-mL volumetric flask. Add 25 mL of methanol, and sonicate to disperse the powder. Add 1 mL of phosphoric acid, dilute with water to volume, and filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; 5-µm packing L11
Flow rate: 2 mL/min
Injection volume: 10 µL

System suitability
Samples: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chlorpheniramine from the *Sample solution*
r_S = peak response of chlorpheniramine from the *Standard solution*
C_S = concentration of USP Chlorpheniramine Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of chlorpheniramine maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$)

- **DEXTROMETHORPHAN HYDROBROMIDE**

Mobile phase, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* for *Chlorpheniramine Maleate*.

Standard stock solution: 0.6 mg/mL of USP Dextromethorphan Hydrobromide RS in water

Standard solution: 0.06 mg/mL of USP Dextromethorphan Hydrobromide RS in 0.1% phosphoric acid, from *Standard stock solution*

Sample solution: Nominally 0.06 mg/mL of dextromethorphan hydrobromide prepared as follows. Transfer a portion of powdered Tablets (NLT 20), equivalent to 6 mg of dextromethorphan hydrobromide, to a 100-mL volumetric flask. Add 10 mL of methanol, and sonicate to disperse the powder. Add 0.4 mL of phosphoric acid, dilute with water to volume, and filter.

System suitability
Samples: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the percentage of the labeled amount of dextromethorphan hydrobromide monohydrate ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of dextromethorphan from the *Sample solution*
r_S = peak response of dextromethorphan from the *Standard solution*
C_S = concentration of USP Dextromethorphan Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dextromethorphan hydrobromide in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dextromethorphan hydrobromide monohydrate, 370.32
M_{r2} = molecular weight of anhydrous dextromethorphan hydrobromide, 352.32

Acceptance criteria: 90.0%–110.0% of the labeled amount of dextromethorphan hydrobromide monohydrate ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms, Procedure for a pooled sample for immediate-release dosage forms*

Test 1
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Sample solution: Mix 9.0 mL of a filtered portion of the solution with 1.0 mL of 1% phosphoric acid solution.
Analysis: Determine the percentage of the labeled amount of acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide dissolved, using the *Analysis* set forth in the *Assay* for *Acetaminophen*, the *Assay* for *Chlorpheniramine Maleate*, and the *Assay* for *Dextromethorphan Hydrobromide*, respectively, making any necessary volumetric adjustments.
Tolerances: NLT 75% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$), chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), and dextromethorphan hydrobromide monohydrate ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 M hydrochloric acid; 900 mL
Apparatus 2, Time, Sample solution, Analysis, and **Tolerances:** Proceed as directed in *Test 1*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: pH 5.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL
Apparatus 2, Time, Sample solution, Analysis, and **Tolerances:** Proceed as directed in *Test 1*.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **LABELING:** The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article. When more than one *Dissolution Test* is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetaminophen RS
USP Chlorpheniramine Maleate RS
USP Dextromethorphan Hydrobromide RS

Acetaminophen and Codeine Phosphate Capsules

DEFINITION

Acetaminophen and Codeine Phosphate Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$) and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$).

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
• **B. THIN-LAYER CHROMATOGRAPHY**
Standard solution: 12 mg/mL each of USP Acetaminophen RS and USP Codeine Phosphate RS in methanol
Sample solution: Transfer a portion of Capsule contents, equivalent to 12 mg of codeine phosphate, to a separator. Add 5 mL of water, 1 mL of ammonium hydroxide, and 5 mL of methylene chloride. Shake for 1 min, and allow the layers to separate. Use the clear lower layer.
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Methanol and ammonium hydroxide (49:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry after applying each sample to the adsorbent. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light.
Acceptance criteria: The R_f values of the two principal spots of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY

• **PROCEDURE**
Solution A: Dissolve 2.04 g of monobasic potassium phosphate in 950 mL of water. Add 2 mL of triethylamine, adjust with phosphoric acid to a pH of 2.35, and dilute with water to 1000 mL.
Mobile phase: Methanol and *Solution A* (8:92)
Codeine phosphate standard stock solution: 0.3 mg/mL of USP Codeine Phosphate RS in *Mobile phase*
Standard solution: 0.3 mg/mL of USP Acetaminophen RS and 0.3*J* mg/mL of codeine phosphate in *Mobile phase*, prepared as follows. Transfer an appropriate amount of USP Acetaminophen RS and a suitable volume (multiplied by *J*) of *Codeine phosphate standard stock solution* (*J* being the ratio of the labeled amount, in mg, of codeine phosphate to that of acetaminophen) to a suitable volumetric flask. Dilute with *Mobile phase* to volume.

USP Monographs

www.webofpharma.com

Sample stock solution: Nominally 3.0 mg/mL of acetaminophen and 3.0*J* mg/mL of codeine phosphate (equivalent to 2.93*J* mg/mL of anhydrous codeine phosphate) in *Mobile phase,* prepared as follows. Transfer a portion of the combined contents, equivalent to 300 mg of acetaminophen, from NLT 20 Capsules, to a 100-mL volumetric flask. Add 75 mL of *Mobile phase*, and sonicate for 10 min. Dilute with *Mobile phase* to volume.

Sample solution: Dilute 5.0 mL of the *Sample stock solution* with *Mobile phase* to 50 mL, and pass a portion through a filter of 1-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 30 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between acetaminophen and codeine

Relative standard deviation: NMT 2.0% for acetaminophen; NMT 3.0% for codeine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*

r_S = peak response of acetaminophen from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of codeine from the *Sample solution*

r_S = peak response of codeine from the *Standard solution*

C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of codeine phosphate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of codeine phosphate, 406.37

M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37

Acceptance criteria

Acetaminophen: 90.0%–110.0%

Codeine phosphate: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Analysis: Determine the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$) dissolved by using the method set forth in the *Assay*, except use 0.01 N hydrochloric acid to prepare the *Codeine phosphate standard stock solution*, and make any other necessary volumetric adjustments.

Tolerances: NLT 75% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$) and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩

Procedure for content uniformity

Solution A, Mobile phase, Codeine phosphate standard stock solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Sample stock solution: Transfer the contents of 1 Capsule to a 100-mL volumetric flask. Add 75 mL of *Mobile phase*, and sonicate for 10 min. Dilute with *Mobile phase* to volume.

Sample solution: Dilute 5.0 mL of the *Sample stock solution* with *Mobile phase* to 50 mL, and pass a portion through a suitable filter of 1-µm pore size.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in mg, of acetaminophen ($C_8H_9NO_2$) in the Capsule taken:

$$\text{Result} = (r_U/r_S) \times C_S \times F$$

r_U = peak response of acetaminophen from the *Sample solution*

r_S = peak response of acetaminophen from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

F = dilution volume, 1000 mL

Calculate the quantity, in mg, of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$) in the Capsule taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times F$$

r_U = peak response of codeine from the *Sample solution*

r_S = peak response of codeine from the *Standard solution*

C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of codeine phosphate, 406.37

M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37

F = dilution volume, 1000 mL

Acceptance criteria: Meet the requirements

IMPURITIES

• **4-Aminophenol in Acetaminophen-Containing Drug Products** ⟨227⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩

USP Acetaminophen RS

USP Codeine Phosphate RS

www.webofpharma.com

Acetaminophen and Codeine Phosphate Oral Solution

DEFINITION

Acetaminophen and Codeine Phosphate Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$) and codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$).

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solutions* correspond to those of the *Standard solutions*, as obtained in the *Assays* for *Acetaminophen* and *Codeine Phosphate*.

• **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 12 mg/mL each of USP Acetaminophen RS and USP Codeine Phosphate RS in methanol

Sample solution: Transfer a volume of Oral Solution, equivalent to 12 mg of codeine phosphate, to a separator. Add 1 mL of ammonium hydroxide and 5 mL of methylene chloride. Shake for 1 min, and allow the layers to separate. Use the clear lower layer.

Developing solvent system: Methanol and ammonium hydroxide (49:1)

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Locate the spots on the plate by examination under short-wavelength UV light.

Acceptance criteria: The R_F values of the two principal spots of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY

• **ACETAMINOPHEN**

Mobile phase: Methanol and water (3:7)

Standard solution: 0.48 mg/mL of USP Acetaminophen RS in *Mobile phase*

Sample solution: Nominally 0.48 mg/mL of acetaminophen in *Mobile phase*, prepared by adding a volume of Oral Solution, equivalent to 120 mg of acetaminophen, to a 250-mL volumetric flask. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*

r_S = peak response of acetaminophen from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

• **CODEINE PHOSPHATE**

Mobile phase: Dissolve 4.44 g of docusate sodium in 1000 mL of a mixture of methanol, tetrahydrofuran, phosphoric acid, and water (600:40:1:360) with stirring, and pass through a membrane filter of 0.45-µm or finer pore size.

Diluent: Methanol and water (3:7)

Standard solution: 0.12 mg/mL of USP Codeine Phosphate RS in *Diluent*

Sample solution: Nominally 0.12 mg/mL of codeine phosphate hemihydrate in *Diluent*, prepared by adding a volume of Oral Solution, equivalent to 12 mg of codeine phosphate hemihydrate, to a 100-mL volumetric flask. Dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of codeine phosphate from the *Sample solution*

r_S = peak response of codeine phosphate from the *Standard solution*

C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of codeine phosphate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of codeine phosphate hemihydrate, 406.37

M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For single-unit containers

Acceptance criteria: Meets the requirements

• **DELIVERABLE VOLUME** ⟨698⟩

For multiple-unit containers

Acceptance criteria: Meets the requirements

IMPURITIES

• **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩: Meets the requirements

USP Monographs

www.webofpharma.com

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.0–6.1
- **Alcohol Determination** ⟨611⟩, *Method II* (if present): 90.0%–120.0% of the labeled quantity of alcohol (C_2H_5OH), acetone being used as the internal standard

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Acetaminophen RS
  USP Codeine Phosphate RS

Acetaminophen and Codeine Phosphate Oral Suspension

DEFINITION
Acetaminophen and Codeine Phosphate Oral Suspension is a suspension of Acetaminophen and Codeine Phosphate in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$) and codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$).

IDENTIFICATION
- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B. Thin-Layer Chromatography**
  Standard solution: 12 mg/mL each of USP Acetaminophen RS and USP Codeine Phosphate RS in methanol
  Sample solution: Transfer a volume of Oral Suspension, equivalent to 12 mg of codeine phosphate, to a separator. Add 1 mL of ammonium hydroxide and 5 mL of methylene chloride, shake for 1 min, and allow the layers to separate. Use the clear lower layer.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Developing solvent system: Methanol and ammonium hydroxide (49:1)
  Application volume: 10 µL
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Locate the spots on the plate by examination under short-wavelength UV light.
  Acceptance criteria: The R_F values of the two principal spots of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY
- **Procedure**
  Diluent: Methanol and 0.01 N sodium hydroxide (30:70)
  Mobile phase: Dissolve 4.9 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 3.9, and add 216 mg of sodium 1-octanesulfonate. Add 100 mL of acetonitrile, and filter.
  Codeine phosphate standard stock solution: 0.5 mg/mL of USP Codeine Phosphate RS in *Diluent*
  Standard stock solution: Transfer a quantity of $5J$ mg of USP Acetaminophen RS (J being the ratio of the labeled amount, in mg, of acetaminophen to the labeled amount, in mg, of codeine phosphate hemihydrate) and 10.0 mL of *Codeine phosphate standard stock solution* to a 100-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume.
  System suitability stock solution: 0.02 mg/mL of sodium benzoate and 0.03 mg/mL of methylparaben in *Diluent*
  System suitability solution: Transfer 10.0 mL of the *System suitability stock solution* to a 50-mL volumetric flask, add 10.0 mL of *Standard stock solution*, and dilute with *Mobile phase* to volume.
  Standard solution: 0.01 mg/mL of USP Codeine Phosphate RS and $0.01J$ mg/mL of USP Acetaminophen RS in *Mobile phase*. Prepare by diluting 10.0 mL of the *Standard stock solution* with *Mobile phase* to 50 mL in a volumetric flask.
  Sample stock solution: Nominally 0.5 mg/mL of acetaminophen and 0.5 mg/mL of codeine phosphate hemihydrate in *Diluent* prepared as follows. Transfer a measured volume of well-mixed Oral Suspension, equivalent to 50 mg of acetaminophen, to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and mix by mechanical means for 30 min. Dilute with *Diluent* to volume. Foaming may be minimized by adding a few drops of acetonitrile before diluting with *Diluent* to volume. Centrifuge a portion of this mixture.
  Sample solution: Dilute 10.0 mL of the clear supernatant from the *Sample stock solution* with *Mobile phase* to 50 mL in a volumetric flask.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 220 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L11
  Flow rate: 2 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [Note—The relative retention times for acetaminophen, benzoate, codeine, and methylparaben are about 0.25, 0.5, 1.0, and 1.3, respectively.]
  Suitability requirements
  Resolution: NLT 2 between each pair of adjacent peaks, *System suitability solution*
  Tailing factor: NMT 2 for each analyte peak, *Standard solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*
r_S = peak response of acetaminophen from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%
Calculate the percentage of the labeled amount of codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

www.webofpharma.com

r_U = peak response of codeine from the *Sample solution*
r_S = peak response of codeine from the *Standard solution*
C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of codeine phosphate hemihydrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of codeine phosphate hemihydrate, 406.37
M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
For single-unit containers
Acceptance criteria: Meets the requirements
• **DELIVERABLE VOLUME** ⟨698⟩
For multiple-unit containers
Acceptance criteria: Meets the requirements

IMPURITIES

• **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩: Meets the requirements

SPECIFIC TESTS

• **PH** ⟨791⟩: 4.0–6.1

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetaminophen RS
USP Codeine Phosphate RS

Acetaminophen and Codeine Phosphate Tablets

DEFINITION

Acetaminophen and Codeine Phosphate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$) and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$).

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
• **B. THIN-LAYER CHROMATOGRAPHY**
Standard solution: 12 mg/mL each of USP Acetaminophen RS and USP Codeine Phosphate RS in methanol
Sample solution: Transfer a quantity of finely powdered Tablets, equivalent to 12 mg of codeine phosphate, to a separator. Add 5 mL of water, 1 mL of ammonium hydroxide, and 5 mL of methylene chloride. Shake for 1 min, and allow the layers to separate. Use the clear lower layer.
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Methanol and ammonium hydroxide (49:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry after applying each sample to the adsorbent. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light.
Acceptance criteria: The R_F values of the two principal spots of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY

• **PROCEDURE**
Solution A: Dissolve 2.04 g of monobasic potassium phosphate in about 950 mL of water. Add 2 mL of triethylamine, adjust with phosphoric acid to a pH of 2.35, and dilute with water to 1000 mL.
Mobile phase: Methanol and *Solution A* (8:92)
Codeine phosphate standard stock solution: 0.3 mg/mL of USP Codeine Phosphate RS in *Mobile phase*
Standard solution: 0.3 mg/mL of USP Acetaminophen RS and 0.3*J* mg/mL of codeine phosphate in *Mobile phase*, prepared as follows. Transfer an appropriate amount of USP Acetaminophen RS and a suitable volume (multiplied by *J*) of *Codeine phosphate standard stock solution* (*J* being the ratio of the labeled amount, in mg, of codeine phosphate to that of acetaminophen) to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.
Sample stock solution: Nominally 3.0 mg/mL of acetaminophen and 3.0*J* mg/mL of codeine phosphate (equivalent to 2.93*J* mg/mL of anhydrous codeine phosphate) in *Mobile phase*, prepared as follows. Transfer a portion of the powder (equivalent to 300 mg of acetaminophen, from NLT 20 finely powdered Tablets) to a 100-mL volumetric flask. Add 75 mL of *Mobile phase*, and sonicate for 10 min. Dilute with *Mobile phase* to volume.
Sample solution: Dilute 5.0 mL of the *Sample stock solution* with *Mobile phase* to 50 mL, and pass a portion of the solution through a suitable filter of 1-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 30 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between acetaminophen and codeine
Relative standard deviation: NMT 2.0% for acetaminophen; NMT 3.0% for codeine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*
r_S = peak response of acetaminophen from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

USP Monographs

www.webofpharma.com

Calculate the percentage of the labeled amount of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of codeine from the *Sample solution*
r_S = peak response of codeine from the *Standard solution*
C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of codeine phosphate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of codeine phosphate, 406.37
M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37

Acceptance criteria
Acetaminophen: 90.0%–110.0%
Codeine phosphate: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Analysis: Determine the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) dissolved by using the method set forth in the *Assay*, except use 0.01 N hydrochloric acid to prepare the *Codeine phosphate standard stock solution* and to make any other necessary volumetric adjustments.
Tolerances: NLT 75% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$) and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Solution A, Mobile phase, Codeine phosphate standard stock solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample stock solution: Transfer 1 Tablet to a 100-mL volumetric flask. Add 75 mL of *Mobile phase*, and sonicate for 10 min. Dilute with *Mobile phase* to volume.
Sample solution: Dilute 5.0 mL of the *Sample stock solution* with *Mobile phase* to 50 mL, and pass a portion through a suitable filter of 1-µm pore size.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in mg, of acetaminophen ($C_8H_9NO_2$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times C_S \times F$$

r_U = peak response of acetaminophen from the *Sample solution*
r_S = peak response of acetaminophen from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
F = dilution volume, 1000 mL

Calculate the quantity, in mg, of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times F$$

r_U = peak response of codeine from the *Sample solution*
r_S = peak response of codeine from the *Standard solution*
C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of codeine phosphate, 406.37
M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37
F = dilution volume, 1000 mL

Acceptance criteria: Meet the requirements

IMPURITIES
• **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetaminophen RS
USP Codeine Phosphate RS

Acetaminophen, Dextromethorphan Hydrobromide, Doxylamine Succinate, and Pseudoephedrine Hydrochloride Oral Solution

DEFINITION
Acetaminophen, Dextromethorphan Hydrobromide, Doxylamine Succinate, and Pseudoephedrine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$), dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$), doxylamine succinate ($C_{17}H_{22}N_2O \cdot C_4H_6O_4$), and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the acetaminophen peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Acetaminophen*.
• **B.** The retention time of the dextromethorphan peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Dextromethorphan Hydrobromide*.
• **C.** The retention time of the doxylamine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Doxylamine Succinate*.
• **D.** The retention time of the pseudoephedrine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Pseudoephedrine Hydrochloride*.

ASSAY
• **ACETAMINOPHEN**
Mobile phase: Methanol and water (45:55)
Standard solution: 0.2 mg/mL of USP Acetaminophen RS in *Mobile phase*
Sample solution: Nominally 0.2 mg/mL of acetaminophen from a volume of Oral Solution in *Mobile phase* prepared as follows. Dilute a volume of Oral Solution, equivalent to about 200 mg of acetaminophen, in *Mobile phase*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1

www.webofpharma.com

Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the acetaminophen peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*
r_S = peak response of acetaminophen from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$)

• **DEXTROMETHORPHAN HYDROBROMIDE**
Solution A: 6.8 g/L of monobasic potassium phosphate in water
Mobile phase: Acetonitrile and *Solution A* (45:55)
Standard solution: 0.1 mg/mL of USP Dextromethorphan Hydrobromide RS, 0.04 mg/mL of USP Doxylamine Succinate RS, and 0.2 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Mobile phase*
Sample solution: Nominally 0.1 mg/mL of dextromethorphan hydrobromide from a volume of Oral Solution in *Mobile phase* prepared as follows. Dilute a volume of Oral Solution, equivalent to about 5 mg of dextromethorphan hydrobromide, in *Mobile phase*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L9
Flow rate: 2.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for pseudoephedrine, dextromethorphan, and doxylamine are 0.38, 0.65, and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.5 for the dextromethorphan, doxylamine, and pseudoephedrine peaks
Relative standard deviation: NMT 2.0% for dextromethorphan, doxylamine, and pseudoephedrine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of dextromethorphan from the *Sample solution*
r_S = peak response of dextromethorphan from the *Standard solution*
C_S = concentration of USP Dextromethorphan Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dextromethorphan hydrobromide in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dextromethorphan hydrobromide monohydrate, 370.32
M_{r2} = molecular weight of anhydrous dextromethorphan hydrobromide, 352.32

Acceptance criteria: 90.0%–110.0% of the labeled amount of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$)

• **DOXYLAMINE SUCCINATE**
Solution A, Mobile phase, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay* for *Dextromethorphan Hydrobromide*.
Sample solution: Nominally 0.04 mg/mL of doxylamine succinate from a volume of Oral Solution in *Mobile phase* prepared as follows. Dilute a volume of Oral Solution, equivalent to about 2 mg of doxylamine succinate, in *Mobile phase*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxylamine succinate ($C_{17}H_{22}N_2O \cdot C_4H_6O_4$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of doxylamine from the *Sample solution*
r_S = peak response of doxylamine from the *Standard solution*
C_S = concentration of USP Doxylamine Succinate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxylamine succinate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of doxylamine succinate ($C_{17}H_{22}N_2O \cdot C_4H_6O_4$)

• **PSEUDOEPHEDRINE HYDROCHLORIDE**
Solution A, Mobile phase, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay* for *Dextromethorphan Hydrobromide*.
Sample solution: Nominally 0.2 mg/mL of pseudoephedrine hydrochloride from a volume of Oral Solution in *Mobile phase* prepared as follows. Dilute a volume of Oral Solution, equivalent to about 10 mg of pseudoephedrine hydrochloride, in *Mobile phase*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pseudoephedrine from the *Sample solution*
r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

www.webofpharma.com

Acceptance criteria: 90.0%–110.0% of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$)

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
For single-unit containers
Acceptance criteria: Meets the requirements
• **DELIVERABLE VOLUME** ⟨698⟩
For multiple-unit containers
Acceptance criteria: Meets the requirements

IMPURITIES

• **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩**:** Meets the requirements

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total bacterial count does not exceed 100 cfu/g, the total combined molds and yeasts count does not exceed 10 cfu/g, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli.*
• **pH** ⟨791⟩**:** 4.5–6.3
• **ALCOHOL DETERMINATION** ⟨611⟩, *Method II* (if present)**:** 90.0%–110.0% of the labeled amount of alcohol (C_2H_5OH)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetaminophen RS
USP Dextromethorphan Hydrobromide RS
USP Doxylamine Succinate RS
USP Pseudoephedrine Hydrochloride RS

Acetaminophen and Diphenhydramine Citrate Tablets

DEFINITION

Acetaminophen and Diphenhydramine Citrate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$) and diphenhydramine citrate ($C_{17}H_{21}NO \cdot C_6H_8O_7$).

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution,* obtained in the *Assay* for *Acetaminophen* and in the *Assay* for *Diphenhydramine Citrate,* relative to the retention times of the respective internal standards, correspond to those of the respective *Standard solution.*

ASSAY

• **ACETAMINOPHEN**
Mobile phase: Methanol and water (40:60)
Diluent: Methanol and water (1:4)
Internal standard solution: 8.0 mg/mL of guaifenesin in *Diluent*
Standard stock solution: 0.5 mg/mL of USP Acetaminophen RS, prepared as follows. Transfer 50 mg of USP Acetaminophen RS to a 100-mL volumetric flask. Dissolve in 2.5 mL of methanol, and dilute with water to volume.
Standard solution: 0.02 mg/mL of acetaminophen from *Standard stock solution* and 0.8 mg/mL of guaifenesin from *Internal standard solution,* in *Mobile phase*
Sample stock solution: Nominally 0.5 mg/mL of acetaminophen prepared as follows. Transfer a portion of the powder from NLT 20 finely powdered Tablets, nominally equivalent to an appropriate amount of acetaminophen, to a suitable volumetric flask. Add 25% of the total volume of methanol, and shake by mechanical means for 10 min. Dilute with water to volume.
Sample solution: Nominally 0.02 mg/mL of acetaminophen prepared as follows. Transfer 2.0 mL of *Sample stock solution* to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution,* and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35 ± 0.5°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for acetaminophen and guaifenesin are 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6.0 between the analyte and internal standard peaks
Tailing factor: NMT 2 for the analyte peak
Relative standard deviation: NMT 2.5% for the peak response ratios
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of acetaminophen to the internal standard from the *Sample solution*
R_S = peak response ratio of acetaminophen to the internal standard from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$)

• **DIPHENHYDRAMINE CITRATE**
Diluent: Methanol and water (1:1)
Mobile phase: Methanol, water, and glacial acetic acid (61:38:1) containing 1.0813 g of sodium 1-octanesulfonate in each 1000 mL of solution
Internal standard solution: 8 mg/mL of xylometazoline hydrochloride in water
Standard solution: 0.38 mg/mL of USP Diphenhydramine Citrate RS and 0.8 mg/mL of xylometazoline hydrochloride from *Internal standard solution* in *Diluent*
Sample solution: Nominally 0.38 mg/mL of diphenhydramine citrate prepared as follows. Transfer a portion of the powder from NLT 20 finely powdered Tablets, nominally equivalent to 38 mg of diphenhydramine citrate, to a 100-mL volumetric flask. Add 65 mL of *Diluent,* and shake by mechanical means for 15 min. Add 5.0 mL of *Internal standard solution,* and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 265 nm
Column: 3.9-mm × 30-cm; packing L1

www.webofpharma.com

Column temperature: 35 ± 0.5°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for diphenhydramine citrate and xylometazoline hydrochloride are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.5 between the analyte and internal standard peaks
Tailing factor: NMT 1.7 for the analyte peak
Relative standard deviation: NMT 2.0% for the peak response ratios
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of diphenhydramine citrate ($C_{17}H_{21}NO \cdot C_6H_8O_7$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of diphenhydramine citrate to the internal standard from the *Sample solution*
R_S = peak response ratio of diphenhydramine citrate to the internal standard from the *Standard solution*
C_S = concentration of USP Diphenhydramine Citrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of diphenhydramine citrate ($C_{17}H_{21}NO \cdot C_6H_8O_7$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms, Procedure for a pooled sample for immediate-release dosage forms*
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Analysis: Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) and diphenhydramine citrate ($C_{17}H_{21}NO \cdot C_6H_8O_7$) dissolved using the procedures set forth in the *Assay* for *Acetaminophen* and the *Assay* for *Diphenhydramine Citrate*, respectively, and make any necessary volumetric adjustments.
Tolerances: NLT 75% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$) and diphenhydramine citrate ($C_{17}H_{21}NO \cdot C_6H_8O_7$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*: Meet the requirements with respect to acetaminophen and diphenhydramine citrate

IMPURITIES

• **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetaminophen RS
USP Diphenhydramine Citrate RS

Acetaminophen, Diphenhydramine Hydrochloride, and Pseudoephedrine Hydrochloride Tablets

DEFINITION

Acetaminophen, Diphenhydramine Hydrochloride, and Pseudoephedrine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$), diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$), and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

IDENTIFICATION

• **A.** The retention time of the acetaminophen peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Acetaminophen*.
• **B.** The retention time of the diphenhydramine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Diphenhydramine Hydrochloride*.
• **C.** The retention time of the pseudoephedrine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Pseudoephedrine Hydrochloride*.

ASSAY

• **ACETAMINOPHEN**
Solution A: Transfer 6.8 g of monobasic potassium phosphate to a 1000-mL volumetric flask, and add water to dissolve. Add 2.0 mL of triethylamine, and dilute with water to volume. Adjust with phosphoric acid to a pH of 4.0.
Diluent: Acetonitrile and *Solution A* (11:89)
Mobile phase: Acetonitrile and *Solution A* (6:94)
Standard solution: 25 µg/mL of USP Acetaminophen RS, 12.5 µg/mL of USP Diphenhydramine Hydrochloride RS, and 30 µg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*
Sample stock solution: Nominally 5 mg/mL of acetaminophen in *Diluent* prepared as follows. Transfer an amount nominally equivalent to 500 mg of acetaminophen from NLT 20 finely powdered Tablets to a 100-mL volumetric flask, add 75 mL of *Diluent*, shake, and sonicate for 15 min. Dilute with *Diluent* to volume.
Sample solution: Nominally 25 µg/mL of acetaminophen from the *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; packing L10
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the acetaminophen, diphenhydramine, and pseudoephedrine peaks
Relative standard deviation: NMT 2.0% determined from the acetaminophen, diphenhydramine hydrochloride, and pseudoephedrine hydrochloride responses for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

www.webofpharma.com

r_U = peak response of acetaminophen from the *Sample solution*
r_S = peak response of acetaminophen from the *Standard solution*
C_S = concentration of USP Acetaminophen RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of acetaminophen in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$)

• **DIPHENHYDRAMINE HYDROCHLORIDE**

Solution A, Diluent, Mobile phase, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* for *Acetaminophen*.

Sample stock solution: Nominally 0.125 mg/mL of diphenhydramine hydrochloride in *Diluent* prepared as follows. Transfer an amount nominally equivalent to 12.5 mg of diphenhydramine hydrochloride from a portion of finely powdered Tablets (NLT 20) to a 100-mL volumetric flask, add 75 mL of *Diluent*, and sonicate for 15 min. Dilute with *Diluent* to volume.

Sample solution: Nominally 12.5 µg/mL of diphenhydramine hydrochloride from the *Sample stock solution* in *Diluent*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diphenhydramine from the *Sample solution*
r_S = peak response of diphenhydramine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$)

• **PSEUDOEPHEDRINE HYDROCHLORIDE**

Solution A, Diluent, Mobile phase, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* for *Acetaminophen*.

Sample stock solution: Nominally 0.3 mg/mL of pseudoephedrine hydrochloride in *Diluent* prepared as follows. Transfer an amount nominally equivalent to 30 mg of pseudoephedrine hydrochloride from a portion of finely powdered Tablets (NLT 20) to a 100-mL volumetric flask, add 75 mL of *Diluent*, and sonicate for 15 min. Dilute with *Diluent* to volume.

Sample solution: Nominally 30 µg/mL of pseudoephedrine hydrochloride from the *Sample stock solution* in *Diluent*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pseudoephedrine from the *Sample solution*
r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms, Procedure for a pooled sample for immediate-release dosage forms*

Medium: pH 5.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Solution A, Diluent, Mobile phase, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay* for *Acetaminophen*.

Sample solution A: Combine equal volumes of the filtered solutions, and use the pooled sample.

Sample solution B: Transfer 5.0 mL of *Sample solution A* to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.

Analysis: Using *Sample solution A* and the *Standard solution*, and making any necessary volumetric adjustments, proceed as directed in the *Assay* for *Diphenhydramine Hydrochloride* and the *Assay* for *Pseudoephedrine Hydrochloride*, and determine the percentage of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved. Using *Sample solution B* and the *Standard solution*, and making any necessary volumetric adjustments, proceed as directed in the *Assay* for *Acetaminophen*, and determine the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) dissolved.

Tolerances: NLT 75% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$), diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$), and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) is dissolved.

For Tablets labeled as chewable

Medium: pH 5.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 75 rpm

Time: 45 min

Tolerances: NLT 75% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$), diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$), and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Acetaminophen RS
USP Diphenhydramine Hydrochloride RS
USP Pseudoephedrine Hydrochloride RS

www.webofpharma.com

Acetaminophen and Pseudoephedrine Hydrochloride Tablets

DEFINITION

Acetaminophen and Pseudoephedrine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

IDENTIFICATION

• **A.** The retention times of the acetaminophen and pseudoephedrine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Acetonitrile and water (10:90)

Solution A: 0.005 M ethanesulfonic acid and 0.05 M monobasic potassium phosphate

Mobile phase: Acetonitrile and *Solution A* (100:900). Adjust with 5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.6.

Pseudoephedrine hydrochloride standard stock solution: 0.6 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*

Standard solution: Transfer 6*J* mg of USP Acetaminophen RS to a 100-mL volumetric flask, *J* being the ratio of the labeled quantity (mg) of acetaminophen to the labeled quantity (mg) of pseudoephedrine hydrochloride in each Tablet. Add 2.0 mL of 1 N hydrochloric acid and 20 mL of *Diluent*, and mix to dissolve. Add 10.0 mL of *Pseudoephedrine hydrochloride standard stock solution* and dilute with *Diluent* to volume. This solution contains 0.06*J* mg/mL of USP Acetaminophen RS and 0.06 mg/mL of USP Pseudoephedrine Hydrochloride RS.

Sample solution: Nominally 0.06 mg/mL of pseudoephedrine hydrochloride prepared as follows. Transfer a portion of finely powdered Tablets (NLT 20), equivalent to 30 mg of pseudoephedrine hydrochloride, to a 500-mL volumetric flask, add 10.0 mL of 1 N hydrochloric acid and 100 mL of *Diluent*, and sonicate for 30 min, with occasional shaking. Allow to cool, and dilute with *Diluent* to volume. Pass a portion of this solution through a glass fiber filter, and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; base-deactivated or end-capped packing L1

Flow rate: 3 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for acetaminophen and pseudoephedrine are about 0.55 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.5 between acetaminophen and pseudoephedrine

Tailing factor: NMT 2 for the pseudoephedrine peak

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*

r_S = peak response of the corresponding analyte from the *Standard solution*

C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of the appropriate analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms, Procedure for a pooled sample for immediate-release dosage forms*

Medium: pH 5.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Determine the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved by using the following method.

Mobile phase: Proceed as directed in the *Assay*.

Standard solution: (*L*/900) mg/mL of USP Pseudoephedrine Hydrochloride RS and (*LJ*/900) mg/mL of USP Acetaminophen RS in *Medium*. [NOTE—*L* is the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Tablet; and *J* is the ratio of the labeled quantity, in mg, of acetaminophen to the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Tablet.]

Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium*, if necessary

Chromatographic system and **System suitability:** Proceed as directed in the *Assay*, except to inject the *Standard solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Inject 20 µL of the *Samples*, and measure the responses for the acetaminophen and pseudoephedrine peaks.]

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times V \times (C_S/L) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*

r_S = peak response of the corresponding analyte from the *Standard solution*

V = volume of *Medium*, 900 mL

C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

L = label amount of the corresponding analyte in a Tablet (mg)

Tolerances: NLT 75% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) is dissolved.

For Tablets labeled as chewable

Medium: pH 5.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 75 rpm

Time: 45 min

www.webofpharma.com

Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed above in *Procedure for a pooled sample for immediate-release dosage forms.*

Tolerances: NLT 75% (*Q*) of the labeled amount of acetaminophen ($C_8H_9NO_2$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

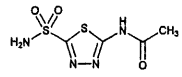
IMPURITIES

- **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** ⟨227⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Acetaminophen RS
  USP Pseudoephedrine Hydrochloride RS

Acetazolamide



$C_4H_6N_4O_3S_2$ 222.25

Acetamide, *N*-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]-; *N*-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide [59-66-5].

DEFINITION

Acetazolamide contains NLT 98.0% and NMT 102.0% of acetazolamide ($C_4H_6N_4O_3S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, add 20 mL of methanol and 30 mL of acetonitrile, and mix. Adjust with glacial acetic acid to a pH of 4.0.

Standard solution: 0.1 mg/mL of USP Acetazolamide RS prepared as follows. Transfer USP Acetazolamide RS into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the final volume, and dilute with water to volume.

Sample solution: 0.1 mg/mL of Acetazolamide prepared as follows. Transfer Acetazolamide into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the final volume, and dilute with water to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetazolamide ($C_4H_6N_4O_3S_2$) in the portion of Acetazolamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetazolamide from the *Sample solution*
r_S = peak response of acetazolamide from the *Standard solution*
C_S = concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Acetazolamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*

Sample solution: Digest 1.5 g with 75 mL of water at about 70° for 5 min. Cool to room temperature, and filter.
Acceptance criteria: A 25-mL portion of the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).

- **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*

Sample solution: A 25-mL portion of the filtrate prepared in the test for *Chloride and Sulfate, Chloride*
Acceptance criteria: It shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.04%).

- **SELENIUM** ⟨291⟩

Sample: 200 mg
Acceptance criteria: NMT 30 ppm

Delete the following:

▲• **SILVER-REDUCING SUBSTANCES**
Sample: 5 g
Analysis: Thoroughly wet the *Sample* with alcohol. Add 125 mL of water, 10 mL of nitric acid, and 5.0 mL of 0.1 N silver nitrate VS. Stir with a mechanical stirrer for 30 min. Filter, add 5 mL of ferric ammonium sulfate TS to the filtrate, and titrate with 0.1 N ammonium thiocyanate VS to a reddish-brown endpoint.
Acceptance criteria: NLT 4.8 mL of 0.1 N ammonium thiocyanate is required.▲ (USP 1-Aug-2019)

Delete the following:

▲• **ORDINARY IMPURITIES** ⟨466⟩
Standard solution: Acetone and methanol (1:1)
Test solution: Acetone and methanol (1:1)
Eluant: *n*-Propyl alcohol and 1 N ammonium hydroxide (88:12)
Visualization: 1▲ (USP 1-Aug-2019)

Add the following:

▲• **ORGANIC IMPURITIES**
Solution A: 13.61 g/L of monobasic potassium phosphate in water
Mobile phase: Methanol and *Solution A* (10:90)

www.webofpharma.com

Standard stock solution: 0.4 mg/mL of USP Acetazolamide RS in *Mobile phase* prepared as follows. Transfer a weighed amount of USP Acetazolamide RS to a suitable volumetric flask and add methanol to fill 20% of the final volume. Sonicate for 15 min with occasional shaking to dissolve. Dilute with *Mobile phase* to volume.
Standard solution: 0.005 mg/mL of USP Acetazolamide RS in *Mobile phase* from *Standard stock solution*
Sensitivity solution: 0.5 µg/mL each of USP Acetazolamide RS from *Standard solution* in *Mobile phase*
Sample solution: 1 mg/mL of Acetazolamide in *Mobile phase* prepared as follows. Transfer a weighed amount of Acetazolamide to a suitable volumetric flask and add methanol to fill 20% of the final volume. Sonicate for 15 min with occasional shaking to dissolve. Dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L10
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 30 µL
Run time: NLT 9.5 times the retention time of acetazolamide
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 14, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Acetazolamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of acetazolamide from the *Standard solution*
C_S = concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Acetazolamide in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desacetyl acetazolamide[a]	0.45	0.48	0.3
Acetazolamide acid analog[b]	0.54	1.0	0.5
Acetamidothiadiazole[c]	0.82	1.5	0.5
Acetazolamide	1.0	1.0	—
Mercaptothiadiazole analog[d]	1.6	0.46	0.5
Chlorothiadiazole analog[e]	1.9	1.4	0.5
Acetazolamide dimer[f]	6.3	1.0	0.5
Any unspecified impurity	—	1.0	0.1
Total impurities	—	—	1.0

[a] 5-Amino-1,3,4-thiadiazole-2-sulfonamide.
[b] 5-Acetamido-1,3,4-thiadiazole-2-sulfonic acid.
[c] *N*-(1,3,4-Thiadiazol-2-yl)acetamide.
[d] *N*-(5-Mercapto-1,3,4-thiadiazol-2-yl)acetamide.
[e] *N*-(5-Chloro-1,3,4-thiadiazol-2-yl)acetamide.
[f] *N,N'*-{5,5'-[(Hydrosulfonylamino)sulfonyl]bis(1,3,4-thiadiazole-5,2-diyl)}diacetamide. ▲ (USP 1-Aug-2019)

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetazolamide RS

Acetazolamide for Injection

DEFINITION
Acetazolamide for Injection is prepared from Acetazolamide with the aid of sodium hydroxide. It is suitable for parenteral use. The contents of each container, when constituted as directed in the labeling, yield a solution containing NLT 95.0% and NMT 110.0% of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Dissolve 500 mg in 5 mL of water, add 2 drops of hydrochloric acid, and allow the mixture to stand for about 15 min. Filter through a fine sintered-glass funnel, wash with several small portions of water, and dry under vacuum over silica gel for 3 h.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*: Meets the requirements

ASSAY
• **PROCEDURE**
Mobile phase: Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, add 20 mL of methanol and 30 mL of acetonitrile, and mix. Adjust with glacial acetic acid to a pH of 4.0.
Standard solution: 0.1 mg/mL of USP Acetazolamide RS prepared as follows. Transfer USP Acetazolamide RS to a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the final volume, and dilute with water to volume.
Sample solution: Nominally 0.1 mg/mL of acetazolamide from Acetazolamide for Injection prepared as follows. Dissolve the contents of one container of Acetazolamide for Injection in a volume of water corresponding to the volume

USP Monographs

www.webofpharma.com

of solvent specified in the labeling. Dilute with water as needed.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) in the portion of Acetazolamide for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetazolamide from the *Sample solution*
r_S = peak response of acetazolamide from the *Standard solution*
C_S = concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetazolamide in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–110.0%

PERFORMANCE TESTS

• **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements

IMPURITIES

• **Organic Impurities**
Buffer: 6.8 g/L of monobasic potassium phosphate in water
Mobile phase: Acetonitrile and *Buffer* (10:90)
System suitability solution: 0.16 µg/mL each of USP Acetazolamide Related Compound D RS and USP Acetazolamide Related Compound E RS in *Mobile phase*
Standard stock solution: 0.1 mg/mL of USP Acetazolamide RS prepared as follows. Transfer a weighed amount of USP Acetazolamide RS to a suitable volumetric flask and add acetonitrile equivalent to 10% of the final volume and *Buffer* equivalent to 20% of the final volume. Sonicate to dissolve and dilute with *Buffer* to volume.
Standard solution: 0.8 µg/mL of USP Acetazolamide RS from *Standard stock solution* in *Mobile phase*
Sample solution: Nominally 0.4 mg/mL of acetazolamide from Acetazolamide for Injection prepared as follows. Dissolve the contents of one container of Acetazolamide for Injection in water corresponding to the volume of solvent specified in the labeling. Dilute with *Mobile phase* as needed.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 265 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L11
Flow rate: 1.0 mL/min
Injection volume: 25 µL
Run time: NLT 3.5 times the retention time of acetazolamide

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between acetazolamide related compound E and acetazolamide related compound D, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Acetazolamide for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of acetazolamide from the *Standard solution*
C_S = concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetazolamide in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Acetazolamide related compound E[a]	0.38	—	—
Acetazolamide related compound D	0.43	0.70	2.0
Aminothiadiazole mercaptan[a, b]	0.55	—	—
Acetamidothiadiazole[a, c]	0.77	—	—
Acetazolamide	1.0	—	—
Mercaptothiadiazole analog[a, d]	1.4	—	—
Chlorothiadiazole analog[a, e]	2.2	—	—
Acetazolamide dimer[a, f]	2.8	—	—
Any unspecified degradation product	—	1.0	0.20
Total degradation products	—	—	3.0

[a] This process impurity is controlled in the drug substance. It is included in the table for identification only and is not to be reported in the total degradation products.
[b] 5-Amino-1,3,4-thiadiazole-2-thiol.
[c] *N*-(1,3,4-Thiadiazol-2-yl)acetamide.
[d] *N*-(5-Mercapto-1,3,4-thiadiazol-2-yl)acetamide.
[e] *N*-(5-Chloro-1,3,4-thiadiazol-2-yl)acetamide.
[f] *N,N'*-{5,5'-[(Hydrosulfonylamino)sulfonyl]*bis*(1,3,4-thiadiazole-5,2-diyl)} diacetamide.

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: Freshly prepared solution (1 in 10)
Acceptance criteria: 9.0–10.0
• **Bacterial Endotoxins Test** ⟨85⟩: It contains NMT 0.5 USP Endotoxin Units/mg of acetazolamide.
• **Injections and Implanted Drug Products** ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and Clarity of Solutions*: Meets the requirements at the time of use

USP Monographs

www.webofpharma.com

• **STERILITY TESTS** ⟨71⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, preferably in containers of Type III glass, and store at room temperature.

• **LABELING** ⟨7⟩, *Labels and Labeling for Injectable Products*: Meets the requirements

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Acetazolamide RS

USP Acetazolamide Related Compound D RS
5-Amino-1,3,4-thiadiazole-2-sulfonamide.
$C_2H_4N_4O_2S_2$ 180.21

USP Acetazolamide Related Compound E RS
5-Acetamido-1,3,4-thiadiazole-2-sulfonic acid potassium salt.
$C_4H_4KN_3O_4S_2$ 261.32

Acetazolamide Compounded Oral Suspension

DEFINITION

Acetazolamide Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$).

Prepare Acetazolamide Compounded Oral Suspension, 25 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Acetazolamide	2.5 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, *NF* (regular or sugar-free), and Vehicle for Oral Suspension, *NF*, or Cherry Syrup, *NF*, a sufficient quantity to make	100 mL

If using tablets, place in a mortar and comminute to a fine powder, or add *Acetazolamide* powder. Add about 20 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough liquid *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, and add 20 mL of methanol and 30 mL of acetonitrile. Adjust with glacial acetic acid to a pH of 4.0.

Standard stock solution: Transfer about 25 mg of USP Acetazolamide RS, accurately weighed, to a 50-mL volumetric flask, add 5.0 mL of 0.5 N sodium hydroxide, and mix to dissolve. Dilute with water to volume, and mix.

Standard solution: 250 µg/mL of USP Acetazolamide RS from the *Standard stock solution* in water

Sample solution: 250 µg/mL of acetazolamide from Oral Suspension in *Mobile phase*. Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for the acetazolamide peak is about 3 min.]

Suitability requirements

Relative standard deviation: NMT 1.1% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Acetazolamide RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of acetazolamide in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.0–5.0 (Vehicle for Oral Solution and Vehicle for Oral Suspension), 3.1–3.9 (Cherry Syrup)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.

• **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded when stored at controlled room temperature, or in a refrigerator

• **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Acetazolamide RS

Acetazolamide Tablets

DEFINITION

Acetazolamide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Extract a quantity of finely powdered Tablets, equivalent to about 500 mg of acetazolamide, with 50 mL of acetone. Filter, and add sufficient solvent hexane to the filtrate to cause formation of a heavy, white precipitate. Collect the precipitate on a medium-porosity, sintered-glass funnel, and dry with suction.

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Mobile phase: Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, add 20 mL of methanol and 30 mL of acetonitrile, and mix. Adjust with glacial acetic acid to a pH of 4.0.

Standard solution: 0.1 mg/mL of USP Acetazolamide RS prepared as follows. Transfer USP Acetazolamide RS into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the final volume, and dilute with water to volume.

Sample stock solution: Nominally equivalent to 1.0 mg/mL of acetazolamide prepared as follows. Transfer a portion of the powder, from NLT 20 Tablets, equivalent to 100 mg acetazolamide into a 100-mL volumetric flask. Add 10 mL of 0.5 N sodium hydroxide, sonicate for 5 min, cool to room temperature, and dilute with water to volume. Filter a portion of this solution, discarding the first 20 mL of the filtrate.

Sample solution: Nominally equivalent to 0.1 mg/mL of acetazolamide prepared as follows. Transfer 10.0 mL of *Sample stock solution* and 10 mL of 0.5 N sodium hydroxide to a 100-mL volumetric flask, and dilute with water to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = acetazolamide peak response from the *Sample solution*
r_S = acetazolamide peak response from the *Standard solution*
C_S = concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetazolamide in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 60 min
Standard solution: USP Acetazolamide RS in *Medium*
Sample solution: Dilute with *Medium* if necessary.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 265 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) dissolved:

$$(A_U/A_S) \times C_S \times D \times (V/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution*, if needed
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetazolamide RS

Glacial Acetic Acid



$C_2H_4O_2$ 60.05
Acetic acid [64-19-7].

DEFINITION

Glacial Acetic Acid contains NLT 99.5% and NMT 100.5%, by weight, of $C_2H_4O_2$.

IDENTIFICATION

• **IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Acetate*: Meets the requirements
Sample solution (for test A): Glacial Acetic Acid and water (1:100)

ASSAY

• **PROCEDURE**
Sample solution: Measure 2 mL of Glacial Acetic Acid into a glass-stoppered flask, previously tared while containing about 20 mL of water, and weigh again to obtain the weight of the substance under assay.
Analysis: Add 20 mL of water, then add phenolphthalein TS. Titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 60.05 mg of $C_2H_4O_2$.
Acceptance criteria: 99.5%–100.5%

IMPURITIES

INORGANIC IMPURITIES
• **Limit of Nonvolatile Residue:** Evaporate 20 mL in a tared dish, and dry at 105° for 1 h: the weight of the residue does not exceed 1.0 mg.
• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample solution: Dilute 1.0 mL with 20 mL of water.
Analysis: Add 5 drops of silver nitrate TS.
Acceptance criteria: No opalescence is produced.
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Sample solution: Dilute 1.0 mL with 10 mL of water.
Analysis: Add 1 mL of barium chloride TS.
Acceptance criteria: No turbidity is produced.

ORGANIC IMPURITIES
• **Procedure: Readily Oxidizable Substances**
Sample solution: Dilute 2.0 mL in a glass-stoppered vessel with 10 mL of water.
Analysis: Add 0.10 mL of 0.10 N potassium permanganate.

USP Monographs

www.webofpharma.com

Acceptance criteria: The pink color is not changed to brown within 2 h.

SPECIFIC TESTS
• **CONGEALING TEMPERATURE** ⟨651⟩**:** NLT 15.6°

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.

Acetic Acid Irrigation

DEFINITION
Acetic Acid Irrigation is a sterile solution of Glacial Acetic Acid in Water for Injection. It contains, in each 100 mL, NLT 237.5 mg and NMT 262.5 mg of $C_2H_4O_2$.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Acetate* ⟨191⟩
Sample: 100 mL of Acetic Acid Irrigation
Analysis: Evaporate the *Sample* to about 10 mL.
Acceptance criteria: The resulting solution meets the requirements.

ASSAY
• **PROCEDURE**
Sample: 50 mL of Acetic Acid Irrigation
Analysis: Pipet the *Sample* into a 150-mL conical flask, add 2 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 6.005 mg of acetic acid ($C_2H_4O_2$).
Acceptance criteria: 237.5–262.5 mg of $C_2H_4O_2$ in each 100 mL of Acetic Acid Irrigation

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 2.8–3.4
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.5 USP Endotoxin Unit/mL.
• **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the container in which it is packaged may be designed to empty rapidly and may exceed 1000 mL in capacity.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I or Type II glass, and store at controlled room temperature. It may be packaged in suitable plastic containers.

Acetic Acid Otic Solution

DEFINITION
Acetic Acid Otic Solution is a solution of Glacial Acetic Acid in a suitable nonaqueous solvent. It contains NLT 85.0% and NMT 130.0% of the labeled amount of $C_2H_4O_2$.

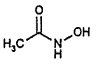
IDENTIFICATION
• **A.**
Sample solution: Dilute 5 mL of Acetic Acid Otic Solution with 10 mL of water.
Analysis: Adjust the *Sample solution* with 1 N sodium hydroxide to a pH of 7. Add ferric chloride TS.
Acceptance criteria: A deep red color is produced, and it is destroyed by the addition of hydrochloric acid.
• **B.**
Analysis: Warm it with sulfuric acid and alcohol.
Acceptance criteria: Ethyl acetate, recognizable by its characteristic odor, is evolved.

ASSAY
• **PROCEDURE**
Sample: A quantity of Acetic Acid Otic Solution containing 100 mg of glacial acetic acid
Analysis: Transfer the *Sample* to a 250-mL conical flask, and add 5 mL of saturated sodium chloride solution, 40 mL of water, and 3 drops of phenolphthalein TS. Titrate with 0.1 N sodium hydroxide VS to a faint pink endpoint. Each mL of 0.1 N sodium hydroxide is equivalent to 6.005 mg of acetic acid ($C_2H_4O_2$).
Acceptance criteria: 85.0%–130.0%

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: Acetic Acid Otic Solution and water (1:1)
Acceptance criteria: 2.0–4.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

Acetohydroxamic Acid



$C_2H_5NO_2$ 75.07
N-Acetyl hydroxyacetamide;
Acetohydroxamic acid [546-88-3].

DEFINITION
Acetohydroxamic Acid, dried over phosphorus pentoxide for 16 h, contains NLT 98.0% and NMT 101.0% of acetohydroxamic acid ($C_2H_5NO_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.**
Sample solution: 20 mg/mL in water
Analysis: To 10 mL of the *Sample solution* add 2 drops of potassium permanganate TS.
Acceptance criteria: The pink color of the permanganate disappears.

ASSAY
• **PROCEDURE**
Ferric chloride solution: 20 mg/mL of ferric chloride in 0.1 N hydrochloric acid
Standard solution: 500 µg/mL of USP Acetohydroxamic Acid RS in 0.1 N hydrochloric acid
Sample solution: 500 µg/mL of Acetohydroxamic Acid, previously dried, in 0.1 N hydrochloric acid
Blank: 0.1 N hydrochloric acid
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Transfer 10.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to separate 100-mL volumetric flasks. To each flask add 50 mL of 0.1 N hydrochloric acid and 10.0 mL of *Ferric chloride solution*, and dilute with 0.1 N hydrochloric acid to volume. Without delay,

www.webofpharma.com

concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 502 nm using the *Blank* to set the instrument.

Calculate the percentage of acetohydroxamic acid ($C_2H_5NO_2$) in the portion of Acetohydroxamic Acid taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Acetohydroxamic Acid RS in the *Standard solution* (µg/mL)
C_U = concentration of Acetohydroxamic Acid in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–101.0% on the previously dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **LIMIT OF HYDROXYLAMINE**

Buffer: 1.36 g/L of monobasic potassium phosphate in water, adjusted with 1 M potassium hydroxide to a pH of 7.4

Solution A: 1 mg/mL of pyridoxal 5-phosphate monohydrate in *Buffer*, prepared in a low-actinic flask fresh before use

Standard stock solution: 2.0 mg/mL of hydroxylamine hydrochloride in water

Standard solutions: Transfer 5.0, 10.0, and 15.0 mL of the *Standard stock solution* to separate 100-mL volumetric flasks, and dilute with water to volume.

Sample solution: Transfer 1500 mg of Acetohydroxamic Acid, previously dried, to a 100-mL beaker, and dissolve in a sufficient amount of water to cover the electrode of a calibrated pH meter (about 60 mL). While stirring, adjust with 0.05 M potassium hydroxide to a pH of 7.4. Transfer the contents of the beaker, with the aid of small portions of water, to a 100-mL volumetric flask, and dilute with water to volume.

Blank: Water

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Transfer 2.0 mL of each *Standard solution*, the *Sample solution*, and *Blank* into separate 100-mL volumetric flasks. To each flask add 4.0 mL of *Solution A*. After 8 min, accurately timed, dilute the contents of each flask with *Buffer* to volume.

Immediately determine the fluorescence intensities of the solutions from the *Standard solutions* and the *Sample solution* in a fluorometer at an excitation wavelength of 350 nm and an emission wavelength of 450 nm, setting the instrument to zero with the *Blank*. Determine the best-fit straight line from the fluorescence intensities of the three *Standard solutions* versus the hydroxylamine hydrochloride concentrations, in µg/mL. From the best-fit straight line, determine the concentration, in µg/mL, of hydroxylamine hydrochloride in the *Sample solution*.

Calculate the percentage of hydroxylamine in the portion of Acetohydroxamic Acid taken:

$$\text{Result} = (C_U/C) \times (M_{r1}/M_{r2}) \times 100$$

C_U = concentration of hydroxylamine hydrochloride in the *Sample solution* (mg/mL)
C = concentration of Acetohydroxamic Acid in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of hydroxylamine, 33.03
M_{r2} = molecular weight of hydroxylamine hydrochloride, 69.50

Acceptance criteria: NMT 0.5%

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample over phosphorus pentoxide for 16 h.

Acceptance criteria: NMT 1.0%

• **COMPLETENESS OF SOLUTION** ⟨641⟩: A 1.0-g portion dissolves in 10 mL of water to yield a clear solution.

• **COLOR OF SOLUTION**

Sample solution: 200 mg/mL in water

Blank: Water

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: Between 400 and 750 nm

Cell: 1 cm

Acceptance criteria: The absorbance is NMT 0.050.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a cool, dry place.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Acetohydroxamic Acid RS

Acetohydroxamic Acid Tablets

DEFINITION

Acetohydroxamic Acid Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetohydroxamic acid ($C_2H_5NO_2$).

IDENTIFICATION

• **A.** Tablets produce a purple color when mixed with an acidic solution of ferric chloride.

ASSAY

• **PROCEDURE**

Ferric chloride solution: 20 mg/mL of ferric chloride in 0.1 N hydrochloric acid

Standard solution: 500 µg/mL of USP Acetohydroxamic Acid RS in 0.1 N hydrochloric acid

Sample solution: Weigh, and finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of acetohydroxamic acid, to a 1000-mL volumetric flask, add about 500 mL of 0.1 N hydrochloric acid, and shake for 1 min. Dilute with 0.1 N hydrochloric acid to volume, and mix. Filter, discarding the first 40 mL of the filtrate. Use the clear filtrate.

Blank: 0.1 N hydrochloric acid

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Transfer 10.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to separate 100-mL volumetric flasks. To each flask add 50 mL of 0.1 N hydrochloric acid and 10.0 mL of *Ferric chloride solution*, and dilute with 0.1 N hydrochloric acid to volume. Without delay, concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 502 nm, using the *Blank* to set the instrument.

Calculate the percentage of labeled amount of acetohydroxamic acid ($C_2H_5NO_2$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Acetohydroxamic Acid RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of acetohydroxamic acid in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Analysis: Calculate the percentage of the labeled amount of acetohydroxamic acid ($C_2H_5NO_2$) dissolved, using the procedure in the *Assay*, using a filtered portion of the solution under test as *Sample solution* in comparison with a *Standard solution* having a known concentration of USP Acetohydroxamic Acid RS in *Medium*.
Tolerances: NLT 85% (*Q*) of the labeled amount of acetohydroxamic acid ($C_2H_5NO_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **LIMIT OF HYDROXYLAMINE**
Buffer: 1.36 g/L of monobasic potassium phosphate in water, adjusted with 1 M potassium hydroxide to a pH of 7.4
Solution A: 1 mg/mL of pyridoxal 5-phosphate monohydrate in *Buffer*, prepared in a low-actinic flask fresh before use
Standard stock solution: 2.0 mg/mL of hydroxylamine hydrochloride in water
Standard solutions: Transfer 5.0, 10.0, and 15.0 mL of the *Standard stock solution* to separate 100-mL volumetric flasks, and dilute with water to volume.
Sample solution: Weigh, and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to about 1500 mg of acetohydroxamic acid to a 50-mL stoppered centrifuge tube. Add 30.0 mL of water, shake for about 2 min, and centrifuge. Pipet 15.0 mL of the clear solution into a 50-mL beaker, add just enough water to cover the electrode of a calibrated pH meter, and while stirring, adjust with 0.5 M potassium hydroxide to a pH of 7.4. Quantitatively transfer the contents of the beaker with the aid of small portions of water to a 50-mL volumetric flask, dilute with water to volume, and mix.
Blank: Water
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Transfer 2.0 mL of each *Standard solution*, the *Sample solution*, and *Blank* into separate 100-mL volumetric flasks. To each flask add 4.0 mL of *Solution A*. After 8 min, accurately timed, dilute the contents of each flask with *Buffer* to volume.
Immediately determine the fluorescence intensities of the solutions from the *Standard solutions* and the *Sample solution* in a fluorometer at an excitation wavelength of 350 nm and an emission wavelength of 450 nm, setting the instrument to zero with the *Blank*. Determine the best-fit straight line from the fluorescence intensities of the three *Standard solutions* versus the hydroxylamine hydrochloride concentrations, in µg/mL. From the best-fit straight line, determine the concentration, in µg/mL, of hydroxylamine hydrochloride in the *Sample solution*.
Calculate the percentage of hydroxylamine in the portion of Tablets taken:

$$\text{Result} = (C_U/C) \times (M_{r1}/M_{r2}) \times 100$$

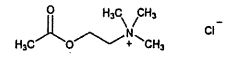
C_U = concentration of hydroxylamine hydrochloride in the *Sample solution* (µg/mL)
C = nominal concentration of acetohydroxamic acid in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of hydroxylamine, 33.03
M_{r2} = molecular weight of hydroxylamine hydrochloride, 69.50

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetohydroxamic Acid RS

Acetylcholine Chloride



$C_7H_{16}ClNO_2$ 181.66
Ethanaminium, 2-(acetyloxy)-*N*,*N*,*N*-trimethyl-, chloride; Choline acetate (ester) chloride [60-31-1].

DEFINITION

Acetylcholine Chloride contains NLT 98.0% and NMT 102.0% of acetylcholine chloride ($C_7H_{16}ClNO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.**
Sample solution: 100 mg/mL in water
Analysis: To 5 mL of *Sample solution* add 5 mL of silver nitrate TS.
Acceptance criteria: A white, curdy precipitate, which is soluble in ammonium hydroxide but insoluble in nitric acid, is formed.

ASSAY

• **PROCEDURE**
Sample: 400 mg of Acetylcholine Chloride
Analysis: Dissolve in 15 mL of water in a glass-stoppered conical flask, add 40.0 mL of 0.1 N sodium hydroxide VS, and heat on a steam bath for 30 min. Insert the stopper, allow to cool, add phenolphthalein TS, and titrate the excess alkali with 0.1 N sulfuric acid VS. Perform a blank determination (see *Titrimetry* ⟨541⟩, *Residual Titrations*). Each mL of 0.1 N sodium hydroxide is equivalent to 18.17 mg of $C_7H_{16}ClNO_2$.
Acceptance criteria: 98.0%–102.0% on the dried basis

OTHER COMPONENTS

• **CONTENT OF CHLORIDE**
Sample: 280 mg of Acetylcholine Chloride
Analysis: Dissolve the *Sample* in 140 mL of water, and add 1 mL of dichlorofluorescein TS. Titrate with 0.1 N silver nitrate VS until the silver chloride flocculates and the mixture acquires a faint pink color. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl.
Acceptance criteria: 19.3%–19.8% of Cl on the dried basis

www.webofpharma.com

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩: 149°–152°

• **ACIDITY**

Sample: 100 mg of Acetylcholine Chloride

Analysis: Dissolve the *Sample* in 10 mL of recently boiled water, and add at once 1 drop of bromothymol blue TS.

Acceptance criteria: NMT 0.50 mL of 0.010 N sodium hydroxide is required to produce a color change.

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 105° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in a tight container, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Acetylcholine Chloride RS

Acetylcholine Chloride for Ophthalmic Solution

DEFINITION

Acetylcholine Chloride for Ophthalmic Solution is a sterile mixture of Acetylcholine Chloride with Mannitol or other suitable diluent, prepared by freeze-drying. Each container contains NLT 90.0% and NMT 115.0% of the labeled amount of acetylcholine chloride ($C_7H_{16}ClNO_2$).

IDENTIFICATION

• **A.**

Standard solution: 10 mg/mL of USP Acetylcholine Chloride RS

Sample solution: 10 mg/mL of acetylcholine chloride

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

Adsorbent: 0.25-mm layer of aluminum oxide

Application volume: 2 µL

Developing solvent system: Mix butyl alcohol, glacial acetic acid, and water (40:10:50). Allow the layers to separate completely. Use the upper layer.

Spray reagent A: Freshly prepared solution of 5 mg/mL of cobaltous chloride prepared as follows. Dissolve the required amount of cobaltous chloride in 50% of the final volume of water, and dilute with 50% alcohol. [NOTE—This solution is freshly prepared.]

Spray reagent B: Freshly prepared potassium ferrocyanide solution prepared as follows. Dissolve 1.0 g of potassium ferrocyanide in 100 mL of water, and dilute with 50 mL of alcohol.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram, without delay, in a vapor-saturated chamber containing the *Developing solvent system.* Allow the solvent front to move about 10 cm beyond the initial spotting line. Dry the plate with a current of warm air. Immediately spray the plate with *Spray reagent A.* Dry the plate as before, and immediately spray the plate with *Spray reagent B.* Dry the plate with a current of warm air.

Acceptance criteria: The R_F value and color of the principal spot from the *Sample solution* correspond to those from the *Standard solution.*

• **B.**

Sample solution: Nominally 10 mg/mL of acetylcholine chloride

Analysis: To 2 mL of *Sample solution* add 1 drop of nitric acid and 1 mL of silver nitrate TS.

Acceptance criteria: A curdy, white precipitate, soluble in an excess of 6 N ammonium hydroxide, is formed.

ASSAY

• **PROCEDURE**

Mobile phase: Add 1.03 g of sodium 1-heptanesulfonate to a mixture of 900 mL of water and 10 mL of methanol. Adjust with ammonium hydroxide or glacial acetic acid to a pH of 4.0. Add 50 mL of acetonitrile. Dilute with water to 1 L. [NOTE—A slight variation of the amount of acetonitrile may be required to improve resolution or adjust retention time.]

Standard solution: A quantity of USP Acetylcholine Chloride RS in *Mobile phase,* to obtain a solution having a known concentration equal to that of the acetylcholine chloride in the *Sample solution*

Sample solution: Transfer the contents of 1 container of Acetylcholine Chloride for Ophthalmic Solution to a 10-mL volumetric flask with the aid of *Mobile phase,* and dilute with *Mobile phase* to volume.

System suitability solution: 0.2% each of acetylcholine chloride and choline chloride

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: Refractive index

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 50 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between acetylcholine chloride and choline chloride, *System suitability solution*

Relative standard deviation: NMT 3.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acetylcholine chloride ($C_7H_{16}ClNO_2$) in the container taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Acetylcholine Chloride RS in the *Standard solution* (mg/mL)

V = volume of the *Sample solution,* 10 mL

L = label claim (mg/vial)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩: Meets the requirements

• **ACIDITY**

Analysis: Dissolve an amount of Acetylcholine Chloride for Ophthalmic Solution equivalent to 100 mg of acetylcholine chloride in 10 mL of recently boiled water. Add at once 1 drop of bromothymol blue TS.

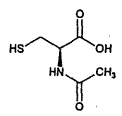
Acceptance criteria: NMT 0.50 mL of 0.010 N sodium hydroxide is required to produce a color change.

www.webofpharma.com

• **WATER DETERMINATION,** *Method I* ⟨921⟩
Analysis: Perform the titration in the original container, observing precautions against contact with water or moist atmosphere. Adjust the concentration of the reagent so that the titration volume approaches but does not exceed the capacity of the container. Titrate to an amber color that persists for 15 s after mixing.
Acceptance criteria: NMT 1.0%
• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution,* and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetylcholine Chloride RS

Acetylcysteine



$C_5H_9NO_3S$ 163.19
L-Cysteine, *N*-acetyl-;
N-Acetyl-L-cysteine [616-91-1].

DEFINITION
Acetylcysteine contains NLT 98.0% and NMT 102.0% of $C_5H_9NO_3S$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Mobile phase: 6.8 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.
Sodium metabisulfite solution: 0.5 mg/mL of sodium metabisulfite in water, freshly prepared
Internal standard solution: 5 mg/mL of USP L-Phenylalanine RS in *Sodium metabisulfite solution*
Standard stock solution: 10 mg/mL of USP Acetylcysteine RS in *Sodium metabisulfite solution*
Standard solution: 0.5 mg/mL of USP Acetylcysteine RS and 0.25 mg/mL of USP L-Phenylalanine RS in *Sodium metabisulfite solution* from *Standard stock solution* and *Internal standard solution*
Sample stock solution: 10 mg/mL of Acetylcysteine in *Sodium metabisulfite solution*
Sample solution: 0.5 mg/mL of Acetylcysteine and 0.25 mg/mL of USP L-Phenylalanine RS in *Sodium metabisulfite solution* from *Sample stock solution* and *Internal standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 214 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for acetylcysteine and L-phenylalanine are about 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6 between acetylcysteine and L-phenylalanine
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetylcysteine ($C_5H_9NO_3S$) in the portion of Acetylcysteine taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of acetylcysteine to L-phenylalanine from the *Sample solution*
R_S = peak response ratio of acetylcysteine to L-phenylalanine from the *Standard solution*
C_S = concentration of USP Acetylcysteine RS in the *Standard solution* (mg/mL)
C_U = concentration of acetylcysteine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Buffer: Mix 29.5 mL of 1 N sodium hydroxide, 50 mL of 1 M monobasic potassium phosphate, and sufficient water to make 100 mL. Adjust to a pH of 7.0 ± 0.1 by adding more of either solution, as necessary.
Sample solution: In a 25-mL volumetric flask, mix 1.25 g with 1 mL of edetate disodium solution (1 in 100), add 7.5 mL of sodium hydroxide solution (1 in 25), and mix to dissolve. Dilute with *Buffer* to volume.
Acceptance criteria: +21° to +27°
• **pH** ⟨791⟩: 2.0–2.8 in a solution (1 in 100)
• **LOSS ON DRYING** ⟨731⟩: Dry a sample at a pressure of about 50 mm of mercury at 70° for 4 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetylcysteine RS
USP L-Phenylalanine RS

Acetylcysteine Solution

DEFINITION
Acetylcysteine Solution is a sterile solution of Acetylcysteine in water, prepared with the aid of Sodium Hydroxide. It contains NLT 90.0% and NMT 110.0% of the labeled amount of acetylcysteine ($C_5H_9NO_3S$).

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample solution: Place 10 mL in a suitable beaker, and adjust to a pH of 2 (pH indicator paper), using 3 N hydrochloric acid. Add up to 2 g of finely powdered sodium chloride, in two portions of 200 mg each initially and then in smaller portions of 25 mg, stirring after each addition until the sodium chloride dissolves and a precipitate is formed. The precipitate appears as a very fine powder, and the solution turns cloudy. If no precipitate forms, add an additional drop of 3 N hydrochloric acid, and stir until the precipitate forms. Allow to stand at room temperature for 15 min, and collect the residue by suction filtration. Use the acetylcysteine so obtained after being dried at a pressure of 50 mm of mercury at 70° for 4 h.

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: 0.5 mg/mL of sodium metabisulfite solution in water, freshly prepared

Solution B: 0.5 mg/mL of sodium bisulfite solution

Mobile phase: 6.8 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.

Internal standard solution: 5 mg/mL of USP L-Phenylalanine RS in *Solution A*

Standard stock solution: 10 mg/mL of USP Acetylcysteine RS in *Solution A*

Standard solution: 0.5 mg/mL of USP Acetylcysteine RS and 0.25 mg/mL of USP L-Phenylalanine RS in *Solution A* from *Standard stock solution* and *Internal standard solution*

Sample stock solution: Equivalent to 10 mg/mL of acetylcysteine from the volume of Solution in *Solution B*

Sample solution: 0.5 mg/mL of acetylcysteine and 0.25 mg/mL of USP L-Phenylalanine RS in *Solution A* from *Sample stock solution* and *Internal standard solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 6 between acetylcysteine and L-phenylalanine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—The relative retention times for acetylcysteine and L-phenylalanine are about 0.5 and 1.0, respectively.]

Calculate the percentage of the labeled amount of acetylcysteine ($C_5H_9NO_3S$) in the portion of Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of acetylcysteine to L-phenylalanine from the *Sample solution*

R_S = peak response ratio of acetylcysteine to L-phenylalanine from the *Standard solution*

C_S = concentration of USP Acetylcysteine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetylcysteine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 6.0–7.5
- **STERILITY TESTS** ⟨71⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-unit or multiple-unit tight containers that effectively exclude oxygen, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Acetylcysteine RS

USP L-Phenylalanine RS

Acetylcysteine Compounded Solution

DEFINITION

Acetylcysteine Compounded Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of acetylcysteine ($C_5H_9NO_3S$). Prepare Acetylcysteine Compounded Solution 20% as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Acetylcysteine	2 g
Edetate Disodium Dihydrate	5.5 mg
Sodium Hydroxide 10% Solution	To adjust pH to 6.5–7.5
Sterile Water for Injection, a sufficient quantity to make	10 mL[a]

[a] It is necessary to adjust the formula and compound an additional amount to completely fill each single-unit container to minimize exposure to oxygen because the preparation is susceptible to oxidation.

Dissolve *Edetate Disodium Dihydrate* in 7 mL of *Sterile Water for Injection*. Slight heating may be necessary. Allow to cool. Dissolve *Acetylcysteine* in the edetate disodium solution. Add *Sodium Hydroxide 10% Solution* dropwise with mixing to adjust the pH to between 6.5 and 7.5. Bring to final volume with *Sterile Water for Injection* and mix well. Pass through a sterile filter of 0.22-µm pore size into single-unit sterile containers. It is necessary to completely fill the container to minimize the amount of oxygen present because the preparation is susceptible to oxidation.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, phosphoric acid, and water (3: 0.5: 96.5)

Standard solution: 0.4 mg/mL of acetylcysteine prepared from USP Acetylcysteine RS in *Mobile phase*

Sample solution: Transfer 0.4 mL of Solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix well.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 25-cm; 5-µm packing L96

Column temperature: 15°

Flow rate: 2.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

USP Monographs

www.webofpharma.com

[NOTE—The retention time for acetylcysteine is about 3.8 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetylcysteine ($C_5H_9NO_3S$) in the portion of Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetylcysteine from the *Sample solution*

r_S = peak response of acetylcysteine from the *Standard solution*

C_S = concentration of USP Acetylcysteine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetylcysteine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 6.5–7.5
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in single-unit sterile glass containers and store at controlled room temperature.

Change to read:

- **BEYOND-USE DATE:** In the absence of ▲passing a sterility test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test is passed, NMT 60 days after the date on which it was compounded when stored at controlled room temperature▲ (CN 1-May-2020).
- **LABELING:** Label it to state the *Beyond-Use Date*. The label indicates that the Solution is not to be used if it contains a precipitate. Label it to state that it is a single-unit container, that it is overfilled with an excess that should be discarded after a measured single dose is used, and to store at controlled room temperature. Label it for inhalation or oral administration only. Label it to state that the preparation may have a disagreeable odor and light purple color that is a result of a chemical reaction that does not affect the strength of the preparation.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Acetylcysteine RS

Acetylcysteine and Isoproterenol Hydrochloride Inhalation Solution

» Acetylcysteine and Isoproterenol Hydrochloride Inhalation Solution is a sterile solution of Acetylcysteine and Isoproterenol Hydrochloride in water. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetylcysteine ($C_5H_9NO_3S$), and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of isoproterenol hydrochloride ($C_{11}H_{17}NO_3 \cdot HCl$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, tightly closed with a glass or polyethylene closure, and store at controlled room temperature.

Labeling—The label indicates that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

USP Reference standards ⟨11⟩—
USP Acetylcysteine RS
USP Isoproterenol Hydrochloride RS
USP L-Phenylalanine RS

Color and clarity—Using the Inhalation Solution as the *Test solution*, proceed as directed for *Color and clarity* under *Isoproterenol Inhalation Solution*.

Identification—

A: Place 2 mL in a 10-mL beaker, and adjust with 3 N hydrochloric acid to a pH of about 3 (pH indicator paper). Add 500 mg to 1 g of finely powdered sodium chloride, in two portions of about 200 mg each initially, and then in smaller portions (about 25 mg), stirring after each addition, until a precipitate is formed. Allow to stand at room temperature for 15 minutes, and collect the residue by suction filtration: the acetylcysteine so obtained, after being dried as directed in the test for *Loss on drying* under *Acetylcysteine*, responds to the *Identification* test under *Acetylcysteine*.

B: Ferro-Citrate Solution and *Buffer Solution*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Procedure—Place a volume of Inhalation Solution, equivalent to about 0.26 mg of isoproterenol hydrochloride, in a test tube with 3 mL of 0.1 M mercuric chloride, and mix. Add 100 µL of *Ferro-Citrate Solution* and 1.0 mL of *Buffer Solution*, and mix: the presence of isoproterenol hydrochloride is confirmed by the development of a purple color.

Sterility Tests ⟨71⟩ **:** meets the requirements.

pH ⟨791⟩**:** between 6.0 and 7.0.

Assay for acetylcysteine—

Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Acetylcysteine*.

Assay preparation—Pipet a volume of Inhalation Solution, equivalent to about 1000 mg of acetylcysteine, into a 100-mL volumetric flask, dilute with sodium metabisulfite solution (1 in 2000) to volume, and mix. Pipet 10 mL of this solution and 10 mL of *Internal standard solution* into a 200-mL volumetric flask, dilute with sodium metabisulfite solution (1 in 2000) to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Acetylcysteine*. Calculate the quantity, in mg, of $C_5H_9NO_3S$ in each mL of the Inhalation Solution taken by the formula:

$$2000(C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Acetylcysteine RS in the *Standard preparation*; V is the volume, in mL, of Inhalation Solution taken; and R_U and R_S are the ratios of the peak response of acetylcysteine to that of DL-phenylalanine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for isoproterenol hydrochloride—

Mobile phase—Dissolve 13.6 g of monobasic potassium phosphate in 1000 mL of water, and pass through a membrane filter having a 0.45-µm porosity. Add 20.0 mL of methanol, mix, and degas.

USP Monographs

www.webofpharma.com

Internal standard solution—Place about 150 mg of acetaminophen in a 500-mL volumetric flask, add 5 mL of glacial acetic acid, dilute with water to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in 0.05 M sodium metabisulfite to obtain a solution having a known concentration of 0.15 mg per mL. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with 0.2 M acetic acid to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Inhalation Solution, equivalent to about 1.5 mg of isoproterenol hydrochloride, and 10 mL of *Internal standard solution* to a 25-mL volumetric flask, add dilute glacial acetic acid (1 in 100) to volume, and mix.

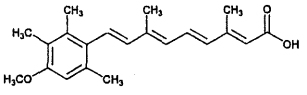
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 40-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.5 for isoproterenol hydrochloride and 1.0 for acetaminophen; the resolution, *R*, between isoproterenol hydrochloride and acetaminophen is not less than 6; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of isoproterenol hydrochloride ($C_{11}H_{17}NO_3 \cdot HCl$) in each mL of the Inhalation Solution taken by the formula:

$$(25C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation; V* is the volume, in mL, of Inhalation Solution taken; and R_U and R_S are the ratios of the peak responses of isoproterenol hydrochloride to those of acetaminophen obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Acitretin



$C_{21}H_{26}O_3$ 326.43

2,4,6,8-Nonatetraenoic acid, 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-, (*all-E*)-;
(*all-E*)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid [55079-83-9].

DEFINITION

Acitretin contains NLT 98.0% and NMT 102.0% of $C_{21}H_{26}O_3$, calculated on the dried basis.

[**CAUTION**—Acitretin is a teratogen. Great care should be taken when handling to avoid inhalation of dust or contact with skin.]

[NOTE—Use low-actinic glassware and perform all tests under yellow and subdued light.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Store the solutions at 4° before injection.]

Mobile phase: Alcohol, glacial acetic acid, and water (92:0.3:8)

System suitability stock solution: 0.01 mg/mL each of USP Acitretin RS and USP Tretinoin RS in alcohol. [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]

System suitability solution: 0.25 µg/mL each of USP Acitretin RS and USP Tretinoin RS in alcohol, from *System suitability stock solution*

Standard solution: 0.1 mg/mL of USP Acitretin RS in alcohol. [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol. The final concentration of tetrahydrofuran in the preparation will be 2%.]

Sample stock solution: 0.25 mg/mL of Acitretin in tetrahydrofuran and alcohol (1:19). [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]

Sample solution: 0.1 mg/mL of Acitretin in alcohol, from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 360 nm

Column: 4-mm × 25-cm; packing L1

Flow rate: 0.6 mL/min

Injection size: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for tretinoin and acitretin are 0.84 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between tretinoin and acitretin, *System suitability solution*

Relative standard deviation: NMT 1.0% of acitretin, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{21}H_{26}O_3$ in the portion of Acitretin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Acitretin RS in the *Standard solution* (mg/mL)

C_U = concentration of Acitretin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

[NOTE—Store the solutions at 4° before injection.]

USP Monographs

www.webofpharma.com

• **Procedure**
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.8 µg/mL each of USP Acitretin RS, USP Acitretin Related Compound A RS, and USP Acitretin Related Compound B RS in alcohol. [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]
Sample solution: 0.25 mg/mL of Acitretin in tetrahydrofuran and alcohol (1:19). [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]
System suitability
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between acitretin related compound A and acitretin; NLT 1.5 between acitretin related compound B and acitretin
Relative standard deviation: NMT 10.0% for acitretin related compound A and NMT 10.0% for acitretin related compound B
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acitretin related compound A and acitretin related compound B in the portion of Acitretin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the relevant impurity from the *Sample solution*
r_S = peak response from the relevant impurity from the *Standard solution*
C_S = concentration of USP Acitretin Related Compound A RS or USP Acitretin Related Compound B RS in the *Standard solution* (µg/mL)
C_U = concentration of Acitretin in the *Sample solution* (µg/mL)

Calculate the percentage of impurities other than acitretin related compounds A and B in the portion of Acitretin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual unspecified impurity from the *Sample solution*
r_S = peak response of USP Acitretin RS in the *Standard solution*
C_S = concentration of USP Acitretin RS in the *Standard solution* (µg/mL)
C_U = concentration of Acitretin in the *Sample solution* (µg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acitretin related compound A	0.78	0.3
Acitretin	1.0	—
Acitretin related compound B	1.61	0.3
Any unspecified impurity	—	0.1
Total unspecified impurities	—	0.4

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩: Dry a sample in a vacuum at a pressure not exceeding 19 mm of mercury at 100° for 4 h: it loses NMT 0.2% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acitretin RS
USP Acitretin Related Compound A RS
(2*Z*,4*E*,6*E*,8*E*)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid.
$C_{21}H_{26}O_3$ 326.43
USP Acitretin Related Compound B RS
Ethyl (all-*E*)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoate.
$C_{23}H_{30}O_3$ 354.48
USP Tretinoin RS

Acitretin Capsules

DEFINITION
Acitretin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of acitretin ($C_{21}H_{26}O_3$).
[**CAUTION**—Acitretin is a teratogen. Great care should be taken when handling to avoid inhalation of dust or contact with skin.]
[NOTE—Use low-actinic glassware and perform all tests under yellow and subdued light. Make all injections within 1 h of the *Sample solution* preparation.]

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Diluent: Methanol and tetrahydrofuran (13:10)
Mobile phase: Methanol, alcohol, glacial acetic acid, and water (74: 5: 0.5: 21)
Standard solution: 0.1 mg/mL of USP Acitretin RS in a mixture of *Diluent* and water (23:2). Dissolve USP Acitretin RS in *Diluent* equivalent to 80% of the final volume, sonicate for 5 min, add water equivalent to 8% of the final volume, and dilute with *Diluent* to volume.
System suitability solution: Transfer 2 mL of the *Standard solution* to a clear 4-mL glass vial. After sealing the vial with a Teflon-lined silicone septum and cap, place the vial on its side in a light chamber, expose it to 400 foot-candles of fluorescent light for 5 min, and then completely wrap the vial with aluminum foil.
[NOTE—Exposure to the fluorescent light allows for the formation of two degradation products: acitretin related compound A and 6*Z*-isomer ((2*E*,4*E*,6*Z*,8*E*)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid).]
Sample solution: Nominally 0.1 mg/mL of acitretin in a mixture of *Diluent* and water (23:2). Open NLT 20 Capsules, composite the Capsule fill, and mix well. Transfer the Capsule fill to a volumetric flask, add water equivalent to 8% of the final volume to wet the sample, and sonicate for 5 min. Dilute with *Diluent* to volume, and sonicate for 5 min. Cool to room temperature, pass the suspension

www.webofpharma.com

through a suitable filter of 0.5-µm pore size, and use the clear filtrate. [NOTE—Inject the *Sample solution* within 1 h of preparation.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 365 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for acitretin related compound A (2*Z*-isomer), acitretin, and the 6*Z*-isomer are 0.84, 1.0, and 1.09, respectively.]
Suitability requirements
Resolution: NLT 3.0 between acitretin related compound A and acitretin; NLT 1.8 between the 6*Z*-isomer and acitretin, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acitretin ($C_{21}H_{26}O_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acitretin from the *Sample solution*
r_S = peak response of acitretin from the *Standard solution*
C_S = concentration of USP Acitretin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acitretin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1
Medium: 3% sodium lauryl sulfate in deaerated water, pH 9.6–10.0; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Determine the amount of acitretin ($C_{21}H_{26}O_3$) dissolved using the following method.
Standard solution: Transfer about 14 mg of USP Acitretin RS to a 500-mL volumetric flask. Dissolve in 50 mL of alcohol, and dilute with *Medium* to volume.
For Capsules labeled to contain 10 mg: Transfer 20 mL of this solution to a 50-mL volumetric flask, and dilute with *Medium* to volume.
Sample solution: Use portions of the solution under test passed through a suitable filter of 0.45-µm pore size.
Capsule shell solution: Dissolve 6 clean empty-shell Capsules in 900 mL of *Medium*.
Instrumental conditions
Mode: UV
Analytical wavelength: 347 nm
Cell: 2 mm
Blank: *Medium*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Capsule shell solution*
Calculate the percentage of the labeled amount of acitretin ($C_{21}H_{26}O_3$) dissolved:

$$\text{Result} = [(A_U - A_{CS})/A_S] \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_{CS} = Capsule shell correction, calculated as shown below
A_S = absorbance of the *Standard solution*
C_S = concentration of the appropriate *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

The Capsule shell correction, A_{CS}, is calculated:

$$A_{CS} = A_{CSS}/N$$

A_{CSS} = absorbance of the *Capsule shell solution*
N = number of Capsule shells used to prepare the *Capsule shell solution*

Tolerances: NLT 85% (*Q*) of the labeled amount of acitretin ($C_{21}H_{26}O_3$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Tier 1
Medium: 3% sodium lauryl sulfate in deaerated water, pH 9.6–10.0 (adjusted with 1 N sodium hydroxide); 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Tier 2
Medium A: Prepare a solution containing pancreatin with NMT 2000 USP Units/L of protease activity in deaerated water, pH 8.0 (adjusted with 1% sodium hydroxide); 450 mL. Use immediately.
Medium B: 6% sodium lauryl sulfate in deaerated water, pH 10.5 (adjusted with 1% sodium hydroxide); 450 mL
Apparatus 1: 100 rpm
Time: 15 min, *Medium A*; 15 min, *Medium A* with the addition of *Medium B*
Determine the amount of acitretin ($C_{21}H_{26}O_3$) dissolved using the following method.
Mobile phase: Methanol, water, and glacial acetic acid (750:250:1)
Standard stock solution: 280 µg/mL of USP Acitretin RS in absolute alcohol. Use sonication to dissolve.
Standard solution: 20 µg/mL of USP Acitretin RS in *Medium* under *Tier 1*, from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable glass filter with 1-µm pore size, discard the first few mL, and use the filtrate for analysis.
Dissolution procedure: Perform the test using the conditions under *Tier 1*. In the presence of cross-linking repeat the test with new Capsules using the conditions under *Tier 2* as follows. After 15 min, stop the dissolution bath and timer (do not lift the baskets), and add 450 mL of *Medium B* pre-equilibrated at 37 ± 0.5°. Restart the timer and bath, and after 5 min check the pH of the medium and adjust with 1% sodium hydroxide to a range of 9.6–10.0. Continue dissolution for an additional 10 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 360 nm
Columns
Guard: 4-mm × 1-cm; 5-µm packing L1
Analytical: 4.6-mm × 5-cm; 5-µm packing L1
Temperatures
Autosampler: 40°

www.webofpharma.com

Column: 35°
Flow rate: 2.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acitretin ($C_{21}H_{26}O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of acitretin from the *Sample solution*
r_S = peak response of acitretin from the *Standard solution*
C_S = concentration of USP Acitretin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of acitretin ($C_{21}H_{26}O_3$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities: Limit of Degradation Products**
Diluent, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acitretin related compound A (2*Z*-isomer)[a]	0.84	0.5
Acitretin	1.0	—
Any unspecified impurity	—	0.4
Total unspecified impurities	—	0.8

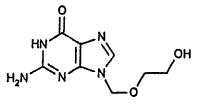
[a] (2*Z*,4*E*,6*E*,8*E*)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid ($C_{21}H_{26}O_3$ 326.43).

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
• **Labeling:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

• **USP Reference Standards** ⟨11⟩
USP Acitretin RS

Acyclovir



$C_8H_{11}N_5O_3$ 225.20
6*H*-Purin-6-one, 2-amino-1,9-dihydro-9-[(2-hydroxyeth oxy) methyl]-.
9-[(2-Hydroxyethoxy)methyl]guanine [59277-89-3].

» Acyclovir contains not less than 98.0 percent and not more than 101.0 percent of $C_8H_{11}N_5O_3$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers. Store at room temperature. Protect from light and moisture.
USP Reference standards ⟨11⟩—
USP Acyclovir RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay and limit for guanine.*
Water Determination, *Method I* ⟨921⟩**:** not more than 6.0%.

Ordinary impurities ⟨466⟩—
Test solution: dimethyl sulfoxide.
Standard solution: dimethyl sulfoxide.
Eluant: a mixture of chloroform, methanol, and ammonium hydroxide (80:20:2).
Visualization: 1.
Application volume: 5 µL.
Limit: 1%.

Assay and limit for guanine—
Mobile phase—Prepare a filtered and degassed solution of glacial acetic acid in water (1 in 1000). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution 1—Dissolve accurately weighed quantities of USP Acyclovir RS and guanine in 0.1 N sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having known concentrations of about 0.1 mg of each per mL.
System suitability solution 2—Dissolve an accurately weighed quantity of guanine in 0.1 N sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.7 µg per mL.
Guanine standard preparation—Transfer about 8.75 mg of guanine, accurately weighed, to a 500-mL volumetric flask. Dissolve in 50 mL of 0.1 N sodium hydroxide, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.01 N sodium hydroxide to

www.webofpharma.com

volume, and mix to obtain a solution having a concentration of about 0.7 µg per mL.

Standard preparation—Dissolve about 25 mg of USP Acyclovir RS, accurately weighed, in 5 mL of 0.1 N sodium hydroxide in a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.01 N sodium hydroxide to volume, and mix to obtain a solution having a known concentration of about 0.1 mg of USP Acyclovir RS per mL.

Assay preparation—Dissolve about 100 mg of Acyclovir, accurately weighed, in 20 mL of 0.1 N sodium hydroxide in a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.01 N sodium hydroxide to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 3 mL per minute. Chromatograph *System suitability solution 1,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between acyclovir and guanine is not less than 2.0; the tailing factor for the analyte peak is not more than 2; and the relative standard deviation for replicate injections for the acyclovir peak is not more than 2.0%. Chromatograph *System suitability solution 2,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation,* the *Guanine standard preparation,* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in µg, of guanine in the portion of Acyclovir taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of guanine in the *Guanine standard preparation;* and r_U and r_S are the peak responses due to guanine in the *Assay preparation* and the *Guanine standard preparation,* respectively: not more than 0.7% of guanine is found. Calculate the quantity, in mg, of $C_8H_{11}N_5O_3$ in the portion of Acyclovir taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Acyclovir RS in the *Standard preparation;* and r_U and r_S are the peak responses due to acyclovir in the *Assay preparation* and the *Standard preparation,* respectively.

Acyclovir Capsules

DEFINITION

Acyclovir Capsules contain NLT 93.0% and NMT 107.0% of the labeled amount of acyclovir ($C_8H_{11}N_5O_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Mobile phase: 0.02 M acetic acid

System suitability solution A: 0.1 mg/mL each of USP Acyclovir RS and guanine. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

System suitability solution B: 2.0 µg/mL of guanine. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

Standard solution: 0.1 mg/mL of USP Acyclovir RS. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

Sample solution: Nominally 0.1 mg/mL of acyclovir prepared as follows. Transfer the contents of Capsules equivalent to 10 mg of acyclovir (NLT 10 Capsules) to a 100-mL volumetric flask. Dissolve in 10 mL of 0.1 N sodium hydroxide, dilute to volume with water, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 4.2-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution A* and *System suitability solution B*

[NOTE—The relative retention times for guanine and acyclovir are about 0.6 and 1.0, respectively, in *System suitability solution A.*]

Suitability requirements

Resolution: NLT 2.0 between guanine and acyclovir, *System suitability solution A*

Relative standard deviation: NMT 2.0% for the acyclovir peak, *System suitability solution A*

Relative standard deviation: NMT 2.0%, *System suitability solution B*

Analysis: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acyclovir ($C_8H_{11}N_5O_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*

r_S = peak response of the *Standard solution*

C_S = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Detector: UV 254 nm

Standard solution: USP Acyclovir RS in *Medium*

Sample solutions: Dilute with *Medium* to a concentration that is similar to the *Standard solution.*

Analysis: Determine the amount of acyclovir ($C_8H_{11}N_5O_3$) dissolved from UV absorption at the wavelength of maximum absorption on filtered portions of the solution under test.

Tolerances: NLT 75% (*Q*) of the labeled amount of acyclovir ($C_8H_{11}N_5O_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements for *Content Uniformity*

IMPURITIES

- **PROCEDURE**

Mobile phase, System suitability solution A, System suitability solution B, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay.*

Analysis: *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of the responses for all of the peaks

Acceptance criteria
Guanine: NMT 2.0%
Any individual impurity: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25°. Protect from light and moisture.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Acyclovir RS

Acyclovir for Injection

DEFINITION
Acyclovir for Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of acyclovir ($C_8H_{11}N_5O_3$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: 0.02 M acetic acid
System suitability solution A: 0.1 mg/mL each of USP Acyclovir RS and guanine in 0.1 N sodium hydroxide
System suitability solution B: 2.0 µg/mL of guanine in 0.1 N sodium hydroxide
Standard solution: 0.1 mg/mL of USP Acyclovir RS in 0.1 N sodium hydroxide
Sample solution: Nominally 0.1 mg/mL of acyclovir prepared as follows. Constitute 1 vial of Acyclovir for Injection with water. Transfer an amount, equivalent to 10 mg of acyclovir, to a 100-mL volumetric flask, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.2-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution A* and *System suitability solution B*
[NOTE—The relative retention times for guanine and acyclovir are 0.6 and 1.0, respectively, in *System suitability solution A*.]
Suitability requirements
Resolution: NLT 2.0 between guanine and acyclovir, *System suitability solution A*
Relative standard deviation: NMT 2.0% for the acyclovir peak, *System suitability solution A*
Relative standard deviation: NMT 2.0%, *System suitability solution B*
Analysis
Calculate the percentage of acyclovir ($C_8H_{11}N_5O_3$) in the portion of Acyclovir for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
- **PROCEDURE**

Solution A: 0.17 M acetic acid and methanol (125:8)
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
45	65	35
46	100	0
56	100	0

System suitability solution: 0.5 µg/mL each of purine and USP Acyclovir RS in *Solution A*
Acyclovir standard solution: 5 µg/mL of USP Acyclovir RS in *Solution A*
Guanine solution: 0.05 mg/mL of guanine prepared as follows. Dissolve 25 mg of guanine in 50 mL of 0.1 N sodium hydroxide in a 500-mL volumetric flask, and bring the solution to volume with water.
Standard solution A: 0.5 µg/mL of *Acyclovir standard solution* in *Solution A*
Standard solution B: 5 µg/mL of *Guanine solution* in *Solution A*
Sample solution: Equivalent to 0.5 mg/mL of acyclovir from a mixture of NLT 10 reconstituted vials of Acyclovir for Injection in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution*, *Standard solution A*, and *Standard solution B*
[NOTE—Typical retention times for guanine and acyclovir of *Standard solution A* and *Standard solution B* are 5.8 and 14 min, respectively.]
Suitability requirements
Resolution: NLT 2.0 between purine and acyclovir, *System suitability solution*
Relative standard deviation: NMT 1% for the acyclovir and the guanine peaks, *Standard solution A* and *Standard solution B*
Analysis 1
Calculate the percentage of guanine in the Acyclovir for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for guanine, if present, in the *Sample solution*

USP Monographs

www.webofpharma.com

r_S = peak response of guanine in the *Standard solution*
C_S = concentration of guanine in the *Standard solution* (mg/mL)
C_U = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

Acceptance criteria 1: NMT 1.0% guanine

Analysis 2

Calculate the percentage of each other impurity in the portion of Acyclovir for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity
r_S = peak response of acyclovir in the *Standard solution*
C_S = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

Acceptance criteria 2: NMT 0.15% for any peak having a relative retention time of about 0.7 compared to the acyclovir peak; NMT 0.5% for any other individual impurity; and NMT 1.0% for the total of all other impurities

SPECIFIC TESTS

- **pH** ⟨791⟩: 11.0–12.5, 50 mg/mL of acyclovir
- **Water Determination,** *Method I* ⟨921⟩: NMT 5.5%
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 0.174 USP Endotoxin Unit/mg of acyclovir
- **Other Requirements:** Meets the requirements for labeling in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store between 15° and 25°. Protect from light.
- **USP Reference Standards** ⟨11⟩
  USP Acyclovir RS

Acyclovir Ointment

DEFINITION

Acyclovir Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of acyclovir ($C_8H_{11}N_5O_3$), in a suitable ointment base.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Mobile phase: 0.02 M acetic acid
System suitability solution A: 0.1 mg/mL each of USP Acyclovir RS and guanine in 0.1 N sodium hydroxide
System suitability solution B: 2.0 µg/mL of guanine in 0.1 N sodium hydroxide
Standard solution: 0.1 mg/mL of USP Acyclovir RS in 0.1 N sodium hydroxide
Sample solution: Nominally 0.1 mg/mL of acyclovir prepared as follows. Transfer an amount of Ointment, equivalent to 10 mg of acyclovir, to a 100-mL volumetric flask. Dissolve in and dilute with 0.1 N sodium hydroxide to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 3 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution A* and *System suitability solution B*
[Note—The relative retention times for guanine and acyclovir are about 0.6 and 1.0, respectively, in *System suitability solution A*.]
Suitability requirements
Resolution: NLT 2.0 between guanine and acyclovir, *System suitability solution A*
Relative standard deviation: NMT 2.0% for the acyclovir peak, *System suitability solution A*; NMT 2.0%, *System suitability solution B*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acyclovir ($C_8H_{11}N_5O_3$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Minimum Fill** ⟨755⟩: Meets the requirements

IMPURITIES

- **Limit of Guanine**

Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 2.0 µg/mL of guanine in 0.1 M sodium hydroxide
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of guanine in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of guanine from the *Sample solution*
r_S = peak response of guanine from the *Standard solution*
C_S = concentration of guanine in the *Standard solution* (mg/mL)
C_U = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.0%

SPECIFIC TESTS

- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25° in a dry place.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Acyclovir RS

Acyclovir Oral Suspension

DEFINITION
Acyclovir Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of acyclovir ($C_8H_{11}N_5O_3$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: 0.02 M acetic acid
System suitability solution A: 0.1 mg/mL each of USP Acyclovir RS and guanine in 0.1 N sodium hydroxide
System suitability solution B: 2.0 µg/mL of guanine in 0.1 N sodium hydroxide
Standard solution: 0.1 mg/mL of USP Acyclovir RS in 0.1 N sodium hydroxide
Sample stock solution: Nominally 1 mg/mL of acyclovir prepared as follows. Transfer an amount of well-shaken Oral Suspension equivalent to 200 mg of acyclovir to a 200-mL volumetric flask. Add 100 mL of 0.1 N sodium hydroxide, shake by mechanical means for 15 min, and sonicate, if necessary, to dissolve the Oral Suspension completely. Dilute with 0.1 N sodium hydroxide to volume.
Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*).
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 3 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution A* and *System suitability solution B*
[NOTE—The relative retention times for guanine and acyclovir are about 0.6 and 1.0, respectively, in *System suitability solution A*.]
Suitability requirements
Resolution: NLT 2.0 between guanine and acyclovir, *System suitability solution A*
Relative standard deviation: NMT 2.0% for replicate injections for the acyclovir peak, *System suitability solution A*
Relative standard deviation: NMT 2.0% for replicate injections, *System suitability solution B*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acyclovir ($C_8H_{11}N_5O_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
- **LIMIT OF GUANINE**

Mobile phase, System suitability solution A, System suitability solution B, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Standard solution: 2.0 µg/mL of guanine in 0.1 M sodium hydroxide
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of guanine in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for guanine from the *Sample solution*
r_S = peak response for guanine from the *Standard solution*
C_S = concentration of guanine in the *Standard solution* (mg/mL)
C_U = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.0%

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** Its total count does not exceed 10^1 cfu/mL, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.
- **PH** ⟨791⟩**:** 4.5–7.0

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements for Oral Suspension packaged in single-unit containers
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements for Oral Suspension packaged in multiple-unit containers

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25°. Protect from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Acyclovir RS

Acyclovir Tablets

DEFINITION
Acyclovir Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acyclovir ($C_8H_{11}N_5O_3$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: 0.02 M acetic acid

USP Monographs

www.webofpharma.com

System suitability solution A: 0.1 mg/mL each of USP Acyclovir RS and guanine. Dissolve in 0.1 N sodium hydroxide, and dilute with water.
System suitability solution B: 2.0 µg/mL of guanine. Dissolve in 0.1 N sodium hydroxide, and dilute with water.
Standard solution: 0.1 mg/mL of USP Acyclovir RS. Dissolve in 0.1 N sodium hydroxide, and dilute with water.
Sample solution: Nominally 0.1 mg/mL of acyclovir prepared as follows. Transfer an amount of finely powdered Tablets equivalent to 10 mg of acyclovir (NLT 10 Tablets) to a 100-mL volumetric flask. Dissolve in 10 mL of 0.1 N sodium hydroxide, dilute with water to volume, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution A* and *System suitability solution B*
[NOTE—The relative retention times for guanine and acyclovir are about 0.6 and 1.0, respectively, in *System suitability solution A*.]
Suitability requirements
Resolution: NLT 2.0 between guanine and acyclovir, *System suitability solution A*
Relative standard deviation: NMT 2.0% for the acyclovir peak, *System suitability solution A*; NMT 2.0%, *System suitability solution B*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acyclovir ($C_8H_{11}N_5O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Instrumental conditions
Mode: UV
Wavelength: 254 nm
Standard solution: USP Acyclovir RS in *Medium*
Sample solutions: Dilute with *Medium* to a concentration that is similar to the *Standard solution*.
Analysis: Determine the amount of acyclovir ($C_8H_{11}N_5O_3$) dissolved from UV absorption at the wavelength of maximum absorbance on filtered portions of the solution under test.
Tolerances: NLT 80% (Q) of the labeled amount of acyclovir ($C_8H_{11}N_5O_3$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements for *Weight Variation*

IMPURITIES
• **PROCEDURE**
Mobile phase, System suitability solution A, System suitability solution B, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

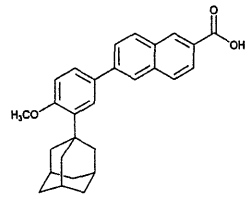
$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of the responses for all of the peaks

Acceptance criteria
Guanine: NMT 2.0%
Any other impurity: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25°. Protect from light and moisture.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acyclovir RS

Adapalene



$C_{28}H_{28}O_3$ 412.52
2-Naphthalenecarboxylic acid, 6-(4-methoxy-3-tricyclo [3.3.1.1^{3,7}]dec-1-ylphenyl)-;
6-[3-(1-Adamantyl)-4-methoxyphenyl]-2-naphthoic acid. [106685-40-9].

DEFINITION
Adapalene contains NLT 98.0% and NMT 102.0% of adapalene ($C_{28}H_{28}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, tetrahydrofuran, trifluoroacetic acid, and water (21:16:0.01:13)
Standard stock solution: 0.2 mg/mL of USP Adapalene RS in *Mobile phase*. Dissolve USP Adapalene RS in a minimal amount of tetrahydrofuran (about 1%–5% of the final volume), using sonication as needed, and dilute with *Mobile phase* to volume.

www.webofpharma.com

Standard solution: 40 µg/mL of USP Adapalene RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: 0.2 mg/mL of Adapalene in *Mobile phase*. Dissolve Adapalene in a minimal amount of tetrahydrofuran (about 1%–5% of the final volume), using sonication as needed, and dilute with *Mobile phase* to volume.
Sample solution: 40 µg/mL of Adapalene in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of adapalene ($C_{28}H_{28}O_3$) in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Adapalene RS in the *Standard solution* (µg/mL)
C_U = concentration of Adapalene in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.20%

[Note—On the basis of the synthetic route, perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*.]

• **Organic Impurities, Procedure 1**

Procedure 1 is recommended if adapalene related compounds A and B may be present.
Mobile phase: Proceed as directed in the *Assay*.
Standard stock solution: 0.2 mg/mL of USP Adapalene RS, 0.3 mg/mL of USP Adapalene Related Compound A RS, and 0.2 mg/mL of USP Adapalene Related Compound B RS in *Mobile phase*. Dissolve USP Adapalene RS, USP Adapalene Related Compound A RS, and USP Adapalene Related Compound B RS in a minimal amount of tetrahydrofuran (about 1%–5% of the final volume), using sonication as needed, and dilute with *Mobile phase* to volume.
Standard solution: 0.2 µg/mL of USP Adapalene RS, 0.3 µg/mL of USP Adapalene Related Compound A RS, and 0.2 µg/mL of USP Adapalene Related Compound B RS in *Mobile phase* from the *Standard stock solution*
Sample solution: 0.2 mg/mL of Adapalene in *Mobile phase*. Dissolve Adapalene in a minimal amount of tetrahydrofuran (about 1%–5% of the final volume), using sonication as needed, and dilute with *Mobile phase* to volume.
Chromatographic system: Proceed as directed in the *Assay*, except use a run time of NLT two times the retention time of adapalene peak for *Standard solution* and NLT six times the retention time of adapalene peak for *Sample solution*.
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0% for the adapalene peak
Column efficiency: NLT 3000 theoretical plates for the adapalene peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of adapalene related compounds A and B in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of corresponding adapalene related compound A or adapalene related compound B from the *Standard solution*
C_S = concentration of corresponding USP Adapalene Related Compound A RS or USP Adapalene Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Adapalene in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each unspecified impurity from the *Sample solution*
r_S = peak area of adapalene from the *Standard solution*
C_S = concentration of USP Adapalene RS in the *Standard solution* (mg/mL)
C_U = concentration of Adapalene in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Adapalene related compound A[a]	0.52	0.10
Adapalene	1.0	—
Adapalene related compound B[b]	1.57	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	0.50

[a] Methyl 6-bromo-2-naphthoate.
[b] Methyl 6-[3-(1-Adamantyl)-4-methoxyphenyl]-2-naphthoate.

• **Organic Impurities, Procedure 2**

Procedure 2 is recommended if adapalene related compounds E, C, and D may be present.
Solution A: Glacial acetic acid and water (0.1:100)
Solution B: Acetonitrile and tetrahydrofuran (65:35)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	50	50
2.5	50	50
40	28	72

Supplement 3

www.webofpharma.com

Table 2 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
42	28	72
42.1	50	50
50	50	50

Diluent: Acetonitrile, tetrahydrofuran, and water (37:20:43)
Standard stock solution: 0.2 mg/mL of USP Adapalene RS in tetrahydrofuran
Standard solution: 2.0 µg/mL of USP Adapalene RS in *Diluent* from the *Standard stock solution*
System suitability solution: 0.2 mg/mL of USP Adapalene RS and 1.2 µg/mL each of USP Adapalene Related Compound C RS, USP Adapalene Related Compound D RS, and USP Adapalene Related Compound E RS prepared by dissolving the standards in tetrahydrofuran equivalent to 50% of the final volume, and diluting with *Diluent* to volume
Sample solution: 2.0 mg/mL of Adapalene prepared by dissolving in tetrahydrofuran equivalent to 50% of the final volume, and diluting with *Diluent* to volume
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11 with 7.5% carbon loading
Column temperature: 30°
Flow rate: 1.2 mL/min
Injection volume: 25 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 4.5 between the adapalene and adapalene related compound C peaks
Signal-to-noise ratio: NLT 10 for the adapalene related compound C peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of adapalene from the *Standard solution*
C_S = concentration of adapalene in the *Standard solution* (mg/mL)
C_U = concentration of Adapalene in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any impurity peaks less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Adapalene related compound E[a]	0.3	1.4	0.3
Hydroxyadapalene[b]	0.5	0.91	0.1

Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Adapalene related compound C[c]	0.9	0.14	0.1
Adapalene	1.0	—	—
Adapalene related compound D[d]	1.9	0.71	0.2
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] 2,2′-Binaphthyl-6,6′-dicarboxylic acid.
[b] 6-[3-(3-Hydroxyadamant-1-yl)-4-methoxyphenyl]-2-naphthoic acid.
[c] 2-(Adamant-1-yl)methoxybenzene.
[d] 4,4′-Dimethoxy-3,3′-di(adamant-1-yl)biphenyl.

• **RESIDUAL SOLVENT: LIMIT OF TRIETHYLAMINE**
[NOTE—This test should be performed if triethylamine is used in the manufacturing process.]
Diluent: Dimethyl sulfoxide
Standard solution: 4.0 µg/mL of USP Triethylamine RS in *Diluent*. Transfer 4.0 mL of this solution to a 20-mL headspace vial, and add 1.0 mL of 1 N NaOH solution.
Sample solution: 50 mg/mL of Adapalene in *Diluent*. Transfer 4.0 mL of this solution to a 20-mL headspace vial, and add 1.0 mL of 1 N NaOH solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 30-m × 0.53-mm; 3.0-µm coating of G27
Temperatures
Injection port: 250°
Detector: 300°
Column: See *Table 4*.

Table 4

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	5
40	40	240	5

Headspace operating parameters
[NOTE—Headspace operating parameters can be modified in order to optimize the performance.]
Equilibration temperature: 95°
Equilibration time: 15 min
Transfer line temperature: 125°
Pressurization time: 3 min
Carrier gas: Nitrogen
Flow rate: 4.8 mL/min
Injection volume: 1 mL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 15%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the content, in ppm, of triethylamine in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 10^6$$

USP Monographs

www.webofpharma.com

r_U = peak response of triethylamine from the *Sample solution*
r_S = peak response of triethylamine from the *Standard solution*
C_S = concentration of triethylamine in the *Standard solution* (mg/mL)
C_U = concentration of Adapalene in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 80 ppm

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 4 h.
Acceptance criteria: NMT 0.6%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.
• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Adapalene RS
USP Adapalene Related Compound A RS
Methyl 6-bromo-2-naphthoate.
$C_{12}H_9BrO_2$ 265.10
USP Adapalene Related Compound B RS
Methyl 6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoate.
$C_{29}H_{30}O_3$ 426.55
USP Adapalene Related Compound C RS
2-(Adamant-1-yl)methoxybenzene.
$C_{17}H_{22}O$ 242.36
USP Adapalene Related Compound D RS
4,4′-Dimethoxy-3,3′-di(adamant-1-yl)biphenyl.
$C_{34}H_{42}O_2$ 482.70
USP Adapalene Related Compound E RS
2,2′-Binaphthyl-6,6′-dicarboxylic acid.
$C_{22}H_{14}O_4$ 342.34
USP Triethylamine RS
Triethylamine.
$C_6H_{15}N$ 101.19

Adapalene Gel

DEFINITION
Adapalene Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of adapalene ($C_{28}H_{28}O_3$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Diluent: Use *Mobile phase* in the *Assay*.
Sample stock solution: Use *Sample stock solution* in the *Assay*.
Sample solution: Nominally equivalent to 0.4 µg/mL of adapalene, prepared as follows. Dilute 2.0 mL of *Sample stock solution* with *Diluent* to 100.0 mL. Pass a portion through a Teflon filter of 0.45-µm pore size and use the filtrate.
Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, tetrahydrofuran, trifluoroacetic acid, and water (43: 36: 0.02: 21)
Standard stock solution: 0.25 mg/mL of USP Adapalene RS, prepared as follows. Transfer USP Adapalene RS to a suitable volumetric flask, add tetrahydrofuran equivalent to 1% of the final volume, and sonicate to dissolve. Dilute with *Mobile phase* to volume.
Standard solution: 20 µg/mL of USP Adapalene RS in *Mobile phase*, from *Standard stock solution*
Sample stock solution: Nominally equivalent to 20 µg/mL of adapalene, prepared as follows. Transfer 2.0 g of Gel to a 100-mL volumetric flask, add 25 mL of tetrahydrofuran, and sonicate to dissolve. Add 25 mL of acetonitrile and sonicate for 20 min. Cool to room temperature and dilute with *Mobile phase* to volume.
Sample solution: Pass a portion of *Sample stock solution* through a Teflon filter of 0.45-µm pore size and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of adapalene ($C_{28}H_{28}O_3$) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Adapalene RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of adapalene in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **ORGANIC IMPURITIES**
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.
Solution A: Use *Mobile phase* in the *Assay*.
Solution B: *Buffer* and *Solution A* (50:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	0	100
4	0	100
30	55	45
65	55	45

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
68	0	100
80	0	100

Diluent: Acetonitrile and tetrahydrofuran (3:2)
System suitability stock solution: 0.5 mg/mL of USP Adapalene RS, prepared as follows. Transfer USP Adapalene RS to a suitable volumetric flask, add tetrahydrofuran equivalent to 40% of the final volume, and sonicate to dissolve. Dilute with acetonitrile to volume.
System suitability solution: 0.2 mg/mL of USP Adapalene RS in *Diluent*, from *System suitability stock solution*
Standard solution: 1.0 µg/mL of USP Adapalene RS in *Diluent*, from *System suitability solution*
Sample solution: Nominally equivalent to 0.2 mg/mL of adapalene, prepared as follows. Transfer 5.0 g of Gel to a 25-mL volumetric flask. Add 10 mL of tetrahydrofuran and sonicate to disperse for 10 min. Add 10 mL of acetonitrile and sonicate for 10 min. Cool to room temperature and dilute with acetonitrile to volume. Pass a portion through a Teflon filter of 0.45-µm pore size and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of adapalene from the *Standard solution*
C_S = concentration of USP Adapalene RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of adapalene in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.1%.

Table 2

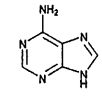
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Adapalene related compound A[a,b]	0.5	—
Adapalene	1.0	—
Adapalene related compound B[b,c]	1.3	—
Any unspecified impurity	—	0.2
Total impurities	—	1.0

[a] Methyl 6-bromo-2-naphthoate.
[b] This process impurity is controlled in the drug substance monograph. It is included in the table for identification only and it is not to be reported in the total impurities.
[c] Methyl 6-[3-(adamant-1-yl)-4-methoxyphenyl]-2-naphthoate.

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.0–6.0
- **Minimum Fill** ⟨755⟩: Meets the requirements
- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total aerobic microbial count is NMT 10^2 cfu/g. The total yeasts and molds count is NMT 10^1 cfu/g. It meets the requirements of the tests for the absence of *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* species.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature and protect from freezing.
- **USP Reference Standards** ⟨11⟩
  USP Adapalene RS

Adenine



$C_5H_5N_5$ 135.13
9H-Purin-6-amine;
1,6-Dihydro-6-iminopurine [73-24-5].

DEFINITION
Adenine contains NLT 98.0% and NMT 102.0% of adenine ($C_5H_5N_5$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**

Buffer solution: Dissolve 6.90 g of monobasic ammonium phosphate in about 800 mL of water. Adjust with ammonium hydroxide to a pH of 6.2, and dilute with water to 1 L.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer Solution (%)	Acetonitrile (%)	Water (%)
0	5	5	90

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Buffer Solution (%)	Acetonitrile (%)	Water (%)
20	5	5	90
20.1	10	10	80
30	10	10	80
30.1	5	5	90
40	5	5	90

System suitability solution: 50 µg/mL each of USP Adenine RS and 7-methyladenine in water
Standard solution: 0.1 mg/mL of USP Adenine RS in water. If necessary, sonicate the solution at 30° until the substance is completely dissolved.
Sample solution: 0.1 mg/mL of Adenine in water. If necessary, sonicate the solution at 30° until the substance is completely dissolved.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 25-cm; 5-µm packing L85
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for 7-methyladenine and adenine are 0.88 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the 7-methyladenine and adenine peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of adenine ($C_5H_5N_5$) in the portion of Adenine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Adenine RS in the *Standard solution* (mg/mL)
C_U = concentration of Adenine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **RELATED COMPOUNDS**

Buffer solution, Mobile phase, System suitability solution, Standard solution, and **System suitability:** Proceed as directed in the *Assay.*
Sample solution: Dissolve 25 mg of Adenine in approximately 15 mL of boiling water. Cool, quantitatively transfer to a 25-mL volumetric flask, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L85
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Adenine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

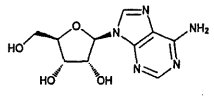
r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria
Individual impurity: NMT 0.1%
Total impurities: NMT 2.0%

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 110° for 4 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Adenine RS

Adenosine



$C_{10}H_{13}N_5O_4$ 267.24
6-Amino-9-β-D-ribofuranosyl-9*H*-purine;
9-β-D-Ribofuranosyladenine [58-61-7].

DEFINITION
Adenosine contains NLT 98.0% and NMT 102.0% of adenosine ($C_{10}H_{13}N_5O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **PROCEDURE**

Buffer: 6.8 g/L of potassium hydrogen sulfate and 3.4 g/L of tetrabutylammonium hydrogen sulfate in a solution prepared as follows. Transfer suitable quantities of potassium hydrogen sulfate and tetrabutyl ammonium hydrogen sulfate to an appropriate volumetric flask, and dissolve in 90% of the flask volume of water. Adjust with 2 N potassium hydroxide to a pH of 6.5, and dilute with water to volume.
Mobile phase: *Buffer* and water (60:40)
System suitability solution: 4 µg/mL each of USP Adenine RS and inosine in *Mobile phase*
Standard solution: 0.2 mg/mL of USP Adenosine RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Adenosine in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)

USP Monographs

www.webofpharma.com

Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of the adenosine peak

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between adenine and inosine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.7%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of adenosine ($C_{10}H_{13}N_5O_4$) in the portion of Adenosine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Adenosine RS in the *Standard solution* (mg/mL)
C_U = concentration of Adenosine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.001 mg/mL of USP Adenosine RS in *Mobile phase*
Sample solution: 1 mg/mL of Adenosine in *Mobile phase*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between adenine and inosine, *System suitability solution*
Relative standard deviation: NMT 5%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Adenosine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Adenosine RS in the *Standard solution* (mg/mL)
C_U = concentration of Adenosine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard peaks that are less than 0.05% of the adenosine peak.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Uridine[a]	0.29	0.73	0.10
Adenine	0.34	1.6	0.2
Inosine[b]	0.42	0.73	0.1
Guanosine[c]	0.51	0.86	0.10
Adenosine	1.0	—	—
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] 1-β-D-Ribofuranosylpyrimidine-2,4(1*H*,3*H*)-dione.
[b] 9-β-D-Ribofuranosylpurine-6(1*H*)-one.
[c] 2-Amino-9-β-D-ribofuranosylpurine-6(1*H*)-one.

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩: −68° to −72°

Test solution: 20 mg/mL in sodium hydroxide solution (1 in 20), determined on a sample previously dried at 105° for 2 h
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Adenine RS
USP Adenosine RS

Adenosine Injection

DEFINITION
Adenosine Injection is a sterile solution of Adenosine in Water for Injection. It may contain Sodium Chloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of adenosine ($C_{10}H_{13}N_5O_4$).

IDENTIFICATION
- The retention time of the adenosine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Dissolve 2.0 g of monobasic potassium phosphate in 800 mL of water. Add 5 mL of 1.0 M tetrabutylammonium dihydrogen phosphate, dilute with water to 980 mL, and mix. Add 20 mL of acetonitrile.
System suitability solution: 0.03 mg/mL each of USP Adenosine RS and inosine dissolved in warm water (50° to 55°), and diluted with water
Standard solution: 0.03 mg/mL of USP Adenosine RS dissolved in warm water (50° to 55°), and diluted with water to volume. Before addition of the warm water, if sodium chloride is present in the Injection, add 0.01 mL of a solution of sodium chloride (0.9 in 100) per mL of the anticipated final volume of the *Standard solution*.
Sample solution: Nominally 0.03 mg/mL of adenosine, from a suitable volume of Injection in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

USP Monographs

www.webofpharma.com

Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2.5 mL/min
Injection volume: 10 µL
Run time: 2.5 times the retention time of adenosine
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times of inosine and adenosine are 0.43 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6.0 between adenosine and inosine, *System suitability solution*
Tailing factor: NMT 2.0 for the adenosine peak, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of adenosine ($C_{10}H_{13}N_5O_4$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Adenosine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of adenosine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase, System suitability solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: Nominally 0.3 mg/mL of adenosine from a volume of Injection, in water
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the volume of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of the responses of all of the peaks

Acceptance criteria
Any individual impurity: NMT 1.0%
Total impurities: NMT 1.5%

SPECIFIC TESTS

• **PH** ⟨791⟩**:** 4.5–7.5
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** It meets the requirements for small-volume injections.
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** When the product is used for rapid intravenous injection, it contains NMT 11.62 USP Endotoxin Units/mg of adenosine. When the product is used for continuous peripheral intravenous infusion, it contains NMT 5.95 USP Endotoxin Units/mg of adenosine.
• **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, single-dose containers, preferably of Type I glass, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Adenosine RS

Medical Air

DEFINITION

Medical Air is a natural or synthetic mixture of gases consisting largely of nitrogen and oxygen. It contains NLT 19.5% and NMT 23.5%, by volume, of oxygen (O_2).

IDENTIFICATION

• **A.** The paramagnetic signal exhibited by the *Sample gas* in the *Assay* confirms the presence of oxygen.
• **B.** The *Sample gas* in the *Assay* meets the assay *Acceptance criteria*.

ASSAY

• **PROCEDURE**
The certified standards called for in the following test are listed in *Reagents, Indicators, and Solutions*.
Zero gas: Nitrogen certified standard
Span gas: 21% Oxygen certified standard. [NOTE—See *Reagents, Indicators, and Solutions*.]
Sample gas: Medical Air
Mode: Paramagnetic oxygen measurement (see *Medical Gases Assay* ⟨415⟩)
Analysis: Determine the concentration of oxygen in percentage by volume of Medical Air using a suitable paramagnetic analyzer.
Acceptance criteria: 19.5%–23.5% of oxygen by volume

IMPURITIES

See *Impurities Testing in Medical Gases Assay* ⟨413⟩. The detector tubes called for in the following tests are listed in *Reagents, Indicators, and Solutions*.
If the label indicates that Medical Air is a synthetic mixture of oxygen and nitrogen, and where oxygen complies to *Oxygen USP* and Nitrogen complies to *Nitrogen NF*, then the *Impurities* tests are not required.
• **LIMIT OF CARBON DIOXIDE**
Sample: Detector tube manufacturer's recommended volume ±5% of Medical Air
Analysis: Pass the *Sample* through a carbon dioxide detector tube at the rate specified for the tube by the detector tube manufacturer.
Acceptance criteria: NMT 500 ppm
• **LIMIT OF CARBON MONOXIDE**
Sample: Detector tube manufacturer's recommended volume ±5% of Medical Air
Analysis: Pass the *Sample* through a carbon monoxide detector tube at the rate specified for the tube by the detector tube manufacturer.
Acceptance criteria: NMT 10 ppm
• **LIMIT OF SULFUR DIOXIDE**
Sample: Detector tube manufacturer's recommended volume ±5% of Medical Air
Analysis: Pass the *Sample* through a sulfur dioxide detector tube at the rate specified for the tube by the detector tube manufacturer.
Acceptance criteria: NMT 5 ppm
• **LIMIT OF NITRIC OXIDE AND NITROGEN DIOXIDE**
Sample: Detector tube manufacturer's recommended volume ±5% of Medical Air
Analysis: Pass the *Sample* through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube by the detector tube manufacturer.
Acceptance criteria: NMT 2.5 ppm

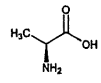
Supplement 1 to

www.webofpharma.com

• **LIMIT OF WATER AND OIL**
Analysis: Support one container in an inverted position (with the valve at the bottom) for 5 min. Cautiously open the valve slightly, maintaining the container in an inverted position. Vent the gas with a barely audible flow against a stainless steel mirror for a few seconds.
Acceptance criteria: No liquid is discernible on the mirror.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in pressurized containers. Container connections shall be appropriate for air. Adaptors shall not be used to connect containers to patient use supply system piping or equipment.
• **LABELING:** Label states if Medical Air is a synthetic mixture of *Oxygen USP* and *Nitrogen NF*. Where it is piped directly from the collecting tank to the patient point of use, label each outlet "Medical Air".

Alanine



$C_3H_7NO_2$ 89.09
L-Alanine [56-41-7].

DEFINITION
Alanine contains NLT 98.5% and NMT 101.5% of L-alanine ($C_3H_7NO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Sample: 80 mg of Alanine
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Blank: 3 mL of formic acid in 50 mL of glacial acetic acid
Analysis: Dissolve the *Sample* in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with *Titrant*.
Calculate the percentage of L-alanine ($C_3H_7NO_2$) in the portion of Alanine taken:

$$\text{Result} = [(V_S - V_B) \times N \times F \times 100]/W$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 89.09 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.15%
• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Standard solution: 0.70 mL of 0.020 N hydrochloric acid
Sample: 1.0 g of Alanine
Acceptance criteria: NMT 0.05%
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Standard solution: 0.30 mL of 0.020 N sulfuric acid
Sample: 1.0 g of Alanine
Acceptance criteria: NMT 0.03%
• **IRON** ⟨241⟩: NMT 30 ppm
• **RELATED COMPOUNDS**
Mobile phase: 0.008 N sulfuric acid solution
System suitability solution: A mixture of 0.05 mg/mL of USP Fumaric Acid RS, 0.05 mg/mL of USP Maleic Acid RS, and 3 mg/mL of USP Malic Acid RS in water
Maleic acid standard solution: 0.05 mg/mL of USP Maleic Acid RS in water
Malic acid standard solution: 3 mg/mL of USP Malic Acid RS in water
Fumaric acid standard solution: 0.05 mg/mL of USP Fumaric Acid RS in water
Sample solution: 100 mg/mL of Alanine in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 7.8-mm × 30-cm; 9-µm packing L17
Column temperature: 30°
Flow rate: 0.6 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between maleic acid and malic acid
Relative standard deviation: NMT 5.0% for each of fumaric acid, maleic acid, and malic acid
Analysis
Samples: Standard solutions and *Sample solution*
Calculate the percentage of each specified acid in the portion of Alanine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of maleic acid, malic acid, or fumaric acid from the *Sample solution*
r_S = peak response of maleic acid, malic acid, or fumaric acid from the corresponding Standard solution
C_S = concentration of USP Maleic Acid RS, USP Malic Acid RS, or USP Fumaric Acid RS in the corresponding Standard solution (mg/mL)
C_U = concentration of Alanine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Alanine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of fumaric acid from the *Fumaric acid standard solution*
C_S = concentration of USP Fumaric Acid RS in the *Fumaric acid standard solution* (mg/mL)
C_U = concentration of Alanine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

www.webofpharma.com

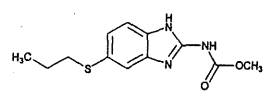
Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Maleic acid	0.5	0.05
Malic acid	0.6	0.05
Fumaric acid	1.0	0.05
Alanine	Not observed	—
Any unspecified impurity	—	0.05
Total unspecified impurities	—	0.20

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 100 mg/mL in 6 N hydrochloric acid
  Acceptance criteria: +13.7° to +15.1°
- **pH** ⟨791⟩**:** 5.5–7.0, in a solution (1 in 20)
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP L-Alanine RS
  USP Fumaric Acid RS
  USP Maleic Acid RS
  USP Malic Acid RS

Albendazole



$C_{12}H_{15}N_3O_2S$ 265.33

Carbamic acid, [5-(propylthio)-1*H*-benzimidazol-2-yl]-, methyl ester;
Methyl 5-(propylthio)-2-benzimidazolecarbamate [54965-21-8].

DEFINITION
Albendazole contains NLT 98.0% and NMT 102.0% of albendazole ($C_{12}H_{15}N_3O_2S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The R_F value of the principal spot of the *Sample solution* corresponds to that of the principal spot of the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY
- **PROCEDURE**
  Sample: 250 mg of Albendazole
  Analysis: Transfer the *Sample* to a suitable flask, and dissolve in 100 mL of glacial acetic acid, warming gently if necessary. Cool, and titrate with 0.1 N perchloric acid VS to a potentiometric endpoint (see *Titrimetry* ⟨541⟩). Perform a blank determination. Each mL of 0.1 N perchloric acid is equivalent to 26.53 mg of $C_{12}H_{15}N_3O_2S$.
  Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%
- **ORGANIC IMPURITIES**
  Standard stock solution: 5 mg/mL of USP Albendazole RS in glacial acetic acid
  Standard solution: 0.05 mg/mL of USP Albendazole RS in glacial acetic acid from *Standard stock solution*
  Sample solution: 10 mg/mL in glacial acetic acid
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbant: 0.25-mm layer of silica gel mixture
  Application volume: 10 µL
  Developing solvent system: Chloroform, ether, and glacial acetic acid (60:10:10)
  Analysis: Proceed as directed for *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.
  Samples: *Standard stock solution*, *Standard solution*, and *Sample solution*
  Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow the solvent to evaporate from the plate, and examine the plate under short-wavelength UV light.
  Acceptance criteria: 0.5%; no spot, other than the principal spot of the *Sample solution*, is larger or more intense than the principal spot of the *Standard solution*.

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 4 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Albendazole RS

Albendazole Oral Suspension

DEFINITION
Albendazole Oral Suspension is Albendazole in an aqueous vehicle. It contains one or more preservatives and dispersing or suspending agents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of albendazole ($C_{12}H_{15}N_3O_2S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Sample stock solution: 1 mg/mL of albendazole from a quantity of Suspension, in a mixture of methanol and hydrochloric acid (99:1). Filter the mixture, if necessary, to obtain a clear solution.
  Sample solution: 0.01 mg/mL of albendazole in 0.1 N sodium hydroxide from *Sample stock solution*
  Acceptance criteria: Meets the requirements

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: Methanol and hydrochloric acid (99:1)

Solution B: 13.75 g/L of monobasic sodium phosphate

Mobile phase: Methanol and *Solution B* (60:40)

Standard stock solution: 1 mg/mL of USP Albendazole RS in *Solution A*

Standard solution: 100 µg/mL of USP Albendazole RS from *Standard stock solution* in *Mobile phase*

Sample stock solution: Equivalent to 1 mg/mL of albendazole from a volume of Oral Suspension in *Solution A*

Sample solution: Nominally 100 µg/mL of albendazole from *Sample stock solution* in *Mobile phase*. [NOTE—Filter, if necessary, to obtain a clear solution.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 308 nm

Column: 4-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of albendazole ($C_{12}H_{15}N_3O_2S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Albendazole RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of albendazole in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **PH** ⟨791⟩**:** 4.5–5.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **LABELING:** Label it to indicate that it is for veterinary use only.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Albendazole RS

Albendazole Tablets

» Albendazole Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of albendazole ($C_{12}H_{15}N_3O_2S$).

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.

Labeling—Tablets intended for veterinary use only are so labeled.

USP Reference standards ⟨11⟩—

USP Albendazole RS

USP Parbendazole RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy:* 197U▲ (CN 1-May-2020)—

Solution: Dilute a portion of the clear filtrate used to prepare the *Assay preparation* and a portion of the stock solution used to prepare the *Standard preparation* prepared in the *Assay* with *Acidified methanol*, prepared as directed for *Dissolution*, to obtain solutions containing about 10 µg of albendazole per mL.

B: The retention time of the major peak for albendazole in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{12}H_{15}N_3O_2S$ dissolved using the following procedure.

Acidified methanol—To about 50 mL of methanol in a 100-mL volumetric flask add 2 mL of hydrochloric acid, dilute with methanol to volume, and mix.

Standard solution—Transfer about 90 mg of USP Albendazole RS, accurately weighed, to a 250-mL volumetric flask, add 10 mL of *Acidified methanol*, and shake to dissolve. Dilute with 0.1 N hydrochloric acid to volume, and mix. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.

Procedure—Transfer 10.0 mL of a filtered portion of the solution under test to a 250-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix. Concomitantly determine the absorbances of this solution and the *Standard solution* at the wavelengths of maximum and minimum absorbance at about 308 nm and 350 nm, using 0.1 N sodium hydroxide as the blank. Calculate the quantity, in mg, of $C_{12}H_{15}N_3O_2S$ dissolved by the formula:

$$22.5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Albendazole RS in the *Standard solution;* and A_U and A_S are the differences in absorbance between 308 nm and 350 nm obtained from the solution under test and the *Standard solution,* respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{12}H_{15}N_3O_2S$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Procedure for content uniformity—

Acidified methanol and *Standard solution*—Prepare as directed under *Dissolution*.

Test solution—Place 1 Tablet in a 500-mL volumetric flask, add about 300 mL of *Acidified methanol*, and shake by mechanical means for about 30 minutes. Dilute with *Acidified methanol* to volume, and mix. Filter a portion of this solution, discarding the first 20 mL of the filtrate. Transfer 4.0 mL of the clear filtrate to a 200-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the wavelengths of maximum and minimum absorbance at about 308 nm and 350 nm, using 0.1 N sodium hydroxide as the blank. Calculate

USP Monographs

www.webofpharma.com

the quantity, in mg, of $C_{12}H_{15}N_3O_2S$ in the Tablet taken by the formula:

$$25C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Albendazole RS in the Standard preparation; and A_U and A_S are the differences in absorbance between 308 nm and 350 nm obtained from the *Test solution* and the *Standard solution,* respectively.

Assay—

Mobile phase—Dissolve 0.50 g of monobasic ammonium phosphate in 400 mL of water. Add 600 mL of methanol, mix, and filter, discarding the first 15 mL of the filtrate. Degas the clear filtrate before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Sulfuric acid in methanol—Prepare a mixture of 1 mL of sulfuric acid and 99 mL of methanol.

Internal standard solution—Transfer about 150 mg of USP Parbendazole RS to a 50-mL volumetric flask. Add 5 mL of *Sulfuric acid in methanol,* 25 mL of methanol, and shake to dissolve. Dilute with methanol to volume, and mix.

Standard preparation—Transfer about 100 mg of USP Albendazole RS, accurately weighed, to a 50-mL volumetric flask. Add 5 mL of *Sulfuric acid in methanol* and 25 mL of methanol, and shake to dissolve. Dilute with methanol to volume, and mix. Transfer 5.0 mL of this stock solution and 5.0 mL of *Internal standard solution* to a second 50-mL volumetric flask, dilute with methanol to volume, and mix.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of albendazole, to a 50-mL volumetric flask. Add 5 mL of *Sulfuric acid in methanol* and 20 mL of methanol, and shake by mechanical means for about 15 minutes. Dilute with methanol to volume, mix, and filter, discarding the first 15 mL of the filtrate. Transfer 5.0 mL of the clear filtrate and 5.0 mL of *Internal standard solution* to a second 50-mL volumetric flask, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; the column efficiency is not less than 1000 theoretical plates; the resolution between the albendazole peak and the parbendazole peak is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— [NOTE—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{12}H_{15}N_3O_2S$ in the portion of Tablets taken by the formula:

$$500C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Albendazole RS in the *Standard preparation;* and R_U and R_S are the peak response ratios of the albendazole peak to the parbendazole peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Albumin Human

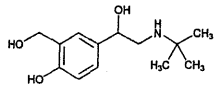
DEFINITION

Albumin Human conforms to the regulations of the federal Food and Drug Administration concerning biologics (640.80 to 640.86) (see *Biologics* ⟨1041⟩). It is a sterile, nonpyrogenic preparation of serum albumin obtained by fractionating material (source blood, plasma, serum, or placentas) from healthy human donors, the source material being tested for the absence of hepatitis B surface antigen. It is made by a process that yields a product that is safe for intravenous use. NLT 96% of its total protein is albumin. It is a solution containing, in each 100 mL, either 25 g of serum albumin osmotically equivalent to 500 mL of normal human plasma, or 20 g equivalent to 400 mL, or 5 g equivalent to 100 mL, or 4 g equivalent to 80 mL, and contains NLT 93.75% and NMT 106.25% of the labeled amount in the case of the solution containing 4 g in each 100 mL, and NLT 94.0% and NMT 106.0% of the labeled amount in the other cases. It contains no added antimicrobial agent, but may contain sodium acetyltryptophanate with or without sodium caprylate as a stabilizing agent. It has a sodium content of NLT 130 mEq/L and NMT 160 mEq/L. It has a heme content such that the absorbance of a solution, diluted to contain 1% of protein, in a 1-cm holding cell, measured at a wavelength of 403 nm, is NMT 0.25. It meets the requirements of the test for heat stability and for pH.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at the temperature recommended by the manufacturer or indicated on the label.
- **EXPIRATION DATE:** The expiration date is not later than 5 years after issue from manufacturer's cold storage (5°, 3 years) if labeling recommends storage between 2° and 10°; not later than 3 years after issue from manufacturer's cold storage (5°, 3 years) if labeling recommends storage at temperatures not higher than 37°; and not later than 10 years after date of manufacture if in a hermetically sealed metal container and labeling recommends storage between 2° and 10°.
- **LABELING:** Label it to state that it is not to be used if it is turbid and that it is to be used within 4 h after the container is entered. Label it also to state the osmotic equivalent in terms of plasma, the sodium content, and the type of source material (venous plasma, placental plasma, or both) from which it was prepared. Label it also to indicate that additional fluids are needed when the 20-g/100-mL or 25-g/100-mL product is administered to a markedly dehydrated patient.

Albuterol



$C_{13}H_{21}NO_3$ 239.31

1,3-Benzenedimethanol, α¹-[[(1,1-dimethylethyl)amino] methyl]-4-hydroxy-;
α¹-[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene-α,α′-diol [18559-94-9].

DEFINITION

Albuterol contains NLT 98.5% and NMT 101.0% of albuterol ($C_{13}H_{21}NO_3$), calculated on the anhydrous basis.

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 80 µg/mL in 0.1 N hydrochloric acid
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Sample solution: 8 mg/mL of Albuterol in glacial acetic acid
Analysis: To 50 mL of the *Sample solution* add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 23.93 mg of $C_{13}H_{21}NO_3$.
Acceptance criteria: 98.5%–101.0% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **ORGANIC IMPURITIES**
Standard solution: 0.10 mg/mL of USP Albuterol RS in methanol
Sample solution: 20 mg/mL of Albuterol in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 10 µL
Developing solvent system: Methyl isobutyl ketone, isopropyl alcohol, ethyl acetate, ammonium hydroxide, and water (50:45:35:3:18)
Visualization: Iodine vapor
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in the chapter, applying aliquots of the *Standard solution* and the *Sample solution*. Develop in the *Developing solvent system* until the solvent front has moved three-fourths the length of the plate. Remove the plate from the developing chamber, air-dry, and expose it to iodine vapor.
Acceptance criteria: Any spot, other than the principal spot, obtained from the *Sample solution* is not greater in size and intensity than the spot produced by the *Standard solution* (0.5%), and the sum of the impurities is not greater than 2.0%.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Albuterol RS

Albuterol Tablets

DEFINITION
Albuterol Tablets contain an amount of albuterol sulfate $[(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4]$ equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of albuterol ($C_{13}H_{21}NO_3$).

IDENTIFICATION
• **A.** The R_F value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*, obtained as directed in the *Procedure* for *Organic Impurities*.
• **B. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩
Sample solution: Shake a quantity of powdered Tablets equivalent to 4 mg of albuterol with 10 mL of water, and filter. Use the filtrate.
Acceptance criteria: Meet the requirements

ASSAY
• **PROCEDURE**
Solution A: 10 mL/L of glacial acetic acid in water
Solution B: 1.13 g of sodium 1-hexanesulfonate in 1200 mL of water. Add 12 mL of glacial acetic acid.
Diluent: Methanol and water (40:60)
Mobile phase: Methanol and *Solution B* (40:60)
Standard stock solution: 0.12 mg/mL of USP Albuterol Sulfate RS, prepared as follows. Transfer USP Albuterol Sulfate RS to a suitable volumetric flask, and add a volume of *Solution A* corresponding to 60% of the flask volume. Sonicate for 5 min, and dilute with methanol to volume.
Standard solution: 0.03 mg/mL of USP Albuterol Sulfate RS in Diluent, from *Standard stock solution*
Sample solution: Nominally 0.025 mg/mL of albuterol, prepared as follows. Transfer a number of whole Tablets, equivalent to 50 mg of albuterol, to a suitable volumetric flask. Add 60% of the flask volume of *Solution A*, shake by mechanical means for 45 min, sonicate for 10 min, allow to cool to room temperature, and dilute with methanol to volume. Pass through a suitable filter of 0.45-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 276 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 800 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of albuterol ($C_{13}H_{21}NO_3$) in the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times M \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of albuterol in the *Sample solution* (mg/mL)
M = number of moles of albuterol per mole of albuterol sulfate, 2
M_{r1} = molecular weight of albuterol, 239.31
M_{r2} = molecular weight of albuterol sulfate, 576.70

USP Monographs

www.webofpharma.com

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: Water; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
Diluent, Mobile phase, and **Standard stock solution:** Proceed as directed in the *Assay*.
Standard solution: 0.03 mg/mL of USP Albuterol Sulfate RS in Diluent, from *Standard stock solution*. If necessary, dilute with *Diluent* to a concentration corresponding to the *Sample solution*.
Sample solution: Pass a portion of the solution under test through a nylon filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 276 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 800 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of albuterol ($C_{13}H_{21}NO_3$) dissolved by comparing the major peak response from the *Sample solution* to that from the *Standard solution*.
Acceptance criteria: NLT 80% (*Q*) of the labeled amount of albuterol ($C_{13}H_{21}NO_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Standard solution A: 0.580 mg/mL of USP Albuterol Sulfate RS in water, equivalent to 0.483 mg/mL of albuterol
Standard solution B: 0.218 mg/mL of USP Albuterol Sulfate RS in water, equivalent to 0.183 mg/mL of albuterol
Standard solution C: 0.073 mg/mL of USP Albuterol Sulfate RS in water, equivalent to 0.061 mg/mL of albuterol
Sample solution: Place a quantity of finely powdered Tablets, equivalent to 48 mg of albuterol, into a suitable container. Add 60 mL of diluted alcohol (1 in 2), and shake by mechanical means for 30 min. Filter the mixture, and wash the filter with small portions of alcohol, combining this with the filtrate. Evaporate the filtrate to dryness under reduced pressure below 40°. Dissolve the residue as completely as possible in 2 mL of water.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 10 µL. Apply two successive 5-µL aliquots, allowing the solvent to evaporate between applications.
Developing solvent system: Methyl isobutyl ketone, isopropyl alcohol, ethyl acetate, ammonium hydroxide, and water (50:45:35:3:18)
Spray reagent A: 3-Methyl-2-benzothiazolinone hydrazone hydrochloride TS
Spray reagent B: Ammoniacal potassium ferricyanide TS
Analysis
Samples: *Standard solution A, Standard solution B, Standard solution C,* and *Sample solution*
Air-dry the plate. Develop the chromatograms until the solvent front has moved about 17 cm. Spray the plate first with *Spray reagent A*, and then *Spray reagent B*, and finally again with *Spray reagent A*. Examine the plate and estimate the responses of any secondary spots observed in the lane of the *Sample solution* by comparison with those of *Standard solutions A, B,* and *C*.
Acceptance criteria
1. 2.0%; no major secondary spot is greater in size or intensity than the principal spot produced by *Standard solution A*.
2. 0.75%; no other secondary spot is greater in size or intensity than the principal spot produced by *Standard solution B*.
3. 0.25%; no more than two other secondary spots are equal in size or intensity to the principal spot produced by *Standard solution C*.
4. The sum of the intensities of all secondary spots obtained from the *Sample solution* corresponds to NMT 3.5%.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Albuterol Sulfate RS

Albuterol Extended-Release Tablets

DEFINITION

Albuterol Extended-Release Tablets contain albuterol sulfate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of albuterol ($C_{13}H_{21}NO_3$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Standard solution: 80 µg/mL of albuterol from USP Albuterol Sulfate RS in methanol
Sample solution: 80 µg/mL of albuterol in methanol, prepared as follows. Transfer a suitable number of Tablets to a volumetric flask, and dilute with methanol to volume. Stir for 30 min, and centrifuge.
Wavelength range: 210–350 nm
Cell path: 0.2 cm
Acceptance criteria: The *Sample solution* exhibits maxima and minima only at the same wavelengths as the *Standard solution*.

ASSAY

• **PROCEDURE**
Buffer: 0.65 g/L of sodium 1-octane sulfonate and 21.7 g/L of ammonium acetate in water
Mobile phase: Glacial acetic acid, 2-propanol, methanol, and *Buffer* (4:3:1:92)
Diluent: 10 mL/L of triethylamine in water
Standard stock solution: 0.2 mg/mL of USP Albuterol Sulfate RS in *Diluent*

USP Monographs

www.webofpharma.com

Standard solution: 0.02 mg/mL of USP Albuterol Sulfate RS in *Diluent*, from the *Standard stock solution*. Transfer an aliquot of the *Standard stock solution* to a suitable volumetric flask, and add 4% of the flask volume of methanol. Allow to cool to room temperature, and dilute with *Diluent* to volume.

Sample solution: Nominally 0.016 mg/mL of albuterol, prepared as follows. Transfer Tablets (NLT 10) to a suitable volumetric flask, add 10% of the flask volume of methanol, and sonicate for 30 min with regular swirling. Add 60% of the flask volume of *Diluent*, and sonicate for 30 min with swirling. Stir for 60 min, allow the solution to cool to room temperature, and dilute with *Diluent* to volume. Centrifuge a portion of this solution at 2500 rpm for 15 min. Transfer 10 mL of the supernatant into a 50-mL volumetric flask, add 1 mL of methanol, cool to room temperature, and dilute with *Diluent* to volume. Pass the solution though a 1-µm glass fiber or equivalent filter, and discard the first 3 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 40 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of albuterol ($C_{13}H_{21}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M \times M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of albuterol in the *Sample solution* (mg/mL)
M = moles of albuterol per mole of albuterol sulfate, 2
M_{r1} = molecular weight of albuterol, 239.31
M_{r2} = molecular weight of albuterol sulfate, 576.70

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm, with helix sinker

Time: 1, 2, 4, and 9 h

Buffer, Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay*, except to use an *Injection volume* of 50 µL.

Standard stock solution: 0.2 mg/mL of albuterol, using USP Albuterol Sulfate RS, in *Medium*

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (L/1000) mg/mL of albuterol, where L is the label claim in mg/Tablet of albuterol.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of albuterol ($C_{13}H_{21}NO_3$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of albuterol in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of albuterol ($C_{13}H_{21}NO_3$) released at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times (V - (2 \times V_S))] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times (V - (3 \times V_S))] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of albuterol in the portion of sample withdrawn at time point i (mg/mL)
V = volume of the *Medium* (900 mL)
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 1*.

Table 1

Time point (i)	Time (h)	Amount released (%)
1	1	25–45
2	2	45–65
3	4	65–85
4	9	NLT 80

The cumulative percentages of the labeled amount of albuterol ($C_{13}H_{21}NO_3$), released at the times specified, conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES, PROCEDURE 1**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with hydrochloric acid to a pH of 3.0.

Mobile phase: Methanol and *Buffer* (5:95)

System suitability solution: 2 µg/mL each of USP Albuterol Sulfate RS and USP Albuterol Related Compound B RS in *Mobile phase*

Standard solution: 2.4 µg/mL of USP Albuterol Sulfate RS, 0.80 µg/mL of USP Levalbuterol Related Compound C RS, and 0.25 µg/mL of USP Levalbuterol Related Compound D RS (equivalent to 0.20 µg/mL free base) in *Mobile phase*

Sensitivity solution: 0.06 µg/mL of USP Albuterol Sulfate RS in *Mobile phase*

Sample solution: Transfer an amount of powder from NLT 20 Tablets to a suitable volumetric flask to obtain a solution with a final nominal concentration of 0.2 mg/mL of albuterol. Add 70% of the flask volume of *Mobile phase*, and

www.webofpharma.com

sonicate for 10 min. Stir for 30 min, and dilute with *Mobile phase* to volume. Pass the solution though a 1-µm glass fiber or equivalent filter, and discard the first 3 mL of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 80 µL
Run time: 5 times the retention time of albuterol

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—Identify the impurities using the relative retention times shown in *Table 2*.]
Suitability requirements:
Tailing factor: NMT 2.0 for each compound, *Standard solution*
Resolution: NLT 2 between albuterol and albuterol related compound B, *System suitability solution*
Relative standard deviation: NMT 10.0% for each compound, *Standard solution*

Analysis
Samples: *Standard solution*, *Sensitivity solution*, and *Sample solution*
[NOTE—Identify the impurities using the relative retention times shown in *Table 2*.]
Calculate the percentage of levalbuterol related compound C in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levalbuterol related compound C from the *Sample solution*
r_S = peak response of levalbuterol related compound C from the *Standard solution*
C_S = concentration of USP Levalbuterol Related Compound C RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of albuterol in the *Sample solution* (mg/mL)

Calculate the percentage of levalbuterol related compound D in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levalbuterol related compound D from the *Sample solution*
r_S = peak response of the levalbuterol related compound D from the *Standard solution*
C_S = concentration of USP Levalbuterol Related Compound D RS (as the free base) in the *Standard solution* (mg/mL)
C_U = nominal concentration of albuterol in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M \times M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the impurity from the *Sample solution*
r_S = peak response of albuterol from the *Standard solution*
C_S = concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of albuterol in the *Sample solution* (mg/mL)
M = moles of albuterol per mole of albuterol sulfate, 2
M_{r1} = molecular weight of albuterol, 239.31
M_{r2} = molecular weight of albuterol sulfate, 576.70

Acceptance criteria: See *Table 2*. Disregard peaks eluting after levalbuterol related compound C or with areas less than that of the *Sensitivity solution*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Albuterol related compound B[a]	0.88	—[b]
Albuterol	1.0	—
Chloroalbuterone[c]	1.7	—[b]
Chloroalbuterol[d]	2.5	—[b]
Albuterol related compound A[e]	2.7	—[b]
Levalbuterol related compound D[f]	3.2	0.1
Levalbuterol related compound C[g,h]	3.5	0.4
Any other individual unspecified impurity	—	0.2

[a] 2-(*tert*-Butylamino)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone.
[b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.
[c] 2-(*tert*-Butylamino)-1-[3-chloro-4-hydroxy-5-(hydroxymethyl)phenyl] ethanone.
[d] 4-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-chloro-6-(hydroxymethyl)phenol.
[e] 4-{2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl}-2-methylphenol.
[f] 5-[2-{(1,1-Dimethylethyl)amino)-1-hydroxyethyl]-2-hydroxy-benzaldehyde.
[g] α-[{(1,1-Dimethylethyl)amino}methyl]-4-hydroxy-3-(methoxymethyl)-benzenemethanol.
[h] Disregard peaks eluting after levalbuterol related compound C.

• **ORGANIC IMPURITIES, PROCEDURE 2**
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with hydrochloric acid to a pH of 3.0.
Mobile phase: Methanol and *Buffer* (30:70)
Diluent: Methanol and *Buffer* (5:95)
Peak identification solution: 2.4 µg/mL of USP Albuterol Sulfate RS, 1.0 µg/mL of USP Levalbuterol Related Compound C RS, and 1.2 µg/mL of USP Levalbuterol Related Compound D RS in *Diluent*
Standard solution: 2.4 µg/mL of USP Albuterol Sulfate RS and 1.0 µg/mL of USP Albuterol Related Compound E RS in *Diluent*
Sensitivity solution: 0.06 µg/mL of USP Albuterol Sulfate RS in *Diluent* from *Standard solution*
Sample solution: Transfer an amount of powder from NLT 20 Tablets to a suitable volumetric flask to obtain a solution with a final concentration of 0.2 mg/mL of albuterol. Add 70% of the flask volume of *Diluent*, and sonicate for 10 min. Stir for 30 min, and dilute with *Diluent* to volume. Pass the solution though a glass fiber or equivalent filter of 1-µm pore size, and discard the first 3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Flow rate: 0.7 mL/min

www.webofpharma.com

Injection volume: 80 µL
Run time: 5 times the retention time of albuterol
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for each compound
Relative standard deviation: NMT 10.0% for each compound
Analysis
Samples: *Standard solution, Peak identification solution, Sensitivity solution,* and *Sample solution*
Calculate the percentage of albuterol related compound E in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of albuterol related compound E from the *Sample solution*
r_S = peak response of albuterol related compound E from the *Standard solution*
C_S = concentration of USP Albuterol Related Compound E RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of albuterol in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M \times M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the impurity from the *Sample solution*
r_S = peak response of albuterol from the *Standard solution*
C_S = concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of albuterol in the *Sample solution* (mg/mL)
M = moles of albuterol per mole of albuterol sulfate, 2
M_{r1} = molecular weight of albuterol, 239.31
M_{r2} = molecular weight of albuterol sulfate, 576.70

Acceptance criteria: See *Table 3*. Disregard the levalbuterol related compound C peak and any peak eluting before it. Disregard peaks with areas less than that of the *Sensitivity solution*.

Table 3

Compound	Relative Retention Time	Acceptance Criteria, NMT (%)
Albuterol	1.0	—
Levalbuterol related compound C	1.79	—
Levalbuterol related compound D	1.83	—
Albuterol related compound E[a]	3.67	0.5
Any other individual unspecified impurity	—	0.2
Total impurities	—	1.5[b]

[a] 2,2′-Oxybis(methylene)bis{4-[2-(*tert*-butylamino)-1-hydroxyethyl]phenol}.
[b] From the sum of the impurities in *Procedure 1* and *Procedure 2*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Albuterol Sulfate RS
  USP Albuterol Related Compound B RS
  2-(*tert*-Butylamino)-1-[4-hydroxy-3-(hydroxymethyl) phenyl]ethanone.
  $C_{13}H_{19}NO_3$ 237.29
  USP Albuterol Related Compound E RS
  2,2′-Oxybis(methylene)bis{4-[2-(*tert*-butylamino)-1-hydroxyethyl]phenol}.
  $C_{26}H_{40}N_2O_5$ 460.61
  USP Levalbuterol Related Compound C RS
  α-[{(1,1-Dimethylethyl)amino}methyl]-4-hydroxy-3-(methoxymethyl)-benzenemethanol.
  $C_{14}H_{23}NO_3$ 253.34
  USP Levalbuterol Related Compound D RS
  5-[2-{(1,1-Dimethylethyl)amino}-1-hydroxyethyl]-2-hydroxy-benzaldehyde sulfate salt.
  $(C_{13}H_{19}NO_3)_2 \cdot H_2SO_4$ 572.67

Albuterol Sulfate

$(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$ 576.70
1,3-Benzenedimethanol, α¹-[[(1,1-dimethylethyl)amino] methyl]-4-hydroxy-, sulfate (2:1) (salt).
α¹-[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene-α,α′-diol sulfate (2:1) (salt) [51022-70-9].

» Albuterol Sulfate contains not less than 98.5 percent and not more than 101.0 percent of $(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Albuterol Related Compound A RS
4-[2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-2-methylphenol sulfate.
USP Albuterol Sulfate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 80 µg per mL.
Medium: 0.1 N hydrochloric acid.
C: Shake a quantity of it, equivalent to 4 mg of albuterol, with 10 mL of water, and filter: the filtrate so obtained meets the requirements of the tests for *Sulfate* ⟨191⟩.
D: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%.
Residue on ignition ⟨281⟩: not more than 0.1%.

www.webofpharma.com

Chromatographic purity—It meets the requirements of the test for *Organic Impurities* under *Albuterol*, except to read Albuterol Sulfate in place of Albuterol and to use water instead of methanol as the solvent to prepare the *Standard solution* and the *Sample solution*.

Assay—

0.05 ± 0.01 M Ammonium acetate solution—Dissolve 3.85 g of ammonium acetate in 1000 mL of water, and mix.

Mobile phase—Prepare a degassed mixture of water, *0.05 ± 0.01 M Ammonium acetate solution*, and isopropanol [65: 30: (5 ± 1)], and adjust dropwise with acetic acid to a pH of 4.5 ± 0.3.

Resolution solution—Dissolve accurately weighed quantities of USP Albuterol Sulfate RS and USP Albuterol Related Compound A RS in water, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.140 mg per mL and 0.030 mg per mL, respectively.

Standard preparation—Dissolve an accurately weighed quantity of USP Albuterol Sulfate RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 0.6 mg per mL.

Assay preparation—Transfer about 60 mg of Albuterol Sulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

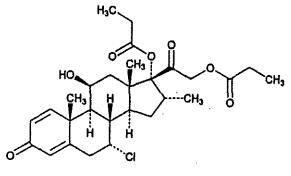
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 276-nm detector and a 4.6-mm × 20-cm column that contains packing L10. The flow rate is about 2.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between albuterol and albuterol related compound A is not less than 1.5; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$ in the portion of Albuterol Sulfate taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Albuterol Sulfate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Alclometasone Dipropionate



$C_{28}H_{37}ClO_7$ 521.04

Pregna-1,4-diene-3,20-dione, 7-chloro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, (7α,11β,16α)-; 7α-Chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [66734-13-2].

DEFINITION

Alclometasone Dipropionate contains NLT 97.0% and NMT 102.0% of $C_{28}H_{37}ClO_7$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the *Internal standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 6.80 mg/mL of monobasic potassium phosphate (0.05 M)

Mobile phase: Methanol and *Solution A* (2:1)

Internal standard solution: 2 mg/mL of betamethasone dipropionate in methanol

Standard stock solution: 1.2 mg/mL of USP Alclometasone Dipropionate RS in methanol

Standard solution: 4.0 mL of *Standard stock solution* and 4.0 mL of *Internal standard solution*. Dilute with methanol to 25 mL. [NOTE—This solution contains approximately 0.2 mg/mL of USP Alclometasone Dipropionate RS.]

Sample stock solution: 1.2 mg/mL of Alclometasone Dipropionate in methanol

Sample solution: 4 mL of *Sample stock solution* and 4 mL of *Internal standard solution*. Dilute with methanol to 25 mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for alclometasone dipropionate and betamethasone dipropionate are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the analyte and the internal standard peaks

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{28}H_{37}ClO_7$ in the portion of Alclometasone Dipropionate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio from the *Sample solution*

R_S = peak height ratio from the *Standard solution*

C_S = concentration of USP Alclometasone Dipropionate RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

- **Procedure**

Mobile phase: Acetonitrile and water (3:2)

Diluent: Acetonitrile and water (2:1)

System suitability solution: 1.5 mg/mL of USP Alclometasone Dipropionate RS and 0.015 mg/mL of USP

www.webofpharma.com

Alclometasone Dipropionate Related Compound A RS in *Diluent*
Sample solution: 1.5 mg/mL of Alclometasone Dipropionate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 5 µL
Run time: Three times the retention time of alclometasone
System suitability
Sample: *System suitability solution*
Suitability requirements
Tailing factor: NMT 1.5 for alclometasone dipropionate
Relative standard deviation: NMT 2.0% for alclometasone dipropionate
Resolution: NLT 2.0 between alclometasone dipropionate and alclometasone dipropionate related compound A
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Alclometasone Dipropionate taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area for each impurity from the *Sample solution*
r_T = sum of all the peaks from the *Sample solution*
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 2.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Alclometasone dipropionate	1.0	—	—
Alclometasone dipropionate related compound A[a]	1.2	0.93	1.0
2-Bromo alclometasone dipropionate[b]	1.7	0.91	0.5
Any individual, unspecified impurity	—	1.0	0.10

[a] 11β,17,21-Trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate.
[b] 2-Bromo-7α-chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate.

SPECIFIC TESTS
• **Optical Rotation,** *Specific Rotation* ⟨781S⟩
Sample solution: 30 mg/mL in dioxane
Acceptance criteria: +21° to +25°
• **Loss on Drying** ⟨731⟩**:** Dry a sample in a vacuum at a pressure not exceeding 5 mm of mercury at 105° for 3 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Alclometasone Dipropionate RS
USP Alclometasone Dipropionate Related Compound A RS
11β,17,21-Trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate.
$C_{28}H_{38}O_7$ 486.60

Alclometasone Dipropionate Cream

DEFINITION
Alclometasone Dipropionate Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of alclometasone dipropionate ($C_{28}H_{37}ClO_7$) in a suitable cream base.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.
• **B. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Standard solution: 0.08 mg/mL of USP Alclometasone Dipropionate RS in methanol
Sample solution: Place a quantity of Cream, equivalent to 1.25 mg of alclometasone dipropionate, in a 50-mL centrifuge tube, and add 15 mL of methanol. Insert a stopper securely into the tube, and place the tube in a water bath maintained at 60° until the semisolid components melt. Remove the tube from the bath, shake vigorously until the specimen components resolidify, and place the tube in an ice–methanol bath for 15 min. Remove the tube from the bath, and centrifuge at 2500 rpm for 5 min. Transfer the clear supernatant to a vial, and allow to equilibrate to room temperature.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: Chloroform and acetone (7:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Dry the applications with the aid of a stream of nitrogen, and develop the chromatograms in a saturated, unlined chromatographic chamber. When the solvent front has moved three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal spot obtained from the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY
• **Procedure**
Buffer: 6.80 g/L of monobasic potassium phosphate (0.05 M)
Mobile phase: Methanol and *Buffer* (2:1)
Internal standard solution: 0.4 mg/mL of betamethasone dipropionate in methanol
Standard stock solution: 0.25 mg/mL of USP Alclometasone Dipropionate RS in methanol
Standard solution: 0.08 mg/mL of USP Alclometasone Dipropionate RS obtained by combining, in a small stoppered flask, 5.0 mL of *Standard stock solution*, 5.0 mL of methanol, and 5.0 mL of *Internal standard solution*
Sample solution: Transfer a quantity of Cream, equivalent to 1.25 mg of alclometasone dipropionate, to a 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution*

www.webofpharma.com

and 10.0 mL of methanol. Insert a stopper securely into the tube, and place it in a water bath maintained at 60° until the semisolid components melt. Remove the tube from the bath, shake vigorously until the specimen components resolidify, and return the tube to the 60° water bath until the semisolid components melt. Remove the tube from the bath, shake vigorously until the specimen components resolidify, and place the tube in an ice–methanol bath for 15 min. Remove the tube from the bath, and centrifuge at 2500 rpm for 5 min. Transfer the clear supernatant to a small stoppered flask, and allow to equilibrate to room temperature.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for alclometasone dipropionate and betamethasone dipropionate are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the analyte and internal standard peaks
Relative standard deviation: NMT 2%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alclometasone dipropionate ($C_{28}H_{37}ClO_7$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio of alclometasone dipropionate to the internal standard from the *Sample solution*
R_S = peak height ratio of alclometasone dipropionate to the internal standard from the *Standard solution*
C_S = concentration of USP Alclometasone Dipropionate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of alclometasone dipropionate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Alclometasone Dipropionate RS

Alclometasone Dipropionate Ointment

DEFINITION
Alclometasone Dipropionate Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of alclometasone dipropionate ($C_{28}H_{37}ClO_7$) in a suitable ointment base.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.
• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 0.25 mg/mL USP Alclometasone Dipropionate RS in methanol
Sample solution: Place a quantity of Ointment, equivalent to 1.25 mg of alclometasone dipropionate, in a 50-mL centrifuge tube, add 10 mL of 2,2,4-trimethylpentane, insert a stopper securely into the tube, and disperse the specimen using a vortex mixer. Add 5.0 mL of a solution of methanol in water (45 in 50), insert the stopper securely, shake vigorously for 2 min, and centrifuge at 2500 rpm for 3 min. Remove the lower, aqueous alcohol phase, and transfer to a stoppered vial.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: Chloroform and acetone (7:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Dry the applications with the aid of a stream of nitrogen, and develop the chromatograms in a saturated, unlined chromatographic chamber. When the solvent front has moved three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal spot obtained from the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY
• **PROCEDURE**
Buffer: 6.80 g/L of monobasic potassium phosphate (0.05 M)
Solution A: Dilute 450 mL of methanol with water to 500 mL.
Mobile phase: Methanol and *Buffer* (2:1)
Internal standard solution: 0.15 mg/mL of betamethasone dipropionate in *Solution A*
Standard stock solution: 0.1 mg/mL of USP Alclometasone Dipropionate RS in *Solution A*
Standard solution: 0.05 mg/mL of USP Alclometasone Dipropionate RS obtained by combining, in a small stoppered flask, 5.0 mL of *Standard stock solution* and 5.0 mL of *Internal standard solution*
Sample solution: Transfer a quantity of Ointment, equivalent to 0.5 mg of alclometasone dipropionate, to a 50-mL centrifuge tube, add 10 mL of 2,2,4-trimethylpentane, insert a stopper securely into the tube, and disperse the specimen using a vortex mixer. Add 5.0 mL of *Internal standard solution* and 5.0 mL of *Solution A*, insert the stopper securely, shake vigorously for 2 min, and centrifuge at 2500 rpm for 3 min. Remove the lower, aqueous alcohol phase, and transfer this *Sample solution* to a stoppered vial.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for alclometasone dipropionate and betamethasone dipropionate are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the analyte and internal standard peaks
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alclometasone dipropionate ($C_{28}H_{37}ClO_7$) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio of alclometasone dipropionate to the internal standard from the *Sample solution*
R_S = peak height ratio of alclometasone dipropionate to the internal standard from the *Standard solution*
C_S = concentration of USP Alclometasone Dipropionate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of alclometasone dipropionate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Alclometasone Dipropionate RS

Alcohol

Portions of this monograph that are national *USP* text, and are not part of the harmonized text, are marked with symbols (•,•) to specify this fact.



C_2H_6O 46.07
Ethanol;
Ethyl alcohol [64-17-5].

DEFINITION
•Alcohol contains NLT 92.3% and NMT 93.8%, by weight, corresponding to NLT 94.9% and NMT 96.0%, by volume, at 15.56°, of C_2H_5OH.•

IDENTIFICATION
• **A.** It meets the requirements of the test for *Specific Gravity* ⟨841⟩.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F OR 197S**▲ (CN 1-May-2020)**:** Neat

IMPURITIES
• **LIMIT OF NONVOLATILE RESIDUE**
Sample: 100 mL of Alcohol
Analysis: Evaporate the *Sample* in a tared dish on a water bath, and dry at 100°–105° for 1 h.
Acceptance criteria: The weight of the residue is NMT 2.5 mg.
• **ORGANIC IMPURITIES**
Sample solution A: Alcohol (substance under test)
Sample solution B: 300 µL/L of 4-methylpentan-2-ol in *Sample solution A*
Standard solution A: 200 µL/L of methanol in *Sample solution A*
Standard solution B: 10 µL/L of methanol and 10 µL/L of acetaldehyde in *Sample solution A*
Standard solution C: 30 µL/L of acetal in *Sample solution A*
Standard solution D: 2 µL/L of benzene in *Sample solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m fused-silica capillary; bonded with a 1.8-µm layer of phase G43
Split ratio: 20:1
Temperatures
Injection port: 200°
Detector: 280°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	12
40	10	240	10

Linear velocity: 35 cm/s
Carrier gas: Helium
Injection volume: 1.0 µL
System suitability
Sample: *Standard solution B*
Suitability requirements
Resolution: NLT 1.5 between the first major peak (acetaldehyde) and the second major peak (methanol)
Analysis
Samples: *Sample solution A, Sample solution B, Standard solution A, Standard solution B, Standard solution C,* and *Standard solution D*
Methanol calculation

$$\text{Result} = (r_U/r_S)$$

r_U = peak area of methanol from *Sample solution A*
r_S = peak area of methanol from *Standard solution A*

USP Monographs

www.webofpharma.com

Acetaldehyde calculation (sum of acetaldehyde and acetal)

$$\text{Result} = \{[A_E/(A_T - A_E)] \times C_A\} + \{[D_E/(D_T - D_E)] \times C_D \times (M_{r1}/M_{r2})\}$$

A_E = peak area of acetaldehyde from *Sample solution A*
A_T = peak area of acetaldehyde from *Standard solution B*
C_A = concentration of acetaldehyde in *Standard solution B* (µL/L)
D_E = peak area of acetal from *Sample solution A*
D_T = peak area of acetal from *Standard solution C*
C_D = concentration of acetal in *Standard solution C* (µL/L)
M_{r1} = molecular weight of acetaldehyde, 44.05
M_{r2} = molecular weight of acetal, 118.2

Benzene calculation

$$\text{Result} = [B_E/(B_T - B_E)] \times C_B$$

B_E = peak area of benzene from *Sample solution A*
B_T = peak area of benzene from *Standard solution D*
C_B = concentration of benzene in *Standard solution D* (µL/L)

[NOTE—If necessary, the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).]

Any other impurity calculation

$$\text{Result} = (r_U/r_M) \times C_M$$

r_U = peak area of each impurity in *Sample solution B*
r_M = peak area of 4-methylpentan-2-ol in *Sample solution B*
C_M = concentration of 4-methylpentan-2-ol in *Sample solution B* (µL/L)

Acceptance criteria: See *Table 2*.

Table 2

Name	Acceptance Criteria
Methanol	NMT 0.5, corresponding to 200 µL/L
Acetaldehyde and acetal	NMT 10 µL/L, expressed as acetaldehyde
Benzene	NMT 2 µL/L
Sum of all other impurities[a]	NMT 300 µL/L

[a] Disregard any peaks of less than 9 µL/L (0.03 times the area of the peak corresponding to 4-methylpentan-2-ol in the chromatogram obtained with *Sample solution B*).

SPECIFIC TESTS

- **• SPECIFIC GRAVITY** ⟨841⟩: 0.812–0.816 at 15.56°, indicating 92.3%–93.8%, by weight, or 94.9%–96.0%, by volume, of C_2H_5OH. •
- **ULTRAVIOLET ABSORPTION**

Analytical wavelength: 235–340 nm
Cell: 5 cm
Reference: Water
Acceptance criteria
Absorbance: NMT 0.40 at 240 nm; NMT 0.30 between 250 nm and 260 nm; NMT 0.10 between 270 nm and 340 nm
Curve: The spectrum shows a steadily descending curve with no observable peaks or shoulders.

Change to read:

- **• CLARITY OF SOLUTION**

[NOTE—The *Sample solution* is to be compared to *Standard suspension A* and to water in diffused daylight 5 min after preparation of *Standard suspension A*.]

Hydrazine solution: 10 mg/mL of hydrazine sulfate in water. Allow to stand for 4–6 h.
Methenamine solution: Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.
Primary opalescent suspension: Transfer 25.0 mL of *Hydrazine solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.
Opalescence standard: Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. This suspension should not be used beyond 24 h after preparation.
Standard suspension A: *Opalescence standard* and water (1 in 20)
Standard suspension B: *Opalescence standard* and water (1 in 10)
Sample solution A: Substance to be examined
Sample solution B: Dilute 1.0 mL of *Sample solution A* with water to 20 mL, and allow to stand for 5 min before testing.
Blank: Water
Analysis: Transfer a sufficient portion of *Sample solution A* and *Sample solution B* to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension A*, *Standard suspension B*, and *Blank* to separate matching test tubes. Compare *Sample solution A*, *Sample solution B*, *Standard suspension A*, *Standard suspension B*, and *Blank* in diffused daylight, viewing vertically against a black background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)). The diffusion of light must be such that *Standard suspension A* can readily be distinguished from water, and *Standard suspension B* can readily be distinguished from *Standard suspension A*.
Acceptance criteria: *Sample solution A* and *Sample solution B* show the same clarity as that of water or their opalescence is not more pronounced than that of *Standardsuspension A*. •

- **• ACIDITY OR ALKALINITY**

Phenolphthalein solution: Dissolve 0.1 g of phenolphthalein in 80 mL of alcohol, and dilute with water to 100 mL.
Sample: 20 mL of Alcohol
Analysis: To the *Sample* add 20 mL of freshly boiled and cooled water and 0.1 mL of *Phenolphthalein solution*. The solution is colorless. Add 1.0 mL of 0.01 N sodium hydroxide.
Acceptance criteria: The solution is pink (30 µL/L, expressed as acetic acid).

USP Monographs

www.webofpharma.com

Change to read:

• **•COLOR OF SOLUTION**
Standard stock solution: Combine 3.0 mL of ferric chloride CS, 3.0 mL of cobaltous chloride CS, 2.4 mL of cupric sulfate CS, and 1.6 mL of dilute hydrochloric acid (10 g/L).
Standard solution: Transfer 1.0 mL of *Standard stock solution* to a 100-mL volumetric flask, and dilute with dilute hydrochloric acid (10 g/L). Prepare the *Standard solution* immediately before use.
Sample solution: Substance to be examined
Blank: Water
Analysis: Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of the *Standard solution* and *Blank* to separate, matching test tubes. Compare the *Sample solution, Standard solution,* and *Blank* in diffused daylight, viewing vertically against a white background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)).
Acceptance criteria: The *Sample solution* has the appearance of water or is not more intensely colored than the *Standard solution*.•

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Alcohol RS

Dehydrated Alcohol

Portions of this monograph that are national *USP* text, and are not part of the harmonized text, are marked with symbols (•,) to specify this fact.



C_2H_6O 46.07
Ethanol;
Ethyl alcohol [64-17-5].

DEFINITION
•Dehydrated Alcohol contains NLT 99.2% by weight, corresponding to NLT 99.5% by volume, at 15.56°, of C_2H_5OH.•

IDENTIFICATION
• **A.** It meets the requirements of the test for *Specific Gravity* ⟨841⟩.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*:**197F OR 197S**▲ (CN 1-May-2020): Neat

IMPURITIES
• **LIMIT OF NONVOLATILE RESIDUE**
Sample: 100 mL of Dehydrated Alcohol
Analysis: Evaporate the *Sample* in a tared dish on a water bath, and dry at 100°–105° for 1 h.
Acceptance criteria: The weight of the residue is NMT 2.5 mg.
• **ORGANIC IMPURITIES**
Sample solution A: Substance to be examined
Sample solution B: 300 µL/L of 4-methylpentan-2-ol in *Sample solution A*
Standard solution A: 200 µL/L of methanol in *Sample solution A*
Standard solution B: 10 µL/L of methanol and 10 µL/L of acetaldehyde in *Sample solution A*
Standard solution C: 30 µL/L of acetal in *Sample solution A*
Standard solution D: 2 µL/L of benzene in *Sample solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m fused-silica capillary; bonded with a 1.8-µm layer of phase G43
Split ratio: 20:1
Temperatures
Injection port: 200°
Detector: 280°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	12
40	10	240	10

Flow rate: 35 cm/s
Carrier gas: Helium
Injection volume: 1.0 µL
System suitability
Sample: *Standard solution B*
Suitability requirements
Resolution: NLT 1.5 between the first major peak (acetaldehyde) and the second major peak (methanol)
Analysis
Samples: *Sample solution A, Sample solution B, Standard solution A, Standard solution B, Standard solution C,* and *Standard solution D*
Methanol calculation

$$\text{Result} = r_U/r_S$$

r_U = peak area of methanol from *Sample solution A*
r_S = peak area of methanol from *Standard solution A*

Acetaldehyde calculation (sum of acetaldehyde and acetal)

$$\text{Result} = \{[A_E/(A_T - A_E)] \times C_A\} + \{[D_E/(D_T - D_E)] \times C_D \times (M_{r1}/M_{r2})\}$$

A_E = peak area of acetaldehyde from *Sample solution A*
A_T = peak area of acetaldehyde from *Standard solution B*
C_A = concentration of acetaldehyde in *Standard solution B* (µL/L)
D_E = peak area of acetal from *Sample solution A*
D_T = peak area of acetal from *Standard solution C*
C_D = concentration of acetal in *Standard solution C* (µL/L)
M_{r1} = molecular weight of acetaldehyde, 44.05
M_{r2} = molecular weight of acetal, 118.2

Benzene calculation

$$\text{Result} = [B_E/(B_T - B_E)] \times C_B$$

USP Monographs

www.webofpharma.com

B_E = peak area of benzene from *Sample solution A*
B_T = peak area of benzene from *Standard solution D*
C_B = concentration of benzene in *Standard solution D* (µL/L)

[NOTE—If necessary, the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).]

Any other impurity calculation

$$\text{Result} = (r_U/r_M) \times C_M$$

r_U = peak area of each impurity from *Sample solution B*
r_M = peak area of 4-methylpentan-2-ol from *Sample solution B*
C_M = concentration of 4-methylpentan-2-ol in *Sample solution B* (µL/L)

Acceptance criteria: See *Table 2*.

Table 2

Name	Acceptance Criteria
Methanol	NMT 0.5, corresponding to 200 µL/L
Acetaldehyde and acetal	NMT 10 µL/L, expressed as acetaldehyde
Benzene	NMT 2 µL/L
Sum of all other impurities[a]	NMT 300 µL/L

[a] Disregard any peaks of less than 9 µL/L (0.03 times the area of the peak corresponding to 4-methylpentan-2-ol in the chromatogram obtained with *Sample solution B*).

SPECIFIC TESTS

• **‣ SPECIFIC GRAVITY ⟨841⟩:** NMT 0.7962 at 15.56°, indicating NLT 99.2% of C_2H_5OH by weight.‣

• **ULTRAVIOLET ABSORPTION**
Analytical wavelength: 235–340 nm
Cell: 5 cm
Reference: Water
Acceptance criteria
Absorbance: NMT 0.40 at 240 nm; NMT 0.30 between 250 and 260 nm; NMT 0.10 between 270 and 340 nm
Curve: The spectrum shows a steadily descending curve with no observable peaks or shoulders.

Change to read:

• **‣CLARITY OF SOLUTION**
[NOTE—The *Sample solution* is to be compared to *Standard suspension A* and to water in diffused daylight 5 min after preparation of *Standard suspension A*.]
Hydrazine solution: 10 mg/mL of hydrazine sulfate in water. Allow to stand for 4–6 h.
Methenamine solution: Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.
Primary opalescent suspension: Transfer 25.0 mL of *Hydrazine solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.
Opalescence standard: Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. This suspension should not be used beyond 24 h after preparation.
Standard suspension A: Dilute 5.0 mL of the *Opalescence standard* with water to 100.0 mL.
Standard suspension B: Dilute 10.0 mL of the *Opalescence standard* with water to 100.0 mL.
Sample solution A: Substance to be examined
Sample solution B: 1.0 mL of *Sample solution A* diluted with water to 20 mL. Allow to stand for 5 min before testing.
Blank: Water
Analysis
Samples: *Standard suspension A, Standard suspension B, Sample solution A, Sample solution B,* and *Blank*
Transfer a sufficient portion of *Sample solution A* and *Sample solution B* to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension A, Standard suspension B,* and *Blank* to separate matching test tubes. Compare samples in diffused daylight, viewing vertically against a black background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)). The diffusion of light must be such that *Standard suspension A* can be readily distinguished from water, and *Standard suspension B* can be readily distinguished from *Standard suspension A*.
Acceptance criteria: *Sample solution A* and *Sample solution B* show the same clarity as that of water, or their opalescence is not more pronounced than that of *Standard suspension A*.‣

• **ACIDITY OR ALKALINITY**
Phenolphthalein solution: Dissolve 0.1 g of phenolphthalein in 80 mL of alcohol, and dilute with water to 100 mL.
Sample: 20 mL of Dehydrated Alcohol
Analysis: To the *Sample* add 20 mL of freshly boiled and cooled water and 0.1 mL of *Phenolphthalein solution*. The solution is colorless. Add 1.0 mL of 0.01 N sodium hydroxide.
Acceptance criteria: The solution is pink (30 µg/g, expressed as acetic acid).

Change to read:

• **‣COLOR OF SOLUTION**
Standard stock solution: Combine 3.0 mL of ferric chloride CS, 3.0 mL of cobaltous chloride CS, 2.4 mL of cupric sulfate CS, and 1.6 mL of dilute hydrochloric acid (10 mg/mL).
Standard solution: 1.0 mL of *Standard stock solution*, diluted with dilute hydrochloric acid (10 mg/mL) to 100 mL. Prepare the *Standard solution* immediately before use.
Sample solution: Substance to be examined
Blank: Water
Analysis
Samples: *Standard solution, Sample solution,* and *Blank*
Transfer a sufficient portion of each of the *Samples* to individual test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Compare the *Samples* in diffused daylight, viewing vertically against a white background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)).
Acceptance criteria: The *Sample solution* has the appearance of water or is not more intensely colored than the *Standard solution*.‣

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dehydrated Alcohol RS

Dehydrated Alcohol Injection

» Dehydrated Alcohol Injection is Dehydrated Alcohol suitable for parenteral use.

Packaging and storage—Preserve in tight, single-dose containers, preferably of Type I glass, and store at controlled room temperature. The container may contain an inert gas in the headspace.

Identification—
A: Mix 5 drops in a small beaker with 1 mL of potassium permanganate solution (1 in 100) and 5 drops of 2 N sulfuric acid, and cover the beaker immediately with a filter paper moistened with a solution recently prepared by dissolving 0.1 g of sodium nitroferricyanide and 0.25 g of piperazine in 5 mL of water: an intense blue color is produced on the filter paper, the color becoming paler after a few minutes.
B: To 5 mL of a solution (1 in 10) add 1 mL of 1.0 N sodium hydroxide, then slowly (over a period of 3 minutes) add 2 mL of 0.1 N iodine: the odor of iodoform develops, and a yellow precipitate is formed within 30 minutes.
Specific gravity ⟨841⟩**:** not more than 0.8035 at 15.56°, indicating not less than 96.8%, by weight, of C_2H_5OH.
Acidity—To 50 mL, in a glass-stoppered flask, add 50 mL of recently boiled water. Add phenolphthalein TS, and titrate with 0.020 N sodium hydroxide to a pink color that persists for 30 seconds: not more than 10.0 mL of 0.020 N sodium hydroxide is required for neutralization.
Limit of nonvolatile residue—Evaporate 40 mL in a tared dish on a water bath, and dry at 105° for 1 hour: the weight of the residue does not exceed 1 mg.
Water-insoluble substances—Dilute it with an equal volume of water: the mixture is clear and remains clear for 30 minutes after cooling to 10°.
Aldehydes and other foreign organic substances—Place 20 mL in a glass-stoppered cylinder that has been thoroughly cleaned with hydrochloric acid, then rinsed with water and finally with the dehydrated alcohol to be tested. Cool the contents to approximately 15°, and add, by means of a carefully cleaned pipet, 0.10 mL of 0.10 N potassium permanganate, noting accurately the time of addition. Mix at once by inverting the stoppered cylinder, and allow it to stand at 15° for 5 minutes: the pink color does not entirely disappear.
Amyl alcohol and nonvolatile, carbonizable substances—Allow 25 mL to evaporate spontaneously from a porcelain dish, carefully protected from dust, until the surface of the dish is barely moist: no red or brown color is produced immediately upon the addition of a few drops of sulfuric acid.
Ultraviolet absorbance—Record the UV absorption spectrum between 340 nm and 235 nm in a 1-cm cell, with water in a matched cell in the reference beam: the absorbance is not more than 0.08 at 240 nm, and 0.02 between 270 nm and 340 nm, and the curve drawn through these points is smooth.
Limit of acetone and isopropyl alcohol—To 1.0 mL add 1 mL of water, 1 mL of a saturated solution of dibasic sodium phosphate, and 3 mL of a saturated solution of potassium permanganate. Warm the mixture to 45° to 50°, and allow to stand until the permanganate color is discharged. Add 3 mL of 2.5 N sodium hydroxide, and pass, without washing, through a sintered-glass filter. Prepare a control containing 1 mL of the saturated solution of dibasic sodium phosphate, 3 mL of 2.5 N sodium hydroxide, and 80 µg of acetone in 9 mL. To each solution add 1 mL of furfural solution (1 in 100), and allow to stand for 10 minutes, then to 1.0 mL of each solution add 3 mL of hydrochloric acid: any pink color produced in the test solution is not more intense than that in the control.
Methanol—To 1 drop add 1 drop of water, 1 drop of dilute phosphoric acid (1 in 20), and 1 drop of potassium permanganate solution (1 in 20). Mix, allow to stand for 1 minute, and add sodium metabisulfite solution (1 in 20), dropwise, until the permanganate color is discharged. If a brown color remains, add 1 drop of the dilute phosphoric acid. To the colorless solution add 5 mL of freshly prepared chromotropic acid TS, and heat on a water bath at 60° for 10 minutes: no violet color appears.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Alcohol in Dextrose Injection

DEFINITION
Alcohol in Dextrose Injection is a sterile solution of Alcohol and Dextrose in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of alcohol (C_2H_5OH), and NLT 95.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$).

IDENTIFICATION
- **A.**
  Sample solution: A few drops of Injection (1 in 20) in water
  Analysis: Add the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.
  Acceptance criteria: A copious red precipitate of cuprous oxide is formed.
- **B. SPECIFIC GRAVITY** ⟨841⟩**:** NMT 0.7962 at 15.56°, indicating NLT 99.2% of alcohol (C_2H_5OH) by weight

Change to read:

- **C. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S** or **197F**▲ (CN 1-May-2020)**:** Meets the requirements

ASSAY
- **ALCOHOL DETERMINATION,** *Method 1—Distillation Method* ⟨611⟩
  Sample solution: 50.0 mL
  Acceptance criteria: 90.0%–110.0%
- **DEXTROSE**
  Sample: Equivalent to 2–5 g of dextrose from a suitable volume of injection
  Analysis: Transfer the *Sample solution* to a 100-mL volumetric flask, add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume. Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).
  Calculate the percentage of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(M_{r1}/M_{r2})/R_{mid}]A\ R \times 100$$

M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

USP Monographs

www.webofpharma.com

R_{mid} = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
A = 100 mm divided by the length of the polarimeter tube (mm)
R = observed rotation (°)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Sample solution: Equivalent to 2 mg/mL of dextrose in water from a suitable volume of Injection

Instrumental conditions

Mode: UV

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Acceptance criteria: The absorbance is NMT 0.25.

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.5–6.5

Sample solution: A portion of Injection to which 0.30 mL of saturated potassium chloride solution has been added for each 100 mL and which previously has been diluted with water, if necessary, to a concentration of NMT 5% of dextrose

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin unit/mL

• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, single-dose containers, preferably of Type I or Type II glass, and store at controlled room temperature.

• **LABELING:** The label states the total osmolarity of the solution expressed in mOsmol/L.

Rubbing Alcohol

DEFINITION

Rubbing Alcohol and all preparations under the classification of Rubbing Alcohols are manufactured in accordance with the requirements of the U.S. Treasury Department, Bureau of Alcohol, Tobacco, and Firearms, Formula 23-H (8 parts by volume of acetone, 1.5 parts by volume of methyl isobutyl ketone, and 100 parts by volume of ethyl alcohol) being used. It contains NLT 68.5% and NMT 71.5% by volume of dehydrated alcohol, the remainder consisting of water and the denaturants, with or without color additives, and perfume oils. Rubbing Alcohol contains, in each 100 mL, NLT 355 mg of sucrose octaacetate or NLT 1.40 mg of denatonium benzoate. The preparation may be colored with one or more color additives, listed by the FDA for use in drugs. A suitable stabilizer may be added. Rubbing Alcohol complies with the requirements of the Bureau of Alcohol, Tobacco, and Firearms of the U.S. Treasury Department.

[NOTE—Rubbing Alcohol is packaged, labeled, and sold in accordance with the regulations issued by the U.S. Treasury Department, Bureau of Alcohol, Tobacco, and Firearms.]

ASSAY

• **DENATONIUM BENZOATE**

Buffer: 9.23 g of anhydrous dibasic sodium phosphate in 800 mL of water. Adjust with saturated citric acid solution to a pH of 4 ± 0.1, dilute with water to 1000 mL, and mix.

Standard solution: 50 µg/mL of USP Denatonium Benzoate RS in water

Sample solution: Dissolve the residue obtained in the test for *Limit of Nonvolatile Residue* in 50.0 mL of water, and transfer to a suitable flask.

Instrumental conditions

Analytical wavelength: Maximum absorbance at about 410 nm

Cell: 1 cm

Analysis

Samples: *Buffer*, *Standard solution*, and *Sample solution*

Transfer 10.0 mL each of *Buffer*, *Standard solution*, and *Sample solution* to individual 250-mL separators. Add to each 40 mL of *Buffer* 10 mL of a 1-in-1000 solution of bromophenol blue in chloroform and 60 mL of chloroform. Shake the separators vigorously for 2 min, allow to stand for 15 min, then withdraw the chloroform layers through chloroform-washed cotton into 100-mL volumetric flasks. Repeat the extraction with 20 mL of chloroform, adding the filtered chloroform extracts to the respective volumetric flasks, and dilute with chloroform to volume. Without delay, concomitantly determine the absorbances of the solutions, using the blank to set a suitable spectrophotometer.

Calculate the quantity, in mg, of denatonium benzoate ($C_{28}H_{34}N_2O_3 \cdot H_2O$) in 100 mL of Rubbing Alcohol:

$$\text{Result} = (A_U/A_S) \times C_S \times 0.025$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Denatonium Benzoate RS in the *Standard solution* (µg/mL)

Acceptance criteria: NLT 1.40 mg

• **SUCROSE OCTAACETATE**

Sample solution: Using about 50 mL of 70% alcohol, transfer the residue obtained in the test for *Limit of Nonvolatile Residue* to a 500-mL conical flask.

Analysis: Neutralize the *Sample solution* with 0.1 N sodium hydroxide VS, using phenolphthalein TS as the indicator. Add 25.0 mL of 0.1 N sodium hydroxide, attach an air condenser to the flask, and reflux on a steam bath for 1 h. Remove from the steam bath, cool quickly, and titrate the excess alkali with 0.1 N sulfuric acid VS, using phenolphthalein TS as the indicator. Perform a blank determination (see *Titrimetry* ⟨541⟩, *Residual Titrations*). Each mL of 0.1 N sodium hydroxide is equivalent to 8.483 mg of sucrose octaacetate ($C_{28}H_{38}O_{19}$).

Acceptance criteria: NLT 355 mg of sucrose octaacetate per 100 mL of Rubbing Alcohol

IMPURITIES

• **METHANOL**

Sample solution: Dilute 0.50 mL of Rubbing Alcohol with water to 1.0 mL.

Analysis: To 0.50 mL of the *Sample solution* add 1 drop of dilute phosphoric acid (1 in 20) and 1 drop of potassium permanganate solution (1 in 20). Mix, allow to stand for 1 min, and add dropwise sodium metabisulfite solution (1 in 20) until the permanganate color is discharged. If a brown color remains, add 1 drop of dilute phosphoric acid (1 in 20). To the colorless solution add 5 mL of freshly prepared chromotropic acid TS, and heat in a water bath at 60° for 10 min.

Acceptance criteria: No violet color appears.

SPECIFIC TESTS

• **SPECIFIC GRAVITY** ⟨841⟩: 0.8691–0.8771 at 15.56° (the U.S. government standard temperature for alcohol

USP Monographs

www.webofpharma.com

determination) for Rubbing Alcohol manufactured with specially denatured alcohol Formula 23-H

• **LIMIT OF NONVOLATILE RESIDUE**

Where the denaturant is denatonium benzoate

Sample: 200.0 mL of Rubbing Alcohol

Analysis: Evaporate the *Sample*, transferred in convenient portions, in a suitable tared dish on a steam bath, and dry the residue at 105° for 1 h. Retain the residue for the *Assay* for *Denatonium Benzoate*.

Acceptance criteria: The weight of the residue is NLT 2.8 mg.

Where the denaturant is sucrose octaacetate

Sample: 25.0 mL of Rubbing Alcohol

Analysis: Evaporate the *Sample* in a suitable tared dish on a steam bath, and dry the residue at 105° for 1 h. Retain the residue for the *Assay* for *Sucrose Octaacetate*.

Acceptance criteria: The weight of the residue is NLT 89 mg.

ADDITIONAL REQUIREMENTS

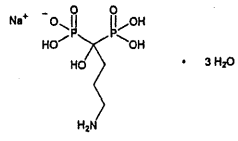
• **PACKAGING AND STORAGE:** Preserve in tight containers, remote from fire, and store at controlled room temperature.

• **LABELING:** Label it to indicate that it is flammable.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Denatonium Benzoate RS

Alendronate Sodium



$C_4H_{12}NNaO_7P_2 \cdot 3H_2O$ 325.12

Phosphonic acid, (4-amino-1-hydroxybutylidene) bis-, monosodium salt, trihydrate;

Sodium trihydrogen (4-amino-1-hydroxybutylidene) diphosphonate, trihydrate [121268-17-5].

DEFINITION

Alendronate Sodium contains NLT 98.0% and NMT 102.0% of alendronate sodium ($C_4H_{12}NNaO_7P_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*: Meets the requirements of test *A*

ASSAY

• **PROCEDURE**

Buffer solution: 14.7 g/L of sodium citrate dihydrate and 7.05 g/L of anhydrous dibasic sodium phosphate. Adjust with phosphoric acid to a pH of 8.

Mobile phase: Acetonitrile, methanol, and *Buffer solution* (25:5:70)

Diluent: 29.4 g/L of sodium citrate dihydrate

Borate solution: 19.1 g/L of sodium borate

Solution A: 0.5 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [NOTE—Prepare this solution fresh just before use.]

Standard stock solution: 0.1 mg/mL of USP Alendronate Sodium RS in *Diluent*

Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of *Solution A*, and shake for 30 s. Allow to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min. Use a portion of the clear upper aqueous layer.

Reagent blank: Transfer 5.0 mL of *Diluent* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of *Solution A*, and shake for 30 s. Allow to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min. Use a portion of the clear upper aqueous layer.

Sample stock solution: 0.1 mg/mL of Alendronate Sodium in *Diluent*

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of *Solution A*, and shake for 30 s. Allow to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min. Use a portion of the clear upper aqueous layer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 266 nm

Column: 4.1-mm × 25-cm; packing L21

Column temperature: 35°

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution*, *Reagent blank*, and *Sample solution*

Calculate the percentage of alendronate sodium ($C_4H_{12}NNaO_7P_2$) in the portion of Alendronate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Alendronate Sodium RS in the *Standard stock solution* (mg/mL)

C_U = concentration of Alendronate Sodium in the *Sample stock solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer solution: 2.94 g/L of sodium citrate dihydrate and 1.42 g/L of anhydrous dibasic sodium phosphate. Adjust with phosphoric acid to a pH of 8 and pass through a filter of 0.5-µm or finer pore size.

Solution A: Acetonitrile and *Buffer solution* (3:17)

Solution B: Acetonitrile and *Buffer solution* (7:3)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
15	50	50
25	0	100
27	100	0
32	100	0

Diluent and **Borate solution:** Proceed as directed in the *Assay*.
Solution C: 4 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [NOTE—Prepare this solution fresh just before use.]
Standard stock solution: 0.6 mg/mL of USP Alendronate Sodium RS in *Diluent*
Standard solution A: Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *Solution C*, and shake for 45 s. Allow to stand at room temperature for 30 min. Add 20 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min, and use a portion of the clear upper aqueous layer.
Standard solution B: 0.6 µg/mL of USP Alendronate Sodium RS in *Diluent* from *Standard stock solution*. Transfer 5 mL of this diluted solution (0.6 µg/mL) to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *Solution C*, and shake for 45 s. Allow to stand at room temperature for 30 min. Add 20 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min, and use a portion of the clear upper aqueous layer.
Reagent blank: Transfer 5.0 mL of *Diluent* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *Solution C*, and shake for 45 s. Allow to stand at room temperature for 30 min. Add 20 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min, and use a portion of the clear upper aqueous layer.
Sample stock solution: 0.6 mg/mL of Alendronate Sodium in *Diluent*
Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *Solution C*, and shake for 45 s. Allow to stand at room temperature for 30 min. Add 20 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min, and use a portion of the clear upper aqueous layer.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 266 nm
Column: 4.1-mm × 25-cm; packing L21
Column temperature: 45°
Flow rate: 1.8 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution A* and *Standard solution B*
Suitability requirements
Tailing factor: NMT 2.0 for the main peak, *Standard solution A*
Signal-to-noise ratio: NLT 3 for the main peak, *Standard solution B*
Analysis
Samples: *Reagent blank* and *Sample solution*
[NOTE—Disregard any peak corresponding to those obtained from the *Reagent blank*.]

Calculate the percentage of each impurity in the portion of Alendronate Sodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each impurity
r_T = sum of all impurity peaks and the main peak

Acceptance criteria
Individual impurities: NMT 0.1%
Total impurities: NMT 0.5%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Sample: Dry at a pressure of NMT 5 mm of mercury at 140° to constant weight.
Acceptance criteria: 16.1%–17.1%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Alendronate Sodium RS

Alendronate Sodium Tablets

DEFINITION
Alendronate Sodium Tablets contain an amount of Alendronate Sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of alendronic acid ($C_4H_{13}NO_7P_2$).

IDENTIFICATION
• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 14.7 g/L of sodium citrate dihydrate and 7.05 g/L of anhydrous dibasic sodium phosphate in water. [NOTE—Adjust with phosphoric acid to a pH of 8.0 before bringing the solution to volume.]
Solution B: 38.1 g/L of sodium borate in water
Solution C: 1 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [NOTE—Prepare this solution fresh just before use.]
Mobile phase: Acetonitrile, methanol, and *Solution A* (20:5:75)
Diluent: 29.4 g/L of sodium citrate dihydrate in water
Standard stock solution: 0.03 mg/mL of anhydrous alendronate sodium in *Diluent*, from USP Alendronate Sodium RS
Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene screw-cap centrifuge tube containing 5 mL of *Solution B*, and mix for 3 min. Add 4 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 10 min. Use the clear upper aqueous layer.
Sample stock solution: Transfer NLT 10 Tablets to a 1000-mL volumetric flask. Add 500 mL of *Diluent*, shake by mechanical means for 30 min, and sonicate for 5 min. Dilute with *Diluent* to volume, and centrifuge a portion of this solution. Quantitatively dilute a portion of the clear supernatant to a concentration of 0.02–0.03 mg/mL of alendronic acid.

USP Monographs

www.webofpharma.com

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 50-mL polypropylene screw-cap centrifuge tube containing 5 mL of *Solution B*, and mix for 3 min. Add 4 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 10 min. Use the clear upper aqueous layer.

Blank: Transfer 5 mL of *Diluent* to a 50-mL polypropylene screw-cap centrifuge tube containing 5 mL of *Solution B*, and mix for 3 min. Add 4 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 10 min. Use the clear upper aqueous layer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 266 nm

Column: 4.1-mm × 25-cm; packing L21

Column temperature: 35°

Flow rate: 1 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor: NLT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of the label claim in the portion of $C_4H_{13}NO_7P_2$ taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of anhydrous USP Alendronate Sodium RS in the *Standard stock solution* (mg/mL)

C_U = nominal concentration of alendronic acid in the *Sample stock solution* (mg/mL)

M_{r1} = molecular weight of alendronic acid, 249.10

M_{r2} = molecular weight of anhydrous alendronate sodium, 271.09

Acceptance criteria: $C_4H_{12}NNaO_7P_2$ equivalent to 90.0%–110.0% of the labeled amount of $C_4H_{13}NO_7P_2$

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 15 min

Determine the amount of $C_4H_{13}NO_7P_2$ dissolved by using the following method.

Solution A and **Mobile phase:** Proceed as directed in the *Assay*.

Diluent: 176.4 g/L of sodium citrate in *Medium*

Solution B: Dissolve 6.2 g of boric acid in approximately 950 mL of water. Adjust with 1 N sodium hydroxide to a pH of 9.0, and dilute with water to 1 L.

Solution C: 0.5 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [NOTE—Prepare this solution fresh.]

Standard stock solution: USP Alendronate Sodium RS in *Medium* to make a concentration equivalent to dissolving 1 Tablet in 900 mL of the same *Medium*. Calculate the concentration, C (mg/mL), of anhydrous alendronate sodium in this solution.

Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene screw-cap centrifuge tube containing 1.0 mL of *Diluent* and 5.0 mL of *Solution B*, and mix for 3 min. Add 4.0 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 5 min. Use a portion of the clear, upper aqueous layer.

Blank: Transfer 5 mL of water to a 50-mL polypropylene screw-cap centrifuge tube containing 1.0 mL of *Diluent* and 5.0 mL of *Solution B*, and mix for 3 min. Add 4.0 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 5 min. Use a portion of the clear, upper aqueous layer.

Sample solution: Withdraw a portion of the solution under test, and centrifuge immediately. Transfer 5.0 mL of the supernatant to a 50-mL polypropylene screw-cap centrifuge tube containing 1.0 mL of *Diluent* and 5.0 mL of *Solution B*, and mix for 3 min. Add 4.0 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 5 min. Use a portion of the clear, upper aqueous layer.

Chromatographic system and **System suitabilitity:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_4H_{13}NO_7P_2$ dissolved:

$$\text{Result} = (r_U/r_S) \times C \times (M_{r1}/M_{r2}) \times V \times (100/L)$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C = defined under the *Standard stock solution*

M_{r1} = molecular weight of alendronic acid, 249.10

M_{r2} = molecular weight of alendronate sodium, 271.09

V = volume of the *Medium*, 900 mL

L = Tablet label claim (mg)

Tolerances: NLT 80% (Q) of the labeled amount of $C_4H_{13}NO_7P_2$ is dissolved; for tablets labeled for weekly dosing, NLT 75% (Q) of the labeled amount of $C_4H_{13}NO_7P_2$ is dissolved.

Test 2

If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Determine the amount of $C_4H_{12}NNaO_7P_2 \cdot 3H_2O$ dissolved using the following method.

Solution B and **Solution C:** Proceed as directed in the *Assay*.

0.6 M citrate buffer: 176.4 g/L of sodium citrate dihydrate in water

0.05 M buffer: Transfer 14.7 g of sodium citrate dihydrate and 7.05 g of anhydrous dibasic sodium phosphate to a 1000-mL volumetric flask, dissolve in about 900 mL of water, adjust with phosphoric acid to a pH of 8.0, and dilute with water to volume.

Mobile phase: *0.05 M buffer*, acetonitrile, and methanol (76:19:5)

Standard stock solution: Prepare a solution of USP Alendronate Sodium RS in *Medium* with a final concentration corresponding to the concentration

www.webofpharma.com

obtained by dissolving 1 tablet in 900 mL of *Medium*. Calculate the concentration, C (mg/mL), of anhydrous alendronate sodium in this solution.

Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 50-mL screw-cap polypropylene centrifuge tube containing 1.0 mL of *0.6 M citrate buffer* and 5.0 mL of *Solution B*, and mix for about 3 min. Add 4.0 mL of *Solution C*, and agitate for about 30 s. Allow the solution to stand at room temperature for about 30 min. Add 25 mL of methylene chloride, and agitate vigorously for about 40 s. Centrifuge the mixture for 10 min. Use a portion of the clear upper aqueous layer.

Blank: Using 5 mL of water, proceed as directed for the *Standard solution*, beginning with "to a 50-mL screw-cap polypropylene centrifuge tube".

Sample solution

For Tablets labeled to contain 5 mg, 10 mg, 35 mg, or 40 mg: After 30 min, withdraw 30 mL of the solution under test, and pass through a suitable 0.45-µm filter, discarding the first 10 mL. Using 5.0 mL of the filtrate, proceed as directed for the *Standard solution*, beginning with "to a 50-mL screw-cap polypropylene centrifuge tube".

For Tablets labeled to contain 70 mg: After 30 min, withdraw 30 mL of the solution under test, and pass through a suitable 0.45-µm filter, discarding the first 10 mL. Transfer 6.0 mL of the filtrate to a 10-mL volumetric flask, and dilute with water to volume. Using 5.0 mL of this dilution, proceed as directed for the *Standard solution*, beginning with "to a 50-mL screw-cap polypropylene centrifuge tube".

Chromatographic system and **System suitability:** Proceed as directed in the *Assay*.

Analysis: Proceed as directed in *Test 1*.

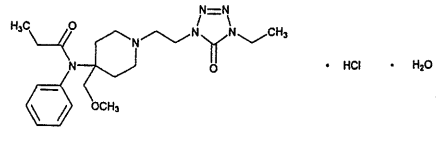
Tolerances: NLT 80% (Q) of the labeled amount of alendronate sodium ($C_4H_{12}NNaO_7P_2 \cdot 3H_2O$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 30°.
- **LABELING:** The labeling indicates weekly dosing where appropriate. When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Alendronate Sodium RS

Alfentanil Hydrochloride



$C_{21}H_{32}N_6O_3 \cdot HCl \cdot H_2O$ 470.99

$C_{21}H_{32}N_6O_3 \cdot HCl$ 452.98

Propanamide, *N*-[1-[2-(4-ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-(methoxymethyl)-4-piperidinyl]-*N*-phenyl, monohydrochloride, monohydrate;
N-[1-[2-(4-Ethyl-5-oxo-2-tetrazolin-1-yl)-ethyl]-4-(methoxymethyl)-4-piperidyl]propionanilide monohydrochloride monohydrate [70879-28-6].
Anhydrous [69049-06-5].

DEFINITION

Alfentanil Hydrochloride contains NLT 98.0% and NMT 102.0% of alfentanil hydrochloride ($C_{21}H_{32}N_6O_3 \cdot HCl$), calculated on the anhydrous basis.

[**CAUTION**—Handle Alfentanil Hydrochloride with great care because it is a potent opioid analgesic. Great care should be taken to prevent inhaling particles of Alfentanil Hydrochloride and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and 0.01 M tetrabutylammonium hydrogen sulfate (14:86)

Standard solution: 0.54 mg/mL of USP Alfentanil Hydrochloride RS in *Mobile phase*

Sample solution: 0.54 mg/mL of Alfentanil Hydrochloride in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.0-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 25 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5400 theoretical plates
Tailing factor: NMT 1.3
Relative standard deviation: NMT 0.73%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of alfentanil hydrochloride ($C_{21}H_{32}N_6O_3 \cdot HCl$) in the portion of Alfentanil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alfentanil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Alfentanil Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase: Acetonitrile and 0.01 M tetrabutylammonium hydrogen sulfate (14:86)

Standard solution: 0.54 mg/mL of USP Alfentanil Hydrochloride RS in *Mobile phase*

Sample solution: 0.54 mg/mL of Alfentanil Hydrochloride in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

www.webofpharma.com

Detector: UV 235 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5400 theoretical plates
Tailing factor: NMT 1.3
Relative standard deviation: NMT 0.73%
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Alfentanil Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria
Any single impurity: NMT 0.5%
Total impurities: NMT 1.0%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 4.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Alfentanil Hydrochloride RS

Alfentanil Injection

DEFINITION
Alfentanil Injection is a sterile solution of Alfentanil Hydrochloride in Water for Injection. It contains an amount of Alfentanil Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of alfentanil ($C_{21}H_{32}N_6O_3$).
[**CAUTION**—Handle Alfentanil Injection with great care because it is a potent opioid analgesic.]

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 0.54 mg/mL of USP Alfentanil Hydrochloride RS
Sample solution: 0.5 mg/mL of alfentanil in water
Chromatographic system
Application volume: 200 µL
Developing solvent system: Chloroform, methanol, and formic acid (85:10:5)
Visualizing agent: Dragendorff's reagent
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in the chapter.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and 0.01 M tetrabutylammonium hydrogen sulfate (14:86)
Standard solution: 0.54 mg/mL of USP Alfentanil Hydrochloride RS in saline TS
Sample solution: Equivalent to 0.50 mg/mL of alfentanil from a suitable volume of Injection in saline TS
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5400 theoretical plates
Tailing factor: NMT 1.3
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of alfentanil ($C_{21}H_{32}N_6O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alfentanil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of alfentanil in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of alfentanil, 416.52
M_{r2} = molecular weight of alfentanil hydrochloride, 452.98

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase: Acetonitrile and 0.01 M tetrabutylammonium hydrogen sulfate (14:86)
Standard solution: 0.54 mg/mL of USP Alfentanil Hydrochloride RS in saline TS
Sample solution: Equivalent to 0.50 mg/mL of alfentanil from a suitable volume of Injection in saline TS
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5400 theoretical plates
Tailing factor: NMT 1.3
Relative standard deviation: NMT 1.0%
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity
r_T = sum of all of the peaks

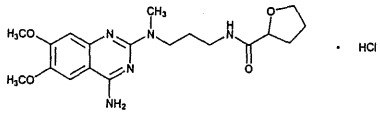
Acceptance criteria: The sum of all impurities is NMT 2.0%.

www.webofpharma.com

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.0–6.0
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 10 USP Endotoxin Units/mL
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, single-dose or multiple-dose containers, preferably of Type I glass, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Alfentanil Hydrochloride RS

Alfuzosin Hydrochloride



$C_{19}H_{27}N_5O_4 \cdot HCl$ 425.91

2-Furancarboxamide, *N*-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl)methylamino]propyl]tetrahydro-, monohydrochloride (±);
(±)-*N*-[3-[(4-Amino-6,7-dimethoxy-2-quinazolinyl) methylamino]propyl]tetrahydro-2-furamide monohydrochloride [81403-68-1].

DEFINITION
Alfuzosin Hydrochloride contains NLT 98.0% and NMT 102.0% of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements
- **C.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

[NOTE—Use low-actinic glassware.]

Solution A: 2 M sodium hydroxide
Solution B: 5.0 mL of perchloric acid in 900 mL of water. Adjust with *Solution A* to a pH of 3.5, and dilute with water to 1000 mL.
Mobile phase: Acetonitrile and *Solution B* (30:70)
Diluent: Methanol and *Solution A* (500:3)
Standard stock solution: 0.5 mg/mL of USP Alfuzosin Hydrochloride RS in *Diluent*
Standard solution: 0.04 mg/mL of USP Alfuzosin Hydrochloride RS in *Mobile phase* from the *Standard stock solution*; the solution is stable for 14 h at 5°.
Sample stock solution: 0.5 mg/mL of Alfuzosin Hydrochloride in *Diluent*
Sample solution: 0.04 mg/mL of Alfuzosin Hydrochloride in *Mobile phase* from the *Sample stock solution*; the solution is stable for 14 h at 5°.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Autosampler temperature: 5°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0%
Tailing factor: NMT 1.5
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) in the portion of Alfuzosin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Alfuzosin Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer: Dilute 5 mL of perchloric acid in 900 mL of water. Adjust with 2 M sodium hydroxide to a pH of 3.5, and dilute with water to 1 L.
Mobile phase: Acetonitrile, tetrahydrofuran, and *Buffer* (20:1:80)
System suitability solution: 0.4 mg/mL of USP Alfuzosin System Suitability Mixture RS in *Mobile phase*
Sample solution: 0.40 mg/mL of Alfuzosin Hydrochloride in *Mobile phase*
Reference solution: 0.40 µg/mL of Alfuzosin Hydrochloride in *Mobile phase* from the *Sample solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Peak-to-valley ratio: The ratio of the height of the furamide analog peak to the height of the valley between the furamide analog peak and alfuzosin is NLT 5.
Analysis
Samples: *Sample solution* and *Reference solution*
Calculate the percentage of each impurity in the portion of Alfuzosin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (1/D) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of alfuzosin from the *Reference solution*
D = dilution factor between the *Sample solution* and the *Reference solution*, 1000

www.webofpharma.com

Acceptance criteria: See *Table 1*. [NOTE—Disregard any peak with an area less than 0.05%.]

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deacylatedalfuzosin[a]	0.5	0.20
Alfuzosin	1.0	—
Furamide analog[b]	1.2	—[c]
Any other individual, unidentified impurity	—	0.10
Total impurities	—	0.30

[a] N^2-(3-Aminopropyl)-6,7-dimethoxy-N^2-methylquinazoline-2,4-diamine.
[b] *N*-{3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl}furan-2-carboxamide.
[c] Furamide analog, a component of USP Alfuzosin System Suitability Mixture RS, is not a specified impurity.

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781⟩
  Sample solution: 20 mg/mL in carbon dioxide-free water
  Acceptance criteria: −0.10° to +0.10°
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.0%
- **pH** ⟨791⟩
  Sample solution: 20 mg/mL in carbon dioxide-free water
  Acceptance criteria: 4.0–5.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from light and humidity, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Alfuzosin Hydrochloride RS
  USP Alfuzosin System Suitability Mixture RS
  Alfuzosin Hydrochloride containing approximately 0.4% furamide analog (*N*-{3-[(4-amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl}furan-2-carboxamide), and about 0.4% deacylated alfuzosin (N^2-(3-aminopropyl)-6,7-dimethoxy-N^2-methylquinazoline-2,4-diamine).

Alfuzosin Hydrochloride Extended-Release Tablets

DEFINITION

Alfuzosin Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K.▲ (CN 1-May-2020)
  Sample: Grind 4 Tablets, and add 20 mL of water. [NOTE—When analyzing multi-layer Tablets, isolate the layer containing alfuzosin hydrochloride using a suitable tool.] Add 20 mL of strong ammonia solution. Extract with 20 mL of methylene chloride, and separate the organic layer. Repeat the extraction successively with 20 mL, then with 10 mL of methylene chloride. Wash the combined organic layers with 20 mL of water. Dry the organic solution using a phase separation filter. Take 2.0 mL of the dried organic solution, and mix with 200 mg of finely ground potassium bromide. Evaporate the methylene chloride at 60°, then at 105° for 30 min. Make a disk. Alternatively, evaporate methylene chloride from the dried organic solution at 60°, then at 105° for 30 min. Perform the IR spectrum.
  Acceptance criteria: The maxima of the spectrum obtained from the *Sample* correspond in position and relative intensity to those obtained from USP Alfuzosin Hydrochloride RS, treated in the same manner as the *Sample*, beginning with "add 20 mL of water."
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Solution A: 5.0 mL of perchloric acid in 900 mL of water. Adjust with 2 M sodium hydroxide to a pH of 3.5, and dilute with water to 1000 mL.
  Mobile phase: Acetonitrile, tetrahydrofuran, and *Solution A* (20:1:80)
  Diluent: 0.01 N hydrochloric acid
  Standard stock solution: 0.15 mg/mL of USP Alfuzosin Hydrochloride RS in methanol
  Standard solution: 0.03 mg/mL of USP Alfuzosin Hydrochloride RS in *Diluent* from the *Standard stock solution*
  Sample stock solution: Place a suitable number of Tablets into a suitable volumetric flask to obtain a solution having a concentration of 0.16 mg/mL of alfuzosin hydrochloride. Add 80% of the flask volume of methanol, and stir for at least 1 h using a magnetic stirrer. Add 10% of the flask volume of *Diluent*, mix, and allow it to cool to room temperature. Dilute the resulting suspension with methanol to volume, stir, and allow to settle for 30 min.
  Sample solution: 0.03 mg/mL of alfuzosin hydrochloride in *Diluent* from the *Sample stock solution* supernatant. Pass through a suitable filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: 0.8–1.5 for alfuzosin
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

www.webofpharma.com

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.01 N hydrochloric acid; 500 mL

Apparatus 2: 100 rpm, with Tablet holder (see *Figure 1. 37.5-mm (l) × 20-mm (d) stainless steel cylinders are used as sample holders. The cylinders contain screw caps drilled with seven 4.5-mm holes. Seven 4.5-mm holes are drilled in the bottom, and 12 longitudinal series of five 5-mm holes are drilled on the cylinders, alternatively starting and ending with one 1.7-mm hole.*)

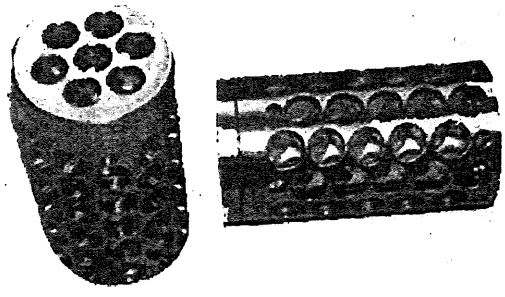


Figure 1. 37.5-mm (l) × 20-mm (d) stainless steel cylinders are used as sample holders. The cylinders contain screw caps drilled with seven 4.5-mm holes. Seven 4.5-mm holes are drilled in the bottom, and 12 longitudinal series of five 5-mm holes are drilled on the cylinders, alternatively starting and ending with one 1.7-mm hole.

Times: 1, 6, 12, and 20 h

Sample solution: Pass a portion of the solution under test through a suitable filter.

Standard solution: (*L*/500) mg/mL of USP Alfuzosin Hydrochloride RS in *Medium*, where *L* is the Tablet label claim in mg

Detector: UV 330 nm

Blank: *Medium*

Path length: 1 cm

Tolerances: See *Table 1*.

Table 1

Level	Time (h)	Amount Dissolved (%)
		Each Tablet:
	1	10–20
	6	40–55
	12	65–85
L1	20	NLT 85
		Average of 12 Tablets complies with L1 and each Tablet:
	1	9–22
	6	36–61
	12	59–94
L2	20	NLT 77

Table 1 *(continued)*

Level	Time (h)	Amount Dissolved (%)
		Average of 24 Tablets complies with L1, NMT 2 Tablets outside L2, and all Tablets within:
	1	8–24
	6	32–66
	12	52–102
L3	20	NLT 68

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 100 rpm

Times: 1, 3, 12, and 24 h

Buffer: Dilute 5.0 mL of perchloric acid in 900 mL of water, adjust with diluted sodium hydroxide (0.1 g/mL) to a pH of 3.5 ± 0.5, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (25:75)

Standard stock solution: 0.28 mg/mL of USP Alfuzosin Hydrochloride RS, prepared as follows. In a 200-mL volumetric flask dissolve 55.5 mg of USP Alfuzosin Hydrochloride RS in 5 mL of methanol, sonicate to dissolve, and dilute with *Medium* to volume.

Standard solution: 0.011 mg/mL of USP Alfuzosin Hydrochloride RS in *Medium* from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter. Replace the portion of solution withdrawn with an equal volume of *Medium*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 244 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 3000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

Supplement 1

www.webofpharma.com

$$Result_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of alfuzosin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	NMT 20
2	3	15–35
3	12	50–70
4	24	NLT 80

The percentages of the labeled amount of alfuzosin hydrochloride dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.25% sodium dodecyl sulfate in 0.05 M sodium phosphate buffer, pH 6.8 (2.5 g/L of sodium dodecyl sulfate, 6.9 g/L of monobasic sodium phosphate monohydrate, and 0.83 g/L of sodium hydroxide in water previously degassed with helium. Adjust with either phosphoric acid or 1 N sodium hydroxide to a pH of 6.8 ± 0.05); 900 mL
Apparatus 1: 100 rpm
Times: 1, 6, 12, and 24 h
Standard stock solution: 1.1 mg/mL of USP Alfuzosin Hydrochloride RS in methanol
Standard solution: 0.011 mg/mL of USP Alfuzosin Hydrochloride RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 331 nm, background correction at 490 nm
Blank: *Medium*
Cell: 1.0 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) dissolved at each time point (*i*):

$$Result_i = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium* (mL)
L = label claim (mg/Tablet)

Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	NMT 15
2	6	20–40
3	12	45–70
4	24	NLT 80

The percentages of the labeled amount of alfuzosin hydrochloride dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 100 rpm
Times: 1, 6, 12, and 20 h
Standard solution: 0.01 mg/mL of USP Alfuzosin Hydrochloride RS in *Medium*
Sample solution: Centrifuge a portion of the solution under test.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 245 nm
Blank: *Medium*
Path length: 0.2 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$Result_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) dissolved at each time point (*i*):

$$Result_1 = C_1 \times V \times (1/L) \times 100$$

$$Result_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$Result_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of alfuzosin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 4*.

www.webofpharma.com

Table 4

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	NMT 30
2	6	40–60
3	12	65–85
4	20	NLT 80

The percentages of the labeled amount of alfuzosin hydrochloride dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 100 rpm

Times: 1, 3, 6, 12, and 20 h

Buffer: Add 1 mL of triethylamine in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.5 ± 0.05. Pass through a suitable filter of 0.45-µm pore size.

Mobile phase: Methanol and *Buffer* (40:60)

Standard stock solution: 0.55 mg/mL of USP Alfuzosin Hydrochloride RS. Prepare by transferring a portion of USP Alfuzosin Hydrochloride RS to a suitable flask. Add methanol to 20% of the flask volume, and sonicate at room temperature to dissolve. Dilute with *Medium* to volume.

Standard solution: 0.011 mg/mL of USP Alfuzosin Hydrochloride RS in *Medium* from the *Standard stock solution*. Pass through a suitable filter of 0.45-µm pore size.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 2000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_5 = (\{C_5 \times [V - (4 \times V_S)]\} + [(C_4 + C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of alfuzosin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 5*.

Table 5

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	NMT 20
2	3	20–40
3	6	35–55
4	12	60–80
5	20	NLT 80

The percentages of the labeled amount of alfuzosin hydrochloride dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 100 rpm. Adjust the paddle height to 4.5 cm above the bottom of the vessel and use a 10# mesh basket as sinker.

Times: 1, 6, 12, and 20 h

Buffer: 2.3 g/L of anhydrous dibasic sodium phosphate and 1.75 g/L of monobasic potassium phosphate

Mobile phase: Acetonitrile and *Buffer* (50:50)

Standard stock solution: 0.45 mg/mL of USP Alfuzosin Hydrochloride RS. Prepare by transferring a portion of USP Alfuzosin Hydrochloride RS to a suitable flask. Add 20% of the flask volume of water. Sonicate to dissolve, and dilute with *Medium* to volume.

Standard solution: 0.011 mg/mL of USP Alfuzosin Hydrochloride RS in *Medium* from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter. Replace with the same volume of *Medium*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 3500 theoretical plates

Relative standard deviation: NMT 2.0%

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of alfuzosin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 6*.

Table 6

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 30
2	6	45–65
3	12	70–90
4	20	NLT 85

The percentages of the labeled amount of alfuzosin hydrochloride dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 100 rpm with sinker; see *Dissolution* ⟨711⟩, *Figure 2a*.
Times: 1, 6, 12, and 20 h
Solution A: 500 g/L of sodium hydroxide
Solution B: 100 g/L of sodium hydroxide
Buffer: Add 5 mL of perchloric acid in 1000 mL of water. Adjust with *Solution A* to a pH of 2.5, then adjust with *Solution B* to a pH of 3.50 ± 0.05. Pass through a suitable filter of 0.45-µm pore size.
Mobile phase: Acetonitrile and *Buffer* (24:76)
Standard solution: 0.01 mg/mL of USP Alfuzosin Hydrochloride RS in *Medium*
Sample solution: Centrifuge or pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 245 nm
Column: 4.6-mm × 5-cm; 3-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 1.5 times the retention time of alfuzosin
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alfuzosin hydrochloride ($C_{19}H_{27}N_5O_4 \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of alfuzosin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 7*.

Table 7

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 25
2	6	40–60
3	12	65–85
4	20	NLT 85

The percentages of the labeled amount of alfuzosin hydrochloride dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A, Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

USP Monographs

www.webofpharma.com

System suitability stock solution: 0.4 mg/mL of USP Alfuzosin System Suitability Mixture A RS in methanol
System suitability solution: 0.03 mg/mL of USP Alfuzosin System Suitability Mixture A RS in *Diluent* from the *System suitability stock solution*
Standard stock solution: 0.15 mg/mL of USP Alfuzosin Hydrochloride RS in methanol
Standard solution: 0.03 mg/mL of USP Alfuzosin Hydrochloride RS in *Diluent* from the *Standard stock solution*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between alfuzosin and the furamide analog; NLT 1.0 between deacylated alfuzosin and the *N*-formyl analog, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of alfuzosin from the *Sample solution*
C_S = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of alfuzosin hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 8*

Table 8

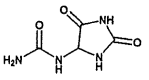
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deacylated alfuzosin[a]	0.46	0.40
N-Formyl analog[b]	0.50	0.30
Alfuzosin	1.0	—
Furamide analog[c]	1.18	—[d]
Any individual unspecified impurity	—	0.20
Total impurities	—	0.80

[a] *N*²-(3-Aminopropyl)-6,7-dimethoxy-*N*²-methylquinazoline-2,4-diamine.
[b] *N*-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl] formamide.
[c] *N*-{3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl}furan-2-carboxamide.
[d] Furamide analog, a component of USP Alfuzosin System Suitability Mixture A RS, is not a specified impurity.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Protect from light and moisture. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Alfuzosin Hydrochloride RS
  USP Alfuzosin System Suitability Mixture A RS
  Furamide analog: *N*-{3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl}furan-2-carboxamide.
  $C_{19}H_{23}N_5O_4$ 385.42
  Deacylated alfuzosin: *N*²-(3-Aminopropyl)-6,7-dimethoxy-*N*²-methylquinazoline-2,4-diamine.
  $C_{14}H_{21}N_5O_2$ 291.35
  N-Formyl analog: *N*-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl] formamide.
  $C_{15}H_{21}N_5O_3$ 319.36

Allantoin



$C_4H_6N_4O_3$ 158.12
Urea, (2,5-dioxo-4-imidazolidinyl)-;
Allantoin [97-59-6].

DEFINITION

Allantoin contains NLT 98.5% and NMT 101.0% of $C_4H_6N_4O_3$.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩: The R_F value of the principal spot from *Sample solution B* corresponds to that from *Standard solution A*, as described in the test for *Organic Impurities*.

ASSAY

- **PROCEDURE**
  Sample: 120 mg
  Analysis: Transfer the *Sample* to a 100-mL beaker, dissolve by stirring in 40 mL of water, and titrate with 0.1 M sodium hydroxide. Use a suitable electrode system (see *Titrimetry* ⟨541⟩). Each mL of 0.1 M sodium hydroxide is equivalent to 15.81 mg of $C_4H_6N_4O_3$.
  Acceptance criteria: 98.5%–101.0%

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Adsorbent: Cellulose
  Diluent: Methanol and water (1:1)
  Urea stock solution: 1 mg/mL of USP Urea RS in water
  Standard solution A: 1 mg/mL of USP Allantoin RS in *Diluent*
  Standard solution B: 0.1 mg/mL of USP Urea RS in methanol, from *Urea stock solution*
  Standard solution C: *Standard solution A* and *Standard solution B* (1:1)
  Sample solution A: Transfer 0.10 g of Allantoin to a 10-mL volumetric flask, add 5 mL of water, dissolve by heating, and allow to cool. Dilute with methanol to volume. [NOTE—Use immediately after preparation.]
  Sample solution B: Transfer 1 mL of *Sample solution A* to a 10-mL volumetric flask, and dilute with *Diluent* to volume.
  Spray reagent: 5 mg/mL of *p*-dimethylaminobenzaldehyde in a mixture of methanol and hydrochloric acid (3:1)
  Application volume
  Standard solution A: 5 µL
  Standard solution B: 5 µL
  Standard solution C: 5 µL
  Sample solution A: 10 µL
  Sample solution B: 5 µL

USP Monographs

www.webofpharma.com

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (60:15:25)
Analysis: Proceed as directed for *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*. Develop the chromatogram until the solvent front has moved about 10 cm. Spray the plate with *Spray reagent*, dry in a current of hot air, and after 30 min examine under visible light.
Acceptance criteria: Any spot from *Sample solution A*, except for the principal spot, is not more intense than the spot from *Standard solution B* (NMT 0.5%). The test is not valid unless the principal spots from *Standard solution C* are clearly separated.

SPECIFIC TESTS

- **ACIDITY OR ALKALINITY**

Sample solution: 5 mg/mL in carbon dioxide-free water
Analysis: To 5 mL of the *Sample solution* add 5 mL of water, 0.1 mL of methyl red TS, and 0.2 mL of 0.01 M sodium hydroxide.
Acceptance criteria: A yellow color is observed. The solution turns red upon the addition of 0.4 mL of 0.01 M hydrochloric acid.

- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° to constant weight: it loses NMT 0.1% of its weight.
- **REDUCING SUBSTANCES**

Sample solution: 1.0 g of Allantoin in 10 mL of water. Shake for 2 min, and filter.
Analysis: To the *Sample solution* add 1.5 mL of 0.02 M potassium permanganate.
Acceptance criteria: The solution remains violet for at least 10 min.

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Allantoin RS
USP Urea RS

Allopurinol



$C_5H_4N_4O$ 136.11
4*H*-Pyrazolo[3,4-*d*]pyrimidin-4-one, 1,5-dihydro-;
1,5-Dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one;
1*H*-Pyrazolo[3,4-*d*]pyrimidin-4-ol [315-30-0].

DEFINITION

Allopurinol contains NLT 98.0% and NMT 102.0% of allopurinol ($C_5H_4N_4O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

▲• **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**

[NOTE—Store and inject the *System suitability solution*, *Standard solution*, and *Sample solution* at 8°, using a cooled autosampler.]
Mobile phase: 1.25-g/L solution of monobasic potassium phosphate in water, filtered and degassed
System suitability solution: 0.5 µg/mL each of USP Allopurinol RS, USP Allopurinol Related Compound B RS, and USP Allopurinol Related Compound C RS, prepared as follows. Transfer weighed quantities of USP Allopurinol RS, USP Allopurinol Related Compound B RS, and USP Allopurinol Related Compound C RS to three separate suitable volumetric flasks, dissolve in a small volume of 0.1 N sodium hydroxide, and immediately dilute with *Mobile phase* to volume to obtain solutions containing 0.05 mg/mL each. Transfer 1.0 mL of each of these three solutions to a 100-mL volumetric flask and dilute with *Mobile phase* to volume.
Standard stock solution: 0.5 mg/mL of USP Allopurinol RS, prepared as follows. Transfer a weighed quantity of USP Allopurinol RS to a suitable volumetric flask, dissolve in a small volume of 0.1 N sodium hydroxide, and immediately dilute with *Mobile phase* to volume.
Standard solution: 0.08 mg/mL of USP Allopurinol RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: 0.5 mg/mL of Allopurinol, prepared as follows. Transfer 50 mg of Allopurinol to a 100-mL volumetric flask, dissolve in 5.0 mL of 0.1 N sodium hydroxide, and immediately dilute with *Mobile phase* to volume.
Sample solution: 0.08 mg/mL of Allopurinol in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.8 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for allopurinol related compound B, allopurinol related compound C, and allopurinol are about 0.7, 0.8, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.1 between allopurinol related compound B and allopurinol related compound C; NLT 6.0 between allopurinol related compound C and allopurinol, *System suitability solution*
Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of allopurinol ($C_5H_4N_4O$) in the portion of Allopurinol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Allopurinol RS in the *Standard solution* (mg/mL)
C_U = concentration of Allopurinol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES**

[NOTE—Store and inject the *Standard solution* and the *Sample solution* at 8°, using a cooled autosampler.]
Solution A: 1.25-g/L solution of monobasic potassium phosphate in water, filtered and degassed
Solution B: Methanol
Mobile phase: See *Table 1*.

www.webofpharma.com

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
30	70	30
35	70	30
36	90	10
46	90	10

Diluent: *Solution A* and *Solution B* (90:10)
Standard stock solution: 0.05 mg/mL each of USP Allopurinol RS, USP Allopurinol Related Compound A RS, USP Allopurinol Related Compound B RS, USP Allopurinol Related Compound C RS, USP Allopurinol Related Compound D RS, and USP Allopurinol Related Compound E RS, prepared as follows. Transfer 5 mg each of USP Allopurinol RS, USP Allopurinol Related Compound A RS, USP Allopurinol Related Compound B RS, USP Allopurinol Related Compound C RS, USP Allopurinol Related Compound D RS, and USP Allopurinol Related Compound E RS to a 100-mL volumetric flask. Add 2.0 mL of 0.1 N sodium hydroxide, and promptly sonicate with swirling for NMT 1 min to dissolve. Add 80 mL of *Diluent*, and sonicate for an additional 5 min. Dilute with *Diluent* to volume. [NOTE—This solution is stable for 48 h when stored at 8°.]
Standard solution: 0.5 µg/mL each of USP Allopurinol RS, USP Allopurinol Related Compound A RS, USP Allopurinol Related Compound B RS, USP Allopurinol Related Compound C RS, USP Allopurinol Related Compound D RS, and USP Allopurinol Related Compound E RS in *Diluent* from the *Standard stock solution*
Sample solution: 0.25 mg/mL of Allopurinol, prepared as follows. Transfer 25 mg of Allopurinol to a 100-mL volumetric flask. Add 5.0 mL of 0.1 N sodium hydroxide to dissolve, promptly sonicate with swirling for NMT 1 min, add 80 mL of *Diluent*, and sonicate for an additional 5 min. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 40 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 0.8 between allopurinol related compound C and allopurinol related compound B
Tailing factor: NMT 1.5 for the allopurinol peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of allopurinol related compounds A, B, C, D, and E in the portion of Allopurinol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of each individual impurity from the *Standard solution*
C_S = concentration of each individual impurity in the *Standard solution* (mg/mL)
C_U = concentration of Allopurinol in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Allopurinol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of allopurinol from the *Standard solution*
C_S = concentration of USP Allopurinol RS in the *Standard solution* (mg/mL)
C_U = concentration of Allopurinol in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Allopurinol related compound A	0.62	0.2
Allopurinol related compound C	0.79	0.2
Allopurinol related compound B	0.81	0.2
Allopurinol	1.0	—
Allopurinol related compound D	4.4	0.2
Allopurinol related compound E	4.8	0.2
Ethyl-(*E*/*Z*)-3-(2-carbethoxy-2-cyanoethenyl) amino-1*H*-pyrazole-4-carboxylate	6.5	0.2
Unspecified impurity	—	0.1
Total impurities	—	1.0

• **LIMIT OF HYDRAZINE**
[NOTE—Under the following conditions, any hydrazine present in the sample will react with benzaldehyde to form benzalazine.]
Mobile phase: Hexane and isopropyl alcohol (95:5)
2 N sodium hydroxide solution: Dissolve 8.5 g of sodium hydroxide in water, and dilute with the same solvent to 100 mL. Alternatively, a commercially available 2 N sodium hydroxide solution can be used.
Diluent: Methanol and *2 N sodium hydroxide solution* (1:1)
Benzaldehyde solution: 40 mg/mL of benzaldehyde in *Diluent*. [NOTE—Prepare immediately before use.]
Hydrazine solution: 2.0 µg/mL of hydrazine sulfate in *Diluent*. Use sonication if necessary.
Standard solution: Transfer 5.0 mL of *Hydrazine solution* to a suitable flask and add 4 mL of *Benzaldehyde solution*. Mix and allow to stand for 2.5 h at room temperature. Add 5.0 mL of hexane, and shake for 1 min. Allow the layers to separate, and use the upper (hexane) layer.
Allopurinol solution: Dissolve 250 mg of Allopurinol in 5 mL of *Diluent*.
Sample solution: Transfer the *Allopurinol solution* to a suitable flask, and add 4 mL of *Benzaldehyde solution*. Mix, and allow to stand for 2.5 h at room temperature. Add 5.0

www.webofpharma.com

mL of hexane, and shake for 1 min. Allow the layers to separate, and use the upper (hexane) layer.

Blank solution: Mix 5.0 mL of *Diluent* and 4 mL of *Benzaldehyde solution*, and allow to stand for 2.5 h at room temperature. Add 5.0 mL of hexane, and shake for 1 min. Allow the layers to separate, and use the upper (hexane) layer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 310 nm

Column: 4.0-mm × 25-cm; 5-µm packing L10

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for benzalazine and benzaldehyde are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between benzalazine and benzaldehyde

Relative standard deviation: NMT 15.0% for the benzalazine peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount, in ppm, of hydrazine in the portion of Allopurinol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times F$$

r_U = peak response of benzalazine from the *Sample solution*

r_S = peak response of benzalazine from the *Standard solution*

C_S = concentration of hydrazine sulfate in the *Hydrazine solution* (µg/mL)

C_U = concentration of Allopurinol in the *Allopurinol solution* (mg/mL)

M_{r1} = molecular weight of hydrazine, 32.05

M_{r2} = molecular weight of hydrazine sulfate, 130.12

F = unit conversion factor (from µg/mg to ppm), 1000

Acceptance criteria: NMT 10 ppm of hydrazine

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 105° for 5 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Allopurinol RS

USP Allopurinol Related Compound A RS
3-Amino-4-carboxamidopyrazole hemisulfate.
▲$(C_4H_6N_4O)_2 \cdot H_2SO_4$ 350.31▲ (ERR 1-Dec-2018)

USP Allopurinol Related Compound B RS
5-(Formylamino)-1*H*-pyrazole-4-carboxamide.
$C_5H_6N_4O_2$ 154.13

USP Allopurinol Related Compound C RS
5-(4*H*-1,2,4-Triazol-4-yl)-1*H*-pyrazole-4-carboxamide.
$C_6H_6N_6O$ 178.15

USP Allopurinol Related Compound D RS
Ethyl 5-amino-1*H*-pyrazole-4-carboxylate.
$C_6H_9N_3O_2$ 155.15

USP Allopurinol Related Compound E RS
Ethyl 5-(formylamino)-1*H*-pyrazole-4-carboxylate.
$C_7H_9N_3O_3$ 183.16

Allopurinol Compounded Oral Suspension

DEFINITION

Change to read:

Allopurinol Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of allopurinol ($C_5H_4N_4O$).

Prepare Allopurinol Compounded Oral Suspension 20 mg/mL ▲in Vehicle for Oral Suspension and Vehicle for Oral Solution▲ (USP 1-May-2020) as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Allopurinol tablets equivalent to	2 g of allopurinol
Glycerin	5 mL
Vehicle for Oral Suspension ▲▲ (USP 1-May-2020)	45 mL
Vehicle for Oral Solution, ▲▲ (USP 1-May-2020) a sufficient quantity to make	100 mL

Select the number of tablets that contain the specified amount of allopurinol, and calculate the quantity of each ingredient required for the total amount to be prepared. Count, weigh, or measure each ingredient. Thoroughly pulverize the tablets. Mix the powdered *Allopurinol tablets* and *Glycerin* to form a smooth paste. Incorporate ▲in▲ (USP 1-May-2020) *Vehicle for Oral Suspension*▲ and *Vehicle for Oral Solution*.▲ (USP 1-May-2020) Add sufficient *Vehicle for Oral Solution* to volume, and mix well. Adjust the pH, if necessary. Package and label.

▲Prepare Allopurinol Compounded Oral Suspension 20 mg/mL in *SyrSpend SF PH4* as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

Allopurinol powder	2 g
SyrSpend SF PH4,[a] a sufficient quantity to make	100 mL

[a] Fagron, St. Paul, MN.

Transfer the *Allopurinol powder* into a suitable container and triturate to form a fine powder. Wet the powder with a small amount of *SyrSpend SF PH4*, and triturate to make a smooth paste. Add increasing volumes of *SyrSpend SF PH4* to make the contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the remainder of *SyrSpend SF PH4*. Add sufficient *SyrSpend SF PH4* to bring to final volume, and mix well.▲ (USP 1-May-2020)

www.webofpharma.com

ASSAY

Add the following:

▲• **ORAL SUSPENSION IN SYRSPEND SF PH4**
Mobile phase: 0.05 M solution of monobasic ammonium phosphate
Standard solution: 0.02 mg/mL of USP Allopurinol RS in acetonitrile
Sample solution: Transfer 1.0 mL of Oral Suspension into a 1000-mL volumetric flask and dilute with acetonitrile to final volume. Mix well.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Columns
Guard: 4.0-mm × 3.0-mm; 5-µm packing L1
Analytical: 4.0-mm × 30-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for allopurinol is about 6.5 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of allopurinol ($C_5H_4N_4O$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of allopurinol from the *Sample solution*
r_S = peak response of allopurinol from the *Standard solution*
C_S = concentration of USP Allopurinol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of allopurinol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% ▲(USP 1-May-2020)

SPECIFIC TESTS

Change to read:

• **PH** ⟨791⟩
▲**Oral Suspension in Vehicle for Oral Suspension and Vehicle for Oral Solution:** ▲(USP 1-May-2020) 6.5–7.5
▲**Oral Suspension in *SyrSpend SF PH4*:** 3.5–4.5 ▲(USP 1-May-2020)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** ▲Package in tight, light-resistant containers. Store the Oral Suspension in Vehicle for Oral Suspension and Vehicle for Oral Solution at controlled room temperature. Store the Oral Suspension in *SyrSpend SF PH4* at controlled room temperature or in a refrigerator. ▲(USP 1-May-2020)

Change to read:

• **BEYOND-USE DATE**
▲**Oral Suspension in Vehicle for Oral Suspension and Vehicle for Oral Solution:** ▲(USP 1-May-2020) NMT 60 days after the date on which it was compounded when stored at controlled room temperature
▲**Oral Suspension in *SyrSpend SF PH4*:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator ▲(USP 1-May-2020)
• **LABELING:** Label it to state that it is to be shaken well before use, and to state the *Beyond-Use Date.*

Add the following:

▲• **USP REFERENCE STANDARDS** ⟨11⟩
USP Allopurinol RS ▲(USP 1-May-2020)

Allopurinol Tablets

» Allopurinol Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of allopurinol ($C_5H_4N_4O$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Allopurinol RS
Identification—Extract a quantity of finely powdered Tablets, equivalent to about 50 mg of allopurinol, by trituration with 10 mL of 0.1 N sodium hydroxide. Filter, acidify the filtrate with 1 N acetic acid, collect the precipitated allopurinol (allow 10 to 15 minutes for sufficient precipitation to occur), wash the precipitate with 3 mL of dehydrated alcohol, in portions, and finally wash with 4 mL of anhydrous ethyl ether. Allow to dry in air for 15 minutes, then dry at 105° for 3 hours: the residue so obtained meets the requirements for the *Identification* test under *Allopurinol.*

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 2: 75 rpm.
Time: 45 minutes.
Standard stock solution—Prepare a stock solution by transferring about 40 mg of USP Allopurinol RS, accurately weighed, to a 200-mL volumetric flask. Add 10 mL of 0.1 N sodium hydroxide, sonicate for about 2 minutes, shake by mechanical means for about 10 minutes, dilute with *Dissolution Medium* to volume, and mix.
Standard solution—Dilute the *Standard stock solution* with *Dissolution Medium* to obtain a solution having a concentration similar to that expected in the solution under test.
Procedure—Determine the amount of $C_5H_4N_4O$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 250 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium,* in comparison with the *Standard solution.*
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_5H_4N_4O$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay— [NOTE—Do not allow the *Mobile phase* to remain in the column overnight. After performing the procedure, flush

www.webofpharma.com

the system with water for not less than 20 minutes, and then flush with methanol for 20 minutes.]

Mobile phase—Prepare a filtered and degassed 0.05 M solution of monobasic ammonium phosphate.

Internal standard solution—On the day of use, dissolve about 50 mg of hypoxanthine in 10 mL of 0.1 N sodium hydroxide, shake by mechanical means until dissolved (about 10 minutes), dilute with water to 50 mL, and mix.

Standard preparation—On the day of use, transfer about 50 mg of USP Allopurinol RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of 0.1 N sodium hydroxide, shake by mechanical means for 10 minutes, dilute with water to volume, and mix. Transfer 4.0 mL of this solution and 2.0 mL of *Internal standard solution* to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of allopurinol, to a 50-mL volumetric flask, add 10 mL of 0.1 N sodium hydroxide, shake by mechanical means for 10 minutes, add water to volume, and mix. [NOTE—From this point, conduct the remainder of the *Assay* without delay.] Filter, rejecting the first 10 mL of the filtrate. Transfer 4.0 mL of the filtrate and 2.0 mL of *Internal standard solution* to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

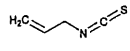
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for hypoxanthine and 1.0 for allopurinol; the resolution, *R,* between the analyte and internal standard is not less than 5; and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of allopurinol ($C_5H_4N_4O$) in the portion of Tablets taken by the formula:

$$2.5C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Allopurinol RS in the *Standard preparation;* and R_U and R_S are the peak response ratios of allopurinol to hypoxanthine obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Allyl Isothiocyanate



C_4H_5NS 99.15

3-Isothiocyanato-1-propene;
Isothiocyanic acid allyl ester [57-06-7].

DEFINITION

Allyl Isothiocyanate contains NLT 93.0% and NMT 105.0% of allyl isothiocyanate (C_4H_5NS).

[CAUTION—Allyl Isothiocyanate is a potent lachrymator, with a pungent, irritating odor. Care should be taken to protect the eyes, to prevent inhalation of fumes, and to avoid tasting.]

IDENTIFICATION

Change to read:

- **A.** **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197F**▲ (CN 1-May-2020)**:** The spectrum exhibits pronounced peaks at about 700, 950, 980, 1300, 1340, 1350, 1410, 1420, 1650, 2100, and 2200 cm^{-1}.

ASSAY

- **PROCEDURE**

Sample solution: Transfer 4 mL into a 100-mL volumetric flask, and dilute with alcohol to volume.

Analysis: Transfer 5.0 mL of the *Sample solution* to a 100-mL conical flask, and add 50.0 mL of 0.1 N silver nitrate VS and 5 mL of ammonia TS. Connect the flask to a reflux condenser, heat on a water bath for 1 h, and allow to cool to room temperature. Disconnect the flask from the condenser, transfer the contents of the conical flask to a 100-mL volumetric flask with the aid of water, and dilute with water to volume. Pass through a dry filter, discarding the first 10 mL of the filtrate. To 50.0 mL of the subsequent filtrate add 5 mL of nitric acid and 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Perform a blank determination, using 5 mL of alcohol in place of the *Sample solution,* and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 4.958 mg of allyl isothiocyanate (C_4H_5NS).

Acceptance criteria: 93.0%–105.0%

IMPURITIES

- **LIMIT OF PHENOLS**

Sample solution: Dilute a 1-mL sample with 5 mL of alcohol.

Analysis: Add 1 drop of ferric chloride TS to the *Sample solution.*

Acceptance criteria: A blue color is not produced immediately.

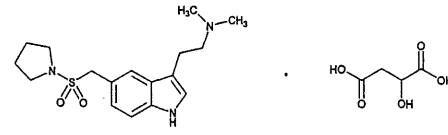
SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩**:** 1.013–1.020
- **REFRACTIVE INDEX** ⟨831⟩**:** 1.527–1.531, determined at 20°
- **DISTILLING RANGE,** *Method I* ⟨721⟩**:** 148°–154°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Almotriptan Malate



$C_{17}H_{25}N_3O_2S \cdot C_4H_6O_5$ 469.55

Pyrrolidine, 1-[[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl] methyl]sulfonyl]-, hydroxybutanedioate (1:1);
1-[({3-[2-(Dimethylamino)ethyl]indol-5-yl}methyl)sulfonyl] pyrrolidine malate (1:1) [181183-52-8].

DEFINITION

Almotriptan Malate contains NLT 98.0% and NMT 102.0% of almotriptan malate ($C_{17}H_{25}N_3O_2S \cdot C_4H_6O_5$), calculated on the anhydrous and solvent-free basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 2.72 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Methanol and *Buffer* (40:60)
System suitability solution: 0.14 mg/mL each of USP Almotriptan Malate RS and USP Almotriptan Related Compound B RS in *Mobile phase*. Sonication may be used to promote dissolution.
Standard solution: 0.14 mg/mL of USP Almotriptan Malate RS in *Mobile phase*. Sonication may be used to promote dissolution.
Sample solution: 0.14 mg/mL of Almotriptan Malate in *Mobile phase*. Sonication may be used to promote dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 5-µm packing L10
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of almotriptan
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for almotriptan related compound B and almotriptan are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between almotriptan and almotriptan related compound B, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.85% for six injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of almotriptan malate ($C_{17}H_{25}N_3O_2S \cdot C_4H_6O_5$) in the portion of Almotriptan Malate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of almotriptan from the *Sample solution*
r_S = peak response of almotriptan from the *Standard solution*
C_S = concentration of USP Almotriptan Malate RS in the *Standard solution* (mg/mL)
C_U = concentration of Almotriptan Malate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.10%

- **LIMIT OF ALMOTRIPTAN RELATED COMPOUND D AND ALMOTRIPTAN *N*-DIMER**

Run buffer: 23.5 g/L of phosphoric acid in water. Adjust with triethanolamine to a pH of 3.0 and pass through a suitable filter of 0.45-µm pore size.
Diluent: Methanol and water (50:50)
Internal standard solution: 0.01 mg/mL of 4-hydroxy-4-phenylpiperidine in *Diluent*
System suitability solution: 0.005 mg/mL each of USP Almotriptan Related Compound B RS, USP Almotriptan Related Compound D RS, and USP Almotriptan Malate RS in the *Internal standard solution*. Pass through a suitable filter of 0.45-µm pore size.
Standard stock solution: 0.5 mg/mL of USP Almotriptan Malate RS in *Diluent*
Standard solution: 0.005 mg/mL of USP Almotriptan Malate RS from the *Standard stock solution* in the *Internal standard solution*. Pass through a suitable filter of 0.45-µm pore size.
Sample solution: 2.5 mg/mL of Almotriptan Malate in the *Internal standard solution*. Sonication may be used to promote dissolution. Pass the solution through a suitable filter of 0.45-µm pore size.
Capillary rinsing procedure: Use separate *Run buffer* vials for the capillary rinse and sample analysis. Condition the capillary by rinsing with water, 0.1 N sodium hydroxide, water, and the *Run buffer* before each injection. [NOTE—It may be suitable to rinse with water, 0.1 N sodium hydroxide, and water using a pressure of 20 psi for NLT 2 min each and then to rinse with the *Run buffer* using a pressure of 20 psi for NLT 5 min.]
Instrumental conditions
Mode: CE
Detector: UV 214 nm
Capillary, Capillary effective length, Capillary temperature, and **Voltage:** Use parameters described under *A* or *B* as indicated in *Table 1*.

Table 1

Parameter	A	B
Capillary	75-µm × 48.5-cm; uncoated fused silica	75-µm × 60-cm; uncoated fused silica
Capillary effective length	40 cm	47 cm
Capillary temperature	15°	25°
Voltage	A voltage of 15.5 kV may be suitable.	A voltage of 15.0 kV may be suitable.

Injection sequence: 0.5 psi for 8 s for the *Sample solution*, followed by 0.5 psi for 1 s for the *Run buffer*
Run time: NLT 2.5 times the migration time of almotriptan
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative migration times.]
Suitability requirements
Resolution: NLT 2.0 between almotriptan related compound B and almotriptan; NLT 2.0 between almotriptan and almotriptan related compound D, *System suitability solution*
Relative standard deviation: NMT 5.0% for the ratio of the peak response of almotriptan to the peak response of the internal standard, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the corrected peak response:

$$\text{Result} = (r/m)$$

www.webofpharma.com

r = peak response
m = migration time of the peak (min)

Calculate the percentage of almotriptan related compound D, almotriptan *N*-dimer, and other impurities in the portion of Almotriptan Malate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = corrected peak response ratio of the impurity to the internal standard from the *Sample solution*
R_S = corrected peak response ratio of almotriptan to the internal standard from the *Standard solution*
C_S = concentration of USP Almotriptan Malate RS in the *Standard solution* (mg/mL)
C_U = concentration of Almotriptan Malate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Migration Time	Acceptance Criteria, NMT (%)
Almotriptan *N*-dimer[a]	0.71	0.3
Internal standard[b]	0.78	—
Almotriptan related compound B[c]	0.92	—
Almotriptan	1.0	—
Almotriptan related compound C[c]	1.02	—
Almotriptan related compound D	1.22	0.1
Any individual unspecified impurities	—	0.1

[a] 2-{1-({3-[2-(Dimethylamino)ethyl]-1*H*-indol-5-yl}methyl)-5-[(pyrrolidin-1-ylsulfonyl)methyl]-1*H*-indol-3-yl}-*N*,*N*-dimethylethan-1-amine.
[b] 4-Hydroxy-4-phenylpiperidine.
[c] If present, this impurity may not be fully resolved from almotriptan. It is quantified using the test for *Organic Impurities*.

• **ORGANIC IMPURITIES**

Buffer: Add 10 mL of triethylamine to every 1000 mL of 0.01 M phosphoric acid. Adjust with phosphoric acid to a pH of 6.5.
Mobile phase: Acetonitrile and *Buffer* (15:85)
System suitability stock solution: 0.5 mg/mL each of USP Almotriptan Related Compound B RS, USP Almotriptan Related Compound C RS, and USP Almotriptan Related Compound D RS in methanol
System suitability solution: 0.005 mg/mL each of USP Almotriptan Related Compound B RS, USP Almotriptan Related Compound C RS, and USP Almotriptan Related Compound D RS from the *System suitability stock solution* in water
Standard solution: 0.007 mg/mL of USP Almotriptan Malate RS in water
Sample solution: 3.5 mg/mL of Almotriptan Malate in water. Sonication may be used to promote dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 30-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 3 times the retention time of almotriptan
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.0 between almotriptan related compound C and almotriptan related compound D, *System suitability solution*
Relative standard deviation: NMT 5.0% for six replicate injections, *Standard solution*
Analysis
Samples: *System suitability solution*, *Standard solution*, and *Sample solution*
Chromatograph the *System suitability solution* and identify the components on the basis of their relative retention times, as shown in *Table 3*.
Calculate the percentage of each impurity in the portion of Almotriptan Malate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of almotriptan from the *Standard solution*
C_S = concentration of USP Almotriptan Malate RS in the *Standard solution* (mg/mL)
C_U = concentration of Almotriptan Malate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Malic acid[a]	0.10	—
Almotriptan related compound B	0.62	0.1
Almotriptan related compound C	0.77	0.5
Almotriptan related compound D[b]	0.92	—
Almotriptan	1.00	—
Any other individual impurity	—	0.1
Total impurities[c]	—	1.0

[a] This peak is due to the malate counterion; hence it is not an impurity. It is not to be reported or included in the total impurities.
[b] This impurity is quantified using the *Limit of Almotriptan Related Compound D and Almotriptan N-Dimer* test.
[c] The sum of all impurities from the test for *Organic Impurities* and the *Limit of Almotriptan Related Compound D and Almotriptan N-Dimer* test.

• **LIMIT OF FUMARIC ACID**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.8.
Mobile phase: Methanol and *Buffer* (5:95)
Standard solution: 0.0085 mg/mL of USP Fumaric Acid RS and 0.0017 mg/mL of USP Maleic Acid RS in water. Sonication may be used to promote dissolution.
Sample solution: 2.8 mg/mL of Almotriptan Malate in water. Sonication may be used to promote dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7

www.webofpharma.com

Flow rate: 0.7 mL/min
Injection volume: 10 µL
Run time: NLT 1.6 times the retention time of fumaric acid
System suitability
Sample: *Standard solution*
[NOTE—See *Table 4* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between fumaric acid and maleic acid
Relative standard deviation: NMT 5.0% for fumaric acid from six injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fumaric acid ($C_4H_4O_4$) in the portion of Almotriptan Malate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fumaric acid from the *Sample solution*
r_S = peak response of fumaric acid from the *Standard solution*
C_S = concentration of USP Fumaric Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of Almotriptan Malate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Malic acid[a]	0.60	—
Maleic acid[a]	0.80	—
Fumaric acid	1.0	0.2

[a] Included for identification purposes only.

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia*: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Almotriptan Malate RS
USP Almotriptan Related Compound B RS
2-{5-[(Pyrrolidin-1-ylsulfonyl)methyl]-1*H*-indol-3-yl} ethanamine hemifumarate.
$C_{15}H_{21}N_3O_2S \cdot ½C_4H_4O_4$ 365.46
USP Almotriptan Related Compound C RS
N-Methyl-2-{5-[(pyrrolidin-1-ylsulfonyl)methyl]-1*H*-indol-3-yl}ethanamine.
$C_{16}H_{23}N_3O_2S$ 321.44
USP Almotriptan Related Compound D RS
1-[({3-[2-(Dimethylamino)ethyl]indol-5-yl}methyl) sulfonyl]pyrrolidine *N*-oxide hydrochloride.
$C_{17}H_{25}N_3O_3S \cdot HCl$ 387.92
USP Fumaric Acid RS
USP Maleic Acid RS

Almotriptan Tablets

DEFINITION
Almotriptan Tablets contain an amount of almotriptan malate ($C_{17}H_{25}N_3O_2S \cdot C_4H_6O_5$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of almotriptan ($C_{17}H_{25}N_3O_2S$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F▲** (CN 1-May-2020)
Sample: Sonicate 5 powdered Tablets in 25 mL of water. Extract the suspension with 25 mL of methylene chloride, and discard the organic phase. Add another 25 mL of methylene chloride and 3 mL of 1 N sodium hydroxide. Extract the precipitated base into the organic layer. Dry with anhydrous sodium sulfate, and evaporate the organic solvent. Prepare the residue oil as a film on a sodium chloride pellet.
Acceptance criteria: The IR spectrum obtained from the *Sample* is consistent with that of similarly prepared USP Almotriptan Malate RS.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Protect samples, the Reference Standards, and solutions containing them from light.
Buffer: Add 10 mL of triethylamine to every 1000 mL of 0.01 M phosphoric acid. Adjust with phosphoric acid to a pH of 6.0.
Mobile phase: Acetonitrile and *Buffer* (10:90)
Standard solution: 0.5 mg/mL of USP Almotriptan Malate RS in *Mobile phase*. Sonication may be used to aid in dissolution.
System suitability stock solution: 0.1 mg/mL each of USP Almotriptan Related Compound B RS, USP Almotriptan Related Compound C RS, and USP Almotriptan Related Compound D RS in methanol. Sonication may be used to aid in dissolution.
System suitability solution: 0.001 mg/mL each of USP Almotriptan Related Compound B RS, USP Almotriptan Related Compound C RS, and USP Almotriptan Related Compound D RS prepared from the *System suitability stock solution* in *Standard solution*
Sample solution: Nominally 0.5 mg/mL of almotriptan from Tablets prepared as follows. Transfer NLT 8 Tablets into a suitable volumetric flask, and add 80% of the flask volume of *Mobile phase*. Sonicate for NLT 10 min, and dilute with *Mobile phase* to volume. Stir for 30 min, and centrifuge. Pass a portion of the supernatant through a suitable filter of 0.45-µm pore size. Use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 2.1-mm × 10-cm; 1.8-µm packing L1
Column temperature: 40°
Flow rate: 0.55 mL/min
Injection volume: 3 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—See *Table 1* for the relative retention times.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 1.5 between almotriptan related compound C and almotriptan peaks, *System suitability solution*
Tailing factor: NMT 3.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of almotriptan ($C_{17}H_{25}N_3O_2S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of almotriptan from the *Sample solution*
r_S = peak response of almotriptan from the *Standard solution*
C_S = concentration of USP Almotriptan Malate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of almotriptan in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of almotriptan, 335.46
M_{r2} = molecular weight of almotriptan malate, 469.55

Acceptance criteria: 90.0%–110.0% of the labeled amount of almotriptan

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 15 min
Standard solution: (*L*/600) mg/mL of USP Almotriptan Malate RS in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
Mode: UV
Analytical wavelength
For Tablets labeled to contain 6.25 mg: 228 nm
For Tablets labeled to contain 12.5 mg: 284 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of almotriptan ($C_{17}H_{25}N_3O_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Almotriptan Malate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of almotriptan, 335.46
M_{r2} = molecular weight of almotriptan malate, 469.55

Tolerances: NLT 80% (*Q*) of the labeled amount of almotriptan ($C_{17}H_{25}N_3O_2S$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard stock solution: 0.425 mg/mL of USP Almotriptan Malate RS, equivalent to 0.30 mg/mL of almotriptan, in water
Standard solution: 0.021 mg/mL of USP Almotriptan Malate RS, equivalent to 0.015 mg/mL of almotriptan, from the *Standard stock solution* in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and discard the first 5 mL. Use the remaining filtrate.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 283 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of almotriptan ($C_{17}H_{25}N_3O_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Almotriptan Malate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of almotriptan, 335.46
M_{r2} = molecular weight of almotriptan malate, 469.55

Tolerances: NLT 80% (*Q*) of the labeled amount of almotriptan ($C_{17}H_{25}N_3O_2S$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase, Standard solution, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution, System suitability solution,* and *Sample solution*
Chromatograph the *System suitability solution* and identify the components on the basis of their relative retention times, as shown in *Table 1*.
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of almotriptan from the *Standard solution*
C_S = concentration of USP Almotriptan Malate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of almotriptan in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of almotriptan, 335.46
M_{r2} = molecular weight of almotriptan malate, 469.55

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Spiroalmotriptan[a]	0.32	0.4

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2-Hydroxyalmotriptan[b]	0.47	0.2
Almotriptan related compound B[c]	0.82	—
Almotriptan related compound C[c]	0.93	—
Almotriptan	1.0	—
Almotriptan related compound D	1.39	0.2
Any individual unspecified degradation product	—	0.2
Total degradation products	—	1.0

[a] 1′-Methyl-5-[(pyrrolidin-1-ylsulfonyl)methyl]spiro[indoline-3,3′-pyrrolidin]-2-ol.
[b] 3-[2-(Dimethylamino)ethyl]-5-[(pyrrolidin-1-ylsulfonyl)methyl]-1*H*-indol-2-ol.
[c] This is a process impurity that is included in this table for identification only. This impurity is controlled in the drug substance. This impurity is not to be reported for the drug product and is not to be included in the total degradation products.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **LABELING:** The labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Almotriptan Malate RS
  USP Almotriptan Related Compound B RS
  2-{5-[(Pyrrolidin-1-ylsulfonyl)methyl]-1*H*-indol-3-yl} ethanamine hemifumarate.
  $C_{15}H_{21}N_3O_2S \cdot \frac{1}{2}C_4H_4O_4$ 365.46
  USP Almotriptan Related Compound C RS
  N-Methyl-2-{5-[(pyrrolidin-1-ylsulfonyl)methyl]-1*H*-indol-3-yl}ethanamine.
  $C_{16}H_{23}N_3O_2S$ 321.44
  USP Almotriptan Related Compound D RS
  1-[({3-[2-(Dimethylamino)ethyl]indol-5-yl}methyl) sulfonyl]pyrrolidine *N*-oxide hydrochloride.
  $C_{17}H_{25}N_3O_3S \cdot HCl$ 387.92

Aloe

DEFINITION

Aloe is the dried latex of the leaves of *Aloe vera* (L.) Burm. f. (syn. *Aloe barbadensis* Mill.), known in commerce as aloe vera, Curaçao aloe, or Barbados aloe; or of *Aloe ferox* Mill., or of hybrids of *Aloe ferox* Mill. with *Aloe africana* Mill. and *Aloe spicata* L.f., known in commerce as Cape aloe (Fam. Liliaceae). Aloe vera contains NLT 16.0% of aloin, and Cape aloe and its hybrids contain NLT 6.0% of aloin, both calculated on the dried basis.

IDENTIFICATION

- **A.**
  Sample: 1 g, finely powdered
  Analysis: Mix the *Sample* with 25 mL of cold water. Shake the mixture occasionally for 2 h, filter, and wash the filter and residue with sufficient cold water to make the filtrate measure 100 mL.
  Acceptance criteria: The color of the filtrate, viewed in the bulb of a 100-mL volumetric flask, is dark orange with Curaçao aloe and greenish-yellow with Cape aloe. The filtrate darkens on standing. [NOTE—Reserve the filtrate for *Identification B*.]
- **B.**
  Sample: 5 mL of the filtrate obtained in *Identification A*
  Analysis: Add 2 mL of nitric acid to the *Sample*, and mix.
  Acceptance criteria: The mixture exhibits a reddish-orange color with aloe vera and a reddish-brown color that changes rapidly to green with Cape aloe.
- **C. THIN-LAYER CHROMATOGRAPHY**
  Standard solution: 1.0 mg/mL of USP Aloin RS in methanol
  Sample solution: 0.5 g of finely powdered Aloe in 10 mL of methanol. Sonicate for 15 min, centrifuge or filter, and use the supernatant or the filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
  Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates)
  Application volume: 2 µL of the *Standard solution* and 5 µL of the *Sample solution* as 8-mm bands
  Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.
  Developing solvent system: Ethyl acetate, methanol, and water (100:17:13)
  Developing distance: 6 cm
  Derivatization reagent: 10% potassium hydroxide solution in methanol (prepare in an ice bath)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Apply the *Samples* as bands to a suitable HPTLC plate. Use a saturated chamber. Develop the chromatograms, dry in air, derivatize with *Derivatization reagent*, and heat at 110° for 5 min. Examine under visible light and UV light at 365 nm.
  Acceptance criteria: Under visible light, the *Sample solution* exhibits a brown band due to aloin at about the middle of the chromatogram, corresponding in color and R_F to the band exhibited by the *Standard solution*. The *Sample solution* containing aloe vera exhibits an additional violet band due to 7-hydroxyaloin right below the aloin band. The *Sample solution* containing Cape aloe lacks the violet band due to 7-hydroxyaloin. Under UV light at 365 nm, the *Sample solution* exhibits a yellow fluorescence band due to aloin, corresponding in color and R_F to the band exhibited by the *Standard solution*, and a light blue fluorescence band due to aloesine at about one-third of the chromatogram.

COMPOSITION

- **CONTENT OF ALOIN**
  Mobile phase: A mixture of acetonitrile and water (3:7)
  Standard solution: 0.1 mg/mL of USP Aloin RS in methanol and water (1:1)
  Sample solution: Transfer about 0.1 g of aloe vera or 0.2 g of Cape aloe, finely powdered and accurately weighed, to a 100-mL volumetric flask, and add about 75 mL of methanol. Sonicate for 30 min, cool to room temperature, adjust with methanol to volume, and mix. Before injection, pass through a PTFE membrane filter of 0.45-µm pore size, discarding the first few mL of the filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 295 nm
  Column: 4.6-mm × 25-cm; end-capped 5-µm packing L1
  Column temperature: 43 ± 1°
  Flow rate: 1.0 mL/min

www.webofpharma.com

Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates for the aloin peak
Tailing factor: NMT 2.0 for the aloin peak
Relative standard deviation: NMT 2.0% determined from the aloin peak in repeated injections
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The *Standard solution* and *Sample solution* are stable for 8 h at room temperature.]
Using the chromatogram of the *Standard solution*, identify the retention time of the peak corresponding to aloin in the *Sample solution*.
Calculate the percentage of aloin in the portion of Aloe taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of aloin from the *Sample solution*
r_S = peak area of aloin from the *Standard solution*
C_S = concentration of USP Aloin RS in the *Standard solution* (mg/mL)
V = final volume of the *Sample solution* (mL)
W = weight of Aloe taken to prepare the *Sample solution* (mg)

Acceptance criteria: Aloe vera contains NLT 16.0% of aloin, and Cape aloe and its hybrids contain NLT 6.0% of aloin, both on the dried basis.

• **WATER-SOLUBLE EXTRACTIVE**
Sample: 2 g of powdered Aloe
Analysis: Macerate the *Sample* in 70 mL of water in a suitable flask. Shake the mixture for 8 h at 30-min intervals, and allow it to stand for 16 h without shaking. Filter, and wash the flask and residue with small portions of water, passing the washings through the filter until the filtrate measures 100.0 mL. Evaporate a 50-mL aliquot of the filtrate in a tared dish on a steam bath to dryness, and dry at 110° to constant weight.
Acceptance criteria: The weight of water-soluble extractive is NLT 50% of the weight of Aloe taken.

CONTAMINANTS

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Pesticide Residue Analysis*: Meets the requirements

SPECIFIC TESTS

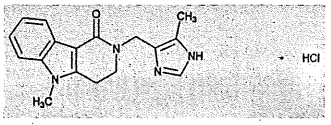
• **LOSS ON DRYING** ⟨731⟩
Sample: Use a powdered sample. If the Aloe is not powdered, crush it in a mortar until it passes through a no. 40 sieve, and mix the ground material before weighing the sample.
Analysis: Dry the *Sample* at 105° for 5 h.
Acceptance criteria: NMT 12.0%
• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Total Ash*
Acceptance criteria: NMT 4.0%
• **ALCOHOL-INSOLUBLE SUBSTANCES**
Sample: 1 g of powdered Aloe
Analysis: Add the *Sample* to 50 mL of alcohol in a flask. Heat the mixture to boiling, and maintain at incipient boiling for 15 min, replacing any loss due to evaporation. Remove from the heat, and shake the mixture at intervals for 1 h. Pass through a small dried and tared filter paper or a dried and tared filtering crucible, and wash the residue on the filter with alcohol until the last washing is colorless. Dry the residue at 105° to constant weight.
Acceptance criteria: The weight of the residue is NMT 10.0% of the weight of Aloe taken.
• **BOTANICAL CHARACTERISTICS**
Curaçao aloe: Brownish-black, opaque masses. Its fractured surface is uneven, waxy, and somewhat resinous.
Cape aloe: Dusky to dark brown irregular masses, the surfaces of which are often covered with a yellowish powder. Its fracture is smooth and glassy.
Powdered Aloe: Yellow, yellowish brown to olive-brown in color. When mounted in olive oil, it appears as greenish-yellow to reddish-brown irregular fragments, the hues of which depend to some extent upon the thickness of the fragments.

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Aloin RS

Add the following:

▲Alosetron Hydrochloride



$C_{17}H_{18}N_4O \cdot HCl$ 330.82
1*H*-Pyrido[4,3-*b*]indol-1-one, 2,3,4,5-tetrahydro-5-methyl-2-[(5-methyl-1*H*-imidazol-4-yl)methyl]-, hydrochloride;
2,3,4,5-Tetrahydro-5-methyl-2-[(5-methylimidazol-4-yl) methyl]-1*H*-pyrido[4,3-*b*]indol-1-one hydrochloride [122852-69-1].

DEFINITION

Alosetron Hydrochloride contains NLT 98.0% and NMT 102.0% of alosetron hydrochloride ($C_{17}H_{18}N_4O \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A OR 197K▲** (CN 1-May-2020) Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*: Meets the requirements of the silver nitrate precipitation test

ASSAY

• **PROCEDURE**
Protect solutions containing alosetron hydrochloride from light.
Buffer: 0.02 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: *Buffer* and acetonitrile (80:20)
Standard solution: 0.05 mg/mL of USP Alosetron Hydrochloride RS in *Mobile phase*
Sample solution: 0.05 mg/mL of Alosetron Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm

www.webofpharma.com

Column: 4.6-mm × 7.5-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of alosetron hydrochloride ($C_{17}H_{18}N_4O \cdot HCl$) in the portion of Alosetron Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alosetron Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Alosetron Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.2
- **ORGANIC IMPURITIES**

Protect solutions containing alosetron hydrochloride from light.
Buffer and **Chromatographic system:** Proceed as directed in the *Assay*.
Solution A: *Buffer*
Solution B: Acetonitrile
Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
20	50	50
25	50	50

Diluent: *Buffer* and acetonitrile (90:10)
System suitability solution: 0.5 mg/mL of USP Alosetron Hydrochloride RS and 0.5 µg/mL of USP Alosetron Related Compound A RS in *Diluent*
Standard solution: 0.5 µg/mL of USP Alosetron Hydrochloride RS in *Diluent*
Sensitivity solution: 0.25 µg/mL of USP Alosetron Hydrochloride RS in *Diluent* from the *Standard solution*
Sample solution: 500 µg/mL of Alosetron Hydrochloride in *Diluent*
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 7 between alosetron and alosetron related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Alosetron Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of alosetron from the *Standard solution*
C_S = concentration of USP Alosetron Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Alosetron Hydrochloride in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Alosetron	1.0	—	—
Alosetron related compound A	1.1	1.3	0.15
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.8

SPECIFIC TESTS

- **WATER DETERMINATION ⟨921⟩,** *Method I*, *Method Ia*: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 30°, protected from light.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Alosetron Hydrochloride RS
USP Alosetron Related Compound A RS
Dealkyl alosetron;
5-Methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indol-1-one.
$C_{12}H_{12}N_2O$ 200.24 ▲(USP 1-Dec-2019)

Add the following:

▲Alosetron Tablets

DEFINITION

Alosetron Tablets contains an amount of Alosetron Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of alosetron ($C_{17}H_{18}N_4O$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

[NOTE—The UV spectra of the major peaks of the *Sample solution* and the *Standard solution* as obtained in the *Assay* may also be used to meet the *Acceptance criteria*.]

USP Monographs

www.webofpharma.com

Standard stock solution: 0.115 mg/mL of USP Alosetron Hydrochloride RS in water
Standard solution: 2.3 µg/mL of USP Alosetron Hydrochloride RS in water, from the *Standard stock solution*
Sample solution: Transfer a number of Tablets, nominally equivalent to 1 mg of alosetron, to a 250-mL volumetric flask. Fill the flask to about ¾ full with water and shake for 30 min. Dilute with water to volume, mix, and pass through a PVDF filter or other suitable filter of 0.45-µm pore size, discarding at least 15 mL of the filtrate.
Wavelength range: 200–400 nm
Cell: 1 cm
Blank: Water
Acceptance criteria: The spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, concomitantly measured.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE** Protect solutions containing alosetron hydrochloride from light.
Buffer: 0.02 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: *Buffer* and acetonitrile (80:20)
Diluent: Mix 1 mL of phosphoric acid with 1000 mL of water.
Standard stock solution: 0.115 mg/mL of USP Alosetron Hydrochloride RS in *Diluent*
Standard solution: 0.0115 mg/mL of USP Alosetron Hydrochloride RS in *Diluent*, from the *Standard stock solution*
Sample solution: Nominally 0.01 mg/mL of alosetron prepared as follows. Transfer 10 Tablets to a suitable volumetric flask. Fill the flask to about ¾ full with *Diluent*, shake for 10 min, and then sonicate for another 10 min. Dilute with *Diluent* to volume, mix, let the solids settle, and pass through a PVDF filter or other suitable filter of 0.45-µm pore size, discarding at least 6 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 7.5-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 15 µL
Autosampler temperature: 5°
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alosetron ($C_{17}H_{18}N_4O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alosetron Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of alosetron in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of alosetron, 294.36
M_{r2} = molecular weight of alosetron hydrochloride, 330.82

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Protect solutions containing alosetron hydrochloride from light.
Medium: Water (deaerated); 500 mL
Apparatus 2: 75 rpm
Time: 20 min
Mobile phase: Prepare as directed in the *Assay*.
Sample solution: Pass a portion of the solution under test through a PVDF filter or other suitable filter of 0.45-µm pore size, discarding at least 15 mL of the filtrate.
Standard stock solution: 0.115 mg/mL of USP Alosetron Hydrochloride RS in *Medium*
Standard solution: 2.3 µg/mL of USP Alosetron Hydrochloride RS in water, from the *Standard stock solution*. Further dilute with *Medium*, if needed, to a concentration that is similar to that of the *Sample solution*.
Chromatographic system: Proceed as directed in the *Assay*, except for *Injection volume*.
Injection volume: 75 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of the labeled amount of alosetron ($C_{17}H_{18}N_4O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of alosetron from the *Sample solution*
r_S = peak response of alosetron from the *Standard solution*
C_S = concentration of USP Alosetron Hydrochloride RS in the *Standard solution* (mg/mL)
L = labeled amount of alosetron (mg/Tablet)
V = volume of *Medium*, 500 mL
M_{r1} = molecular weight of alosetron, 294.36
M_{r2} = molecular weight of alosetron hydrochloride, 330.82

Tolerances: NLT 80% (Q) of the labeled amount of alosetron ($C_{17}H_{18}N_4O$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Protect solutions containing alosetron hydrochloride from light.
Buffer: Prepare as directed in the *Assay*.
Solution A: *Buffer*
Solution B: Acetonitrile
Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for at least 10 min.

www.webofpharma.com

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
20	50	50
25	50	50

Diluent: *Buffer* and acetonitrile (90:10)
System suitability solution: 200 µg/mL of USP Alosetron Hydrochloride RS and 0.5 µg/mL of USP Alosetron Related Compound A RS in *Diluent*
Standard stock solution: 0.115 mg/mL of USP Alosetron Hydrochloride RS in *Diluent*
Standard solution: 0.46 µg/mL of USP Alosetron Hydrochloride RS in *Diluent*, from the *Standard stock solution*
Sample solution: Nominally 200 µg/mL of alosetron prepared as follows. Transfer a number of Tablets, equivalent to 5 mg of alosetron, to a suitable container. Add 25 mL of *Diluent*, shake for 10 min, and then sonicate for another 20 min. Mix, let the solids settle, and pass through a PVDF filter or other suitable filter of 0.45-µm pore size, discarding at least 6 mL of the filtrate.
Chromatographic system: Proceed as directed in the *Assay*, except for *Injection volume*.
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 7 between alosetron and alosetron related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of alosetron from the *Standard solution*
C_S = concentration of USP Alosetron Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of alosetron in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)
M_{r1} = molecular weight of alosetron, 294.36
M_{r2} = molecular weight of alosetron hydrochloride, 330.82

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Alosetron	1.0	—	—
Alosetron related compound A	1.1	1.3	0.3

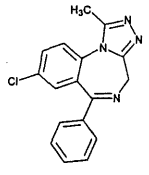
Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other individual impurity	—	1.0	0.5
Total impurities	—	—	2.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature, protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Alosetron Hydrochloride RS
  USP Alosetron Related Compound A RS
  Dealkyl alosetron;
  5-Methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indol-1-one.
  $C_{12}H_{12}N_2O$ 200.24▲ (USP 1-Dec-2019)

Alprazolam



$C_{17}H_{13}ClN_4$ 308.76
4*H*-[1,2,4]Triazolo[4,3-α][1,4]benzodiazepine, 8-chloro-1-methyl-6-phenyl-;
8-Chloro-1-methyl-6-phenyl-4*H*-*s*-triazolo[4,3-α][1,4]benzodiazepine [28981-97-7].

DEFINITION

Alprazolam contains NLT 98.0% and NMT 102.0% of $C_{17}H_{13}ClN_4$.
[**CAUTION**—Care should be taken to prevent inhaling particles of Alprazolam and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: Acetonitrile and water (1:1)
Buffer: 1.4 g/L of monobasic potassium phosphate in water
Mobile phase: Acetonitrile and *Buffer* (1:1)
Standard solution: 25 µg/mL of USP Alprazolam RS in *Diluent*. [NOTE—The solution is stable for 48 h at room temperature when stored in closed containers.]
Sample solution: 25 µg/mL of Alprazolam in *Diluent*. Sonicate for about 1 min. [NOTE—The solution is stable for 48 h at room temperature when stored in closed containers.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 231 nm

www.webofpharma.com

Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of alprazolam ($C_{17}H_{13}ClN_4$) in the portion of Alprazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)
C_U = concentration of Alprazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%
• **ORGANIC IMPURITIES**
Diluent, Buffer, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 20 µg/mL each of USP Alprazolam RS, USP Alprazolam Related Compound A RS, and USP 2-Amino-5-chlorobenzophenone RS in *Diluent*
Standard solution: 0.25 µg/mL of USP Alprazolam RS in *Diluent*. [NOTE—When stored in closed containers, the solution is stable for 48 h at room temperature.]
Sample solution: 250 µg/mL of Alprazolam in *Diluent*. Sonicate for about 1 min. [NOTE—When stored in closed containers, the *Sample solution* is stable for 24 h at room temperature.]
System suitability
Samples: *Standard solution* and *System suitability solution* [NOTE—For relative retention times, see *Table 1*.]
Suitability requirements
Resolution: NLT 2.0 between alprazolam related compound A and alprazolam, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Alprazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity in the *Sample solution*
r_S = peak response for alprazolam from the *Standard solution*
C_S = concentration of USP Alprazolam RS in the *Standard solution* (µg/mL)
C_U = concentration of Alprazolam in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Alprazolam related compound A	0.8	0.76	0.15
Alprazolam	1.0	1.0	—
2-Amino-5-chlorobenzophenone	4.0	1.0	0.15
Individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 1 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Alprazolam RS
USP Alprazolam Related Compound A RS
2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3*H*-1,4-benzodiazepine.
USP 2-Amino-5-chlorobenzophenone RS
2-Amino-5-chlorobenzophenone.
$C_{13}H_{10}ClNO$ 231.68

Alprazolam Compounded Oral Suspension

DEFINITION
Alprazolam Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$).
Prepare Alprazolam Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Alprazolam	100 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Comminute tablets in a suitable mortar to a fine powder, or add *Alprazolam* powder. Add about 20 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY
• **PROCEDURE**
Buffer: 0.04 M sodium acetate solution. Adjust with glacial acetic acid to a pH of 2.4.
Mobile phase: Methanol, acetonitrile, and *Buffer* (45:8:47)
Standard solution: 20 µg/mL of USP Alprazolam RS in *Mobile phase*
Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to

www.webofpharma.com

reach room temperature, and mix with a vortex mixer for 30 s. Dilute a suitable volume of the Oral Suspension in *Mobile phase* to obtain a nominal concentration of 20 µg/mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 0.6 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time of alprazolam is about 10 min.]
Suitability requirements
Relative standard deviation: NMT 1.4% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alprazolam RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of alprazolam in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 4.0–5.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
• **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded when stored at controlled room temperature or in a refrigerator
• **LABELING:** Label it to state that it is to be well-shaken before use, and to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Alprazolam RS

Alprazolam Tablets

DEFINITION
Alprazolam Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$).

IDENTIFICATION

Delete the following:

▲• **A. INFRARED ABSORPTION**
Sample: An amount of finely powdered Tablets, equivalent to 15 mg of alprazolam, prepared as follows. Dissolve the *Sample* in 10 mL of 10 mg/mL of sodium carbonate solution. Add 15 mL of chloroform, and shake vigorously for 30 min. Centrifuge, withdraw the aqueous layer, and transfer the chloroform to a clean container. Add 200 mg of potassium bromide. Evaporate the chloroform from this mixture to dryness, and dry the dispersion in vacuum at 60° for 24 h. Grind this dispersion into a fine powder. Prepare a suitable pellet for testing by placing 100 mg of dried potassium bromide into a die. Sprinkle 20 mg of the finely ground alprazolam–potassium bromide dispersion onto the dried potassium bromide layer, and cover with another specimen of 100 mg of dried potassium bromide.
Acceptance criteria: The IR absorption spectrum of the potassium bromide dispersion so obtained exhibits maxima characteristic of alprazolam, as compared to that of a similar preparation of USP Alprazolam RS, at the following wavenumbers: at 1609, 1578, 1566, 1539, 1487, and 1379 wavenumbers in the region of 1650– 1300 cm^{-1}; at 932, 891, 826, 779, 746, 696, and 658 wavenumbers in the region of 975–600 cm^{-1}. ▲2S (USP41)

Add the following:

▲• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲2S (USP41)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲2S (USP41)

ASSAY

Change to read:

• **PROCEDURE**
▲**Solution A:** 0.77 g/L of ammonium acetate prepared as follows. Dissolve 0.77 g of ammonium acetate in each liter of water and adjust with acetic acid to a pH of 4.7.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
2.5	60	40
9.0	5	95
9.1	60	40
11.0	60	40

Diluent: Acetonitrile and water (40:60)
System suitability solution: 50 µg/mL of USP Alprazolam RS, 1 µg/mL of USP Alprazolam Related Compound A RS, and 1 µg/mL of USP Chlordiazepoxide Related Compound A RS in *Diluent*
Standard solution: 50 µg/mL of USP Alprazolam RS in *Diluent*
Sample solution: Nominally 50 µg/mL of alprazolam from Tablets prepared as follows. Transfer a suitable portion of powder from NLT 10 Tablets to an appropriate volumetric flask. Add 80% of the total flask volume of *Diluent*. Sonicate for NLT 10 min. Dilute with *Diluent* to volume. Centrifuge a portion and use the clear supernatant. [NOTE—The use of a centrifuge speed of 3500 rpm for 10 min may be suitable.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

www.webofpharma.com

Detector: UV 260 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 3-µm packing L7
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between chlordiazepoxide related compound A and alprazolam related compound A, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alprazolam RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of alprazolam in the *Sample solution* (µg/mL) ▲2S (USP41)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Immediate-release dosage forms, Procedure for a pooled sample for immediate-release dosage forms*
Buffer stock solution: Dissolve 80 g of monobasic potassium phosphate and 20 g of dibasic potassium phosphate in 1 L of water. Add, with mixing, phosphoric acid or potassium hydroxide solution (45 in 100), as necessary to adjust the solution, such that the resulting solution has a pH of 6.0 ± 0.1.
Buffer: Prepare a 1-in-10 dilution of the *Buffer stock solution* to obtain a solution that has a pH of 6.0 ± 0.1.
Medium: *Buffer*, 500 mL
Apparatus 1: 100 rpm
Time: 30 min
Mobile phase: Acetonitrile, tetrahydrofuran, and *Buffer* (35:5:60)
Standard stock solution: 0.05 mg/mL of USP Alprazolam RS in methanol
Standard solution: Add 50 mL of *Buffer stock solution* and 250 mL of water to a 500-mL flask. Add to the flask 5.0 mL of *Standard stock solution* for every 0.25 mg of alprazolam contained in the Tablet being assayed. Dilute with water to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 10-cm; packing L7
Flow rate: 1 mL/min
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 500 theoretical plates
Relative standard deviation: NMT 3.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) is dissolved.

Change to read:

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
▲**Mobile phase:** Acetonitrile, chloroform, butyl alcohol, glacial acetic acid, and water (850: 80: 50: 0.5: 20) ▲2S (USP41)
Internal standard solution: 0.032 mg/mL of triazolam in acetonitrile
Standard solution: 0.025 mg/mL of USP Alprazolam RS in *Internal standard solution*
Sample solution: Transfer 1 Tablet to a container. Add 0.4 mL of water directly onto the Tablet, allow the Tablet to stand for 2 min, and then swirl the container to disperse the Tablet. For every 0.25 mg of alprazolam contained in the Tablet, add 10.0 mL of *Internal standard solution* to the container. Shake, and centrifuge if necessary.
▲**Chromatographic system**
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 30-cm; packing L3
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between triazolam and alprazolam
Relative standard deviation: NMT 2.0% for replicate injections ▲2S (USP41)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) in the Tablet taken:

$$\text{Result} = (R_U/R_S) \times C \times V \times (100/L)$$

R_U = peak area response ratio of alprazolam relative to the internal standard from the *Sample solution*
R_S = peak area response ratio of alprazolam relative to the internal standard from the *Standard solution*
C = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)
V = volume of the *Internal standard solution* used to prepare the *Sample solution* (mL)
L = label claim (mg/Tablet)

Acceptance criteria: Meet the requirements

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**
Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.05 µg/mL of USP Alprazolam RS in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]

www.webofpharma.com

Suitability requirements
Resolution: NLT 1.5 between chlordiazepoxide related compound A and alprazolam related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of alprazolam from the *Standard solution*
C_S = concentration of USP Alprazolam RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of alprazolam in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chlordiazepoxide related compound A[a]	0.7	1.1	0.5
Alprazolam related compound A[a]	0.8	1.7	0.5
Alprazolam	1.0	—	—
Nordazepam[a, b]	1.2	1.3	0.5
Alprazolam quinoline derivative[a, c]	1.4	1.1	0.5
2-Amino-5-chlorobenzophenone[a]	1.6	1.4	0.5
Any individual unspecified degradation product	—	1.0	0.5
Total degradation products	—	—	1.0

[a] If present, it is controlled as an unspecified degradation product.
[b] 7-Chloro-5-phenyl-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one.
[c] 7-Chloro-1-methyl-5-phenyl[1,2,4]triazolo[4,3-*a*]quinolin-4-amine. ▲(Postponed on 1-Mar-2019)

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
USP Alprazolam RS
▲USP Alprazolam Related Compound A RS
2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3*H*-1,4-benzodiazepine.
$C_{17}H_{15}ClN_4O$ 326.78
USP Chlordiazepoxide Related Compound A RS
7-Chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine 4-oxide;
Also known as 7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.
$C_{15}H_{11}ClN_2O_2$ 286.71 ▲2S (USP41)

Alprazolam Extended-Release Tablets

DEFINITION
Alprazolam Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Mobile phase: Acetonitrile, water, and phosphoric acid (350:650:1)
Standard solution: 0.05 mg/mL of USP Alprazolam RS in methanol
Sample solution: Nominally 0.05 mg/mL of alprazolam prepared as follows. Transfer an appropriate number of Tablets to a suitable volumetric flask. Sonicate in 80% of the flask volume of methanol for 15 min, and shake mechanically for 30 min. Dilute with methanol to final volume, filter a portion of the solution, and discard the first 3 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Column efficiency: NLT 3000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of alprazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

USP Monographs

www.webofpharma.com

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL

Apparatus 1: 100 rpm

Times: 1, 4, 8, and 12 h

Mobile phase: Acetonitrile, tetrahydrofuran, and *Medium* (7:1:12)

Standard stock solution: 0.5 mg/mL of USP Alprazolam RS in acetonitrile

Standard solution: (*L*/500) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 10-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 3000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 500 mL

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved		
	0.5-mg Tablet (%)	2-mg Tablet (%)	3-mg Tablet (%)
1	NMT 25	NMT 20	NMT 20
4	40–60	30–55	30–55
8	70–90	65–90	65–90
12	NLT 85	NLT 85	NLT 85

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL

Apparatus 1: 100 rpm

Times: 1, 4, 8, and 16 h

Mobile phase: Acetonitrile, tetrahydrofuran, and *Medium* (35:5:60)

Standard stock solution: 0.05 mg/mL of USP Alprazolam RS in methanol

Standard solution: (*L*/500) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 7.5-cm; 5-µm packing L7

Flow rate: 1.3 mL/min

Injection volume: 80 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of alprazolam ($C_{17}H_{13}ClN_4$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of alprazolam from the *Sample solution* at each time point

r_S = peak response of alprazolam from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (h)	Amount Dissolved			
		0.5-mg Tablet (%)	1-mg Tablet (%)	2-mg Tablet (%)	3-mg Tablet (%)
1	1	NMT 25	NMT 25	NMT 20	NMT 20
2	4	45–60	40–55	30–50	25–45
3	8	70–90	65–85	55–75	50–70
4	16	NLT 85	NLT 85	NLT 85	NLT 80

USP Monographs

www.webofpharma.com

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL, deaerated

Apparatus 1: 100 rpm

Times: 1, 4, and 8 h for Tablets labeled to contain 0.5 mg or 1 mg; 1, 4, 8, and 16 h for Tablets labeled to contain 2 mg or 3 mg

Mobile phase: Acetonitrile and *Medium* (40:60)

Standard stock solution: 0.5 mg/mL of USP Alprazolam RS in methanol

Standard solution: (*L*/500) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 1-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 10-cm; 3-µm or 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of alprazolam ($C_{17}H_{13}ClN_4$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of alprazolam from the *Sample solution* at each time point

r_S = peak response of alprazolam from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved			
		0.5-mg Tablet (%)	1-mg Tablet (%)	2-mg Tablet (%)	3-mg Tablet (%)
1	1	15–35	10–30	10–30	5–25
2	4	50–75	45–65	30–55	25–50
3	8	NLT 75	NLT 70	60–80	50–75
4	16	—	—	NLT 85	NLT 80

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of 6.0); 500 mL

Apparatus 1 (20-mesh basket): 100 rpm

Times: 1, 4, 8, and 16 h

Mobile phase: Acetonitrile and *Medium* (32:68)

Standard stock solution: 0.4 mg/mL of USP Alprazolam RS in methanol

Standard solution: (*L*/500) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet. Pass through a suitable filter of 0.45-µm pore size, and use the filtrate.

Sample solution: At the end of specified time intervals, withdraw a known volume (V_S) of the solution from the dissolution vessel, and replace an equal volume of fresh *Medium* into the dissolution vessel. Pass the withdrawn sample through a suitable filter of 0.45-µm pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of alprazolam ($C_{17}H_{13}ClN_4$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of alprazolam from the *Sample solution* at each time point

r_S = peak response of alprazolam from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

www.webofpharma.com

$$Result_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$Result_4 = \{[C_4 \times V] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (*i*)	Time (h)	Amount Dissolved			
		0.5-mg Tablet (%)	1-mg Tablet (%)	2-mg Tablet (%)	3-mg Tablet (%)
1	1	NMT 40	NMT 35	NMT 35	NMT 35
2	4	50–75	45–65	35–55	30–55
3	8	NLT 75	70–90	55–75	50–70
4	16	NLT 85	NLT 85	NLT 85	NLT 75

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.0); 500 mL
Apparatus 1: 100 rpm
Times: 1, 4, 8, and 16 h
Mobile phase: Acetonitrile, water, and phosphoric acid (350:650:1)
Standard stock solution: 0.5 mg/mL of USP Alprazolam RS in methanol
Standard solution: (L/500) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of alprazolam ($C_{17}H_{13}ClN_4$) in the sample withdrawn from the vessel at each time point (i):

$$Result_i = (r_U/r_S) \times C_S$$

r_U = peak response of alprazolam from the *Sample solution* at each time point
r_S = peak response of alprazolam from the *Standard solution*
C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved at each time point (i):

$$Result_1 = C_1 \times V \times (1/L) \times 100$$

$$Result_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$Result_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 5*.

Table 5

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	NMT 25
2	4	40–65
3	8	65–95
4	16	NLT 85

The percentages of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer: 5.4 g/L of monobasic potassium phosphate (KH_2PO_4) in water. Adjust with phosphoric acid to a pH of 3.4.
Solution A: Acetonitrile, methanol, and *Buffer* (27:10:63)
Solution B: Acetonitrile, methanol, and *Buffer* (7:3:10)
Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	95	5
22	95	5
25	15	85
60	15	85
60.1	95	5
70	95	5

System suitability solution: 1 µg/mL each of USP Chlordiazepoxide Related Compound A RS, USP Alprazolam Related Compound A RS, and USP

www.webofpharma.com

Nordazepam RS; and 0.4 µg/mL of USP Alprazolam RS in methanol

Standard solution: 0.4 µg/mL of USP Alprazolam RS in methanol

Sample solution: From NLT 20 Tablets ground to a fine powder, transfer an amount of powder to a suitable flask to obtain a nominal concentration of 0.2 mg/mL of alprazolam in methanol. [NOTE—Sonicate for 15 min to dissolve the contents.] Filter a portion, and discard the first 1 mL of filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution* [NOTE—The relative retention times are listed in *Table 7*.]

Suitability requirements

Resolution: NLT 1.5 between nordazepam and alprazolam; NLT 1.5 between chlordiazepoxide related compound A and alprazolam related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for the alprazolam peak, *System suitability solution*

Relative standard deviation: NMT 5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of alprazolam in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 7*)

Acceptance criteria: See *Table 7*.

Table 7

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chlordiazepoxide related compound A[a]	0.36	1.0	0.2
Alprazolam related compound A	0.45	0.7	0.5
Nordazepam[a, b]	0.8	1.0	0.2
Alprazolam	1.0	—	—
2-Amino-5-chloro-benzophenone	1.8	0.9	0.5
Amino-derivative[c]	2.2	1.2	0.5
Any other individual degradation product	—	1.0	0.2
Total impurities	—	—	2.0

[a] If possible from the manufacturing process.

[b] 7-Chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

[c] 7-Chloro-1-methyl-5-phenyl[1,2,4]triazolo[4,3-*a*]quinolin-4-amine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.
- **LABELING:** The labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Alprazolam RS

USP Alprazolam Related Compound A RS

2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3*H*-1,4-benzodiazepine.

$C_{17}H_{15}ClN_4O$ 326.78

USP Chlordiazepoxide Related Compound A RS

7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.

$C_{15}H_{11}ClN_2O_2$ 286.71

USP Nordazepam RS

Alprazolam Orally Disintegrating Tablets

DEFINITION

Alprazolam Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Diluent: Acetonitrile and water (60:40)

Mobile phase: Acetonitrile, methanol, and *Buffer* (35:10:55)

Standard solution: 10 µg/mL of USP Alprazolam RS in *Diluent*

Sample solution: Nominally 10 µg/mL of alprazolam from Tablets prepared as follows. Transfer 10 Tablets to a suitable volumetric flask. Add *Diluent* to volume and pass through a suitable filter. [NOTE—Sonicate with intermittent shaking to help dissolve, if necessary.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 221 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 30 µL

www.webofpharma.com

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alprazolam RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of alprazolam in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩
Test 1
Time: NMT 60 s
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Disintegration Test 2*.
Time: NMT 30 s

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: pH 6.0 phosphate buffer (8 g/L of monobasic potassium phosphate and 2 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid or diluted potassium hydroxide to a pH of 6.0 ± 0.1); 900 mL
Apparatus 2: 50 rpm
Time: 10 min
Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay*, except use an *Injection volume* of 100 µL.
Standard stock solution: 0.05 mg/mL of USP Alprazolam RS in methanol. [NOTE—Sonicate to help dissolve, if necessary.]
Standard solution: (*L*/1000) mg/mL of USP Alprazolam RS from the *Standard stock solution* in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a nylon membrane filter of 0.45-µm pore size, discarding the first few mL.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: pH 6.0 phosphate buffer (8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL
Apparatus 2: 50 rpm
Time: 10 min
Buffer: 1.36 g/L of monobasic potassium phosphate. Adjust with dilute sodium hydroxide to a pH of 6.0.
Mobile phase: Acetonitrile and *Buffer* (35:65)
Standard stock solution: 0.05 mg/mL of USP Alprazolam RS in methanol. [NOTE—Sonicate to help dissolve, if necessary.]
Standard solution: (*L*/500) mg/mL of USP Alprazolam RS from the *Standard stock solution* in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a 5-mL aliquot of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 3 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 7.5-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 40 µL
Run time: 3 times the retention time of alprazolam
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)

Tolerances: NLT 70% (*Q*) of the labeled amount of alprazolam ($C_{17}H_{13}ClN_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Diluent: Prepare as directed in the *Assay*.
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Solution A: Acetonitrile, methanol, and *Buffer* (25:20:55)
Solution B: Acetonitrile, methanol, and *Buffer* (40:5:55)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
12	100	0
15	0	100
60	0	100
65	100	0
70	100	0

www.webofpharma.com

Standard solution: 0.6 µg/mL of USP Alprazolam RS in *Diluent*
Sample solution: Nominally 200 µg/mL of alprazolam in *Diluent*. Prepare using 10 Tablets, and pass through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.2 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Theoretical plates: NLT 2000
Tailing factor: NMT 1.5
Relative standard deviation: NMT 6.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of alprazolam from the *Standard solution*
C_S = concentration of USP Alprazolam RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of alprazolam in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peaks less than 0.05%.

Table 2

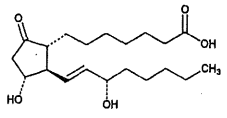
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Alprazolam related compound A[a,b]	0.8	—	—
Alprazolam	1.0	—	—
2-Amino-5-chlorobenzophenone	2.9	1.9	0.5
Any other unknown impurity	—	1.0	0.5
Total impurities	—	—	2.0

[a] 2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3*H*-1,4-benzodiazepine.
[b] Disregard the peak due to alprazolam related compound A, because it is a process impurity in alprazolam.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** When more than one *Disintegration* test is given, the labeling states the *Disintegration* test used only if *Test 1* is not used. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Alprazolam RS

Alprostadil



$C_{20}H_{34}O_5$ 354.48
Prost-13-en-1-oic acid, 11,15-dihydroxy-9-oxo-, (11α,13*E*,15*S*)-;
(1*R*,2*R*,3*R*)-3-Hydroxy-2-[(*E*)-(3*S*)-3-hydroxy-1-octenyl]-5-oxocyclopentaneheptanoic acid [745-65-3].

DEFINITION

Alprostadil contains NLT 95.0% and NMT 105.0% of alprostadil ($C_{20}H_{34}O_5$), calculated on the anhydrous basis.
[**CAUTION**—Great care should be taken to prevent inhaling particles of Alprostadil and exposing the skin to it.]

IDENTIFICATION

Change to read:

▲• **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**
Use freshly prepared solutions.
Mobile phase: Methanol, acetonitrile, and 0.1 M monobasic potassium phosphate (2:1:2). Adjust with phosphoric acid to a pH of 3.0.
Diluent: Methanol and water (90:10)
Internal standard solution: 0.05 mg/mL of ethylparaben in *Diluent*
Standard stock solution: 0.3 mg/mL of USP Alprostadil RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Alprostadil RS prepared by combining 2.0 mL of *Standard stock solution* with 1.0 mL of *Internal standard solution*
System suitability stock solution: 4.5 µg/mL of USP Prostaglandin A1 RS in *Standard solution*
System suitability solution: Combine 2.0 mL of *System suitability stock solution* with 1.0 mL of *Internal standard solution*.
Sample stock solution: 0.3 mg/mL of Alprostadil in *Diluent*
Sample solution: 0.2 mg/mL of Alprostadil prepared by combining 2.0 mL of *Sample stock solution* and 1.0 mL of *Internal standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Photodiode array detector or equivalent capable of detecting UV wavelengths of 200–300 nm
Analytical wavelength: 200 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 7.5 between prostaglandin A_1 and alprostadil, and NLT 2.0 between prostaglandin A_1 and ethylparaben
Relative standard deviation: NMT 2.0%, determined from the peak area ratio of alprostadil to ethylparaben
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the percentage of alprostadil ($C_{20}H_{34}O_5$) in the portion of Alprostadil taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of alprostadil to the internal standard from the *Sample solution*
R_S = peak area ratio of alprostadil to the internal standard from the *Standard solution*
C_S = concentration of USP Alprostadil RS in the *Standard solution* (mg/mL)
C_U = concentration of Alprostadil in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the anhydrous basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩
Sample: 0.3 g
Acceptance criteria: NMT 0.5%

• **Limit of Chromium**
Standard stock solution: 3.04 µg/mL of chromium trichloride in 0.05 M nitric acid
Standard solution: 20 ng/mL of chromium (Cr) in alcohol, prepared as follows. Transfer 2 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with alcohol to volume.
Sample solution: 1.0 mg/mL of Alprostadil in alcohol
Blank: Alcohol
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectroscopy
Lamp: Chromium hollow-cathode
Analytical wavelength: 357.9 nm
Atomization type: Graphite furnace
Temperatures
Drying: 100°
Ashing: 1000°
Atomization: 2700°
Injection volume: 20 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of chromium (Cr) in the portion of Alprostadil taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of chromium in the *Standard solution* (mg/mL)
C_U = concentration of Alprostadil in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.002%

• **Limit of Rhodium**
Standard stock solution: 100 µg/mL of rhodium in 1.2 M hydrochloric acid, prepared by diluting rhodium chloride hydrate
Standard solution: 50 ng/mL of rhodium (Rh) in alcohol from *Standard stock solution*
Sample solution: 2.0 mg/mL of Alprostadil in alcohol
Blank: Alcohol
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectroscopy
Lamp: Rhodium hollow-cathode
Analytical wavelength: 343.5 nm
Atomization type: Graphite furnace
Temperatures
Drying: 100°
Ashing: 1000°
Atomization: 2800°
Injection volume: 20 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of rhodium (Rh) in the portion of Alprostadil taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of rhodium in the *Standard solution* (mg/mL)
C_U = concentration of Alprostadil in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.002%

• **Limit of Foreign Prostaglandins, Test 1**
Use freshly prepared solutions.
Mobile phase: Methanol, acetonitrile, and 0.1 M monobasic potassium phosphate (2:1:2). Adjust with phosphoric acid to a pH of 3.0.
Diluent: Methanol and water (90:10)
Standard solution: 6 µg/mL of USP Alprostadil RS,15 µg/mL of USP Prostaglandin A1 RS, and 6 µg/mL of USP Prostaglandin B1 RS in *Diluent*
Sample solution: 3.0 mg/mL of Alprostadil in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Photodiode array detector or equivalent capable of detecting UV wavelengths of 200–300 nm
Analytical wavelengths
Prostaglandin A_1: 224 nm
Prostaglandin B_1: 280 nm
Other foreign prostaglandins: 200 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 7.5 between prostaglandin A_1 and alprostadil
Relative standard deviation: NMT 4.0%, determined from the peaks at their respective wavelength for replicate injections
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of prostaglandin A_1 and prostaglandin B_1 in the portion of Alprostadil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of prostaglandin A_1 or prostaglandin B_1 from the *Sample solution*
r_S = peak response of prostaglandin A_1 or prostaglandin B_1 from the *Standard solution*
C_S = concentration of USP Prostaglandin A1 RS or USP Prostaglandin B1 RS in the *Standard solution* (mg/mL)
C_U = concentration of Alprostadil in the *Sample solution* (mg/mL)

www.webofpharma.com

Calculate the percentage of each impurity occurring at 200 nm and eluting before prostaglandin A_1 in the portion of Alprostadil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for alprostadil from the *Standard solution*
C_S = concentration of USP Alprostadil RS in the *Standard solution* (mg/mL)
C_U = concentration of Alprostadil in the *Sample solution* (mg/mL)

Calculate the percentage of the impurity having a relative retention time of 0.6, relative to the prostaglandin A_1 peak detected at 224 nm, in the portion of Alprostadil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for any impurity having a relative retention time of 0.6, relative to the prostaglandin A_1 peak, from the *Sample solution*
r_S = peak response for prostaglandin A_1 from the *Standard solution*
C_S = concentration of USP Prostaglandin A1 RS in the *Standard solution* (mg/mL)
C_U = concentration of Alprostadil in the *Sample solution* (mg/mL)

Acceptance criteria
Prostaglandin A_1: NMT 1.5%
Prostaglandin B_1: NMT 0.1%
Any foreign prostaglandin impurity eluting before prostaglandin A_1: NMT 0.9%
Impurity at relative retention time 0.6, relative to prostaglandin A_1: NMT 0.9%

• **LIMIT OF FOREIGN PROSTAGLANDINS, TEST 2**
Mobile phase: Methanol, acetonitrile, and 0.02 M monobasic potassium phosphate (2:1:1). Adjust with phosphoric acid to a pH of 3.
System suitability solution: 6 µg/mL of USP Alprostadil RS, 15 µg/mL of USP Prostaglandin A1 RS, and 6 µg/mL of USP Prostaglandin B1 RS in methanol and water (9:1)
Standard solution: 10 µg/mL of USP Alprostadil RS in acetonitrile and water (1:1)
Sample solution: 5.0 mg/mL of Alprostadil in acetonitrile and water (1:1). [NOTE—Sonicate if necessary.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Photodiode array detector or equivalent, capable of detecting UV wavelengths of 200–300 nm
Analytical wavelengths
Prostaglandin A_1: 224 nm
Prostaglandin B_1: 280 nm
Other foreign prostaglandins: 200 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for alprostadil and prostaglandin A_1 are 1.0 and 1.2, respectively, *System suitability solution*.]
Suitability requirements
Resolution: NLT 4.0 between prostaglandin A_1 and alprostadil, *System suitability solution*
Relative standard deviation: NMT 2.0%, determined from the main peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity, excluding prostaglandin B_1, observed at 200 nm and eluting after prostaglandin A_1 in the portion of Alprostadil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for alprostadil from the *Standard solution*
C_S = concentration of USP Alprostadil RS in the *Standard solution* (mg/mL)
C_U = concentration of Alprostadil in the *Sample solution* (mg/mL)

Acceptance criteria
Sum of the impurities at relative retention times 2.0 and 2.3: NMT 0.6%
Any other foreign prostaglandin impurity eluting after prostaglanin A_1: NMT 0.9%
Total impurities: The sum of the impurities from *Limit of Foreign Prostaglandins, Test 1* and *Test 2*, is NMT 2.0%.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩
Sample: 0.5 g
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a refrigerator.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Alprostadil RS
USP Prostaglandin A1 RS
USP Prostaglandin B1 RS
(13*E*,15*S*)-15-Hydroxy-9-oxoprosta-8(12),13-dien-1-oic acid.
$C_{20}H_{32}O_4$ 336.47

Alprostadil Injection

DEFINITION
Alprostadil Injection is a sterile solution of Alprostadil in Dehydrated Alcohol. It contains NLT 90.0% and NMT 115.0% of the labeled amount of alprostadil ($C_{20}H_{34}O_5$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Dry an amount of Injection, equivalent to 2 mg of alprostadil, on 500 mg of spectroscopic grade potassium bromide at 40°–50° under vacuum. Prepare a pellet from this mixture.
Standard: A solution of USP Alprostadil RS dissolved in dehydrated alcohol and processed as described in the *Sample*
Acceptance criteria: Meets the requirements

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: 40 mg/mL of α-bromo-2′-acetonaphthone in acetonitrile, freshly prepared

Solution B: 5 mg/mL of diisopropylethylamine in acetonitrile, freshly prepared

Mobile phase: Methylene chloride, 1,3-butanediol, and water (1000: 6: 0.5)

Internal standard solution: 50 µg/mL of ethylparaben in methylene chloride

Standard stock solution: 0.5 mg/mL of USP Alprostadil RS in dehydrated alcohol

Standard solution: 0.13 mg/mL of USP Alprostadil RS, prepared as follows. Gently evaporate a 0.5-mL portion of the *Standard stock solution* to dryness with a stream of nitrogen. Add 150 µL of *Solution A*, rinse the inside of the container with this solution, and swirl. Add 150 µL of *Solution B* to the container, rinse the inside of the container with this solution, and swirl. Cap and sonicate to dissolve. Heat the container at 45° for 45 min, swirling occasionally. Sonicate again after heating is complete. Discard the specimen if the entire sample does not dissolve. Evaporate the solution using a stream of nitrogen, add 2.0 mL of *Internal standard solution*, and sonicate to dissolve. Discard the specimen if the entire sample does not dissolve.

Sample solution: Nominally 0.13 mg/mL of alprostadil, prepared as follows. Pool the contents of several containers of the Injection. Gently evaporate a volume, equivalent to 0.25 mg of alprostadil, to dryness using a stream of nitrogen. Proceed as directed for the *Standard solution*, beginning with "Add 150 µL of *Solution A*".

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.4-mm × 25-cm; packing L18

Flow rate: 1.5 mL/min

Injection volume: Equal volumes of *Standard solution* and *Sample solution*

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ethylparaben and alprostadil are about 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 9.0 between alprostadil and the internal standard

Relative standard deviation: NMT 2.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprostadil ($C_{20}H_{34}O_5$) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of alprostadil to the internal standard from the *Sample solution*

R_S = peak response ratio of alprostadil to the internal standard from the *Standard solution*

C_S = concentration of USP Alprostadil RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of alprostadil the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 5 USP Endotoxin Units/100 µg of alprostadil.

• **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.4%

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

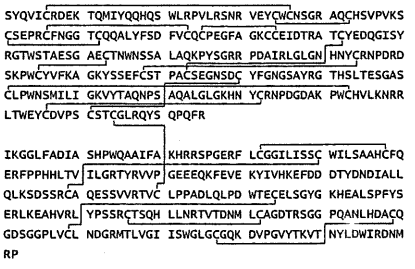
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, single-dose containers, preferably of Type I glass. Store in a refrigerator.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Alprostadil RS

Alteplase



$C_{2569}H_{3894}N_{746}O_{781}S_{40}$ 59,007.61

[105857-23-6].

DEFINITION

Alteplase is a highly purified glycosylated serine protease with fibrin-binding properties and plasminogen-specific proteolytic activities. It is produced by recombinant DNA synthesis in mammalian cell culture. It has a biological potency of NLT 90.0% and NMT 115.0% of the potency stated on the label, the potency being 580,000 USP Alteplase Units/mg of protein.

The presence of host cell DNA and host cell protein impurities in Alteplase is process specific; the limits of these impurities are determined by validated methods.

IDENTIFICATION

• **A.**

Standard solution: 1.0–2.5 mg/mL of USP Alteplase RS

Sample solution: Prepare similarly to the *Standard solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

To each of three test tubes transfer 1 mL of 0.5-mg/mL H-D-isoleucyl-prolyl-arginyl-*p*-nitroaniline dihydrochloride. Separately transfer 200 µL of the *Standard solution* and 200 µL of the *Sample solution* to two of the test tubes. To the third test tube add 200 µL of 0.2 M arginine solution that has been adjusted with phosphoric acid to a pH of 7.3 (negative control). Mix the solutions in the three test tubes, and allow to stand for 1 min.

Acceptance criteria: A yellow color is produced in the solutions from the *Standard solution* and the *Sample solution*, while no yellow color is produced in the negative control.

• **B. PEPTIDE MAPPING**

Solution A: 6.9 mg/mL of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.85. Filter, and degas.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

USP Monographs

www.webofpharma.com

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
91	70	30
121	40	60
131	40	60

Dialysis solution: 480 mg/mL of urea, 44 mg/mL of tris(hydroxymethyl)aminomethane, and 0.88 mg/mL of edetic acid in water. Adjust with hydrochloric acid to a pH of 8.6.

Standard solution: Prepare a solution containing 1.0 mg/mL of USP Alteplase RS in water. Dialyze 2.0 mL of this solution into the *Dialysis solution* at room temperature for NLT 12 h. Measure the volume of the solution, and transfer it to a clean test tube. For each mL of solution in the tube, add 10 µL of 1 M dithiothreitol. Incubate at room temperature for 4 h, then add 25 µL of 1 M iodoacetic acid per mL of the solution, and incubate in the dark for 30 min. Quench the reaction by adding 50 µL of 1 M dithiothreitol per mL of the solution. Dialyze the solution against 0.1 M ammonium bicarbonate for 24 h, replacing the 0.1 M ammonium bicarbonate twice during the dialysis period. To 2.0 mL of the dialyzed solution, add 20 µg of trypsin, and incubate for 6–8 h at room temperature. Again add 20 µg of trypsin, and incubate for 16–18 h for a total of 24 h of incubation of the trypsin-treated solution. [NOTE—Store the *Standard solution* in a freezer.]

Sample solution: Using an accurately weighed quantity of Alteplase, proceed as directed in the *Standard solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between peaks 6 and 7 as defined by the USP Alteplase RS Data Sheet. The times for peaks 6 and 7 baseline widths are NMT 0.5 min.

Analysis

Samples: *Standard solution*, *Sample solution*, and a mixture of the *Standard solution* and the *Sample solution* (1:1)

Measure the responses for NLT 20 major peaks as defined in the USP Alteplase RS Data Sheet.

Acceptance criteria: The retention times of the corresponding peaks of the *Standard solution* and the *Sample solution* do not differ by more than 0.4 min, and the peak area ratios relative to peak 19 (as shown on the USP Alteplase RS Data Sheet) do not differ by more than 20%. No additional significant peaks or shoulders are found, a significant peak or shoulder being defined as one having a peak response of NLT 5% of peak 19.

ASSAY

• **BIOLOGICAL POTENCY**

Buffer: 1.38 mg/mL of monobasic sodium phosphate, 7.10 mg/mL of anhydrous dibasic sodium phosphate, 0.20 mg/mL of sodium azide, and 0.10 mg/mL of polysorbate 80 in water

Human thrombin solution: 33 U.S. Units in terms of the U.S. Standard Thrombin/mL in *Buffer*

Human fibrinogen solution: 2 mg/mL of human fibrinogen in *Buffer*

Human plasminogen solution: 1 mg/mL of human plasminogen in *Buffer*

Standard stock solution: 1.0 mg/mL (580,000 USP Alteplase Units) of USP Alteplase RS in water

Standard solutions: Dilute volumes of *Standard stock solution* with water to obtain a series of five *Standard solutions* having known concentrations ranging from 145 to 9.3 USP Alteplase Units/mL.

Sample stock solution: 1.0 mg/mL of Alteplase in water

Sample solutions: Dilute a volume of *Sample stock solution* with *Buffer* to obtain a series of dilutions of about 1:20,000; 1:10,000; and 1:5,000.

Analysis

Samples: *Standard solutions* and *Sample solutions*

To a set of labeled glass test tubes add 0.5 mL of *Human thrombin solution*. To separate test tubes add 0.5 mL of each *Standard solution* or *Sample solution*, and store on ice. To a second set of labeled glass tubes, add 20 µL of *Human plasminogen solution* and 1 mL of *Human fibrinogen solution*, and store on ice. Beginning with the thrombin–*Standard solution* mixture containing the *Standard solution* with the lowest number of USP Units/mL, record the time, and separately add 200 µL of each of the thrombin–*Standard solution* mixtures to the test tubes containing the plasminogen-fibrinogen mixture. Using a vortex mixer, intermittently mix the contents of each tube for a total of 15 s, and carefully place into a rack in a 37° circulating water bath. A visually turbid clot forms within 30 s, followed by the formation of bubbles within the clot. Record the clot lysis time (t_{cl}) from the first addition of the Alteplase solution to the last bubble to rise to the surface.

Using a least squares fit, determine the equation of the line using the log values of the standard concentration, in USP Alteplase Units/mL, versus the log values of their clot lysis times in s taken:

$$\log t = m(\log U_S) + b$$

t = time to bubble release (s)
m = slope of the line
U_S = activity of the *Standard solution* (USP Alteplase Units/mL)
b = y-intercept of the line

The correlation coefficient is NLT −0.9900. From the line equation and using the log of the clot lysis time for the *Sample solution*, calculate the log of the activity (U_A):

$$\log U_A = \{[(\log t) - b]/m\}$$

Calculate the alteplase activity in USP Alteplase Units/mL taken:

$$\text{Result} = D(10^{\log U})$$

D = dilution factor for the *Sample solution*

Calculate the specific activity in the portion of Alteplase taken:

$$\text{Result} = (U_A/P)$$

P = concentration of protein obtained in the test for *Protein Content*

Acceptance criteria: 90.0%–115.0% of the potency stated on the label, the potency being 580,000 USP Alteplase Units/mg of protein

USP Monographs

www.webofpharma.com

OTHER COMPONENTS

• **PROTEIN CONTENT**

Arginine solution: 34.8 mg/mL of arginine in water. Adjust with phosphoric acid to a pH of 7.3.

Sample stock solution: 1 mg/mL of Alteplase in water

Sample solution: Dilute a volume of *Sample stock solution* with a volume of *Arginine solution* to obtain a solution having an absorbance value of 0.5–1.0 at the wavelength of maximum absorbance at about 280 nm. Determine the dilution volume (*V*).

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Wavelength range: 240–500 nm

Analytical wavelengths: 320 nm and maximum absorbance at about 280 nm

Cell: 1 cm

Blank: *Arginine solution*

Analysis

Samples: *Sample solution* and *Blank*

Calculate the protein content in the portion of Alteplase taken:

$$\text{Result} = [(A_{max} - A_{320})/\varepsilon] \times V$$

A_{max} = absorbance value at the wavelength of maximum absorbance

A_{320} = absorbance of the *Sample solution* at 320 nm

ε = molar absorptivity of Alteplase, 1.9

V = volume of *Arginine solution* required to prepare the *Sample solution*

IMPURITIES

• **CHROMATOGRAPHIC PURITY**

SDS buffer: 400 mg/mL of glycerol, 5.52 mg/mL of tris(hydroxymethyl)aminomethane hydrochloride, 3.28 mg/mL of tris(hydroxymethyl)aminomethane, 0.20 mg/mL of bromophenol blue, and 0.20 mg/mL of xylene cyanole FF in sodium dodecyl sulfate solution (8 in 100)

Diluted SDS buffer: Dilute 1 volume of *SDS buffer* with 4 volumes of water.

Running buffer: 3.03 mg/mL of tris(hydroxymeth yl) aminomethane and 14.26 mg/mL of glycine in sodium dodecyl sulfate (1 in 1000)

Carboxymethylation buffer: 480 mg/mL of urea, 44 mg/mL of tris(hydroxymethyl)aminomethane, and 1.2 mg/mL of edetic acid in water. Adjust with hydrochloric acid, if necessary, to a pH of 8.6.

Ammoniacal silver nitrate solution: Transfer 105 mL of sodium hydroxide solution (0.36 in 100) and 7.0 mL of ammonium hydroxide to a 500-mL volumetric flask, and add slowly, with stirring, 20.0 mL of silver nitrate solution (20 in 100). Dilute with water to volume.

[NOTE—Prepare this solution immediately before use, and protect it from light. This amount of solution is sufficient for two slab gels.]

Citric acid–formaldehyde solution: To 500 mL of water add 25 mg of citric acid, 0.25 mL of formaldehyde, and 0.025 mL of methanol, omitting the methanol if the formaldehyde is preserved with methanol.

[NOTE—Prepare this solution fresh at the time of use. This amount of solution is sufficient for two slab gels.]

Gel: Prepare a 10% T (total acrylamide)–0.25% C (cross-linked bisacrylamide) resolving gel containing 0.1% sodium dodecyl sulfate, 0.375 M tris(hydroxymethyl) aminomethane hydrochloride, and 0.05 M tris(hydroxymethyl)aminomethane.

Arginine solution: 34.8 mg/mL of arginine in water. Adjust with phosphoric acid to a pH of 7.3.

Molecular weight standard solution: Use a commercially available preparation of low molecular weight protein standards (10,000–100,000 Da) at 2 mg/mL. Mix 990 µL of *Diluted SDS buffer* and 10 µL of the molecular weight standard mixture.

Control solution: Prepare a control solution of bovine serum albumin containing 10 µg/mL. For a 10 ng/25 µL load, mix 600 µL of *Diluted SDS buffer* and 25 µL of the control solution, and heat at 90° for 2 min. For a 2.5 ng/25 µL load, mix 594 µL of *Diluted SDS buffer* and 6 µL of the control solution, and heat at 90° for 2 min.

Standard stock solution: 1 mg/mL of USP Alteplase RS in water

Standard solution: Dilute an accurately measured volume of *Standard stock solution* in *Arginine solution* to obtain a solution having a final concentration of 0.25 mg/mL. Heat 0.5 mL of this solution with 116 µL of *SDS buffer* and 10 µL of 1 M dithiothreitol at 80° for 2 min.

Carboxymethylated standard solution: Dilute 1.0 mL of *Standard stock solution* with 1 mL of *Carboxymethylation buffer*, and adjust with 1 M sodium hydroxide to a pH of 8.5. Add 20 µL of 1 M dithiothreitol, and incubate at 37° for 60 min. Add 100 µL of 1 M iodoacetic acid, and incubate in the dark for 20 min. Desalt by passing the solution through a chromatographic column containing fine gel chromatographic packing equilibrated with a buffer solution containing 20 mg/mL of sodium dodecyl sulfate, 100 mg/mL of glycerol, 1.42 mg/mL of tris(hydroxymethyl) aminomethane hydrochloride, and 0.85 mg/mL of tris(hydroxymeth yl)aminomethane. Collect the protein fraction of the preparation by elution with the same buffer, and add 20 µL of 1 M dithiothreitol. Adjust the protein concentration to about 0.2 mg/mL with a buffer solution containing 20 mg/mL of sodium dodecyl sulfate, 100 mg/mL of glycerol, 1.42 mg/mL of tris(hydroxymeth yl) aminomethane hydrochloride, 0.85 mg/mL of tris(hydroxymethyl)aminomethane, 1.06 mg/mL of dithiothreitol, 0.05 mg/mL of bromophenol blue, and 0.05 mg/mL of xylene cyanole FF.

Sample stock solution, Sample solution, and **Carboxymethylated sample solution:** Using an accurately weighed quantity of Alteplase, proceed as directed for *Standard stock solution*, *Standard solution*, and *Carboxymethylated standard solution*.

Blank: Mix 500 µL of water, 126 µL of *SDS buffer*, and 10 µL of 1 M dithiothreitol.

Analysis

Samples: *Molecular weight standard solution*, *Control solution*, *Standard solution*, *Carboxymethylated standard solution*, *Sample solution*, *Carboxymethylated sample solution*, and *Blank*

Separately apply equal volumes (about 25 µL) of the *Standard solution*, *Carboxymethylated standard solution*, *Sample solution*, and *Carboxymethylated sample solution* at the 5-µg load; apply equal volumes (about 38 µL) of the *Standard solution* and the *Carboxymethylated standard solution* at the 7.5-µg load; and apply the *Control solutions* at the 10- and 2.5-ng load onto separate lanes of the gel. Apply about 25 µL of the *Molecular weight standard solution* to each side of the gel, and apply about 25 µL of the *Blank* onto a separate lane. Apply the *Standard solution* and the *Sample solution* on one half and the *Carboxymethylated standard solution* and *Carboxymethylated sample solution* on the other half. Perform the electrophoresis using a constant current of 1.3–1.5 mAmp/cm of gel length and the *Running buffer*. Remove the gel from the apparatus 10–20 min after the tracking dye starts to move. Place the gel in 250 mL of a solution of 20% alcohol and 6% glacial acetic acid for NLT

www.webofpharma.com

1 h, and change the solution every 20 min, leaving the gel to soak overnight following the last change.

Perform silver staining of the gel by placing the gel in 250 mL of a 10% glutaraldehyde solution (v/v) in a shallow dish, and shake for about 30 min. Replace the glutaraldehyde solution with distilled water, allow the gel to soak for about 20 min, and then change the water. Repeat for a total of three washings. Transfer the gel to a dish, and cover with 250 mL of *Ammoniacal silver nitrate solution*. Place the dish on a shaker for about 15 min. Rinse four times with 250 mL of water, rocking the dish for 1 min between rinses. Continue rocking to prevent the gel from sticking and to facilitate washing.

Transfer the gel to a clear dish containing 250 mL of *Citric acid–formaldehyde solution*, and rock the dish. Protein bands become visible. When the gel is visibly stained, wash immediately with water, and rinse it repeatedly with water to remove the *Citric acid–formaldehyde solution*. Rinse the gel for NLT 1 h, and dry. Soak cellophane membranes in glycerol solution (2 in 100). Roll a membrane onto a rigid sheet of plastic. Roll the gel onto the membrane, and cover with another membrane. Lay a frame on the edges of the membranes, and clamp it to the rigid plastic sheet. Dismantle the dryer, and cut off excess cellophane when dry (about 24 h). Visually examine the gel under light.

System suitability: The 2.5- and 10-ng *Control solutions* must be visible. The nonreduced *Control solutions* migrate with an apparent molecular weight of slightly less than 66,000 Da, as compared with the *Molecular weight standard solution*.

Acceptance criteria: The *Sample solution* exhibits three major bands in the region between 66,000 Da and 31,000 Da, corresponding to the major bands from the *Standard solution*. The *Carboxymethylated sample solution* exhibits six major bands in the region between 92,500 Da and 45,000 Da, corresponding to the major bands from the *Carboxymethylated standard solution*.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 1 USP Endotoxin Unit/mg of Alteplase
- **SINGLE-CHAIN CONTENT**

Mobile phase: 27.6 g of monobasic sodium phosphate in 1000 mL of sodium dodecyl sulfate solution (1 in 1000). Adjust with sodium hydroxide to a pH of 6.8. Filter, and degas.

Dithiothreitol solution: 3.12 mg/mL of dithiothreitol in *Mobile phase*

Standard stock solution: Using an accurately weighed quantity of USP Alteplase RS, make a 1-mg/mL solution in water.

Standard solution: Pipet 1 mL of the *Standard stock solution* into a glass tube, add 3 mL of *Dithiothreitol solution*, cap the tube, and invert to mix. Heat for 3–5 min at about 80°.

Sample stock solution: Using an accurately weighed quantity of Alteplase, make a 1-mg/mL solution in water.

Sample solution: Pipet 1 mL of the *Sample stock solution* into a glass tube, add 3 mL of *Dithiothreitol solution*, cap the tube, and invert to mix. Heat for 3–5 min at about 80°.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 7.5-mm × 60-cm; packing L25
Flow rate: 0.5 mL/min
Injection volume: 50 µL

System suitability
Sample: *Standard solution*

Suitability requirements
Resolution: NLT 1.1 between the single-chain and two-chain alteplase peaks

Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The major peaks are from single-chain and two-chain alteplase and from higher and lower molecular weight species.]
Calculate the percentage of single-chain alteplase in the portion of Alteplase taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for single-chain alteplase
r_T = sum of all the peak responses of alteplase

Acceptance criteria: No peaks or shoulders in the *Sample solution* that are not present in the *Standard solution* are found; NLT 60%.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store in the frozen state at a temperature of –20° or below.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Alteplase RS

Alteplase for Injection

DEFINITION

Alteplase for Injection is a sterile lyophilized preparation of Alteplase. Its biological activity is NLT 90% and NMT 115% of that stated on the label in USP Alteplase Units. It contains NLT 95% and NMT 111% of the total protein content stated on the label.

IDENTIFICATION

- **A.**

Standard solution: 1.0–2.5 mg/mL of USP Alteplase RS in water

Sample solution: Prepare similarly to the *Standard solution*.

Analysis
Samples: *Standard solution* and *Sample solution*
To each of three test tubes transfer 1 mL of 0.5-mg/mL H-D-isoleucyl-prolyl-arginyl-*p*-nitroaniline dihydrochloride. Separately transfer 200 µL of the *Standard solution* and 200 µL of the *Sample solution* to two of the test tubes. To the third test tube add 200 µL of 0.2 M arginine solution that has been adjusted with phosphoric acid to a pH of 7.3 (negative control). Mix the solutions in the three test tubes, and allow to stand for 1 min.

Acceptance criteria: A yellow color is produced in the solutions from the *Standard solution* and the *Sample solution*, while no yellow color is produced in the negative control.

- **B. PEPTIDE MAPPING**

Solution A: 6.9 mg/mL of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.85. Filter, and degas.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
91	70	30
121	40	60
131	40	60

Dialysis solution: 480 mg/mL of urea, 44 mg/mL of tris(hydroxymethyl)aminomethane, and 0.88 mg/mL of edetic acid in water. Adjust with hydrochloric acid to a pH of 8.6.

Standard solution: Prepare a solution containing 1.0 mg/mL of USP Alteplase RS in water. Dialyze 2.0 mL of this solution into the *Dialysis solution* at room temperature for NLT 12 h. Measure the volume of the solution, and transfer it to a clean test tube. For each mL of solution in the tube, add 10 µL of 1 M dithiothreitol. Incubate at room temperature for 4 h, then add 25 µL of 1 M iodoacetic acid per mL of the solution, and incubate in the dark for 30 min. Quench the reaction by adding 50 µL of 1 M dithiothreitol per mL of the solution. Dialyze the solution against 0.1 M ammonium bicarbonate for 24 h, replacing the 0.1 M ammonium bicarbonate twice during the dialysis period. To 2.0 mL of the dialyzed solution, add 20 µg of trypsin, and incubate for 6–8 h at room temperature. Again add 20 µg of trypsin, and incubate for 16–18 h for a total of 24 h of incubation of the trypsin-treated solution. [NOTE—Store the *Standard solution* in a freezer.]

Sample solution: Using a quantity of Alteplase for Injection, proceed as directed in the *Standard solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between peaks 6 and 7 as defined by the USP Alteplase RS Data Sheet. The times for peaks 6 and 7 baseline widths are NMT 0.5 min.

Analysis

Samples: *Standard solution*, *Sample Solution*, and a mixture of the *Standard solution* and the *Sample solution* (1:1)

Measure the responses for NLT 20 major peaks as defined in the USP Alteplase RS Data Sheet.

Acceptance criteria: The retention times of corresponding peaks of the *Standard solution* and the *Sample solution* do not differ by more than 0.4 min, and the peak area ratios relative to peak 19 (as shown on the USP Alteplase RS Data Sheet) do not differ by more than 20%. No additional significant peaks or shoulders are found, a significant peak or shoulder being defined as one having a peak response of NLT 5% of peak 19.

ASSAY

• **BIOLOGICAL POTENCY**

Buffer: 1.38 mg/mL of monobasic sodium phosphate, 7.10 mg/mL of anhydrous dibasic sodium phosphate, 0.20 mg/mL of sodium azide, and 0.10 mg/mL of polysorbate 80 in water

Human thrombin solution: 33 U.S. Units in terms of the U.S. Standard Thrombin/mL in *Buffer*

Human fibrinogen solution: 2 mg/mL of human fibrinogen in *Buffer*

Human plasminogen solution: 1 mg/mL of human plasminogen in *Buffer*

Standard stock solution: 1.0 mg/mL (580,000 USP Alteplase Units) of USP Alteplase RS in water

Standard solutions: Dilute volumes of *Standard stock solution* with water to obtain a series of five *Standard solutions* having known concentrations ranging from 145 to 9.3 USP Alteplase Units/mL.

Sample stock solution: 1.0 mg/mL of Alteplase for Injection in water

Sample solutions: Dilute a volume of *Sample stock solution* with *Buffer* to obtain a series of dilutions of about 1:20,000; 1:10,000; and 1:5,000.

Analysis

Samples: *Standard solutions* and *Sample solutions*

To a set of labeled glass test tubes add 0.5 mL of *Human thrombin solution*. To separate test tubes add 0.5 mL of each *Standard solution* or *Sample solution*, and store on ice. To a second set of labeled glass tubes, add 20 µL of *Human plasminogen solution* and 1 mL of *Human fibrinogen solution*, and store on ice. Beginning with the thrombin–*Standard solution* mixture containing the *Standard solution* with the lowest number of USP Units/mL, record the time, and separately add 200 µL of each of the thrombin–*Standard solution* mixtures to the test tubes containing the plasminogen-fibrinogen mixture. Using a vortex mixer, intermittently mix the contents of each tube for a total of 15 s, and carefully place into a rack in a 37° circulating water bath. A visually turbid clot forms within 30 s, followed by the formation of bubbles within the clot. Record the clot lysis time (t_{cl}) from the first addition of the alteplase solution to the last bubble to rise to the surface.

Using a least squares fit, determine the equation of the line using the log values of the standard concentration, in USP Alteplase Units/mL, versus the log values of their clot lysis times in seconds taken:

$$\log t = m(\log U_S) + b$$

t = time to bubble release (s)
m = slope of the line
U_S = activity of the *Standard solution* (USP Alteplase Units/mL)
b = *y*-intercept of the line

The correlation coefficient is NLT –0.9900. From the line equation and using the log of the clot lysis time for the *Sample solution*, calculate the log of the activity (U_A):

$$\log U_A = \{[(\log t) - b]/m\}$$

Calculate the alteplase activity in USP Alteplase Units/mL taken:

$$\text{Result} = D(10^{\log U})$$

D = dilution factor for the *Sample solution*

Calculate the specific activity in the portion of Alteplase for Injection taken:

$$\text{Result} = (U_A/P)$$

P = concentration of protein obtained in the test for *Protein Content*

Acceptance criteria: 90%–115% of the potency stated on the label in USP Alteplase Units

www.webofpharma.com

OTHER COMPONENTS

• **PROTEIN CONTENT**

Arginine solution: 34.8 mg/mL of arginine in water. Adjust with phosphoric acid to a pH of 7.3.

Sample stock solution: 1 mg/mL of Alteplase for Injection in water

Sample solution: Dilute a volume of *Sample stock solution* with a volume of *Arginine solution* to obtain a solution having an absorbance value of 0.5–1.0 at the wavelength of maximum absorbance at about 280 nm. Determine the dilution volume (*V*).

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Wavelength range: 240–500 nm

Analytical wavelengths: 320 nm and maximum absorbance at about 280 nm

Cell: 1 cm

Blank: *Arginine solution*

Analysis

Samples: *Sample solution* and *Blank*

Calculate the protein content in the portion of Alteplase for Injection taken:

$$\text{Result} = [(A_{max} - A_{320})/\varepsilon] \times V$$

A_{max} = absorbance value at the wavelength of maximum absorbance

A_{320} = absorbance of the *Sample solution* at 320 nm

ε = molar absorptivity of alteplase, 1.9

V = volume of *Arginine solution* required to prepare the *Sample solution*

Acceptance criteria: 95%–111% of the total protein content stated on the label

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Acceptance criteria: Meets the requirements for *Content Uniformity*

SPECIFIC TESTS

• **PERCENT MONOMER**

Mobile phase: 34.84 mg/mL of arginine, 158.56 mg/mL of ammonium sulfate, and 100 mL/L of isopropyl alcohol in water. Adjust with phosphoric acid to a pH of 7.3, degas, and pass through a filter of 0.45-µm pore size.

System suitability solution: 1 mg/mL each of chicken ovalbumin and bovine gamma globulin

Standard solution: 1 mg/mL of USP Alteplase RS in water

Sample solution: 1 mg/mL of Alteplase for Injection in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 7.5-mm × 30-cm; packing L25

Flow rate: 0.5–1.0 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.6 between gamma globulin and ovalbumin, *System suitability solution*

Column efficiency: NLT 1200 theoretical plates, determined from the alteplase peak, *Standard solution*

Analysis

Sample: *Sample solution*

Calculate, as a percentage, the monomer in the portion of Alteplase for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of the alteplase monomer

r_T = sum of all the peak responses related to alteplase

Acceptance criteria: NLT 95.0%

• **SINGLE-CHAIN CONTENT**

Mobile phase: 27.6 mg/mL of monobasic sodium phosphate in sodium dodecyl sulfate solution (1 in 1000). Adjust with sodium hydroxide to a pH of 6.8. Filter, and degas.

Dithiothreitol solution: 3.12 mg/mL of dithiothreitol in *Mobile phase*

Standard stock solution: Using an accurately weighed quantity of USP Alteplase RS, make a 1-mg/mL solution in water.

Standard solution: Pipet 1 mL of the *Standard stock solution* into a glass tube. Add 3 mL of *Dithiothreitol solution*, cap the tube, and invert to mix. Heat for 3–5 min at about 80°.

Sample stock solution: Using an accurately weighed quantity of Alteplase for Injection, make a 1-mg/mL solution in water.

Sample solution: Pipet 1 mL of the *Sample stock solution* into a glass tube. Add 3 mL of *Dithiothreitol solution*, cap the tube, and invert to mix. Heat for 3–5 min at about 80°.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 7.5-mm × 60-cm; packing L25

Flow rate: 0.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.1 between the single-chain and two-chain alteplase peaks

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—The major peaks are from single-chain and two-chain alteplase and from higher and lower molecular weight species.]

Calculate the percentage of single-chain alteplase in the portion of Alteplase for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for single-chain alteplase

r_T = sum of all the peak responses of alteplase

Acceptance criteria: No peaks or shoulders in the *Sample solution* that are not present in the *Standard solution* are found; NLT 60%.

• **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩**:** Meets the requirements of constituted solutions at the time of use

• **PH** ⟨791⟩

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 7.1–7.5

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 4.0%

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 1 USP Endotoxin Unit/mg

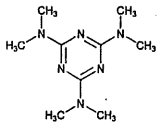
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*

• **BIOLOGICAL REACTIVITY TESTS, IN VIVO** ⟨88⟩**:** Meets the requirements for *Safety Tests—Biologicals*

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in hermetic, light-resistant containers, and store in a refrigerator.
- **LABELING:** Label it to state the biological activity in USP Alteplase Units/vial and the amount of protein/vial.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Alteplase RS

Altretamine



$C_9H_{18}N_6$ 210.28
1,3,5-Triazine-2,4,6-triamine, *N,N,N′,N′,N″,N″*-hexamethyl-; Hexamethylmelamine [645-05-6].

DEFINITION
Altretamine contains NLT 98.0% and NMT 102.0% of altretamine ($C_9H_{18}N_6$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy* 197A or 197K▲ (CN 1-May-2020)**:** Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: 1.7 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 7.0 ± 0.1.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
25	50	50
28	50	50
29	100	0
35	100	0

Standard stock solution: 1.0 mg/mL of USP Altretamine RS prepared as follows. Dissolve a suitable amount of USP Altretamine RS in about 40% of the flask volume of acetonitrile. Sonicate as needed to dissolve. Add water almost to volume and allow the solution to reach room temperature. Dilute with water to volume and mix.
Standard solution: 0.1 mg/mL of USP Altretamine RS in water from *Standard stock solution*
Sample stock solution: 1.0 mg/mL of Altretamine prepared as follows. Dissolve a suitable amount of Altretamine in about 40% of the flask volume of acetonitrile. Sonicate as needed to dissolve. Add water almost to volume and allow the solution to reach room temperature. Dilute with water to volume and mix.
Sample solution: 0.1 mg/mL of Altretamine in water from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 4.6-mm × 25-cm; 5-µm packing L96
Flow rate: 1.5 mL/min
Injection volume: 20 µL. [NOTE—An in-line filter may be needed to reduce system pressure.]
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of altretamine ($C_9H_{18}N_6$) in the portion of Altretamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Altretamine RS in the *Standard solution* (mg/mL)
C_U = concentration of Altretamine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Standard solution, and **Sample solution:** Prepare as directed in the *Assay*.
Sensitivity solution: 0.06 µg/mL of USP Altretamine RS in water from *Standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 and 242 nm (see *Table 2*)
Column: 4.6-mm × 25-cm; 5-µm packing L96
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10 for the altretamine peak, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Altretamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of altretamine from the *Standard solution*
C_S = concentration of USP Altretamine RS in the *Standard solution* (mg/mL)

www.webofpharma.com

C_U = concentration of Altretamine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is NMT 0.06%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Detector (nm)	Acceptance Criteria, NMT (%)
Altretamine diketo analog[a]	0.21	0.44	242	0.1
Altretamine chloro keto analog[b]	0.35	0.60	215	0.1
Altretamine keto analog[c]	0.56	1.3	242	0.1
Altretamine dichloro analog[d]	0.87	0.87	242	0.1
Altretamine monochloro analog[e]	0.96	1.3	242	0.1
Altretamine	1.00	1.0	215, 242[f]	—
Any other individual impurities	—	—	242	0.1
Total impurities	—	—	—	0.3

[a] 6-(Dimethylamino)-1,3,5-triazine-2,4[1*H*,3*H*]-dione.
[b] 4-Chloro-6-(dimethylamino)-1,3,5-triazine-2[1*H*]-one.
[c] 4,6-Bis(dimethylamino)-1,3,5-triazine-2[1*H*]-one.
[d] 4,6-Dichloro-*N*,*N*-dimethyl-1,3,5-triazine-2-amine.
[e] 6-Chloro-N^2,N^2,N^4,N^4-tetramethyl-1,3,5-triazine-2,4-diamine.
[f] Use the detector consistent with respective impurity.

SPECIFIC TESTS
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 1%
- **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count is NMT 10^3 cfu/g. The total combined molds and yeasts count is NMT 10^2 cfu/g.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Altretamine RS

Altretamine Capsules

DEFINITION
Altretamine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of altretamine ($C_9H_{18}N_6$).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Remove as completely as possible the contents of 5 Capsules, and dissolve, with shaking, in 10 mL of chloroform. Filter, and evaporate the chloroform solution to dryness.
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**
  ▲**Solution A:** 1.7 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 7.0 ± 0.1.
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
25	50	50
28	50	50
29	100	0
35	100	0

Standard stock solution: 1.0 mg/mL of USP Altretamine RS prepared as follows. Dissolve a suitable amount of USP Altretamine RS in about 40% of the flask volume of acetonitrile. Sonicate as needed to dissolve. Add water almost to volume and allow it to reach room temperature. Dilute with water to volume and mix.
Standard solution: 0.1 mg/mL of USP Altretamine RS in water from *Standard stock solution*
Sample stock solution: Nominally 1.0 mg/mL of altretamine from Capsules prepared as follows. Transfer NLT 20 Capsules to a suitable volumetric flask. Dissolve in about 40% of the flask volume of acetonitrile. Sonicate as needed to dissolve. Add water almost to volume and allow the solution to reach room temperature. Dilute with water to volume and mix.
Sample solution: Nominally 0.1 mg/mL of altretamine in water from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 4.6-mm × 25-cm; 5-µm packing L96
Flow rate: 1.5 mL/min
Injection volume: 20 µL. [NOTE—In-line filter may be needed to reduce system pressure.]▲ (USP 1-May-2019)
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of altretamine ($C_9H_{18}N_6$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

USP Monographs

www.webofpharma.com

r_S = peak response from the *Standard solution*
C_S = concentration of USP Altretamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of altretamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Standard solution, and Sample solution: Prepare as directed in the *Assay*.

Sensitivity solution: 0.1 µg/mL of USP Altretamine RS in water from *Standard solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 and 242 nm (see *Table 2*)

Column: 4.6-mm × 25-cm; 5-µm packing L96

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10 for the altretamine peak, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of altretamine from the *Standard solution*
C_S = concentration of USP Altretamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of altretamine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is NMT 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Detector (nm)	Acceptance Criteria, NMT (%)
Altretamine diketo analog[a]	0.21	0.44	242	0.4
Altretamine chloro keto analog[b]	0.35	0.60	215	0.4
Altretamine keto analog[c]	0.56	1.3	242	0.4
Altretamine dichloro analog[d]	0.87	0.87	242	0.4
Altretamine monochloro analog[e]	0.96	1.3	242	0.4
Altretamine	1.00	1.0	215, 242[f]	—
Any other individual impurity	—	—	242	0.4
Total impurities	—	—	—	1.0

[a] 6-(Dimethylamino)-1,3,5-triazine-2,4[1*H*,3*H*]-dione.
[b] 4-Chloro-6-(dimethylamino)-1,3,5-triazine-2[1*H*]-one.
[c] 4,6-Bis(dimethylamino)-1,3,5-triazine-2[1*H*]-one.
[d] 4,6-Dichloro-*N*,*N*-dimethyl-1,3,5-triazine-2-amine.
[e] 6-Chloro-N^2,N^2,N^4,N^4-tetramethyl-1,3,5-triazine-2,4-diamine.
[f] Use the detector consistent with the respective impurity. ▲ (USP 1-May-2019)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Detector: UV 242 nm

Standard solution: USP Altretamine RS in *Medium*

Analysis: Determine the amount of altretamine ($C_9H_{18}N_6$) dissolved from UV absorbances on filtered portions of the solution under test, suitably diluted if necessary with *Medium*, compared with the *Standard solution*.

Tolerances: NLT 80% (*Q*) of the labeled amount of altretamine ($C_9H_{18}N_6$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Altretamine RS

Ammonium Alum

$AlNH_4(SO_4)_2 \cdot 12H_2O$ 453.33

$AlNH_4(SO_4)_2$ 237.15

Sulfuric acid, aluminum ammonium salt (2:1:1), dodecahydrate;
Aluminum ammonium sulfate (1:1:2), dodecahydrate [7784-26-1].
Anhydrous [7784-25-0].

DEFINITION

Ammonium Alum contains NLT 99.0% and NMT 100.5% of ammonium alum [$AlNH_4(SO_4)_2$], calculated on the dried basis.

IDENTIFICATION

• **A.**

Sample solution: 50 mg/mL

Analysis: Add 1 N sodium hydroxide dropwise to the *Sample solution*.

Acceptance criteria: A precipitate is formed, and it dissolves in an excess of the reagent with the evolution of ammonia, recognizable by its alkaline effect upon moistened red litmus paper exposed to the vapor.

• **B. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

USP Monographs

www.webofpharma.com

• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sulfate*
Sample solution: 50 mg/mL
Analysis: Proceed as directed in *Identification Tests—General, Sulfate* ⟨191⟩, except centrifuge the neutral solutions of sulfates and use the supernatants for test *B.*
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M).*
Sample: 800 mg of Ammonium Alum
Analysis: Transfer the *Sample* to a 400-mL beaker, moisten with 1 mL of glacial acetic acid, and add 50 mL of water, 50.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS. Warm on a steam bath until the solution is complete, and boil gently for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and titrate the excess edetate disodium with 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 11.86 mg of $AlNH_4(SO_4)_2$.
Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES
• **IRON**
Sample solution: 6.7 mg/mL
Analysis: Add 5 drops of potassium ferrocyanide TS to 20 mL of the *Sample solution.*
Acceptance criteria: No blue color is produced immediately.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Sample: 2.0 g
Analysis: Transfer the *Sample,* in a tared porcelain crucible, to a muffle furnace at 200°. Increase the temperature to 300°, and dry at 300° to a constant weight. Cool in a desiccator, and weigh.
Acceptance criteria: 45.0%–48.0%
• **LIMIT OF ALKALIES AND ALKALINE EARTHS**
Sample: 1 g
Analysis: Completely precipitate the aluminum from a boiling solution of the *Sample* in 100 mL of water by the addition of sufficient 6 N ammonium hydroxide to render the solution distinctly alkaline to methyl red TS, and filter. Evaporate the filtrate to dryness, and ignite.
Acceptance criteria: The weight of the residue is NMT 5 mg (0.5%).

Potassium Alum

$AlK(SO_4)_2 \cdot 12H_2O$ 474.39

$AlK(SO_4)_2$ 258.21

Sulfuric acid, aluminum potassium salt (2:1:1), dodecahydrate;
Aluminum potassium sulfate (1:1:2) dodecahydrate [7784-24-9].
Anhydrous [10043-67-1].

DEFINITION
Potassium Alum contains NLT 99.0% and NMT 100.5% of potassium alum [$AlK(SO_4)_2$], calculated on the dried basis.

IDENTIFICATION
• **A.**
Sample solution: 50 mg/mL in water
Analysis: Add 1 N sodium hydroxide dropwise to the *Sample solution.*
Acceptance criteria: A precipitate is formed that dissolves in an excess of the reagent. Ammonia is not evolved (distinction from ammonium alum).
• **B.**
Analysis: Hold it in a nonluminous flame.
Acceptance criteria: A violet color is imparted to the flame.
• **C.**
Sample solution: Saturated solution in water
Analysis: Add 10 mL of sodium bitartrate TS to 5 mL of the *Sample solution.*
Acceptance criteria: A white, crystalline precipitate is formed within 30 min.
• **D. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩ **AND** *Sulfate* ⟨191⟩
Sample solution: 50 mg/mL in water
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M).*
Sample: 800 mg
Analysis: Transfer the *Sample* to a 400-mL beaker, moisten with 1 mL of glacial acetic acid, and add 50 mL of water, 50.0 mL of *Edetate disodium titrant,* and 20 mL of acetic acid–ammonium acetate buffer TS. Warm on a steam bath until solution is complete, and boil gently for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and titrate 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 12.91 mg of potassium alum [$AlK(SO_4)_2$].
Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES
• **IRON**
Sample solution: Potassium alum in water (1 in 150)
Analysis: Add 5 drops of potassium ferrocyanide TS to 20 mL of the *Sample solution.*
Acceptance criteria: No blue color is produced immediately.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Sample: 2.0 g
Analysis: Transfer the *Sample* in a tared porcelain crucible to a muffle furnace at 200°. Increase the temperature to 400°, and dry at 400° to constant weight. Cool in a desiccator, and weigh.
Acceptance criteria: 43.0%–46.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.

Alumina and Magnesia Oral Suspension

DEFINITION
Alumina and Magnesia Oral Suspension is a mixture containing aluminum hydroxide [$Al(OH)_3$] and Magnesium Hydroxide [$Mg(OH)_2$]. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amounts of

USP Monographs

www.webofpharma.com

aluminum hydroxide [$Al(OH)_3$] and magnesium hydroxide [$Mg(OH)_2$]. It may contain a flavoring agent, and may contain suitable antimicrobial agents.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
Sample solution: To a solution of 5 g in 10 mL of 3 N hydrochloric acid add 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 min, and filter.
Acceptance criteria: The filtrate meets the requirements.

• **B. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩
Sample solution: Wash the precipitate obtained in *Identification* test *A* with a hot solution containing 20 mg/mL of ammonium chloride, and dissolve the precipitate in hydrochloric acid.
Acceptance criteria: The solution meets the requirements.

ASSAY

• **ALUMINUM HYDROXIDE**
Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M).*
Sample solution: Transfer a volume of Oral Suspension, previously well shaken in its original container, equivalent to 1200 mg of aluminum hydroxide, to a suitable beaker. Add 20 mL of water, stir, and slowly add 10 mL of hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, and add water to volume.
Analysis: Pipet 10 mL of the *Sample solution* into a beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat near the boiling point for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 3.900 mg of aluminum hydroxide [$Al(OH)_3$].
Acceptance criteria: 90.0%–110.0%

• **MAGNESIUM HYDROXIDE**
Sample solution: Prepare as directed in the *Assay* for *Aluminum Hydroxide*.
Analysis: Pipet a volume of the *Sample solution*, equivalent to 40 mg of magnesium hydroxide, into a 400-mL beaker. Add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol), and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of magnesium hydroxide [$Mg(OH)_2$].
Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample solution: Dissolve 5.0 g in the minimum volume of nitric acid required to achieve complete solution, add 1 mL of acid in excess, then add water to make 100 mL, and filter.
Acceptance criteria: NMT 0.14%; a 10-mL portion of the *Sample solution* shows no more chloride than corresponds to 1.0 mL of 0.020 N hydrochloric acid.

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Sample solution: Dissolve 5.0 g in 5 mL of 3 N hydrochloric acid, with gentle heating. Cool, add water to make 250 mL, and filter.
Acceptance criteria: NMT 0.1%; a 20-mL portion of the *Sample solution* shows no more sulfate than corresponds to 0.40 mL of 0.020 N sulfuric acid.

• **ARSENIC,** *Method I* ⟨211⟩
Standard preparation: Prepare as directed in *Arsenic* ⟨211⟩, except prepare it to contain 5 µg of arsenic instead of 3 µg.
Test preparation: Dissolve a portion of Oral Suspension, equivalent to 0.5 g of aluminum hydroxide [$Al(OH)_3$], in 20 mL of 7 N sulfuric acid.
Acceptance criteria: NMT 10 ppm, based on the aluminum hydroxide [$Al(OH)_3$] content

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** Its total aerobic microbial count does not exceed 10^2 cfu/mL, and it meets the requirements for the absence of *Escherichia coli.*

• **pH** ⟨791⟩**:** 7.3–8.5

• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩
Acceptance criteria: The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M)$$

F_A = theoretical acid-neutralizing capacity of aluminum hydroxide [$Al(OH)_3$], 0.0385 mEq
A = amount of aluminum hydroxide [$Al(OH)_3$] in the specimen tested, based on the labeled quantity (mg)
F_M = theoretical acid-neutralizing capacity of magnesium hydroxide [$Mg(OH)_2$], 0.0343 mEq
M = amount of magnesium hydroxide [$Mg(OH)_2$] in the specimen tested, based on the labeled quantity (mg)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.

• **LABELING:** Oral Suspension may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of aluminum hydroxide [$Al(OH)_3$].

Alumina and Magnesia Tablets

DEFINITION

Alumina and Magnesia Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum hydroxide [$Al(OH)_3$] and magnesium hydroxide [$Mg(OH)_2$].

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
Sample solution: To a 0.7-g portion of finely powdered Tablets add 10 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution changes to deep yellow. Continue boiling for 2 min, and filter.
Acceptance criteria: The filtrate meets the requirements.

www.webofpharma.com

• **B. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩
Sample solution: Wash the precipitate obtained in *Identification* test *A* with hot ammonium chloride solution (1 in 50), and dissolve the precipitate in hydrochloric acid.
Acceptance criteria: The solution meets the requirements.

ASSAY

• **ALUMINUM HYDROXIDE**
Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M).*
Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 1200 mg of aluminum hydroxide, to a 150-mL beaker. Add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, and add water to volume.
Analysis: Pipet 10 mL of the *Sample solution* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat near the boiling point for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* is equivalent to 3.900 mg of aluminum hydroxide [$Al(OH)_3$].
Acceptance criteria: 90.0%–110.0%

• **MAGNESIUM HYDROXIDE**
Sample solution: Prepare as directed in the *Assay* for *Aluminum Hydroxide.*
Analysis: Pipet a volume of the *Sample solution*, equivalent to 40 mg of magnesium hydroxide, into a 400-mL beaker. Add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black TS in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol), and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of magnesium hydroxide [$Mg(OH)_2$].
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩
Time: 10 min, simulated gastric fluid TS being substituted for water in the test
Acceptance criteria: Meet the requirements

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Weight Variation* with respect to alumina and to magnesia

SPECIFIC TESTS

• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩**:** The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M)$$

F_A = theoretical acid-neutralizing capacity of aluminum hydroxide [$Al(OH)_3$], 0.0385 mEq
A = amount of aluminum hydroxide [$Al(OH)_3$] in the specimen tested, based on the labeled quantity (mg)
F_M = theoretical acid-neutralizing capacity of magnesium hydroxide [$Mg(OH)_2$], 0.0343 mEq
M = amount of magnesium hydroxide [$Mg(OH)_2$] in the specimen tested, based on the labeled quantity (mg)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** Tablets prepared with the use of Dried Aluminum Hydroxide Gel may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of aluminum hydroxide [$Al(OH)_3$].

Alumina, Magnesia, and Calcium Carbonate Oral Suspension

DEFINITION

Alumina, Magnesia, and Calcium Carbonate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum hydroxide [$Al(OH)_3$], magnesium hydroxide [$Mg(OH)_2$], and calcium carbonate ($CaCO_3$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩
Sample solution: To 5 g of Oral Suspension add 25 mL of 2 N sulfuric acid, stir, and allow to stand for 5 min. Add 25 mL of alcohol, stir, and place in an ice bath for 30 min. Filter while cold, retaining the filtrate for *Identification* test *B.* Wash the precipitate with 50 mL of 0.75 N sulfuric acid, and discard the washings. Dissolve the precipitate in 3 N hydrochloric acid, filter, and use the filtrate.
Acceptance criteria: Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩
Sample solution: To the filtrate obtained in *Identification* test *A*, add 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, continue boiling for 2 min, and filter through hardened filter paper. (Retain the filtrate for *Identification* test C.) Wash the precipitate with 350 mL of a hot solution containing 20 mg/mL of ammonium chloride, discarding the washings. Dissolve the precipitate so obtained in 3 N hydrochloric acid.
Acceptance criteria: Meets the requirements

• **C. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
Sample solution: The filtrate obtained in *Identification* test *B.*
Acceptance criteria: Meets the requirements

ASSAY

• **ALUMINUM HYDROXIDE**
Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M).*
Sample solution: Transfer an amount of Oral Suspension, previously shaken in its original container, equivalent to 600 mg of aluminum hydroxide, to a tared beaker, and weigh. Add 20 mL of water, stir, and slowly add 40 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and transfer to a 200-mL volumetric flask. Wash the

USP Monographs

www.webofpharma.com

beaker with water, adding the washings to the flask, and add water to volume.

Analysis: Pipet 10 mL of the *Sample solution* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 3.900 mg of aluminum hydroxide [$Al(OH)_3$].

Acceptance criteria: 90.0%–110.0%

- **MAGNESIUM HYDROXIDE**

Sample solution: Prepare as directed in the *Assay* for *Aluminum Hydroxide*.

Analysis: Pipet a volume of the *Sample solution*, equivalent to 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of trolamine, and mix. Add 50 mL of ammonia–ammonium chloride buffer TS and 2 drops of an eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of trolamine and 5 mL of dehydrated alcohol, and mixing). Cool the solution to between 3° and 4° by immersing the beaker in an ice bath, and titrate with 0.05 M edetate disodium VS until the color changes to pure blue. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. From the volume of 0.05 M edetate disodium consumed, subtract the volume of 0.05 M edetate disodium consumed in the *Assay* for *Calcium Carbonate*. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of magnesium hydroxide [$Mg(OH)_2$].

Acceptance criteria: 90.0%–110.0%

- **CALCIUM CARBONATE**

Sample solution: Prepare as directed in the *Assay* for *Aluminum Hydroxide*.

Analysis: Pipet a volume of the *Sample solution*, equivalent to 50 mg of calcium carbonate, into a 400-mL beaker, and add 200 mL of water, 5 mL of sodium hydroxide solution (1 in 2), and 250 mg of hydroxy naphthol blue. Stir with a magnetic stirrer, and titrate immediately with 0.05 M edetate disodium VS until the solution is distinctly blue. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of calcium carbonate ($CaCO_3$).

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Sample solution: Dissolve 5.0 g in 3 mL of nitric acid, add water to make 100 mL, and filter.

Acceptance criteria: NMT 0.14%; a 10.0-mL portion of the *Sample solution* shows no more chloride than corresponds to 1.0 mL of 0.020 N hydrochloric acid.

- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Sample solution: Dissolve 5.0 g in 7 mL of 3 N hydrochloric acid, and gently heat. Cool, add water to make 250 mL, and filter.

Acceptance criteria: NMT 0.1%; a 20.0-mL portion of the *Sample solution* shows no more sulfate than corresponds to 0.40 mL of 0.020 N sulfuric acid.

- **ARSENIC,** *Method I* ⟨211⟩

Standard preparation: Prepare as directed in *Arsenic* ⟨211⟩, except prepare it to contain 5 µg of arsenic instead of 3 µg.

Test preparation: Dissolve a portion of Oral Suspension, equivalent to 0.5 g of aluminum hydroxide [$Al(OH)_3$], in 20 mL of 7 N sulfuric acid.

Acceptance criteria: NMT 10 ppm, based on the aluminum hydroxide [$Al(OH)_3$] content

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** Its total aerobic microbial count does not exceed 10^2 cfu/mL, and it meets the requirements of the test for the absence of *Escherichia coli*.
- **pH** ⟨791⟩**:** 7.5–8.5
- **ACID-NEUTRALIZING CAPACITY** ⟨301⟩

Acceptance criteria: The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M) + 0.9 \times (F_C \times C)$$

F_A = theoretical acid-neutralizing capacity of aluminum hydroxide [$Al(OH)_3$], 0.0385 mEq

A = amount of aluminum hydroxide [$Al(OH)_3$] in the specimen tested, based on the labeled quantity (mg)

F_M = theoretical acid-neutralizing capacity of magnesium hydroxide [$Mg(OH)_2$], 0.0343 mEq

M = amount of magnesium hydroxide [$Mg(OH)_2$] in the specimen tested, based on the labeled quantity (mg)

F_C = theoretical acid-neutralizing capacity of calcium carbonate ($CaCO_3$), 0.02 mEq

C = amount of calcium carbonate ($CaCO_3$) in the specimen tested, based on the labeled quantity (mg)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.
- **LABELING:** Oral Suspension may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of aluminum hydroxide [$Al(OH)_3$].

Alumina, Magnesia, and Calcium Carbonate Chewable Tablets

DEFINITION

Alumina, Magnesia, and Calcium Carbonate Chewable Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum hydroxide [$Al(OH)_3$], magnesium hydroxide [$Mg(OH)_2$], and calcium carbonate ($CaCO_3$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩

Sample solution: To 3 g of finely powdered Chewable Tablets add 25 mL of water and 25 mL of 2 N sulfuric acid, stir, and heat on a steam bath for 10 min. Cool, add 50 mL of alcohol, stir, and place in an ice bath for 30 min. Filter while cold, retaining the filtrate for *Identification* test *B*. Wash the precipitate with 50 mL of 0.75 N sulfuric acid, and discard the washings. Dissolve the precipitate in 3 N hydrochloric acid, filter, and use the filtrate.

Acceptance criteria: Meet the requirements

USP Monographs

www.webofpharma.com

• **B. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩
Sample solution: To the filtrate obtained in *Identification* test *A* add 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, continue boiling for 2 min, and filter through hardened filter paper. (Retain the filtrate for *Identification* test *C*.) Wash the precipitate with 350 mL of a hot solution containing 20 mg/mL of ammonium chloride, discarding the washings. Dissolve the precipitate so obtained in 3 N hydrochloric acid.
Acceptance criteria: Meet the requirements
• **C. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
Sample solution: The filtrate obtained in *Identification* test *B*
Acceptance criteria: Meet the requirements

ASSAY

• **ALUMINUM HYDROXIDE**
Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.
Sample solution: Weigh and finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent to 600 mg of aluminum hydroxide, to a beaker, add 20 mL of water, and slowly add 40 mL of 3 N hydrochloric acid, with mixing. Heat the mixture to boiling, cool, and filter into a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the filter. Add water to volume.
Analysis: Pipet 10 mL of the *Sample solution* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 3.900 mg of aluminum hydroxide [$Al(OH)_3$].
Acceptance criteria: 90.0%–110.0%
• **MAGNESIUM HYDROXIDE**
Sample solution: Prepare as directed in the *Assay* for *Aluminum Hydroxide*.
Analysis: Pipet a volume of the *Sample solution*, equivalent to 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of trolamine, and mix. Add 50 mL of ammonia–ammonium chloride buffer TS and 2 drops of an eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of trolamine and 5 mL of dehydrated alcohol, and mixing). Cool the solution to between 3° and 4° by immersing the beaker in an ice bath, and titrate with 0.05 M edetate disodium VS until the color changes to pure blue. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. From the volume of 0.05 M edetate disodium consumed, subtract the volume of 0.05 M edetate disodium consumed in the *Assay* for *Calcium Carbonate*. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of magnesium hydroxide [$Mg(OH)_2$].
Acceptance criteria: 90.0%–110.0%
• **CALCIUM CARBONATE**
Sample solution: Prepare as directed in the *Assay* for *Aluminum Hydroxide*.
Analysis: Pipet a volume of the *Sample solution*, equivalent to 50 mg of calcium carbonate, into a 400-mL beaker, and add 200 mL of water, 5 mL of sodium hydroxide solution (1 in 2), and 250 mg of hydroxy naphthol blue. Stir with a magnetic stirrer, and titrate immediately with 0.05 M edetate disodium VS until the solution is distinctly blue. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of calcium carbonate ($CaCO_3$).
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩
Time: 45 min
Acceptance criteria: Meet the requirements
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Weight Variation* with respect to aluminum hydroxide, to magnesium hydroxide, and to calcium carbonate.

SPECIFIC TESTS

• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩
Acceptance criteria: The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M) + 0.9 \times (F_C \times C)$$

F_A = theoretical acid-neutralizing capacity of aluminum hydroxide [$Al(OH)_3$], 0.0385 mEq
A = amount of aluminum hydroxide [$Al(OH)_3$] in the specimen tested, based on the labeled quantity (mg)
F_M = theoretical acid-neutralizing capacity of magnesium hydroxide [$Mg(OH)_2$], 0.0343 mEq
M = amount of magnesium hydroxide [$Mg(OH)_2$] in the specimen tested, based on the labeled quantity (mg)
F_C = theoretical acid-neutralizing capacity of calcium carbonate ($CaCO_3$), 0.02 mEq
C = amount of calcium carbonate ($CaCO_3$) in the specimen tested, based on the labeled quantity (mg)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** Label the Chewable Tablets to indicate that they are to be chewed before being swallowed. Chewable Tablets prepared using dried aluminum hydroxide gel may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of aluminum hydroxide [$Al(OH)_3$].

Alumina, Magnesia, Calcium Carbonate, and Simethicone Chewable Tablets

DEFINITION

Alumina, Magnesia, Calcium Carbonate, and Simethicone Chewable Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum hydroxide [$Al(OH)_3$], magnesium hydroxide [$Mg(OH)_2$], and calcium carbonate ($CaCO_3$), and an amount of polydimethylsiloxane ([$-(CH_3)_2SiO-]_n$) that is NLT 85.0% and NMT 115.0% of the labeled amount of simethicone.

USP Monographs

www.webofpharma.com

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩
Sample solution: Cut a Chewable Tablet into pieces, add 50 mL of 1 N sulfuric acid, stir until the pieces disintegrate, and heat on a steam bath for 10 min. Cool, add 50 mL of alcohol, and stir. Place in an ice bath for 30 min. Filter while cold, retaining the filtrate for *Identification* test *B*. Wash the precipitate with 50 mL of 0.75 N sulfuric acid, and discard the washings. Dissolve the precipitate in 3 N hydrochloric acid, filter, and use the filtrate.
Acceptance criteria: Meet the requirements

• **B. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩
Sample solution: To the filtrate obtained in *Identification* test *A*, add 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, continue boiling for 2 min, and filter through hardened filter paper. (Retain the filtrate for *Identification* test *C*.) Wash the precipitate with 350 mL of a hot solution containing 20 mg/mL of ammonium chloride, discarding the washings. Dissolve the precipitate so obtained in 3 N hydrochloric acid.
Acceptance criteria: Meet the requirements

• **C. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
Sample solution: The filtrate obtained in *Identification* test *B*
Acceptance criteria: Meet the requirements

Change to read:

• **D. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)
Sample solution: Prepare as directed in the *Assay* for *Polydimethylsiloxane*.
Analysis: Proceed as directed using a 0.5-mm cell.
Acceptance criteria: Meet the requirements

ASSAY

• **ALUMINUM HYDROXIDE**
Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.
Sample solution: Transfer a number of Chewable Tablets, equivalent of about 665 mg of aluminum hydroxide, to a suitable beaker. Add 15 mL of hydrochloric acid, and swirl to dissolve the Chewable Tablets. Add 80 mL of water, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, and add water to volume.
Analysis: Pipet 20 mL of the *Sample solution* into a 250-mL beaker, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 20 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 3.900 mg of $Al(OH)_3$.
Acceptance criteria: 90.0%–110.0%

• **MAGNESIUM HYDROXIDE**
Lanthanum chloride solution: Transfer 17.6 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, and carefully add 50 mL of hydrochloric acid. Allow to cool, and dilute with water to volume.
Dilute hydrochloric acid: Dilute 226 mL of hydrochloric acid to 1000 mL with water.
Potassium chloride solution: 30 mg/mL in water
Magnesium stock solution: Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water, slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with water to volume. Transfer 1.0 mL of this solution to a 100-mL volumetric flask to obtain a solution containing 10 µg/mL of magnesium (Mg).
Standard solutions: To three separate 100-mL volumetric flasks each containing 5.0 mL of *Lanthanum chloride solution*, add 1.0, 2.0, and 3.0 mL, respectively, of the *Magnesium stock solution*. Dilute each with water to volume. These solutions contain 0.1, 0.2, and 0.3 µg/mL of magnesium (Mg), respectively.
Sample stock solution: Transfer a number of Chewable Tablets, equivalent of 250 mg of magnesium hydroxide (100 mg of magnesium), to a 1000-mL volumetric flask. Add 500 mL of *Dilute hydrochloric acid*, and swirl to dissolve the Chewable Tablets. Add 100.0 mL of *Potassium chloride solution*, and dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.
Sample solution: Transfer 2.0 mL of *Sample stock solution* to a second 100-mL volumetric flask, add 5.0 mL of *Lanthanum chloride solution*, and dilute with water to volume.
Blank: Add 50 mL of *Dilute hydrochloric acid* and 10.0 mL of *Potassium chloride solution* to a 100-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, and dilute with water to volume. Transfer 2.0 mL of this solution to a third 100-mL volumetric flask, add 5.0 mL of *Lanthanum chloride solution*, and dilute with water to volume.
Analysis: Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* at the magnesium emission line at 285.2 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a magnesium hollow-cathode lamp and an air–acetylene flame, using the *Blank* to set the instrument. Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of magnesium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, *C*, in µg/mL, of magnesium in the *Sample solution*.
Calculate the percentage of the labeled amount of magnesium hydroxide $[Mg(OH)_2]$ in the portion of Tablets taken:

$$\text{Result} = (C/C_U) \times (M_r/A_r) \times 100$$

C = concentration of magnesium in the *Sample solution*, as determined above (µg/mL)
C_U = nominal concentration of magnesium hydroxide in the *Sample solution* (µg/mL)
M_r = molecular weight of magnesium hydroxide, 58.34
A_r = atomic weight of magnesium, 24.305

Acceptance criteria: 90.0%–110.0%

• **CALCIUM CARBONATE**
Sample solution: Transfer a number of Chewable Tablets, equivalent of about 665 mg of aluminum hydroxide, to a suitable beaker. Add 15 mL of hydrochloric acid, and swirl to dissolve the Chewable Tablets. Add 80 mL of water, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, and add water to volume.
Analysis: Pipet a volume of the *Sample solution*, equivalent to 50 mg of calcium carbonate, into a 400-mL beaker, and add 200 mL of water, a volume of sodium hydroxide solution (1 in 2) equivalent to the volume of the *Sample solution* taken, and 250 mg of hydroxy naphthol blue. Stir with a magnetic stirrer, and titrate immediately with 0.05

www.webofpharma.com

M edetate disodium VS until the solution is distinctly blue. Perform a blank determination, substituting a volume of water equivalent to the volume of the *Sample solution* taken, and make any necessary correction. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of calcium carbonate ($CaCO_3$).

Acceptance criteria: 90.0%–110.0%

• **POLYDIMETHYLSILOXANE**

Dilute hydrochloric acid: Dilute 400 mL of hydrochloric acid with sufficient water to make 1000 mL.

Standard solution: Transfer 60 mg of USP Polydimethylsiloxane RS to a separator, add 30.0 mL of chloroform and 60 mL of *Dilute hydrochloric acid*, shake for 30 s, and allow the phases to separate. Remove 10 mL of the lower, organic layer to a screw-capped, 15-mL test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture to obtain a clear supernatant.

Sample solution: Weigh and finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent of 60 mg of simethicone, to a suitable screw-capped bottle. Add 30.0 mL of chloroform and 60 mL of *Dilute hydrochloric acid*, and allow to stand, with frequent shaking, until the Chewable Tablets are dissolved. Transfer the contents of the bottle to a separator, shake, and allow the phases to separate. Remove 10 mL of the lower, organic layer to a screw-capped, 15-mL test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture to obtain a clear supernatant.

Blank: Place 30.0 mL of chloroform and 60 mL of *Dilute hydrochloric acid* in a separator, shake for 30 s, and allow the phases to separate. Remove 10 mL of the lower, organic layer to a screw-capped, 15-mL test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap that has an inert liner, agitate vigorously, and centrifuge the mixture to obtain a clear supernatant.

Analysis: Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution* in 0.5-mm cells at the wavelength of maximum absorbance at about 7.9 µm, with a suitable IR spectrophotometer, using the *Blank* to set the instrument.

Calculate the percentage of polydimethylsiloxane ($[-(CH_3)_2\ SiO-]_n$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (W_S/W_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
W_S = weight of USP Polydimethylsiloxane RS taken to prepare the *Standard solution* (mg)
W_U = nominal amount of simethicone in the portion of the Tablets taken to prepare the *Sample solution* (mg)

Acceptance criteria: 85.0%–115.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Weight Variation* with respect to aluminum hydroxide, to magnesium hydroxide, and to calcium carbonate.

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 2×10^2 cfu/g, the total combined molds and yeasts count is NMT 2×10^2 cfu/g, and the Chewable Tablets meet the requirements of the test for the absence of *Salmonella* species and *Escherichia coli*.

• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩

Sample solution: Dissolve an accurately counted number of Chewable Tablets, equivalent to about 120 mEq of acid-neutralizing capacity, in 400 mL of water. Transfer the mixture to a 500-mL volumetric flask, and dilute with water to volume.

Analysis: Proceed as directed in the section *Procedure for Powders, Effervescent Solids, Suspensions and Other Liquids, Lozenges Nonchewable Tablets, Chewable Tablets, and Capsules* using 75.0 mL of the *Sample solution*.

Acceptance criteria: The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M) + 0.9 \times (F_C \times C)$$

F_A = theoretical acid-neutralizing capacity of aluminum hydroxide [$Al(OH)_3$], 0.0385 mEq
A = amount of aluminum hydroxide [$Al(OH)_3$] in the specimen tested, based on the labeled quantity (mg)
F_M = theoretical acid-neutralizing capacity of magnesium hydroxide [$Mg(OH)_2$], 0.0343 mEq
M = amount of magnesium hydroxide [$Mg(OH)_2$] in the specimen tested, based on the labeled quantity (mg)
F_C = theoretical acid-neutralizing capacity of calcium carbonate ($CaCO_3$), 0.02 mEq
C = amount of calcium carbonate ($CaCO_3$) in the specimen tested, based on the labeled quantity (mg)

• **SODIUM CONTENT**

Potassium chloride solution: 30 mg/mL of potassium chloride

Dilute hydrochloric acid: Dilute 226 mL of hydrochloric acid with sufficient water to make 1000 mL.

Standard stock solution: Transfer 2.5420 g of sodium chloride, previously dried at 105° for 2 h, to a 1000-mL volumetric flask, and dissolve in and dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, and dilute with water to volume.

Standard solutions: To three separate 100-mL volumetric flasks, each containing 10.0 mL of *Potassium chloride solution* and 3.0 mL of *Dilute hydrochloric acid*, add 10.0, 20.0, and 30.0 mL, respectively, of the *Standard stock solution*. The resulting *Standard solutions* contain 1.0, 2.0, and 3.0 µg/mL of sodium (Na), respectively.

Sample stock solution: Weigh 10 Chewable Tablets, and determine the average weight, *A*, in mg. Cut 4 Chewable Tablets into pieces, combine the pieces, and weigh them. Transfer the combined pieces to a 500-mL volumetric flask, add 150 mL of *Dilute hydrochloric acid*, and swirl gently to dissolve the pieces. Dilute with water to volume.

Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 10.0 mL of *Potassium chloride solution*, and dilute with water to volume.

Blank solution: Combine 3.0 mL of *Dilute hydrochloric acid* and 10.0 mL of *Potassium chloride solution* in a 100-mL volumetric flask, and dilute with water to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* at the sodium emission line at 589.0 nm with a suitable atomic

www.webofpharma.com

absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a sodium hollow-cathode lamp and an air–acetylene flame, using the *Blank solution* as the blank. Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of sodium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, *C*, in µg/mL, of sodium in the *Sample solution*.

Calculate the quantity, in mg, of sodium (Na) in each Chewable Tablet taken:

$$\text{Result} = (A/W) \times C \times D \times F$$

A = average weight of each Tablet (mg)
W = weight of the portion of Chewable Tablets taken to prepare the *Sample solution* (mg)
C = concentration of sodium in the *Sample solution* (µg/mL)
D = dilution factor for the *Sample solution*, 5000
F = conversion factor, 0.001 mg/µg

Acceptance criteria: Chewable Tablets contain NMT 5 mg/Tablet of sodium, except when labeled as containing more than 5 mg/Tablet of sodium; then they contain NMT 110% of the labeled amount.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** The labeling indicates that the Chewable Tablets are to be chewed before swallowing. Label the Chewable Tablets to state the sodium content, if it is greater than 5 mg/Chewable Tablet.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Polydimethylsiloxane RS

Alumina, Magnesia, and Simethicone Oral Suspension

DEFINITION

Alumina, Magnesia, and Simethicone Oral Suspension contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amounts of aluminum hydroxide [$Al(OH)_3$] and magnesium hydroxide [$Mg(OH)_2$], and an amount of polydimethylsiloxane ([$-(CH_3)_2\ SiO-]_n$) that is NLT 85.0% and NMT 115.0% of the labeled amount of simethicone.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)
  Sample solution: Prepare as directed in the *Assay* for *Polydimethylsiloxane*.
  Analysis: Proceed as directed using a 0.5-mm cell.
  Acceptance criteria: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
  Sample solution: Add 5 g of Oral Suspension to 10 mL of 3 N hydrochloric acid, then add 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution just changes to deep yellow, then continue boiling for 2 min, and filter.
  Acceptance criteria: Meets the requirements
- **C. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩
  Sample solution: Wash the precipitate from *Identification* test *B* with hot ammonium chloride solution (1 in 50), and dissolve the precipitate in hydrochloric acid. Divide this solution into two portions.
  Analysis 1: Add, dropwise, 6 N ammonium hydroxide to one portion of the *Sample solution*.
  Acceptance criteria 1: A gelatinous white precipitate, which does not dissolve in an excess of 6 N ammonium hydroxide, is obtained.
  Analysis 2: Add, dropwise, 1 N sodium hydroxide to the second portion of the *Sample solution*.
  Acceptance criteria 2: A gelatinous white precipitate, which dissolves in an excess of 1 N sodium hydroxide, leaving some turbidity, is obtained.

ASSAY

- **ALUMINUM HYDROXIDE**
  Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.
  Sample solution: Transfer a measured amount of Oral Suspension, previously well shaken in its original container, equivalent to 800 mg of aluminum hydroxide, to a suitable beaker. Add 20 mL of water, stir, and slowly add 10 mL of hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, and add water to volume.
  Analysis: Pipet 10 mL of the *Sample solution* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 3.900 mg of aluminum hydroxide [$Al(OH)_3$].
  Acceptance criteria: 90.0%–115.0%
- **MAGNESIUM HYDROXIDE**
  Sample solution: Prepare as directed in the *Assay* for *Aluminum Hydroxide*.
  Analysis: Pipet a volume of the *Sample solution*, equivalent to 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mixing). Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting water for the *Sample solution*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of magnesium hydroxide [$Mg(OH)_2$].
  Acceptance criteria: 90.0%–115.0%
- **POLYDIMETHYLSILOXANE**
  Standard solution: Prepare similarly to the *Sample solution* below, except dissolve 50 mg of USP Polydimethylsiloxane RS in 25.0 mL of toluene, add 40 mL of 0.1 N sodium hydroxide, and add a volume of water equal to that of the specimen of Oral Suspension taken for the *Sample solution*.
  Sample solution: Transfer an amount of Oral Suspension, equivalent to 50 mg of simethicone, to a suitable round,

USP Monographs

www.webofpharma.com

narrow-mouth, screw-capped, 120-mL bottle, add 40 mL of 0.1 N sodium hydroxide, and swirl to disperse. Add 25.0 mL of toluene, close the bottle securely with a cap having an inert liner, and shake for 15 min on a reciprocating shaker (e.g., about 200 oscillations/min and a stroke of 38 ± 2 mm). Transfer the mixture to a 125-mL separator. Remove about 5 mL of the upper, organic layer to a screw-capped, 15-mL test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained.

Blank: Mix 10 mL of toluene with 0.5 g of anhydrous sodium sulfate, and centrifuge to obtain a clear supernatant.

Analysis: Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution* in 0.5-mm cells at the wavelength of maximum absorbance at about 7.9 µm, with a suitable IR spectrophotometer, using the *Blank* to set the instrument.

Calculate the percentage of polydimethylsiloxane ($[-(CH_3)_2\ SiO-]_n$) in the portion of Oral Suspension taken:

$$\text{Result} = (A_U/A_S) \times (W_S/W_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
W_S = weight of USP Polydimethylsiloxane RS taken to prepare the *Standard solution* (mg)
W_U = nominal amount of simethicone in the portion of the Oral Suspension taken to prepare the *Sample solution* (mg)

Acceptance criteria: 85.0%–115.0%

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 10^2 cfu/g, and it meets the requirements of the test for the absence of *Escherichia coli*.
- **ACID-NEUTRALIZING CAPACITY** ⟨301⟩

Acceptance criteria: The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M)$$

F_A = theoretical acid-neutralizing capacity of aluminum hydroxide [$Al(OH)_3$], 0.0385 mEq
A = amount of aluminum hydroxide [$Al(OH)_3$] in the specimen tested, based on the labeled quantity (mg)
F_M = theoretical acid-neutralizing capacity of magnesium hydroxide [$Mg(OH)_2$], 0.0343 mEq
M = amount of magnesium hydroxide [$Mg(OH)_2$] in the specimen tested, based on the labeled quantity (mg)

- **PH** ⟨791⟩**:** 7.0–8.6
- **SODIUM CONTENT**

Potassium chloride solution: 38 mg/mL of potassium chloride

Sodium chloride stock solution: 25.42 µg/mL of sodium chloride (previously dried at 105° for 2 h) in water. The solution contains 10 µg/mL of sodium.

Standard solutions: On the day of use, transfer 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* to each of two 100-mL volumetric flasks. To the respective flasks add 5.0 and 10.0 mL of *Sodium chloride stock solution*. Dilute with water to volume. The resulting *Standard solutions* contain 0.5 and 1.0 µg/mL of sodium (Na), respectively.

Sample solution: Transfer 5.0 mL of Oral Suspension, previously well shaken in its original container, to a 100-mL volumetric flask. Add 50 mL of 1 N hydrochloric acid, boil for 15 min, cool to room temperature, and dilute with water to volume. Filter, discarding the first few mL of the filtrate. Transfer 5.0 mL of the filtrate to a 100-mL volumetric flask containing 10.0 mL of *Potassium chloride solution*, and dilute with water to volume.

Blank solution: Combine 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* in a 100-mL volumetric flask, and dilute with water to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* at the sodium emission line at 589.0 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a sodium hollow-cathode lamp and an air–acetylene flame, using the *Blank solution* as the blank. Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of sodium, and draw a straight line between the plotted points. From the graph so obtained, determine the concentration, C, in µg/mL, of sodium in the *Sample solution*.

Calculate the quantity, in mg, of sodium (Na) in each mL of Oral Suspension taken:

$$\text{Result} = (1/N) \times C \times D \times F$$

N = volume of the Oral Suspension taken to prepare the *Sample solution*, 5.0 mL
C = concentration of sodium in the *Sample solution* (µg/mL)
D = dilution factor for the *Sample solution*, 2000
F = conversion factor, 0.001 mg/µg

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.
- **LABELING:** Oral Suspension may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of aluminum hydroxide ($Al(OH)_3$). Label it to state the sodium content if it is greater than 1 mg/mL.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Polydimethylsiloxane RS

Alumina, Magnesia, and Simethicone Chewable Tablets

DEFINITION

Alumina, Magnesia, and Simethicone Chewable Tablets contain the equivalent of NLT 90.0% and NMT 115.0% of the labeled amounts of aluminum hydroxide [$Al(OH)_3$] and magnesium hydroxide [$Mg(OH)_2$], and an amount of polydimethylsiloxane ($[-(CH_3)_2\ SiO-]_n$) that is NLT 85.0% and NMT 115.0% of the labeled amount of simethicone.

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)
Sample solution: Prepare as directed in the *Assay* for *Polydimethylsiloxane*.
Analysis: Proceed as directed using a 0.5-mm cell.
Acceptance criteria: Meet the requirements
• **B. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
Sample solution: To a portion of finely powdered Chewable Tablets, equivalent to 600 mg of magnesium hydroxide, add 25 mL of 3 N hydrochloric acid and 25 mL of water, and mix. Boil gently for 2 min. Allow to cool, and filter. Add 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution just turns to deep yellow. Continue boiling for 2 min, and filter.
Acceptance criteria: Meet the requirements
• **C. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩
Sample solution: Wash the precipitate from *Identification* test *B* with hot ammonium chloride solution (1 in 50), and dissolve the precipitate in hydrochloric acid. Divide this solution into two portions.
Analysis 1: Add, dropwise, 6 N ammonium hydroxide to one portion of the *Sample solution*.
Acceptance criteria 1: A gelatinous white precipitate, which does not dissolve in an excess of 6 N ammonium hydroxide, is obtained.
Analysis 2: Add, dropwise, 1 N sodium hydroxide to the second portion of the *Sample solution*.
Acceptance criteria 2: A gelatinous white precipitate, which dissolves in an excess of 1 N sodium hydroxide, leaving some turbidity, is obtained.

ASSAY

• **ALUMINUM HYDROXIDE**
Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.
Sample solution: Weigh and finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent of 800 mg of aluminum hydroxide, to a 150-mL beaker. Add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool to room temperature, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, and add water to volume.
Analysis: Pipet 10 mL of the *Sample solution* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 3.900 mg of aluminum hydroxide [$Al(OH)_3$].
Acceptance criteria: 90.0%–115.0%
• **MAGNESIUM HYDROXIDE**
Sample solution: Prepare as directed in the *Assay* for *Aluminum Hydroxide*.
Analysis: Pipet a volume of the *Sample solution*, equivalent to 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mixing). Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting water for the *Sample solution*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of magnesium hydroxide [$Mg(OH)_2$].
Acceptance criteria: 90.0%–115.0%
• **POLYDIMETHYLSILOXANE**
Standard solution: Prepare similarly to the *Sample solution* below, using 33 mg of USP Polydimethylsiloxane RS.
Sample solution: Weigh and finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent of 33 mg of simethicone, to a suitable round, narrow-mouth, screw-capped, 120-mL bottle. Add 40 mL of 0.1 N sodium hydroxide, and swirl to disperse. Add 20.0 mL of toluene, close the bottle securely with a cap having an inert liner, and shake for 30 min on a reciprocating shaker (e.g., 200 oscillations/min and a stroke of 38 ± 2 mm). Transfer the mixture to a 125-mL separator, and allow to separate. Remove the upper, organic layer to a screw-capped, centrifuge tube containing 2 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained.
Blank: Mix 10 mL of toluene with 1 g of anhydrous sodium sulfate, and centrifuge to obtain a clear supernatant.
Analysis: Concomitantly determine the absorbances of the *Standard solution* and *Sample solution* in 0.5-mm cells at the wavelength of maximum absorbance at about 7.9 µm (1265.8 cm^{-1}), with a suitable IR spectrophotometer, using the *Blank* to set the instrument.
Calculate the percentage of polydimethylsiloxane ($[-(CH_3)_2\ SiO-]_n$) in the portion of Chewable Tablets taken:

$$\text{Result} = (A_U/A_S) \times (W_S/W_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
W_S = weight of USP Polydimethylsiloxane RS taken to prepare the *Standard solution* (mg)
W_U = nominal amount of simethicone in the portion of the Chewable Tablets taken to prepare the *Sample solution* (mg)

Acceptance criteria: 85.0%–115.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium hydroxide

SPECIFIC TESTS

• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩
Acceptance criteria: The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M)$$

F_A = theoretical acid-neutralizing capacity of aluminum hydroxide [$Al(OH)_3$], 0.0385 mEq
A = amount of aluminum hydroxide [$Al(OH)_3$] in the specimen tested, based on the labeled quantity (mg)

www.webofpharma.com

F_M = theoretical acid-neutralizing capacity of magnesium hydroxide [$Mg(OH)_2$], 0.0343 mEq
M = amount of magnesium hydroxide [$Mg(OH)_2$] in the specimen tested, based on the labeled quantity (mg)

• **SODIUM CONTENT**

Potassium chloride solution: 38 mg/mL of potassium chloride

Sodium chloride stock solution: 25.42 µg/mL of sodium chloride (previously dried at 105° for 2 h) in water. The solution contains 10 µg/mL of sodium.

Standard solutions: On the day of use, transfer 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* to each of two 100-mL volumetric flasks. To the respective flasks add 5.0 and 10.0 mL of *Sodium chloride stock solution*. Dilute with water to volume. The resulting *Standard solutions* contain 0.5 and 1.0 µg/mL of sodium (Na), respectively.

Sample solution: Weigh and finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent to the average weight of 1 Chewable Tablet, to a 100-mL volumetric flask. Add 50 mL of 1 N hydrochloric acid, boil for 15 min, cool to room temperature, and dilute with water to volume. Filter, discarding the first few mL of the filtrate. Transfer 5.0 mL of the filtrate to a 100-mL volumetric flask containing 10.0 mL of *Potassium chloride solution*, and dilute with water to volume.

Blank solution: Combine 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* in a 100-mL volumetric flask, and dilute with water to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* at the sodium emission line at 589.0 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a sodium hollow-cathode lamp and an air–acetylene flame, using the *Blank solution* as the blank. Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of sodium, and draw a straight line between the plotted points. From the graph so obtained, determine the concentration, C, in µg/mL, of sodium in the *Sample solution*.

Calculate the quantity, in mg, of sodium (Na) in each Chewable Tablet taken:

$$\text{Result} = C \times D \times F$$

C = concentration of sodium in the *Sample solution* (µg/mL)
D = dilution factor for the *Sample solution*, 2000
F = conversion factor, 0.001 mg/µg

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** Label the Chewable Tablets to indicate that they are to be chewed before being swallowed. Label the Chewable Tablets to state the sodium content if it is greater than 5 mg/Tablet. The Chewable Tablets may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of aluminum hydroxide [$Al(OH)_3$].

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Polydimethylsiloxane RS

Alumina and Magnesium Carbonate Oral Suspension

» Alumina and Magnesium Carbonate Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide [$Al(OH)_3$] and magnesium carbonate ($MgCO_3$).

Packaging and storage—Preserve in tight containers, and avoid freezing.

Identification—

A: Place about 1 g in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

B: To a solution of 5 g in 10 mL of 3 N hydrochloric acid add 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* ⟨191⟩.

C: Wash the precipitate obtained in *Identification* test *B* with a hot solution of ammonium chloride (1 in 50), and dissolve the precipitate in hydrochloric acid: the solution responds to the tests for *Aluminum* ⟨191⟩.

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for absence of *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than the number of mEq calculated by the formula:

$$0.55(0.0385A) + 0.8(0.024M)$$

in which 0.0385 and 0.024 are the theoretical acid-neutralizing capacities, in mEq, of $Al(OH)_3$ and $MgCO_3$, respectively; and A and M are the respective quantities, in mg, of $Al(OH)_3$ and $MgCO_3$ in the specimen tested, based on the labeled quantities.

pH ⟨791⟩: between 7.5 and 9.5.

Assay for aluminum hydroxide—

Potassium chloride solution—Prepare a solution containing 4.5 g of potassium chloride in each 100 mL.

Aluminum stock solution—Transfer 1.000 g of aluminum wire to a 1000-mL volumetric flask, and add 50 mL of 6 N hydrochloric acid. Swirl to ensure contact of the aluminum and the acid, and allow the reaction to proceed until all of the aluminum has dissolved. Dilute with water to volume, and mix.

Standard preparations—To separate 100-mL volumetric flasks, each containing 10 mL of *Potassium chloride solution*, transfer 9.0, 10.0, and 11.0 mL, respectively, of *Aluminum stock solution*, dilute with water to volume, and mix. These *Standard preparations* contain 90.0, 100.0, and 110.0 µg of aluminum per mL, respectively.

Assay preparation—Transfer an accurately measured quantity of Oral Suspension, previously shaken in its original container, equivalent to about 75 mg of aluminum hydroxide, to a suitable beaker. Add 25 mL of 6 N hydrochloric acid, and heat on a steam bath for 30 minutes, with occasional swirling. Cool, and transfer with the aid of water to a 250-mL volumetric

USP Monographs

www.webofpharma.com

flask containing 25 mL of *Potassium chloride solution*. Dilute with water to volume, mix, and filter.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the aluminum emission line at 309.3 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with an aluminum hollow-cathode lamp and a nitrous oxide–acetylene flame, using water as the blank. Calculate the quantity, in mg, of $Al(OH)_3$ in the portion of Oral Suspension taken by the formula:

$$(78.00/26.98)(0.25)(A_U/R_S)$$

in which 78.00 is the molecular weight of aluminum hydroxide; 26.98 is the atomic weight of aluminum; A_U is the absorbance of the *Assay preparation;* and R_S is the average of the ratios of the absorbances of the *Standard preparations* to their respective concentrations, in µg of aluminum per mL.

Assay for magnesium carbonate—

Lanthanum chloride solution—Prepare a solution of lanthanum chloride in water containing 5 mg per mL.

Magnesium stock solution—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 50 mL of water, and slowly add 10 mL of hydrochloric acid. Dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard preparations—To separate 100-mL volumetric flasks, each containing 10 mL of *Lanthanum chloride solution*, transfer 1.70 mL and 1.80 mL, respectively, of *Magnesium stock solution*, dilute with water to volume, and mix. These *Standard preparations* contain 1.7 µg of magnesium per mL and 1.8 µg of magnesium per mL, respectively.

Assay preparation—Quantitatively dilute an accurately measured volume of the *Assay preparation* prepared as directed in the *Assay for aluminum hydroxide* with water to obtain a solution having a concentration of about 6 µg of magnesium carbonate per mL.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a magnesium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Calculate the quantity, in mg, of magnesium carbonate ($MgCO_3$) in the portion of Oral Suspension taken by the formula:

$$(84.31/24.31)(L/D)(A_U/R_S)$$

in which 84.31 is the molecular weight of magnesium carbonate; 24.31 is the atomic weight of magnesium; L is the labeled quantity, in mg, of magnesium carbonate in the portion of Oral Suspension taken; D is the concentration, in µg of magnesium carbonate per mL, of the *Assay preparation*, based on the labeled amount of magnesium carbonate in the portion of Oral Suspension taken and the extent of dilution; A_U is the absorbance of the *Assay preparation;* and R_S is the average of the ratios of the absorbances of the *Standard preparations* to their respective concentrations, in µg of magnesium per mL.

Alumina and Magnesium Carbonate Tablets

» Alumina and Magnesium Carbonate Tablets contain the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide [$Al(OH)_3$] and magnesium carbonate ($MgCO_3$).

Packaging and storage—Preserve in tight containers.

Identification—

A: Place about 1 g of finely powdered Tablets in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

B: To a 7-g portion of finely powdered Tablets add 10 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution changes to deep yellow. Continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* ⟨191⟩.

C: Wash the precipitate obtained in *Identification* test *B* with a hot solution of ammonium chloride (1 in 50), and dissolve the precipitate in hydrochloric acid: the solution responds to the tests for *Aluminum* ⟨191⟩.

Disintegration ⟨701⟩: 10 minutes, simulated gastric fluid TS being substituted for water in the test.

Uniformity of dosage units ⟨905⟩: meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium carbonate.

Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

Assay for aluminum hydroxide—

Potassium chloride solution—Prepare a solution containing 38.1 g of potassium chloride in each 1000 mL.

Digestion fluid—Mix 5 mL of hydrochloric acid, 10 mL of nitric acid, and 10 mL of water, and use promptly.

Aluminum stock solution—Transfer 1.000 g of aluminum metal to a 1000-mL volumetric flask, and add 50 mL of 6 N hydrochloric acid. Swirl to ensure contact of the aluminum and the acid, and allow the reaction to proceed until all of the aluminum has dissolved. Dilute with water to volume, and mix.

Standard preparations—To separate 100-mL volumetric flasks transfer 3.0 mL, 4.0 mL, and 5.0 mL of *Aluminum stock solution*, respectively. To each flask add 10 mL of *Potassium chloride solution* and 7.5 mL of *Digestion fluid*, dilute with water to volume, and mix. These *Standard preparations* contain 30, 40, and 50 µg of aluminum per mL, respectively.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 30 mg of aluminum hydroxide, to a 100-mL volumetric flask, add 25-mL of *Digestion fluid*, and heat on a steam bath for 30 minutes or on a hot plate until the volume is reduced by about one-half. Cool, dilute with water to volume, and mix. Filter, discarding the first 20 mL of the filtrate. Transfer 15.0 mL of the filtrate to a 50-mL volumetric flask, add 5.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix. [NOTE—Reserve a portion of the filtrate for use in the *Assay for magnesium carbonate.*]

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the

www.webofpharma.com

aluminum emission line at 309.3 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with an aluminum hollow-cathode lamp and a nitrous oxide–acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of aluminum, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, *C*, in µg per mL, of aluminum in each mL of the *Assay preparation*. Calculate the quantity, in mg, of aluminum hydroxide [$Al(OH)_3$] in the portion of Tablets taken by the formula:

$$(78.00/26.98)(C/3)$$

in which 78.00 is the molecular weight of aluminum hydroxide, and 26.98 is the atomic weight of aluminum.

Assay for magnesium carbonate—

Lanthanum chloride solution—Transfer 17.6 g of lanthanum chloride to a 1000-mL volumetric flask, add 500 mL of water, and carefully add 50 mL of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume, and mix.

Digestion fluid—Mix 5 mL of hydrochloric acid, 10 mL of nitric acid, and 10 mL of water, and use promptly.

Magnesium stock solution—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 50 mL of water, and slowly add 10 mL of hydrochloric acid. Dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Standard preparations—To separate 100-mL volumetric flasks transfer 4.0, 6.0, and 8.0 mL of *Magnesium stock solution*, respectively. To each flask add 0.5 mL of *Digestion fluid* and 10 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix. These *Standard preparations* contain 0.40, 0.60, and 0.80 µg of magnesium per mL, respectively.

Assay preparation—Transfer an accurately measured volume of the filtrate used to prepare the *Assay preparation* in the *Assay for aluminum hydroxide*, equivalent to about 0.4 mg of magnesium carbonate, to a 200-mL volumetric flask, add 20 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a magnesium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of magnesium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, *C*, in µg per mL, of magnesium in each mL of the *Assay preparation*. Calculate the quantity, in mg, of magnesium carbonate ($MgCO_3$) in the portion of Tablets taken by the formula:

$$(84.31/24.31)(20C/V)$$

in which 84.31 is the molecular weight of magnesium carbonate; 24.31 is the atomic weight of magnesium; and *V* is the volume taken, in mL, of the *Assay preparation* prepared as directed in the *Assay for aluminum hydroxide*.

Alumina, Magnesium Carbonate, and Magnesium Oxide Tablets

» Alumina, Magnesium Carbonate, and Magnesium Oxide Tablets contain the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide [$Al(OH)_3$] and magnesium carbonate ($MgCO_3$), and not less than 85.0 percent and not more than 115.0 percent of the labeled amount of magnesium oxide (MgO).

Packaging and storage—Preserve in tight containers.

Identification—

A: Place about 3 g of finely powdered Tablets in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

B: To the solution in the flask obtained in *Identification* test *A* add 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, continue boiling for 2 minutes, and filter through hardened filter paper. (Retain the filtrate for *Identification* test *C*.) Wash the precipitate with 350 mL of a hot ammonium chloride solution (1 in 50), discarding the washings: the precipitate so obtained, dissolved in 3 N hydrochloric acid, responds to the tests for *Aluminum* ⟨191⟩.

C: The filtrate obtained in *Identification* test *B* responds to the tests for *Magnesium* ⟨191⟩.

Disintegration ⟨701⟩: 10 minutes, simulated gastric fluid TS being substituted for water in the test.

Uniformity of dosage units ⟨905⟩: meet the requirements for *Weight Variation* with respect to alumina, to magnesium carbonate, and to magnesium oxide.

Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

Assay for aluminum hydroxide—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1200 mg of aluminum hydroxide, to a 150-mL beaker, add 20 mL of water, stir, and slowly add 30 mL 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, add water to volume, and mix.

Procedure—Pipet 10 mL of the *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat near the boiling point for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of $Al(OH)_3$.

Assay for magnesium carbonate—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately

USP Monographs

www.webofpharma.com

weighed portion of the powder, equivalent to about 750 mg of magnesium carbonate, to a 250-mL conical flask fitted with a two-hole stopper. Fill the lower transverse section of a U-shaped drying tube of about 15-mm internal diameter and 15-cm height with loosely packed glass wool. Place in one arm of the tube about 5 g of anhydrous calcium chloride, and accurately weigh the tube and the contents. Into the other arm of the tube place 9.5 g to 10.5 g of soda lime, and again weigh accurately. Insert stoppers in the open arms of the U-tube, and connect the side tube of the arm filled with soda lime to a calcium chloride drying tube, which in turn is connected to one of the holes in the stopper of the 250-mL conical flask. Attach a dropping funnel to the other hole in the stopper of the 250-mL conical flask. Add 100 mL of water and 10 mL of a mixture of hydrochloric acid and nitric acid (4:1) to the 250-mL conical flask through the dropping funnel, and close the dropping funnel. Heat the 250-mL conical flask at 95° for 1 hour, and allow the evolved carbon dioxide to pass through the U-tube. Replace the dropping funnel with a source of carbon dioxide-free air, and pass the carbon dioxide-free air through the apparatus at a rate of about 75 mL per minute for 30 minutes. Disconnect the U-tube, cool to room temperature, remove the stoppers, and weigh. The increase in weight corresponds to the quantity of carbon dioxide evolved. Calculate the quantity, in mg, of magnesium carbonate in each Tablet taken by the formula:

$$(84.31/44.01)(I)(W_A/W_P)$$

in which 84.31 and 44.01 are the molecular weights of magnesium carbonate and carbon dioxide, respectively; I is the quantity, in mg, of carbon dioxide evolved from the portion of Tablets taken; W_A is the average weight, in g, of 1 Tablet; and W_P is the weight, in g, of the portion of Tablets taken.

Assay for magnesium oxide—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1000 mg of magnesium carbonate and magnesium oxide combined, to a beaker, add 20 mL of water, and slowly add 40 mL of 3 N hydrochloric acid, with mixing. Heat the mixture to boiling, cool, and filter into a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the filter. Add water to volume, and mix. Transfer 20.0 mL of this solution to a 400-mL beaker, add 180 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 20 mL of water for the assay solution, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 1.216 mg of Mg. Calculate the quantity, in mg, of magnesium equivalent in each Tablet taken by the formula:

$$10T(W_A/W_P)$$

in which T is the magnesium equivalent obtained in the titration; W_A is the average weight, in g, of 1 Tablet; and W_P is the weight, in g, of the portion of Tablets taken. Calculate the quantity, in mg, of magnesium oxide in each Tablet taken by the formula:

$$(40.30/24.31)(A - 0.2883B)$$

in which 40.30 and 24.31 are the molecular weight of magnesium oxide and the atomic weight of magnesium, respectively; A is the quantity, in mg, of magnesium equivalent in each Tablet; and B is the quantity, in mg, of magnesium carbonate in each Tablet, as determined in the *Assay for magnesium carbonate*.

Alumina and Magnesium Trisilicate Oral Suspension

» Alumina and Magnesium Trisilicate Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide [$Al(OH)_3$] and magnesium trisilicate ($Mg_2Si_3O_8$).

Packaging and storage—Preserve in tight containers.

Identification—

A: To a mixture of 5 mL in 10 mL of 3 N hydrochloric acid add 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* ⟨191⟩.

B: Wash the solids on the filter obtained in *Identification* test *A* with hot ammonium chloride solution (1 in 50), add 10 mL of 3 N hydrochloric acid, and filter: the filtrate responds to the tests for *Aluminum* ⟨191⟩.

C: Transfer the filter paper and contents from *Identification* test *B* to a small platinum dish, ignite, cool in a desiccator, and weigh. Moisten the residue with water and add 6 mL of hydrofluoric acid. Evaporate to dryness, ignite for 5 minutes, cool in a desiccator, and weigh: a loss of more than 10% in relation to the weight of the residue from the initial ignition indicates SiO_2.

Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

pH ⟨791⟩: between 7.5 and 8.5.

Assay for aluminum hydroxide—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Assay preparation—Transfer about 10 g of well-shaken Oral Suspension to a tared beaker, and weigh accurately. Add 50 mL of water and 10 mL of hydrochloric acid, and digest on a steam bath for 1 hour. Cool, and filter into a 200-mL volumetric flask, washing the filter with water into the flask. Dilute with water to volume, and mix.

Procedure—Pipet 20 mL of *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat near the boiling point for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 20 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of $Al(OH)_3$.

Assay for magnesium trisilicate—

Assay preparation—Prepare as directed in the *Assay for aluminum hydroxide*.

USP Monographs

www.webofpharma.com

Procedure—Pipet 20 mL of *Assay preparation* into a 400-mL beaker, add 180 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 20 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 6.521 mg of $Mg_2Si_3O_8$.

Alumina and Magnesium Trisilicate Tablets

» Alumina and Magnesium Trisilicate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide [$Al(OH)_3$] and magnesium trisilicate ($Mg_2Si_3O_8$).

Packaging and storage—Preserve in well-closed containers.

Labeling—Tablets prepared with the use of *Dried Aluminum Hydroxide Gel* may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of $Al(OH)_3$. Tablets intended for the temporary relief of heartburn (acid indigestion) due to acid reflux are so labeled. Tablets that must be chewed before swallowing are so labeled.

Identification—One powdered Tablet responds to the *Identification* tests under *Alumina and Magnesium Trisilicate Oral Suspension*.

Disintegration ⟨701⟩: 10 minutes, simulated gastric fluid TS being substituted for water in the test. [NOTE—Tablets that must be chewed before swallowing are exempt from this requirement.]

Uniformity of dosage units ⟨905⟩: meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium trisilicate.

Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling. [NOTE—Tablets labeled for the temporary relief of heartburn (acid indigestion) due to acid reflux are exempt from this requirement.]

Foam [where Tablets are labeled for the temporary relief of heartburn (acid indigestion) due to acid reflux]—Finely powder a number of Tablets, accurately counted, equivalent to the minimum single dose recommended in the labeling, and transfer the powder to a 100-mL beaker having an inside diameter of 45 mm. Add 5 mL of alcohol and sufficient water to make 40 mL. Mix at 300 rpm for 60 seconds, using a magnetic stirrer and a 9.5- × 38-mm polytef-coated stirring bar. Stop the stirrer, and carefully add 10 mL of 0.5 N hydrochloric acid down the side of the beaker. Stir for 30 seconds at 300 rpm. Allow to stand for 10 minutes, and measure the thickness of the foam layer above the liquid in the beaker: the thickness of the foam is not less than 10 mm.

pH ⟨791⟩ [where Tablets are labeled for the temporary relief of heartburn (acid indigestion) due to acid reflux]: not less than 4.5, determined on the foam layer obtained in the *Foam* test. [NOTE—Take care that the electrodes do not touch the liquid beneath the foam.]

Assay for aluminum hydroxide—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 600 mg of aluminum hydroxide, to a beaker, add 20 mL of water, stir, and slowly add 40 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and transfer to a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the flask, add water to volume, and mix.

Procedure—Pipet 10 mL of *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of 0.05 M *Edetate disodium titrant* and 20 mL of acetic acid-ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of $Al(OH)_3$.

Assay for magnesium trisilicate—

Potassium chloride solution—Prepare a solution in water containing 5 g of potassium chloride per 100 mL.

Magnesium standard solution—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 50 mL of water, and slowly add 10 mL of hydrochloric acid. Dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Standard preparations—Transfer 16.0 mL, 18.0 mL, and 20.0 mL of *Magnesium standard solution* to separate 100-mL volumetric flasks, add 2.0 mL of *Potassium chloride solution* to each flask, dilute with water to volume, and mix. These *Standard preparations* contain 1.6, 1.8, and 2.0 µg of magnesium per mL, respectively. [NOTE—Prepare these solutions on the day of use.]

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of magnesium trisilicate, to a 100-mL volumetric flask, and add 10 mL of 18 N sulfuric acid. Heat on a steam bath for 30 minutes with occasional swirling. Allow to cool, dilute with water to volume, and mix. Filter this solution, discarding the first 20 mL of the filtrate. Transfer 20.0 mL of the filtrate to a second 100-mL volumetric flask, add 2.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbance of the *Standard preparations* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with an atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩), equipped with a magnesium hollow-cathode lamp and a nitrous oxide–acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations*, in µg per mL, of magnesium, and draw the line best fitting the three plotted points. From the graph so obtained determine the concentration, *C*, in µg per mL, of magnesium in the *Assay preparation*. Calculate the quantity, in mg, of magnesium trisilicate ($Mg_2Si_3O_8$) in the portion of Tablets taken by the formula:

$$0.5C(260.86/48.62)$$

USP Monographs

www.webofpharma.com

in which 260.86 is the molecular weight of anhydrous magnesium trisilicate and 48.62 is twice the atomic weight of magnesium.

Aluminum Acetate Topical Solution

DEFINITION

Aluminum Acetate Topical Solution yields NLT 1.20 g and NMT 1.45 g of aluminum oxide (Al_2O_3) and NLT 4.24 g and NMT 5.12 g of acetic acid ($C_2H_4O_2$), corresponding to NLT 4.8 g and NMT 5.8 g of aluminum acetate ($C_6H_9AlO_6$) in each 100 mL. Aluminum Acetate Topical Solution may be stabilized by the addition of NMT 0.6% of boric acid (H_3BO_3).

Aluminum Subacetate Topical Solution	545 mL
Glacial Acetic Acid	15 mL
Purified Water, a sufficient quantity to make	1000 mL

Add the *Glacial Acetic Acid* to the *Aluminum Subacetate Topical Solution* and sufficient *Purified Water* to bring to final volume. Mix, and filter if necessary. Dispense only clear Aluminum Acetate Topical Solution.

IDENTIFICATION

• **A. Identification Tests—General** ⟨191⟩**:** It meets the requirements of the test for *Aluminum* and for the test *B* under *Acetate*. Ferric chloride TS produces a deep red color that is destroyed by the addition of a mineral acid.

ASSAY

• **Aluminum Oxide**

Edetate disodium titrant: Prepare and standardize 0.05 M edetate disodium titrant as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.
Sample: 25 mL
Blank: 25 mL of water
Titrimetric system
Mode: Residual titration
Back-titrant: 0.05 M zinc sulfate VS
Endpoint detection: Visual
Analysis: Pipet the *Sample* into a 250-mL volumetric flask, add 5 mL of hydrochloric acid, and dilute with water to volume. Pipet 25 mL of this solution into a 250-mL beaker, and add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, then heat the solution near the boiling point for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the solution with *Back-titrant* to a bright rose-pink color. Perform a blank determination, and make any necessary correction. Each mL of *Edetate disodium titrant* is equivalent to 2.549 mg of aluminum oxide (Al_2O_3).
Acceptance criteria: 1.20–1.45 g of aluminum oxide (Al_2O_3) in 100 mL

• **Acetic Acid**

Sample: 20 mL
Titrimetric system
Mode: Residual titration
Titrant: 0.5 N sodium hydroxide VS
Back-titrant: 0.5 N sulfuric acid VS
Endpoint detection: Visual
Analysis: Pipet the *Sample* into a Kjeldahl flask containing a mixture of 20 mL of phosphoric acid and 150 mL of water. Connect the flask to a condenser, the delivery tube from which dips beneath the surface of 50.0 mL of *Titrant* contained in a receiving flask. Distill about 160 mL, then remove the delivery tube from below the surface of the liquid. Allow the distilling flask to cool, add 50 mL of water, and distill an additional 40–45 mL into the receiving flask. Add phenolphthalein TS to the distillate, and titrate the excess *Titrant* with *Back-titrant*. Each mL of *Titrant* is equivalent to 30.03 mg of acetic acid ($C_2H_4O_2$).
Acceptance criteria: 4.24–5.12 g of acetic acid ($C_2H_4O_2$) in 100 mL

OTHER COMPONENTS

• **Limit of Boric Acid**

Sample: 25 mL
Titrimetric system
Mode: Direct titration
Titrant: 0.5 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Pipet the *Sample* into 75 mL of water in a conical flask. Add 3 mL of phenolphthalein TS, and add *Titrant* from a buret until a faint pink color is obtained. Heat to boiling, and again neutralize. Add 150 mL of glycerin to the neutralized solution, and titrate with *Titrant*. Perform a blank determination in a similar manner. Subtract the volume of *Titrant* used in the blank from the volume of *Titrant* used after the addition of the glycerin. Each mL of *Titrant* is equivalent to 30.92 mg of boric acid (H_3BO_3).
Acceptance criteria: NMT 0.6% of boric acid (H_3BO_3)

IMPURITIES

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 3.6–4.4

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Package in tight containers.

Aluminum Chloride

$AlCl_3 \cdot 6H_2O$ 241.43

$AlCl_3$ 133.34

Aluminum chloride, hexahydrate;
Aluminum chloride hexahydrate [7784-13-6].
Anhydrous [7446-70-0].

DEFINITION

Aluminum Chloride contains NLT 95.0% and NMT 102.0% of aluminum chloride ($AlCl_3$), calculated on the anhydrous basis.

IDENTIFICATION

• **A. Identification Tests—General,** *Aluminum* ⟨191⟩ and *Chloride* ⟨191⟩
Sample solution: 100 mg/mL
Acceptance criteria: Meets the requirements

ASSAY

• **Procedure**

Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.
Sample solution: 20 mg/mL of aluminum chloride in water
Titrimetric system
Mode: Back-titration
Titrant: 0.05 M zinc sulfate VS
Endpoint detection: Visual
Analysis: Transfer 10.0 mL of the *Sample solution* into a 250-mL beaker, and add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and

USP Monographs

www.webofpharma.com

20 mL of acetic acid–ammonium acetate buffer TS, and boil gently for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the excess edetate disodium with *Titrant* to a bright rose-pink color. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 6.667 mg of aluminum chloride ($AlCl_3$).

Acceptance criteria: 95.0%–102.0% on the anhydrous basis

IMPURITIES

• **LIMIT OF SULFATE**

Sample solution: 10 mg/mL

Analysis: Add 0.2 mL of barium chloride TS to 10 mL of the *Sample solution*.

Acceptance criteria: No turbidity is produced within 1 min.

• **IRON** ⟨241⟩

Sample: 1.0 g

Analysis: Dissolve the *Sample* in 45 mL of water, and add 2 mL of hydrochloric acid.

Acceptance criteria: NMT 10 ppm

• **LIMIT OF ALKALIES AND ALKALINE EARTHS**

Sample: 1.0 g

Analysis: To a boiling solution of the *Sample* in 150 mL of water add a few drops of methyl red TS, then add 6 N ammonium hydroxide until the color of the solution just changes to a distinct yellow. Add hot water to restore the volume to 150 mL, and filter while hot. Evaporate 75 mL of the filtrate to dryness, and ignite to a constant weight.

Acceptance criteria: 0.5%; the weight of the residue is NMT 2.5 mg.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** 42.0%–48.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Aluminum Chlorohydrate

$Al_y(OH)_{3y-z}Cl_z \cdot H_2O$

$Al_y(OH)_{3y-z}Cl_z \cdot 2H_2O$ 210.48

$Al_y(OH)_{3y-z}Cl_z$ 174.45

Aluminum chlorohydroxide, dihydrate;
Aluminum hydroxychloride, dihydrate;
Aluminum chlorohydroxide;
Aluminum hydroxychloride;
Dihydrate [12359-72-7].
Anhydrous [12042-91-0].

DEFINITION

Aluminum Chlorohydrate consists of complex basic aluminum chloride that is polymeric and loosely hydrated and encompasses a range of aluminum-to-chloride atomic ratios between 1.91:1 and 2.10:1. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous aluminum chlorohydrate [$Al_y(OH)_{3y-z}Cl_z$].

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩ and *Chloride* ⟨191⟩

Sample solution: 100 mg/mL

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE 1: CONTENT OF CHLORIDE**

Sample: 700 mg

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Electrode system: A glass silver–silver chloride electrode and a silver billet electrode system

Endpoint detection: Potentiometric

Analysis: Transfer the *Sample* to a 250-mL beaker and add 100 mL of water and 10 mL of diluted nitric acid with stirring. Titrate with *Titrant* and determine the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Use the chloride content thus obtained to calculate the aluminum:chloride atomic ratio.

• **PROCEDURE 2: CONTENT OF ALUMINUM**

Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*, except use 37.2 g of edetate disodium.

Sample solution: Transfer 200 mg of Aluminum Chlorohydrate to a 250-mL beaker, add 20 mL of water and 5 mL of hydrochloric acid, boil on a hot plate for NLT 5 min, and allow to cool.

Titrimetric system

Mode: Back-titration

Titrant: 0.1 M zinc sulfate VS

Endpoint detection: Visual

Analysis: To the *Sample solution* add 25.0 mL of *Edetate disodium titrant*, and adjust with 2.5 N ammonium hydroxide or 1 N acetic acid to a pH of 4.7 ± 0.1. Add 20 mL of acetic acid–ammonium acetate buffer TS, 50 mL of alcohol, and 5 mL of dithizone TS. The pH of this solution should be 4.7 ± 0.1. Titrate the excess edetate disodium with *Titrant* until the color changes from green-violet to rose-pink. Perform a blank titration, and make any necessary correction. Each mL of 0.1 M *Edetate disodium titrant* consumed is equivalent to 2.698 mg of aluminum (Al). Use the aluminum content thus obtained to calculate the aluminum:chloride atomic ratio.

• **PROCEDURE 3: ALUMINUM:CHLORIDE ATOMIC RATIO**

Analysis: Use the percentage of aluminum found in the test for *Content of Aluminum* and the percentage of chloride found in the test for *Content of Chloride*.

Calculate the aluminum:chloride atomic ratio (X) as follows:

$$\text{Result} = (p_{Al}/p_{Cl}) \times (A_{Cl}/A_{Al})$$

p_{Al} = percentage of aluminum found in *Content of Aluminum*

p_{Cl} = percentage of chloride found in *Content of Chloride*

A_{Cl} = atomic weight of chlorine (Cl), 35.453

A_{Al} = atomic weight of aluminum (Al), 26.98

Acceptance criteria: Between 1.91:1 and 2.10:1

• **PROCEDURE 4**

Analysis: Calculate the percentage of anhydrous aluminum chlorohydrate [$Al_y(OH)_{3y-z}Cl_z$] in the portion of Aluminum Chlorohydrate taken:

$$\text{Result} = P_{Al}(\{A_{Al}X + [M(3X - 1)] + A_{Cl}\}/A_{Al}X)$$

P_{Al} = percentage of aluminum as obtained in the test for *Content of Aluminum*

A_{Al} = atomic weight of aluminum (Al), 26.98

X = aluminum:chloride atomic ratio, as determined in the test for *Aluminum:Chloride Atomic Ratio*

M = molecular weight of the hydroxide anion (OH), 17.01

A_{Cl} = atomic weight of chlorine (Cl), 35.453

www.webofpharma.com

Acceptance criteria: 90.0%–110.0% on the anhydrous basis

IMPURITIES

• **ARSENIC,** *Method I* ⟨211⟩**:** NMT 2 ppm

• **LIMIT OF IRON**

Standard solution: Transfer 2.0 mL of *Standard Iron Solution*, prepared as directed in *Iron* ⟨241⟩, to a 50-mL beaker.

Sample solution: Transfer 2.7 g of Aluminum Chlorohydrate to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL beaker.

Analysis: To each of the beakers containing the *Standard solution* and the *Sample solution*, add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3–5 min. Allow to cool. Add 5 mL of *Ammonium Thiocyanate Solution* (prepared as directed in *Iron* ⟨241⟩), transfer to separate 50-mL color comparison tubes, and dilute with water to volume.

Acceptance criteria: 150 ppm; the color of the solution from the *Sample solution* is not darker than that of the solution from the *Standard solution*.

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: 15 g of Aluminum Chlorohydrate in 100 g of water

Acceptance criteria: 3.0–5.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** The label states the content of anhydrous aluminum chlorohydrate.

Aluminum Chlorohydrate Solution

DEFINITION

Aluminum Chlorohydrate Solution consists of complex basic aluminum chloride that is polymeric and encompasses a range of aluminum-to-chloride ratios between 1.91:1 and 2.10:1. The following solvents may be used: water, propylene glycol, dipropylene glycol, or alcohol. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled concentration of anhydrous aluminum chlorohydrate ($Al_y(OH)_{3y-z}Cl_z$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩ and *Chloride* ⟨191⟩

Sample solution: Nominally equivalent to 100 mg/mL of anhydrous aluminum chlorohydrate

Acceptance criteria: Meets the requirements

• **B. IDENTIFICATION OF PROPYLENE GLYCOL**

Perform this test where propylene glycol is stated on the label.

Sample solution: 2 g of Solution in 10 mL of isopropyl alcohol. Mix, filter, and evaporate the filtrate to 1 mL on a steam bath.

Acceptance criteria: The IR spectrum of a film of the *Sample solution* on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

• **C. IDENTIFICATION OF DIPROPYLENE GLYCOL**

Perform this test where dipropylene glycol is stated on the label.

Sample solution: 2 g of Solution in 10 mL of isopropyl alcohol. Mix, filter, and evaporate the filtrate to 1 mL on a steam bath.

Acceptance criteria: The IR spectrum of a film of the *Sample solution* on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

• **D. IDENTIFICATION OF ALCOHOL**

Perform this test where alcohol is stated on the label.

Analysis: Mix 5 drops of Solution in a small beaker with 1 mL of potassium permanganate solution (1 in 100) and 5 drops of 2 N sulfuric acid, and cover the beaker immediately with filter paper moistened with a freshly prepared solution of 0.1 g of sodium nitroferricyanide and 0.25 g of piperazine in 5 mL of water.

Acceptance criteria: An intense blue color is produced on the filter paper, the color becoming paler after a few min.

ASSAY

• **PROCEDURE 1: CONTENT OF CHLORIDE**

Sample: 1.4 g of Solution

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Electrode system: A silver–silver chloride glass electrode and a silver billet electrode system

Endpoint detection: Potentiometric

Analysis: Transfer the *Sample* to a 250-mL beaker, and add 100 mL of water and 10 mL of diluted nitric acid with stirring. Titrate with *Titrant*, and determine the endpoint.

Acceptance criteria: Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Use the chloride content thus obtained to calculate the aluminum/chloride atomic ratio.

• **PROCEDURE 2: CONTENT OF ALUMINUM**

Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*, except use 37.2 g of edetate disodium.

Sample solution: Transfer 400 mg of Solution to a 250-mL beaker, add 20 mL of water and 5 mL of hydrochloric acid, boil on a hot plate for NLT 5 min, and allow to cool.

Titrimetric system

Mode: Back titration

Titrant: 0.1 M zinc sulfate VS

Endpoint detection: Visual

Analysis: To the *Sample solution* add 25.0 mL of *Edetate disodium titrant*, and adjust with 2.5 N ammonium hydroxide or 1 N acetic acid to a pH of 4.7 ± 0.1. Add 20 mL of acetic acid–ammonium acetate buffer TS, 50 mL of alcohol, and 5 mL of dithizone TS. The pH of this solution should be 4.7 ± 0.1. Titrate excess edetate disodium with *Titrant* until the color changes from green-violet to rose-pink. Perform a blank titration, and make any necessary correction.

Acceptance criteria: Each mL of 0.1 M *Edetate disodium titrant* consumed is equivalent to 2.698 mg of aluminum (Al). Use the aluminum content thus obtained to calculate the aluminum/chloride atomic ratio.

• **PROCEDURE 3: ALUMINUM/CHLORIDE ATOMIC RATIO**

Analysis: Use the percentage of aluminum found in *Content of Aluminum* and the percentage of chloride found in *Content of Chloride*.

Calculate the aluminum/chloride atomic ratio (X) as follows:

$$\text{Result} = (P_{Al}/P_{Cl}) \times (A_{Cl}/A_{Al})$$

P_{Al} = percentage of aluminum found in *Content of Aluminum*

USP Monographs

www.webofpharma.com

P_{Cl} = percentage of chloride found in *Content of Chloride*
A_{Cl} = atomic weight of chlorine (Cl), 35.453
A_{Al} = atomic weight of aluminum (Al), 26.98

Acceptance criteria: 1.91:1 to 2.10:1

• **PROCEDURE 4**

Analysis: Calculate the percentage of the labeled concentration of anhydrous aluminum chlorohydrate ($Al_y(OH)_{3y-z}Cl_z$) in the portion of Solution taken:

$$\text{Result} = P_{Al} \times \{[A_{Al}X + (M(3X - 1)) + A_{Cl}]/A_{Al}X\}$$

P_{Al} = percentage of aluminum found in *Content of Aluminum*
A_{Al} = atomic weight of aluminum (Al), 26.98
X = aluminum/chloride atomic ratio, as determined in *Aluminum/Chloride Atomic Ratio*
M = molecular weight of the hydroxide anion (OH), 17.01
A_{Cl} = atomic weight of chlorine (Cl), 35.453

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ARSENIC,** *Method I* ⟨211⟩**:** NMT 2 ppm

• **LIMIT OF IRON**

Standard preparation: 2.0 mL of *Standard Iron Solution*, prepared as directed in *Iron* ⟨241⟩

Test preparation: Transfer 5.3 g of Solution to a 100-mL volumetric flask, and dilute with water to volume.

Analysis: Transfer 2.0 mL of the *Standard preparation* into a 50-mL beaker. Transfer 5.0 mL of the *Test preparation* into a second 50-mL beaker. To each of the beakers add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3–5 min. Allow to cool. Add 5 mL of *Ammonium Thiocyanate Solution*, prepared as directed in *Iron* ⟨241⟩, transfer to separate 50-mL color-comparison tubes, and dilute with water to volume.

Acceptance criteria: 75 ppm; the color of the solution from the *Test preparation* is not darker than that from the *Standard preparation*.

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: Dilute 3 g of Solution with water to 10 mL.

Acceptance criteria: 3.0–5.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum chlorohydrate contained therein.

Aluminum Chlorohydrex Polyethylene Glycol

$Al_y(OH)_{3y-z}Cl_z \cdot nH_2O \cdot mH(OCH_2CH_2)_nOH$

Aluminum chlorohydroxide polyethylene glycol complex.
Aluminum hydroxychloride polyethylene glycol complex.

» Aluminum Chlorohydrex Polyethylene Glycol consists of aluminum chlorohydrate in which some of the waters of hydration have been replaced by polyethylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 1.91:1 and 2.10:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum chlorohydrate.

Packaging and storage—Preserve in well-closed containers.

Labeling—The label states the content of anhydrous aluminum chlorohydrate.

Identification—

A: A solution (1 in 10) responds to the tests for *Aluminum* ⟨191⟩ and for *Chloride* ⟨191⟩.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197F*▲ (CN 1-May-2020)—

Test specimen—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of 9.55 ± 0.05. Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

Standard specimen: a similar preparation of polyethylene glycol.

pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

Arsenic, *Method I* ⟨211⟩: 2 µg per g.

Limit of iron—Using Aluminum Chlorohydrex Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

Content of aluminum—Using Aluminum Chlorohydrex Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

Content of chloride—Using Aluminum Chlorohydrex Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

Aluminum/chloride atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.91:1 and 2.10:1.

Assay—Calculate the percentage of anhydrous aluminum chlorohydrate in the Aluminum Chlorohydrex Polyethylene Glycol by the formula:

$$Al(\{26.98x + [17.01(3x - 1)] + 35.453\}/26.98x)$$

in which Al is the percentage of aluminum found in the test for *Content of aluminum*, x is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

USP Monographs

www.webofpharma.com

Aluminum Chlorohydrex Propylene Glycol

$Al_y(OH)_{3y-z}Cl_z \cdot nH_2O \cdot mC_3H_8O_2$

$Al_2(H_2O)_{y-z}(OH)_{6-n}(Cl)_n(C_3H_8O_2)_z$

Aluminum chlorohydroxide, hydrate: propylene glycol complex (1:1).

Aluminum hydroxychloride, hydrate: propylene glycol complex (1:1) [53026-85-0].

» Aluminum Chlorohydrex Propylene Glycol is a complex of aluminum chlorohydrate and propylene glycol in which some of the waters of hydration of the aluminum chlorohydrate have been replaced by propylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum chlorohydrate.

Packaging and storage—Preserve in well-closed containers.

Labeling—The label states the content of anhydrous aluminum chlorohydrate.

Identification—

A: A solution (1 in 10) responds to the tests for *Aluminum* ⟨191⟩ and for *Chloride* ⟨191⟩.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197F*▲ (CN 1-May-2020)—

Test specimen—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of 9.55 ± 0.05. Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

Standard specimen: a similar preparation of propylene glycol.

pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

Arsenic, *Method I* ⟨211⟩: 2 µg per g.

Limit of iron—Using Aluminum Chlorohydrex Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

Content of aluminum—

Edetate disodium titrant—Prepare and standardize as directed in the *ASSAY* under *Ammonium Alum*, except to use 37.2 g of edetate disodium instead of 18.6 g.

Test solution—Transfer about 1.6 g of Aluminum Chlorohydrex Propylene Glycol, accurately weighed, to a 100-mL beaker, add 15 to 20 mL of water and 5 to 6 mL of hydrochloric acid, and boil on a hot plate for 15 to 20 minutes. Cool the solution, and with the aid of water transfer to a 100-mL volumetric flask. Dilute with water to volume, and mix.

Procedure—Transfer 5.0 mL of the *Test solution* to a 250-mL beaker, add 10 to 15 mL of water, and adjust with 1 N sodium hydroxide to a pH of 1.5 ± 0.5. Add 10.0 mL of *Edetate disodium titrant*, and heat to boiling. Cool the solution and carefully introduce a magnetic stirring bar into the beaker. Add 10 to 15 mL of acetic acid–ammonium acetate buffer TS, 40 to 50 mL of alcohol, and while stirring adjust with glacial acetic acid to a pH of 4.6 ± 0.1. Add 1 to 2 mL of dithizone TS and 40 to 50 mL of alcohol, and titrate with 0.1 M zinc sulfate VS until the color changes from a green-violet to a rose-pink. Perform a blank titration, and make any necessary correction. Each mL of 0.1 M *Edetate disodium titrant* consumed is equivalent to 2.698 mg of aluminum (Al). Use the aluminum content thus obtained to calculate the *Aluminum/chloride atomic ratio*.

Content of chloride—Using Aluminum Chlorohydrex Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Procedure 1: Content of Chloride* under *Aluminum Chlorohydrate*. Use the chloride content thus obtained to calculate the *Aluminum/chloride atomic ratio*.

Aluminum/chloride atomic ratio—Divide the percentage of aluminum found in the *Assay* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.91:1 and 2.1:1.

Assay—Calculate the percentage of anhydrous aluminum chlorohydrate in the Aluminum Chlorohydrex Propylene Glycol by the formula:

$$Al(\{26.98x + [17.01(3x - 1)] + 35.453\}/26.98x)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide ion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Dichlorohydrate

$Al_y(OH)_{3y-z}Cl_z \cdot nH_2O$

Aluminum chlorohydroxide;
Aluminum hydroxychloride.

DEFINITION

Aluminum Dichlorohydrate consists of complex basic aluminum chloride that is polymeric and loosely hydrated and encompasses a range of aluminum-to-chloride atomic ratios between 0.90:1 and 1.25:1. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous aluminum dichlorohydrate [$Al_y(OH)_{3y-z}Cl_z$].

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩ and *Chloride* ⟨191⟩
  Sample solution: 100 mg/mL
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE 1: CONTENT OF CHLORIDE**
  Sample: 700 mg
  Titrimetric system
  Mode: Direct titration
  Titrant: 0.1 N silver nitrate VS
  Electrode system: A glass silver–silver chloride electrode and a silver billet electrode system
  Endpoint detection: Potentiometric
  Analysis: Transfer the *Sample* to a 250-mL beaker, and add 100 mL of water and 10 mL of diluted nitric acid with stirring. Titrate with *Titrant*, and determine the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Use the chloride content thus obtained to calculate the aluminum:chloride atomic ratio.

www.webofpharma.com

• **PROCEDURE 2: CONTENT OF ALUMINUM**

Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*, except use 37.2 g of edetate disodium.

Sample solution: Transfer 200 mg of Aluminum Dichlorohydrate to a 250-mL beaker, add 20 mL of water and 5 mL of hydrochloric acid, boil on a hot plate for NLT 5 min, and allow to cool.

Titrimetric system

Mode: Back-titration

Titrant: 0.1 M zinc sulfate VS

Endpoint detection: Visual

Analysis: To the *Sample solution* add 25.0 mL of *Edetate disodium titrant*, and adjust with 2.5 N ammonium hydroxide or 1 N acetic acid to a pH of 4.7 ± 0.1. Add 20 mL of acetic acid–ammonium acetate buffer TS, 50 mL of alcohol, and 5 mL of dithizone TS. The pH of this solution should be 4.7 ± 0.1. Titrate the excess edetate disodium with *Titrant* until the color changes from green-violet to rose-pink. Perform a blank titration, and make any necessary correction. Each mL of 0.1 M *Edetate disodium titrant* consumed is equivalent to 2.698 mg of aluminum (Al). Use the aluminum content thus obtained to calculate the aluminum:chloride atomic ratio.

• **PROCEDURE 3: ALUMINUM:CHLORIDE ATOMIC RATIO**

Analysis: Use the percentage of aluminum found in the test for *Content of Aluminum* and the percentage of chloride found in the test for *Content of Chloride*.

Calculate the aluminum:chloride atomic ratio (X) as follows:

$$\text{Result} = (p_{Al}/p_{Cl}) \times (A_{Cl}/A_{Al})$$

p_{Al} = percentage of aluminum found in *Content of Aluminum*

p_{Cl} = percentage of chloride found in *Content of Chloride*

A_{Cl} = atomic weight of chlorine (Cl), 35.453

A_{Al} = atomic weight of aluminum (Al), 26.98

Acceptance criteria: Between 0.90:1 and 1.25:1

• **PROCEDURE 4**

Analysis: Calculate the percentage of anhydrous aluminum dichlorohydrate [$Al_y(OH)_{3y-z}Cl_z$] in the portion of Aluminum Dichlorohydrate taken:

$$\text{Result} = P_{Al}(\{A_{Al}X + [M(3X - 1)] + A_{Cl}\}/A_{Al}X)$$

P_{Al} = percentage of aluminum as obtained in the test for *Content of Aluminum*

A_{Al} = atomic weight of aluminum (Al), 26.98

X = aluminum:chloride atomic ratio, as determined in the test for *Aluminum:Chloride Atomic Ratio*

M = molecular weight of the hydroxide anion (OH), 17.01

A_{Cl} = atomic weight of chlorine (Cl), 35.453

Acceptance criteria: 90.0%–110.0% on the anhydrous basis

IMPURITIES

• **ARSENIC,** *Method I* ⟨211⟩**:** NMT 2 ppm

• **LIMIT OF IRON**

Standard solution: Transfer 2.0 mL of *Standard Iron Solution*, prepared as directed in *Iron* ⟨241⟩, to a 50-mL beaker.

Sample solution: Transfer 2.7 g of Aluminum Dichlorohydrate to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL beaker.

Analysis: To each of the beakers containing the *Standard solution* and the *Sample solution*, add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3–5 min. Allow to cool. Add 5 mL of *Ammonium Thiocyanate Solution* (prepared as directed in *Iron* ⟨241⟩), transfer to separate 50-mL color comparison tubes, and dilute with water to volume.

Acceptance criteria: 150 ppm; the color of the solution from the *Sample solution* is not darker than that of the solution from the *Standard solution*.

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: 15 g of Aluminum Dichlorohydrate in 100 g of water

Acceptance criteria: 3.0–5.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** The label states the content of anhydrous aluminum dichlorohydrate.

Aluminum Dichlorohydrate Solution

DEFINITION

Aluminum Dichlorohydrate Solution consists of complex basic aluminum chloride that is polymeric and encompasses a range of aluminum-to-chloride atomic ratios between 0.90:1 and 1.25:1. The following solvents may be used: water, propylene glycol, dipropylene glycol, or alcohol. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled concentration of anhydrous aluminum dichlorohydrate ($Al_y(OH)_{3y-z}Cl_z$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩ and *Chloride* ⟨191⟩

Sample solution: Nominally equivalent to 100 mg/mL of anhydrous aluminum dichlorohydrate

Acceptance criteria: Meets the requirements

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)**:** (where propylene glycol is indicated on the label)

Sample solution: Add 10 mL of isopropyl alcohol to 2 g of Solution, and filter. Evaporate the filtrate to 1 mL on a steam bath. Deposit this solution on a silver chloride disk.

Standard solution: A similar preparation of propylene glycol

Acceptance criteria: Meets the requirements

Change to read:

• **C. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)**:** (where dipropylene glycol is indicated on the label)

Sample solution: Add 10 mL of isopropyl alcohol to 2 g of Solution, and filter. Evaporate the filtrate to 1 mL on a steam bath. Deposit this solution on a silver chloride disk.

Standard solution: A similar preparation of dipropylene glycol

Acceptance criteria: Meets the requirements

• **D. IDENTIFICATION OF ALCOHOL**

Perform this test where alcohol is stated on the label.

www.webofpharma.com

Analysis: Mix 5 drops of Solution in a small beaker with 1 mL of potassium permanganate solution (1 in 100) and 5 drops of 2 N sulfuric acid, and cover the beaker immediately with filter paper moistened with a freshly prepared solution of 0.1 g of sodium nitroferricyanide and 0.25 g of piperazine in 5 mL of water.
Acceptance criteria: An intense blue color is produced on the filter paper, the color becoming paler after a few min.

ASSAY

- **PROCEDURE 1: CONTENT OF CHLORIDE**

Sample: 1.4 g of Solution
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Electrode system: A silver–silver chloride glass electrode and a silver billet electrode system
Endpoint detection: Potentiometric
Analysis: Transfer the *Sample* to a 250-mL beaker, and add 100 mL of water and 10 mL of diluted nitric acid with stirring. Titrate with *Titrant*, and determine the endpoint.
Acceptance criteria: Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Use the chloride content thus obtained to calculate the aluminum/chloride atomic ratio.

- **PROCEDURE 2: CONTENT OF ALUMINUM**

Edetate disodium titrant: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*, except use 37.2 g of edetate disodium.
Sample solution: Transfer 400 mg of Solution to a 250-mL beaker, add 20 mL of water and 5 mL of hydrochloric acid, boil on a hot plate for NLT 5 min, and allow to cool.
Titrimetric system
Mode: Back titration
Titrant: 0.1 M zinc sulfate VS
Endpoint detection: Visual
Analysis: To the *Sample solution* add 25.0 mL of *Edetate disodium titrant*, and adjust with 2.5 N ammonium hydroxide or 1 N acetic acid to a pH of 4.7 ± 0.1. Add 20 mL of acetic acid–ammonium acetate buffer TS, 50 mL of alcohol, and 5 mL of dithizone TS. The pH of this solution should be 4.7 ± 0.1. Titrate excess edetate disodium with *Titrant* until the color changes from green-violet to rose-pink. Perform a blank titration, and make any necessary correction.
Acceptance criteria: Each mL of 0.1 M *Edetate disodium titrant* consumed is equivalent to 2.698 mg of aluminum (Al). Use the aluminum content thus obtained to calculate the aluminum/chloride atomic ratio.

- **PROCEDURE 3: ALUMINUM/CHLORIDE ATOMIC RATIO**

Analysis: Use the percentage of aluminum found in *Content of Aluminum* and the percentage of chloride found in *Content of Chloride*.
Calculate the aluminum/chloride atomic ratio (X) as follows:

$$\text{Result} = (P_{Al}/P_{Cl}) \times (A_{Cl}/A_{Al})$$

P_{Al} = percentage of aluminum found in *Content of Aluminum*
P_{Cl} = percentage of chloride found in *Content of Chloride*
A_{Cl} = atomic weight of chlorine (Cl), 35.453
A_{Al} = atomic weight of aluminum (Al), 26.98

Acceptance criteria: 0.90:1 to 1.25:1

- **PROCEDURE 4**

Analysis: Calculate the percentage of the labeled concentration of anhydrous aluminum dichlorohydrate ($Al_y(OH)_{3y-z}Cl_z$) in the portion of Solution taken:

$$\text{Result} = P_{Al} \times \{[A_{Al}X + (M(3X - 1)) + A_{Cl}]/A_{Al}X\}$$

P_{Al} = percentage of aluminum found in *Content of Aluminum*
A_{Al} = atomic weight of aluminum (Al), 26.98
X = aluminum/chloride atomic ratio, as determined in *Aluminum/Chloride Atomic Ratio*
M = molecular weight of the hydroxide anion (OH), 17.01
A_{Cl} = atomic weight of chlorine (Cl), 35.453

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ARSENIC,** *Method I* ⟨211⟩**:** NMT 2 ppm
- **LIMIT OF IRON**

Standard preparation: 2.0 mL of *Standard Iron Solution*, prepared as directed in *Iron* ⟨241⟩
Test preparation: Transfer 5.3 g of Solution to a 100-mL volumetric flask, and dilute with water to volume.
Analysis: Transfer 2.0 mL of the *Standard preparation* into a 50-mL beaker. Transfer 5.0 mL of the *Test preparation* into a second 50-mL beaker. To each of the beakers add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3–5 min. Allow to cool. Add 5 mL of *Ammonium Thiocyanate Solution*, prepared as directed in *Iron* ⟨241⟩, transfer to separate 50-mL color-comparison tubes, and dilute with water to volume.
Acceptance criteria: 75 ppm; the color of the solution from the *Test preparation* is not darker than that from the *Standard preparation*.

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: Dilute 3 g of Solution with water to 10 mL.
Acceptance criteria: 3.0–5.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum dichlorohydrate contained therein.

Aluminum Dichlorohydrex Polyethylene Glycol

$Al_y(OH)_{3y-z}Cl_z \cdot nH_2O \cdot mH(OCH_2CH_2)_nOH$
Aluminum chlorohydroxide polyethylene glycol complex.
Aluminum hydroxychloride polyethylene glycol complex.

» Aluminum Dichlorohydrex Polyethylene Glycol consists of aluminum dichlorohydrate in which some of the waters of hydration have been replaced by polyethylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 0.90:1 and 1.25:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum dichlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—The label states the content of anhydrous aluminum dichlorohydrate.

www.webofpharma.com

Identification—
A: A solution (1 in 10) responds to the tests for *Aluminum* ⟨191⟩ and for *Chloride* ⟨191⟩.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197F*▲ (CN 1-May-2020)—
Test specimen—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of 9.55 ± 0.05. Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.
Standard specimen: a similar preparation of polyethylene glycol.
pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].
Arsenic, *Method I* ⟨211⟩: 2 µg per g.
Limit of iron—Using Aluminum Dichlorohydrex Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.
Content of aluminum—Using Aluminum Dichlorohydrex Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.
Content of chloride—Using Aluminum Dichlorohydrex Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.
Aluminum/chloride atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 0.90:1 and 1.25:1.
Assay—Calculate the percentage of anhydrous aluminum dichlorohydrate in the Aluminum Dichlorohydrex Polyethylene Glycol by the formula:

$$Al(\{26.98x + [17.01(3x - 1)] + 35.453\}/26.98x)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum-to-chloride atomic ratio, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Dichlorohydrex Propylene Glycol

$Al_y(OH)_{3y-z}Cl_z \cdot nH_2O \cdot mC_3H_8O_2$
Aluminum chlorohydroxide propylene glycol complex.
Aluminum hydroxychloride propylene glycol complex.

» Aluminum Dichlorohydrex Propylene Glycol consists of aluminum dichlorohydrate in which some of the waters of hydration have been replaced by propylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 0.90:1 and 1.25:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum dichlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—The label states the content of anhydrous aluminum dichlorohydrate.

Identification—
A: A solution (1 in 10) responds to the tests for *Aluminum* ⟨191⟩ and for *Chloride* ⟨191⟩.
B: Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of 9.55 ± 0.05. Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.
pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].
Arsenic, *Method I* ⟨211⟩: 2 µg per g.
Limit of iron—Using Aluminum Dichlorohydrex Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.
Content of aluminum—Using Aluminum Dichlorohydrex Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.
Content of chloride—Using Aluminum Dichlorohydrex Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.
Aluminum/chloride atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 0.90:1 and 1.25:1.
Assay—Calculate the percentage of anhydrous aluminum dichlorohydrate in the Aluminum Dichlorohydrex Propylene Glycol by the formula:

$$Al(\{26.98x + [17.01(3x - 1)] + 35.453\}/26.98x)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum-to-chloride atomic ratio, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Hydroxide Gel

$Al(OH)_3$ 78.00
Aluminum hydroxide.
Aluminum hydroxide [21645-51-2].

» Aluminum Hydroxide Gel is a suspension of amorphous aluminum hydroxide in which there is a partial substitution of carbonate for hydroxide. It contains the equivalent of not less than 90.0

www.webofpharma.com

percent and not more than 110.0 percent of the labeled amount of aluminum hydroxide [$Al(OH)_3$]. It may contain Peppermint Oil, Glycerin, Sorbitol, Sucrose, Saccharin, or other suitable flavors, and it may contain suitable antimicrobial agents.

Packaging and storage—Preserve in tight containers, and avoid freezing.

Identification—

A: Place about 1 g in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

B: The solution remaining in the flask responds to the tests for *Aluminum* ⟨191⟩.

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for the absence of *Escherichia coli*.

Acid-neutralizing capacity ⟨301⟩—Not less than 65.0% of the expected mEq value, calculated from the results of the *Assay*, is obtained. Each mg of $Al(OH)_3$ has an expected acid-neutralizing capacity value of 0.0385 mEq.

pH ⟨791⟩: between 5.5 and 8.0, determined potentiometrically.

Chloride—Transfer an accurately measured quantity of the Gel, equivalent to 0.6 g of $Al(OH)_3$, to a porcelain dish. Add 0.1 mL of potassium chromate TS and 25 mL of water. Stir, and add 0.10 N silver nitrate until a faint, persistent pink color is obtained: not more than 8.0 mL of 0.10 N silver nitrate is required [4.7%, based on the $Al(OH)_3$ content].

Sulfate ⟨221⟩—Add 5.0 mL of 3 N hydrochloric acid to an accurately measured quantity of the Gel, equivalent to 0.3 g of $Al(OH)_3$, and heat to dissolve the specimen under test. Cool, dilute with water to 250 mL, and filter if necessary: a 20-mL portion of the filtrate shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid [0.8%, based on the $Al(OH)_3$ content].

Arsenic, *Method I* ⟨211⟩—Prepare a *Standard Preparation* as directed in the test for *Arsenic* ⟨211⟩, except to prepare it to contain 5 µg of arsenic instead of 3 µg. Prepare the *Test Preparation* as follows. Dissolve an accurately measured quantity of the Gel, equivalent to 0.5 g of $Al(OH)_3$, in 20 mL of 7 N sulfuric acid. The limit is 0.001%, based on the $Al(OH)_3$ content.

Assay—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Procedure—Transfer an accurately measured quantity of Gel, equivalent to about 1.5 g of $Al(OH)_3$, to a beaker, add 15 mL of hydrochloric acid, and heat gently until solution is complete. Cool, transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. Pipet 20 mL of this solution into a 250-mL beaker, and add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, then heat the solution near the boiling point for 5 minutes. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the solution with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 20 mL of water for the sample, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of $Al(OH)_3$.

Dried Aluminum Hydroxide Gel

$Al(OH)_3$ 78.00
Aluminum hydroxide [21645-51-2].

» Dried Aluminum Hydroxide Gel is an amorphous form of aluminum hydroxide in which there is a partial substitution of carbonate for hydroxide. It contains the equivalent of not less than 76.5 percent of $Al(OH)_3$, and it may contain varying quantities of basic aluminum carbonate and bicarbonate.

Packaging and storage—Preserve in tight containers.

Labeling—Where the quantity of dried aluminum hydroxide gel equivalent is stated in the labeling of any preparation, this shall be understood to be on the basis that each mg of dried gel is equivalent to 0.765 mg of $Al(OH)_3$.

USP Reference standards ⟨11⟩—
USP Dried Aluminum Hydroxide Gel RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Dissolve 500 mg in 10 mL of 3 N hydrochloric acid, with gentle warming: the solution responds to the tests for *Aluminum* ⟨191⟩.

Acid-neutralizing capacity ⟨301⟩: not less than 25.0 mEq per g, 400 mg being tested as directed for *Powders* under *Test Preparation*.

pH ⟨791⟩: not higher than 10.0, in an aqueous dispersion (1 in 25).

Chloride ⟨221⟩—Dissolve 1.0 g in 30 mL of 2 N nitric acid, heat to boiling, add water to make 100 mL, and filter: a 5.0-mL portion of the filtrate, diluted with an equal volume of water, shows no more chloride than corresponds to 0.60 mL of 0.020 N hydrochloric acid (0.85%).

Sulfate ⟨221⟩—Dissolve 330 mg in 15 mL of 3 N hydrochloric acid, heat to boiling, add water to make 250 mL, and filter: a 25-mL portion of the filtrate shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.6%).

Arsenic, *Method I* ⟨211⟩—Dissolve 1.5 g in 80 mL of 7 N sulfuric acid, and dilute with water to 220 mL: 55 mL of the resulting solution meets the requirements of the test, the addition of 20 mL of 7 N sulfuric acid specified under *Procedure* being omitted. The limit is 8 ppm.

Assay—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Procedure—Weigh accurately about 2 g of Gel, and dissolve in 15 mL of hydrochloric acid, with the aid of heat. Cool, transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. Pipet 20 mL of this solution into a 250-mL beaker, and add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, then heat the solution near the boiling point for 5 minutes. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the solution with 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination, substituting 20 mL of water for the sample solution, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of $Al(OH)_3$.

www.webofpharma.com

Dried Aluminum Hydroxide Gel Capsules

» Dried Aluminum Hydroxide Gel Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aluminum hydroxide [$Al(OH)_3$].

Packaging and storage—Preserve in well-closed containers.
Labeling—The Capsules may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of $Al(OH)_3$.

Identification—
A: Place a portion of Capsule contents, equivalent to about 500 mg of aluminum hydroxide, in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 10 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.
B: The solution remaining in the flask responds to the tests for *Aluminum* ⟨191⟩.
Disintegration ⟨701⟩: 10 minutes, simulated gastric fluid TS being substituted for water in the test.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than 55.0% of the expected mEq value, calculated from the labeled quantity of $Al(OH)_3$, is obtained. Each mg of $Al(OH)_3$ has an expected acid-neutralizing capacity value of 0.0385 mEq.

Assay—
Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.
Procedure—Weigh accurately the contents of not fewer than 20 Capsules, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 1.2 g of aluminum hydroxide, to a beaker, add 15 mL of hydrochloric acid, and heat until dissolved. Dilute with water to about 100 mL, mix, and filter quantitatively into a 500-mL volumetric flask, washing the filter with water. Proceed as directed in the *Assay* under *Dried Aluminum Hydroxide Gel* beginning with "dilute with water to volume." Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of $Al(OH)_3$.

Dried Aluminum Hydroxide Gel Tablets

» Dried Aluminum Hydroxide Gel Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aluminum hydroxide [$Al(OH)_3$].

Packaging and storage—Preserve in well-closed containers.
Labeling—Tablets may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of $Al(OH)_3$.

Identification—
A: Place a quantity of finely ground Tablets, equivalent to about 500 mg of aluminum hydroxide, in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.
B: The solution remaining in the flask responds to the tests for *Aluminum* ⟨191⟩.
Disintegration ⟨701⟩: 10 minutes, simulated gastric fluid TS being substituted for water in the test.
Uniformity of dosage units ⟨905⟩: meet the requirements for *Weight Variation*.
Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than 55.0% of the expected mEq value, calculated from the labeled quantity of $Al(OH)_3$, is obtained. Each mg of $Al(OH)_3$ has an expected acid-neutralizing capacity value of 0.0385 mEq.

Assay—
Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.
Procedure—Weigh and finely powder not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 1.2 g of aluminum hydroxide, add 15 mL of hydrochloric acid, and heat until dissolved. Dilute with water to about 100 mL, mix, and filter quantitatively into a 500-mL volumetric flask, washing the filter with water. Proceed as directed in the *Assay* under *Dried Aluminum Hydroxide Gel*, beginning with "dilute with water to volume." Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of $Al(OH)_3$.

Aluminum Phosphate Gel

Phosphoric acid, aluminum salt (1:1).
Aluminum phosphate (1:1) [7784-30-7].

» Aluminum Phosphate Gel is a water suspension containing not less than 4.0 percent and not more than 5.0 percent (w/w) of aluminum phosphate ($AlPO_4$). It may contain sodium benzoate, benzoic acid, or other suitable agent, in an amount not exceeding 0.5 percent, as a preservative.

Packaging and storage—Preserve in tight containers.

Identification—
A: A solution of it in hydrochloric acid meets the requirements of the tests for *Aluminum* ⟨191⟩.
B: A solution of it in 2 N nitric acid meets the requirements of the tests for *Phosphate* ⟨191⟩.
pH ⟨791⟩: between 6.0 and 7.2.
Soluble phosphate—Filter 20 g, and wash the residue with 30 mL of water. Add to the filtrate 2 mL of nitric acid, heat to 60°, and add 20 mL of ammonium molybdate TS. Heat at 50° for 30 minutes, filter, wash the precipitate with dilute nitric acid (1 in 36), then wash with potassium nitrate solution (1 in 100) until the last portion of the filtrate is not acid to litmus paper. Dissolve the precipitate in 50.0 mL of 0.5 N sodium hydroxide VS, add phenolphthalein TS, and titrate the excess alkali with 0.5 N hydrochloric acid VS. Each mL of 0.5 N

www.webofpharma.com

sodium hydroxide is equivalent to 2.065 mg of PO_4. The soluble phosphate, calculated as PO_4, does not exceed 0.30%.
Sulfate ⟨221⟩—Add 10 mL of 3 N hydrochloric acid to 10 g of Gel, and heat to boiling. Cool, dilute with water to 250 mL, and filter, if necessary. A 10-mL portion of the solution shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid: not more than 0.05% is found.
Arsenic, *Method I* ⟨211⟩—Prepare the *Test Preparation* by dissolving 5.0 g of Gel in the smallest necessary volume of 3 N hydrochloric acid. The limit is 0.6 ppm.
Chloride—Transfer 25 g to a beaker with the aid of about 50 mL of water, add 5 mL of nitric acid, mix, then add, with stirring, 30.0 mL of 0.1 N silver nitrate VS. Warm on a steam bath for 30 minutes, filter, and wash the precipitate with water acidified with nitric acid. To the filtrate add ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Not more than 0.16% of chloride is found.
Assay—To about 20 g of Gel, accurately weighed, in a 100-mL volumetric flask, add nitric acid to effect solution, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 400-mL beaker, dilute with water to 100 mL, heat to 60°, add an excess of ammonium molybdate TS, and maintain at 50° for 30 minutes. Filter, and wash the precipitate with dilute nitric acid (1 in 36), then with potassium nitrate solution (1 in 100) until the last portion of the filtrate is not acid to litmus paper. Dissolve the precipitate in 50.0 mL of 0.5 N sodium hydroxide VS, add phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 N sulfuric acid VS. Each mL of 0.5 N sodium hydroxide is equivalent to 2.651 mg of $AlPO_4$.

Aluminum Sesquichlorohydrate

$Al_y(OH)_{3y-z}Cl_z \cdot nH_2O$
Aluminum chlorohydroxide.
Aluminum hydroxychloride [11097-68-0].

» Aluminum Sesquichlorohydrate consists of complex basic aluminum chloride that is polymeric and loosely hydrated and encompasses a range of aluminum-to-chloride atomic ratios between 1.26:1 and 1.90:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum sesquichlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—The label states the content of anhydrous aluminum sesquichlorohydrate.
Identification—A solution (1 in 10) responds to the tests for *Aluminum* ⟨191⟩ and for *Chloride* ⟨191⟩.
pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].
Arsenic, *Method I* ⟨211⟩: 2 µg per g.
Limit of iron—Using Aluminum Sesquichlorohydrate instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.
Content of aluminum—Using Aluminum Sesquichlorohydrate instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.
Content of chloride—Using Aluminum Sesquichlorohydrate instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.
Aluminum/chloride atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.26:1 and 1.90:1.
Assay—Calculate the percentage of anhydrous aluminum sesquichlorohydrate in the Aluminum Sesquichlorohydrate by the formula:

$$Al(\{26.98x + [17.01(3x - 1)] + 35.453\}/26.98x)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Sesquichlorohydrate Solution

» Aluminum Sesquichlorohydrate Solution consists of complex basic aluminum chloride that is polymeric and encompasses a range of aluminum-to-chloride atomic ratios between 1.26:1 and 1.90:1. The following solvents may be used: water, propylene glycol, dipropylene glycol, or alcohol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum sesquichlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum sesquichlorohydrate contained therein.

Identification—
A: A solution containing the equivalent of about 100 mg of anhydrous aluminum sesquichlorohydrate per mL responds to the tests for *Aluminum* ⟨191⟩ and for *Chloride* ⟨191⟩.
B: Identification of propylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.
C: Identification of dipropylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the

www.webofpharma.com

same wavelengths as that of a similar preparation of a film of dipropylene glycol.

D: Identification of alcohol (where stated on the label)—Mix 5 drops of Solution in a small beaker with 1 mL of potassium permanganate solution (1 in 100) and 5 drops of 2 N sulfuric acid, and cover the beaker immediately with filter paper moistened with a freshly prepared solution of 0.1 g of sodium nitroferricyanide and 0.25 g of piperazine in 5 mL of water: an intense blue color is produced on the filter paper, the color becoming paler after a few minutes.

pH ⟨791⟩: between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

Arsenic, *Method I* ⟨211⟩—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

Limit of iron—Using Aluminum Sesquichlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Limit of iron* under *Aluminum Chlorohydrate Solution*. The limit is 75 µg per g.

Content of aluminum—Using Aluminum Sesquichlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Content of aluminum* under *Aluminum Chlorohydrate Solution*. Use the result to calculate the *Aluminum/chloride atomic ratio*.

Content of chloride—Using Aluminum Sesquichlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Content of chloride* under *Aluminum Chlorohydrate Solution*. Use the result to calculate the *Aluminum/chloride atomic ratio*.

Aluminum/chloride atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.26:1 and 1.90:1.

Assay—Calculate the percentage of anhydrous aluminum sesquichlorohydrate in the Solution by the formula:

$$Al(\{26.98x + [17.01(3x - 1)] + 35.453\}/26.98x)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Sesquichlorohydrex Polyethylene Glycol

$Al_y(OH)_{3y-z}Cl_z \cdot nH_2O \cdot mH(OCH_2CH_2)_nOH$

Aluminum chlorohydroxide polyethylene glycol complex.
Aluminum hydroxychloride polyethylene glycol complex.

» Aluminum Sesquichlorohydrex Polyethylene Glycol consists of aluminum sesquichlorohydrate in which some of the waters of hydration have been replaced by polyethylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 1.26:1 and 1.90:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum sesquichlorohydrate.

Packaging and storage—Preserve in well-closed containers.

Labeling—The label states the content of anhydrous aluminum sesquichlorohydrate.

Identification—

A: A solution (1 in 10) responds to the tests for *Aluminum* ⟨191⟩ and for *Chloride* ⟨191⟩.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197F*▲ (CN 1-May-2020)—

Test specimen—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of 9.55 ± 0.05. Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

Standard specimen: a similar preparation of polyethylene glycol.

pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

Arsenic, *Method I* ⟨211⟩: 2 µg per g.

Limit of iron—Using Aluminum Sesquichlorohydrex Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate* The limit is 150 µg per g.

Content of aluminum—Using Aluminum Sesquichlorohydrex Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate* Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

Content of chloride—Using Aluminum Sesquichlorohydrex Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate* Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

Aluminum/chloride atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.26:1 and 1.90:1.

Assay—Calculate the percentage of anhydrous aluminum sesquichlorohydrex in the Aluminum Sesquichlorohydrex Polyethylene Glycol by the formula:

$$Al(\{26.98x + [17.01(3x - 1)] + 35.453\}/26.98x)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Sesquichlorohydrex Propylene Glycol

$Al_y(OH)_{3y-z}Cl_z \cdot nH_2O \cdot mC_3H_8O_2$

Aluminum chlorohydroxide propylene glycol complex.
Aluminum hydroxychloride propylene glycol complex.

USP Monographs

www.webofpharma.com

» Aluminum Sesquichlorohydrex Propylene Glycol consists of aluminum sesquichlorohydrate in which some of the waters of hydration have been replaced by propylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 1.26:1 and 1.90:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum sesquichlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—The label states the content of anhydrous aluminum sesquichlorohydrate.

Identification—
A: A solution (1 in 10) responds to the tests for *Aluminum* ⟨191⟩ and for *Chloride* ⟨191⟩.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197F*▲ (CN 1-May-2020)—
Test specimen—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of 9.55 ± 0.05. Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.
Standard specimen: a similar preparation of propylene glycol.
pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].
Arsenic, *Method I* ⟨211⟩: 2 µg per g.
Limit of iron—Using Aluminum Sesquichlorohydrex Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.
Content of aluminum—Using Aluminum Sesquichlorohydrex Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.
Content of chloride—Using Aluminum Sesquichlorohydrex Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.
Aluminum/chloride atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.26:1 and 1.90:1.
Assay—Calculate the percentage of anhydrous aluminum sesquichlorohydrate in the Aluminum Sesquichlorohydrex Propylene Glycol by the formula:

$$Al(\{26.98x + [17.01(3x - 1)] + 35.453\}/26.98x)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Subacetate Topical Solution

» Aluminum Subacetate Topical Solution yields, from each 100 mL, not less than 2.30 g and not more than 2.60 g of aluminum oxide (Al_2O_3), and not less than 5.43 g and not more than 6.13 g of acetic acid ($C_2H_4O_2$). It may be stabilized by the addition of not more than 0.9 percent of boric acid.
Aluminum Subacetate Topical Solution may be prepared as follows.

Aluminum Sulfate	145 g
Acetic Acid	160 mL
Calcium Carbonate	70 g
Purified Water, a sufficient quantity, to make	1000 mL

Dissolve the Aluminum Sulfate in 600 mL of cold water, filter the solution, and add the Calcium Carbonate gradually, in several portions, with constant stirring. Then slowly add the Acetic Acid, mix, and set the mixture aside for 24 hours. Filter the product with the aid of vacuum if necessary, returning the first portion of the filtrate to the funnel. Wash the magma on the filter with small portions of cold water, until the total filtrate measures 1000 mL.

Packaging and storage—Preserve in tight containers.
Identification—It responds to the tests for *Aluminum* and for test *B* under *Acetate* under *Identification Tests—General* ⟨191⟩ with a deep red color upon the addition of ferric chloride TS. This color is destroyed by the addition of a mineral acid.
pH ⟨791⟩: between 3.8 and 4.6.
Limit of boric acid—Proceed as directed in the test for *Limit of boric acid* under *Aluminum Acetate Topical Solution*.

Assay for aluminum oxide—
Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.
Procedure—Pipet 20 mL of Topical Solution into a 250-mL volumetric flask, add 5 mL of hydrochloric acid, dilute with water to volume, and mix. Pipet 25 mL of this dilution into a 250-mL beaker, and proceed as directed for *Procedure* in the *Assay for aluminum oxide* under *Aluminum Acetate Topical Solution*, beginning with "add, in the order named." Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 2.549 mg of Al_2O_3.
Assay for acetic acid—Proceed as directed in the *Assay for acetic acid* under *Aluminum Acetate Topical Solution*.

Aluminum Sulfate

$Al_2(SO_4)_3 \cdot xH_2O$ (anhydrous) 342.15
Sulfuric acid, aluminum salt (3:2), hydrate.

www.webofpharma.com

Aluminum sulfate (2:3) hydrate [17927-65-0].
Anhydrous 342.16
[10043-01-3].

» Aluminum Sulfate contains not less than 54.0 percent and not more than 59.0 percent of $Al_2(SO_4)_3$. It contains a varying amount of water of crystallization.

Packaging and storage—Preserve in well-closed containers.
Identification—A solution (1 in 10) responds to the tests for *Aluminum* and for *Sulfate* ⟨191⟩.
pH ⟨791⟩: not less than 2.9, in a solution (1 in 20).
Water Determination, *Method I* ⟨921⟩: not less than 41.0% and not more than 46.0%.
Limit of alkalies and alkaline earths—To a boiling solution of 1.0 g in 150 mL of water add a few drops of methyl red TS and then add 6 N ammonium hydroxide just until the color of the solution changes to a distinct yellow. Add hot water to restore the volume to 150 mL, and filter while hot. Evaporate 75 mL of the filtrate to dryness, and ignite to constant weight: not more than 2 mg of residue remains (0.4%).
Limit of ammonium salts—Heat 1 g with 10 mL of 1 N sodium hydroxide on a steam bath for 1 minute: the odor of ammonia is not perceptible.
Iron—To 20 mL of a solution (1 in 150) add 0.3 mL of potassium ferrocyanide TS: no blue color is produced immediately.

Assay—
Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum.*
Procedure—Transfer about 7.5 g of Aluminum Sulfate, accurately weighed, to a 250-mL volumetric flask, and dissolve in water. Dilute with water to volume, mix, and pipet 10 mL of the solution into a 250-mL beaker. Proceed as directed in the *Assay for aluminum oxide* under *Aluminum Acetate Topical Solution* beginning with "add, in the order named." Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 8.554 mg of $Al_2(SO_4)_3$.

Aluminum Sulfate and Calcium Acetate for Topical Solution

DEFINITION
Aluminum Sulfate and Calcium Acetate for Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum sulfate tetradecahydrate [$Al_2(SO_4)_3 \cdot 14H_2O$] and calcium acetate monohydrate ($C_4H_6CaO_4 \cdot H_2O$).

IDENTIFICATION
• **A.**
Sample: 0.25 g of Aluminum Sulfate and Calcium Acetate for Topical Solution
Analysis: Place the *Sample* in a test tube. Add 10 mL of water and 0.25 g of calcium carbonate. Heat on a steam bath for 10 min, and filter. Add 3–4 drops of ferric chloride TS to the filtrate. [NOTE—After the addition of the ferric chloride TS, the solution may be heated for 1 min to speed the reaction.]
Acceptance criteria: A reddish-brown color or precipitate indicates acetate.

• **B. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩ and *Calcium* ⟨191⟩
Sample solution: Suspend 2 g of sample in 50 mL of water and filter.
Acceptance criteria: Meets the requirements

ASSAY
• **ALUMINUM SULFATE TETRADECAHYDRATE**
Sample solution: Transfer 10 g of Aluminum Sulfate and Calcium Acetate for Topical Solution to a 1000-mL volumetric flask. Add 100 mL of 1.2 N hydrochloric acid and 250 mL of water. Heat on a steam bath or hot plate until dissolved. Cool, and dilute with water to volume. Retain a portion of the *Sample solution* for the *Assay* for *Calcium Acetate Monohydrate.*
Blank: Water
Titrimetric system
Mode: Residual titration
Titrant: 0.02 M zinc sulfate VS
Endpoint detection: Visual
Analysis: Transfer a 5.0-mL aliquot of the *Sample solution* to a 250-mL conical flask. Add, in the order named, 40.0 mL of 0.01 M edetate disodium VS and 20 mL of acetic acid–ammonium acetate buffer TS, and mix by swirling. Add 50 mL of alcohol and 2 mL of dithizone TS, and titrate the excess 0.01 M edetate disodium VS with *Titrant* until the color changes from green-violet to a clear rose-pink. Perform a blank titration, substituting 5.0 mL of water for the *Sample solution.*
Calculate the percentage of aluminum sulfate tetradecahydrate [$Al_2(SO_4)_3 \cdot 14H_2O$] in the portion of Aluminum Sulfate and Calcium Acetate for Topical Solution taken:

$$\text{Result} = \{[D \times (V_B - V_S) \times M \times F]/W\} \times 100$$

D = dilution factor, 1000/5.0
V_B = blank titration volume (mL)
V_S = sample titration volume (mL)
M = molarity of the *Titrant* (mmol/mL)
F = equivalency factor, 297.2 (mg/mmol)
W = weight of sample used (mg)

Acceptance criteria: 90.0%–110.0% of the labeled amount of aluminum sulfate tetradecahydrate [$Al_2(SO_4)_3 \cdot 14H_2O$]

• **CALCIUM ACETATE MONOHYDRATE**
Sample: Transfer a 5.0-mL aliquot of the *Sample solution* retained from the *Assay* for *Aluminum Sulfate Tetradecahydrate* to a 250-mL conical flask.
Titrimetric system
Mode: Direct titration
Titrant: 0.01 M edetate disodium VS
Endpoint detection: Visual
Analysis: Add 1–2 mL of 50% triethanolamine to mask the aluminum, and mix well. With constant stirring, add to the *Sample,* in the order named, 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue, and titrate with *Titrant.* The indicator will change from purple to a clear blue color at the endpoint.
Calculate the percentage of calcium acetate monohydrate ($C_4H_6CaO_4 \cdot H_2O$) in the portion of Aluminum Sulfate and Calcium Acetate for Topical Solution taken:

$$\text{Result} = [(D \times V \times M \times F)/W] \times 100$$

D = dilution factor, 1000/5.0
V = sample titration volume (mL)
M = molarity of the *Titrant* (mmol/mL)
F = equivalency factor, 176.2 (mg/mmol)
W = weight of sample used (mg)

www.webofpharma.com

Acceptance criteria: 90.0%–110.0% of the labeled amount of calcium acetate monohydrate ($C_4H_6CaO_4 \cdot H_2O$)

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: 1 g of Aluminum Sulfate and Calcium Acetate for Topical Solution in 200 mL of water
Acceptance criteria: 4.0–4.8

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-unit containers, and protect from excessive heat.

Aluminum Sulfate and Calcium Acetate Tablets for Topical Solution

DEFINITION

Aluminum Sulfate and Calcium Acetate Tablets for Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum sulfate tetradecahydrate [$Al_2(SO_4)_3 \cdot 14H_2O$] and calcium acetate monohydrate ($C_4H_6CaO_4 \cdot H_2O$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩
Sample solution: Suspend 2 g of ground Tablet powder in 50 mL of water, and filter. Use a portion in the *Analysis*, and retain the remaining filtrate for *Identification* test *B*.
Analysis: Mix 2 mL of the *Sample solution* with 2 mL of water and 2 drops of 3 N hydrochloric acid.
Acceptance criteria: Meets the requirements of test *A*
• **B. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩ and *Calcium* ⟨191⟩
Sample solution: A portion of the filtrate retained from *Identification* test *A*
Acceptance criteria: Meets the requirements

ASSAY

• **ALUMINUM SULFATE TETRADECAHYDRATE**
Sample solution: Finely powder and mix NLT 20 Tablets. Weigh a portion of the powder, equivalent to 2.8 g of aluminum sulfate, and transfer to a 1000-mL volumetric flask. Add 100 mL of 1.2 N hydrochloric acid and 100 mL of water, and heat on a steam bath, with occasional swirling, to dissolve the powder. Allow the solution to cool, and dilute with water to volume. Retain a portion of the *Sample solution* for the *Assay* for *Calcium Acetate Monohydrate*.
Blank: Water
Titrimetric system
Mode: Residual titration
Titrant: 0.02 M zinc sulfate VS
Back-titrant: 0.01 M edetate disodium VS
Endpoint detection: Visual
Analysis: Transfer 25.0 mL of the *Sample solution* to a 250-mL conical flask. Add, in the order named, 40.0 mL of *Back-titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and mix by swirling. Add 50 mL of alcohol and 2 mL of dithizone TS, and titrate the excess *Back-titrant* with *Titrant* until the color changes from green-violet to a clear rose-pink. Perform a blank determination. Each mL of 0.01 M edetate disodium is equivalent to 2.972 mg of the labeled amount of aluminum sulfate tetradecahydrate [$Al_2(SO_4)_3 \cdot 14H_2O$].
Acceptance criteria: 90.0%–110.0%

• **CALCIUM ACETATE MONOHYDRATE**
Sample: Transfer 20.0 mL of the *Sample solution* retained from the *Assay* for *Aluminum Sulfate Tetradecahydrate* to a 125-mL conical flask.
Titrimetric system
Mode: Direct titration
Titrant: 0.01 M edetate disodium VS
Endpoint detection: Visual
Analysis: With constant stirring, add to the *Sample*, in the order named, 0.5 mL of trolamine, 10 mL of ammonia–ammonium chloride buffer TS, and 3 drops of a solution prepared by dissolving 500 mg of eriochrome black T trituration in 10 mL of methanol. Titrate with *Titrant* to a violet endpoint. Each mL of 0.01 M edetate disodium is equivalent to 1.762 mg of the labeled amount of calcium acetate monohydrate ($C_4H_6CaO_4 \cdot H_2O$).
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩**:** 10 min
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements for *Weight Variation*

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: 2 g of ground Tablet powder in 500 mL of water
Acceptance criteria: 4.0–4.8
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry ground Tablet powder at 150° for 15 min.
Acceptance criteria: NMT 18%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid excessive heat.

Aluminum Zirconium Octachlorohydrate

$Al_y Zr(OH)_{3y+4-x} Cl_x \cdot nH_2O$

» Aluminum Zirconium Octachlorohydrate is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium octachlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—The label states the content of anhydrous aluminum zirconium octachlorohydrate.
Identification—A solution (1 in 10) responds to the test for *Chloride* ⟨191⟩.
pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].
Arsenic, *Method I* ⟨211⟩: 2 µg per g.

www.webofpharma.com

Limit of iron—

Standard preparation—Transfer 2.0 mL of *Standard Iron Solution*, prepared as directed under *Iron* ⟨241⟩, to a 50-mL beaker.

Test preparation—Transfer 2.7 g of Aluminum Zirconium Octachlorohydrate to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL beaker.

Procedure—To each of the beakers containing the *Standard preparation* and the *Test preparation* add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3 to 5 minutes. Allow to cool, add 5 mL of *Ammonium Thiocyanate Solution*, prepared as directed under *Iron* ⟨241⟩, transfer to separate 50-mL color comparison tubes, dilute with water to volume, and mix: the color of the solution from the *Test preparation* is not darker than that of the solution from the *Standard preparation* (150 µg per g).

Content of aluminum—Transfer about 0.15 g of Aluminum Zirconium Octachlorohydrate, accurately weighed, to a 150-mL beaker, and add 5 mL of water and 15 mL of hydrochloric acid. Heat this solution to boiling, and continue boiling for 5 minutes. Add 40 mL of water and 15.0 mL of 0.1 *M* edetate disodium VS. Heat the solution to boiling, and continue boiling for 5 minutes. Allow the solution to cool, add 10 to 15 mL of acetic acid–ammonium acetate buffer TS, and adjust with ammonium hydroxide to a pH of 4.5 ± 0.1. Add 20 mL of alcohol, and adjust with ammonium hydroxide to a pH of 4.6 ± 0.1. Add 5 to 10 drops of dithizone TS, and titrate with 0.1 *M* zinc sulfate VS until the first permanent purple-pink color appears. Perform a blank determination, and make any necessary correction. Calculate the percentage of aluminum (Al) in the Aluminum Zirconium Octachlorohydrate by the formula:

$$2.698[15.0M_e - (zM_z + Z_e)]/W$$

in which M_e is the molarity of the edetate disodium VS; z is the volume, in mL, of zinc sulfate VS consumed; M_z is the molarity of the zinc sulfate VS; W is the quantity, in g, of Aluminum Zirconium Octachlorohydrate taken; Z_e is the equivalent volume, in mL, of edetate disodium VS consumed by the zirconium moiety, calculated as follows:

$$(Zr/M_e)(W/92.97)$$

in which *Zr* is the percentage of zirconium as determined in the test for *Content of zirconium*, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and the other terms are as defined above. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

Content of zirconium—Transfer about 250 mg of Aluminum Zirconium Octachlorohydrate, accurately weighed, to a 150-mL beaker, and add 5 mL of water and 15 mL of hydrochloric acid. Heat this solution to boiling, and continue boiling for 6 to 8 minutes. Add 30 to 40 mL of water and 5 mL of hydrochloric acid, and heat to boiling. Add 1 drop of xylenol orange TS, and, while still hot, titrate with 0.1 M edetate disodium VS until the color of the solution changes from pink to yellow. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M edetate disodium is equivalent to 9.297 mg of zirconium (Zr). Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

Content of chloride—Transfer about 250 mg of Aluminum Zirconium Octachlorohydrate, accurately weighed, to a 250-mL beaker, add 100 to 120 mL of water and 20 mL of diluted nitric acid, and swirl to dissolve. Titrate with 0.05 N silver nitrate VS using a calomel electrode and a silver billet electrode system, determining the endpoint potentiometrically. Each mL of 0.05 N silver nitrate is equivalent to 1.773 mg of chloride (Cl). Use the result obtained to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

Assay—Calculate the percentage of anhydrous aluminum zirconium octachlorohydrate in the Aluminum Zirconium Octachlorohydrate by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Octachlorohydrate Solution

» Aluminum Zirconium Octachlorohydrate Solution consists of complex basic aluminum chloride that is polymeric and encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium octachlorohydrate.

Packaging and storage—Preserve in well-closed containers.

USP Monographs

www.webofpharma.com

Labeling—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum zirconium octachlorohydrate.

Identification—

A: A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium octachlorohydrate per mL responds to the test for *Chloride* ⟨191⟩.

B: Identification of propylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

C: Identification of dipropylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

pH ⟨791⟩: between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

Arsenic, *Method I* ⟨211⟩: Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

Limit of iron—Using Aluminum Zirconium Octachlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Limit of iron* under *Aluminum Chlorohydrate Solution*. The limit is 75 µg per g.

Content of aluminum—Using about 0.3 g of Aluminum Zirconium Octachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

Content of zirconium—Using about 500 mg of Aluminum Zirconium Octachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

Content of chloride—Using about 500 mg of Aluminum Zirconium Octachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al, Zr,* and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum, Content of zirconium,* and *Content of chloride,* respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

Assay—Calculate the percentage of anhydrous aluminum zirconium octachlorohydrate in the Solution by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y+1)/z] + 35.453(y+1)/z\}/26.98y)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum, y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio, z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio,* 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Octachlorohydrex Gly

» Aluminum Zirconium Octachlorohydrex Gly is a derivative of Aluminum Zirconium Octachlorohydrate in which some of the water molecules have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium octachlorohydrate.

Packaging and storage—Preserve in well-closed containers.

Labeling—The label states the form of glycine used and the claimed content of anhydrous aluminum zirconium octachlorohydrate.

Identification—

A: A solution (1 in 10) responds to the test for *Chloride* ⟨191⟩.

B: Place about 0.5 g of it in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

Arsenic, *Method I* ⟨211⟩: 2 µg per g.

Limit of iron—Using Aluminum Zirconium Octachlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

Content of aluminum—Using Aluminum Zirconium Octachlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

www.webofpharma.com

Content of zirconium—Using Aluminum Zirconium Octachlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the (*Aluminum plus zirconium*)/*chloride atomic ratio*.

Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

Content of chloride—Using Aluminum Zirconium Octachlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the (*Aluminum plus zirconium*)/*chloride atomic ratio*.

(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

Assay—Calculate the percentage of anhydrous aluminum zirconium octachlorohydrate in the Aluminum Zirconium Octachlorohydrex Gly by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for (*Aluminum plus zirconium*)/*chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Octachlorohydrex Gly Solution

» Aluminum Zirconium Octachlorohydrex Gly Solution is a solution of Aluminum Zirconium Octachlorohydrate in which some of the waters of hydration have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium octachlorohydrate.

Packaging and storage—Preserve in well-closed containers.

Labeling—Label Solution to state the solvent and form of glycine used and the claimed concentration of anhydrous aluminum zirconium octachlorohydrate.

Identification—

A: A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium octachlorohydrate per mL responds to the test for *Chloride* ⟨191⟩.

B: Identification of propylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

C: Identification of dipropylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

D: Identification of glycine—Place about 1 g of Solution in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

pH ⟨791⟩: between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

Arsenic, *Method I* ⟨211⟩—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

Limit of iron—Using about 5.4 g of Aluminum Zirconium Octachlorohydrex Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

Content of aluminum—Using about 0.3 g of Aluminum Zirconium Octachlorohydrex Gly Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the (*Aluminum plus zirconium*)/*chloride atomic ratio*.

Content of zirconium—Using about 500 mg of Aluminum Zirconium Octachlorohydrex Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the (*Aluminum plus zirconium*)/*chloride atomic ratio*.

Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

Content of chloride—Using about 500 mg of Aluminum Zirconium Octachlorohydrex Gly Solution, accurately

USP Monographs

www.webofpharma.com

weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the (*Aluminum plus zirconium*)/*chloride atomic ratio*.
(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.
Assay—Calculate the percentage of anhydrous aluminum zirconium octachlorohydrate in the Solution by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for (*Aluminum plus zirconium*)/*chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Pentachlorohydrate

$Al_y Zr(OH)_{3y+4-x} Cl_x \cdot nH_2O$

» Aluminum Zirconium Pentachlorohydrate is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium pentachlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—The label states the content of anhydrous aluminum zirconium pentachlorohydrate.
Identification—A solution (1 in 10) responds to the test for *Chloride* ⟨191⟩.
pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].
Arsenic, *Method I* ⟨211⟩: 2 µg per g.
Limit of iron—Using Aluminum Zirconium Pentachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).
Content of aluminum—Using Aluminum Zirconium Pentachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the (*Aluminum plus zirconium*)/*chloride atomic ratio*.
Content of zirconium—Using Aluminum Zirconium Pentachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the (*Aluminum plus zirconium*)/*chloride atomic ratio*.
Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.
Content of chloride—Using Aluminum Zirconium Pentachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the (*Aluminum plus zirconium*)/*chloride atomic ratio*.
(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride, as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.
Assay—Calculate the percentage of anhydrous aluminum zirconium pentachlorohydrate in the Aluminum Zirconium Pentachlorohydrate by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for (*Aluminum plus zirconium*)/*chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Pentachlorohydrate Solution

» Aluminum Zirconium Pentachlorohydrate Solution is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1 and a

www.webofpharma.com

range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium pentachlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum zirconium pentachlorohydrate.

Identification—
A: A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium pentachlorohydrate per mL responds to the test for *Chloride* ⟨191⟩.
B: Identification of propylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.
C: Identification of dipropylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.
pH ⟨791⟩: between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.
Arsenic, *Method I* ⟨211⟩—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.
Limit of iron—Using about 5.4 g of Aluminum Zirconium Pentachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate.* The limit is 75 µg per g.
Content of aluminum—Using about 0.3 g of Aluminum Zirconium Pentachlorohydrate Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate.* Use the result to calculate the *Aluminum/zirconium atomic ratio* and the (*Aluminum plus zirconium*)/*chloride atomic ratio.*
Content of zirconium—Using about 500 mg of Aluminum Zirconium Pentachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate.* Use the result to calculate the *Aluminum/zirconium atomic ratio* and the (*Aluminum plus zirconium*)/*chloride atomic ratio.*
Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium,* and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.
Content of chloride—Using about 500 mg of Aluminum Zirconium Pentachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate.* Use the result to calculate the (*Aluminum plus zirconium*)/*chloride atomic ratio.*
(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al, Zr,* and *Cl* are the percentages of aluminum, zirconium, and chloride found in the test for *Content of aluminum, Content of zirconium,* and *Content of chloride,* respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.
Assay—Calculate the percentage of anhydrous aluminum zirconium pentachlorohydrate in the Solution by the formula:

$$Al\{[26.98y + 92.97 + (17.01)(3y + 4 - (y + 1)/z) + 35.453(y + 1)/z]/26.98y\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum, y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio, z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for (*Aluminum plus zirconium*)/*chloride atomic ratio,* 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Pentachlorohydrex Gly

» Aluminum Zirconium Pentachlorohydrex Gly is a derivative of Aluminum Zirconium Pentachlorohydrate in which some of the water molecules have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium pentachlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—The label states the form of glycine used and the claimed content of anhydrous aluminum zirconium pentachlorohydrate.

Identification—
A: A solution (1 in 10) responds to the test for *Chloride* ⟨191⟩.
B: Place about 0.5 g of it in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.
pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

USP Monographs

www.webofpharma.com

Arsenic, *Method I* ⟨211⟩: 2 µg per g.
Limit of iron—Using Aluminum Zirconium Pentachlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate.* The specified result is obtained (150 µg per g limit).
Content of aluminum—Using Aluminum Zirconium Pentachlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate.* Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio.*
Content of zirconium—Using Aluminum Zirconium Pentachlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate.* Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio.*
Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium,* and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.
Content of chloride—Using Aluminum Zirconium Pentachlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate.* Use the result obtained to calculate the *(Aluminum plus zirconium)/chloride atomic ratio.* ,
(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al, Zr,* and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum, Content of zirconium,* and *Content of chloride,* respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.
Assay—Calculate the percentage of anhydrous aluminum zirconium pentachlorohydrate in the Aluminum Zirconium Pentachlorohydrex Gly by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum, y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio, z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio,* 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Pentachlorohydrex Gly Solution

» Aluminum Zirconium Pentachlorohydrex Gly Solution is a solution of Aluminum Zirconium Pentachlorohydrate in which some of the waters of hydration have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium ratios between 6.0:1 and 10.0:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium pentachlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label Solution to state the solvent and form of glycine used and the claimed concentration of anhydrous aluminum zirconium pentachlorohydrate.

Identification—
A: A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium pentachlorohydrate per mL responds to the test for *Chloride* ⟨191⟩.
B: Identification of propylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.
C: Identification of dipropylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.
D: Identification of glycine—Place about 1 g of Solution in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.
pH ⟨791⟩: between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.
Arsenic, *Method I* ⟨211⟩—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.
Limit of iron—Using about 5.4 g of Aluminum Zirconium Pentachlorohydrex Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate.* The limit is 75 µg per g.
Content of aluminum—Using about 0.3 g of Aluminum Zirconium Pentachlorohydrex Gly Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate.* Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio.*

www.webofpharma.com

Content of zirconium—Using about 500 mg of Aluminum Zirconium Pentachlorohydrex Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.
Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.
Content of chloride—Using about 500 mg of Aluminum Zirconium Pentachlorohydrex Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.
(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the test for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.
Assay—Calculate the percentage of anhydrous aluminum zirconium pentachlorohydrate in the Solution by the formula:

$$Al\{[26.98y + 92.97 + (17.01)(3y + 4 - (y + 1)/z) + 35.453(y + 1)/z]/26.98y\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Tetrachlorohydrate

$Al_y Zr(OH)_{3y+4-x} Cl_x \cdot nH_2O$

» Aluminum Zirconium Tetrachlorohydrate is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium tetrachlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—The label states the content of anhydrous aluminum zirconium tetrachlorohydrate.
Identification—A solution (1 in 10) responds to the test for *Chloride* ⟨191⟩.
pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].
Arsenic, *Method I* ⟨211⟩: 2 µg per g.
Limit of iron—Using Aluminum Zirconium Tetrachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate* The specified result is obtained (150 µg per g limit).
Content of aluminum—Using Aluminum Zirconium Tetrachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.
Content of zirconium—Using Aluminum Zirconium Tetrachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate* Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.
Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.
Content of chloride—Using Aluminum Zirconium Tetrachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate* Use the result obtained to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.
(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.
Assay—Calculate the percentage of anhydrous aluminum zirconium tetrachlorohydrate in the Aluminum Zirconium Tetrachlorohydrate by the formula:

$$Al\{\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

USP Monographs

www.webofpharma.com

Aluminum Zirconium Tetrachlorohydrate Solution

» Aluminum Zirconium Tetrachlorohydrate Solution is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium tetrachlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum zirconium tetrachlorohydrate.

Identification—
A: A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium tetrachlorohydrate per mL responds to the test for *Chloride* ⟨191⟩.
B: Identification of propylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.
C: Identification of dipropylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.
pH ⟨791⟩: between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.
Arsenic, *Method I* ⟨211⟩—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.
Limit of iron—Using about 5.4 g of Aluminum Zirconium Tetrachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.
Content of aluminum—Using about 0.3 g of Aluminum Zirconium Tetrachlorohydrate Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.
Content of zirconium—Using about 500 mg of Aluminum Zirconium Tetrachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.
Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium* and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.
Content of chloride—Using about 500 mg of Aluminum Zirconium Tetrachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.
(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the test for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.
Assay—Calculate the percentage of anhydrous aluminum zirconium tetrachlorohydrate in the Solution by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Tetrachlorohydrex Gly

» Aluminum Zirconium Tetrachlorohydrex Gly is a derivative of Aluminum Zirconium Tetrachlorohydrate in which some of the water molecules have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium tetrachlorohydrate.

Packaging and storage—Preserve in well-closed containers.

www.webofpharma.com

Labeling—The label states the form of glycine used and the claimed content of anhydrous aluminum zirconium tetrachlorohydrate.

Identification—

A: A solution (1 in 10) responds to the test for *Chloride* ⟨191⟩.

B: Place about 0.5 g of it in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

Arsenic, *Method I* ⟨211⟩: 2 µg per g.

Limit of iron—Using Aluminum Zirconium Tetrachlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

Content of aluminum—Using Aluminum Zirconium Tetrachlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

Content of zirconium—Using Aluminum Zirconium Tetrachlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

Content of chloride—Using Aluminum Zirconium Tetrachlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

Assay—Calculate the percentage of anhydrous aluminum zirconium tetrachlorohydrate in the Aluminum Zirconium Tetrachlorohydrex Gly by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Tetrachlorohydrex Gly Solution

» Aluminum Zirconium Tetrachlorohydrex Gly Solution is a solution of Aluminum Zirconium Tetrachlorohydrate in which some of the waters of hydration have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium tetrachlorohydrate.

Packaging and storage—Preserve in well-closed containers.

Labeling—Label Solution to state the solvent and form of glycine used and the claimed concentration of anhydrous aluminum zirconium tetrachlorohydrate.

Identification—

A: A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium tetrachlorohydrate per mL responds to the test for *Chloride* ⟨191⟩.

B: Identification of propylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

C: Identification of dipropylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

D: Identification of glycine—Place about 1 g of Solution in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

pH ⟨791⟩: between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

Arsenic, *Method I* ⟨211⟩—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

Limit of iron—Using about 5.4 g of Aluminum Zirconium Tetrachlorohydrex Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as

USP Monographs

www.webofpharma.com

directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.
Content of aluminum—Using about 0.3 g of Aluminum Zirconium Tetrachlorohydrex Gly Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the (*Aluminum plus zirconium*)/*chloride atomic ratio*.
Content of zirconium—Using about 500 mg of Aluminum Zirconium Tetrachlorohydrex Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the (*Aluminum plus zirconium*)/*chloride atomic ratio*.
Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.
Content of chloride—Using about 500 mg of Aluminum Zirconium Tetrachlorohydrex Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the (*Aluminum plus zirconium*)/*chloride atomic ratio*.
(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.
Assay—Calculate the percentage of anhydrous aluminum zirconium tetrachlorohydrate in the Solution by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for (*Aluminum plus zirconium*)/*chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Trichlorohydrate

$Al_y Zr(OH)_{3y+4-x} Cl_x \cdot nH_2O$

» Aluminum Zirconium Trichlorohydrate is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium trichlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—The label states the content of anhydrous aluminum zirconium trichlorohydrate.
Identification—A solution (1 in 10) responds to the test for *Chloride* ⟨191⟩.
pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].
Arsenic, *Method I* ⟨211⟩: 2 µg per g.
Limit of iron—Using Aluminum Zirconium Trichlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).
Content of aluminum—Using Aluminum Zirconium Trichlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the (*Aluminum plus zirconium*)/*chloride atomic ratio*.
Content of zirconium—Using Aluminum Zirconium Trichlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the (*Aluminum plus zirconium*)/*chloride atomic ratio*.
Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.
Content of chloride—Using Aluminum Zirconium Trichlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the (*Aluminum plus zirconium*)/*chloride atomic ratio*.
(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.
Assay—Calculate the percentage of anhydrous aluminum zirconium trichlorohydrate in the Aluminum Zirconium Trichlorohydrate by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y)$$

www.webofpharma.com

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Trichlorohydrate Solution

» Aluminum Zirconium Trichlorohydrate Solution is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium trichlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum zirconium trichlorohydrate.

Identification—
A: A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium trichlorohydrate per mL responds to the test for *Chloride* ⟨191⟩.
B: Identification of propylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.
C: Identification of dipropylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.
pH ⟨791⟩: between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.
Arsenic, *Method I* ⟨211⟩—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.
Limit of iron—Using about 5.4 g of Aluminum Zirconium Trichlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.
Content of aluminum—Using about 0.3 g of Aluminum Zirconium Trichlorohydrate Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.
Content of zirconium—Using about 500 mg of Aluminum Zirconium Trichlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.
Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium* and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.
Content of chloride—Using about 500 mg of Aluminum Zirconium Trichlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.
(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the test for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.
Assay—Calculate the percentage of anhydrous aluminum zirconium trichlorohydrate in the Solution by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio as determined in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride ratio determined in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Trichlorohydrex Gly

» Aluminum Zirconium Trichlorohydrex Gly is a derivative of Aluminum Zirconium Trichlorohydrate in which some of the water molecules have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a

USP Monographs

www.webofpharma.com

range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium trichlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—The label states the form of glycine used and the claimed content of anhydrous aluminum zirconium trichlorohydrate.

Identification—
A: A solution (1 in 10) responds to the test for *Chloride* ⟨191⟩.
B: Place about 0.5 g of it in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.
pH ⟨791⟩: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].
Arsenic, *Method I* ⟨211⟩: 2 µg per g.
Limit of iron—Using Aluminum Zirconium Trichlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).
Content of aluminum—Using Aluminum Zirconium Trichlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.
Content of zirconium—Using Aluminum Zirconium Trichlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.
Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.
Content of chloride—Using Aluminum Zirconium Trichlorohydrex Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.
(Aluminum plus zirconium)/chloride atomic ratio— Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

Assay—Calculate the percentage of anhydrous aluminum zirconium trichlorohydrate in the Aluminum Zirconium Trichlorohydrex Gly by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Aluminum Zirconium Trichlorohydrex Gly Solution

» Aluminum Zirconium Trichlorohydrex Gly Solution is a solution of Aluminum Zirconium Trichlorohydrate in which some of the waters of hydration have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium trichlorohydrate.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label Solution to state the solvent and form of glycine used and the claimed concentration of anhydrous aluminum zirconium trichlorohydrate.

Identification—
A: A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium trichlorohydrate per mL responds to the test for *Chloride* ⟨191⟩.
B: Identification of propylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.
C: Identification of dipropylene glycol (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.
D: Identification of glycine—Place about 1 g of Solution in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve.

www.webofpharma.com

Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.
pH ⟨791⟩: between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.
Arsenic, *Method I* ⟨211⟩—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.
Limit of iron—Using about 5.4 g of Aluminum Zirconium Trichlorohydrex Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate.* The limit is 75 µg per g.
Content of aluminum—Using about 0.3 g of Aluminum Zirconium Trichlorohydrex Gly Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate.* Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio.*
Content of zirconium—Using about 500 mg of Aluminum Zirconium Trichlorohydrex Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate.* Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio.*
Aluminum/zirconium atomic ratio—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium* and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.
Content of chloride—Using about 500 mg of Aluminum Zirconium Trichlorohydrex Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate.* Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio.*
(Aluminum plus zirconium)/chloride atomic ratio—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

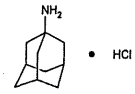
$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al, Zr,* and *Cl* are the percentages of aluminum, zirconium, and chloride found in the tests for *Content of aluminum, Content of zirconium,* and *Content of chloride,* respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.
Assay—Calculate the percentage of anhydrous aluminum zirconium trichlorohydrate in the Solution by the formula:

$$Al(\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y)$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum, y* is the aluminum/zirconium atomic ratio, as determined in the test for *Aluminum/zirconium atomic ratio, z* is the (aluminum plus zirconium)/chloride atomic ratio as determined in the test for *(Aluminum plus zirconium)/chloride atomic ratio,* 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

Amantadine Hydrochloride



$C_{10}H_{17}N \cdot HCl$ 187.71
Tricyclo[3.3.1.1^{3,7}]decan-1-amine, hydrochloride;
1-Adamantanamine hydrochloride [665-66-7].

DEFINITION
Amantadine Hydrochloride contains NLT 98.0% and NMT 102.0% of amantadine hydrochloride ($C_{10}H_{17}N \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, and 197S▲ (CN 1-May-2020)
  Cell: 1 mm
  Sample solution: 50 mg in 10 mL of 0.1 N hydrochloric acid, and filter. Transfer the filtrate to a suitable separator, add 1 mL of 5 N sodium hydroxide, and extract with 5 mL of methylene chloride.
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the amantadine peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*

ASSAY
- **PROCEDURE**
  Internal standard solution: 0.3 mg/mL of adamantane in *n*-heptane
  Standard stock solution: 1 mg/mL of USP Amantadine Hydrochloride RS in water
  Standard solution: Transfer 10 mL of *Standard stock solution* and 10 mL of 5 N sodium hydroxide solution to a separatory funnel. Add 25 mL of *Internal standard solution* and shake for 10 min. Collect the upper layer of *n*-heptane and swirl with anhydrous sodium sulfate to remove traces of water.
  Sample stock solution: 1 mg/mL of Amantadine Hydrochloride in water
  Sample solution: Transfer 10 mL of *Sample stock solution* and 10 mL of 5 N sodium hydroxide solution to a separatory funnel. Add 25 mL of *Internal standard solution* and shake for 10 min. Collect the upper layer of *n*-heptane and swirl with anhydrous sodium sulfate to remove traces of water.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: GC
  Detector: Flame ionization
  Column: 0.53-mm × 30-m base deactivated fused-silica; coated with 1.0-µm film of stationary phase G27
  Temperatures
  Injection port: 220°
  Detector: 300°
  Column: See *Table 1.*

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
120	0	120	3

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
120	8	250	10

Carrier gas: Helium
Flow rate: 4 mL/min
Injection volume: 2 µL
Injection type: Split ratio, 5:1 (deactivated split liner with glass wool)

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for adamantane and amantadine are about 0.7 and 1.0, respectively.]
Suitability requirements
Relative standard deviation: NMT 1.0% for the peak response ratio of amantadine to adamantane

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of amantadine hydrochloride ($C_{10}H_{17}N \cdot HCl$) in the portion of Amantadine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of amantadine to adamantane from the *Sample solution*
R_S = peak response ratio of amantadine to adamantane from the *Standard solution*
C_S = concentration of USP Amantadine Hydrochloride RS in the *Standard stock solution* (mg/mL)
C_U = concentration of Amantadine Hydrochloride in the *Sample stock solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Internal standard solution: 0.1 mg/mL of adamantane in *n*-heptane
Peak identification solution: 0.03 mg/mL each of USP Amantadine Related Compound A RS and USP Amantadine Related Compound B RS in *Internal standard solution* prepared as follows. Transfer suitable amounts of USP Amantadine Related Compound A RS and USP Amantadine Related Compound B RS to a suitable volumetric flask. Add methylene chloride to about 5% of the flask volume to dissolve, and dilute with *Internal standard solution* to volume.
Standard stock solution: 0.03 mg/mL of USP Amantadine Hydrochloride RS in water
Standard solution: Transfer 25 mL of *Standard stock solution* and 10 mL of 5 N sodium hydroxide solution to a separatory funnel. Add 25 mL of *Internal standard solution* and shake for 10 min. Collect the upper layer of *n*-heptane and swirl with anhydrous sodium sulfate to remove traces of water.
Sample stock solution: 10 mg/mL of Amantadine Hydrochloride in water
Sample solution: Transfer 25 mL of *Sample stock solution* and 10 mL of 5 N sodium hydroxide solution to a separatory funnel. Add 25 mL of *Internal standard solution* and shake for 10 min. Collect the upper layer of *n*-heptane and swirl with anhydrous sodium sulfate to remove traces of water.
Chromatographic system: Proceed as directed in the *Assay*.

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for adamantane and amantadine are 0.7 and 1.0, respectively.]
Suitability requirements
Relative standard deviation: NMT 3.0% for the peak response ratio of amantadine to adamantane

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Amantadine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of any individual impurity to adamantane from the *Sample solution*
R_S = peak response ratio of amantadine to adamantane from the *Standard solution*
C_S = concentration of USP Amantadine Hydrochloride RS in the *Standard stock solution* (mg/mL)
C_U = concentration of Amantadine Hydrochloride in the *Sample stock solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amantadine	1.0	—
Amantadine Related Compound A	1.2	0.3
Amantadine Related Compound B	1.9	0.3
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample: 0.2 g/mL in water
Acceptance criteria: 3.0–5.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amantadine Hydrochloride RS
USP Amantadine Related Compound A RS
1-Chloroadamantane.
$C_{10}H_{15}Cl$ 170.68
USP Amantadine Related Compound B RS
N-(Adamantan-1-yl)acetamide.
$C_{12}H_{19}NO$ 193.29

Amantadine Hydrochloride Capsules

DEFINITION

Amantadine Hydrochloride Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of amantadine hydrochloride ($C_{10}H_{17}N \cdot HCl$).

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197S▲ (CN 1-May-2020)
Cell: 1 mm
Sample solution: Place the contents of Capsules, equivalent to 200 mg of amantadine hydrochloride, in a vessel, dissolve in 0.1 N hydrochloric acid, and filter. Transfer the filtrate to a separator, add 1 mL of 5 N sodium hydroxide, and extract with 5 mL of methylene chloride. Filter the extract through anhydrous sodium sulfate, and rinse the anhydrous sodium sulfate with 2 mL of methylene chloride.
• **B.** The retention time of the amantadine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Internal standard solution: 0.4 mg/mL of naphthalene in hexane
Standard stock solution: 2 mg/mL of USP Amantadine Hydrochloride RS in water
Standard solution: Transfer 25.0 mL of *Standard stock solution* into a 250-mL separator, and add 25 mL of 2 N sodium hydroxide and 50.0 mL of *Internal standard solution*. Shake for about 10 min, and collect the hexane layer.
Sample stock solution: Transfer NLT 20 Capsules to a 200-mL volumetric flask. Add 40 mL of 0.1 N hydrochloric acid and 40 mL of water. Sonicate for 20 min with intermittent shaking, and dilute with water to volume. Centrifuge the solution for 10 min and pass through a suitable filter.
Sample solution: Transfer 5.0 mL of the filtrate into a 250-mL separator, and add 40.0 mL of 1 N sodium hydroxide and 50.0 mL of *Internal standard solution*. Shake for about 10 min, and collect the hexane layer.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m; coated with a 0.25-µm film of phase G1
Temperatures
Injection port: 250°
Detector: 300°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	0	100	3
100	10	200	2

Carrier gas: Helium
Flow rate: 1.4 mL/min
Injection volume: 2 µL
Injection type: Split
Split flow rate: 20 mL/min
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for naphthalene and amantadine are 0.90 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between naphthalene and amantadine
Tailing factor: NMT 2.0 for the amantadine peak
Relative standard deviation: NMT 2.0% for the peak response ratio of amantadine to naphthalene
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amantadine hydrochloride ($C_{10}H_{17}N \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of amantadine to naphthalene from the *Sample solution*
R_S = peak response ratio of amantadine to naphthalene from the *Standard solution*
C_S = concentration of USP Amantadine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amantadine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Internal standard solution: 0.054 mg/mL of naphthalene in hexane
Standard stock solution: 0.1 mg/mL of USP Amantadine Hydrochloride RS in water
Standard solution: Transfer 15.0 mL of *Standard stock solution* into a 50-mL screw-capped test tube, add 5.0 mL of 5 N sodium hydroxide and 10.0 mL of *Internal standard solution*, and shake for 60 min. Collect the hexane layer.
Sample solution: Transfer 15.0 mL of the filtered solution under test, and place into a 50-mL screw-capped test tube. Pipet 5.0 mL of 5 N sodium hydroxide and 10.0 mL of the *Internal standard solution* into the test tube, and shake for 60 min. Collect the hexane layer.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m; coated with a 0.25-µm film of phase G1
Temperatures
Injection port: 250°
Detector: 300°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	0	100	3
100	10	200	2

Carrier gas: Helium
Flow rate: 1.4 mL/min
Injection volume: 2 µL
Injection type: Split
Split flow rate: 20 mL/min

www.webofpharma.com

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for naphthalene and amantadine are 0.90 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between naphthalene and amantadine
Tailing factor: NMT 2.0 for the amantadine peak
Relative standard deviation: NMT 2.0% for the peak response ratio of amantadine to naphthalene
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amantadine hydrochloride dissolved:

$$\text{Result} = (R_U/R_S) \times (C_S/L) \times V \times 100$$

R_U = peak response ratio of amantadine to naphthalene from the *Sample solution*
R_S = peak response ratio of amantadine to naphthalene from the *Standard solution*
C_S = concentration of USP Amantadine Hydrochloride RS in the *Standard stock solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of amantadine hydrochloride ($C_{10}H_{17}N \cdot HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 900 mL
Apparatus 2: 75 rpm, with sinkers. [NOTE—A suitable sinker is available as catalog number CAPWHT-2S from www.qla-llc.com or www.tabletdissolution.com or www.labhut.com.]
Time: 45 min
Internal standard solution: 0.06 mg/mL of naphthalene in hexane
Standard stock solution: 0.12 mg/mL of USP Amantadine Hydrochloride RS in *Medium*
Standard solution: Transfer 60.0 mL of the *Standard stock solution* to a 200-mL volumetric flask. Add 20 mL of 5 N sodium hydroxide and 40.0 mL of *Internal standard solution*. Shake the flask for approximately 10 min, and allow the layers to separate. Use the top layer for injection. The final concentration is about 0.18 mg/mL.
Sample solution: Transfer 3.0 mL of the solution under test to a centrifuge tube. Add 1.0 mL of 5 N sodium hydroxide and 2.0 mL of *Internal standard solution*. Shake the tube for approximately 10 min, and allow the layers to separate. Use the top layer for injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m; coated with a 0.25-µm film of phase G1
Temperatures
Injection port: 250°
Detector: 300°
Column: See *Table 3*.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	0	100	3
100	10	200	2

Carrier gas: Helium
Flow rate: 1.4 mL/min
Injection volume: 2 µL
Injection type: Split
Split flow rate: 20 mL/min
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2 between naphthalene and amantadine
Tailing factor: NMT 2.0 for the amantadine peak
Relative standard deviation: NMT 2.0% for the peak response ratio of amantadine to naphthalene
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amantadine hydrochloride dissolved:

$$\text{Result} = (R_U/R_S) \times (C_S/L) \times V \times 100$$

R_U = peak response ratio of amantadine to naphthalene from the *Sample solution*
R_S = peak response ratio of amantadine to naphthalene from the *Standard solution*
C_S = concentration of USP Amantadine Hydrochloride RS in the *Standard stock solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of amantadine hydrochloride ($C_{10}H_{17}N \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Internal standard solution: 0.1 mg/mL of adamantane in *n*-heptane
Peak identification solution: 0.03 mg/mL each of USP Amantadine Related Compound A RS and USP Amantadine Related Compound B RS in *Internal standard solution* prepared as follows. Transfer suitable amounts of USP Amantadine Related Compound A RS and USP Amantadine Related Compound B RS to a suitable volumetric flask. Add methylene chloride to about 5% of the flask volume to dissolve, and dilute with *Internal standard solution* to volume.
Standard stock solution: 0.03 mg/mL of USP Amantadine Hydrochloride RS in water
Standard solution: Transfer 25 mL of *Standard stock solution*, 10 mL of 5 N sodium hydroxide solution, and 25 mL of *Internal standard solution* to a separatory funnel and shake for 10 min. Collect the upper layer of *n*-heptane and swirl with anhydrous sodium sulfate to remove traces of water.
Sample stock solution (for hard gelatin Capsules)**:** Nominally 10 mg/mL of amantadine hydrochloride prepared as follows. Combine the contents of NLT 20 Capsules and transfer a portion equivalent to 500 mg of amantadine hydrochloride to a 100-mL flask. Add 20 mL of 0.1 N hydrochloric acid using a volumetric pipette. Heat gently to dissolve and cool. Add 20 mL of water and sonicate for 15 min. Cool and add 10 mL of water. Centrifuge the solution for 10 min and collect the supernatant.
Sample solution
For hard gelatin Capsules: Transfer 25 mL of *Sample stock solution* and 10 mL of 5 N sodium hydroxide solution to a separatory funnel. Add 25 mL of *Internal standard solution* and shake for 10 min. Collect the upper layer of

www.webofpharma.com

n-heptane and swirl with anhydrous sodium sulfate to remove traces of water.

For soft gelatin Capsules: Transfer 2 Capsules to a 200-mL flask and add 10 mL of water. Heat gently and cool. Add 5.0 mL of 5 N sodium hydroxide, 3.0 mL of concentrated sodium chloride, and 50.0 mL of *Internal standard solution*. Shake the contents for 20 min and collect the upper layer of *n*-heptane.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m; base deactivated fused-silica coated with a 1.0-µm film of stationary phase G27

Temperatures

Injection port: 220°

Detector: 300°

Column: See *Table 4*.

Table 4

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
120	0	120	3
120	8	250	10

Carrier gas: Helium

Flow rate: 4 mL/min

Injection volume: 2 µL

Injection type: Split ratio, 5:1 (deactivated split liner with glass wool)

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for adamantane and amantadine are 0.7 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 3.0% for the peak response ratio of amantadine to adamantane

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified impurity in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of any individual unspecified impurity to adamantane from the *Sample solution*

R_S = peak response ratio of amantadine to adamantane from the *Standard solution*

C_S = concentration of USP Amantadine Hydrochloride RS in the *Standard stock solution* (mg/mL)

C_U = nominal concentration of amantadine hydrochloride in the *Sample stock solution* (mg/mL)

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amantadine	1.0	—
Amantadine related compound A[a]	1.2	—
Amantadine related compound B[a]	1.9	—

Table 5 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	0.2
Total impurities	—	1.5

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Amantadine Hydrochloride RS

USP Amantadine Related Compound A RS

1-Chloroadamantane.

$C_{10}H_{15}Cl$ 170.68

USP Amantadine Related Compound B RS

N-(Adamantan-1-yl)acetamide.

$C_{12}H_{19}NO$ 193.29

Amantadine Hydrochloride Oral Solution

DEFINITION

Amantadine Hydrochloride Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of amantadine hydrochloride ($C_{10}H_{17}N \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197S▲ (CN 1-May-2020)

Cell: 1 mm

Sample solution: Place a volume of Oral Solution, equivalent to 200 mg of amantadine hydrochloride, in a vessel, dissolve in 0.1 N hydrochloric acid, and filter. Transfer the filtrate to a separator, add 10 mL of 0.5 N sodium hydroxide, and extract with 5 mL of methylene chloride. Filter the extract through anhydrous sodium sulfate, and rinse the anhydrous sodium sulfate with 2 mL of methylene chloride.

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Internal standard solution: 0.3 mg/mL of adamantane in *n*-heptane

Standard stock solution: 1 mg/mL of USP Amantadine Hydrochloride RS in water

Standard solution: Transfer 10 mL of *Standard stock solution* to a separatory funnel and add 10 mL of 5 N sodium hydroxide solution. Add 25 mL of *Internal standard solution* and shake for 10 min. Collect the *n*-heptane upper layer and swirl with anhydrous sodium sulfate to remove traces of water.

www.webofpharma.com

Sample stock solution: Nominally 1 mg/mL of amantadine hydrochloride from a portion of Oral Solution in water
Sample solution: Transfer 10 mL of *Sample stock solution* to a separatory funnel and add 10 mL of 5 N sodium hydroxide solution. Add 25 mL of *Internal standard solution* and shake for 10 min. Collect the *n*-heptane upper layer and swirl with anhydrous sodium sulfate to remove traces of water.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m base deactivated fused-silica; coated with 1.0-µm film of stationary phase G27
Temperatures
Injection port: 220°
Detector: 300°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
120	0	120	3
120	8	250	10

Carrier gas: Helium
Flow rate: 4 mL/min
Injection volume: 2 µL
Injection type: Split ratio, 5:1 (deactivated split liner with glass wool)
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for adamantane and amantadine are about 0.7 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0 for the amantadine peak
Relative standard deviation: NMT 2.0% for the peak response ratio of amantadine to adamantane
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amantadine hydrochloride ($C_{10}H_{17}N \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of amantadine to adamantane from the *Sample solution*
R_S = peak response ratio of amantadine to adamantane from the *Standard solution*
C_S = concentration of USP Amantadine Hydrochloride RS in the *Standard stock solution* (mg/mL)
C_U = nominal concentration of amantadine hydrochloride in the *Sample stock solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• ORGANIC IMPURITIES
Internal standard solution: 0.1 mg/mL of adamantane in *n*-heptane
Peak identification solution: 0.03 mg/mL each of USP Amantadine Related Compound A RS and USP Amantadine Related Compound B RS in *Internal standard solution* prepared as follows. Transfer suitable amounts of USP Amantadine Related Compound A RS and USP Amantadine Related Compound B RS to a suitable volumetric flask. Add methylene chloride to about 5% of the flask volume to dissolve, and dilute with *Internal standard solution* to volume.
Standard stock solution: 0.03 mg/mL of USP Amantadine Hydrochloride RS in water
Standard solution: Transfer 25 mL of *Standard stock solution*, 10 mL of 5 N sodium hydroxide solution, and 25 mL of *Internal standard solution* to a separatory funnel and shake for 10 min. Collect the upper layer of *n*-heptane and swirl with anhydrous sodium sulfate to remove traces of water.
Sample stock solution: Nominally 10.0 mg/mL of amantadine hydrochloride from Oral Solution in water
Sample solution: Transfer 25 mL of *Sample stock solution*, equivalent to 250 mg of amantadine hydrochloride, and 10 mL of 5 N sodium hydroxide solution to a separatory funnel. Add 25 mL of *Internal standard solution* and shake for 10 min. Collect the upper layer of *n*-heptane and swirl with anhydrous sodium sulfate to remove traces of water.
Chromatographic system: Proceed as directed in the *Assay*.
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for adamantane and amantadine are 0.7 and 1.0, respectively.]
Suitability requirements
Relative standard deviation: NMT 3.0% for the peak response ratio of amantadine and adamantane
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual unspecified impurity in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of any individual unspecified impurity to adamantane from the *Sample solution*
R_S = peak response ratio of amantadine to adamantane from the *Standard solution*
C_S = concentration of USP Amantadine Hydrochloride RS in the *Standard stock solution* (mg/mL)
C_U = nominal concentration of amantadine hydrochloride in the *Sample stock solution* (mg/mL)

Acceptance criteria: see *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amantadine	1.0	—
Amantadine related compound A[a]	1.2	—
Amantadine related compound B[a]	1.9	—
Any individual unspecified impurity	—	0.2
Total impurities	—	2.0

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

ADDITIONAL REQUIREMENTS

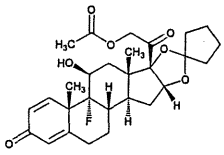
• PACKAGING AND STORAGE: Preserve in tight containers. Store at controlled room temperature.

USP Monographs

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amantadine Hydrochloride RS
USP Amantadine Related Compound A RS
1-Chloroadamantane.
$C_{10}H_{15}Cl$ 170.68
USP Amantadine Related Compound B RS
N-(Adamantan-1-yl)acetamide.
$C_{12}H_{19}NO$ 193.29

Amcinonide



$C_{28}H_{35}FO_7$ 502.57
Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-16,17-[cyclopentylidenebis(oxy)]-9-fluoro-11-hydroxy-, (11β,16α)-;
9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with cyclopentanone, 21-acetate [51022-69-6].

DEFINITION
Amcinonide contains NLT 97.0% and NMT 102.0% of amcinonide ($C_{28}H_{35}FO_7$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

• **PROCEDURE**
Solution A: Acetonitrile and water▲(35:65)▲ (USP 1-Aug-2019)
Solution B: Acetonitrile and water▲(70:30)▲ (USP 1-Aug-2019)
Mobile phase: See *Table 1*. Equilibrate the system with *Solution A*.

▲**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2.5	100	0
10	0	100
25	0	100
26	100	0
32	100	0▲ (USP 1-Aug-2019)

System suitability solution: 12.5 µg/mL of ▲USP Butylparaben RS▲ (USP 1-Aug-2019) and 20 µg/mL of USP Amcinonide RS in *Solution B*
Standard solution: 0.02 mg/mL of USP Amcinonide RS in *Solution B*
Sample solution: 0.02 mg/mL of Amcinonide in *Solution B*. Sonicate for 5 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; ▲5-µm▲ (USP 1-Aug-2019) packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for butylparaben and amcinonide are 0.78 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 8.0 between butylparaben and amcinonide, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of amcinonide ($C_{28}H_{35}FO_7$) in the portion of Amcinonide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amcinonide RS in the *Standard solution* (mg/mL)
C_U = concentration of Amcinonide in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

SPECIFIC TESTS
• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL of Amcinonide in chloroform
Acceptance criteria: +89.4° to +94.0°
• **LOSS ON DRYING** ⟨731⟩
Sample: Amcinonide
Analysis: Dry the *Sample* at 105° for 4 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amcinonide RS
▲USP Butylparaben RS▲ (USP 1-Aug-2019)

Amcinonide Cream

DEFINITION
Amcinonide Cream is Amcinonide in a suitable cream base. It contains NLT 90.0% and NMT 115.0% of the labeled amount of amcinonide ($C_{28}H_{35}FO_7$).

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A.** ▲The retention time of the amcinonide peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

• **PROCEDURE**
Solution A: Acetonitrile and water▲(35:65)▲ (USP 1-Dec-2019)
Solution B: Acetonitrile and water▲(70:30)▲ (USP 1-Dec-2019)
Mobile phase: See *Table 1*. Equilibrate the system with *Solution A*.

▲Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2.5	100	0
10	0	100
25	0	100
26	100	0
32	100	0▲ (USP 1-Dec-2019)

System suitability solution: 12.5 µg/mL of ▲USP Butylparaben RS▲ (USP 1-Dec-2019) and 20 µg/mL of USP Amcinonide RS in *Solution B*
Standard solution: 0.02 mg/mL of USP Amcinonide RS in *Solution B*
Sample stock solution: Nominally 0.2 mg/mL of amcinonide from Cream prepared as follows. Transfer a quantity of Cream, equivalent to 10 mg of amcinonide, to a 50-mL volumetric flask. Add 5 mL of *Solution B* and 15 mL of acetonitrile, and heat over a steam bath until dissolved. Add 20 mL of *Solution B* while hot, cool to room temperature, dilute with *Solution B* to volume, and refrigerate for 30 min. Vigorously shake the solution to disperse the mixture, and filter while cold. Use the filtrate.
Sample solution: Nominally 0.02 mg/mL of amcinonide from the ▲*Sample stock solution*▲ (USP 1-Dec-2019) in *Solution B*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; ▲5-µm▲ (USP 1-Dec-2019) packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for butylparaben and amcinonide are 0.78 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 8.0 between butylparaben and amcinonide, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amcinonide ($C_{28}H_{35}FO_7$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amcinonide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amcinonide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS

Change to read:

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** ▲The total aerobic microbial count is NMT 10^2 cfu/g and the total yeast and mold count is NMT 10^1 cfu/g.▲ (USP 1-Dec-2019) It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
• **pH** ⟨791⟩**:** 3.5–5.2

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight containers.▲Store at controlled room temperature.▲ (USP 1-Dec-2019)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amcinonide RS
▲USP Butylparaben RS▲ (USP 1-Dec-2019)

Amcinonide Ointment

DEFINITION
Amcinonide Ointment is Amcinonide in a suitable ointment base. It contains NLT 90.0% and NMT 115.0% of the labeled amount of amcinonide ($C_{28}H_{35}FO_7$).

IDENTIFICATION

Change to read:

• **A.** ▲The retention time of the amcinonide peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

• **PROCEDURE**
Solution A: Acetonitrile and water▲(35:65)▲ (USP 1-Aug-2019)
Solution B: Acetonitrile and water▲(70:30)▲ (USP 1-Aug-2019)
Mobile phase: See *Table 1*. Equilibrate the system with *Solution A*.

USP Monographs

www.webofpharma.com

▲Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2.5	100	0
10	0	100
25	0	100
26	100	0
32	100	0▲ (USP 1-Aug-2019)

Diluent: Acetonitrile and chloroform (80:20)
System suitability solution: 12.5 µg/mL of ▲USP Butylparaben RS▲ (USP 1-Aug-2019) and 20 µg/mL of USP Amcinonide RS in *Solution B*
Standard solution: 0.02 mg/mL of USP Amcinonide RS in *Solution B*
Sample stock solution: Nominally 0.2 mg/mL of amcinonide prepared as follows. Dissolve a suitable amount of Ointment in a suitable volume of *Diluent* ▲in a volumetric flask▲ (USP 1-Aug-2019) by heating in a hot water bath, cooling, and diluting with *Diluent* to volume. Cool to room temperature, dilute with acetonitrile to volume, and filter.
Sample solution: Nominally 0.02 mg/mL of amcinonide in *Solution B* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; ▲5-µm▲ (USP 1-Aug-2019) packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for butylparaben and amcinonide are 0.78 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 8.0 between butylparaben and amcinonide, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amcinonide ($C_{28}H_{35}FO_7$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amcinonide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amcinonide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS

Change to read:

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** ▲The total aerobic microbial count is NMT 10^2 cfu/g and the total yeasts and molds count is NMT 10^1 cfu/g.▲ (USP 1-Aug-2019) Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

ADDITIONAL REQUIREMENTS

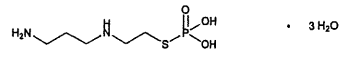
Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight containers. ▲Store at controlled room temperature.▲ (USP 1-Aug-2019)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amcinonide RS
▲USP Butylparaben RS▲ (USP 1-Aug-2019)

Amifostine



$C_5H_{15}N_2O_3PS \cdot 3H_2O$ 268.27
Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester), trihydrate;
S-[2-(3-Aminopropyl)amino]ethyl]dihydrogen phosphorothioate, trihydrate [112901-68-5].

DEFINITION
Amifostine contains NLT 78.0% and NMT 82.0% of $C_5H_{15}N_2O_3PS$, calculated on the as-is basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 0.94 g/L of sodium 1-hexanesulfonate. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Methanol and *Buffer* (7:18)
Standard solution: 3 mg/mL of USP Amifostine RS in water. [NOTE—Inject immediately after preparation.]
Sample solution: 3 mg/mL of Amifostine in water. [NOTE—Inject immediately after preparation.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Autosampler temperature: 4°
Flow rate: 1.0 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*

www.webofpharma.com

Suitability requirements
Tailing factor: NMT 2.0
Column efficiency: NLT 1000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_5H_{15}N_2O_3PS$ in the portion of Amifostine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amifostine RS in the *Standard solution* (mg/mL)
C_U = concentration of Amifostine in the *Sample solution* (mg/mL)

Acceptance criteria: 78.0%–82.0% on the as-is basis

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 70 µg/mL of USP Amifostine Thiol RS and 16 µg/mL of USP Amifostine RS in water. [NOTE—Inject immediately after preparation.]
System suitability solution: Use the *Standard solution* as described in the *Assay*. [NOTE—Inject immediately after preparation.]
Sample solution: 15 mg/mL of Amifostine in water. [NOTE—Inject immediately after preparation.]
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates, *System suitability solution*
Tailing factor: NMT 2.0, *System suitability solution*
Relative standard deviation: NMT 15.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of amifostine thiol in the portion of Amifostine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amifostine thiol from the *Sample solution*
r_S = peak response of amifostine thiol from the *Standard solution*
C_S = concentration of USP Amifostine Thiol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amifostine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of amifostine thiol, 134.24
M_{r2} = molecular weight of amifostine thiol dihydrochloride, 207.17

Calculate the percentage of any other individual impurity in the portion of Amifostine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity in the *Sample solution*
r_S = peak response of amifostine in the *Standard solution*
C_S = concentration of USP Amifostine RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria
Amifostine thiol: NMT 0.3%
Any individual impurity, excluding amifostine thiol: NMT 0.1%
Total impurities including amifostine thiol: NMT 0.3%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 6.5–7.5, in a solution (5 in 100)
• **WATER DETERMINATION,** *Method Ic* ⟨921⟩
Sample solution: To 100.0 mg of Amifostine, contained in a stoppered centrifuge tube, add 10.0 mL of the solution of *N*-ethylmaleimide in methanol (4 in 100), and sonicate for 15 min. Shake to disperse, and sonicate for an additional 15 min. Use 1.0 mL of the supernatant.
Acceptance criteria: 19.2%–21.2%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a refrigerator.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amifostine RS
USP Amifostine Thiol RS
Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrochloride.
$C_5H_{16}N_2SCl_2$ 207.17

Amifostine for Injection

DEFINITION

Amifostine for Injection is a sterile, crystalline substance suitable for parenteral use. It contains NLT 90.0% and NMT 110.0% of the labeled amount of amifostine ($C_5H_{15}N_2O_3PS$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer: 0.94 g/L of sodium 1-hexanesulfonate. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Methanol and *Buffer* (7:18)
Standard solution: 3 mg/mL of USP Amifostine RS in water. [NOTE—Inject immediately after preparation, or refrigerate until use.]
Sample solution: 3 mg/mL of amifostine from Amifostine for Injection, in water. [NOTE—Inject immediately after preparation, or refrigerate until use.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Autosampler temperature: 4°
Flow rate: 1.0 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*

www.webofpharma.com

Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_5H_{15}N_2O_3PS$ in the portion of Amifostine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak responses from the *Sample solution*
r_S = peak responses from the *Standard solution*
C_S = concentration of USP Amifostine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amifostine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements

IMPURITIES

Organic Impurities

• **Procedure 1**
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution 1: 70 µg/mL of USP Amifostine Thiol RS in water
Standard solution 2: 15 µg/mL of sodium thiophosphate and 13 µg/mL of *N,N*-dimethylformamide in water. [Note—The retention times of sodium thiophosphate and *N,N*-dimethylformamide are about 2 min and about 3.6 min, respectively.]
Sample solution: 2.4 mg/mL of amifostine from Amifostine for Injection in water. [Note—Inject immediately after preparation.]
System suitability
Samples: *Standard solution 1* and *Standard solution 2*
Suitability requirements
Relative standard deviation: NMT 10.0%, *Standard solution 1*; NMT 4.0%, *Standard solution 2*
Analysis
Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*
Calculate the percentage of amifostine thiol in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amifostine thiol from the *Sample solution*
r_S = peak response of amifostine thiol from *Standard solution 1*
C_S = concentration of USP Amifostine Thiol RS in *Standard solution 1* (mg/mL)
C_U = concentration of amifostine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of amifostine thiol, 134.24
M_{r2} = molecular weight of amifostine thiol dihydrochloride, 207.17

Calculate the percentage of sodium thiophosphate or *N,N*-dimethylformamide in the portion of sample taken, if present:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sodium thiophosphate or *N,N*-dimethylformamide from the *Sample solution*
r_S = peak response of sodium thiophosphate or *N,N*-dimethylformamide from *Standard solution 2*
C_S = concentration of sodium thiophosphate or *N,N*-dimethylformamide in *Standard solution 2* (mg/mL)
C_U = concentration of amifostine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual, unspecified impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity in the *Sample solution*
r_T = total of all peak responses in the *Sample solution*

Acceptance criteria: NMT 0.1% of sodium thiophosphate; NMT 0.088% of *N,N*-dimethylformamide; NMT 0.1% of any other individual unspecified impurity

• **Procedure 2**
Buffer: 0.4 g/L of sodium 1-octanesulfonate. Adjust with trifluoroacetic acid to a pH of 2.5 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (1:3)
Standard solution: 46 µg/mL of USP Amifostine Disulfide RS in water
Sample solution: Dilute a quantity of Amifostine for Injection in water to prepare a solution equivalent to 10 mg/mL. [Note—Inject immediately after preparation.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 247 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Autosampler temperature: 4°
Flow rate: 1.0 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 4.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of amifostine disulfide in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amifostine disulfide from the *Sample solution*
r_S = peak response of amifostine disulfide from the *Standard solution*
C_S = concentration of USP Amifostine Disulfide RS in the *Standard solution* (mg/mL)
C_U = concentration of amifostine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of amifostine disulfide, 266.47
M_{r2} = molecular weight of amifostine disulfide tetrahydrochloride, 412.31

Acceptance criteria: NMT 2.0% of total impurities, including amifostine thiol and amifostine disulfide

USP Monographs

www.webofpharma.com

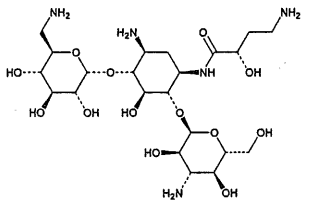
SPECIFIC TESTS

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*. When constituted with 0.9% *Sodium Chloride Injection*, the solution must completely dissolve in 45 s.
- **X-RAY DIFFRACTION** ⟨941⟩: Its X-ray diffraction pattern conforms to that of USP Amifostine RS, similarly determined.
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined, Membrane Filtration*.
- **pH** ⟨791⟩: 6.5–7.5, in a solution constituted as directed in the labeling
- **WATER DETERMINATION,** *Method Ic* ⟨921⟩
  Sample solution: To 100.0 mg of Amifostine for Injection, contained in a stoppered centrifuge tube, add 10.0 mL of a solution of *N*-ethylmaleimide in methanol (4 in 100), and sonicate for 15 min. Shake to disperse, and sonicate for an additional 15 min. Use 1.0 mL of the supernatant.
  Acceptance criteria: 18.0%–22.0%
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Contains NMT 0.2 USP Endotoxin Unit/mg of amifostine
- **OTHER REQUIREMENTS:** Meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amifostine RS
  USP Amifostine Disulfide RS
  1,3-Propanediamine, *N,N*-(dithiodi-2,1-ethanediyl)bis, tetrahydrochloride.
  $C_{10}H_{30}N_4S_2Cl_4$ 412.32
  USP Amifostine Thiol RS
  Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrochloride.
  $C_5H_{16}N_2SCl_2$ 207.17

Amikacin



$C_{22}H_{43}N_5O_{13}$ 585.60

D-Streptamine, *O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→4)]-N^1-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-, (*S*)-;
O-3-Amino-3-deoxy-α-D-glucopyranosyl(1→4)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl(1→6)]-N^3-(4-amino-L-2-hydroxybutyryl)-2-deoxy-L-streptamine [37517-28-5].

DEFINITION

Amikacin has a potency of NLT 900 µg/mg of amikacin ($C_{22}H_{43}N_5O_{13}$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197K or 197A▲ (CN 1-May-2020)
- **B.** The retention time of the peak for amikacin of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: 134 mM sodium hydroxide, prepared as follows. Transfer a volume of deionized water to a suitable plastic container, sonicate, degas, and sparge with helium. While stirring, slowly add sodium hydroxide solution of a suitable concentration.
[NOTE—Prepare fresh daily. The *Mobile phase* readily absorbs carbon dioxide and produces carbonate that changes the retention time of amikacin. The use of a 50% (w/w), low-carbonate sodium hydroxide solution is recommended.]
System suitability solution: 0.02 mg/mL of USP Amikacin RS and 0.008 mg/mL of USP Kanamycin Sulfate RS in water
Standard solution: 0.02 mg/mL of USP Amikacin RS in water
Sample solution: 0.02 mg/mL of Amikacin in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Electrochemical
Mode: Integrated amperometric
Electrodes
Working: Gold
Reference: Silver–silver chloride
Detector settings: See *Table 1*.

Table 1

Step	Time (s)	Potential (V)	Integration
1	0.00	+0.04	—
2	0.30	+0.04	Begins
3	0.50	+0.04	Ends
4	0.51	+0.80	—
5	0.70	+0.80	—
6	0.71	−0.80	—
7	0.90	−0.80	—

Column: 4-mm × 25-cm; 7.5-µm packing L47
[NOTE—A guard column of packing L47 is recommended.]
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for kanamycin and amikacin are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3 between kanamycin and amikacin, *System suitability solution*
Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 3%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the quantity, in µg, of amikacin ($C_{22}H_{43}N_5O_{13}$) in each mg of Amikacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

r_U = peak response of amikacin from the *Sample solution*
r_S = peak response of amikacin from the *Standard solution*
C_S = concentration of USP Amikacin RS in the *Standard solution* (mg/mL)
C_U = concentration of Amikacin in the *Sample solution* (mg/mL)
P = potency of amikacin in USP Amikacin RS (mg/mg)
F = conversion factor, 1000 µg/mg

Acceptance criteria: NLT 900 µg/mg on the anhydrous basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

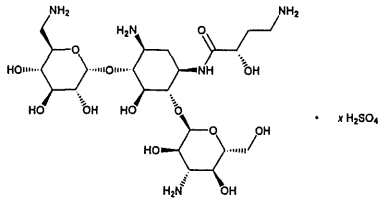
SPECIFIC TESTS

- **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 20 mg/mL in water
  Acceptance criteria: +97° to +105°
- **Crystallinity** ⟨695⟩: Meets the requirements
- **pH** ⟨791⟩
  Sample solution: 10 mg/mL in water
  Acceptance criteria: 9.5–11.5
- **Water Determination** ⟨921⟩, *Method I*: NMT 8.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** ⟨11⟩
  USP Amikacin RS
  USP Kanamycin Sulfate RS

Amikacin Sulfate



$C_{22}H_{43}N_5O_{13} \cdot xH_2SO_4$
D-Streptamine, *O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→4)]-N^1-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-, (*S*)-, sulfate (1:2 or 1:1.8 salt);
O-3-Amino-3-deoxy-α-D-glucopyranosyl-(1→4)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→6)]-N^3-(4-amino-L-2-hydroxybutyryl)-2-deoxy-D-streptamine sulfate (1:2 or 1:1.8 salt);
(2*S*)-4-Amino-*N*-{(1*R*,2*S*,3*S*,4*R*,5*S*)-5-amino-2-[(3-amino-3-deoxy-α-D-glucopyranosyl)oxy]-4-[(6-amino-6-deoxy-α-D-glucopyranosyl)oxy]-3-hydroxycyclohexyl}-2-hydroxybutanamide sulfate (1:2 or 1:1.8 salt).

$C_{22}H_{43}N_5O_{13} \cdot 1.8H_2SO_4$ 762.15
[149022-22-0].

$C_{22}H_{43}N_5O_{13} \cdot 2H_2SO_4$ 781.76
[39831-55-5].

DEFINITION

Amikacin Sulfate having a molar ratio of amikacin to sulfuric acid (H_2SO_4) of 1:2 contains the equivalent of NLT 674 µg/mg and NMT 786 µg/mg of amikacin ($C_{22}H_{43}N_5O_{13}$), calculated on the dried basis. Amikacin Sulfate having a molar ratio of amikacin to sulfuric acid (H_2SO_4) of 1:1.8 contains the equivalent of NLT 691 µg/mg and NMT 806 µg/mg of amikacin ($C_{22}H_{43}N_5O_{13}$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197K or 197A▲ (CN 1-May-2020)
  Acceptance criteria: The IR absorption spectrum conforms to that of USP Amikacin Sulfate RS, similarly obtained.
- **B.** The retention time of the peak for amikacin of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General** ⟨191⟩, *Chemical Identification Tests, Sulfate*: Meets the requirements

ASSAY

- **Procedure**
  Mobile phase: 134 mM sodium hydroxide, prepared as follows. Transfer a volume of deionized water to a suitable plastic container, sonicate, degas, and sparge with helium. While stirring, slowly add sodium hydroxide solution of a suitable concentration.
  [Note—Prepare fresh daily. The *Mobile phase* readily absorbs carbon dioxide and produces carbonate that changes the retention time of amikacin. The use of a 50% (w/w), low-carbonate sodium hydroxide solution is recommended.]
  System suitability solution: 0.02 mg/mL of USP Amikacin RS and 0.008 mg/mL of USP Kanamycin Sulfate RS in water
  Standard solution: 0.02 mg/mL of USP Amikacin RS in water
  Sample solution: Equivalent to 0.02 mg/mL of amikacin, from Amikacin Sulfate, in water
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: Electrochemical
  Mode: Integrated amperometric
  Electrodes
  Working: Gold
  Reference: Silver–silver chloride
  Detector settings: See *Table 1*.

Table 1

Step	Time (s)	Potential (V)	Integration
1	0.00	+0.04	—
2	0.30	+0.04	Begins
3	0.50	+0.04	Ends
4	0.51	+0.80	—
5	0.70	+0.80	—
6	0.71	−0.80	—
7	0.90	−0.80	—

Column: 4-mm × 25-cm; 7.5-µm packing L47

USP Monographs

www.webofpharma.com

[NOTE—A guard column of packing L47 is recommended.]
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for kanamycin and amikacin are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3 between kanamycin and amikacin, *System suitability solution*
Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 3%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg, of amikacin ($C_{22}H_{43}N_5O_{13}$) in each mg of Amikacin Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

r_U = peak response of amikacin from the *Sample solution*
r_S = peak response of amikacin from the *Standard solution*
C_S = concentration of USP Amikacin RS in the *Standard solution* (mg/mL)
C_U = concentration of amikacin in the *Sample solution* (mg/mL)
P = potency of amikacin in USP Amikacin RS (mg/mg)
F = conversion factor, 1000 µg/mg

Acceptance criteria: See *Table 2*.

Table 2

Ratio of Amikacin:Sulfuric Acid	Acceptance Criteria (µg/mg)[a]
1:2	674–786
1:1.8	691–806

[a] Calculated on the dried basis.

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

SPECIFIC TESTS
• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 20 mg/mL in water
Acceptance criteria: +76° to +84°
• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
• **PH** ⟨791⟩
Sample solution: 10 mg/mL in water
Acceptance criteria: See *Table 3*.

Table 3

Ratio of Amikacin:Sulfuric Acid	Acceptance Criteria
1:2	2.0–4.0
1:1.8	6.0–7.3

• **LOSS ON DRYING** ⟨731⟩
Sample: 100 mg
Analysis: Dry the *Sample* under vacuum at a pressure not exceeding 5 mm of mercury at 110° for 3 h.
Acceptance criteria: NMT 13.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** Label it to indicate whether its molar ratio of amikacin to sulfuric acid (H_2SO_4) is 1:2 or 1:1.8.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amikacin RS
USP Amikacin Sulfate RS
USP Kanamycin Sulfate RS

Amikacin Sulfate Injection

DEFINITION
Amikacin Sulfate Injection is a sterile solution of Amikacin Sulfate in Water for Injection, or of Amikacin in Water for Injection prepared with the aid of Sulfuric Acid. It contains NLT 90.0% and NMT 120.0% of the labeled amount of amikacin ($C_{22}H_{43}N_5O_{13}$).

IDENTIFICATION
• **A.** The retention time of the peak for amikacin of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**
Mobile phase: ▲134 mM sodium hydroxide, prepared as follows. Transfer a volume of deionized water to a suitable plastic container, sonicate, degas, and sparge with helium. While stirring, slowly add sodium hydroxide solution of a suitable concentration.
[NOTE—Prepare fresh daily. The *Mobile phase* readily absorbs carbon dioxide and produces carbonate that changes the retention time of amikacin. The use of a 50% (w/w), low-carbonate sodium hydroxide solution is recommended.]
▲ (USP41)
System suitability solution: 0.02 mg/mL of USP Amikacin RS and 0.008 mg/mL of USP Kanamycin Sulfate RS in water
Standard solution: 0.02 mg/mL of USP Amikacin RS in water
Sample solution: Nominally 0.02 mg/mL of amikacin, from Injection in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Electrochemical
Detector mode: Integrated amperometric
Electrodes
Working: Gold
Reference: Silver–silver chloride
Detector settings: See *Table 1*.

▲**Table 1**

Step	Time (s)	Potential (V)	Integration
1	0.00	+0.04	—
2	0.30	+0.04	Begins
3	0.50	+0.04	Ends
4	0.51	+0.80	—

www.webofpharma.com

▲Table 1 *(continued)*

Step	Time (s)	Potential (V)	Integration
5	0.70	+0.80	—
6	0.71	−0.80	—
7	0.90	−0.80	—

Column: 4-mm × 25-cm; 7.5-µm packing L47
[NOTE—A guard column of packing L47 is recommended.]
▲(USP41)
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for kanamycin and amikacin are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3 between kanamycin and amikacin, *System suitability solution*
Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 3%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲the labeled amount of▲ (USP41) amikacin ($C_{22}H_{43}N_5O_{13}$) in ▲the portion▲ (USP41) of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak ▲response of amikacin▲ (USP41) from the *Sample solution*
r_S = peak ▲response of amikacin▲ (USP41) from the *Standard solution*
C_S = concentration of USP Amikacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amikacin in the *Sample solution*▲(mg/mL)▲ (ERR 1-Sep-2018)
P = potency of amikacin in USP Amikacin RS (mg/mg)

Acceptance criteria: 90.0%–120.0%

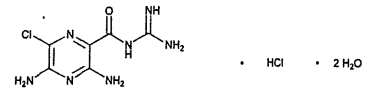
SPECIFIC TESTS
- **pH** ⟨791⟩: 3.5–5.5
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.33 USP Endotoxin Units/mg of amikacin
- **OTHER REQUIREMENTS:** Meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type III glass.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
USP Amikacin RS
▲▲ (CN 1-May-2018)
USP Kanamycin Sulfate RS

Amiloride Hydrochloride



$C_6H_8ClN_7O \cdot HCl \cdot 2H_2O$ 302.12
Pyrazinecarboxamide, 3,5-diamino-*N*-(aminoiminomethyl)-6-chloro-, monohydrochloride dihydrate;
N-Amidino-3,5-diamino-6-chloropyrazinecarboxamide monohydrochloride dihydrate [17440-83-4].

DEFINITION
Amiloride Hydrochloride contains NLT 98.0% and NMT 101.0% of $C_6H_8ClN_7O \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample: 600 µg/mL of water, diluted quantitatively and stepwise with 0.1 N hydrochloric acid to a concentration of about 9.6 µg/mL
Acceptance criteria: Meets the requirements
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY
- **PROCEDURE**
Sample: 450 mg
Analysis: Dissolve the *Sample* in 100 mL of glacial acetic acid, add 10 mL of mercuric acetate TS and 15 mL of dioxane. Add crystal violet TS. Titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 26.61 mg of $C_6H_8ClN_7O \cdot HCl$.
Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
Standard solutions: Prepare a series of solutions, *A*, *B*, *C*, *D*, *E*, and *F*, of USP Amiloride Hydrochloride RS in a mixture of methanol and chloroform (4:1) having concentrations of 4000, 40, 20, 8, 4, and 2 µg/mL, respectively.
Sample solution: 4 mg/mL of Amiloride Hydrochloride in methanol and chloroform (4:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel previously washed with methanol
Application volume: 5 µL
Developing solvent system: Tetrahydrofuran and 3 N ammonium hydroxide (15:2)
Analysis
Samples: *Standard solutions A, B, C, D, E,* and *F* and *Sample solution*
Proceed as directed in the chapter. Dry the spots with a stream of nitrogen, and develop the chromatograms in the solvent system, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the

www.webofpharma.com

solvent front, allow to air-dry, and examine the plate under long-wavelength UV light: the R_F value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*. Estimate the levels of any additional spots observed in the chromatogram of the *Sample solution* by comparison with the principal spots in the chromatograms of *Standard solutions B, C, D, E,* and *F*.

Acceptance criteria: The sum of the intensities of any additional spots observed is NMT that of the principal spot obtained from *Standard solution B* (equivalent to 1%).

SPECIFIC TESTS

• **ACIDITY**

Sample: 1.0 g

Analysis: Dissolve the *Sample* in 100 mL of a mixture of methanol and water (1:1). Titrate with 0.10 N sodium hydroxide to a potentiometric endpoint.

Acceptance criteria: NMT 0.30 mL is required (0.1% as HCl).

• **LOSS ON DRYING**

(See *Thermal Analysis* ⟨891⟩.)

[NOTE—The quantity taken for the determination may be adjusted, if necessary, for instrument sensitivity.]

Sample: 10 mg

Analysis: Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument using the *Sample*. Heat the specimen at the rate of 10°/min between ambient temperature and 225° in an atmosphere of nitrogen at a flow rate of 40 mL/min. From the thermogram determine the accumulated loss in weight between ambient temperature and about 200° on the plateau.

Acceptance criteria: 11.0%–13.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Amiloride Hydrochloride RS

Amiloride Hydrochloride Tablets

DEFINITION

Amiloride Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.**

Standard solution: 0.2 mg/mL of USP Amiloride Hydrochloride RS in methanol

Sample solution: Prepare from finely ground Tablets, equivalent to 0.2 mg/mL of amiloride hydrochloride in methanol. Filter the solution.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Developing solvent system: Tetrahydrofuran and 3 N ammonium hydroxide (88:12)

Application volume: 10 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the plate until the solvent is about three-fourths of the length of the plate from the origin. Remove the plate from the developing chamber, air-dry, and examine under short-wavelength UV light.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 136 g of monobasic potassium phosphate in 800 mL of water. Adjust with phosphoric acid to a pH of 3.0. Dilute with water to 1000 mL.

Mobile phase: Methanol, water, and *Buffer* (25:71:4)

Standard stock solution: 1.0 mg/mL of USP Amiloride Hydrochloride RS in methanol

Standard solution: 0.1 mg/mL of USP Amiloride Hydrochloride RS from *Standard stock solution*, prepared as follows. Transfer 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask. Add 10.0 mL of methanol, 2.0 mL of 0.1 N hydrochloric acid, and dilute with water to volume.

Sample solution: Transfer an amount equivalent to 5 mg of amiloride hydrochloride, from NLT 20 finely powdered Tablets, to a 50-mL volumetric flask containing 15.0 mL of methanol and 2.0 mL of 0.1 N hydrochloric acid. Sonicate for 10 min, dilute with water to volume, sonicate for an additional 10 min, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 286 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of amiloride from the *Sample solution*

r_S = peak response of amiloride from the *Standard solution*

C_S = concentration of USP Amiloride Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amiloride hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Instrumental conditions

Mode: UV

Analytical wavelength: 363 nm

Standard solution: USP Amiloride Hydrochloride RS of known concentration in *Medium*. [NOTE—An amount of methanol not to exceed 2% of the total volume of the

USP Monographs

www.webofpharma.com

Standard solution may be used to dissolve the amiloride hydrochloride.]

Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* as necessary.

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 80% (*Q*) of the labeled amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Standard solution: 10 µg/mL of USP Amiloride Hydrochloride RS in 0.1 N hydrochloric acid

Sample solution: 10 µg/mL of amiloride hydrochloride prepared as follows. Transfer one finely powdered Tablet to a 100-mL volumetric flask, add 60 mL of 0.1 N hydrochloric acid, and shake by mechanical means for 30 min. Dilute with 0.1 N hydrochloric acid to volume, and centrifuge a portion of the mixture. Dilute a portion of the clear supernatant to obtain the required concentration.

Instrumental conditions

Mode: UV

Analytical wavelength: 363 nm

Blank: 0.1 N hydrochloric acid

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of amiloride hydrochloride in the *Sample solution*

A_S = absorbance of amiloride hydrochloride in the *Standard solution*

C_S = concentration of USP Amiloride Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of amiloride hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Diluent: Methanol, 1 N hydrochloric acid, and water (40:4:56)

Buffer: 0.9 g/L of sodium 1-hexanesulfonate in water. Initially add water to about 90% of the volume of the flask, adjust with diluted phosphoric acid to a pH of 3.0 ± 0.1, and dilute with water to volume.

Mobile phase: Acetonitrile and *Buffer* (10:90)

Standard solution: 0.01 mg/mL of USP Amiloride Hydrochloride RS in *Diluent*

Sample solution: Nominally equivalent to 2 mg/mL of amiloride hydrochloride in *Diluent* from NLT 20 powdered Tablets. Initially add methanol to fill about 40% of the volume of the flask and 1 N hydrochloric acid to about 4% of the volume of the flask. Sonicate for 10 min, dilute with water to volume, and sonicate for another 10 min. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 350 nm

Column: 4.6-mm × 15-cm; 4-µm packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of amiloride from the *Standard solution*

C_S = concentration of USP Amiloride Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amiloride hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chloropyrazine acid[a, e]	0.15	—
Chloropyrazine methyl carboxylate[b, e]	0.48	—
Chloropyrazine carboxamide[c, e]	0.56	—
Amiloride	1.00	—
Any other unknown impurity	—	0.5
Total impurities[d]	—	2.0

[a] 3,5,-Diamin-6-chloropyrazine carboxylic acid.
[b] 3,5,-Diamin-6-chloropyrazine-2-carboxylate.
[c] *N*-Ammidine-3-amino-5-hydroxy-6-chloropyrazine-2-carboxamide hydrochloride.
[d] Total impurities is the sum of all the impurities including process-related. Disregard peaks less than 0.05%.
[e] Process-related impurity, controlled in the drug substance.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Amiloride Hydrochloride RS

Amiloride Hydrochloride and Hydrochlorothiazide Tablets

DEFINITION

Amiloride Hydrochloride and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲The UV spectrum of the amiloride peak of the *Sample solution* exhibits maxima and minima at the same

www.webofpharma.com

wavelengths as that of the *Standard solution*, as obtained in the *Assay*. The UV spectrum of the hydrochlorothiazide peak of the *Diluted sample solution* exhibits maxima and minima at the same wavelengths as that of the *Diluted standard solution*, as obtained in the *Assay*. ▲(USP 1-Aug-2019)

ASSAY

Change to read:

• **Procedure**

Buffer: Dissolve 136 g of monobasic potassium phosphate in 800 mL of water. Adjust with phosphoric acid to a pH of 3.0. Dilute with water to 1000 mL.

Mobile phase: Methanol, water, and *Buffer* (25:71:4)

Standard stock solution: 1.0 mg/mL of USP Amiloride Hydrochloride RS in methanol

Standard solution: 0.1 mg/mL of USP Amiloride Hydrochloride RS and 1 mg/mL of USP Hydrochlorothiazide RS, prepared as follows. Transfer 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask containing 100 mg of USP Hydrochlorothiazide RS and 20.0 mL of methanol. Add 4.0 mL of 1 N hydrochloric acid, and dilute with water to volume.

▲**Diluted standard solution:** 0.005 mg/mL of USP Amiloride Hydrochloride RS and 0.05 mg/mL of USP Hydrochlorothiazide RS, from the *Standard solution*, diluted with water▲(USP 1-Aug-2019)

Sample solution: ▲Nominally 0.1 mg/mL of amiloride hydrochloride and 1 mg/mL of hydrochlorothiazide, prepared as follows.▲(USP 1-Aug-2019) Transfer an equivalent to 5 mg of amiloride hydrochloride ▲and 50 mg of hydrochlorothiazide▲(USP 1-Aug-2019) from powdered Tablets (NLT 20) to a 50-mL volumetric flask. Add 15.0 mL of methanol and 2.0 mL of 1 N hydrochloric acid. Sonicate for 10 min, dilute with water to volume, sonicate for an additional 10 min, and filter.

▲**Diluted sample solution:** Nominally 0.005 mg/mL of amiloride hydrochloride and 0.05 mg/mL of hydrochlorothiazide, from the *Sample solution*, diluted with water▲(USP 1-Aug-2019)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 286 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲(USP 1-Aug-2019)

Column: 3.9-mm × 30-cm; ▲10-µm▲(USP 1-Aug-2019) packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

▲**Run time:** NLT 2 times the retention time of amiloride▲(USP 1-Aug-2019)

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for hydrochlorothiazide and amiloride ▲▲(USP 1-Aug-2019) are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between hydrochlorothiazide and amiloride▲▲(USP 1-Aug-2019)

Relative standard deviation: NMT 2.0% for hydrochlorothiazide and amiloride

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of amiloride ▲▲(USP 1-Aug-2019) from the *Sample solution*

r_S = peak response of amiloride ▲▲(USP 1-Aug-2019) from the *Standard solution*

C_S = concentration of USP Amiloride Hydrochloride RS▲▲(USP 1-Aug-2019) in the *Standard solution* (mg/mL)

C_U = nominal concentration of amiloride hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydrochlorothiazide from the *Sample solution*

r_S = peak response of hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$)

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

▲Determine the percentage of the labeled amount of amiloride hydrochloride and hydrochlorothiazide dissolved using the *Spectrophotometric procedure* or the *Chromatographic procedure*.

Spectrophotometric procedure▲(USP 1-Aug-2019)

Amiloride standard solution: ▲0.005 mg/mL of USP Amiloride Hydrochloride RS, prepared as follows. Transfer 52 mg of USP Amiloride Hydrochloride RS to▲(USP 1-Aug-2019) a 200-mL volumetric flask. Dissolve in and dilute with methanol to volume. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume.

Hydrochlorothiazide standard solution: 0.01 mg/mL of USP Hydrochlorothiazide RS, prepared as follows. Transfer 100 mg of USP Hydrochlorothiazide RS to a 100-mL volumetric flask. Dissolve in and dilute with methanol to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, and dilute with *Medium* to volume.

Sample solution A: Pass a portion of the solution under test through a glass fiber filter of 0.45-µm pore size.

Sample solution B: Transfer 5.0 mL of *Sample solution A* to a 25-mL volumetric flask, and dilute with *Medium* to volume.

Blank: *Medium*

Instrumental conditions

Mode: UV-Vis

Analytical wavelengths: 363 nm for amiloride hydrochloride; 270 nm for hydrochlorothiazide

USP Monographs

www.webofpharma.com

Analysis
Samples: *Amiloride standard solution, Hydrochlorothiazide standard solution, Sample solution A,* and *Sample solution B*
Calculate the percentage ▲of the labeled amount▲ (USP 1-Aug-2019) of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) dissolved:

$$\text{Result} = [(A_U \times C_S \times V)/(A_S \times L)] \times 100$$

A_U = absorbance of *Sample solution A*
C_S = concentration of ▲USP Amiloride Hydrochloride RS in▲ (USP 1-Aug-2019) the *Amiloride standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
A_S = absorbance of the *Amiloride standard solution*
L = label claim of amiloride ▲hydrochloride▲ (USP 1-Aug-2019) (mg/Tablet)

▲Calculate *F*, the ratio of absorbance of the *Amiloride standard solution* at 270 nm to that at 363 nm:

$$\text{Result} = A_{S270}/A_{S363}$$

A_{S270} = absorbance of the *Amiloride standard solution* at 270 nm
A_{S363} = absorbance of the *Amiloride standard solution* at 363 nm

Calculate A_{UC}, the absorbance of *Sample solution B* at 270 nm, corrected for the interference of amiloride:

$$\text{Result} = A_{U270} - [(A_{U363} \times F)/D]$$

A_{U270} = absorbance of *Sample solution B* at 270 nm
A_{U363} = absorbance of *Sample solution A* at 363 nm
F = ratio of the absorbance of the *Amiloride standard solution* at 270 nm to that at 363 nm
D = dilution factor of *Sample solution B*, 5▲ (USP 1-Aug-2019)

Calculate the percentage ▲of the labeled amount▲ (USP 1-Aug-2019) of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = [(A_{UC} \times C_S \times V \times D)/(A_S \times L)] \times 100$$

A_{UC} = corrected absorbance of *Sample solution*▲*B*▲ (USP 1-Aug-2019) at 270 nm
C_S = concentration of ▲USP Hydrochlorothiazide RS in▲ (USP 1-Aug-2019) the *Hydrochlorothiazide standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor of *Sample solution B*, 5
A_S = absorbance of the *Hydrochlorothiazide standard solution*
L = label claim of hydrochlorothiazide (mg/Tablet)

▲**Chromatographic procedure**▲ (USP 1-Aug-2019)
Buffer and **Mobile phase:** Prepare as directed in the *Assay*.
Standard stock solution: Use the *Standard solution* from the *Assay*.
Standard solution: 0.005 mg/mL of USP Amiloride Hydrochloride RS and 0.05 mg/mL of USP Hydrochlorothiazide RS, from the *Standard stock solution*, diluted with *Medium*
Sample solution: Pass a portion of the solution under test through a filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 286 nm
Column: 4.6-mm × ▲15-cm; 5-µm▲ (USP 1-Aug-2019) packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 ▲▲ (USP 1-Aug-2019) between hydrochlorothiazide and amiloride
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the ▲percentage of the labeled▲ (USP 1-Aug-2019) amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of amiloride or hydrochlorothiazide from the *Sample solution*
r_S = peak response of amiloride or hydrochlorothiazide from the *Standard solution*
C_S = concentration of ▲USP Amiloride Hydrochloride RS or USP Hydrochlorothiazide RS▲ (USP 1-Aug-2019) in the *Standard solution* (mg/mL)
L = label claim of amiloride hydrochloride or hydrochlorothiazide (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) and NLT 75% (*Q*) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.

Change to read:

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*: Meet the requirements▲▲ (USP 1-Aug-2019)

IMPURITIES

Change to read:

- ▲**LIMIT OF BENZOTHIADIAZINE RELATED COMPOUND A**▲▲ (USP 1-Aug-2019)

Buffer, Mobile phase, and **Sample solution:** Prepare as directed in the *Assay*.
▲**System suitability solution:** Use the *Standard solution* from the *Assay*.▲ (USP 1-Aug-2019)
Standard solution: ▲0.01 mg/mL▲ (USP 1-Aug-2019) of USP Benzothiadiazine Related Compound A RS in *Mobile phase*
Chromatographic system: ▲Proceed as directed in the *Assay*, except for the *Injection volume*.▲ (USP 1-Aug-2019)
Injection volume: 20 µL. ▲For *System suitability*, use 10 µL.▲ (USP 1-Aug-2019)
System suitability
Sample: ▲*System suitability solution*▲ (USP 1-Aug-2019)
[NOTE—The relative retention times for hydrochlorothiazide and amiloride ▲▲ (USP 1-Aug-2019) are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between hydrochlorothiazide and amiloride▲▲ (USP 1-Aug-2019)

www.webofpharma.com

Relative standard deviation: NMT 2.0% ▲for hydrochlorothiazide and amiloride▲ (USP 1-Aug-2019)

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

▲ (USP 1-Aug-2019)

r_U = peak response of benzothiadiazine related compound A from the *Sample solution*

r_S = peak response of benzothiadiazine related compound A from the *Standard solution*

C_S = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* ▲(mg/mL)▲ (USP 1-Aug-2019)

C_U = nominal concentration of ▲hydrochlorothiazide▲ (USP 1-Aug-2019) in the *Sample solution* (mg/mL)

▲ ▲ (USP 1-Aug-2019)

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. ▲Store at controlled room temperature. Protect from light.▲ (USP 1-Aug-2019)
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Amiloride Hydrochloride RS

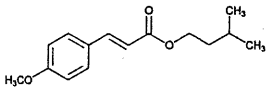
USP Benzothiadiazine Related Compound A RS

4-Amino-6-chloro-1,3-benzenedisulfonamide.

$C_6H_8ClN_3O_4S_2$ 285.73

USP Hydrochlorothiazide RS

Amiloxate



$C_{15}H_{20}O_3$ 248.32

4-Methoxycinnamic acid, isoamyl ester;

3-Methylbutyl 3-(4-methoxyphenyl)-(*E*)-2-propenoate [71617-10-2].

DEFINITION

Amiloxate contains NLT 98.0% and NMT 102.0% of amiloxate ($C_{15}H_{20}O_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 5.0 µg/mL in alcohol

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Standard solution: 20 mg/mL of USP Amiloxate RS in *tert*-butyl methyl ether

Sample solution: 20 mg/mL of Amiloxate in *tert*-butyl methyl ether

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 25-m; coated with a 0.1-µm film of phase G1

Temperatures

Injection port: 240°

Detector: 260°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	8	240	10

Carrier gas: Helium

Flow rate: 6 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amiloxate ($C_{15}H_{20}O_3$) in the portion of Amiloxate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Amiloxate RS in the *Standard solution* (mg/mL)

C_U = concentration of Amiloxate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Amiloxate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses, excluding the solvent peak, from the *Sample solution*

Acceptance criteria

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩: 1.037–1.041

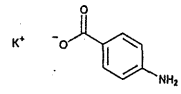
USP Monographs

www.webofpharma.com

• **REFRACTIVE INDEX** ⟨831⟩: 1.556–1.560 at 20°
• **ACIDITY**
Sample solution: Transfer 50 mL of alcohol to a suitable container. Add 1 mL of phenolphthalein TS and sufficient 0.1 N sodium hydroxide to obtain a persistent pink color. Transfer 50 mL of this solution to a suitable container, and add 5.0 mL of Amiloxate.
Analysis: Titrate with 0.1 N sodium hydroxide VS.
Acceptance criteria: NMT 0.2 mL of titrant per mL of Amiloxate is required for neutralization.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amiloxate RS

Aminobenzoate Potassium



$C_7H_6KNO_2$ 175.23
Benzoic acid, 4-amino-, potassium salt;
Potassium 4-aminobenzoate [138-84-1].

DEFINITION
Aminobenzoate Potassium contains NLT 98.0% and NMT 102.0% of aminobenzoate potassium ($C_7H_6KNO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C.**
Sample solution: 10 mg/mL of Aminobenzoate Potassium in water
Acceptance criteria: The *Sample solution* imparts a violet color to a nonluminous flame. Since the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

ASSAY
• **PROCEDURE**
Solution A: 1.5% acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid and passing through a suitable filter of 0.45-µm pore size
Mobile phase: Methanol and *Solution A* (15:85)
Standard solution: 0.1 mg/mL of USP Aminobenzoate Potassium RS in *Mobile phase*. Sonicate to dissolve.
Sample solution: 0.1 mg/mL of Aminobenzoate Potassium in *Mobile phase*. Sonicate to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.0-mm × 15-cm; 3.5-µm packing L11
Flow rate: 0.35 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminobenzoate potassium ($C_7H_6KNO_2$) in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Delete the following:

• ▲**HEAVY METALS** ⟨231⟩, *Method II*: NMT 0.002%▲ (Official 1-Jan-2018)

Change to read:

• **LIMIT OF ANILINE AND *p*-TOLUIDINE**
Diluent: Methylene chloride
Standard stock solution: 0.1 mg/mL each of USP Aniline RS and USP p-Toluidine RS in *Diluent*
Standard solution: 1 µg/mL each of USP Aniline RS and USP p-Toluidine RS in *Diluent* from *Standard stock solution*
Sample solution: 100 mg/mL of Aminobenzoate Potassium in *Diluent* prepared as follows. Add an appropriate quantity of Aminobenzoate Potassium to a suitable volumetric flask and dilute with *Diluent* to volume. Agitate for 10 min on a shaker and centrifuge at 3000 rpm for 5 min. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detectors
Flame ionization: 300°
Hydrogen: 40 mL/min
Air: 400 mL/min
Column: 30-m × 0.32-mm fused silica capillary; coated with 0.5-µm film of phase G27
Temperatures
Injection port: 280°
Detector: 300°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	—	130	4
130	20	180	5

Carrier gas: Helium
Flow rate: 1.0 mL/min
Injection volume: 2 µL

www.webofpharma.com

Injection type: Split ratio, 1:10
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times of aniline and *p*-toluidine are ▲0.8 and 1.0,▲ (ERR 1-Sep-2018) respectively.]
Suitability requirements
Resolution: NLT 7.0 between aniline and *p*-toluidine
Tailing factor: NMT 1.5 for aniline and *p*-toluidine
Relative standard deviation: NMT 6.0% for aniline and *p*-toluidine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of *p*-toluidine or aniline in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *p*-toluidine or aniline from the *Sample solution*
r_S = peak response of *p*-toluidine or aniline from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)

Acceptance criteria
Aniline: NMT 10 ppm
***p*-Toluidine:** NMT 20 ppm

• **ORGANIC IMPURITIES**
Solution A: 1.5% acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid
Solution B: Methanol
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	85	15
4.0	85	15
4.1	45	55
10.0	45	55
10.1	85	15
13	85	15

Diluent: Methanol and water (85:15)
System suitability solution: 1 mg/mL of USP Aminobenzoate Potassium RS, 0.01 mg/mL of USP 4-Nitrobenzoic Acid RS, and 0.01 mg/mL of USP Benzocaine RS in *Diluent* prepared as follows. Transfer 1 mL each of 0.1 mg/mL of USP 4-Nitrobenzoic Acid RS in methanol and 0.1 mg/mL of USP Benzocaine RS in *Diluent* to a 10-mL volumetric flask containing the appropriate amount of USP Aminobenzoate Potassium RS, and dilute with *Diluent* to volume.
Standard solution: 1 µg/mL each of USP Aminobenzoate Potassium RS, USP 4-Nitrobenzoic Acid RS, and USP Benzocaine RS in *Diluent*
Sample solution: 1 mg/mL of Aminobenzoate Potassium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.0-mm × 15-cm; 3.5-µm packing L11
Flow rate: 0.4 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between benzocaine and 4-nitrobenzoic acid, *System suitability solution*
Relative standard deviation: NMT 3% for the aminobenzoate potassium, 4-nitrobenzoic acid, and benzocaine peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 4-nitrobenzoic acid or benzocaine in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 4-nitrobenzoic acid or benzocaine from the *Sample solution*
r_S = peak response of 4-nitrobenzoic acid or benzocaine from the *Standard solution*
C_S = concentration of USP 4-Nitrobenzoic Acid RS or USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of aminobenzoate from the *Standard solution*
C_S = concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	1.0	—
Benzocaine	2.0	0.2
4-Nitrobenzoic acid	2.1	0.2
Any individual unspecified impurity	—	0.10

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 50 mg/mL of Aminobenzoate Potassium in water
Acceptance criteria: 8.0–9.0
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 1.0%

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Aminobenzoate Potassium RS
USP Aniline RS
Aniline.
C_6H_7N 93.13
USP Benzocaine RS
USP 4-Nitrobenzoic Acid RS
4-Nitrobenzoic acid.
$C_7H_5NO_4$ 167.12
USP p-Toluidine RS
4-Methylaniline.
C_7H_9N 107.15

Aminobenzoate Potassium Capsules

DEFINITION

Aminobenzoate Potassium Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of aminobenzoate potassium ($C_7H_6KNO_2$).

IDENTIFICATION

- **A.**

Sample: 1 g of the Capsule contents
Analysis: Dissolve the *Sample* in 25 mL of water, add 5 mL of 3 N hydrochloric acid, and wash the precipitate with two 5-mL portions of cold water. Recrystallize from alcohol the precipitate so obtained, and dry at 110° for 1 h.
Acceptance criteria: The *p*-aminobenzoic acid melts between 186° and 189°.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 1.5% acetic acid prepared as follows. Mix 690 mL of water with 10 mL of acetic acid and pass through a filter of 0.45-µm pore size.
Mobile phase: Methanol and *Solution A* (15:85)
Standard solution: 0.1 mg/mL of USP Aminobenzoate Potassium RS in *Mobile phase*
Sample solution: Nominally 0.1 mg/mL of aminobenzoate potassium prepared as follows. Remove as completely as possible, and combine the contents of NLT 10 Capsules. Transfer a portion of the combined contents, equivalent to 10 mg of aminobenzoate potassium to a 100-mL volumetric flask, dissolve in 70 mL of *Mobile phase*, sonicate for 3–4 min, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 280 nm
Column: 3.0-mm × 15-cm; 3.5-µm packing L11
Flow rate: 0.35 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aminobenzoate potassium ($C_7H_6KNO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aminobenzoate potassium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 270 nm
Standard solution: A known concentration of USP Aminobenzoate Potassium RS in *Medium*
Sample solution: Filter portions of the solution under test, and dilute with *Medium*, if necessary, in comparison with the *Standard solution* concentration.
Analysis: Calculate the percentage of the labeled amount of aminobenzoate potassium ($C_7H_6KNO_2$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of aminobenzoate potassium ($C_7H_6KNO_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Mobile phase, and **Chromatographic system:** Prepare as directed in the *Assay*.
Standard solution: 1 µg/mL each of USP Aminobenzoate Potassium RS, USP 4-Nitrobenzoic Acid RS, and USP Benzocaine RS in *Mobile phase*
Sensitivity solution: 0.1 µg/mL of USP Aminobenzoate Potassium RS in *Mobile phase* from the *Standard solution*
Sample solution: Nominally 1 mg/mL of aminobenzoate potassium in *Mobile phase* prepared as follows. Remove as completely as possible, and combine, the contents of NLT 10 Capsules. Transfer a portion of the combined contents, equivalent to 10 mg of aminobenzoate potassium, to a 10-mL volumetric flask. Dissolve in 7 mL of *Mobile phase*, sonicate for 3–4 min, and dilute with *Mobile phase* to volume.
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between benzocaine and 4-nitrobenzoic acid, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak response of any individual unspecified degradation product from the *Sample solution*
r_S = peak response of aminobenzoate from the *Standard solution*
C_S = concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aminobenzoate potassium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

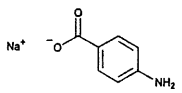
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	1.0	—
Benzocaine[a]	2.0	—
4-Nitrobenzoic acid[a]	2.1	—
Any individual unspecified degradation product	—	0.10
Total impurities	—	1.0

[a] These are process impurities controlled in the API and are included in this table for identification purposes only. They are not reported in the drug product and should not be included in the total impurities.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aminobenzoate Potassium RS
  USP Benzocaine RS
  USP 4-Nitrobenzoic Acid RS
  4-Nitrobenzoic acid.
  $C_7H_5NO_4$ 167.12

Aminobenzoate Sodium



$C_7H_6NNaO_2$ 159.12
Benzoic acid, 4-amino-, sodium salt;
Sodium 4-aminobenzoate [555-06-6].

DEFINITION

Aminobenzoate Sodium contains NLT 98.0% and NMT 102.0% of aminobenzoate sodium ($C_7H_6NNaO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*: A solution (1 in 100) meets the requirements of the flame test.

ASSAY

- **PROCEDURE**

Solution A: 1.5% acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid, and passing through a suitable filter of 0.45-µm pore size
Mobile phase: Methanol and *Solution A* (15:85)
Standard solution: 0.1 mg/mL of USP Aminobenzoate Sodium RS in *Mobile phase*. Sonicate to dissolve.
Sample solution: 0.1 mg/mL of Aminobenzoate Sodium in *Mobile phase*. Sonicate to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.0-mm × 15-cm; 3.5-µm packing L11
Flow rate: 0.35 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminobenzoate sodium ($C_7H_6NNaO_2$) in the portion of Aminobenzoate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aminobenzoate Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminobenzoate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **LIMIT OF ANILINE AND *p*-TOLUIDINE**

Diluent: Methylene chloride
Standard stock solution: 0.1 mg/mL each of USP Aniline RS and USP p-Toluidine RS in *Diluent*
Standard solution: 1.0 µg/mL each of USP Aniline RS and USP p-Toluidine RS in *Diluent* from *Standard stock solution*
Sample solution: 100 mg/mL of Aminobenzoate Sodium in *Diluent* prepared as follows. Add an appropriate quantity of Aminobenzoate Sodium to a suitable volumetric flask and dilute with *Diluent* to volume. Agitate for 10 min on a shaker and centrifuge at 3000 rpm for 5 min. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detectors
Flame ionization: 300°
Hydrogen: 40 mL/min
Air: 400 mL/min
Column: 30-m × 0.32-mm fused silica capillary; coated with 0.5-µm film of phase G27
Temperatures
Injection port: 280°
Detector: 300°
Column: See *Table 1*.

USP Monographs

www.webofpharma.com

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	—	130	4
130	20	180	5

Carrier gas: Helium
Flow rate: 1 mL/min
Injection volume: 2 µL
Injection type: Split ratio, 1:10
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times of aniline and *p*-toluidine are about 4.1 and 5.1 min, respectively.]
Suitability requirements
Resolution: NLT 7.0 between aniline and *p*-toluidine
Tailing factor: NMT 1.5 for aniline and *p*-toluidine
Relative standard deviation: NMT 6.0% for aniline and *p*-toluidine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of *p*-toluidine or aniline in the portion of Aminobenzoate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *p*-toluidine or aniline from the *Sample solution*
r_S = peak response of *p*-toluidine or aniline from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = concentration of Aminobenzoate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria
Aniline: NMT 10 ppm
***p*-Toluidine:** NMT 20 ppm

• **ORGANIC IMPURITIES**
Solution A: 1.5% acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid
Solution B: Methanol
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	85	15
4.0	85	15
4.1	45	55
10.0	45	55
10.1	85	15
13	85	15

Diluent: Methanol and water (85:15)
System suitability solution: 1 mg/mL of USP Aminobenzoate Sodium RS, 0.01 mg/mL of USP 4-Nitrobenzoic Acid RS, and 0.01 mg/mL of USP Benzocaine RS in *Diluent* prepared as follows. Transfer 1 mL each of 0.1 mg/mL of USP 4-Nitrobenzoic Acid RS in methanol and 0.1 mg/mL of USP Benzocaine RS in *Diluent* to a 10-mL volumetric flask containing the appropriate amount of USP Aminobenzoate Sodium RS, and dilute with *Diluent* to volume.
Standard solution: 1 µg/mL each of USP Aminobenzoate Sodium RS, USP 4-Nitrobenzoic Acid RS, and USP Benzocaine RS in *Diluent*
Sample solution: 1 mg/mL of Aminobenzoate Sodium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.0-mm × 15-cm; 3.5-µm packing L11
Flow rate: 0.4 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between benzocaine and 4-nitrobenzoic acid, *System suitability solution*
Relative standard deviation: NMT 3% for the aminobenzoate sodium, 4-nitrobenzoic acid, and benzocaine peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 4-nitrobenzoic acid or benzocaine in the portion of Aminobenzoate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 4-nitrobenzoic acid or benzocaine from the *Sample solution*
r_S = peak response of 4-nitrobenzoic acid or benzocaine from the *Standard solution*
C_S = concentration of USP 4-Nitrobenzoic Acid RS or USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminobenzoate Sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Aminobenzoate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of aminobenzoate from the *Standard solution*
C_S = concentration of USP Aminobenzoate Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminobenzoate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	1.0	—
Benzocaine	2.0	0.2
4-Nitrobenzoic acid	2.1	0.2
Any individual unspecified impurity	—	0.10

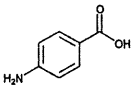
USP Monographs

www.webofpharma.com

SPECIFIC TESTS
- **pH** ⟨791⟩
  Sample solution: 50 mg/mL
  Acceptance criteria: 8.0–9.0
- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨11⟩
  USP Aminobenzoate Sodium RS
  USP Aniline RS
  Aniline.
  C_6H_7N 93.13
  USP Benzocaine RS
  USP 4-Nitrobenzoic Acid RS
  4-Nitrobenzoic acid.
  $C_7H_5NO_4$ 167.12
  USP p-Toluidine RS
  4-Methylaniline.
  C_7H_9N 107.16

Aminobenzoic Acid



$C_7H_7NO_2$ 137.14
Benzoic acid, 4-amino;
p-Aminobenzoic acid [150-13-0].

DEFINITION
Aminobenzoic Acid contains NLT 98.0% and NMT 102.0% of aminobenzoic acid ($C_7H_7NO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** **▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**
  Acetic acid solution: Glacial acetic acid and water (1:69)
  Mobile phase: Methanol and *Acetic acid solution* (15:85)
  Standard solution: 0.1 mg/mL of USP Aminobenzoic Acid RS in *Mobile phase*. Sonicate to aid dissolution.
  Sample solution: 0.1 mg/mL of Aminobenzoic Acid in *Mobile phase*. Sonicate to aid dissolution.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 3.0-mm × 15-cm; 3.5-µm packing L11
  Flow rate: 0.4 mL/min
  Injection volume: 5 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing: NMT 1.5
  Relative standard deviation: NMT 1.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of aminobenzoic acid ($C_7H_7NO_2$) in the portion of Aminobenzoic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.1%
- **Organic Impurities**
  Solution A: Acetonitrile and methanol (70:80)
  Buffer: 1.5 g/L of monobasic potassium phosphate and 2.5 g/L of octanesulfonic acid sodium salt in water. Adjust with phosphoric acid to a pH of 2.2.
  Mobile phase: *Solution A* and *Buffer* (20:80)
  Standard stock solution: 0.25 mg/mL each of USP Benzocaine RS and 4-nitrobenzoic acid in methanol
  Standard solution: 0.5 µg/mL each of USP Benzocaine RS and 4-nitrobenzoic acid in *Mobile phase*, from the *Standard stock solution*
  Sample solution: 0.25 mg/mL of Aminobenzoic Acid in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 270 nm
  Column: 4.0-mm × 15-cm; 5-µm packing L7
  Flow rate: 1.0 mL/min
  Injection volume: 20 µL
  Run time: 11 times the retention time of the aminobenzoic acid peak
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of 4-nitrobenzoic acid or any unspecified impurity in the portion of Aminobenzoic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 4-nitrobenzoic acid or any unspecified impurity from the *Sample solution*
r_S = peak response of 4-nitrobenzoic acid from the *Standard solution*
C_S = concentration of 4-nitrobenzoic acid in the *Standard solution* (mg/mL)
C_U = concentration of Aminobenzoic Acid in the *Sample solution* (mg/mL)

Calculate the percentage of benzocaine in the portion of Aminobenzoic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzocaine from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminobenzoic Acid in the *Sample solution* (mg/mL)

www.webofpharma.com

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.02%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	1.0	—
4-Nitrobenzoic acid	4.0	0.2
Benzocaine	9.0	0.2
Any individual unspecified impurity	—	0.1
Total impurities	—	0.5

• **LIMIT OF ANILINE AND *p*-TOLUIDINE**
Diluent: 84 g/L of sodium hydroxide in water
Standard solution: 1.0 µg/mL each of aniline and *p*-toluidine in methylene chloride
Sample solution: Dissolve 1 g of Aminobenzoic Acid in 10.0 mL of *Diluent*, and extract with two quantities each of 10.0 mL of methylene chloride. Combine, and wash with 5 mL of water. Filter through anhydrous sodium sulfate, and wash the filter with methylene chloride. Evaporate in a water bath at 50°–60° to obtain a volume of about 1–5 mL. Transfer to a 10-mL volumetric flask, and dilute with methylene chloride to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m fused silica capillary, coated with a 0.5-µm film of phase G27
Temperatures
Injection port: 280°
Detector: 300°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	0	130	4
130	20	180	5

Carrier gas: Helium
Flow rate: 1.0 mL/min
Injection volume: 2 µL
Split ratio: 1:10
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for aniline and *p*-toluidine are about 0.8 and 1.0, respectively.]
Calculate, in ppm, the amount of aniline and *p*-toluidine in the portion of Aminobenzoic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 10^6$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of the corresponding impurity from the *Standard solution*
C_S = concentration of the corresponding impurity in the *Standard solution* (mg/mL)
C_U = concentration of Aminobenzoic Acid in the *Sample solution* (mg/mL)

Acceptance criteria
Aniline: NMT 10 ppm
***p*-Toluidine:** NMT 10 ppm

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 2 h.
Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Aminobenzoic Acid RS
USP Benzocaine RS
Benzoic acid, 4-amino-, ethyl ester.
$C_9H_{11}NO_2$ 165.19

Aminobenzoic Acid Gel

DEFINITION
Aminobenzoic Acid Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of aminobenzoic acid ($C_7H_7NO_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 5 µg/mL in alcohol
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Mobile phase: Methanol, glacial acetic acid, and water (30:1:69). Allow the mixture to cool, and pass, if necessary, through a suitable microporous membrane filter, and degas.
Internal standard solution: 7 mg/mL of salicylic acid in methanol; dissolve by sonicating.
Standard stock solution: 0.42 mg/mL of USP Aminobenzoic Acid RS in methanol; dissolve by sonicating.
Standard solution: 0.042 mg/mL of USP Aminobenzoic Acid RS in methanol prepared as follows. Transfer 5 mL each of *Standard stock solution* and *Internal standard solution* into a 50-mL volumetric flask, and dilute with methanol to volume. Pass through 0.6-µm filter paper. Throughout the preparation, protect against actinic light.
Sample solution: Nominally 0.042 mg/mL of aminobenzoic acid in methanol prepared as follows. Transfer a quantity of Gel, equivalent to 4.2 mg of aminobenzoic acid, into a 100-mL volumetric flask. Add 10.0 mL of *Internal standard solution* and 50 mL of methanol. Shake or sonicate, as necessary, and dilute with methanol to volume. Filter, if necessary, through filter paper (Whatman No. 41 or equivalent). Pass through 0.6-µm filter paper. Throughout this preparation, protect against actinic light.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

www.webofpharma.com

Detector: UV 280 nm
Column: 3.9-mm × 30-cm; packing L11
Flow rate: 1.0 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
Chromatograph replicate 15-µL injections of the *Standard solution* until the response ratio variability is within 1.0% of average.
[NOTE—The relative retention times for aminobenzoic acid and salicylic acid are about 1.0 and 3.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between aminobenzoic acid and salicylic acid peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aminobenzoic acid ($C_7H_7NO_2$) in the portion of Gel taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of aminobenzoic to salicylic acid from the *Sample solution*
R_S = peak response ratio of aminobenzoic to salicylic acid from the *Standard solution*
C_S = concentration of USP Aminobenzoic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aminobenzoic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

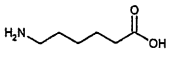
OTHER COMPONENTS
• **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩**:** 42.3%–54.0% (w/w) of C_2H_5OH

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 4.0–6.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Aminobenzoic Acid RS

Aminocaproic Acid



$C_6H_{13}NO_2$ 131.17
Hexanoic acid, 6-amino-;
6-Aminohexanoic acid [60-32-2].

DEFINITION
Aminocaproic Acid contains NLT 98.5% and NMT 101.5% of aminocaproic acid ($C_6H_{13}NO_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Solution A: 0.55 g/L of sodium 1-heptanesulfonate in water
Mobile phase: Dissolve 10 g of monobasic potassium phosphate in 300 mL of *Solution A*, and add 250 mL of methanol, followed by another 300 mL of *Solution A*. Adjust the mixture with phosphoric acid to a pH of 2.2, and dilute with *Solution A* to 1 L.
Internal standard solution: 1.25 mg/mL of methionine in water
Standard stock solution: 12.5 mg/mL of USP Aminocaproic Acid RS in water
Standard solution: 5.0 mL of *Standard stock solution* and 2.0 mL of *Internal standard solution* in a 100-mL volumetric flask. Dilute with water to volume.
Sample stock solution: 12.5 mg/mL of Aminocaproic Acid in water
Sample solution: 5.0 mL of *Sample stock solution* and 2.0 mL of *Internal standard solution* in a 100-mL volumetric flask. Dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 30°
Flow rate: 0.7 mL/min
Run time: NLT 2 times the retention time of aminocaproic acid
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for aminocaproic acid and methionine are about 0.76 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between aminocaproic acid and methionine
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminocaproic acid ($C_6H_{13}NO_2$) in the portion of Aminocaproic Acid taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of aminocaproic acid to the internal standard from the *Sample solution*
R_S = peak response ratio of aminocaproic acid to the internal standard from the *Standard solution*
C_S = concentration of USP Aminocaproic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminocaproic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aminocaproic Acid RS

Aminocaproic Acid Injection

DEFINITION
Aminocaproic Acid Injection is a sterile solution of Aminocaproic Acid in Water for Injection. It contains NLT 95.0% and NMT 107.5% of the labeled amount of aminocaproic acid ($C_6H_{13}NO_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Mix 2 mL of Injection, added dropwise, with 100 mL of acetone, rapidly stirring the mixture with a glass rod to induce crystallization. Allow the mixture to stand for 15 min, and pass through a sintered-glass filter of medium pore size. Wash the crystals with 25 mL of acetone, apply a vacuum to remove the solvent, dry at 105° for 30 min, and cool. Use the residue.
Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**

Mobile phase: Transfer 11 g of sodium 1-pentanesulfonate and 40 g of anhydrous sodium sulfate to a 2-L volumetric flask, and dissolve in about 500 mL of water. Add 20 mL of 1 N sulfuric acid and 30 mL of acetonitrile, and dilute with water to volume.
Standard solution: 2.5 mg/mL of USP Aminocaproic Acid RS in *Mobile phase*
System suitability solution: Mix 20 µL of benzyl alcohol with 100 mL of water. Dilute 1.0 mL of this solution with *Standard solution* to 10 mL.
Sample stock solution: Nominally equivalent to 25 mg/mL of aminocaproic acid from a suitable volume of Injection in water
Sample solution: 2.5 mg/mL of *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 7.0 between benzyl alcohol and aminocaproic acid, *System suitability solution*
[NOTE—The aminocaproic acid peak elutes before the benzyl alcohol peak.]
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminocaproic acid ($C_6H_{13}NO_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Aminocaproic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aminocaproic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–107.5%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 6.0–7.6
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩ **:** NMT 0.05 USP Endotoxin Unit/mg of aminocaproic acid
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aminocaproic Acid RS

Aminocaproic Acid Oral Solution

DEFINITION
Aminocaproic Acid Oral Solution contains NLT 95.0% and NMT 115.0% of the labeled amount of aminocaproic acid ($C_6H_{13}NO_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Mix 1 g of ion-exchange resin (strongly acidic styrene–divinylbenzene cation-exchange resin) with 10 mL of 1 N hydrochloric acid in a 100-mL beaker. Decant and discard the hydrochloric acid, and wash the resin with five 10-mL portions of water, decanting and discarding the liquid following each washing. Place the washed resin in a 125-mL glass-stoppered, conical flask, and add a volume of Oral Solution, nominally equivalent to 250 mg of aminocaproic acid, and 10 mL of water. Insert the stopper in the flask, and shake by mechanical means for 30 min. Transfer the resin slurry to a sintered-glass funnel of medium pore size. Wash with 100 mL of water, filter by applying suction, and discard the washing. Place a beaker under the stem of the funnel, add 10 mL of 1 N hydrochloric acid to the resin, stir for 4–5 min, and filter by applying suction. Evaporate the filtrate on a steam bath to dryness, dry at 105° for 1 h, and cool.
Acceptance criteria: The residue meets the requirements.

ASSAY
- **PROCEDURE**

Sample solution: Place an amount nominally equivalent to 250 mg of aminocaproic acid from a volume of Oral Solution in about 80 mL of glacial acetic acid.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N perchloric acid in dioxane VS
Endpoint detection: Visual
Analysis: To the *Sample solution* add 10 drops of a 1-in-500 solution of crystal violet in chlorobenzene. Titrate with *Titrant* to a blue endpoint, and perform a blank

www.webofpharma.com

determination. Each mL of 0.1 N perchloric acid is equivalent to 13.12 mg of aminocaproic acid ($C_6H_{13}NO_2$).
Acceptance criteria: 95.0%–115.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 6.0–6.5

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aminocaproic Acid RS

Aminocaproic Acid Tablets

DEFINITION
Aminocaproic Acid Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of aminocaproic acid ($C_6H_{13}NO_2$).

IDENTIFICATION

Change to read:

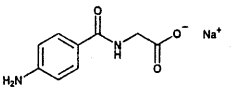
- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
  Sample: Triturate 2 Tablets with 10 mL of water, and filter into 100 mL of acetone. Swirl the mixture, and allow to stand for 15 min to complete crystallization. Pass the solution through a sintered-glass filter of medium pore size, and wash the crystals with 25 mL of acetone. Apply vacuum to remove the solvent, dry at 105° for 30 min, and cool. Use the residue.
  Acceptance criteria: Meet the requirements

ASSAY
- **PROCEDURE**
  Sample solution: Nominally equivalent to about 500 mg of aminocaproic acid from NLT 20 finely powdered Tablets taken in a beaker in about 100 mL of glacial acetic acid. Heat gently to effect solution, and cool.
  Titrimetric system
  Mode: Direct titration
  Titrant: 0.1 N perchloric acid in dioxane VS
  Endpoint detection: Visual
  Analysis: To the *Sample solution* add 10 drops of a 1-in-500 solution of crystal violet in chlorobenzene. Titrate with *Titrant* to a blue endpoint, and perform a blank determination. Each mL of 0.1 N perchloric acid is equivalent to 13.12 mg of aminocaproic acid ($C_6H_{13}NO_2$).
  Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: Water; 900 mL
  Apparatus 1: 100 rpm
  Time: 45 min
  Buffer: Dissolve 6.185 g of boric acid and 7.930 g of potassium chloride in about 1000 mL of water, and add 60 mL of 1.0 N sodium hydroxide. Dilute with water to 2000 mL, and adjust if necessary with 1.0 N sodium hydroxide to a pH of 9.5 ± 0.1.
  Standard solution: 0.5 mg/mL of USP Aminocaproic Acid RS in water
  Sample solution: Filter a portion of the solution under test.
  Blank: Water
  Analysis: Into three separate 50-mL volumetric flasks pipet 1 mL each of *Sample solution*, *Standard solution*, and *Blank*. Add 20.0 mL of *Buffer* and 3.0 mL of a freshly prepared 1-in-500 solution of β-naphthoquinone-4-sodium sulfonate to each flask. Swirl to mix, and place the three flasks in a water bath maintained at a temperature of 65 ± 5° for 45 min. Cool, and dilute the contents of each flask with water to volume. Determine the percentage of the labeled amount of aminocaproic acid ($C_6H_{13}NO_2$) dissolved from absorbances, at the wavelength of maximum absorbance at about 460 nm, from the *Sample solution* in comparison with those from the *Standard solution*, using the *Blank* to set the instrument.
  Tolerances: NLT 75% (*Q*) of the labeled amount of aminocaproic acid ($C_6H_{13}NO_2$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aminocaproic Acid RS

Aminohippurate Sodium Injection



$C_9H_9N_2NaO_3$ 216.17
Glycine, *N*-(4-aminobenzoyl)-, monosodium salt; Monosodium *p*-aminohippurate [94-16-6].

DEFINITION
Aminohippurate Sodium Injection is a sterile solution of Aminohippuric Acid in Water for Injection prepared with the aid of Sodium Hydroxide. It contains NLT 95.0% and NMT 105.0% of the labeled amount of aminohippurate sodium ($C_9H_9N_2NaO_3$).

IDENTIFICATION
- **A.**
  Sample solution: A volume of Injection equivalent to 100 mg of aminohippuric acid
  Analysis: Dilute the *Sample solution* with water to 50 mL. To 5 mL of this solution add 0.5 mL of 3 N hydrochloric acid, 0.5 mL of sodium nitrite solution (1 in 10), and a solution of 0.20 g of 2-naphthol in 10 mL of 6 N ammonium hydroxide.
  Acceptance criteria: A red color is produced.
- **B.**
  Sample solution: A volume of Injection equivalent to about 200 mg of aminohippurate sodium
  Analysis: In the order named, add to the *Sample solution* 2 mL of potassium iodide TS, 10 mL of water, and 5 mL of sodium hypochlorite TS.
  Acceptance criteria: A red color is produced.
- **C.** A sample imparts an intense yellow color to a nonluminous flame.

ASSAY
- **PROCEDURE**
  Sample solution: A volume of Injection equivalent to 1 g of aminohippurate sodium
  Analysis: Transfer the *Sample solution* to a 200-mL volumetric flask, and dilute with water to volume. Transfer 50.0 mL of the solution to a suitable container, and add 5 mL of hydrochloric acid. Proceed as directed in *Nitrite Titration* ⟨451⟩, beginning with "cool to about 15°". Each

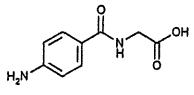
www.webofpharma.com

mL of 0.1 M sodium nitrite is equivalent to 21.62 mg of aminohippurate sodium ($C_9H_9N_2NaO_3$).
Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 6.7–7.6
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.04 USP Endotoxin Units/mg of aminohippurate sodium
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

Aminohippuric Acid



$C_9H_{10}N_2O_3$ 194.19
Glycine, *N*-(4-aminobenzoyl)-;
p-Aminohippuric acid [61-78-9].

DEFINITION
Aminohippuric Acid contains NLT 98.0% and NMT 100.5% of aminohippuric acid ($C_9H_{10}N_2O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.**
  Sample: 10 mg of Aminohippuric Acid
  Analysis: Dissolve the *Sample* in 5 mL of water, add 0.5 mL of 3 N hydrochloric acid, 0.5 mL of sodium nitrite solution (1 in 10), and a solution of 0.20 g of 2-naphthol in 10 mL of 6 N ammonium hydroxide.
  Acceptance criteria: A red color is produced.

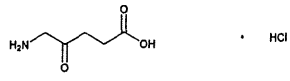
ASSAY
- **PROCEDURE**
  Sample: 150 mg of Aminohippuric Acid
  Analysis: To the *Sample* add 5 mL of hydrochloric acid and 50 mL of water. Proceed as directed in *Nitrite Titration* ⟨451⟩, beginning with "stir until dissolved." Each mL of 0.1 M sodium nitrite is equivalent to 19.42 mg of aminohippuric acid ($C_9H_{10}N_2O_3$).
  Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.25%

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 0.25%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aminohippuric Acid RS

Aminolevulinic Acid Hydrochloride



$C_5H_9NO_3 \cdot HCl$ 167.59
5-Aminolevulinic acid hydrochloride;
5-Amino-4-oxopentanoic acid hydrochloride [5451-09-2].

DEFINITION
Aminolevulinic Acid Hydrochloride contains NLT 98.0% and NMT 102.0% of aminolevulinic acid hydrochloride ($C_5H_9NO_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A** or **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY
- **PROCEDURE**
  Solution A: Water adjusted with 2 M sulfuric acid to a pH of 2.2
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Methanol (%)
0	95	5
2	95	5
6	60	40
8	60	40
9	95	5
23	95	5

Diluent: Methanol and *Solution A* (1:3)
Standard solution: 4 mg/mL of USP Aminolevulinic Acid Hydrochloride RS in *Diluent*
Sample solution: 4 mg/mL of Aminolevulinic Acid Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Flow rate: 0.4 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.6
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminolevulinic acid hydrochloride ($C_5H_9NO_3 \cdot HCl$) in the portion of Aminolevulinic Acid Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

www.webofpharma.com

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aminolevulinic Acid Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminolevulinic Acid Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%
- **ORGANIC IMPURITIES**

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.04 mg/mL each of USP Aminolevulinic Acid Hydrochloride RS, USP Aminolevulinic Acid Related Compound A RS, and USP Aminolevulinic Acid Related Compound B RS in *Diluent*
Sample solution: 40 mg/mL of Aminolevulinic Acid Hydrochloride in *Diluent*
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 10%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminolevulinic acid related compound A or aminolevulinic acid related compound B in the portion of Aminolevulinic Acid Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = concentration of Aminolevulinic Acid Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity eluting before aminolevulinic acid related compound A in the portion of Aminolevulinic Acid Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity eluting before aminolevulinic acid related compound A from the *Sample solution*
r_S = peak response of aminolevulinic acid from the *Standard solution*
C_S = concentration of USP Aminolevulinic Acid Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminolevulinic Acid Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity eluting after aminolevulinic acid related compound A in the portion of Aminolevulinic Acid Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity eluting after aminolevulinic acid related compound A from the *Sample solution*
r_S = peak response of aminolevulinic acid related compound A from the *Standard solution*
C_S = concentration of USP Aminolevulinic Acid Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminolevulinic Acid Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminolevulinic acid	1.0	—
Aminolevulinic acid related compound A	7.8	0.15
Aminolevulinic acid related compound B	12.0	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

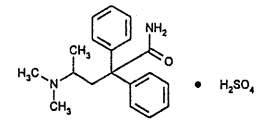
SPECIFIC TESTS

- **PH** ⟨791⟩
Sample solution: 10 mg/mL in carbon dioxide–free water
Acceptance criteria: 2.5–2.9
- **LOSS ON DRYING** ⟨731⟩
Sample: 1 g
Analysis: Dry the *Sample* over phosphorous pentoxide under vacuum at 100°–105° for 5 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Aminolevulinic Acid Hydrochloride RS
USP Aminolevulinic Acid Related Compound A RS
3,3′-(Pyrazine-2,5-diyl)dipropionic acid.
$C_{10}H_{12}N_2O_4$ 224.22
USP Aminolevulinic Acid Related Compound B RS
Methyl 5-(1,3-dioxoisoindolin-2-yl)-4-oxopentanoate.
$C_{14}H_{13}NO_5$ 275.26

Aminopentamide Sulfate



$C_{19}H_{24}N_2O \cdot H_2SO_4$ 394.49
α-[2-(Dimethylamino)propyl]-α-phenylbenzeneacetamide sulfate [60-46-8].

» Aminopentamide Sulfate contains not less than 95.0 percent and not more than 103.0 percent of $C_{19}H_{24}N_2O \cdot H_2SO_4$.

USP Monographs

www.webofpharma.com

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Aminopentamide Sulfate RS
Clarity and color of solution—Dissolve 0.5 g in 10 mL of water: the solution is clear and colorless.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: It meets the requirements of the tests for *Sulfate* ⟨191⟩.
Melting range ⟨741⟩: between 179° and 186°.
pH ⟨791⟩: between 1.2 and 3.0, in a solution (2.5 in 100).
Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 4.4% of its weight.
Residue on ignition ⟨281⟩: not more than 0.5%.
Assay—Dissolve about 500 mg of Aminopentamide Sulfate, accurately weighed, in 100 mL of dimethylformamide in a suitable container. Add 5 drops of thymol blue TS, and titrate with 0.1 N lithium methoxide VS in toluene to a deep blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N lithium methoxide is equivalent to 19.72 mg of $C_{19}H_{24}N_2O \cdot H_2SO_4$.

Aminopentamide Sulfate Injection

» Aminopentamide Sulfate Injection is a sterile solution of Aminopentamide Sulfate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aminopentamide sulfate ($C_{19}H_{24}N_2O \cdot H_2SO_4$).

Packaging and storage—Preserve in tight, single-dose or multiple-dose *Containers for Injections*, as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Store at controlled room temperature.
Labeling—Label Injection to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Aminopentamide Sulfate RS
Identification—Transfer 10 mL of the Injection to a separator, add sodium hydroxide TS until alkaline to litmus, and extract with 25 mL of chloroform. Transfer a few drops of the chloroform extract to a KRS-5 plate, and allow to dry. Record the IR absorption spectrum by the attenuated total reflectance technique (see *Mid-Infrared Spectroscopy* ⟨854⟩). The spectrum thus obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Aminopentamide Sulfate RS, concomitantly measured.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 25 USP Endotoxin Units per mg of aminopentamide sulfate.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
pH ⟨791⟩: between 2.5 and 4.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—
Mobile phase—Transfer 14.4 g of sodium lauryl sulfate to a 500-mL volumetric flask, add 100 mL of glacial acetic acid, dilute with water to volume, mix, and pass through a filter having a 0.5-µm or finer porosity. Transfer 50 mL of this solution to a 1000-mL volumetric flask, add 350 mL of methanol and 350 mL of acetonitrile, dilute with water to volume, and mix. Filter and degas before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Aminopentamide Sulfate RS in water to obtain a solution having a known concentration equivalent to the labeled concentration of aminopentamide sulfate in the Injection.
Assay preparation—Use the undiluted Injection.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of aminopentamide sulfate ($C_{19}H_{24}N_2O \cdot H_2SO_4$) in each mL of the Injection taken by the formula:

$$C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Aminopentamide Sulfate RS in the *Standard preparation;* and r_U and r_S are the aminopentamide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Aminopentamide Sulfate Tablets

» Aminopentamide Sulfate Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of aminopentamide sulfate ($C_{19}H_{24}N_2O \cdot H_2SO_4$).

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
Labeling—Label Tablets to indicate that they are for veterinary use only.
USP Reference standards ⟨11⟩—
USP Aminopentamide Sulfate RS
Identification—Transfer a portion of ground Tablet powder, equivalent to about 2 mg of aminopentamide, to a separator, add 20 mL of water and 3 mL of 10 N sodium hydroxide, and mix. Extract with two 20-mL portions of methylene chloride, and evaporate the combined methylene chloride extracts to a volume of about 0.5 mL. Transfer a few drops of the chloroform concentrate to a KRS-5 plate, and allow to dry. Record the IR absorption spectrum by the attenuated total reflectance technique (see *Mid-Infrared Spectroscopy* ⟨854⟩). The spectrum thus obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Aminopentamide Sulfate RS, concomitantly measured.

USP Monographs

www.webofpharma.com

Disintegration ⟨701⟩: not more than 10 minutes, simulated gastric fluid TS being substituted for water in the test.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Loss on drying ⟨731⟩—Dry about 1 g of powdered Tablets, accurately weighed, in vacuum at a pressure of 5 mm of mercury or less at 60° for 3 hours: it loses not more than 4.0% of its weight.

Assay—

Mobile phase—Transfer 14.4 g of sodium lauryl sulfate to a 500-mL volumetric flask, add 100 mL of glacial acetic acid, dilute with water to volume, mix, and pass through a filter having a 0.5-µm or finer porosity. Transfer 50 mL of this solution to a 1000-mL volumetric flask, add 350 mL of methanol and 350 mL of acetonitrile, dilute with water to volume, and mix. Filter and degas before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Aminopentamide Sulfate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.02 mg per mL.

Assay preparation—Weigh and finely powder not fewer than 10 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 0.2 mg of aminopentamide, to a suitable flask. Add 10.0 mL of *Mobile phase*, sonicate for 5 minutes, and stir by mechanical means for about 10 minutes. Pass this mixture through a filter having a 0.5-µm or finer porosity, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation.*

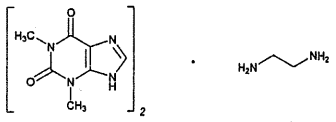
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1, and is maintained at a constant temperature of about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 900 theoretical plates; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of aminopentamide sulfate ($C_{19}H_{24}N_2O \cdot H_2SO_4$) in the portion of Tablets taken by the formula:

$$10C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Aminopentamide Sulfate RS in the *Standard preparation;* and r_U and r_S are the aminopentamide peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Aminophylline



$C_{16}H_{24}N_{10}O_4$ 420.43

$C_{16}H_{24}N_{10}O_4 \cdot 2H_2O$ 456.46

1*H*-Purine-2,6-dione, 3,7-dihydro-1,3-dimethyl-, compd. with 1,2-ethanediamine (2:1);
Theophylline compound with ethylenediamine (2:1).

Anhydrous [317-34-0].
Dihydrate [5897-66-5].

DEFINITION

Aminophylline is anhydrous or contains NMT two molecules of water of hydration. It contains NLT 84.0% and NMT 87.4% of anhydrous theophylline ($C_7H_8N_4O_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K.▲ (CN 1-May-2020)
  Sample: 500 mg of Aminophylline
  Analysis: Dissolve the *Sample* in 20 mL of water, add, with constant stirring, 1 mL of 3 N hydrochloric acid, filter (retain the filtrate), wash the precipitate with small portions of cold water, and dry at 105° for 1 h.
  Acceptance criteria: The IR spectrum of theophylline so obtained corresponds to that of USP Theophylline RS.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **C.**
  Sample: The filtrate obtained in *Identification A.*
  Analysis: To the *Sample* add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline. Shake by mechanical means for 10 min, add 5 mL of 3 N hydrochloric acid to acidify, chill, collect the precipitated disulfonamide of ethylenediamine, wash with water, recrystallize from water, and dry at 105° for 1 h.
  Acceptance criteria: The dried precipitate melts at 164°–171°.

ASSAY

- **PROCEDURE**

Solution A: 10 mM ammonium acetate prepared as follows. Transfer 770.8 mg of ammonium acetate to a 1-L volumetric flask, and dissolve in water to 80% of the flask volume. Adjust with glacial acetic acid to a pH of 5.5, and dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.
Solution B: Methanol
Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

Impurity stock solution: 25 µg/mL of USP Theophylline Related Compound F RS in water
System suitability solution: 0.8 mg/mL of USP Theophylline RS and 1 µg/mL of USP Theophylline Related Compound F RS in water prepared as follows. Transfer 21 mg of USP Theophylline RS to a 25-mL volumetric flask and add 15 mL of water. Sonicate to dissolve, add 1 mL of *Impurity stock solution,* and dilute with water to volume.
Standard solution: 0.17 mg/mL of USP Theophylline RS in water. Sonicate to dissolve as needed.

www.webofpharma.com

Sample solution: 0.2 mg/mL of Aminophylline in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 40°
Flow rate: 0.4 mL/min
Injection volume: 1 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of theophylline ($C_7H_8N_4O_2$) in the portion of Aminophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminophylline in the *Sample solution* (mg/mL)

Acceptance criteria: 84.0%–87.4% of theophylline on the anhydrous basis

OTHER COMPONENTS

• **CONTENT OF ETHYLENEDIAMINE**
Sample: 500 mg of Aminophylline
Diluent: Water
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N hydrochloric acid VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 30 mL of *Diluent*, add methyl orange TS, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine ($C_2H_8N_2$).
Acceptance criteria: 157–175 mg of ethylenediamine ($C_2H_8N_2$) per gram of theophylline ($C_7H_8N_4O_2$) found in the *Assay*

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.15%
• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 25.0 µg/mL each of USP Caffeine RS, USP Theophylline RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, USP Theophylline Related Compound D RS, and USP Theophylline Related Compound F RS in water
Standard solution: 1.0 µg/mL each of USP Caffeine RS, USP Theophylline RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, USP Theophylline Related Compound D RS, and USP Theophylline Related Compound F RS in water, from *Standard stock solution*
Sample solution: 1.0 mg/mL of Aminophylline in water
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*
Relative standard deviation: NMT 3.0% for each peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of caffeine, theophylline related compound B, theophylline related compound C, theophylline related compound D, and theophylline related compound F in the portion of Aminophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of caffeine, theophylline related compound B, theophylline related compound C, theophylline related compound D, or theophylline related compound F from the *Sample solution*
r_S = peak response of the corresponding Reference Standard from the *Standard solution*
C_S = concentration of the corresponding Reference Standard in the *Standard solution* (mg/mL)
C_U = concentration of Aminophylline in the *Sample solution* (mg/mL)

Calculate the percentage of dimethyl uric acid, theobromine, and any other individual unspecified impurity in the portion of Aminophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of dimethyl uric acid, theobromine, or any other individual unspecified impurity from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminophylline in the *Sample solution* (mg/mL)
F = relative response factor

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Theophylline related compound C	0.36	—	0.10
Theophylline related compound B	0.63	—	0.10
Theophylline related compound D	0.69	—	0.10
Dimethyl uric acid[a]	0.76	0.55	0.10
Theobromine[b]	0.82	1.0	0.10
Theophylline	1.0	—	—
Theophylline related compound F	1.09	—	0.10
Caffeine	1.20	—	0.10

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.3

[a] 1,3-Dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione.
[b] 3,7-Dihydro-3,7-dimethylpurine-2,6(1*H*)-dione.

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*
Sample: 1.5 g of Aminophylline
Solvent: 50 mL of chloroform and anhydrous methanol (50:50) in place of anhydrous methanol
Acceptance criteria
Anhydrous: NMT 0.75%
Hydrous: NMT 7.9%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **LABELING:** Label it to indicate whether it is anhydrous or hydrous, and also to state the content of anhydrous theophylline.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Caffeine RS
USP Theophylline RS
USP Theophylline Related Compound B RS
3-Methyl-1*H*-purine-2,6-dione;
Also known as 3-Methyl-3,7-dihydro-1*H*-purine-2,6-dione.
$C_6H_6N_4O_2$ 166.14
USP Theophylline Related Compound C RS
N-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide.
$C_7H_{10}N_4O_3$ 198.18
USP Theophylline Related Compound D RS
Theophyllidine;
N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide hydrochloride monohydrate.
$C_6H_{10}N_4O \cdot HCl \cdot H_2O$ 208.65
USP Theophylline Related Compound F RS
7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.
$C_9H_{12}N_4O_3$ 224.22

Aminophylline Injection

DEFINITION

Aminophylline Injection is a sterile solution of Aminophylline in Water for Injection, or is a sterile solution of Theophylline in Water for Injection prepared with the aid of Ethylenediamine. It contains, in each mL, an amount of aminophylline ($C_{16}H_{24}N_{10}O_4$) equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).
Aminophylline Injection may contain an excess of Ethylenediamine, but no other substance may be added for the purpose of pH adjustment.
[NOTE—Do not use the Injection if crystals have separated.]

IDENTIFICATION

• **A.**
Analysis: Dilute a volume of Injection equivalent to 500 mg of aminophylline with water to 20 mL, and add, with constant stirring, 1 mL of 3 N hydrochloric acid or enough to completely precipitate the theophylline, and filter. To the filtrate add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline. Shake by mechanical means for 10 min, add 5 mL of 3 N hydrochloric acid to acidify, chill, collect the precipitated disulfonamide of ethylenediamine, wash with water, recrystallize from water, and dry at 105° for 1 h.
Acceptance criteria: The precipitate melts at 164°–171°.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Analysis: Wash the precipitated theophylline from *Identification A* with small portions of cold water, and dry at 105° for 1 h.
Acceptance criteria: The IR spectrum of theophylline so obtained corresponds to that of USP Theophylline RS.
• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Solution A: 10 mM ammonium acetate prepared as follows. Transfer 770.8 mg of ammonium acetate to a 1-L volumetric flask, and dissolve in water to 80% of the flask volume. Adjust with glacial acetic acid to a pH of 5.5 and dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

Impurity stock solution: 25 µg/mL of USP Theophylline Related Compound F RS in water
System suitability solution: 0.8 mg/mL of USP Theophylline RS and 1 µg/mL of USP Theophylline Related Compound F RS in water prepared as follows. Transfer 21 mg of USP Theophylline RS to a 25-mL volumetric flask, and add 15 mL of water. Sonicate to dissolve, add 1 mL of *Impurity stock solution*, and dilute with water to volume.
Standard solution: 0.17 mg/mL of USP Theophylline RS in water. Sonicate to dissolve as needed.
Sample solution: Nominally 0.17 mg/mL of anhydrous theophylline in water prepared as follows. Transfer 8.5 mg of anhydrous theophylline from a volume of Injection to a 50-mL volumetric flask. Dissolve and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

www.webofpharma.com

Detector: UV 270 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 40°
Flow rate: 0.4 mL/min
Injection volume: 1 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

OTHER COMPONENTS

• **CONTENT OF ETHYLENEDIAMINE**
Sample: A volume of Injection equivalent to 500 mg of aminophylline
Diluent: Water
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N hydrochloric acid VS
Endpoint detection: Visual
Analysis: If necessary, dilute the *Sample* with *Diluent* to 30 mL, add methyl orange TS, and titrate with *Titrant*. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine ($C_2H_8N_2$).
Acceptance criteria: 166–192 mg of ethylenediamine ($C_2H_8N_2$) per gram of theophylline ($C_7H_8N_4O_2$) found in the *Assay*

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Impurity stock solution, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 2.0 µg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS in water
Sample solution: Nominally 1.0 mg/mL of anhydrous aminophylline in water prepared as follows. Transfer 25 mg of anhydrous aminophylline from a volume of Injection to a 25-mL volumetric flask. Dissolve and dilute with water to volume.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*
Relative standard deviation: NMT 3.0% for each peak, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of theophylline related compound D in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline related compound D from the *Sample solution*
r_S = peak response of theophylline related compound D from the *Standard solution*
C_S = concentration of USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aminophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified degradation product from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aminophylline in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound C[a, b]	0.36	—
Theophylline related compound B[a, c]	0.63	—
Theophylline related compound D	0.69	0.2
Dimethyl uric acid[a, d]	0.76	—
Theobromine[a, e]	0.82	—
Theophylline	1.0	—
Theophylline related compound F[a]	1.09	—
Caffeine[a]	1.20	—
Any other individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

[a] Process impurity included for identification only and not to be included in the calculation of total degradation products.
[b] *N*-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl) formamide.
[c] 3-Methyl-1*H*-purine-2,6-dione.
[d] 1,3-Dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione.
[e] 3,7-Dihydro-3,7-dimethylpurine-2,6(1*H*)-dione.

SPECIFIC TESTS

• **pH** ⟨791⟩: 8.6–9.0
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

www.webofpharma.com

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 1.0 USP Endotoxin Unit/mg of aminophylline.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers from which carbon dioxide has been excluded, preferably of Type I glass, protected from light. Store at controlled room temperature.

• **LABELING:** Label the Injection to state the content of anhydrous theophylline.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Theophylline RS

USP Theophylline Related Compound D RS
Theophyllidine;
N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide hydrochloride monohydrate.
$C_6H_{10}N_4O \cdot HCl \cdot H_2O$ 208.65

USP Theophylline Related Compound F RS
7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.
$C_9H_{12}N_4O_3$ 224.22

Aminophylline Oral Solution

DEFINITION

Aminophylline Oral Solution is an aqueous solution of Aminophylline, prepared with the aid of Ethylenediamine. It contains an amount of aminophylline ($C_{16}H_{24}N_{10}O_4$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

Aminophylline Oral Solution may contain an excess of ethylenediamine, but no other substance may be added for the purpose of pH adjustment.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Analysis: Transfer a volume of Oral Solution equivalent to 500 mg of aminophylline to a suitable container and add, with constant stirring, 1 mL of 3 N hydrochloric acid or enough to completely precipitate the theophylline. Filter (retain the filtrate), wash the precipitate with small portions of cold water until free from chloride, and dry at 105° for 1 h.

Acceptance criteria: The IR spectrum of theophylline so obtained matches that of USP Theophylline RS.

• **B.**

Analysis: To the filtrate obtained in *Identification A* add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline. Shake by mechanical means for 10 min, add 5 mL of 3 N hydrochloric acid to acidify, chill, collect the precipitated disulfonamide of ethylenediamine, wash with water, recrystallize from water, and dry at 105° for 1 h.

Acceptance criteria: The dried precipitate melts at 164°–171°.

ASSAY

• **PROCEDURE**

Solution A: 10 mM ammonium acetate prepared as follows. Transfer an appropriate amount of ammonium acetate to a volumetric flask and dissolve in water (about 80% of the flask volume). Adjust with glacial acetic acid to a pH of 5.5 and dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

Impurity stock solution: 25 µg/mL of USP Theophylline Related Compound F RS in water

System suitability solution: 0.8 mg/mL of USP Theophylline RS and 2 µg/mL of USP Theophylline Related Compound F RS in water prepared as follows. Transfer 1 mg of USP Theophylline RS to a 25-mL volumetric flask and add 15 mL of water. Sonicate to dissolve, add 2 mL of *Impurity stock solution*, and dilute with water to volume.

Standard solution: 0.17 mg/mL of USP Theophylline RS in water. Sonicate to dissolve, as needed.

Sample solution: Nominally 0.17 mg/mL of anhydrous theophylline in water prepared as follows. Transfer an appropriate amount of anhydrous theophylline from a volume of Oral Solution to a suitable volumetric flask. Dissolve and dilute with water to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 40°
Flow rate: 0.4 mL/min
Injection volume: 1 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

USP Monographs

www.webofpharma.com

OTHER COMPONENTS

• **CONTENT OF ETHYLENEDIAMINE**

Sample: A volume of Oral Solution equivalent to 500 mg of aminophylline

Diluent: Water

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N hydrochloric acid VS

Endpoint detection: Visual

Analysis: If necessary, dilute the *Sample* with *Diluent* to 30 mL, add methyl orange TS, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine ($C_2H_8N_2$).

Acceptance criteria: 176–283 mg of ethylenediamine ($C_2H_8N_2$) per gram of theophylline ($C_7H_8N_4O_2$) found in the *Assay*

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Impurity stock solution, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 2.0 µg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS in water

Sample solution: Nominally 1.0 mg/mL of anhydrous aminophylline in water prepared as follows. Transfer an appropriate amount of anhydrous aminophylline from a volume of Oral Solution to a suitable volumetric flask. Dissolve and dilute with water to volume.

System suitability

Samples: *System suitability solution* and *Standard solution* [NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

Relative standard deviation: NMT 3.0% for each peak present in the *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of theophylline related compound D in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline related compound D from the *Sample solution*

r_S = peak response of theophylline related compound D from the *Standard solution*

C_S = concentration of USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of anhydrous theophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified degradation product from the *Sample solution*

r_S = peak response of theophylline from the *Standard solution*

C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of anhydrous theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.086%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound C[a, b]	0.36	—
Theophylline related compound B[a, c]	0.63	—
Theophylline related compound D	0.69	0.2
Dimethyl uric acid[a, d]	0.76	—
Theobromine[a, e]	0.82	—
Theophylline	1.0	—
Theophylline related compound F[a]	1.09	—
Caffeine[a]	1.20	—
Any other individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

[a] Process impurity included for identification only and not to be included in the calculation of total degradation products.

[b] *N*-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl) formamide.

[c] 3-Methyl-1*H*-purine-2,6-dione.

[d] 1,3-Dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione.

[e] 3,7-Dihydro-3,7-dimethylpurine-2,6(1*H*)-dione.

SPECIFIC TESTS

• **pH** ⟨791⟩: 8.5–9.7

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label the Oral Solution to state the content of anhydrous theophylline.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Theophylline RS

USP Theophylline Related Compound D RS

Theophyllidine;

N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide hydrochloride monohydrate.

$C_6H_{10}N_4O \cdot HCl \cdot H_2O$ 208.65

USP Theophylline Related Compound F RS

7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

$C_9H_{12}N_4O_3$ 224.22

Aminophylline Rectal Solution

DEFINITION

Aminophylline Rectal Solution is an aqueous solution of Aminophylline, prepared with the aid of Ethylenediamine. It contains an amount of aminophylline equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

Rectal Solution may contain an excess of ethylenediamine, but no other substance may be added for the purpose of pH adjustment.

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Dilute an amount of Rectal Solution, equivalent to 500 mg of aminophylline, with water to 20 mL. Add, with constant stirring, 1 mL of 3 N hydrochloric acid or enough to precipitate the theophylline completely, and filter (save the filtrate). Wash the precipitate with a small portion of cold water until free from chloride, and dry at 105° for 4 h.
Acceptance criteria: The dried precipitate meets the requirements.

• **B.**
Analysis: To the filtrate obtained in *Identification* test *A* add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline, shake by mechanical means for 10 min, and add 5 mL of 3 N hydrochloric acid to acidify. Chill, collect the precipitated disulfonamide of ethylenediamine, and wash with water. Recrystallize the washed precipitate from water, and dry at 105° for 1 h.
Acceptance criteria: The dried precipitate melts at 164°–171°.

ASSAY

• **PROCEDURE**
Standard solution: 8 µg/mL of USP Theophylline RS in dilute hydrochloric acid (1:100)
Sample solution: Pipet a volume of Rectal Solution equivalent to 500 mg of aminophylline into a 500-mL volumetric flask, and dilute with water to volume. Pipet 5 mL of this solution to a second 500-mL volumetric flask, add 50-mL of dilute hydrochloric acid (1:10), and dilute with water to volume.
Instrumental conditions
Mode: UV
Analytical wavelength: About 270 nm
Cell: 1 cm
Blank: Dilute hydrochloric acid (1:100)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$) in the portion of Rectal Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **CONTENT OF ETHYLENEDIAMINE**
Sample solution: Measure a volume of Rectal Solution, equivalent to 500 mg of aminophylline, and dilute with water if necessary to make 30 mL.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N hydrochloric acid VS
Endpoint detection: Visual
Analysis: Add methyl orange TS to the *Sample solution*, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylene diamine ($C_2H_8N_2$).
Acceptance criteria: 218–267 mg of ethylenediamine ($C_2H_8N_2$) per g of theophylline ($C_7H_8N_4O_2$) found in the *Assay*

SPECIFIC TESTS

• **pH** ⟨791⟩: 9.0–9.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, single-dose or multiple-dose containers at controlled room temperature.
• **LABELING:** Label the Rectal Solution to state the content of anhydrous theophylline.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Theophylline RS

Aminophylline Suppositories

DEFINITION

Aminophylline Suppositories contain an amount of aminophylline equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

IDENTIFICATION

• **A.**
Analysis: Evaporate on a steam bath a portion of *Sample stock solution* from the *Assay*, equivalent to 500 mg of aminophylline, to about one-half its volume. Adjust with 1 N sodium hydroxide to a pH of 7.0, chill, and filter the crystals of theophylline. Retain the filtrate, free from washings. Wash the crystals of theophylline with small portions of ice-cold water, and dry at 105° for 1 h.
Acceptance criteria: The recrystallized theophylline melts at 270°–274°.

• **B.**
Analysis: Transfer 10 mg of the dried precipitate from *Identification* test *A* to a porcelain dish, and add 1 mL of hydrochloric acid and 100 mg of potassium chlorate. Evaporate on a steam bath to dryness, and invert the dish over a vessel containing a few drops of 6 N ammonium hydroxide.
Acceptance criteria: The residue acquires a purple color, which is destroyed by solutions of fixed alkalies.

• **C.**
Analysis: To the filtrate obtained in *Identification* test *A* add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline, shake by mechanical means for 10 min, and add 5 mL of 3 N hydrochloric acid to acidify. Chill, collect the precipitated disulfonamide of ethylenediamine, and wash with water. Recrystallize the washed precipitate from water, and dry at 105° for 1 h.
Acceptance criteria: The dried precipitate melts at 164°–171°.

ASSAY

• **PROCEDURE**
Sample stock solution: Transfer NLT 5 Suppositories to a tared small dish and a glass rod, and heat on a steam bath until the suppositories are melted. Mix the melt by stirring it with the rod, and cool while stirring. Transfer a portion of the cooled melt, equivalent to 1 g of aminophylline, into a beaker, add 60 mL of hot water and 3 mL of nitric acid, and heat on a steam bath for 15 min with frequent stirring. Cool, transfer to a separator with the aid of 40 mL of ether, shake well, and allow to separate, using a few mL of alcohol if necessary to bring about separation of any emulsion that has formed. Draw the water layer into a 100-mL volumetric flask; wash the ether with two 15-mL portions of water,

www.webofpharma.com

adding the washings to the volumetric flask; and dilute with water to volume.

Sample solution: Transfer a portion of the *Sample stock solution*, equivalent to 250 mg of aminophylline, to a 250-mL conical flask. Add 10 mL of 6 N ammonium hydroxide and 20 mL of 0.1 N silver nitrate VS, and heat on a steam bath for 15 min. Cool to between 5° and 10° for 20 min; filter, preferably through a filtering crucible of fine porosity under reduced pressure; and wash the precipitate with small portions of water until the last washing gives NMT a faint opalescence with hydrochloric acid. Dissolve the precipitate by pouring over it small volumes of warm 2 N nitric acid, collecting the solution in a conical flask. Wash the filtering crucible a few times with warm water acidified with nitric acid, receiving the washings in the same flask. Cool, and add 2 mL of ferric ammonium sulfate TS.

Analysis: Titrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N ammonium thiocyanate is equivalent to 18.02 mg of theophylline ($C_7H_8N_4O_2$).

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **CONTENT OF ETHYLENEDIAMINE**

Sample solution: Weigh a portion of the stirred, congealed mass of the Suppositories from the *Assay*, equivalent to 500 mg of aminophylline, and place in a 500-mL conical flask. Add 150 mL of a mixture of equal volumes of alcohol and ether, and warm gently under reflux for 30 min, with occasional swirling. Cool to room temperature.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N hydrochloric acid VS

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* using a glass-modified calomel electrode system (replace the saturated potassium chloride solution of the calomel electrode with methanol saturated with lithium chloride). Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine ($C_2H_8N_2$).

Acceptance criteria: 152–190 mg of ethylenediamine ($C_2H_8N_2$) per g of theophylline ($C_7H_8N_4O_2$) found in the *Assay*

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, in a cold place.

• **LABELING:** Label the Suppositories to state the content of anhydrous theophylline.

Aminophylline Tablets

DEFINITION

Aminophylline Tablets contain an amount of aminophylline equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

[NOTE—The ammoniacal odor present in the vapor space above the Tablets is often quite strong, especially when bottles having suitably tight closures are newly opened. This is due to ethylenediamine vapor pressure build-up, a natural condition in the case of aminophylline.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)

Analysis: Macerate a quantity of Tablets, equivalent to 500 mg of aminophylline, with 25 mL of water, and filter. The filtrate is alkaline to litmus. To the filtrate add 1 mL of 3 N hydrochloric acid, stir, and if necessary, chill to precipitate the theophylline. Filter, and retain the filtrate, free from washings. Use the filtrate in *Identification C*. Wash the theophylline crystals so obtained with small quantities of ice-cold water, and dry at 105° for 1 h.

Acceptance criteria: The IR spectrum of theophylline so obtained corresponds to that of USP Theophylline RS.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C.**

Sample: The filtrate obtained in *Identification A*

Analysis: To the *Sample* add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline, shake by mechanical means for 10 min, and add 5 mL of 3 N hydrochloric acid to acidify. Chill, collect the precipitated disulfonamide of ethylenediamine, and wash with water. Recrystallize the washed precipitate from water, and dry at 105° for 1 h.

Acceptance criteria: The dried precipitate melts at 164°–171°.

ASSAY

• **PROCEDURE**

Solution A: 10 mM ammonium acetate prepared as follows. Transfer 770.8 mg of ammonium acetate to a 1-L volumetric flask, and dissolve in water to 80% of the flask volume. Adjust with glacial acetic acid to a pH of 5.5 and dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

Impurity stock solution: 25 µg/mL of USP Theophylline Related Compound F RS in water

System suitability solution: 0.8 mg/mL of USP Theophylline RS and 1 µg/mL of USP Theophylline Related Compound F RS in water prepared as follows. Transfer 21 mg of USP Theophylline RS to a 25-mL volumetric flask and add 15 mL of water. Sonicate to dissolve, add 1 mL of *Impurity stock solution*, and dilute with water to volume.

Standard solution: 0.17 mg/mL of USP Theophylline RS in water. Sonicate to dissolve as needed.

Sample solution: Nominally 0.17 mg/mL of anhydrous theophylline from NLT 20 finely powdered Tablets in water prepared as follows. Transfer 34 mg of anhydrous theophylline from a portion of the powder to a 200-mL

USP Monographs

www.webofpharma.com

volumetric flask. Add 20 mL of water and mix for 1 min. Add an additional 140 mL of water and sonicate for 30 min. Dilute with water to volume. Pass through a suitable filter of 0.22-µm pore size, discarding the first 2–3 mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 40°
Flow rate: 0.4 mL/min
Injection volume: 1 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

OTHER COMPONENTS

• **CONTENT OF ETHYLENEDIAMINE**

Sample solution: Transfer a portion of the powdered Tablets, equivalent to 350 mg of aminophylline, prepared in the *Assay*, into a 100-mL conical flask. Add 20 mL of water, and digest at 50°, with frequent shaking, for 30 min. Cool, filter into a 250-mL conical flask, and wash with water until the last washing is neutral to litmus. Use the combined filtrate and washings.

Titrimetric system
Mode: Direct titration
Titrant: 0.1 N hydrochloric acid VS
Endpoint detection: Visual

Analysis: Add methyl orange TS to the *Sample solution*, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine ($C_2H_8N_2$).

Acceptance criteria: 140–190 mg of ethylenediamine ($C_2H_8N_2$) per gram of theophylline ($C_7H_8N_4O_2$) found in the *Assay*

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

For uncoated or plain coated tablets
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: USP Theophylline RS in *Medium*
Sample solution: Proceed as directed in the chapter for sample. Dilute with water to a concentration that is similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: UV about 269 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of theophylline ($C_7H_8N_4O_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity
Standard solution: 10 µg/mL of USP Theophylline RS
Sample solution: Place 1 Tablet in a 250-mL volumetric flask, add 200 mL of water, and shake by mechanical means until disintegration is complete. Add water to volume. Filter a portion of the mixture, discarding the first 20 mL of the filtrate.
Instrumental conditions
Mode: UV
Analytical wavelength: About 269 nm
Cell: 1 cm
Blank: Water
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of theophylline in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Impurity stock solution, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 2.0 µg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS in water
Sample solution: Nominally 1.0 mg/mL of anhydrous aminophylline from NLT 20 finely powdered Tablets in water prepared as follows. Transfer 10 mg of anhydrous aminophylline from a portion of the powder to a 10-mL volumetric flask. Add 5 mL of water and sonicate for 30 min. Dilute with water to volume. Pass through a suitable filter of 0.22-µm pore size, discarding the first 2–3 mL.

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*
Relative standard deviation: NMT 3.0% for each peak, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of theophylline related compound D in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of theophylline related compound D from the *Sample solution*

USP Monographs

www.webofpharma.com

r_S = peak response of theophylline related compound D from the *Standard solution*
C_S = concentration of USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aminophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified degradation product from the *Sample solution*
r_S = peak response of theophylline from the *Standard solution*
C_S = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aminophylline in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound C[a,b]	0.36	—
Theophylline related compound B[a,c]	0.63	—
Theophylline related compound D	0.69	0.2
Dimethyl uric acid[a,d]	0.76	—
Theobromine[a,e]	0.82	—
Theophylline	1.0	—
Theophylline related compound F[a]	1.09	—
Caffeine[a]	1.20	—
Any other individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

[a] Process impurity included for identification only and not to be included in the calculation of total degradation products.
[b] *N*-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl) formamide.
[c] 3-Methyl-1*H*-purine-2,6-dione.
[d] 1,3-Dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione.
[e] 3,7-Dihydro-3,7-dimethylpurine-2,6(1*H*)-dione.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** Label the Tablets to state the content of anhydrous theophylline.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Theophylline RS
  USP Theophylline Related Compound D RS
  Theophyllidine;
  N-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide hydrochloride monohydrate.
  $C_6H_{10}N_4O \cdot HCl \cdot H_2O$ 208.65
  USP Theophylline Related Compound F RS
  7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.
  $C_9H_{12}N_4O_3$ 224.22

Aminophylline Delayed-Release Tablets

DEFINITION

Aminophylline Delayed-Release Tablets contain an amount of aminophylline equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).
[NOTE—The ammoniacal odor present in the vapor space above Tablets is often quite strong, especially when bottles having suitably tight closures are newly opened. This is due to ethylenediamine vapor pressure build-up, a natural condition in the case of aminophylline.]

IDENTIFICATION

- **A.**
  Analysis: Macerate a quantity of Tablets, equivalent to 500 mg of aminophylline, with 25 mL of water, and filter: the filtrate is alkaline to litmus. To the filtrate add 1 mL of 3 N hydrochloric acid, stir, and if necessary, chill to precipitate the theophylline. Filter, and retain the filtrate, free from washings. Wash the theophylline crystals so obtained with small quantities of ice-cold water, and dry at 105° for 1 h. Transfer 10 mg of the dried theophylline crystals to a porcelain dish, add 1 mL of hydrochloric acid and 100 mg of potassium chlorate, evaporate on a steam bath to dryness, and invert the dish over a vessel containing a few drops of 6 N ammonium hydroxide.
  Acceptance criteria: The residue acquires a purple color, which is destroyed by solutions of fixed alkalies.
- **B.**
  Analysis: Recrystallize the dried theophylline crystals from *Identification* test *A* from water, and dry at 105° for 1 h.
  Acceptance criteria: The recrystallized theophylline melts at 270°–274°.
- **C.**
  Analysis: To the filtrate obtained in *Identification* test *A* add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline, shake by mechanical means for 10 min, and add 5 mL of 3 N hydrochloric acid to acidify. Chill, collect the precipitated disulfonamide of ethylenediamine, and wash with water. Recrystallize the washed precipitate from water, and dry at 105° for 1 h.
  Acceptance criteria: The dried precipitate melts at 164°–171°.

ASSAY

- **PROCEDURE**
  Sample solution: Transfer an equivalent to 2 g of aminophylline, from powdered Tablets (NLT 20), to a 200-mL volumetric flask with the aid of a mixture of 50 mL of water and 15 mL of 6 N ammonium hydroxide, and allow to stand for 30 min with frequent shaking, warming to 50° if necessary to dissolve the aminophylline. Cool the mixture to room temperature if it has been warmed, and add water to volume. Centrifuge 50 mL of the mixture; pipet a portion of the clear supernatant, equivalent to 250 mg of aminophylline, into a 250-mL conical flask; and dilute with water if necessary to make 40 mL. Add 8 mL of 6 N ammonium hydroxide and 20.0 mL of 0.1 N silver nitrate VS, mix, heat to boiling, and continue boiling for 15 min. Cool to between 5° and 10° for 20 min, then filter, preferably through a filtering crucible under reduced pressure, and wash the precipitate with three 10-mL portions of water. Acidify the combined filtrate and washings with nitric acid, and add an additional 3 mL of the acid. Cool, and add 2 mL of ferric ammonium sulfate TS.
  Titrimetric system
  Mode: Residual titration
  Titrant: 0.1 N ammonium thiocyanate VS
  Endpoint detection: Visual

USP Monographs

www.webofpharma.com

Analysis: Titrate the excess silver nitrate with *Titrant*. Each mL of 0.1 N silver nitrate is equivalent to 18.02 mg of theophylline ($C_7H_8N_4O_2$).

Acceptance criteria: 93.0%–107.0%

OTHER COMPONENTS

• **CONTENT OF ETHYLENEDIAMINE**

Sample solution: Transfer a portion, equivalent to 350 mg of aminophylline, of the powdered Tablets prepared in the *Assay* into a 100-mL conical flask. Add 20 mL of water, and digest at 50°, with frequent shaking, for 30 min. Cool, filter into a 250-mL conical flask, and wash with water until the last washing is neutral to litmus. Use the combined filtrate and washings.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N hydrochloric acid VS

Endpoint detection: Visual

Analysis: Add methyl orange TS to the *Sample solution*, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine ($C_2H_8N_2$).

Acceptance criteria: 140–190 mg of ethylenediamine ($C_2H_8N_2$) per g of theophylline ($C_7H_8N_4O_2$) found in the *Assay*

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩**:** 30 min, determined as directed in *Delayed-Release (Enteric-Coated) Tablets*

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Standard solution: 10 µg/mL of USP Theophylline RS

Sample solution: Place 1 Tablet in a 250-mL volumetric flask, add 200 mL of water, and shake by mechanical means until disintegration is complete. Add water to volume. Filter a portion of the mixture, discarding the first 20 mL of the filtrate.

Instrumental conditions

Mode: UV

Analytical wavelength: About 269 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in each Tablet:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Theophylline RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of theophylline in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

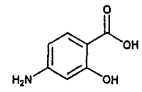
• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label the Tablets to state the content of anhydrous theophylline.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Theophylline RS

Aminosalicylic Acid



$C_7H_7NO_3$ 153.14

Benzoic acid, 4-amino-2-hydroxy-;
4-Aminosalicylic acid [65-49-6].

DEFINITION

Aminosalicylic Acid contains NLT 98.5% and NMT 100.5% of aminosalicylic acid ($C_7H_7NO_3$), calculated on the anhydrous basis.

[**CAUTION**—Under no circumstances use a solution prepared from Aminosalicylic Acid if its color is darker than that of a freshly prepared solution.]

IDENTIFICATION

• **A.**

Sample stock solution: Dissolve 250 mg in 3 mL of 1 N sodium hydroxide, transfer to a 500-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer a 5-mL aliquot of the *Sample stock solution* to a 250-mL volumetric flask containing 12.5 mL of pH 7 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*), and dilute with water to volume.

Analysis: Compare the *Sample solution* in a suitable spectrometer against a blank of the same buffer in the same concentration.

Acceptance criteria: The *Sample solution* exhibits absorbance maxima at 265 ± 2 and 299 ± 2 nm, and the ratio A_{265}/A_{299} is 1.50–1.56.

• **B.**

Sample: 1 g

Analysis: Place the *Sample* in a small, round-bottom flask, and add 10 mL of acetic anhydride. Heat the flask on a steam bath for 30 min, add 40 mL of water, filter, cool, and allow to stand until the diacetyl derivative has crystallized. Collect the precipitate on a filter, wash well with water, and dry at 105° for 1 h.

Acceptance criteria: The diacetyl derivative melts at 191°–197°.

• **C.**

Sample: 0.1 g

Analysis: Shake the *Sample* with 10 mL of water, and filter. To 5 mL of the filtrate add 1 drop of ferric chloride TS.

Acceptance criteria: A violet color is produced.

ASSAY

• **PROCEDURE**

Solution A: 12.7 mg/mL of tetrabutylammonium hydroxide in methanol

Mobile phase: *Solution A*, 0.05 M dibasic sodium phosphate, and 0.05 M monobasic sodium phosphate (150:425:425)

Internal standard solution: 5 mg/mL of acetaminophen in *Mobile phase*

Standard solution: 0.5 mg/mL of aminosalicylic acid prepared as follows. Transfer 12.5 mg of USP Aminosalicylic Acid RS to a 25-mL low-actinic volumetric flask, add 15 mL of *Mobile phase*, and swirl to dissolve. Add 2.5 mL of the *Internal standard solution*, and dilute with *Mobile phase* to volume.

Sample solution: 0.5 mg/mL of Aminosalicylic Acid prepared as follows. Transfer 12.5 mg of Aminosalicylic Acid to a 25-mL low-actinic volumetric flask, add 15 mL of *Mobile phase*, and swirl to dissolve. Add 2.5 mL of the

USP Monographs

www.webofpharma.com

Internal standard solution, and dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for acetaminophen and aminosalicylic acid are 0.83 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.7 between aminosalicylic acid and acetaminophen
Relative standard deviation: NMT 1.0% for the peak response ratio of aminosalicylic acid to acetaminophen
Analysis
Samples: *Standard solution* and *Sample solution*
After use, wash the column for 30 min with a mixture of methanol, water, and phosphoric acid (77: 23: 0.6), and then wash for 30 min with a mixture of methanol and water (50:50).
Calculate the percentage of aminosalicylic acid ($C_7H_7NO_3$) in the portion of Aminosalicylic Acid taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of aminosalicylic acid to acetaminophen from the *Sample solution*
R_S = peak response ratio of aminosalicylic acid to acetaminophen from the *Standard solution*
C_S = concentration of USP Aminosalicylic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of Aminosalicylic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–100.5% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample solution: 25 mg/mL in a mixture of nitric acid and water (5:15)
Acceptance criteria: NMT 0.042%; the solution shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid.
- **LIMIT OF *m*-AMINOPHENOL**
Solution A: 12.7 mg/mL of tetrabutylammonium hydroxide in methanol
Mobile phase: *Solution A*, 0.05 M dibasic sodium phosphate, and 0.05 M monobasic sodium phosphate (150:425:425)
Internal standard solution: 5 µg/mL of sulfanilamide in *Mobile phase*
Standard stock solution: 12 µg/mL of USP *m*-Aminophenol RS in *Mobile phase*
Standard solution: 1.2 µg/mL of USP *m*-Aminophenol RS prepared as follows. Transfer 10.0 mL of the *Standard stock solution* and 10.0 mL of the *Internal standard solution* to a 100-mL low-actinic volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: 0.5 mg/mL of Aminosalicylic Acid prepared as follows. Transfer 50 mg of Aminosalicylic Acid to a 100-mL low-actinic volumetric flask, add 50 mL of *Mobile phase*, and swirl to dissolve. Add 10.0 mL of the *Internal standard solution*, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 10-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for sulfanilamide and *m*-aminophenol are about 0.66 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.5 between *m*-aminophenol and sulfanilamide
Relative standard deviation: NMT 7%
Analysis
Samples: *Standard solution* and *Sample solution*
After use, wash the column for 30 min with a mixture of methanol, water, and phosphoric acid (77: 23: 0.6), and then wash for 30 min with a mixture of methanol and water (50:50).
Calculate the percentage of *m*-aminophenol in the portion of Aminosalicylic Acid taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of *m*-aminophenol to sulfanilamide from the *Sample solution*
R_S = peak response ratio of *m*-aminophenol to sulfanilamide from the *Standard solution*
C_S = concentration of USP *m*-Aminophenol RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution*, as determined in the *Assay* (mg/mL)

Acceptance criteria: NMT 0.25%

SPECIFIC TESTS

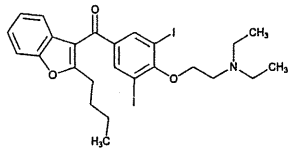
- **pH** ⟨791⟩: 3.0–3.7, in a saturated solution
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%
- **HYDROGEN SULFIDE, SULFUR DIOXIDE, AND AMYL ALCOHOL**
Sample: 500 mg
Analysis: Dissolve the *Sample* in 5 mL of 1 N sodium hydroxide, add 6 mL of 3 N hydrochloric acid, and stir vigorously.
Acceptance criteria: No odor of hydrogen sulfide or sulfur dioxide is perceptible, and NMT a faint odor of amyl alcohol is perceptible. A piece of moistened lead acetate test paper held over the mixture does not become discolored.
- **CLARITY AND COLOR OF SOLUTION**
Sample 1: 1 g
Analysis 1: Dissolve *Sample 1* in 10 mL of sodium bicarbonate solution (1 in 15).
Acceptance criteria 1: The resulting solution is clear and has NMT a faint yellow color.
Sample 2: 1 g
Analysis 2: Dissolve *Sample 2* in 50 mL of freshly prepared 1.6 M nitric acid.
Acceptance criteria 2: The resulting solution is clear and has NMT a slight color.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature not exceeding 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP *m*-Aminophenol RS
USP Aminosalicylic Acid RS

www.webofpharma.com

Amiodarone Hydrochloride



$C_{25}H_{29}I_2NO_3 \cdot HCl$ 681.77

Methanone, (2-butyl-3-benzofuranyl)[4-[2-(diethyl amino) ethoxy]-3,5-diiodophenyl]- hydrochloride;

2-Butyl-3-benzofuranyl 4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone hydrochloride [19774-82-4].

2-Butyl-3-benzofuranyl 4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone [1951-25-3].

DEFINITION

Amiodarone Hydrochloride contains NLT 98.5% and NMT 101.0% of $C_{25}H_{29}I_2NO_3 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: Dissolve 6.80 g of monobasic potassium phosphate in 900 mL of water, and add 1.0 mL of triethylamine. Adjust with phosphoric acid to a pH of 6.00 ± 0.05, and dilute with water to 1000 mL.

Diluent: Acetonitrile and water (1:1)

Mobile phase: Acetonitrile and *Buffer* (1:1)

Standard stock solution: 0.5 mg/mL of USP Amiodarone Hydrochloride RS in methanol

Standard solution: 0.1 mg/mL USP Amiodarone Hydrochloride RS in *Diluent* from *Standard stock solution*

Sample stock solution: 0.5 mg/mL of Amiodarone Hydrochloride in methanol

Sample solution: 0.1 mg/mL of Amiodarone Hydrochloride in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 3.9-mm × 15-cm; 5-µm packing L26

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{25}H_{29}I_2NO_3 \cdot HCl$ in the portion of Amiodarone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of amiodarone in the *Sample solution*

r_S = peak response of amiodarone in the *Standard solution*

C_S = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of Amiodarone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0%, on the dried basis

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1% on a 1-g sample

ORGANIC IMPURITIES

[NOTE—The product meets the requirements for both *Procedure 1* and *Procedure 2*.]

- **Procedure 1**

Potassium iodobismuthate solution: Dissolve 100 g of tartaric acid in 400 mL of water, and add 8.5 g of bismuth subnitrate. Shake for 1 h, add 200 mL of a 400 g/L solution of potassium iodide, and shake well. Allow to stand for 24 h, filter, and protect from light.

Standard solution A: 0.02 mg/mL of USP Amiodarone Related Compound H RS in methylene chloride

Standard solution B: *Standard solution A* and *Sample solution* (1:1).

Sample solution: 100 mg/mL of Amiodarone Hydrochloride in methylene chloride

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: Suitable layer of chromatographic silica gel and fluorescent indicator with maximum absorbance at 254 nm

Application volume

Standard solution A: 50 µL

Standard solution B: 100 µL

Sample solution: 50 µL

Developing solvent system: Methylene chloride, methanol, and anhydrous formic acid (17:2:1)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the plate in the *Developing solvent system* until the solvent front has moved NLT two-thirds the length of the plate, and dry in a current of cold air. Spray the plate with *Potassium iodobismuthate solution* and then with 3% hydrogen peroxide solution. Examine immediately in daylight: the spot from *Standard solution B* due to amiodarone related compound H is clearly visible.

Acceptance criteria: Any spot with the same R_F as the spot due to amiodarone related compound H from the *Sample solution* is not more intense than the spot from *Standard solution A* (0.02%).

- **Procedure 2**

Buffer: Add 3 mL of glacial acetic acid to 800 mL of water. Adjust with diluted ammonia solution to a pH of 4.9, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile: methanol: *Buffer* (4:3:3 v/v/v).

Diluent: Acetonitrile and water (1:1)

Standard stock solution: Dissolve equal quantities of USP Amiodarone Related Compound D RS, USP Amiodarone Related Compound E RS, and USP Amiodarone Hydrochloride RS in a known amount of methanol.

Standard solution: 0.01 mg/mL each of USP Amiodarone Related Compound D RS, USP Amiodarone Related Compound E RS, and USP Amiodarone Hydrochloride RS, in *Diluent* from *Standard stock solution*

Sample solution: 5 mg/mL of Amiodarone Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

USP Monographs

www.webofpharma.com

Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 10 µL
Run time: 2 times the retention time of amiodarone
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.5 between amiodarone related compound D and amiodarone related compound E
Analysis
[NOTE—Disregard any peak that is less than 0.05%.]
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Amiodarone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity in the *Sample solution*
r_S = peak response of amiodarone in the *Standard solution*
C_S = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Amiodarone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amiodarone related compound A[a]	0.26	0.2
Amiodarone related compound D[b]	0.29	0.2
Amiodarone related compound E[c]	0.37	0.2
Amiodarone related compound B[d]	0.49	0.2
Amiodarone related compound C[e]	0.55	0.2
Amiodarone related compound G[f]	0.62	0.2
Amiodarone related compound F[g]	0.69	0.2
Amiodarone hydrochloride	1.00	—
Any other individual impurity	—	0.10

[a] (2-Butylbenzofuran-3-yl){4-[2-(diethylamino)ethoxy]phenyl}methanone.
[b] (2-Butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl)methanone.
[c] (2-Butylbenzofuran-3-yl)(4-hydroxyphenyl)methanone.
[d] (2-Butylbenzofuran-3-yl){4-[2-(ethylamino)ethoxy]-3,5-diiodophenyl}methanone.
[e] (2-Butylbenzofuran-3-yl){4-[2-(diethylamino)ethoxy]-3-iodophenyl}methanone.
[f] [2-[(1*RS*)-1-Methoxybutyl]benzofuran-3-yl][4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]methanone.
[g] (2-Butylbenzofuran-3-yl)(4-hydroxy-3-iodophenyl)methanone.

SPECIFIC TESTS
• **LIMIT OF IODIDES**
Solution A: Add 1.50 g of Amiodarone Hydrochloride to 40 mL of water at 80°, and shake until completely dissolved. Cool, and dilute with water to 50.0 mL.
Standard solution: To 15.0 mL of *Solution A* add 1.0 mL of 0.1 M hydrochloric acid, 1.0 mL of an 88.2 mg/L solution of potassium iodide, and 1.0 mL of 0.05 M potassium iodate. Dilute with water to 20.0 mL. Allow to stand protected from light for 4 h.
Sample solution: To 15.0 mL of *Solution A* add 1.0 mL of 0.1 M hydrochloric acid and 1.0 mL of 0.05 M potassium iodate. Dilute with water to 20.0 mL. Allow to stand protected from light for 4 h.
Analysis: Measure the absorbances of the *Standard solution* and the *Sample solution* at 420 nm, using a mixture of 15.0 mL of *Solution A* and 1.0 mL of 0.1 M hydrochloric acid diluted with water to 20.0 mL to serve as the blank. The absorbance of the *Sample solution* is NMT half the absorbance of the *Standard solution*.
Acceptance criteria: NMT 150 ppm
• **pH** ⟨791⟩**:** 3.2–3.8. Dissolve 1 g of Amiodarone Hydrochloride in water by heating at 80°. Cool, and dilute with water to 20 mL.
• **LOSS ON DRYING** ⟨731⟩**:** Use 1 g of sample, and dry under vacuum (NMT 0.3 kPa) at 50° for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in light-resistant, tight containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amiodarone Hydrochloride RS
USP Amiodarone Related Compound D RS
(2-Butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl) methanone.
$C_{19}H_{16}I_2O_3$ 546.14
USP Amiodarone Related Compound E RS
(2-Butylbenzofuran-3-yl)(4-hydroxyphenyl) methanone.
$C_{19}H_{18}O_3$ 294.34
USP Amiodarone Related Compound H RS
2-Chloro-*N*,*N*-diethylethanamine.
$C_6H_{14}ClN$ 135.64

Amiodarone Hydrochloride Injection

DEFINITION
Amiodarone Hydrochloride Injection is a sterile solution of Amiodarone Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3 \cdot HCl$). It may contain suitable preservatives.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

• **PROCEDURE**
Buffer: 1.36 g/L of potassium phosphate, monobasic in water prepared as follows. To 1.36 g of potassium phosphate, monobasic in a 1-L volumetric flask add about 900 mL of water and 1 mL of triethylamine. Adjust with phosphoric acid to a pH of 6.0. Dilute with water to volume.
Mobile phase: Acetonitrile and *Buffer* (80:20)
Diluent: Acetonitrile and water (60:40)
Standard solution: 0.025 mg/mL of USP Amiodarone Hydrochloride RS in *Diluent*

www.webofpharma.com

Sample solution: Nominally ▲0.025▲ (USP 1-Dec-2019) mg/mL of amiodarone hydrochloride in *Diluent* from a suitable volume of Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Dec-2019)
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of amiodarone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak ▲response▲ (USP 1-Dec-2019) of amiodarone from the *Sample solution*
r_S = peak ▲response▲ (USP 1-Dec-2019) of amiodarone from the *Standard solution*
C_S = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amiodarone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

Change to read:

• **CONTENT OF BENZYL ALCOHOL** (if present)
Internal standard solution: 1 mg/mL of phenol in isopropyl alcohol
Standard stock solution: ▲1.6 of▲ (USP 1-Dec-2019) mg/mL of USP Benzyl Alcohol RS in isopropyl alcohol
Standard solution: ▲0.2 mg/mL of phenol and 0.19 mg/mL of USP Benzyl Alcohol RS in isopropyl alcohol from the *Internal standard solution* and the *Standard stock solution*▲ (USP 1-Dec-2019)
Sample stock solution: Nominally ▲1.6▲ (USP 1-Dec-2019) mg/mL of benzyl alcohol in isopropyl alcohol▲from Injection▲ (USP 1-Dec-2019)
Sample solution: Nominally 0.2 mg/mL of phenol and 0.19 mg/mL of benzyl alcohol in isopropyl alcohol▲from the *Internal standard solution* and the *Sample stock solution*▲ (USP 1-Dec-2019)
Blank: 0.2 mg/mL of phenol in isopropyl alcohol▲from the *Internal standard solution*▲ (USP 1-Dec-2019)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m ▲fused silica capillary;▲ (USP 1-Dec-2019) coated with 1-µm ▲film of▲ (USP 1-Dec-2019) phase G16
Temperatures
Injection port: 200°
Column: 150°
Detector: 200°
Carrier gas: Nitrogen
Flow rate: 10 mL/min
Injection volume: 1 µL
Injection type: Split; split ratio, 10:1
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0% for the peak response ratio of benzyl alcohol to phenol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzyl alcohol in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of benzyl alcohol to phenol from the *Sample solution*
R_S = peak response ratio of benzyl alcohol to phenol from the *Standard solution*
C_S = concentration of USP Benzyl Alcohol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzyl alcohol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

• **LIMIT OF IODIDE**
Use freshly prepared solutions in amber glassware.
Amiodarone stock solution: ▲Nominally▲ (USP 1-Dec-2019) 5 mg/mL of amiodarone hydrochloride in water from Injection ▲▲ (USP 1-Dec-2019)
Potassium iodide solution: 88.2 µg/mL of potassium iodide in water
Potassium iodate solution: 10.7 g/L of potassium iodide in water
Standard solution: 4.41 µg/mL ▲of potassium iodide▲ (USP 1-Dec-2019) prepared as follows. Into a suitable flask pipet 15.0 mL of *Amiodarone stock solution*, 1.0 mL of ▲0.1 N▲ (USP 1-Dec-2019)hydrochloric acid, 1.0 mL of *Potassium iodide solution*, 1.0 mL of *Potassium iodate solution*, and 2.0 mL of water. Mix, and allow to stand for 4 h. Protect from light.
Sample solution: ▲Nominally 3.75 mg/mL of amiodarone hydrochloride prepared as follows.▲ (USP 1-Dec-2019) Into a suitable flask pipet 15.0 mL of *Amiodarone stock solution*, 1.0 mL of ▲0.1 N▲ (USP 1-Dec-2019)hydrochloric acid, 1.0 mL of *Potassium iodate solution*, and 3.0 mL of water. Mix, and allow to stand for 4 h. Protect from light.
Blank: Into a suitable flask pipet 15.0 mL of *Amiodarone stock solution*, 1.0 mL of ▲0.1 N▲ (USP 1-Dec-2019)hydrochloric acid, and 4.0 mL of water. Mix, and allow to stand for 4 h. Protect from light.
Instrumental conditions
Mode: ▲▲ (USP 1-Dec-2019)Vis
Analytical wavelength: 420 nm
Cell: 1 cm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the amount of iodide, in ppm, in the portion of Injection taken:

$$\text{Result} = (A_U - A_B)/[(A_S - A_B) - (A_U - A_B)] \times (C_S/C_U) \times (M_{r1}/M_{r2})$$

USP Monographs

www.webofpharma.com

A_U = absorbance of the *Sample solution*
A_B = absorbance of the *Blank*
A_S = absorbance of the *Standard solution*
C_S = concentration of potassium iodide in the *Standard solution* (µg/mL)
C_U = ▲nominal▲ (USP 1-Dec-2019) concentration of amiodarone hydrochloride in the *Sample solution* (g/mL)
M_{r1} = molecular weight of iodide, 126.90
M_{r2} = molecular weight of potassium iodide, 166.00

Acceptance criteria: NMT 250 ppm

Change to read:

- **ORGANIC IMPURITIES**

Buffer: ▲Add 3 mL of acetic acid, glacial to 800 mL of water. Adjust with ammonia TS to a pH of 4.9. Dilute with water to 1000 mL.▲ (USP 1-Dec-2019)
Mobile phase: Acetonitrile, methanol, and *Buffer* (40:30:30)
Diluent: Acetonitrile, methanol, and water (50:30:20)
▲Standard stock solution A: 0.2 mg/mL of USP Amiodarone Hydrochloride RS in *Diluent*
Standard stock solution B: 0.4 mg/mL of USP Amiodarone Related Compound E RS in *Diluent*
Standard solution: 1 µg/mL of USP Amiodarone Hydrochloride RS, 30 µg/mL of USP Amiodarone Related Compound D RS, and 2 µg/mL of USP Amiodarone Related Compound E RS in *Diluent* prepared as follows. Transfer a suitable quantity of USP Amiodarone Related Compound D RS to a suitable volumetric flask, and add a suitable amount of *Standard stock solution A* and *Standard stock solution B*. Dilute with *Diluent* to volume.▲ (USP 1-Dec-2019)
Sample solution: Nominally 1 mg/mL of amiodarone hydrochloride in *Diluent* from a suitable volume of Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
▲Autosampler: 2°–8°▲ (USP 1-Dec-2019)
Column: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 1.5 times the retention time of amiodarone for the *Standard solution*, and NLT 2 times the retention time of amiodarone for the *Sample solution*
System suitability
Sample: *Standard solution*▲
[NOTE—See *Table 1* for relative retention times.]
▲ (USP 1-Dec-2019)
Suitability requirements
▲Resolution: NLT 3.5 between the amiodarone related compound E and amiodarone related compound D peaks▲ (USP 1-Dec-2019)
Tailing factor: NMT 2.0 ▲for the amiodarone, amiodarone related compound D, and amiodarone related compound E peaks▲ (USP 1-Dec-2019)
Relative standard deviation: NMT 5.0% ▲for the amiodarone, amiodarone related compound D, and amiodarone related compound E peaks▲ (USP 1-Dec-2019)
Analysis
Samples: *Standard solution* ▲▲ (USP 1-Dec-2019) and *Sample solution*

Calculate the percentage of amiodarone related compound D or amiodarone related compound E in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak ▲response▲ (USP 1-Dec-2019) of amiodarone related compound D or amiodarone related compound E from the *Sample solution*
r_S = peak ▲response▲ (USP 1-Dec-2019) of amiodarone related compound D or amiodarone related compound E from the *Standard solution* ▲▲ (USP 1-Dec-2019)
C_S = concentration of USP Amiodarone Related Compound D RS or USP Amiodarone Related Compound E RS in the *Standard solution* ▲▲ (USP 1-Dec-2019) (mg/mL)
C_U = nominal concentration of amiodarone hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak ▲response▲ (USP 1-Dec-2019) of any unspecified degradation product from the *Sample solution*
r_S = peak ▲response▲ (USP 1-Dec-2019) of amiodarone from the *Standard solution* ▲▲ (USP 1-Dec-2019)
C_S = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* ▲▲ (USP 1-Dec-2019) (mg/mL)
C_U = nominal concentration of amiodarone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amiodarone related compound E ▲▲ (USP 1-Dec-2019)	0.39	0.2
Amiodarone related compound D ▲▲ (USP 1-Dec-2019)	0.55	3.0
Amiodarone	1.00	—
Any unspecified degradation product	—	0.20
Total ▲degradation products▲ (USP 1-Dec-2019)	—	3.5

SPECIFIC TESTS

Change to read:

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲Meets the requirements▲ (USP 1-Dec-2019)
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **pH** ⟨791⟩: 3.0–5.0
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose glass containers, protected from light and excessive heat. Store at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be diluted to the appropriate strength with a suitable parenteral vehicle prior to administration. Label it to indicate the type and amount of preservative used. Label it to indicate that it is preservative free, if no preservative is present.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amiodarone Hydrochloride RS
  USP Amiodarone Related Compound D RS
  (2-Butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl) methanone.
  $C_{19}H_{16}I_2O_3$ 546.14
  USP Amiodarone Related Compound E RS
  (2-Butylbenzofuran-3-yl)(4-hydroxyphenyl)methanone.
  $C_{19}H_{18}O_3$ 294.34
  USP Benzyl Alcohol RS

Amiodarone Hydrochloride Compounded Oral Suspension

DEFINITION

Amiodarone Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3 \cdot HCl$).

Prepare Amiodarone Hydrochloride Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Amiodarone Hydrochloride tablets[a] equivalent to	600 mg of amiodarone hydrochloride
Vehicle: a 1:1 mixture of Ora-Sweet[b] (regular or sugar-free) and Ora-Plus,[b] a sufficient quantity to make	120 mL

[a] Cordarone 200-mg tablets, Wyeth-Ayerst Laboratories, Philadelphia, PA.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Amiodarone Hydrochloride tablets* in a suitable mortar and comminute to a fine powder with a pestle. Adjust the pH of the *Vehicle* to 6.5 ± 0.5 with a sodium bicarbonate 50-mg/mL solution prepared in Purified Water. Add the *Vehicle* in small portions and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make an amiodarone hydrochloride liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume and mix well.

ASSAY

- **PROCEDURE**
  Mobile phase: Methanol, water, and 50 mM monobasic ammonium phosphate (0.5: 0.5: 99)
  Standard solution: 2.5 mg/mL of USP Amiodarone Hydrochloride RS in *Mobile phase*
  Sample solution: Shake thoroughly by hand each bottle of the Oral Suspension. Prepare 2.5 mg/mL of amiodarone hydrochloride from Oral Suspension and *Mobile phase*.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 230 nm
  Column: 4.6-mm × 25-cm; 10-µm packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The retention time for amiodarone is about 3.6 min.]
  Suitability requirements
  Relative standard deviation: NMT 2.1% for replicate injections
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3 \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amiodarone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 5.8–6.8

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator; NMT 30 days when stored at controlled room temperature
- **LABELING:** Label it to state that it is to be well shaken before use and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amiodarone Hydrochloride RS

Add the following:

▲Amiodarone Hydrochloride Tablets

DEFINITION

Amiodarone Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: Add 3 mL of acetic acid, glacial to 1 L of water. Adjust with ammonia water, 25 percent to a pH of 3.0.
  Mobile phase: Acetonitrile and *Buffer* (40:60)
  Standard solution: 0.1 mg/mL of USP Amiodarone Hydrochloride RS in *Mobile phase*
  Sample stock solution: Nominally 1 mg/mL of amiodarone hydrochloride in *Mobile phase* prepared as follows. Transfer

www.webofpharma.com

a quantity, equivalent to 100 mg of amiodarone hydrochloride, from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add *Mobile phase* to about 50% of the final flask volume. Sonicate with occasional shaking to dissolve. Cool the solution and dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.1 mg/mL of amiodarone hydrochloride in *Mobile phase* from *Sample stock solution*. Pass a portion of the solution through a suitable filter of 0.45-µm pore size, discard the first few milliliters, and collect the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 2.5 times the retention time of amiodarone

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of amiodarone from the *Sample solution*
r_S = peak response of amiodarone from the *Standard solution*
C_S = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amiodarone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 1% (w/v) sodium dodecyl sulfate; 1000 mL
Apparatus 2: 100 rpm
Time: 60 min
Standard stock solution: 0.2 mg/mL of USP Amiodarone Hydrochloride RS prepared as follows. Transfer an appropriate quantity of USP Amiodarone Hydrochloride RS to a suitable volumetric flask and add methanol to 5% of the final flask volume. Sonicate to dissolve and dilute with *Medium* to volume.
Standard solution: 0.01 mg/mL of USP Amiodarone Hydrochloride RS in *Medium* from *Standard stock solution*
Sample solution: Dilute a portion of the solution under test with *Medium* to a concentration similar to that of the *Standard solution*. Pass a portion of the solution through a suitable filter of 0.45-µm pore size, discard the first few milliliters, and collect the filtrate.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 243 nm
Cell: 1 cm
Blank: *Medium*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3 \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of amiodarone from the *Sample solution*
A_S = absorbance of amiodarone from the *Standard solution*
C_S = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 1000 mL
L = label claim of amiodarone hydrochloride (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3 \cdot HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer: Add 3 mL of acetic acid, glacial to 800 mL of water. Adjust with 10% (v/v) ammonia hydroxide solution to a pH of 4.9. Dilute with water to 1000 mL.
Mobile phase: Acetonitrile, methanol, and *Buffer* (40:30:30)
Diluent: Acetonitrile and water (50:50)
Standard solution: 0.01 mg/mL of USP Amiodarone Hydrochloride RS in *Diluent*
Sensitivity solution: 0.3 µg/mL of USP Amiodarone Hydrochloride RS in *Diluent* from *Standard solution*
Sample solution: Nominally 1 mg/mL of amiodarone hydrochloride in *Diluent* prepared as follows. Transfer a quantity equivalent to 50 mg of amiodarone hydrochloride from NLT 20 finely powdered Tablets to a 50-mL volumetric flask. Add *Diluent* to 50% of the final flask volume. Sonicate with occasional shaking to dissolve. Cool the solution and dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size, discard the first few milliliters, and collect the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 1.7 times the retention time of amiodarone for the *Standard solution*; NLT 3.4 times the retention time of amiodarone for the *Sample solution*

System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Relative standard deviation: NMT 10.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of amiodarone related compound D or any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of amiodarone related compound D or any unspecified degradation product from the *Sample solution*
r_S = peak response of amiodarone from the *Standard solution*
C_S = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amiodarone hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.03%.

Table 1

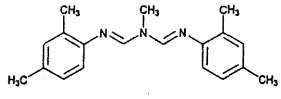
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amiodarone related compound A[a,b]	0.22	—	—
Amiodarone related compound D[c]	0.29	0.90	0.5
Amiodarone related compound C[d,b]	0.52	—	—
Amiodarone	1.00	—	—
Any unspecified degradation product	—	1.00	0.2
Total degradation products	—	—	1.0

[a] (2-Butylbenzofuran-3-yl)(4-[2-(diethylamino)ethoxy]phenyl}methanone.
[b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total degradation products for the drug product.
[c] (2-Butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl)methanone.
[d] (2-Butylbenzofuran-3-yl)(4-[2-(diethylamino)ethoxy]-3-iodophenyl} methanone.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight and light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amiodarone Hydrochloride RS ▲(USP 1-Dec-2019)

Amitraz



$C_{19}H_{23}N_3$ 293.41

Methanimidamide,*N*′-(2,4-dimethylphenyl)-*N*-[[(2,4-dimethylphenyl)imino]methyl]-*N*-methyl-;
N-Methyl-*N*′-2,4-xylyl-*N*-(*N*-2,4-xylylformimidoyl) formamidine;
N-Methylbis(2,4-xylyliminomethyl)amine [33089-61-1].

DEFINITION

Amitraz contains NLT 95.0% and NMT 101.5% of amitraz ($C_{19}H_{23}N_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
- **B.** The retention time of the amitraz peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Internal standard solution: 0.7% v/v solution of squalane in methyl acetate
Standard solution: 5.0 mg/mL of USP Amitraz RS in *Internal standard solution*
Sample solution: 5.0 mg/mL of Amitraz in *Internal standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 15-m fused silica; coated with a 1.5-µm layer of liquid phase G9
Temperatures
Detector: 300°
Inlet: 230°
Column: 220°
Carrier gas: Helium
Flow rate: 12 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—The elution order is amitraz followed by squalane.]
Suitability requirements
Resolution: NLT 3.0 between amitraz and squalane
Relative standard deviation: NMT 2.0% from the peak area ratio of amitraz to squalane
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of amitraz ($C_{19}H_{23}N_3$) in the portion of Amitraz taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of amitraz and squalane from the *Sample solution*
R_S = peak response ratio of amitraz and squalane from the *Standard solution*
C_S = concentration of USP Amitraz RS in the *Standard solution* (mg/mL)
C_U = concentration of Amitraz in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–101.5% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

USP Monographs

www.webofpharma.com

• **ORGANIC IMPURITIES**

Standard solution: 0.05 mg/mL of 2,4-dimethylaniline, 1.0 mg/mL of USP Amitraz Related Compound A RS, 0.5 mg/mL of USP Amitraz Related Compound B RS, and 1.0 mg/mL of USP Amitraz Related Compound C RS in methyl acetate

Sample solution: 50.0 mg/mL of Amitraz in methyl acetate

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 10-m fused silica; coated with a 5-µm layer of liquid phase G27

Temperatures

Detector: 300°

Inlet: 230°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
125	0	125	5
125	5	270	15

Carrier gas: Helium

Flow rate: 12 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between amitraz related compound A and amitraz related compound B

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each of amitraz related compounds A, B, and C in the portion of Amitraz taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of the corresponding related compound from the *Standard solution*

C_S = concentration of the corresponding related compound in the *Standard solution* (mg/mL)

C_U = concentration of Amitraz in the *Sample solution* (mg/mL)

Calculate the percentage of 2,4-dimethylaniline and any other individual impurity in the portion of Amitraz taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of 2,4-dimethylaniline from the *Standard solution*

C_S = concentration of 2,4-dimethylaniline in the *Standard solution* (mg/mL)

C_U = concentration of Amitraz in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2,4-Dimethylaniline	0.11	0.1
Amitraz related compound A	0.35	2
Amitraz related compound B	0.40	1
Amitraz related compound C	0.86	2
Amitraz	1.0	—
Any other individual impurity	—	0.1

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.1%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** Label it to indicate that it is for veterinary use only.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Amitraz RS

USP Amitraz Related Compound A RS
2,4-Dimethylphenyl formamide;
N-(2,4-Dimethylphenyl)formamide.
$C_9H_{11}NO$ 149.19

USP Amitraz Related Compound B RS
2,4-Dimethylphenyl *N*-methyl-formamidine;
N'-(2,4-Dimethylphenyl)-*N*-methylformimidamide.
$C_{10}H_{14}N_2$ 162.23

USP Amitraz Related Compound C RS
Bisformamidine analog;
N,N'-Bis(2,4-dimethylphenyl)formimidamide.
$C_{17}H_{20}N_2$ 252.35

Amitraz Concentrate for Dip

DEFINITION

Amitraz Concentrate for Dip contains amitraz in a suitable vehicle. It may contain a suitable stabilizing agent. It contains NLT 90.0% and NMT 120.0% of the labeled amount of amitraz ($C_{19}H_{23}N_3$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 5 mg/mL of USP Amitraz RS in toluene

Sample solution: Nominally 5 mg/mL of amitraz from Concentrate for Dip diluted with toluene

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 2 µL

Developing solvent system: Cyclohexane, ethyl acetate, and triethylamine (5:3:2)

Spray reagent: 0.5% solution of *N*-(1-naphthyl) ethylenediamine dihydrochloride in methanol

Analysis

Samples: *Standard solution* and *Sample solution*

Stand the plate to a depth of 3.5 cm in a solution prepared by dissolving 35 g of acetamide in 100 mL of methanol, adding 100 mL of triethylamine, and diluting to 250 mL with methanol. Allow the wet plate to stand in a current of cold air for 30 s. Immediately apply the *Samples*

www.webofpharma.com

separately to the plate, at a level about 1 cm below the top of the impregnated zone. Promptly develop the plate until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber and allow to air-dry. Examine the plate under short-wavelength UV light.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

• **B.** The retention time of the amitraz peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Internal standard solution: 0.7% v/v solution of squalane in methyl acetate

Standard solution: 5.0 mg/mL of USP Amitraz RS in *Internal standard solution*

Sample solution: Nominally equivalent to 5.0 mg/mL of amitraz from Concentrate for Dip in *Internal standard solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 15-m fused silica; coated with a 1.5-µm layer of liquid phase G9

Temperatures

Column: 220°

Inlet: 230°

Detector: 300°

Carrier gas: Helium

Flow rate: 12 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

[NOTE—The elution order is amitraz, followed by squalane.]

Suitability requirements

Resolution: NLT 3.0 between amitraz and squalane

Relative standard deviation: NMT 2.0% from the peak area ratio of amitraz to squalane

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amitraz ($C_{19}H_{23}N_3$) in the portion of Concentrate for Dip taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of amitraz and squalane from the *Sample solution*

R_S = peak response ratio of amitraz and squalane from the *Standard solution*

C_S = concentration of USP Amitraz RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amitraz in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.15%

ADDITIONAL REQUIREMENTS

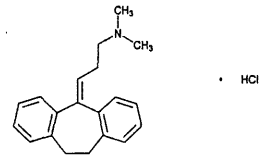
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** Label it to indicate that it is for veterinary use only and that it is to be diluted before use. The label also states the name and quantity of diluent to be used, the directions for dilution, and the conditions for storage of the constituted Concentrate for Dip.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Amitraz RS

Amitriptyline Hydrochloride



$C_{20}H_{23}N \cdot HCl$ 313.86

1-Propanamine, 3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*,*N*-dimethyl-, hydrochloride;

10,11-Dihydro-*N*,*N*-dimethyl-5*H*-dibenzo[*a*,*d*]cycloheptene-Δ^5,γ-propylamine hydrochloride [549-18-8].

DEFINITION

Amitriptyline Hydrochloride contains NLT 98.0% and NMT 102.0% of amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*: Meets the requirements

ASSAY

• **PROCEDURE**

Buffer: 1.4 g/L of anhydrous dibasic sodium phosphate in water, adjusted with 1.5 M phosphoric acid TS to a pH of 7.7

Mobile phase: Methanol and *Buffer* (70:30)

System suitability stock solution A: 1 mg/mL of USP Amitriptyline Related Compound A RS in methanol

System suitability stock solution B: 0.4 mg/mL of USP Amitriptyline Hydrochloride RS, 0.6 mg/mL each of USP Amitriptyline Related Compound B RS, USP Cyclobenzaprine Hydrochloride RS, and USP Nortriptyline Hydrochloride RS in *Mobile phase*

Standard solution: 0.2 mg/mL of USP Amitriptyline Hydrochloride RS in *Mobile phase*

System suitability solution: 0.5 µg/mL of USP Amitriptyline Related Compound A RS, 1 µg/mL of USP Amitriptyline Hydrochloride RS, and 1.5 µg/mL each of USP Amitriptyline Related Compound B RS, USP Cyclobenzaprine Hydrochloride RS, and USP Nortriptyline Hydrochloride RS from suitable volumes of *Standard solution*, *System suitability stock solution A*, and *System suitability stock solution B* in *Mobile phase*

Sample solution: 0.2 mg/mL of Amitriptyline Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 45°

www.webofpharma.com

Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of amitriptyline
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—For relative retention times, see *Table 1*.]
Suitability requirements
Resolution: NLT 1.5 between amitriptyline related compound B and nortriptyline, *System suitability solution*
Relative standard deviation: NMT 0.73% for amitriptyline, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$) in the portion of Amitriptyline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Amitriptyline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

• **ORGANIC IMPURITIES**
Buffer, Mobile phase, ▲and▲ (USP 1-May-2020)
Chromatographic system▲▲ (USP 1-May-2020)**:** Proceed as directed in the *Assay*.
▲**Sensitivity solution:** 0.5 µg/mL of USP Amitriptyline Hydrochloride RS in *Mobile phase*▲ (USP 1-May-2020)
Standard solution: Use the *System suitability solution*, prepared as directed in the *Assay*.
Sample solution: 1000 µg/mL of Amitriptyline Hydrochloride in *Mobile phase*
▲**System suitability**
Samples: *Sensitivity solution* and *Standard solution*
[NOTE—For relative retention times, see *Table 1*.]
Suitability requirements
Resolution: NLT 1.5 between amitriptyline related compound B and nortriptyline, *Standard solution*
Relative standard deviation: NMT 5.0% each for amitriptyline related compound A, amitriptyline related compound B, nortriptyline, cyclobenzaprine, and amitriptyline, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*▲ (USP 1-May-2020)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of amitriptyline related compound A, amitriptyline related compound B, and nortriptyline hydrochloride in the portion of Amitriptyline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of amitriptyline related compound A, amitriptyline related compound B, or nortriptyline from the *Sample solution*
r_S = peak response of amitriptyline related compound A, amitriptyline related compound B, or nortriptyline from the *Standard solution*
C_S = concentration of USP Amitriptyline Related Compound A RS, USP Amitriptyline Related Compound B RS, or USP Nortriptyline Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Amitriptyline Hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of cyclobenzaprine in the portion of Amitriptyline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of cyclobenzaprine from the *Sample solution*
r_S = peak response of cyclobenzaprine from the *Standard solution*
C_S = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Amitriptyline Hydrochloride in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of cyclobenzaprine, 275.39
M_{r2} = molecular weight of cyclobenzaprine hydrochloride, 311.85

Calculate the percentage of each unspecified impurity in the portion of Amitriptyline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of USP Amitriptyline Hydrochloride RS from the *Standard solution*
C_S = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Amitriptyline Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*. Do not include any peak with a relative retention time less than 0.22. ▲The reporting threshold is 0.05%.▲ (USP 1-May-2020)

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amitriptyline related compound A	0.35	0.05
Amitriptyline related compound B	0.52	0.15
Nortriptyline	0.60	0.15
Cyclobenzaprine	0.76	0.15
Amitriptyline	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample: 10 mg/mL in water
Acceptance criteria: 5.0–6.0

www.webofpharma.com

• **Loss on Drying** ⟨731⟩
Analysis: Dry at a pressure not exceeding 5 mm of mercury at 60° to constant weight.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Amitriptyline Hydrochloride RS
USP Amitriptyline Related Compound A RS
10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one;
Also known as Dibenzosuberone.
$C_{15}H_{12}O$ 208.26
USP Amitriptyline Related Compound B RS
5-[3-(Dimethylamino)propyl]-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-ol;
Also known as Amitriptynol.
$C_{20}H_{25}NO$ 295.42
USP Cyclobenzaprine Hydrochloride RS
USP Nortriptyline Hydrochloride RS

Amitriptyline Hydrochloride Tablets

DEFINITION
Amitriptyline Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$).

IDENTIFICATION

Change to read:

• **A.** ▲The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **Procedure**
Buffer: 11.04 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 2.5 ± 0.5, and dilute to make 1000 mL.
Mobile phase: Acetonitrile and *Buffer* (42:58)
Standard solution: 0.2 mg/mL of USP Amitriptyline Hydrochloride RS in *Mobile phase*
Sample solution: Nominally 0.2 mg/mL of amitriptyline hydrochloride in *Mobile phase*, prepared as follows. Transfer NLT 20 Tablets to a suitable volumetric flask, add 50% of the flask volume of *Mobile phase*, and shake the mixture for 1 h or until the Tablets have disintegrated. Dilute with *Mobile phase* to volume, and filter. Dilute the clear filtrate with *Mobile phase* to obtain a solution with a nominal concentration of 0.2 mg/mL of amitriptyline hydrochloride.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. ▲For *Identification A*, use a diode array detector in the range of 220–400 nm.▲ 2S (USP41)
Column: ▲3.9-mm▲ 2S (USP41) × 30-cm; ▲10-µm▲ 2S (USP41) packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
▲Run time: NLT 1.5 times the retention time of amitriptyline▲ 2S (USP41)
System suitability
Sample: *Standard solution*
Suitability requirements
▲▲ 2S (USP41)
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$) in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amitriptyline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: ▲(*L*/900) mg/mL of USP Amitriptyline Hydrochloride RS in *Medium*, where *L* is the Tablet label claim in milligrams. Dilute with *Medium*, if necessary.
Sample solution: Pass a portion of solution under test through a suitable filter. Dilute with *Medium*, if necessary.▲ 2S (USP41)
Instrumental conditions
Analytical wavelength: UV 239 nm
Analysis
Samples: *Standard solution* and *Sample solution*
▲Calculate the percentage of the labeled amount of amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amitriptyline hydrochloride in the *Sample solution* (mg/mL)
D = dilution factor, if necessary▲ 2S (USP41)

Tolerances: NLT 75% (*Q*) of the labeled amount of amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

www.webofpharma.com

IMPURITIES

Change to read:

▲• **ORGANIC IMPURITIES**

Buffer: 1.42 g/L of anhydrous dibasic sodium phosphate in water adjusted with 1.5 M phosphoric acid TS to a pH of 7.7

Mobile phase: Methanol and *Buffer* (70:30)

Diluent: Methanol and water (70:30)

Standard solution: 2 µg/mL each of USP Amitriptyline Hydrochloride RS, USP Amitriptyline Related Compound A RS, USP Amitriptyline Related Compound B RS, and USP Nortriptyline Hydrochloride RS in *Diluent*

Sample solution: Nominally 1000 µg/mL of amitriptyline hydrochloride in *Diluent*, prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask, add 80% of the flask volume of *Diluent*, and shake the mixture for 1 h or until the Tablets have disintegrated. Dilute with *Diluent* to volume. If needed, a portion of this solution can be further diluted with *Diluent*. Centrifuge a portion of the solution with a nominal concentration of 1000 µg/mL of amitriptyline hydrochloride and use the supernatant. [NOTE—A centrifuge speed of 3000 rpm for about 10 min may be suitable.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 45°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Run time: NLT 1.5 times the retention time of amitriptyline

System suitability

Sample: *Standard solution*

[NOTE—For relative retention times, see *Table 1*.]

Suitability requirements

Resolution: NLT 3.0 between amitriptyline related compound B and nortriptyline

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amitriptyline related compound A, amitriptyline related compound B, and nortriptyline hydrochloride in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of amitriptyline related compound A, amitriptyline related compound B, or nortriptyline ▲▲ (ERR 1-Mar-2019) from the *Sample solution*

r_S = peak response of amitriptyline related compound A, amitriptyline related compound B, or nortriptyline ▲▲ (ERR 1-Mar-2019) from the *Standard solution*

C_S = concentration of USP Amitriptyline Related Compound A RS, USP Amitriptyline Related Compound B RS, or USP Nortriptyline Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of amitriptyline hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of any other individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual degradation product from the *Sample solution*

r_S = peak response of amitriptyline from the *Standard solution*

C_S = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of amitriptyline hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amitriptyline related compound A	0.32	0.2
Amitriptyline related compound B	0.48	0.2
Nortriptyline	0.62	0.2
Amitriptyline	1.0	—
Any other individual degradation product	—	0.2
Total degradation products	—	1.0▲ 2S (USP41)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. ▲Store at controlled room temperature.▲ 2S (USP41)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Amitriptyline Hydrochloride RS

▲USP Amitriptyline Related Compound A RS

10,11-Dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-one;

Also known as Dibenzosuberone.

$C_{15}H_{12}O$ 208.26

USP Amitriptyline Related Compound B RS

5-[3-(Dimethylamino)propyl]-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-ol;

Also known as Amitriptynol.

$C_{20}H_{25}NO$ 295.42

USP Nortriptyline Hydrochloride RS▲ 2S (USP41)

Amlodipine Compounded Oral Suspension

DEFINITION

Amlodipine Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$).

Prepare Amlodipine Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

www.webofpharma.com

Amlodipine besylate tablets[a] equivalent to	100 mg of amlodipine
Vehicle: a 1:1 mixture of Ora-Sweet[b] and Ora-Plus,[b] a sufficient quantity to make	100 mL

[a] Norvasc 5-mg tablets, Pfizer, Inc., Groton, CT.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make an amlodipine liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well. [NOTE—To ensure component uniformity, homogenization is recommended.]

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and 40 mM ammonium acetate (50:15:35). Filter through a nylon 66 filter of 0.45-µm pore size, and degas.

Standard stock solution: Dissolve an appropriately weighed amount of USP Amlodipine Besylate RS in methanol, equivalent to 1.0 mg/mL of amlodipine (approximately equal to 1.4 mg/mL of amlodipine besylate).

Standard solution: Transfer 1.0 mL of the *Standard stock solution* into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of about 20 µg/mL of amlodipine. Centrifuge.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of Oral Suspension into a 50-mL volumetric flask, rinse the pipet three times with *Mobile phase*, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of about 20 µg/mL of amlodipine. Centrifuge.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 3.0-mm × 15-cm; 5-µm packing L10
Flow rate: 0.4 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for amlodipine is about 10.1 min.]
Suitability requirements
Column efficiency: NLT 4000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of amlodipine in the *Standard solution* (µg/mL)
C_U = nominal concentration of amlodipine in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.0–5.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator; NMT 60 days when stored at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Amlodipine Besylate RS

Amlodipine and Atorvastatin Tablets

Change to read:

DEFINITION
Amlodipine and Atorvastatin Tablets contain an amount of amlodipine besylate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) and an amount of atorvastatin calcium equivalent to NLT 94.5% and NMT 105.0% of the labeled amount of atorvastatin ▲($C_{33}H_{35}FN_2O_5$)▲ (ERR 1-Dec-2018). It may contain suitable antioxidants.

IDENTIFICATION
- **A.** The UV spectrum of the major peaks of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Solution A: Dissolve 1.54 g of ammonium acetate in 1000 mL of water and add 2 mL of triethylamine. Adjust with acetic acid to a pH of 5.0.

Mobile phase: Acetonitrile, methanol, and *Solution A* (38:15:47)

Buffer: Transfer 7 mL of triethylamine to a 1000-mL volumetric flask containing 900 mL of water and mix. Adjust with dilute phosphoric acid (1 in 100) to a pH of 3.0 and dilute with water to volume.

Diluent: Acetonitrile, methanol, and *Buffer* (3:7:10)

Standard stock solution 1: 0.35 mg/mL of USP Amlodipine Besylate RS in methanol

Standard stock solution 2: 0.44 mg/mL of USP Atorvastatin Calcium RS in methanol

Standard solution: Prepare solutions of USP Amlodipine Besylate RS and USP Atorvastatin Calcium RS in *Mobile phase* at concentrations given in *Table 1* from *Standard stock solution 1* and *Standard stock solution 2*.

USP Monographs

www.webofpharma.com

Table 1

Strength of Tablet Amlodipine/Atorvastatin (mg/mg)	Concentration of Amlodipine Besylate (mg/mL)	Concentration of Atorvastatin Calcium (mg/mL)
2.5/10, 5/20, 10/40	0.028	0.088
2.5/20, 5/40, 10/80	0.014	0.088
5/10, 10/20	0.028	0.044
2.5/40, 5/80	0.014	0.176
10/10	0.028	0.022

Sample solution: Transfer NLT 10 Tablets to a suitable volumetric flask. Add about 20% of the final volume of the volumetric flask size in *Diluent* and sonicate to disperse the Tablets. Add about 40% of the final volume of the volumetric flask size in *Diluent*, sonicate for 20 min, and dilute with *Diluent* to volume. Centrifuge and transfer a suitable quantity of the supernatant to an appropriate suitable volumetric flask. Dilute with *Mobile phase* to volume to obtain the nominal concentrations of amlodipine and atorvastatin similar to that of the *Standard solution*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 237 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 3.5 times the retention time of amlodipine

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both peaks
Relative standard deviation: NMT 2.0% for both peaks

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amlodipine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05

Calculate the percentage of the labeled amount of atorvastatin ▲($C_{33}H_{35}FN_2O_5$)▲ (ERR 1-Dec-2018) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M \times (M_{r1}/M_{r2})] \times 100$$

r_U = peak response of atorvastatin from the *Sample solution*
r_S = peak response of atorvastatin from the *Standard solution*
C_S = concentration of USP Atorvastatin Calcium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of atorvastatin in the *Sample solution* (mg/mL)
M = number of moles of atorvastatin per mole of atorvastatin calcium, 2
M_{r1} = molecular weight of atorvastatin, 558.64
M_{r2} = molecular weight of atorvastatin calcium, 1155.34

Acceptance criteria
Amlodipine: 90.0%–110.0%
Atorvastatin: 94.5%–105.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩
Solution A, Mobile phase, Standard stock solution 1, Standard stock solution 2, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Medium: 0.1% polysorbate 80 in pH 6.8 phosphate buffer; 900 mL
Apparatus 2: 75 rpm
Time: 20 min
Standard solution: (L_1/900) mg/mL of amlodipine and (L_2/900) mg/mL of atorvastatin in *Medium* from *Standard stock solution 1* and *Standard stock solution 2*, where L_1 is the label claim of amlodipine in mg/Tablet, and L_2 is the label claim of atorvastatin in mg/Tablet
Sample solution: Centrifuge the solution under test and use the supernatant.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05
L = label claim of amlodipine (mg/Tablet)

Calculate the percentage of the labeled amount of atorvastatin ▲($C_{33}H_{35}FN_2O_5$)▲ (ERR 1-Dec-2018) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times [M \times (M_{r1}/M_{r2})] \times (1/L) \times 100$$

r_U = peak response of atorvastatin from the *Sample solution*
r_S = peak response of atorvastatin from the *Standard solution*
C_S = concentration of USP Atorvastatin Calcium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M = number of moles of atorvastatin per mole of atorvastatin calcium, 2
M_{r1} = molecular weight of atorvastatin, 558.64
M_{r2} = molecular weight of atorvastatin calcium, 1155.34
L = label claim of atorvastatin (mg/Tablet)

USP Monographs

www.webofpharma.com

Tolerances: NLT 80% (*Q*) of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) and atorvastatin ▲($C_{33}H_{35}FN_2O_5$)▲ (ERR 1-Dec-2018) are dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities Related to Amlodipine**

Buffer 1: Add 7 mL of triethylamine in 1000 mL of water and adjust with phosphoric acid to a pH of 2.5. Add 1.8 g of tetrabutylammonium hydrogen sulfate and mix well.

Solution A: Methanol and *Buffer 1* (40:60)

Solution B: Acetonitrile, methanol, and *Buffer 1* (40:40:20)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
2	90	10
7	75	25
16	70	30
18	55	45
24	25	75
30	10	90
31	0	100
35	0	100
36	90	10
40	90	10

Buffer 2: Add 7 mL of triethylamine in 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0.

Diluent 1: Methanol and water (50:50)

Diluent 2: Methanol and *Buffer 2* (50:50)

Standard stock solution: 0.7 mg/mL of USP Amlodipine Besylate RS in *Diluent 2*, prepared as follows. Transfer a suitable amount of USP Amlodipine Besylate RS to a suitable volumetric flask and dissolve in a quantity of methanol, about 20% of the volume of the flask. Dilute with *Diluent 2* to volume.

Standard solution 1: 5 µg/mL of USP Amlodipine Related Compound A RS in *Diluent 1*

Standard solution 2: 3.5 µg/mL of USP Amlodipine Besylate RS from *Standard stock solution* in *Diluent 2*

Sample solution: Nominally 0.5 mg/mL of amlodipine in *Diluent 2*, prepared as follows. Finely powder NLT 25 Tablets and transfer a portion of the powder, equivalent to 50 mg of amlodipine to a 100-mL volumetric flask. Add about 40 mL of methanol, shake to disperse, and sonicate for 15 min. Add about 40 mL of *Buffer 2* and sonicate for another 10 min. Dilute with *Diluent 2* to volume, centrifuge, and use the supernatant. Pass a portion of the solution through a suitable filter of 0.22-µm pore size. Prepare this solution fresh.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm for amlodipine related compound A; 360 nm for all other impurities

Column: 2.1-mm × 15-cm; 1.8-µm packing L1

Column temperature: 40°

Flow rate: 0.3 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution 2*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of amlodipine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine related compound A from the *Sample solution*

r_S = peak response of amlodipine related compound A from *Standard solution 1*

C_S = concentration of USP Amlodipine Related Compound A RS in *Standard solution 1* (mg/mL)

C_U = nominal concentration of amlodipine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of amlodipine related compound A free base, 406.86

M_{r2} = molecular weight of amlodipine related compound A fumarate, 522.94

Calculate the percentage of atorvastatin–amlodipine adduct or any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of amlodipine from *Standard solution 2*

C_S = concentration of USP Amlodipine Besylate RS in *Standard solution 2* (mg/mL)

C_U = nominal concentration of amlodipine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of amlodipine, 408.88

M_{r2} = molecular weight of amlodipine besylate, 567.05

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard peaks at the relative retention times of 2.18, 2.47 (atorvastatin), and 2.79 min.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amlodipine related compound A	0.59	—	0.50
Amlodipine	1.00	—	—
Atorvastatin–amlodipine adduct[a]	3.49	0.47	0.50
Any unspecified degradation product	—	1.0	0.20

USP Monographs

www.webofpharma.com

Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total degradation products for amlodipine	—	—	1.0

[a] 3-Ethyl 5-methyl 4-(2-chlorophenyl)-2-[(2-{(3*R*,5*R*)-7-[2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanamido}ethoxy)methyl]-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate.

• **ORGANIC IMPURITIES RELATED TO ATORVASTATIN**

Buffer 1: Dissolve 6.8 g of potassium dihydrogen phosphate in 1000 mL of water and adjust with dilute phosphoric acid (1 in 10) to a pH of 3.4.

Buffer 2: Dissolve 6.8 g of potassium dihydrogen phosphate in 1000 mL of water and adjust with triethylamine to a pH of 7.0.

Solution A: Tetrahydrofuran, acetonitrile, and *Buffer 1* (5:25:70)

Solution B: Tetrahydrofuran, acetonitrile, and *Buffer 2* (5:70:25)

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	85	15
30	75	25
70	40	60
75	25	75
80	25	75
85	85	15
90	85	15

Diluent: Acetonitrile and water (50:50)

System suitability solution: Heat a suitable amount of USP Atorvastatin Calcium RS at 60° for 1 h for degradation; 0.55 mg/mL of degraded USP Atorvastatin Calcium RS, 3 µg/mL each of USP Atorvastatin Related Compound A RS, USP Atorvastatin Related Compound B RS, USP Atorvastatin Related Compound C RS, and USP Atorvastatin Related Compound H RS in *Diluent*. Sonication may be necessary for complete dissolution.

Standard solution: 2.7 µg/mL of USP Atorvastatin Calcium RS in *Diluent*

Sample solution: Nominally 0.5 mg/mL of atorvastatin in *Diluent*, prepared as follows. Transfer an amount equivalent to 50 mg of atorvastatin from a portion of NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add about 10 mL of acetonitrile, shake to disperse, and sonicate for 5 min. Add about 70 mL of *Diluent* and sonicate for another 20 min. Dilute with *Diluent* to volume and centrifuge. Prepare this solution fresh.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 246 nm

Column: 4.6-mm × 25-cm; 4-µm packing L11

Column temperature: 45°

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between atorvastatin pyrrolidone analog and atorvastatin related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each atorvastatin specified or unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M \times (M_{r1}/M_{r2})] \times (1/F) \times 100$$

r_U = peak response of each atorvastatin degradation product from the *Sample solution*

r_S = peak response of atorvastatin from the *Standard solution*

C_S = concentration of USP Atorvastatin Calcium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of atorvastatin in the *Sample solution* (mg/mL)

M = number of moles of atorvastatin per mole of atorvastatin calcium, 2

M_{r1} = molecular weight of atorvastatin, 558.64

M_{r2} = molecular weight of atorvastatin calcium, 1155.34

F = relative response factor (see *Table 5*)

Acceptance criteria: See *Table 5*. Disregard any impurity peaks less than 0.05% and the peaks from amlodipine related impurities.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Atorvastatin pyrrolidone analog[a]	0.86	0.67	0.45
Atorvastatin related compound A[b]	0.91	—	—
Atorvastatin related compound B[b]	0.95	—	—
Atorvastatin	1.00	—	—
Atorvastatin related compound C[b]	1.04	—	—
Atorvastatin epoxy pyrrolooxazin 6-hydroxy analog[c]	1.35	0.39	0.5
Atorvastatin epoxy pyrrolooxazin 7-hydroxy analog[d]	1.40	0.52	0.5
Atorvastatin related compound H	1.78	1.0	1.0
Atorvastatin epoxy tetrahydrofuran analog[e]	1.96	0.63	
Atorvastatin oxirane[f]	2.23	1.0	0.5[g]
Atorvastatin *tert*-butyl ester[b,h]	2.55	—	—
Any unspecified degradation product	—	—	0.20

USP Monographs

www.webofpharma.com

Table 5 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total degradation products for atorvastatin	—	—	2.0
Total degradation products[i]	—	—	3.0

[a] (3*R*,5*R*)-7-[5-(4-Fluorophenyl)-3-isopropyl-2-oxo-4-phenyl-3-(phenylcarbamoyl)-2,3-dihydro-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid.
[b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.
[c] 4-{6-(4-Fluorophenyl)-7,8-epoxy-6-hydroxy-8a-isopropyl-7-phenyl-8-(phenylcarbamoyl)hexahydro-2*H*-pyrrolo[2,1-*b*][1,3]oxazin-2-yl}-3-hydroxybutanoic acid.
[d] (3*R*)-4-(1b-(4-Fluorophenyl)-7-hydroxy-7-isopropyl-1a-phenyl-7a-(phenylcarbamoyl)hexahydro-1a*H*-oxireno[2′,3′:3,4]pyrrolo[2,1-*b*][1,3]oxazin-3-yl)-3-hydroxybutanoic acid.
[e] 4-(4-Fluorophenyl)-2,4-dihydroxy-2-isopropyl-*N*,5-diphenyl-3,6-dioxabicyclo[3.1.0]hexane-1-carboxamide.
[f] 3-(4-Fluorobenzoyl)-2-isobutyryl-*N*,3-diphenyloxirane-2-carboxamide.
[g] Sum of atorvastatin epoxy tetrahydrofuran analog and atorvastatin oxirane.
[h] (3*R*,5*R*)-*tert*-Butyl 7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl)-3,5-dihydroxyheptanoate.
[i] Sum of the total degradation products for amlodipine from the test for *Organic Impurities Related to Amlodipine* and the total degradation products for atorvastatin from the test for *Organic Impurities Related to Atorvastatin*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amlodipine Besylate RS
  USP Amlodipine Related Compound A RS
  3-Ethyl 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate] fumarate.
  $C_{20}H_{23}ClN_2O_5 \cdot C_4H_4O_4$ 522.94
  USP Atorvastatin Calcium RS
  USP Atorvastatin Related Compound A RS
  Calcium (3*R*,5*R*)-7-[2-isopropyl-4,5-diphenyl-3-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoate (1:2).
  $C_{66}H_{70}CaN_4O_{10}$ 1119.38
  USP Atorvastatin Related Compound B RS
  (3*S*,5*R*)-7-[3-(Phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid calcium salt.
  $C_{66}H_{68}CaF_2N_4O_{10}$ 1155.34
  USP Atorvastatin Related Compound C RS
  Calcium (3*R*,5*R*)-7-[2,3-Bis(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoate (1:2).
  $C_{66}H_{66}CaF_4N_4O_{10}$ 1191.34
  USP Atorvastatin Related Compound H RS
  5-(4-Fluorophenyl)-1-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-2-isopropyl-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide.
  $C_{33}H_{33}FN_2O_4$ 540.62

Amlodipine and Benazepril Hydrochloride Capsules

DEFINITION

Amlodipine and Benazepril Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of each of amlodipine ($C_{20}H_{25}N_2O_5Cl$) and benazepril hydrochloride ($C_{24}H_{28}N_2O_5 \cdot HCl$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer 1: 0.7% (v/v) of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0, and add 1.2 g of tetrabutyl ammonium hydrogen sulfate per 1 L of *Buffer*. Pass through a suitable filter of 0.45-µm pore size.
Buffer 2: 7.0 mL/L of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0. Pass through a suitable filter of 0.45-µm pore size.
Diluent: Acetonitrile, methanol, and *Buffer 2* (20:30:50)
Mobile phase: Acetonitrile, methanol, and *Buffer 1* (10:30:70)
Standard solution: Prepare the corresponding solutions in *Diluent* as directed in *Table 1*.

Table 1

Strength of Capsule (Amlodipine (mg)/Benazepril Hydrochloride (mg))	Concentration of Amlodipine Besylate/Benazepril Hydrochloride (mg/mL)
2.5/10	0.18/0.5
5/20	0.18/0.5
5/10	0.18/0.25
10/20	0.36/0.5
5/40 and 10/40	0.04/0.16

Pass through a suitable membrane filter of 0.45-µm pore size.
Sample solution: Transfer the contents of five Capsules into a volumetric flask as given in *Table 2*. Add *Diluent* (about 70% of the volume of the flask) and keep on a rotary shaker for about 45 min, sonicate for 30 min with occasional shaking, and dilute with *Diluent* to volume. Centrifuge a portion of the above solution at 3000 rpm for 10 min, and pass through a filter of 0.45-µm pore size.

Table 2

Strength of Capsule (Amlodipine (mg)/Benazepril Hydrochloride (mg))	Concentration of Amlodipine/Benazepril Hydrochloride (mg/mL)
2.5/10	0.125/0.5
5/20	0.125/0.5
5/10	0.125/0.25
10/20	0.25/0.5
5/40	0.02/0.16
10/40	0.04/0.16

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

USP Monographs

www.webofpharma.com

Mode: LC
Detector: UV 237 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
Run time: Two times the retention time of amlodipine

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both amlodipine and benazepril peaks
Relative standard deviation: NMT 2.0% for both amlodipine and benazepril peaks

Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}N_2O_5Cl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = response of the amlodipine peak from the *Sample solution*
r_S = response of the amlodipine peak from the *Standard solution*
C_S = concentration of amlodipine in the *Standard solution* (mg/mL)
C_U = nominal concentration of amlodipine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05

Calculate the percentage of the labeled amount of benazepril hydrochloride ($C_{24}H_{28}N_2O_5 \cdot HCl$), in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response of the benazepril peak from the *Sample solution*
r_S = response of the benazepril peak from the *Standard solution*
C_S = concentration of benazepril hydrochloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of benazepril hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of amlodipine free base and 90.0%–110.0% of the labeled amount of benazepril hydrochloride

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 1: 100 rpm
Time: 30 min
Buffer: 2.72 g/L of potassium dihydrogen phosphate in water. Add 0.2% (v/v) of triethylamine per L. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile, methanol, and *Buffer* (15:35:50)
Amlodipine besylate standard stock solution: 0.385 mg/mL of USP Amlodipine Besylate RS in *Medium*
Benazepril hydrochloride standard stock solution: 0.225 mg/mL of USP Benazepril Hydrochloride RS in *Medium*
Standard solution: Dilute aliquots of the *Amlodipine besylate standard stock solution* and *Benazepril hydrochloride standard stock solution* with *Medium* as per *Table 3*.

Table 3

Strength of the Capsule (Amlodipine/Benazepril Hydrochloride) (mg/mg)	Volume of Amlodipine Besylate Standard Stock Solution/ Volume of Benazepril Hydrochloride Standard Stock Solution (mL)	Volume of the Flask (mL)
2.5/10	1/5	50
5/10	2/5	50
5/20	2/10	50
10/20	2/5	25

For Tablet strengths 5/40 (Amlodipine/Benazepril Hydrochloride, mg/mg): 0.01 mg/mL of USP Amlodipine Besylate RS and 0.08 mg/mL of USP Benazepril Hydrochloride RS in *Medium*.
For Tablet strengths 10/40 (Amlodipine/Benazepril Hydrochloride, mg/mg): 0.02 mg/mL of USP Amlodipine Besylate RS and 0.08 mg/mL of USP Benazepril Hydrochloride RS in *Medium*.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 237 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both amlodipine and benazepril peaks
Relative standard deviation: NMT 2.0% for both amlodipine and benazepril peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}N_2O_5Cl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

r_U = peak response for amlodipine from the *Sample solution*
r_S = peak response for amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution*
L = label claim for amlodipine (mg/Capsule)
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05
V = volume of *Medium*, 500 mL

Calculate the percentage of the labeled amount of benazepril hydrochloride ($C_{24}H_{28}N_2O_5 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response for benazepril from the *Sample solution*
r_S = peak response for benazepril from the *Standard solution*
C_S = concentration of benazepril hydrochloride in the *Standard solution*
L = label claim for benazepril hydrochloride (mg/Capsule)

www.webofpharma.com

V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amounts of amlodipine ($C_{20}H_{25}N_2O_5Cl$) and benazepril hydrochloride ($C_{24}H_{28}N_2O_5 \cdot HCl$) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **PROCEDURE**

Buffer 1, Buffer 2, and **Diluent:** Proceed as directed in the *Assay*.

Solution A: Acetonitrile and *Buffer 1* (20:80)

Solution B: Methanol and *Buffer 1* (80:20)

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	85	15
100	30	70
101	85	15
110	85	15

Standard stock solution: 0.36 mg/mL of amlodipine and amlodipine related compound A and 1 mg/mL each of benazepril hydrochloride and benazepril related compound C solution in *Diluent*

Standard solution: 1 µg/mL of amlodipine and amlodipine related compound A and 3 µg/mL each of benazepril hydrochloride and benazepril related compound C solution in *Diluent*

Sample solution: 0.25 mg/mL of amlodipine from powdered Capsules (NLT 20). [NOTE—The benazepril hydrochloride concentration may vary depending on the ratio of amlodipine to benazepril hydrochloride in the Capsule.] Initially add *Diluent*, about 70% of the volume of the flask, sonicate for 30 min with intermittent shaking, and dilute with *Diluent* to volume. Pass through a membrane filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 237 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 40 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for both amlodipine and benazepril peaks

Resolution: NLT 2.0 between the amlodipine and benazepril peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amlodipine related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = response of amlodipine related compound A from the *Sample solution*

r_S = response of amlodipine related compound A from the *Standard solution*

C_S = concentration of amlodipine related compound A in the *Standard solution* (mg/mL)

C_U = nominal concentration of amlodipine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of amlodipine related compound A, 408.88

M_{r2} = molecular weight of amlodipine related compound A fumarate, 522.93

Calculate the percentage of benazepril related compound C in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response of benazepril related compound C from the *Sample solution*

r_S = response of benazepril related compound C from the *Standard solution*

C_S = concentration of benazepril related compound C in the *Standard solution* (mg/mL)

C_U = nominal concentration of benazepril hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each other impurity from the *Sample solution*

r_T = sum of responses of all peaks from the *Sample solution*

Acceptance criteria: See *Table 5*.

Table 5

Impurity Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benazepril related compound C[a]	0.23	3.0
Amlodipine related compound A[b]	0.44	1.0
Amlodipine	1.00	—
Benazepril	1.20	—
Any other individual unspecified impurity	—	0.2
Total impurities[c]	—	5.0
[NOTE—Disregard the peaks at relative retention times of 0.09 and 0.10.]		

[a] {3-[1-Carboxy-3-phenyl-(1*S*)-propyl]amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine}-1-acetic acid.

[b] 3-Ethyl 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate].

[c] Total impurities include the sum of all impurities. The process-related impurities are not included.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Amlodipine Besylate RS

USP Amlodipine Related Compound A RS

3-Ethyl 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate] fumarate.

$C_{20}H_{23}ClN_2O_5 \cdot C_4H_4O_4$ 522.93

USP Benazepril Hydrochloride RS

www.webofpharma.com

USP Benazepril Related Compound C RS
3-(1-Carboxy-3-phenyl-1(*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid.
$C_{22}H_{24}N_2O_5$ 396.44

Add the following:

▲Amlodipine and Olmesartan Medoxomil Tablets

DEFINITION

Amlodipine and Olmesartan Medoxomil Tablets contain an amount of Amlodipine Besylate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) and NLT 90.0% and NMT 110.0% of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$).

IDENTIFICATION

- **A.** The UV spectra of the amlodipine and olmesartan medoxomil peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention times of the amlodipine and olmesartan medoxomil peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 6.9 g/L of sodium phosphate, monobasic. Adjust with phosphoric acid to a pH of 2.5.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	68	32
12	68	32
15	30	70
21	30	70
23	68	32
25	68	32

Diluent: Acetonitrile and water (50:50)
Standard stock solution: 0.28 mg/mL of USP Amlodipine Besylate RS and 0.8 mg/mL of USP Olmesartan Medoxomil RS in *Diluent*
Standard solution: 0.056 mg/mL of USP Amlodipine Besylate RS and 0.16 mg/mL of USP Olmesartan Medoxomil RS in *Diluent* from *Standard stock solution*
Sample stock solution: Nominal concentrations given in *Table 2* are prepared as follows.

For Tablet strength 5/20, transfer NLT 5 Tablets equivalent to 25 mg of amlodipine and 100 mg of olmesartan medoxomil into a suitable volumetric flask. Add water to 20% of the total volume and sonicate for 5 min. Add acetonitrile to 20% of the total volume and sonicate for 5 min. Add *Diluent* to 30% of the total volume and sonicate for 15 min. Dilute with *Diluent* to volume. Centrifuge a portion of the solution for 10 min and pass through a filter of 0.45-µm pore size.

For Tablet strength 5/40, 10/20, or 10/40, transfer NLT 5 Tablets equivalent to 25 mg of amlodipine and 200 mg of olmesartan medoxomil, 50 mg of amlodipine and 100 mg of olmesartan medoxomil, or 50 mg of amlodipine and 200 mg of olmesartan medoxomil into a suitable volumetric flask. Add water to 10% of the total volume and sonicate for 5 min. Add acetonitrile to 10% of the total volume and sonicate for 5 min. Add *Diluent* to 30% of the total volume and sonicate for 15 min. Dilute with *Diluent* to volume. Centrifuge a portion of the solution for 10 min and pass through a filter of 0.45-µm pore size.

Table 2

Tablet Strength Amlodipine/Olmesartan Medoxomil (mg/mg)	Nominal Concentration of Amlodipine (mg/mL)	Nominal Concentration of Olmesartan Medoxomil (mg/mL)
5/20, 10/40	0.5	2
5/40	0.25	2
10/20	0.5	1

Sample solution: Nominal concentrations in *Diluent* from *Sample stock solution* are given in *Table 3*.

Table 3

Tablet Strength Amlodipine/Olmesartan Medoxomil (mg/mg)	Nominal Concentration of Amlodipine (mg/mL)	Nominal Concentration of Amlodipine/Olmesartan Medoxomil (mg/mL)
5/20, 10/40	0.04	0.16
5/40	0.02	0.16
10/20	0.04	0.08

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L11
Temperatures
Autosampler: 5°
Column: 60°
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for amlodipine and olmesartan medoxomil peaks
Relative standard deviation: NMT 2.0% for amlodipine and olmesartan medoxomil peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)

USP Monographs

www.webofpharma.com

C_U = nominal concentration of amlodipine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05

Calculate the percentage of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of olmesartan medoxomil from the *Sample solution*
r_S = peak response of olmesartan medoxomil from the *Standard solution*
C_S = concentration of USP Olmesartan Medoxomil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of olmesartan medoxomil in the *Sample solution* (mg/mL)

Acceptance criteria
Amlodipine: 90.0%–110.0%
Olmesartan medoxomil: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 6.8 g/L of potassium phosphate, monobasic. Adjust with 0.2 N sodium hydroxide solution to a pH of 6.8; 900 mL.
Apparatus 2: 50 rpm
Times
Amlodipine: 30 min
Olmesartan medoxomil: 45 min
Buffer: 4.08 g/L of potassium phosphate, monobasic. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile and *Buffer* (40:60)
Standard stock solution A: 0.16 mg/mL of USP Amlodipine Besylate RS in *Mobile phase*
Standard stock solution B: 0.44 mg/mL of USP Olmesartan Medoxomil RS in *Mobile phase*
Standard solution: 0.016 mg/mL of USP Amlodipine Besylate RS and 0.044 mg/mL of USP Olmesartan Medoxomil RS in *Medium* from *Standard stock solution A* and *Standard stock solution B*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first 2–3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Autosampler temperature: 5°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
Run time: NLT 1.4 times the retention time of olmesartan medoxomil
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for amlodipine and olmesartan medoxomil peaks
Relative standard deviation: NMT 2.0% for amlodipine and olmesartan medoxomil peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05
L = label claim of amlodipine (mg/Tablet)

Calculate the concentration (C_1 or C_2) of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) in the sample withdrawn from the vessel at the 30- or 45-min time point:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of olmesartan medoxomil from the *Sample solution* at the 30- or 45-min time point
r_S = peak response of olmesartan medoxomil from the *Standard solution*
C_S = concentration of USP Olmesartan Medoxomil RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) dissolved:

$$\text{Result} = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

C_2 = concentration of olmesartan medoxomil in the *Sample solution* at the 45-min time point (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of the *Sample solution* withdrawn at the 30-min time point (mL)
C_1 = concentration of olmesartan medoxomil in the *Sample solution* at the 30-min time point (mg/mL)
L = label claim of olmesartan medoxomil (mg/Tablet)

Tolerances: NLT 80.0% (*Q*) of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) at 30 min and NLT 70.0% (*Q*) of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) at 45 min are dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A, Solution B, Mobile phase, Diluent, Standard stock solution, Sample stock solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution A: 28 µg/mL of USP Amlodipine Besylate RS and 80 µg/mL of USP Olmesartan Medoxomil RS in *Diluent* from *Standard stock solution*
Standard stock solution B: 50 µg/mL of USP Amlodipine Related Compound A RS in *Diluent*
Standard solution: 1.4 µg/mL of USP Amlodipine Besylate RS, 2.5 µg/mL of USP Amlodipine Related Compound A RS, and 4 µg/mL of USP Olmesartan Medoxomil RS in *Diluent* from *Standard stock solution A* and *Standard stock solution B*
Sensitivity solution: 0.28 µg/mL of USP Amlodipine Besylate RS, 0.5 µg/mL of USP Amlodipine Related

www.webofpharma.com

Compound A RS, and 0.8 µg/mL of USP Olmesartan Medoxomil RS in *Diluent* from *Standard solution*

Sample solution: Use the *Sample stock solution*, prepared as directed in the *Assay*.

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[NOTE—See *Table 4* for relative retention times.]

Suitability requirements

Tailing factor: NMT 2.0 for amlodipine related compound A, amlodipine, and olmesartan medoxomil peaks, *Standard solution*

Relative standard deviation: NMT 5.0% for amlodipine related compound A, amlodipine, and olmesartan medoxomil peaks, *Standard solution*

Signal-to-noise ratio: NLT 10 for amlodipine related compound A, amlodipine, and olmesartan medoxomil peaks, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amlodipine related compound A free base in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine related compound A from the *Sample solution*

r_S = peak response of amlodipine related compound A from the *Standard solution*

C_S = concentration of USP Amlodipine Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amlodipine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of amlodipine related compound A free base, 406.86

M_{r2} = molecular weight of amlodipine related compound A, 522.93

Calculate the percentage of any unspecified amlodipine related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of any unspecified amlodipine related impurity from the *Sample solution*

r_S = peak response of amlodipine from the *Standard solution*

C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amlodipine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of amlodipine, 408.88

M_{r2} = molecular weight of amlodipine besylate, 567.05

Calculate the percentage of olmesartan or any unspecified olmesartan medoxomil related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of olmesartan or any unspecified olmesartan medoxomil related impurity from the *Sample solution*

r_S = peak response of olmesartan medoxomil from the *Standard solution*

C_S = concentration of USP Olmesartan Medoxomil RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of olmesartan medoxomil in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzenesulfonic acid[a]	0.13	—
Olmesartan[b]	0.25	2.0
Amlodipine related compound A[c]	0.36	0.5
Amlodipine	0.47	—
Olmesartan medoxomil	1.0	—
Olmesartan medoxomil related compound A[d, e]	1.13	—
Olmesartan olefinic impurity[f, e]	1.50	—
Olmesartan *N*-alkyl impurity[g, e]	2.03	—
Any unspecified amlodipine or olmesartan medoxomil related impurity[h]	—	0.2
Total impurities[i]	—	2.0

[a] This peak is due to the counterion and is not to be reported or included in the total impurities.

[b] 1-{[2′-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carboxylic acid.

[c] 3-Ethyl 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate].

[d] 1-{[2′-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4,4-dimethyl-2-propyl-1*H*-furo[3,4-*d*]imidazol-6(4*H*)-one.

[e] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities for the drug product.

[f] (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 1-((2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl)-4-(prop-1-en-2-yl)-2-propyl-1*H*-imidazole-5-carboxylate.

[g] (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-((2′-(2-trityl-2*H*-tetrazol-5-yl)biphenyl-4-yl)methyl)-1*H*-imidazole-5-carboxylate.

[h] The relative retention times for unspecified amlodipine related impurities are up to 1.0. The relative retention times for unspecified olmesartan medoxomil related impurities are after 1.0 and also at 0.47, 0.60, 0.76, 0.79, and 0.92.

[i] Excluding olmesartan.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Amlodipine Besylate RS

USP Amlodipine Related Compound A RS

3-Ethyl 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate] fumarate.

$C_{20}H_{23}ClN_2O_5 \cdot C_4H_4O_4$ 522.93

USP Olmesartan Medoxomil RS▲ (USP 1-Dec-2019)

www.webofpharma.com

Amlodipine and Valsartan Tablets

DEFINITION

Amlodipine and Valsartan Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) and valsartan ($C_{24}H_{29}N_5O_3$).

IDENTIFICATION

- **A.** The UV absorption spectra of the major peaks of *Sample solution A* and *Sample solution B* and those of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.
- **B.** The retention times of the major peaks of *Sample solution A* and *Sample solution B* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Water and triethylamine (1000:10). Adjust with phosphoric acid to a pH of 2.8.

Solution B: Methanol and acetonitrile (700:300)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	50	50
3	50	50
15	30	70
20	30	70
20.1	50	50
25	50	50

Diluent: *Solution A* and *Solution B* (50:50)

Standard solution: 0.14 mg/mL of USP Amlodipine Besylate RS and 0.16 mg/mL of USP Valsartan RS. Add methanol to 5% of the final volume to dissolve, and dilute with *Diluent* to volume.

Sample stock solution: Transfer NLT 10 Tablets into a suitable volumetric flask. Initially add water to 10% of the final volume, and sonicate to disperse as needed. Add *Diluent*, using about 70% of the final volume, and shake for up to 45 min to disperse. Following dispersion, sonicate for 15 min, and shake for 30 min. Dilute with *Diluent* to volume to obtain a solution containing known nominal concentrations of 0.1–0.2 mg/mL of amlodipine and 1.6–6.4 mg/mL of valsartan. Centrifuge the solution for about 10 min at 3000 rpm.

Sample solution A: Nominally equivalent to 0.1 mg/mL of amlodipine in *Diluent* from the *Sample stock solution*

Sample solution B: Nominally equivalent to 0.16 mg/mL of valsartan in *Diluent* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 237 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.

Column: 3.9-mm × 15-cm; 5-µm packing L1

Temperatures

Autosampler: 10°

Column: 30°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for both amlodipine and valsartan

Relative standard deviation: NMT 2.0% for amlodipine and valsartan

Analysis

Samples: *Standard solution*, *Sample solution A*, and *Sample solution B*

Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine from *Sample solution A*

r_S = peak response of amlodipine from the *Standard solution*

C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amlodipine in *Sample solution A* (mg/mL)

M_{r1} = molecular weight of amlodipine, 408.88

M_{r2} = molecular weight of amlodipine besylate, 567.05

Calculate the percentage of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of valsartan from *Sample solution B*

r_S = peak response of valsartan from the *Standard solution*

C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of valsartan in *Sample solution B* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1

Buffer: Dissolve 6.805 g of monobasic potassium phosphate and 0.896 g of sodium hydroxide in water, and dilute with water to 1000 mL. Adjust with 0.2 N sodium hydroxide or 1 M phosphoric acid to a pH of 6.8.

Medium: *Buffer*, 1000 mL

Apparatus 2: 75 rpm

Time: 30 min

Mobile phase: Acetonitrile, water, and trifluoroacetic acid (500:500:2)

Diluent: 1 mg/mL of polysorbate 80 in *Buffer*

System suitability solution: 0.4 mg/mL each of USP Amlodipine Besylate RS and USP Valsartan RS, prepared as follows. Initially dissolve in methanol to 40% of the total volume, and dilute with *Buffer* to volume.

Standard stock solution A: 0.072 mg/mL of USP Amlodipine Besylate RS, prepared as follows. Initially dissolve in methanol to 4% of the final volume, and dilute with *Diluent* to volume.

Standard stock solution B: 2.2 mg/mL of USP Valsartan RS in methanol

Standard solution: (L_1/1000) mg/mL of amlodipine and (L_2/1000) mg/mL of valsartan in *Diluent* from *Standard stock solution A* and *Standard stock solution B*, where L_1 is the label claim of amlodipine in mg/Tablet, and L_2 is the label claim of valsartan in mg/Tablet

www.webofpharma.com

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard the first 10 mL of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 4-µm packing L11
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of amlodipine

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between amlodipine and valsartan, *System suitability solution*
Tailing factor: NMT 2.0 for amlodipine and valsartan, *Standard solution*
Relative standard deviation: NMT 2.0% for amlodipine and valsartan, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L_1) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05
L_1 = label claim of amlodipine (mg/Tablet)

Calculate the percentage of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_2) \times 100$$

r_U = peak response of valsartan from the *Sample solution*
r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L_2 = label claim of valsartan (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) and valsartan ($C_{24}H_{29}N_5O_3$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium and **Time:** Proceed as directed in *Dissolution Test 1*; 1000 mL.
Apparatus 2: 50 rpm
Buffer: Mix 7.0 mL of triethylamine with 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0.
Solution A: Acetonitrile and *Buffer* (10:90)
Solution B: Acetonitrile and *Buffer* (90:10)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
7	30	70
8	80	20
10	80	20

Standard stock solution A: 0.14 mg/mL of USP Amlodipine Besylate RS, prepared as follows. Initially dissolve in 10% of the final volume of methanol, and dilute with *Medium* to volume.
Standard stock solution B: 1.6 mg/mL of USP Valsartan RS in methanol
Standard solution: (L_1/1000) mg/mL of amlodipine and (L_2/1000) mg/mL of valsartan in *Medium* from *Standard stock solution A* and *Standard stock solution B*, where L_1 is the label claim of amlodipine in mg/Tablet, and L_2 is the label claim of valsartan in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 1-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 237 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 50°
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for amlodipine and valsartan
Relative standard deviation: NMT 2.0% for amlodipine and valsartan

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L_1) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05
L_1 = label claim of amlodipine (mg/Tablet)

Calculate the percentage of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_2) \times 100$$

r_U = peak response of valsartan from the *Sample solution*
r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL

www.webofpharma.com

L_2 = label claim of valsartan (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) is dissolved and NLT 80% (*Q*) of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

Medium, Apparatus 2, and **Time:** Proceed as directed in *Dissolution Test 1*.

Solution A: Acetonitrile, trifluoroacetic acid, and water (10: 0.1: 90)

Solution B: Acetonitrile, trifluoroacetic acid, and water (90: 0.1: 10)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0.01	90	10
2.5	10	90
3.0	90	10
5.0	90	10

Diluent: Acetonitrile and water (50:50)

Standard stock solution A: 0.14 mg/mL of USP Amlodipine Besylate RS, prepared as follows. Initially dissolve in *Diluent* about 4% of the final volume, and dilute with *Medium* to volume.

Standard stock solution B: 1.6 mg/mL of USP Valsartan RS, prepared as follows. Initially dissolve in about 20% of the final volume of *Diluent*, and dilute with *Medium* to volume.

Standard solution: (L_1/1000) mg/mL of amlodipine and (L_2/1000) mg/mL of valsartan in *Medium* from *Standard stock solution A* and *Standard stock solution B*, where L_1 is the label claim of amlodipine in mg/Tablet, and L_2 is the label claim of valsartan in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first few milliliters of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 237 nm for amlodipine and UV 270 nm for valsartan
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for amlodipine and valsartan
Relative standard deviation: NMT 2.0% for amlodipine and valsartan

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L_1) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05
L_1 = label claim of amlodipine (mg/Tablet)

Calculate the percentage of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_2) \times 100$$

r_U = peak response of valsartan from the *Sample solution*
r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L_2 = label claim of valsartan (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) is dissolved and NLT 80% (*Q*) of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**

Mobile phase, Diluent, Sample solution A, Sample solution B, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution A: Prepare as directed for the *Standard solution* in the *Assay*.

System suitability solution: Dissolve a suitable quantity of USP Valsartan Related Compound B RS in *Standard stock solution A* to obtain a solution containing 0.08 mg/mL of USP Valsartan Related Compound B RS, 0.14 mg/mL of USP Amlodipine Besylate RS, and 0.16 mg/mL of USP Valsartan RS.

Sensitivity solution: 0.14 µg/mL of USP Amlodipine Besylate RS and 0.16 µg/mL of USP Valsartan RS in *Diluent* from *Standard stock solution A*

Standard stock solution B: 0.1 mg/mL of USP Amlodipine Related Compound A RS as free base, prepared as follows. Add methanol to 5% of the final volume to dissolve, and dilute with *Diluent* to volume.

Standard solution: 0.0005 mg/mL of USP Amlodipine Related Compound A RS as free base, and 0.0003 mg/mL each of USP Amlodipine Besylate RS and USP Valsartan RS in *Diluent* from *Standard stock solution A* and *Standard stock solution B*, respectively

System suitability
Samples: *System suitability solution, Sensitivity solution,* and *Standard solution*
Suitability requirements
Resolution: More than 4.0 between amlodipine and valsartan related compound B and more than 4.0 between valsartan related compound B and valsartan, *System suitability solution*
Relative standard deviation: NMT 5.0% for amlodipine related compound A, amlodipine, and valsartan, *Standard solution*
Signal-to-noise ratio: NLT 10 for amlodipine and valsartan, *Sensitivity solution*

Analysis
Samples: *Sample solution A, Sample solution B,* and *Standard solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of amlodipine related compound A free base in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine related compound A from *Sample solution A*
r_S = peak response of amlodipine related compound A from the *Standard solution*
C_S = concentration of USP Amlodipine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amlodipine in *Sample solution A* (mg/mL)
M_{r1} = molecular weight of amlodipine related compound A free base, 406.86
M_{r2} = molecular weight of amlodipine related compound A fumarate, 522.93

Calculate the percentage of valsartan related degradation products other than valsartan related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of valsartan related degradation product from *Sample solution B*
r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valsartan in *Sample solution B* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified degradation product from *Sample solution A*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amlodipine in *Sample solution A* (mg/mL)
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05

Acceptance criteria: See *Table 4*. Disregard valsartan related compound B, the benzenesulfonic acid peak at relative retention time 0.19, and any peaks below 0.1%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Devaleryl valsartan[a]	0.24	0.2
Amlodipine related compound A[b]	0.50	0.5
Valsartan related degradation product 1[c]	0.54	0.2
Valsartan related degradation product 2[c]	0.81	0.2
Amlodipine	1.00	—
Valsartan related compound B[d]	1.34	—
Valsartan related degradation product 3[c]	1.44	0.2
Valsartan	1.74	—
Valsartan related degradation product 4[c]	2.06	0.2
Valsartan ethyl ester[e]	2.32	0.2
Any other unspecified degradation product	—	0.2
Total degradation products[f]	—	1.2; 2.0, if valsartan related compound A is a potential degradation product

[a] *N*-{[2′-(1*H*-Tetrazole-5-yl)biphenyl-4-yl]methyl}-L-valine.
[b] 3-Ethyl 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate].
[c] These are specified unidentified degradation products. No information is available about chemical structures or chemical names for these impurities.
[d] *N*-Butyryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl}-L-valine.
[e] *N*-Valeryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl}-L-valine ethyl ester.
[f] If valsartan related compound A is a potential degradation product, the total degradation products limit does not include valsartan related compound A and amlodipine related compound A.

• **LIMIT OF VALSARTAN RELATED COMPOUND A**

[NOTE—Valsartan related compound A is a process impurity and a formulation-specific degradation product.]

Mobile phase: *n*-Hexane, 2-propanol, and trifluoroacetic acid (850:150:1)

System suitability solution: 0.04 mg/mL each of USP Valsartan Related Compound A and USP Valsartan RS in *Mobile phase*

Standard solution: 0.001 mg/mL of USP Valsartan Related Compound A RS in *Mobile phase*

Sample solution: Nominally 0.5 mg/mL of valsartan in *Mobile phase* from a suitable amount of finely crushed powder from NLT 20 Tablets. Sonication may be necessary for complete dissolution. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L40
Temperatures
Autosampler: 10°
Column: 30°
Flow rate: 0.8 mL/min
Injection volume: 20 µL
Run time: NLT 3.5 times the retention time of valsartan related compound A

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for valsartan related compound A and valsartan are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between valsartan and valsartan related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0% for valsartan related compound A, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the percentage of valsartan related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of valsartan related compound A from the *Sample solution*
r_S = peak response of valsartan related compound A from the *Standard solution*
C_S = concentration of USP Valsartan Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valsartan in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store at controlled room temperature, in tight containers, and in a dry place.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amlodipine Besylate RS
  USP Amlodipine Related Compound A RS
  3-Ethyl 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate] fumarate.
  $C_{20}H_{23}ClN_2O_5 \cdot C_4H_4O_4$ 522.93
  USP Valsartan RS
  USP Valsartan Related Compound A RS
  N-Valeryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl] methyl}-D-valine.
  $C_{24}H_{29}N_5O_3$ 435.52
  USP Valsartan Related Compound B RS
  N-Butyryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl] methyl}-L-valine.
  $C_{23}H_{27}N_5O_3$ 421.49

Amlodipine, Valsartan, and Hydrochlorothiazide Tablets

DEFINITION

Amlodipine, Valsartan, and Hydrochlorothiazide Tablets contain NLT 92.5% and NMT 107.5% each of the labeled amounts of amlodipine ($C_{20}H_{25}ClN_2O_5$), valsartan ($C_{24}H_{29}N_5O_3$), and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

- **A.** The UV absorption spectra of the amlodipine, valsartan, and hydrochlorothiazide peaks of *Sample solution A*, *Sample solution B*, and *Sample solution C*, and those of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.
- **B.** The retention times of the amlodipine, valsartan, and hydrochlorothiazide peaks of *Sample solution A*, *Sample solution B*, and *Sample solution C* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Use amber glassware for all solutions containing drug substances.

Solution A: Acetonitrile, water, and phosphoric acid (50:950:1)

Solution B: Acetonitrile, water, and phosphoric acid (950:50:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
3	50	50
6	40	60
10	5	95
10.1	95	5
15	95	5

Diluent: Acetonitrile and water (500:500)

0.1% Phosphoric acid: Water and phosphoric acid (1000:1)

Standard solution: 0.14 mg/mL of USP Amlodipine Besylate RS, 0.064 mg/mL of USP Valsartan RS, and 0.025 mg/mL of USP Hydrochlorothiazide RS in *Diluent*

Sample stock solution: Transfer NLT 10 Tablets into a suitable volumetric flask. Add *0.1% Phosphoric acid* to 4% of the total volume to disperse the Tablets. Sonicate for 10 min. Add 4% of the total volume of acetonitrile, swirl to mix, and add 60% of the total volume of *Diluent*. Sonicate for 20 min. Dilute with *Diluent* to volume to obtain solutions of nominal concentrations stated in *Table 2*. Centrifuge, and use the clear supernatant.

Table 2

Tablet Strength Amlodipine/ Valsartan/ Hydrochlorothiazide (mg/mg/mg)	Nominal Concentration of Amlodipine (mg/mL)	Nominal Concentration of Valsartan (mg/mL)	Nominal Concentration of Hydrochlorothiazide (mg/mL)
5/160/12.5	0.1	3.2	0.25
10/160/12.5	0.2	3.2	0.25
5/160/25	0.1	3.2	0.5
10/160/25	0.2	3.2	0.5
10/320/25	0.1	3.2	0.25

Sample solution A: Nominally equivalent to 0.1 mg/mL of amlodipine in *Diluent* from *Sample stock solution*

Sample solution B: Nominally equivalent to 0.064 mg/mL of valsartan in *Diluent* from *Sample stock solution*

Sample solution C: Nominally equivalent to 0.025 mg/mL of hydrochlorothiazide in *Diluent* from *Sample stock solution*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for amlodipine, valsartan, and hydrochlorothiazide
Relative standard deviation: NMT 2.0% for amlodipine, valsartan, and hydrochlorothiazide

www.webofpharma.com

Analysis
Samples: *Standard solution*, *Sample solution A*, *Sample solution B*, and *Sample solution C*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine from *Sample solution A*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amlodipine in *Sample solution A* (mg/mL)
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05

Calculate the percentage of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of valsartan from *Sample solution B*
r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valsartan in *Sample solution B* (mg/mL)

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydrochlorothiazide from *Sample solution C*
r_S = peak response of hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in *Sample solution C* (mg/mL)

Acceptance criteria: 92.5%–107.5%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Test 1
Buffer: Dissolve 6.805 g of monobasic potassium phosphate and 0.896 g of sodium hydroxide in 1000 mL of water. Adjust with 0.2 N sodium hydroxide or 1 M phosphoric acid to a pH of 6.8.
Medium: *Buffer*; 900 mL
Apparatus 2
For 5/160/12.5, 10/160/12.5, 5/160/25, and 10/160/25 (mg/mg/mg) of Tablet strengths (amlodipine/valsartan/hydrochlorothiazide): 50 rpm
For 10/320/25 (mg/mg/mg) of Tablet strengths (amlodipine/valsartan/hydrochlorothiazide): 55 rpm
Time: 30 min
Solution A: Acetonitrile, water, and phosphoric acid (50:950:1)
Solution B: Acetonitrile, water, and phosphoric acid (950:50:1)
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0.00	67	33
2.50	23	77
2.51	67	33
4.00	67	33

Diluent: 1 mg/mL of polysorbate 80 in *Buffer*
Standard stock solution A: 0.07 mg/mL of USP Amlodipine Besylate and 0.124 mg/mL of USP Hydrochlorothiazide RS. Initially dissolve with 4% of the total volume of methanol, and dilute with *Diluent* to volume.
Standard stock solution B: 3.2 mg/mL of USP Valsartan RS in methanol
Standard solution: 0.014 mg/mL of USP Amlodipine Besylate RS, 0.16 mg/mL of USP Valsartan RS, and 0.0248 mg/mL of USP Hydrochlorothiazide RS in *Diluent* from *Standard stock solution A* and *Standard stock solution B*, respectively
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard at least the first 10 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 5-cm; 3-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume
For 10/320/25 (mg/mg/mg) of Tablet strengths (amlodipine/valsartan/hydrochlorothiazide): 5 µL
For 5/160/12.5, 10/160/12.5, 5/160/25, and 10/160/25 (mg/mg/mg) of Tablet strengths (amlodipine/valsartan/hydrochlorothiazide): 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between amlodipine and valsartan
Tailing factor: NMT 2.0 for amlodipine, valsartan, and hydrochlorothiazide
Relative standard deviation: NMT 2.0% for amlodipine, valsartan, and hydrochlorothiazide
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L_1) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05
L_1 = label claim of amlodipine (mg/Tablet)

www.webofpharma.com

Calculate the percentage of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_2) \times 100$$

r_U = peak response of valsartan from the *Sample solution*
r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L_2 = label claim of valsartan (mg/Tablet)

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_3) \times 100$$

r_U = peak response of hydrochlorothiazide from the *Sample solution*
r_S = peak response of hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L_3 = label claim of hydrochlorothiazide (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) is dissolved, NLT 80% (*Q*) of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) is dissolved, and NLT 80% (*Q*) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: Proceed as directed under *Dissolution Test 1*; 900 mL.
Apparatus 2
For Tablets labeled to contain amlodipine/valsartan/hydrochlorothiazide, 5/160/12.5, 10/160/12.5, 5/160/25, 10/160/25, and 5/80/12.5 (mg/mg/mg): 50 rpm
For Tablets labeled to contain amlodipine/valsartan/hydrochlorothiazide, 10/320/25 (mg/mg/mg): 55 rpm
Times
For valsartan and hydrochlorothiazide: 30 min
For amlodipine: 45 min
Buffer: Mix 7.0 mL of triethylamine with 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0.
Solution A: Acetonitrile and *Buffer* (10:90)
Solution B: Acetonitrile and *Buffer* (90:10)
Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	90	10
7	30	70
8	90	10
15	90	10

Standard stock solution A: 0.35 mg/mL of USP Amlodipine Besylate RS, prepared as follows. Initially dissolve in 10% of the final volume of methanol and dilute with *Medium* to volume.
Standard stock solution B: 1.6 mg/mL of USP Valsartan RS in methanol
Standard stock solution C: 0.7 mg/mL of USP Hydrochlorothiazide RS, prepared as follows. Initially dissolve in 25% of the final volume of methanol and dilute with *Medium* to volume.
Standard solution: (L_1/1000) mg/mL of amlodipine, (L_2/1000) mg/mL of valsartan, and (L_3/1000) mg/mL of hydrochlorothiazide in *Medium* from *Standard stock solution A*, *Standard stock solution B*, and *Standard stock solution C*, where L_1 is the label claim of amlodipine in mg/Tablet, L_2 is the label claim of valsartan in mg/Tablet, and L_3 is the label claim of hydrochlorothiazide in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 1-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 237 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 50°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for each peak
Relative standard deviation: NMT 2.0% for each peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L_1) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05
L_1 = label claim of amlodipine (mg/Tablet)

Calculate the percentage of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_2) \times 100$$

r_U = peak response of valsartan from the *Sample solution*
r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L_2 = label claim of valsartan (mg/Tablet)

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_3) \times 100$$

www.webofpharma.com

r_U = peak response of hydrochlorothiazide from the *Sample solution*
r_S = peak response of hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L_3 = label claim of hydrochlorothiazide (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) is dissolved, NLT 80% (*Q*) of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) is dissolved, and NLT 80% (*Q*) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

Medium: Dissolve 6.80 g of monobasic potassium phosphate in 1000 mL of water. Adjust with 10% sodium hydroxide solution to a pH of 6.8; 1000 mL for valsartan and hydrochlorothiazide; 900 mL for amlodipine.

Apparatus 2

For valsartan and hydrochlorothiazide: 50 rpm

For amlodipine in Tablets labeled to contain amlodipine/valsartan/hydrochlorothiazide, 10/320/25 (mg/mg/mg): 55 rpm

For amlodipine in Tablets labeled to contain amlodipine/valsartan/hydrochlorothiazide, 5/160/12.5, 10/160/12.5, 5/160/25, 10/160/25, and 5/80/12.5 (mg/mg/mg): 50 rpm

Times

For valsartan and hydrochlorothiazide: 30 min

For amlodipine: 45 min

Solution A: Acetonitrile, trifluoroacetic acid and water (10: 0.1: 90)

Solution B: Acetonitrile, trifluoroacetic acid and water (90: 0.1: 10)

Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0.01	90	10
2.5	10	90
3.0	90	10
5.0	90	10

Diluent: Acetonitrile and water (50:50)

Standard stock solution A: 0.15 mg/mL of USP Amlodipine Besylate RS in *Medium*, prepared as follows. Initially dissolve and sonicate in 5% of the final volume of *Diluent*, and dilute with *Medium* to volume.

Standard stock solution B: 1.6 mg/mL of USP Valsartan RS in *Medium*, prepared as follows. Initially dissolve and sonicate in 20% of the final volume of *Diluent*, and dilute with *Medium* to volume.

Standard stock solution C: 0.25 mg/mL of USP Hydrochlorothiazide RS in *Medium*, prepared as follows. Initially dissolve and sonicate in 10% of the final volume of *Diluent*, and dilute with *Medium* to volume.

Standard solution: (L_1/1000) mg/mL of amlodipine, (L_2/1000) mg/mL of valsartan, and (L_3/1000) mg/mL of hydrochlorothiazide in *Diluent* from *Standard stock solution A*, *Standard stock solution B*, and *Standard stock solution C*, where L_1 is the label claim of amlodipine in mg/Tablet, L_2 is the label claim of valsartan in mg/Tablet, and L_3 is the label claim of hydrochlorothiazide in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard at least the first few milliliters of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detectors

For amlodipine: UV 237 nm

For valsartan and hydrochlorothiazide: UV 270 nm

Column: 4.6-mm × 10-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for each peak

Relative standard deviation: NMT 2.0% for each peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L_1) \times 100$$

r_U = peak response of amlodipine from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05
L_1 = label claim of amlodipine (mg/Tablet)

Calculate the percentage of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_2) \times 100$$

r_U = peak response of valsartan from the *Sample solution*
r_S = peak response of valsartan from the *Standard solution*
C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L_2 = label claim of valsartan (mg/Tablet)

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_3) \times 100$$

r_U = peak response of hydrochlorothiazide from the *Sample solution*
r_S = peak response of hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L_3 = label claim of hydrochlorothiazide (mg/Tablet)

Tolerances

For Tablets labeled to contain amlodipine/valsartan/hydrochlorothiazide, 5/160/12.5, 10/160/12.5, 5/160/25, and 10/160/25 (mg/mg/mg): NLT 75%

USP Monographs

www.webofpharma.com

(*Q*) of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) is dissolved, NLT 80% (*Q*) of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) is dissolved, and NLT 80% (*Q*) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

For Tablets labeled to contain amlodipine/valsartan/hydrochlorothiazide, 5/160/25, and 10/320/25 (mg/mg/mg): NLT 70% (*Q*) of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) is dissolved, NLT 80% (*Q*) of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$) is dissolved, and NLT 80% (*Q*) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

- **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

- **Organic Impurities**

Use amber glassware for all solutions containing drug substances.

Mobile phase, Diluent, Sample solution A, Sample solution B, Sample solution C, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 0.02 mg/mL each of USP Benzothiadiazine Related Compound A RS and USP Valsartan Related Compound B RS, 0.005 mg/mL of USP Amlodipine Related Compound A RS, 0.14 mg/mL of USP Amlodipine Besylate RS, 0.064 mg/mL of USP Valsartan RS, and 0.025 mg/mL of USP Hydrochlorothiazide RS in *Diluent*

Sensitivity solution: 0.14 µg/mL of USP Amlodipine Besylate RS, 0.064 µg/mL of USP Valsartan RS, and 0.025 µg/mL of USP Hydrochlorothiazide RS in *Diluent*

Standard solution: 0.0005 mg/mL of USP Amlodipine Related Compound A RS, 0.0001 mg/mL of USP Benzothiadiazine Related Compound A RS, 0.0003 mg/mL of USP Amlodipine Besylate RS, 0.00015 mg/mL of USP Valsartan RS, and 0.00005 mg/mL of USP Hydrochlorothiazide RS in *Diluent*

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between any adjacent peaks of benzothiadiazine related compound A, hydrochlorothiazide, amlodipine related compound A, amlodipine, valsartan related compound B, and valsartan, *System suitability solution*

Relative standard deviation: NMT 5.0% for amlodipine related compound A, benzothiadiazine related compound A, amlodipine, valsartan, and hydrochlorothiazide, *Standard solution*

Signal-to-noise ratio: NLT 10 for amlodipine, valsartan, and hydrochlorothiazide, *Sensitivity solution*

Analysis

Samples: *Sample solution A*, *Sample solution B*, *Sample solution C*, and *Standard solution*

Calculate the percentage of amlodipine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine related compound A from *Sample solution A*

r_S = peak response of amlodipine related compound A from the *Standard solution*

C_S = concentration of USP Amlodipine Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amlodipine in *Sample solution A* (mg/mL)

M_{r1} = molecular weight of amlodipine related compound A free base, 406.86

M_{r2} = molecular weight of amlodipine related compound A fumarate, 522.93

Calculate the percentage of any valsartan related degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any valsartan related degradation product from *Sample solution B*

r_S = peak response of valsartan from the *Standard solution*

C_S = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of valsartan in *Sample solution B* (mg/mL)

Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzothiadiazine related compound A from *Sample solution C*

r_S = peak response of benzothiadiazine related compound A from the *Standard solution*

C_S = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrochlorothiazide in *Sample solution C* (mg/mL)

Calculate the percentage of chlorothiazide and hydrochlorothiazide dimer in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chlorothiazide or hydrochlorothiazide dimer from *Sample solution C*

r_S = peak response of hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrochlorothiazide in *Sample solution C* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified degradation product from *Sample solution A*

r_S = peak response of amlodipine from the *Standard solution*

C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amlodipine in *Sample solution A* (mg/mL)

M_{r1} = molecular weight of amlodipine, 408.88

M_{r2} = molecular weight of amlodipine besylate, 567.05

Acceptance criteria: See *Table 6*. Disregard the amlodipine ethyl analog peak, the valsartan related compound B peak, and any peaks below 0.1%.

USP Monographs

www.webofpharma.com

Table 6

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A[a]	0.60	1.0
Chlorothiazide[b]	0.62	0.50
Hydrochlorothiazide	0.64	—
Devaleryl valsartan[c]	0.71	0.2
Hydrochlorothiazide dimer[d]	0.89	0.50
Amlodipine related compound A[e]	0.96	0.5
Amlodipine	1.00	—
Valsartan related degradation product 1[f]	1.04	0.2
Amlodipine ethyl analog[g]	1.08	—
Valsartan related compound B[h]	1.22	—
Valsartan related degradation product 2[f]	1.27	0.2
Valsartan	1.36	—
Valsartan related degradation product 3[f]	1.51	0.2
Valsartan related degradation product 4[f]	1.62	0.2
Any other unspecified degradation product[i]	—	0.2
Total degradation products	—	2.0

[a] 4-Amino-6-chloro-1,3-benzenedisulfonamide.
[b] 6-Chloro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide.
[c] *N*-{[2′-(1*H*-Tetrazole-5-yl)biphenyl-4-yl]methyl}-L-valine.
[d] 6-Chloro-*N*-[(6-chloro-7-sulfamoyl-2,3-dihydro-4*H*-1,2,4-benzothiadiazine-4-yl 1,1-dioxide)methyl]3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide.
[e] 3-Ethyl 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate].
[f] These are specified unidentified degradation products. No information is available about chemical structures or chemical names for these impurities.
[g] Diethyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. Process related impurity given for information only.
[h] *N*-Butyryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]-methyl}-L-valine. Process related impurity given for information only.
[i] Benzenesulfonic acid is the counter ion to the amlodipine, and peaks at RRT of 0.33 and 0.42 are not considered as degradation products.

- **LIMIT OF VALSARTAN RELATED COMPOUND A**

[NOTE—Valsartan related compound A is a process impurity and a formulation specific degradation product.]

Mobile phase: *n*-Hexane, 2-propanol, and trifluoroacetic acid (850:150:1)

System suitability solution: 0.04 mg/mL each of USP Valsartan Related Compound A and USP Valsartan RS in *Mobile phase*

Standard solution: 0.001 mg/mL of USP Valsartan Related Compound A RS in *Mobile phase*

Sample solution: Nominally 0.5 mg/mL of valsartan in *Mobile phase* from a suitable amount of finely crushed powder from NLT 20 Tablets. Sonication may be necessary for complete dissolution. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L40
Temperatures
Autosampler: 10°
Column: 30°
Flow rate: 0.8 mL/min
Injection volume: 20 µL
Run time: NLT 3.5 times the retention time of valsartan related compound A

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times of valsartan related compound A and valsartan are about 0.65 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between valsartan and valsartan related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0% for valsartan related compound A, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the valsartan related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of valsartan related compound A from the *Sample solution*
r_S = peak response of valsartan related compound A from the *Standard solution*
C_S = concentration of USP Valsartan Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valsartan in the *Sample solution* (mg/mL)

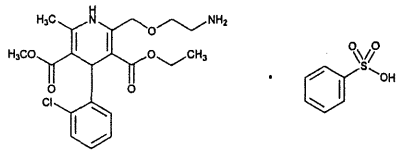
Acceptance criteria: NMT 1.0 %

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store at controlled room temperature in tight containers in a dry place.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Amlodipine Besylate RS
USP Amlodipine Related Compound A RS
3-Ethyl 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate] fumarate.
$C_{20}H_{23}ClN_2O_5 \cdot C_4H_4O_4$ 522.93
USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73
USP Hydrochlorothiazide RS
USP Valsartan RS
USP Valsartan Related Compound A RS
N-Valeryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl] methyl}-D-valine.
$C_{24}H_{29}N_5O_3$ 435.52
USP Valsartan Related Compound B RS

www.webofpharma.com

Amlodipine Besylate



$C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$ 567.05

3,5-Pyridinedicarboxylic acid, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-, 3-ethyl 5-methyl ester, (±)-, monobenzenesulfonate.

3-Ethyl 5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(*o*-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulfonate [111470-99-6].

Monohydrate 585.07

» Amlodipine Besylate is anhydrous or hydrated and contains not less than 97.0 percent and not more than 102.0 percent of $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, protected from light. Store at room temperature.

USP Reference standards ⟨11⟩—
USP Amlodipine Besylate RS

Labeling—Where it is the hydrated form, the label so indicates.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Optical rotation ⟨781A⟩**:** between -0.10° and +0.10°, measured at 20°.

Test solution: 10 mg per mL, in methanol.

Water Determination, *Method I* ⟨921⟩**:** not more than 0.5% for the anhydrous form. If labeled as the hydrated form, the limit is between 3.1% and 5.0%.

Residue on ignition ⟨281⟩**:** not more than 0.2%.

Related compounds—

TEST 1—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Test solution—Transfer 140 mg of Amlodipine Besylate to a 2-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

System suitability solution—Transfer about 14 mg of USP Amlodipine Besylate RS to a suitable container, dissolve in 0.2 mL of methanol, and mix.

Standard stock solution—Dissolve an accurately weighed quantity of USP Amlodipine Besylate RS in methanol to obtain a solution containing 7.0 mg per mL.

Standard solution 1—Transfer 3.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Standard solution 2—Transfer 1.0 mL of the *Standard stock solution* to another 100-mL volumetric flask, dilute with methanol to volume, and mix.

Application volume: 10 µL.

Developing solvent system—Use the upper layer of a mixture of methyl isobutyl ketone, water, and glacial acetic acid (50:25:25).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. Dry the plate for 15 minutes at 80°. Examine the plate under UV light at 254 nm and 365 nm. The chromatogram from the *System suitability solution* shows two clearly separated minor spots with R_F values of about 0.18 and 0.22. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions.* Any spot obtained from the *Test solution,* except for the principal spot, is not greater in size than the spot obtained from *Standard solution 1* (0.3%), and at most two spots are more intense than the spot obtained from *Standard solution 2* (0.1%).

TEST 2—

pH 3.0 Buffer and *Mobile phase*—Prepare as directed in the *Assay.*

System suitability solution—Dissolve about 5 mg of Amlodipine Besylate in 5 mL of hydrogen peroxide, and heat at 70° for 45 minutes.

Standard solution—Dissolve an accurately weighed quantity of USP Amlodipine Besylate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.003 mg per mL.

Test solution—Transfer about 50 mg of Amlodipine Besylate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay.* Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between amlodipine impurity A and amlodipine is not less than 4.5.

[NOTE—For the purpose of identification, the relative retention times are about 0.2 for benzene sulfonate, 0.5 for amlodipine impurity A, and 1.0 for amlodipine. Amlodipine impurity A is 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate.]

Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the standard deviation for replicate injections is not more than 10.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for a period of time that is about 3 times the retention time of amlodipine, and measure the peak responses. Calculate the percentage of each impurity in the portion of Amlodipine Besylate taken by the formula:

$$100(1/F)(C_S/C_T)(r_i/r_S)$$

in which F is the relative response factor, which is equal to 0.5 for amlodipine impurity A and to 1.0 for other impurities; C_S and C_T are the concentrations, in mg per mL, of amlodipine besylate in the *Standard solution* and the *Test solution,* respectively; r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for amlodipine besylate obtained from the *Standard solution:* not more than 0.3% of amlodipine impurity A is found, and not more than 0.3% of total other impurities is found. Disregard any peak less than 0.03%, and disregard any peak due to benzene sulfonate.

www.webofpharma.com

Assay—

pH 3.0 Buffer—Dissolve 7.0 mL of triethylamine in 800 mL of water. Adjust with phosphoric acid to a pH of 3.0 ± 0.1, and dilute with water to 1 L.

Mobile phase—Prepare a filtered and degassed mixture of *pH 3.0 Buffer,* methanol, and acetonitrile (50:35:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Amlodipine Besylate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

Assay preparation—Transfer about 50 mg of Amlodipine Besylate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 237-nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$ in the portion of Amlodipine Besylate taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of amlodipine besylate in the *Standard preparation* and the *Assay preparation,* respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Amlodipine Besylate Tablets

DEFINITION

Amlodipine Besylate Tablets contain NLT 90% and NMT 110% of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet Spectroscopy*: **197U**▲ (CN 1-May-2020)

Standard solution and **Sample solution:** Prepare as directed in the test for *Dissolution.*

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer: Add 7.0 mL of triethylamine into a 1000-mL flask containing 900 mL of water. Adjust the solution with phosphoric acid to a pH of 3.0 ± 0.1. Dilute with water to volume, and mix well.

Mobile phase: Methanol, acetonitrile, and *Buffer* (35:15:50)

Standard solution: 0.0275 mg/mL of USP Amlodipine Besylate RS and 0.0025 mg/mL of USP Amlodipine Related Compound A RS in *Mobile phase*

Sample solution: Nominally 0.02 mg/mL of amlodipine in *Mobile phase* prepared as follows. Place NLT 5 Tablets in a suitable volumetric flask, and add sufficient quantity of *Mobile phase* to disintegrate the Tablets. Shake for 30 min, and dilute with *Mobile phase* to volume. Pass the sample through a syringe tip filter of 0.45-µm pore size. Discard the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 237 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time: NLT 3 times the retention of the amlodipine peak

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for amlodipine and amlodipine related compound A are about 1.0 and 0.5 respectively.]

Suitability requirements

Resolution: NLT 8.5 between amlodipine and amlodipine related compound A

Tailing factor: NMT 2.0 for both amlodipine and amlodipine related compound A

Relative standard deviation: NMT 5.0% for amlodipine related compound A

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amlodipine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of amlodipine, 408.88

M_{r2} = molecular weight of amlodipine besylate, 567.05

Acceptance criteria: 90%–110% of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$)

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

[NOTE—Do not expose any of the solutions to stainless steel because of the degradation of amlodipine.]

Medium: 0.01 N hydrochloric acid; 500 mL

Apparatus 2: 75 rpm. [NOTE—Use paddles covered with Teflon or made of any inert material except stainless steel.]

Time: 30 min

Standard solution: Make appropriate dilutions of USP Amlodipine Besylate RS in *Medium* to obtain the following concentrations: 0.00695 mg/mL for Tablets labeled to contain 2.5 mg, 0.0139 mg/mL for Tablets labeled to contain 5 mg, and 0.0278 mg/mL for Tablets labeled to contain 10 mg. These solutions are stable for 1 day.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved by using UV absorption at the wavelength of maximum absorbance at about 239 nm on portions of the *Sample solution* in comparison with the *Standard solution*, using a 1-cm quartz cell and the *Medium* as the blank.
Calculate the percentage of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution*
M_{r1} = molecular weight of amlodipine, 408.88
M_{r2} = molecular weight of amlodipine besylate, 567.05
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of amlodipine ($C_{20}H_{25}ClN_2O_5$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES
• **ORGANIC IMPURITIES**
Buffer, Mobile phase, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Sample solution: Place a suitable number of Tablets into a 25-mL volumetric flask to obtain a solution with a final nominal concentration of 0.4 mg/mL of amlodipine. Add about 10 mL of *Mobile phase* to the flask. Swirl to disintegrate the Tablets, then sonicate for 5 min to completely dissolve, and cool the sample to room temperature. Dilute with *Mobile phase* to volume. Stir for an additional 15 min using a magnetic stir bar, and pass the sample through a syringe tip filter of 0.45-µm pore size, discarding the first 5 mL.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of amlodipine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amlodipine related compound A from the *Sample solution*
r_S = peak response of amlodipine related compound A from the *Standard solution*
C_S = concentration of USP Amlodipine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amlodipine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of amlodipine related compound A, 406.86
M_{r2} = molecular weight of amlodipine related compound A fumarate, 522.93

Calculate the percentage of amlodipine glucose/galactose adduct or amlodipine lactose adduct, if present, and any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the amlodipine glucose/galactose adduct, amlodipine lactose adduct, or any unspecified degradation product from the *Sample solution*
r_S = peak response of amlodipine from the *Standard solution*
C_S = concentration of USP Amlodipine Besylate RS from the *Standard solution* (mg/mL)
C_U = nominal concentration of amlodipine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of amlodipine, 408.9
M_{r2} = molecular weight of amlodipine besylate, 567.05

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amlodipine related compound A[a]	0.50	1.0
Amlodipine lactose adduct[b]	0.80	0.5
Amlodipine glucose/galactose adduct[b]	0.90	0.5
Amlodipine besylate	1.0	—
Any unspecified degradation product	—	0.2

[a] 3-Ethyl 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate] fumarate.
[b] Formulation-specific impurities.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amlodipine Besylate RS
USP Amlodipine Related Compound A RS
3-Ethyl 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate] fumarate.
$C_{20}H_{23}ClN_2O_5 \cdot C_4H_4O_4$ 522.93

Aromatic Ammonia Spirit

DEFINITION
Aromatic Ammonia Spirit is a hydroalcoholic solution that contains, in each 100 mL, NLT 1.7 g and NMT 2.1 g of total ammonia (NH_3) and Ammonium Carbonate corresponding to NLT 3.5 g and NMT 4.5 g of ammonium carbonate [$(NH_4)_2CO_3$].

ASSAY
• **TOTAL AMMONIA (NH_3)**
Sample: 10.0 mL
Titrimetric system
(See *Titrimetry* ⟨541⟩, *Residual Titrations*.)
Mode: Residual titration
Titrant: 0.5 N sodium hydroxide VS
Analysis: Transfer the *Sample* to a 250-mL conical flask containing 50 mL of water. Add 30.0 mL of 0.5 N sulfuric acid VS, and boil until the solution becomes clear. Cool, add methyl red TS, and titrate the excess acid with *Titrant*.

www.webofpharma.com

Perform a blank determination. Each mL of 0.5 N sulfuric acid is equivalent to 8.515 mg of ammonia (NH_3).

• **AMMONIUM CARBONATE**
Sample: 10.0 mL
Titrimetric system
(See *Titrimetry* ⟨541⟩, *Residual Titrations.*)
Mode: Residual titration
Titrant: 0.5 N sulfuric acid VS
Analysis: Transfer the *Sample* to a 300-mL flask. Add 30 mL of 0.5 N sodium hydroxide, and boil the mixture, replacing the water lost by evaporation, until the vapors no longer turn moistened red litmus paper blue. Cool, dilute with 100 mL of cold, carbon dioxide-free water, add 6 drops of phenolphthalein TS, then add just enough 0.5 N sulfuric acid VS to discharge the color of the phenolphthalein. Add methyl orange TS, and titrate with *Titrant*. Perform a blank determination. Each mL of *Titrant* consumed in the titration with methyl orange TS is equivalent to 48.04 mg of ammonium carbonate [$(NH_4)_2CO_3$].
Acceptance criteria: In each 100 mL, NLT 1.7 g and NMT 2.1 g of total ammonia (NH_3) and Ammonium Carbonate corresponding to NLT 3.5 g and NMT 4.5 g of ammonium carbonate [$(NH_4)_2CO_3$]

OTHER COMPONENTS
• **ALCOHOL DETERMINATION,** *Method I* ⟨611⟩: 62.0%–68.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature not exceeding 30°.

Ammonium Chloride

NH_4Cl 53.49
Ammonium chloride.
Ammonium chloride [12125-02-9].

» Ammonium Chloride contains not less than 99.5 percent and not more than 100.5 percent of NH_4Cl, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
Identification—A solution (1 in 10) responds to the tests for *Ammonium* ⟨191⟩ and for *Chloride* ⟨191⟩.
pH ⟨791⟩: between 4.6 and 6.0, in a solution (1 in 20).
Loss on drying ⟨731⟩—Dry it over silica gel for 4 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩—Add 1 mL of sulfuric acid to about 2 g, accurately weighed, and heat the mixture gently until volatilization is complete: the residue is white, and when ignited, not more than 0.1% of nonvolatile substance remains.
Limit of thiocyanate—Acidify 10 mL of a solution (1 in 10) with hydrochloric acid, and add a few drops of ferric chloride TS: no orange-red color is produced.
Assay—Transfer about 100 mg of Ammonium Chloride, accurately weighed, to a conical flask, add 10 mL of water, and swirl to dissolve. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 5.349 mg of NH_4Cl.

Ammonium Chloride Injection

» Ammonium Chloride Injection is a sterile solution of Ammonium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of NH_4Cl. Hydrochloric acid may be added to adjust the pH.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.
Labeling—The label states the content of ammonium chloride in terms of weight and of milliequivalents in a given volume. The label states also the total osmolar concentration in mOsmol per L or per mL. The label states that the Injection is not for direct injection but is to be diluted with Sodium Chloride Injection to the appropriate strength before use.
Identification—It responds to the tests for *Ammonium* ⟨191⟩ and for *Chloride* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.72 USP Endotoxin Units per mEq of chloride.
pH ⟨791⟩: between 4.0 and 6.0, in a concentration of not more than 100 mg of ammonium chloride per mL.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Chloride content—Transfer an accurately measured volume of Injection, evaporated, if necessary, equivalent to about 2 g of ammonium chloride, to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a conical flask, add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. The content of Cl is between 63.0% and 70.3% of the labeled amount of ammonium chloride.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer an accurately measured volume of Injection, equivalent to about 200 mg of ammonium chloride, to a 500-mL Kjeldahl flask, dilute with water to 200 mL, mix, and add 50 mL of sodium hydroxide solution (2 in 5). Immediately connect the flask by means of a distillation trap to a well-cooled condenser, the delivery tube of which dips into 40 mL of boric acid solution (1 in 25) contained in a suitable receiver. Heat to boiling, and distill about 200 mL. Cool the liquid in the receiver, if necessary, then add methyl red TS, and titrate with 0.1 N sulfuric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sulfuric acid is equivalent to 5.349 mg of NH_4Cl.

Ammonium Chloride Delayed-Release Tablets

» Ammonium Chloride Delayed-Release Tablets contain not less than 94.0 percent and not more than 106.0 percent of the labeled amount of NH_4Cl. Ammonium Chloride Delayed-Release Tablets are enteric-coated.

Packaging and storage—Preserve in tight containers.
Identification—A filtered solution of finely powdered Tablets, equivalent to ammonium chloride solution (1 in 10),

USP Monographs

www.webofpharma.com

responds to the tests for *Ammonium* ⟨191⟩ and for *Chloride* ⟨191⟩.

Disintegration ⟨701⟩: 2 hours, determined as directed for *Enteric-Coated Tablets*.

Limit of thiocyanate—Powder and dissolve in water a sufficient number of Tablets to make about 25 mL of ammonium chloride solution (1 in 10), and filter. Acidify 10 mL of the solution with hydrochloric acid, and add a few drops of ferric chloride TS: no reddish orange color is produced.

Assay—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of ammonium chloride, to a 500-mL Kjeldahl flask, and add 200 mL of water and 50 mL of sodium hydroxide solution (2 in 5). Immediately connect the flask by means of a distillation trap to a well-cooled condenser, the delivery tube of which dips into 40 mL of boric acid solution (1 in 25) contained in a suitable receiver. Heat to boiling, and distill about 200 mL. Cool the liquid in the receiver, if necessary, then add methyl red TS, and titrate with 0.1 N sulfuric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sulfuric acid is equivalent to 5.349 mg of NH_4Cl.

Ferric Ammonium Citrate

» Ferric Ammonium Citrate contains not less than 16.5 percent and not more than 18.5 percent of iron (Fe).

Packaging and storage—Preserve in tight, light-resistant containers, in a cool place.

Identification—

A: Ignite about 0.5 g: it chars, and leaves a residue of iron oxide.

B: To 10 mL of a solution of Ferric Ammonium Citrate (1 in 100) add 6 N ammonium hydroxide dropwise: the solution darkens, but no precipitate forms.

C: To 5 mL of a solution of Ferric Ammonium Citrate (1 in 100) add 0.3 mL of potassium permanganate TS and 4 mL of mercuric sulfate TS, and heat the mixture to boiling: a white precipitate forms.

Ferric citrate—To a solution of Ferric Ammonium Citrate (1 in 100) add potassium ferrocyanide TS: no blue precipitate is formed.

Sulfate ⟨221⟩—Dissolve 100 mg in 1 mL of 2.7 N hydrochloric acid, and dilute with water to 30 mL. Add 3 mL of barium chloride TS, dilute with water to 50 mL, and mix: any turbidity formed after 10 minutes is not greater than that produced in a similarly treated control solution containing 0.31 mL of 0.020 N sulfuric acid (0.3%).

Oxalate—Transfer 1 g to a 125-mL separator, dissolve in 10 mL of water, add 2 mL of hydrochloric acid, and extract successively with one 50-mL portion and one 20-mL portion of ether. Transfer the combined ether extracts to a 150-mL beaker, add 10 mL of water, and remove the ether by evaporation on a steam bath. Add 1 drop of glacial acetic acid and 1 mL of calcium acetate solution (1 in 20): no turbidity is produced within 5 minutes.

Mercury—

Mercury Stock Solution and *Standard Mercury Solution*—Proceed as directed for *Method I* under *Mercury* ⟨261⟩.

Mercury Detection Instrument, Aeration Apparatus, and *Stannous Chloride Solution*—Proceed as directed for *Method IIa* and *Method IIb* under *Mercury* ⟨261⟩.

Standard solutions—Transfer 0.25, 0.50, 1.0, and 3.5 mL of *Standard Mercury Solution* to four separate glass-stoppered bottles, such as biological oxygen-demand bottles, of about 300-mL capacity. Dilute the contents of each bottle with water to 100 mL, and mix. These solutions contain the equivalent of 2.5, 5.0, 10.0, and 35.0 ng of mercury per mL, respectively.

Test solution—Transfer about 1.000 g of Ferric Ammonium Citrate, accurately weighed, to a 200-mL centrifuge bottle with a polytef-lined screw cap, and add 5 mL of nitric acid and 5 mL of hydrochloric acid. Close the bottle tightly, digest on a steam bath for 1 hour, and cool. Quantitatively transfer the solution to a suitable glass-stoppered bottle, dilute with water to 100 mL, and bubble air through the solution for 2 minutes. Prepare a reagent blank in the same manner.

Procedure—Add 5 mL of *Stannous Chloride Solution* to each solution, and immediately insert the bubbler of the *Aeration Apparatus*. Obtain the absorbances as directed by the instrument manufacturer's operating instructions. Perform a blank determination, and make any necessary correction. Plot the absorbances of the *Standard solutions* versus concentrations, in µg per mL, of mercury, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the concentration, in µg per g, of mercury in the *Test solution:* not more than 10 µg per g is found.

Limit of lead—

Standard stock solution—Dissolve about 159.8 mg of lead nitrate, accurately weighed, in 100 mL of water containing 1 mL of nitric acid. Dilute with water to 1000.0 mL, and mix.

Standard solution—[NOTE—Prepare this solution on the day of use.] Transfer 10.0 mL of *Standard stock solution* to a 500-mL volumetric flask, dilute with water to volume, and mix. Each mL contains the equivalent of 2 µg of lead (Pb).

Test solution—Transfer about 15 g of Ferric Ammonium Citrate, accurately weighed, to a 100-mL volumetric flask (previously rinsed with nitric acid and water), dissolve in a mixture of 50 mL of water and 1 mL of nitric acid, dilute with water to volume, and mix.

Procedure—Using a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a deuterium arc background corrector, a digital readout device, and a burner head capable of handling 15% solids content, perform a blank determination with water, following the manufacturer's operating instructions. Separately aspirate portions of the *Standard solution* and the *Test solution*, and record the absorbances. Calculate the lead content, in µg per g, in the portion of Ferric Ammonium Citrate taken by the formula:

$$100(C/W)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of lead in the *Standard solution; W* is the weight, in g, of Ferric Ammonium Citrate taken; and A_U and A_S are the absorbances of the *Test solution* and the *Standard solution*, respectively: not more than 10 µg per g is found.

Assay—Transfer about 1 g of Ferric Ammonium Citrate, accurately weighed, to a 250-mL conical flask, and dissolve in 25 mL of water and 5 mL of hydrochloric acid. Add 4 g of potassium iodide, insert the stopper, and allow to stand protected from light for 15 minutes. Add 100 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, using starch TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of iron (Fe).

www.webofpharma.com

Ferric Ammonium Citrate for Oral Solution

» Ferric Ammonium Citrate for Oral Solution contains Ferric Ammonium Citrate and an effervescent mixture of a suitable organic acid and an alkali metal bicarbonate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of Fe. It may contain one or more suitable flavors, colors, or stabilizing agents.

Packaging and storage—Preserve in tight, light-resistant containers, and store in a cool place.
Identification—A 6-g portion dissolves in 600 mL of water with effervescence. The collected gas meets the requirements of the test for *Bicarbonate* ⟨191⟩, and the resulting solution meets the requirements of the tests for *Iron* ⟨191⟩ and for *Citrate* ⟨191⟩.

Uniformity of dosage units ⟨905⟩—
FOR POWDER PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR POWDER PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.
Assay—Transfer about 6 g of Ferric Ammonium Citrate for Oral Solution, accurately weighed, to a 250-mL conical flask, and dissolve in 100 mL of water. Allow the gas to escape, add 5 mL of hydrochloric acid and 4 g of potassium iodide, insert the stopper, and allow to stand protected from light for 15 minutes. Add 25 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, using starch TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of Fe.

Ammonium Molybdate

$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 1236.00
Molybdate ($Mo_7O_{24}^{6-}$), hexaammonium, tetrahydrate; Hexaammonium molybdate tetrahydrate [12054-85-2].

DEFINITION
Ammonium Molybdate contains NLT 99.3% and NMT 101.8% of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$.

IDENTIFICATION
• **PROCEDURE**
Sample: 0.6 g
Analysis: Dissolve the *Sample* in 1.4 mL of water and 1.45 mL of ammonium hydroxide. Cool this mixture, and add slowly, with mixing, 7.2 mL of a well-cooled mixture of 3.2 mL of nitric acid and 4 mL of water. Allow to stand for 24–48 h, and pass through a sintered-glass filter. To 5 mL of the filtrate add 2 mL of dibasic sodium phosphate TS.
Acceptance criteria: A yellow precipitate is formed, and it is soluble in an excess of 6 N ammonium hydroxide.

ASSAY
• **PROCEDURE**
Sample solution: Dissolve 0.7 g of Ammonium Molybdate in 100 mL water. Adjust with dilute nitric acid to a pH of 4.0. Add saturated hexamethylenetetramine solution to achieve a pH of 5–6.
Analysis: Heat the *Sample solution* to 60°, and add 0.2 mL of 0.1% 4-[2-pyridylazo]resorcinol solution in alcohol. Titrate with 0.1 M lead nitrate VS from the yellow color to the first permanent pink endpoint. Carry out a blank titration. Each mL of 0.1 M lead nitrate is equivalent to 17.66 mg of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$.
Acceptance criteria: 99.3%–101.8%

IMPURITIES
INORGANIC IMPURITIES
• **Arsenate, Phosphate, and Silicate**
Sample solution: Dissolve 2.5 g of the analyte in 70 mL of water in a container other than glass.
Control solution: Dissolve 0.5 g of the analyte in 70 mL of water in a container other than glass, and add an amount of sodium silicate solution equivalent to 0.02 mg of silica (SiO_2).
Analysis
Samples: *Sample solution* and *Control solution*
Adjust with 1.2 N hydrochloric acid to a pH of between 3 and 4, transfer to a glass container, add 2 mL of bromine TS, and adjust with 1.2 N hydrochloric acid to a pH of 1.8 ± 0.1. Heat almost to boiling, and cool to room temperature. Dilute with water to 90 mL, add 10 mL of hydrochloric acid, and transfer to a separator. Add 1 mL of butyl alcohol and 30 mL of 4-methyl-2-pentanone, shake vigorously, and allow the phases to separate. Discard the aqueous phase, and wash the ketone phase with three successive 10-mL portions of 1.2 N hydrochloric acid, discarding the washings. To the washed ketone phase add 10 mL of 1.2 N hydrochloric acid to which has just been added 0.2 mL of a freshly prepared solution (1 in 50) of stannous chloride in hydrochloric acid.
Acceptance criteria: Any blue color in the *Sample solution* does not exceed that in the *Control solution* (NMT 10 ppm).
• **Chloride and Sulfate,** *Chloride* ⟨221⟩: A 0.5-g portion shows no more chloride than 0.30 mL of 0.001 N hydrochloric acid (NMT 20 ppm).
• **Chloride and Sulfate,** *Sulfate* ⟨221⟩: A 0.25-g portion shows no more sulfate than corresponds to 1.0 mL of 0.001 N sulfuric acid (NMT 200 ppm).
• **INSOLUBLE SUBSTANCES**
Sample: 20 g
Analysis: Dissolve the *Sample* in 200 mL of water in a beaker, heat to boiling, cover, and heat on a steam bath for 1 h. Pass the hot solution through a tared filtering crucible, wash the insoluble residue with hot water, and dry at 105° for 2 h.
Acceptance criteria: The weight of the residue is NMT 1 mg (0.005%).
• **NITRATE**
Sample: 1 g
Analysis: Dissolve the *Sample* in 10 mL of water containing 5 mg of sodium chloride, and add 0.10 mL of a solution (1 in 1000) of indigo carmine in 3.6 N sulfuric acid.
Acceptance criteria: The blue color is not completely discharged in 5 min.
• **MAGNESIUM AND ALKALI SALTS**
Sample: 5.0 g
Analysis: Dissolve the *Sample* in 50 mL of water, and filter. To the filtrate add 0.5 g of sodium carbonate and 25 mL of 2.5 N sodium hydroxide. Boil the solution gently for 5 min, cool, and pass through an ignited and tared filter. Wash the filter with 1 N ammonium hydroxide. Ignite the filter at 800 ± 25° for 30 min.
Acceptance criteria: The weight of the residue does not exceed 1 mg (NMT 0.02%).

www.webofpharma.com

• **PHOSPHATE**

Standard solution: Dissolve 143.3 mg of dried monobasic potassium phosphate in water to make 1000 mL, and then dilute 1.0 mL of this solution with 3 N ammonium hydroxide to 100 mL.

Sample solution: Dissolve 20 g of the analyte in 100 mL of 3 N ammonium hydroxide.

Analysis

Samples: *Standard solution* and *Sample solution*

Add 3.5 mL of ferric nitrate solution (1 in 10), and allow to stand for 15 min. Warm gently to coagulate the precipitate, and filter. Wash the filter several times with 1.5 N ammonium hydroxide, then wash the filter with 60 mL of warm 4 N nitric acid to dissolve the residue on the filter, collecting the filtrate in a glass-stoppered, 250-mL conical flask. Add 13 mL of ammonium hydroxide, warm to 40°, add 50 mL of ammonium molybdate TS, shake for 5 min, and allow to stand at 40° for 2 h.

Acceptance criteria: Any yellow precipitate formed from the *Sample solution* does not exceed that from the *Standard solution* (5 ppm).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Ammonium Molybdate Injection

» Ammonium Molybdate Injection is a sterile solution of Ammonium Molybdate in Water for Injection. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of molybdenum (Mo).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.
Labeling—Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.

Identification—

A: The *Assay preparation,* prepared as directed in the *Assay,* exhibits an absorption maximum at about 313 nm when tested as directed for *Procedure* in the *Assay.*

B: Add 0.3 mL of alkaline mercuric-potassium iodide TS to 5 mL of Injection: a reddish-brown color develops.

C: Evaporate 50 mL of Injection on a steam bath to a volume of about 0.3 mL, and add 0.3 mL of ammonium hydroxide. Cool, and add slowly, with mixing, a well-cooled mixture of 1 mL of nitric acid and 1.2 mL of water. Allow to stand for 24 to 48 hours, and pass through a sintered-glass filter. To the filtrate add 0.5 mL of dibasic sodium phosphate TS: a yellow precipitate is formed, and it dissolves in an excess of 6 N ammonium hydroxide.

Pyrogen ⟨151⟩—It meets the requirements, the test dose being 10 mL of Injection per kg.

pH ⟨791⟩: between 3.0 and 6.0.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Ammonium hydroxide diluent—Dilute 40 mL of ammonium hydroxide with water to 1000 mL. Store in a plastic bottle.

Sodium sulfate solution—Dissolve 1 g of sodium sulfate in water to make 100 mL.

Molybdenum stock solution—Transfer about 1.84 g of previously assayed Ammonium Molybdate, accurately weighed, to a 1000-mL volumetric flask, dilute with *Ammonium hydroxide diluent* to volume, and mix. This solution contains the equivalent of 1000 µg of molybdenum per mL.

Standard preparations—Transfer 0, 1.0, 2.0, 3.0, and 4.0 mL, respectively, of *Molybdenum stock solution* to separate 100-mL volumetric flasks, and to the respective flasks add 5.0, 4.0, 3.0, 2.0, and 1.0 mL of *Ammonium hydroxide diluent.* To each flask add 10 mL of *Sodium sulfate solution,* dilute with water to volume, and mix. These *Standard preparations* contain, respectively, 0, 10, 20, 30, and 40 µg of molybdenum per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 500 µg of molybdenum, to a 25-mL volumetric flask, add 1.25 mL of *Ammonium hydroxide diluent* and 2.5 mL of *Sodium sulfate solution,* dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the molybdenum emission line of 313.3 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a molybdenum hollow-cathode lamp and a nitrous oxide–acetylene reducing flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of molybdenum, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, in µg per mL, of molybdenum in the *Assay preparation.* Calculate the quantity, in µg, of molybdenum (Mo) in each mL of the Injection taken by the formula:

$$25C/V$$

in which C is the concentration, in µg per mL, of molybdenum in the *Assay preparation;* and V is the volume, in mL, of Injection taken.

Amobarbital Sodium

$C_{11}H_{17}N_2NaO_3$ 248.25

2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-5-(3-methyl butyl)-, monosodium salt.

Sodium 5-ethyl-5-isopentylbarbiturate [64-43-7].

» Amobarbital Sodium contains not less than 98.5 percent and not more than 100.5 percent of $C_{11}H_{17}N_2NaO_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩—
USP Amobarbital RS

Completeness of solution ⟨641⟩—Mix 1.0 g with 10 mL of carbon dioxide-free water: after 1 minute, the solution is clear and free from undissolved solid.

www.webofpharma.com

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020): of residue obtained in the *Assay*.

B: Ignite about 200 mg: the residue effervesces with acid and responds to the tests for *Sodium* ⟨191⟩.

pH ⟨791⟩: not more than 11.0, in the solution prepared for the test for *Completeness of solution*.

Loss on drying ⟨731⟩—Dry about 1 g, accurately weighed, at 105° for 4 hours: it loses not more than 2.0% of its weight.

Other requirements—Where the label states that Amobarbital Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Amobarbital Sodium for Injection*. Where the label states that Amobarbital Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Amobarbital Sodium for Injection*.

Assay—Dissolve about 500 mg of Amobarbital Sodium, accurately weighed, in about 15 mL of water in a separator. To the solution add 2 mL of hydrochloric acid, shake, and completely extract the liberated amobarbital with 25-mL portions of chloroform. Test for completeness of extraction by extracting with an additional 10-mL portion of chloroform and evaporating the solvent: not more than 0.5 mg of residue remains. Filter the combined extract through a glass filter funnel into a tared beaker, and wash the separator and the filter with several small portions of chloroform. Evaporate the combined filtrate and washings on a steam bath with the aid of a current of air, dry the residue at 105° for 30 minutes, cool, and weigh. The weight of the residue, multiplied by 1.097, represents the weight of $C_{11}H_{17}N_2NaO_3$.

Amobarbital Sodium for Injection

DEFINITION

Amobarbital Sodium for Injection is Amobarbital Sodium suitable for parenteral use. It contains NLT 98.5% and NMT 100.5% of the labeled amount of amobarbital sodium ($C_{11}H_{17}N_2NaO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Residue obtained in the *Assay*
  Acceptance criteria: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩
  Sample: Nominally 200 mg of amobarbital sodium from Amobarbital Sodium for Injection
  Analysis: Ignite the *Sample*.
  Acceptance criteria: The residue effervesces with acid and meets the requirements of the tests.

ASSAY

- **PROCEDURE**
  Sample solution: Transfer nominally 500 mg of amobarbital sodium from Amobarbital Sodium for Injection to a suitable separator, and dissolve in 15 mL of water. Add 2 mL of hydrochloric acid, and shake. Completely extract the amobarbital with 25-mL portions of chloroform. Combine the chloroform extractions, and pass through a glass filter funnel into a tared beaker. Wash the separator and the filter with several small portions of chloroform. Use the combined chloroform extractions and the washings.
  Test for completeness of extraction: Extract the remaining solution in the separator with an additional 10-mL portion of chloroform, and evaporate the solvent; NMT 0.5 mg of residue remains.
  Analysis: Evaporate the *Sample solution* on a steam bath with the aid of a current of air. Dry the residue at 105° for 30 min, cool, and weigh.
  Calculate the percentage of the labeled amount of amobarbital sodium ($C_{11}H_{17}N_2NaO_3$) in the portion of Amobarbital Sodium for Injection taken:

$$\text{Result} = (W_R/W_S) \times (M_{r1}/M_{r2}) \times 100$$

W_R = weight of the residue from the *Analysis* (mg)
W_S = nominal weight of amobarbital sodium in the *Sample solution* (mg)
M_{r1} = molecular weight of amobarbital sodium, 248.25
M_{r2} = molecular weight of amobarbital, 226.27

Acceptance criteria: 98.5%–100.5% on the dried basis

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

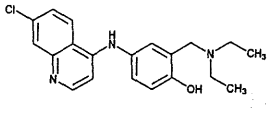
SPECIFIC TESTS

- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*: At the time of use, it meets the requirements.
- **COMPLETENESS OF SOLUTION** ⟨641⟩
  Sample solution: 1 g of amobarbital sodium from Amobarbital Sodium for Injection in 10 mL of carbon dioxide-free water
  Acceptance criteria: After 1 min, the solution is clear and free from undissolved solid.
- **LOSS ON DRYING** ⟨731⟩
  Sample: 1 g
  Analysis: Dry the *Sample* at 105° for 4 h.
  Acceptance criteria: NMT 1.0%
- **PH** ⟨791⟩
  Sample solution: Nominally 100 mg/mL of amobarbital sodium from Amobarbital Sodium for Injection in carbon dioxide-free water
  Acceptance criteria: NMT 11.0
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.4 USP Endotoxin Units/mg of amobarbital sodium.
- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amobarbital RS

Amodiaquine



$C_{20}H_{22}ClN_3O$ 355.86

USP Monographs

www.webofpharma.com

Phenol, 4-[(7-chloro-4-quinolinyl)amino]-2-[(diethylamino)-methyl]-;
4-[(7-Chloro-4-quinolyl)amino]-α-(diethylamino)-*o*-cresol [86-42-0].

DEFINITION
Amodiaquine contains NLT 97.0% and NMT 103.0% of amodiaquine ($C_{20}H_{22}ClN_3O$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

Standard: Dissolve 20 mg of USP Amodiaquine Hydrochloride RS in 10 mL of water in a separator, add 1 mL of ammonium hydroxide, and extract by shaking with 25 mL of chloroform. Draw off and evaporate the chloroform extract, and dry the residue at 105° for 2 h.
Acceptance criteria: Meets the requirements

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

Sample solution: 10 µg/mL in 0.1 N hydrochloric acid
Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**

Standard solution: 15 µg/mL of USP Amodiaquine Hydrochloride RS in 0.1 N hydrochloric acid
Sample solution: 15 µg/mL of Amodiaquine in 0.1 N hydrochloric acid
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 342 nm
Cell: 1 cm
Blank: 0.1 N hydrochloric acid
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of amodiaquine ($C_{20}H_{22}ClN_3O$) in the portion of Amodiaquine taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Amodiaquine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Amodiaquine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of amodiaquine, 355.86
M_{r2} = molecular weight of anhydrous aminodiaquine hydrochloride, 428.79

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.2%
- **ORGANIC IMPURITIES**

Standard solution A: To 20 mg of USP Amodiaquine Hydrochloride RS in a glass-stoppered test tube add 1.0 mL of chloroform (saturated with ammonium hydroxide), and shake vigorously for 2 min. Allow the solids to settle, and decant the liquid into a second test tube.
Standard solution B: *Standard solution A* and chloroform (saturated with ammonium hydroxide) (1 in 200)
Sample solution: 15 mg/mL of Amodiaquine in chloroform (saturated with ammonium hydroxide)
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform (saturated with ammonium hydroxide) and dehydrated alcohol (9:1)
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength UV light.
Acceptance criteria: The chromatograms show principal spots at about the same R_F value; and no secondary spot, if present in the chromatogram from the *Sample solution*, is more intense than the principal spot obtained from *Standard solution B*.

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Amodiaquine Hydrochloride RS

Amodiaquine Hydrochloride

$C_{20}H_{22}ClN_3O \cdot 2HCl \cdot 2H_2O$ 464.81
$C_{20}H_{22}ClN_3O \cdot 2HCl$ 428.79
Phenol, 4-[(7-chloro-4-quinolinyl)amino]-2-[(diethylamino)-methyl]-, dihydrochloride, dihydrate;
4-[(7-Chloro-4-quinolyl)amino]-α-(diethylamino)-*o*-cresol dihydrochloride dihydrate [6398-98-7].
Anhydrous [69-44-3].

DEFINITION
Amodiaquine Hydrochloride contains NLT 97.0% and NMT 103.0% of amodiaquine hydrochloride ($C_{20}H_{22}ClN_3O \cdot 2HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: 197K▲ (CN 1-May-2020)

Sample: Dissolve 20 mg of Amodiaquine Hydrochloride in 10 mL of water in a separator. Add 1 mL of ammonium hydroxide, and extract by shaking with 25 mL of chloroform. Draw off and evaporate the chloroform extract, and dry the residue at 105° for 2 h.
Acceptance criteria: Meets the requirements

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: 197U▲ (CN 1-May-2020)

USP Monographs

www.webofpharma.com

Sample solution: 10 µg/mL in dilute hydrochloric acid (1 in 100)
Acceptance criteria: Meets the requirements
• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements
• **D.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Add 1.0 mL of perchloric acid to each 1 L of solution, adjust with phosphoric acid to a pH of 2.5 ± 0.5, and pass through a filter of 0.45-µm pore size.
Mobile phase: Methanol and *Buffer* (22:78)
System suitability solution: 0.15 mg/mL of USP Amodiaquine Hydrochloride RS and 0.15 mg/mL of USP Chloroquine Phosphate RS in water
Standard solution: 0.15 mg/mL of USP Amodiaquine Hydrochloride RS in water
Sample solution: 0.15 mg/mL in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 224 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Column temperature: 25° ± 5°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for chloroquine phosphate and amodiaquine hydrochloride are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between amodiaquine hydrochloride and chloroquine phosphate
Tailing factor: NMT 1.5 for amodiaquine hydrochloride and chloroquine phosphate
Relative standard deviation: NMT 2.0% for amodiaquine hydrochloride and chloroquine phosphate
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of amodiaquine hydrochloride ($C_{20}H_{22}ClN_3O \cdot 2HCl$) in the portion of Amodiaquine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response from the *Sample solution*
r_S = response from the *Standard solution*
C_S = concentration of USP Amodiaquine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Amodiaquine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%
• **ORGANIC IMPURITIES**
Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Amodiaquine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each impurity from the *Sample solution*
r_T = sum of the responses from the *Sample solution*

Acceptance criteria
Individual impurity: NMT 0.5%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** 7.0%–9.0%
• **COMPLETENESS OF SOLUTION** ⟨641⟩**:** A solution of 200 mg in 10 mL of water is clear.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amodiaquine Hydrochloride RS
USP Chloroquine Phosphate RS

Amodiaquine Hydrochloride Tablets

DEFINITION
Amodiaquine Hydrochloride Tablets contain an amount of amodiaquine hydrochloride ($C_{20}H_{22}ClN_3O \cdot 2HCl \cdot 2H_2O$) equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of amodiaquine ($C_{20}H_{22}ClN_3O$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Powder 1 or more Tablets, and transfer a portion of the powder, equivalent to 50 mg of amodiaquine, to a 125-mL separator. Add 20 mL of water, and shake for 1 min. Add 25 mL of chloroform and 1 mL of ammonium hydroxide, shake for 2 min, and when settled, filter the chloroform extract through cotton that previously has been rinsed with chloroform, collecting the extract in a vessel suitable for evaporation. Evaporate the chloroform, and dry the residue at 105° for 1 h.
Acceptance criteria: Meet the requirements
• **B.** The retention time of the amodiaquine hydrochloride peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Add 1.0 mL of perchloric acid to each 1 L of solution, adjust with phosphoric acid to a pH of 2.5, and pass through a filter of 0.45-µm pore size.
Diluent: 1% (v/v) hydrochloric acid in water
Mobile phase: Methanol and *Buffer* (22:78)
Standard solution: 0.15 mg/mL of USP Amodiaquine Hydrochloride RS and 0.15 mg/mL of USP Chloroquine Phosphate RS in water
Sample solution: Transfer a quantity equivalent to 7.5 mg of amodiaquine hydrochloride from finely powdered Tablets (NLT 20) to a 50-mL volumetric flask, and dissolve in and dilute with *Diluent* to volume. Sonicate for 25 min at 29°. Pass 10 mL through a nylon filter of 0.2-µm pore size, discarding the first 4 mL. Use 2 mL for the analysis.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 224 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for the chloroquine and amodiaquine peaks are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between amodiaquine hydrochloride and chloroquine phosphate
Tailing factor: NMT 1.5 for amodiaquine hydrochloride and chloroquine phosphate
Relative standard deviation: NMT 2.0% for amodiaquine hydrochloride and chloroquine phosphate
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amodiaquine ($C_{20}H_{22}ClN_3O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

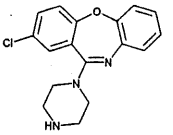
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of amodiaquine in USP Amodiaquine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amodiaquine in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Detector: UV 342 nm
Standard solution: USP Amodiaquine Hydrochloride RS in *Medium*
Sample solution: Filter portions of the solution under test, suitably diluted with water, if necessary, in comparison with a *Standard solution* having a known concentration of USP Amodiaquine Hydrochloride RS.
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amount of amodiaquine hydrochloride ($C_{20}H_{22}ClN_3O \cdot 2HCl \cdot 2H_2O$) dissolved from UV absorbances.
Tolerances: An amount of amodiaquine hydrochloride ($C_{20}H_{22}ClN_3O \cdot 2HCl \cdot 2H_2O$) equivalent to NLT 75% (*Q*) of the labeled amount of amodiaquine ($C_{20}H_{22}ClN_3O$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amodiaquine Hydrochloride RS
USP Chloroquine Phosphate RS

Amoxapine



$C_{17}H_{16}ClN_3O$ 313.78
Dibenz[*b*,*f*][1,4]oxazepine, 2-chloro-11-(1-piperazinyl)-; 2-Chloro-11-(1-piperazinyl)dibenz[*b*,*f*][1,4]oxazepine [14028-44-5].

DEFINITION
Amoxapine contains NLT 98.0% and NMT 102.0% of amoxapine ($C_{17}H_{16}ClN_3O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 3.9 g/L of ammonium acetate in water adjusted with acetic acid or diluted ammonia solution to a pH of 7.3
Mobile phase: Acetonitrile and *Buffer* (30:70)
Diluent: Acetonitrile and *Buffer* (70:30)
System suitability solution: 0.1 mg/mL each of USP Amoxapine RS and USP Amoxapine Related Compound G RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Amoxapine RS in *Diluent*
Sample solution: 0.1 mg/mL of Amoxapine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 7.5-cm; 2.5-µm or 2.7-µm packing L1
Column temperature: 35°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for amoxapine and amoxapine related compound G are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 1.5 between amoxapine and amoxapine related compound G, *System suitability solution*
Tailing factor: 0.8–1.8, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of amoxapine ($C_{17}H_{16}ClN_3O$) in the portion of Amoxapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of amoxapine from the *Sample solution*
r_S = peak response of amoxapine from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Amoxapine RS in the *Standard solution* (mg/mL)
C_U = concentration of Amoxapine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A: 3.9 g/L of ammonium acetate in water adjusted with acetic acid or diluted ammonia solution to a pH of 7.3
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
5	70	30
7.5	60	40
15	60	40
20	20	80
25	20	80
30	70	30
35	70	30

Diluent: *Solution A* and *Solution B* (30:70)
System suitability solution: 1 mg/mL of USP Amoxapine RS and 1.5 µg/mL of USP Amoxapine Related Compound G RS in *Diluent*
Standard solution: 1 µg/mL of USP Amoxapine RS, and 1.5 µg/mL each of USP Amoxapine Related Compound G RS and USP Amoxapine Related Compound D RS in *Diluent*
Sample solution: 1000 µg/mL of Amoxapine in *Diluent*
Chromatographic system: Proceed as directed in the *Assay*.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Peak-to-valley ratio: NLT 3 between amoxapine and amoxapine related compound G, *System suitability solution*
Relative standard deviation: NMT 5.0% each for amoxapine, amoxapine related compound G, and amoxapine related compound D, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of amoxapine related compound G and amoxapine related compound D in the portion of Amoxapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of amoxapine related compound G or amoxapine related compound D from the *Sample solution*
r_S = peak response of amoxapine related compound G or amoxapine related compound D from the *Standard solution*
C_S = concentration of USP Amoxapine Related Compound G RS or USP Amoxapine Related Compound D RS in the *Standard solution* (µg/mL)
C_U = concentration of Amoxapine in the *Sample solution* (µg/mL)

Calculate the percentage of any other impurity in the portion of Amoxapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other impurity from the *Sample solution*
r_S = peak response of amoxapine from the *Standard solution*
C_S = concentration of USP Amoxapine RS in the *Standard solution* (µg/mL)
C_U = concentration of Amoxapine in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.02% of the amoxapine peak.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorophenoxyaniline urea analog[a]	0.57	0.10
Amoxapine	1.0	—
Amoxapine related compound G	1.4	0.15
Amoxapine related compound D	1.7	0.15
Chlorophenoxyaniline[b]	2.9	0.10
Chlorophenoxyaniline carbamate[c]	3.8	0.10
N-Carbamoyl amoxapine[d]	4.3	0.10
Amoxapine dimer[e]	5.0	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	0.50

[a] *N*-[2-(4-Chlorophenoxy)phenyl]piperazine-1-carboxamide.
[b] 2-(4-Chlorophenoxy)aniline.
[c] Ethyl [2-(4-Chlorophenoxy)phenyl]carbamate.
[d] 4-(2-Chlorodibenzo[*b,f*][1,4]oxazepin-11-yl)-*N*-[2-(4-chlorophenoxy)phenyl] piperazine-1-carboxamide.
[e] 1,4-Bis(2-chlorodibenzo[*b,f*][1,4]oxazepine-11-yl)piperazine.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Amoxapine RS
USP Amoxapine Related Compound D RS
2-Chlorodibenzo[*b,f*]-[1,4]-oxazepin-11-one.
$C_{13}H_8ClNO_2$ 245.66
USP Amoxapine Related Compound G RS
3-Chloro-11-(piperazin-1-yl)dibenzo[*b,f*][1,4]oxazepine.
$C_{17}H_{16}ClN_3O$ 313.78

USP Monographs

www.webofpharma.com

Amoxapine Tablets

DEFINITION
Amoxapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of amoxapine ($C_{17}H_{16}ClN_3O$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Triturate a quantity of finely ground Tablets, equivalent to 50 mg of amoxapine, with 10 mL of chloroform, and filter. Evaporate the filtrate on a steam bath to dryness (about 30 min).
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 1.38 g/L of monobasic sodium phosphate in water
  Solution B: 113 g/L of tetramethylammonium chloride in water
  Mobile phase: Transfer 20.0 mL of *Solution B*, 4.0 mL of dilute phosphoric acid (1 in 5), and 720 mL of acetonitrile to a 2000-mL volumetric flask. Dilute with *Solution A* to volume.
  Standard stock solution: 1 mg/mL of USP Amoxapine RS in acetonitrile. Shake by mechanical means to dissolve, and then dilute with acetonitrile to volume.
  Standard solution: 0.1 mg/mL from the *Standard stock solution* diluted with *Mobile phase*
  Sample stock solution: Nominally 1 mg/mL of amoxapine from NLT 20 finely powdered Tablets prepared as follows. Transfer a suitable quantity of the powder to a volumetric flask. Add 80% of the flask volume of *Mobile phase*, and shake vigorously by mechanical means for 20 min. Dilute with *Mobile phase* to volume, and filter.
  Sample solution: 0.1 mg/mL from the *Sample stock solution* diluted with *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 25-cm; packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 1200 theoretical plates
  Tailing factor: NMT 1.8
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of amoxapine ($C_{17}H_{16}ClN_3O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of amoxapine from the *Sample solution*
r_S = peak response of amoxapine from the *Standard solution*
C_S = concentration of USP Amoxapine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amoxapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: Simulated gastric fluid (without enzyme); 900 mL
  Apparatus 2: 50 rpm
  Time: 30 min
  Sample solution: Sample per *Dissolution* ⟨711⟩.
  Standard solution: USP Amoxapine RS having a concentration similar to the expected *Sample solution* in *Medium*
  Instrumental conditions
  Analytical wavelength: 294 nm
  Analysis
  Samples: *Sample solution* and *Standard solution*
  Determine the percentage of the labeled amount of amoxapine ($C_{17}H_{16}ClN_3O$) dissolved from UV absorbances of filtered portions of the *Sample solution*, suitably diluted with *Medium*, if necessary.
  Calculate the percentage of the labeled amount of amoxapine ($C_{17}H_{16}ClN_3O$) dissolved:

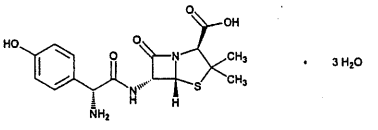
$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)

  Tolerances: NLT 80% (Q) of the labeled amount of amoxapine ($C_{17}H_{16}ClN_3O$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amoxapine RS

Amoxicillin



$C_{16}H_{19}N_3O_5S \cdot 3H_2O$ 419.45
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,6-[[amino(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-, trihydrate [2*S*-[2α,5α,6β(*S**)]]-;
(2*S*,5*R*,6*R*)-6-[(*R*)-(–)-2-Amino-2-(*p*-hydroxyphenyl) acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid trihydrate [61336-70-7].
Anhydrous 365.41
[26787-78-0].

DEFINITION
Amoxicillin contains NLT 900 µg/mg and NMT 1050 µg/mg of amoxicillin ($C_{16}H_{19}N_3O_5S$), calculated on the anhydrous basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: 6.8 g/L of *monobasic potassium phosphate* in *water*. Adjust with a 45% (w/w) solution of *potassium hydroxide* to a pH of 5.0 ± 0.1.
Mobile phase: *Acetonitrile* and *Diluent* (1:24)
Standard solution: 1.2 mg/mL of USP Amoxicillin RS in *Diluent*. [NOTE—Use this solution within 6 h.]
Sample solution: 1.2 mg/mL of Amoxicillin in *Diluent*. [NOTE—Use this solution within 6 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4-mm × 25-cm; packing *L1*
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of amoxicillin ($C_{16}H_{19}N_3O_5S$) in the portion of Amoxicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
C_U = concentration of Amoxicillin in the *Sample solution* (mg/mL)
P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)

Acceptance criteria: 900–1050 µg/mg of amoxicillin ($C_{16}H_{19}N_3O_5S$) on the anhydrous basis

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: 2.72 g/L of *monobasic potassium phosphate*. Adjust with *1 N potassium hydroxide* or *20% phosphoric acid* to a pH of 5.0 ± 0.1.
Solution B: *Methanol*
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	97	3
10	97	3
22	75	25
26	97	3

System suitability solution: 12.5 µg/mL each of USP Amoxicillin Related Compound A RS and USP Amoxicillin Related Compound D RS in *Solution A*
Standard solution: 12.5 µg/mL of USP Amoxicillin RS in *Solution A*
Sample solution: 1.25 mg/mL of Amoxicillin in *Solution A*. [NOTE—Store this solution at 4° and use within 4 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; 5-µm packing *L1*
Temperatures
Autosampler: 4°
Column: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution* [NOTE—Identify peaks by the relative retention times in *Table 2*.]
Suitability requirements
Resolution: NLT 1.5 between amoxicillin related compound A and the second peak for amoxicillin related compound D, *System suitability solution*
Relative standard deviation: NMT 10%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Amoxicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of amoxicillin from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (µg/mL)
C_U = concentration of Amoxicillin in the *Sample solution* (mg/mL)
F = unit conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*. [NOTE—The reporting limit is 0.03 times the amoxicillin peak from the *Standard solution*.]

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amoxicillin related compound I[a] (D-hydroxyphenylglycine)	0.32	1.0
Amoxicillin related compound D[b,c] (amoxicillin open ring)	0.53	1.0
	0.68	1.0
Amoxicillin related compound A[d] (6-aminopenicillanic acid)	0.78	0.5
Amoxicillin related compound B[e,f] (L-amoxicillin)	0.87	—
Amoxicillin	1.0	—
Amoxicillin related compound G[g] (D-hydroxyphenyl glycylamoxicillin)	2.9	1.0
Amoxicillin related compound E[h,i] (amoxicillin penilloic derivative)	4.5	1.0

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amoxicillin related compound M[l] [*N*-(penicillan-6-yl) open ring amoxicillinamide]	6.0	1.0
Amoxicillin related compound F[e,k] (phenylpyrazinediol)	6.3	—
Amoxicillin related compound C[l] (amoxicillin rearrangement product)	6.4	1.0
Amoxicillin related compound E[h,i] (amoxicillin penilloic derivative)	6.7	1.0
Amoxicillin related compound J[m] (amoxicillin open ring dimer)	8.8	1.0
Amoxicillin related compound L[n] [*N*-(penicillan-6-yl)amoxicillinamide]	9.0	1.0
Any unspecified individual impurity	—	1.0
Total impurities	—	5.0

[a] (*R*)-2-Amino-2-(4-hydroxyphenyl)acetic acid.
[b] The chromatographic system resolves two penicilloic acids from each other.
[c] (4*S*)-2-{[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[d] (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[e] These compounds are listed for information only and are not to be reported.
[f] (2*S*,5*R*,6*R*)-6-[(*S*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[g] (2*S*,5*R*,6*R*)-6-{(*R*)-2-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[h] The chromatographic system resolves two penilloic acids from each other.
[i] (4*S*)-2-{[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[j] (2*S*,5*R*,6*R*)-6-(2-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-((4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[k] 3-(4-Hydroxyphenyl)pyrazin-2-ol.
[l] (4*S*)-2-[5-(4-Hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid.
[m] (2*S*,5*R*,6*R*)-6-((2*R*)-2-{2-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido}-2-(4-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[n] (2*S*,5*R*,6*R*)-6-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

SPECIFIC TESTS
- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **DIMETHYLANILINE** ⟨223⟩: Meets the requirements
- **pH** ⟨791⟩
  Sample solution: 2 mg/mL
  Acceptance criteria: 3.5–6.0
- **WATER DETERMINATION** ⟨921⟩, *Method I*: 11.5%–14.5%
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*: Where the label states that Amoxicillin is sterile, it meets the requirements, except to use Fluid Thioglycollate Medium containing polysorbate 80 solution (5 mg/mL) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, to use Soybean-Casein Digest Medium containing polysorbate 80 solution (5 mg/mL) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, and to shake the tubes once daily.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Amoxicillin is sterile or Amoxicillin must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.25 USP Endotoxin Units/mg of amoxicillin.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is intended for veterinary use only and that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Label all other Amoxicillin to indicate that it is to be used in the manufacture of nonparenteral drugs only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amoxicillin RS
  USP Amoxicillin Related Compound A RS
  (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid;
  6-Aminopenicillanic acid.
  $C_8H_{12}N_2O_3S$ 216.26
  USP Amoxicillin Related Compound D RS
  (4*S*)-2-{[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl}-5,5-dimethylthiazolidine-4-carboxylic acid;
  Amoxicillin open ring.
  $C_{16}H_{21}N_3O_6S$ 383.42

Amoxicillin Boluses

» Amoxicillin Boluses contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$).

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
Labeling—Label Boluses to indicate that they are for veterinary use only.
USP Reference standards ⟨11⟩—
USP Amoxicillin RS

Identification—
Test solution—To a portion of powdered Boluses add 0.1 N hydrochloric acid to obtain a *Test solution* containing about 4 mg of amoxicillin per mL. Use within 10 minutes after preparation.
Application volume, Developing solvent system, Procedure—Proceed as directed for the *Identification* test under *Amoxicillin Tablets*.
Disintegration ⟨701⟩: 30 minutes, simulated gastric fluid being used instead of water.
Water Determination, *Method I* ⟨921⟩: not more than 7.5%.

Assay—
Diluent, Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Amoxicillin*.
Assay preparation—Weigh and finely powder not fewer than 5 Boluses. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of amoxicillin, to a 250-mL volumetric flask, add *Diluent* to volume, and mix. Sonicate if necessary to ensure complete dissolution of the amoxicillin. Pass a portion of this solution through a filter of 1-µm or finer porosity, and use the filtrate as the *Assay preparation*. [NOTE—Use this solution within 6 hours.]
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Amoxicillin*. Calculate the quantity, in mg, of

www.webofpharma.com

amoxicillin ($C_{16}H_{19}N_3O_5S$) in the portion of Boluses taken by the formula:

$$0.25CP(r_U/r_S)$$

in which the terms are as defined therein.

Amoxicillin Capsules

DEFINITION

Amoxicillin Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 6.8 g/L of monobasic potassium phosphate in water. Adjust with 45% potassium hydroxide TS to a pH of 5.0 ± 0.1.

Mobile phase: Acetonitrile and *Buffer* (1:24)

Standard solution: 1.2 mg/mL of USP Amoxicillin RS in *Buffer*. [NOTE—Use this solution within 6 h.]

Sample solution: Remove, as completely as possible, the contents of NLT 20 Capsules. Mix the combined contents, and transfer a quantity, equivalent to 200 mg of anhydrous amoxicillin, to a 200-mL volumetric flask. Add *Buffer* to volume. Sonicate if necessary to ensure complete dissolution. [NOTE—Use this solution within 6 h.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4-mm × 25-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)

P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: Water; 900 mL

Apparatus 1: 100 rpm, for Capsules containing 250 mg

Apparatus 2: 75 rpm, for Capsules containing 500 mg

Time: 60 min

Analytical wavelength: UV 272 nm

Standard solution: USP Amoxicillin RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

Tolerances: NLT 80% (*Q*) of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 90 min

Analytical wavelength: UV 272 nm

Standard solution: USP Amoxicillin RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

Tolerances: NLT 80% (*Q*) of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

▲• **ORGANIC IMPURITIES**

Solution A: 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 20% (w/v) solution of sodium hydroxide to a pH of 5.0 ± 0.1.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
25	94	6
40	84	16
50	84	16
51	100	0
60	100	0

Impurity stock solution: 0.15 mg/mL each of USP Amoxicillin Related Compound C RS and USP Amoxicillin Related Compound H RS in *Solution A*, prepared as follows. Transfer a weighed amount of USP Amoxicillin Related Compound C RS and USP Amoxicillin Related Compound H RS to a suitable volumetric flask. Add acetonitrile to fill 10% of the flask volume and *Solution A* to fill 60% of the flask volume. Sonicate to dissolve and dilute with *Solution A* to volume.

System suitability solution: 1.5 mg/mL of USP Amoxicillin RS and 0.015 mg/mL each of USP Amoxicillin Related Compound C RS and USP Amoxicillin Related Compound

USP Monographs

www.webofpharma.com

H RS in *Solution A* prepared as follows. Transfer a weighed amount of USP Amoxicillin RS to a suitable volumetric flask. Add *Solution A* to fill 60% of the flask volume. Add an appropriate volume of *Impurity stock solution* to the volumetric flask. Sonicate to dissolve and dilute with *Solution A* to volume.

Standard solution: 0.017 mg/mL of USP Amoxicillin RS in *Solution A*. Sonicate if necessary to dissolve. Use this solution immediately after preparation.

Sample solution: Nominally 1.5 mg/mL of amoxicillin in *Solution A* from the Capsules, prepared as follows. Transfer Capsule powder equivalent to 75 mg of amoxicillin into a 50-mL volumetric flask. Add *Solution A* to fill 60% of the final flask volume. Sonicate for 15 min and dilute with *Solution A* to volume. Pass through a suitable filter of 0.45-µm pore size. Use this solution immediately after preparation.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between amoxicillin related compound C and amoxicillin related compound H, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of amoxicillin from the *Standard solution*

C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)

P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)

F_1 = conversion factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound I[a,b] (D-hydroxyphenylglycine)	0.19	—	—
Amoxicillin related compound D[c,d] (amoxicillin open ring)	0.36, 0.47	0.8	2.4

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound A[a,e] (6-aminopenicillanic acid)	0.66	—	—
Amoxicillin related compound B[a,f] (L-amoxicillin)	0.82	—	—
Amoxicillin	1.0	1.0	—
Amoxicillin related compound E[d,g]	2.5, 3.32	1.0	3.6
Amoxicillin related compound G[a,h] (D-hydroxyphenylglycylamoxicillin)	3.03	—	—
Amoxicillin related compound C[i] (amoxicillin rearrangement product)	3.63, 3.84	0.98	2.0
Amoxicillin related compound H[a,j] (*N*-pivaloyl pHPG)	4.03	—	—
Amoxicillin related compound F[k] (pyrazine-2-ol)	4.12	1.1	1.0
Amoxicillin related compound K[d,l] (amoxicilloic acid dimers 1 and 2)	4.39, 4.75	0.64	1.0
6-APA amoxicillin amide[a,m]	6.24	—	—
Amoxicilloic amoxilloic acid dimers 1, 2, 3, and 4[d]	6.18, 6.40, 6.56	0.46	1.0
Amoxicillin related compound J[n] (amoxicillin open ring dimer)	7.02	0.64	2.0
N-Pivaloyl amoxicillin	7.96	—	—
Any individual unspecified degradation product	—	—	1.0

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	7.0

[a] These are process impurities that are controlled in the drug substance. They are listed here for reference only and are not to be reported.
[b] (*R*)-2-Amino-2-(4-hydroxyphenyl)acetic acid.
[c] (4*S*)-2-[[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.
[d] Some chromatographic systems may resolve the peaks from isomers, and the limit is for the sum of all the isomers.
[e] (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[f] (2*S*,5*R*,6*R*)-6-[(*S*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[g] (4*S*)-2-[[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid and (4*R*)-2-[[(*S*)-2-amino-2-(4-hydroxyphenyl)acetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.
[h] (2*S*,5*R*,6*R*)-6-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[i] (4*S*)-2-[5-(4-Hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid.
[j] (*R*)-2-(4-Hydroxyphenyl)-2-pivalamidoacetic acid.
[k] 3-(4-Hydroxyphenyl) pyrazin-2-ol.
[l] Oligomers of penicilloic acids of amoxicillin.
[m] (2*S*,5*R*,6*R*)-6-[[(2*S*,5*R*,6*R*)-6-[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[n] Co-oligomers of amoxicillin and penicilloic acids of amoxicillin.▲
(Postponed on 1-May-2018)

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amoxicillin RS
  ▲USP Amoxicillin Related Compound C RS
  (4*S*)-2-[5-(4-Hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid.
  $C_{16}H_{19}N_3O_5S$ 365.40
  USP Amoxicillin Related Compound H RS
  (*R*)-2-(4-Hydroxyphenyl)-2-pivalamidoacetic acid.
  $C_{13}H_{17}NO_4$ 251.28▲ (Postponed on 1-May-2018)

Amoxicillin Intramammary Infusion

» Amoxicillin Intramammary Infusion is a suspension of Amoxicillin in a suitable vegetable oil vehicle. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$). It contains a suitable dispersing agent and preservative.

Packaging and storage—Preserve in well-closed disposable syringes.
Labeling—Label it to indicate that it is intended for veterinary use only.
USP Reference standards ⟨11⟩—
USP Amoxicillin RS
Identification—Transfer a quantity of Intramammary Infusion, equivalent to about 60 mg of amoxicillin, to a 50-mL centrifuge tube, add 25 mL of toluene, mix, and centrifuge. Decant and discard the toluene. Wash the residue with four 25-mL portions of toluene, sonicating for about 30 seconds after each addition of toluene. Dry the residue in vacuum over silica gel. Add 15 mL of 0.1 N hydrochloric acid to the residue, and mix. The solution obtained responds to the *IDENTIFICATION* test under *Amoxicillin Capsules*.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay—Proceed as directed for amoxicillin under *Antibiotics—Microbial Assays* ⟨81⟩. Expel the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of *Buffer B.3* and 1.0 mL of polysorbate 80, and blend for 3 to 5 minutes. Allow to stand for about 10 minutes, and dilute an accurately measured volume of the aqueous phase quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Amoxicillin for Injectable Suspension

» Amoxicillin for Injectable Suspension is a sterile mixture of Amoxicillin and one or more suitable buffers, preservatives, stabilizers, and suspending agents. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Amoxicillin RS
Identification—Prepare a test solution containing the equivalent of 4 mg of amoxicillin per mL by adding 0.1 N hydrochloric acid to Amoxicillin for Injectable Suspension. Allow the solution to stand for 5 minutes before use: the solution responds to the *IDENTIFICATION* test under *Amoxicillin Capsules*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.25 Endotoxin Unit per mg of amoxicillin.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed in the section *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, except to use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, to use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, and to shake the tubes once daily.

www.webofpharma.com

pH ⟨791⟩: between 5.0 and 7.0, in the suspension constituted as directed in the labeling.
Water Determination, *Method I* ⟨921⟩: between 11.0% and 14.0%.

Assay—

Diluent, Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Amoxicillin.*

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Amoxicillin for Injectable Suspension as directed in the labeling. Withdraw all of the withdrawable contents, using a hypodermic needle and syringe, and quantitatively dilute with *Diluent* to obtain a solution containing about 1 mg of anhydrous amoxicillin per mL. Pass a portion of this solution through a suitable filter of 1-µm or finer porosity, and use the filtrate as *Assay preparation 1*. Use this solution within 6 hours.

Assay preparation 2 (where the label states the quantity of amoxicillin in a given volume of constituted suspension)—Constitute Amoxicillin for Injectable Suspension as directed in the labeling. Quantitatively dilute an accurately measured volume of the constituted suspension with *Diluent* to obtain a solution containing about 1 mg of anhydrous amoxicillin per mL. Pass a portion of this solution through a suitable filter of 1-µm or finer porosity, and use the filtrate as *Assay preparation 2*. Use this solution within 6 hours.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Amoxicillin*. Calculate the quantity, in mg, of amoxicillin ($C_{16}H_{19}N_3O_5S$) in the container, or in the portion of constituted Suspension taken by the formula:

$$(L/D)(CP/1000)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of anhydrous amoxicillin in the container, or in the volume of constituted suspension taken; D is the concentration, in mg of anhydrous amoxicillin per mL, of *Assay preparation 1* or of *Assay preparation 2* on the basis of the labeled quantity in the container or in the portion of constituted suspension taken, respectively, and the extent of dilution; and the other terms are as defined therein.

Amoxicillin Oral Suspension

» Amoxicillin Oral Suspension is a suspension of Amoxicillin in Soybean Oil. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$).

Packaging and storage—Preserve in multiple-dose containers equipped with a suitable dosing pump.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Amoxicillin RS
Identification—Shake a portion of Oral Suspension with a mixture of acetone and 0.1 N hydrochloric acid (4:1) to obtain a solution containing about 1 mg of amoxicillin per mL. The solution responds to the *Identification* test under *Amoxicillin Capsules.*
Water Determination, *Method I* ⟨921⟩: not more than 2.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay—

Standard preparation—Prepare as directed for *Standard Preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩, using USP Amoxicillin RS.

Assay preparation—Using the dosing pump, deliver a number of doses of Oral Suspension, equivalent to about 250 mg of amoxicillin, to a separator containing 100 mL of hexanes, and shake vigorously. Add 140 mL of water, and shake for 5 minutes. Allow the layers to separate, and drain the lower, aqueous layer into a 250-mL volumetric flask. Repeat the extraction with two 50-mL portions of water. Combine the aqueous extracts in the volumetric flask, dilute with water to volume, and mix.

Procedure—Proceed with Oral Suspension as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩, using USP Amoxicillin RS. Calculate the quantity, in mg, of amoxicillin ($C_{16}H_{19}N_3O_5S$) in each dose of Oral Suspension taken by the formula:

$$(250/N)(F/2000)(B - I)$$

in which N is the number of doses taken, and the other terms are as defined therein.

Amoxicillin for Oral Suspension

DEFINITION
Amoxicillin for Oral Suspension contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$). It contains one or more suitable buffers, colors, flavors, preservatives, stabilizers, sweeteners, and suspending agents.

IDENTIFICATION
- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **PROCEDURE**

Buffer: Dissolve 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (1:24)
Standard solution: 1.2 mg/mL of USP Amoxicillin RS in *Buffer.* [NOTE—Use this solution within 6 h.]
Sample solution: Dilute a measured volume of Amoxicillin for Oral Suspension, constituted as directed in the labeling, freshly mixed and free from air bubbles, quantitatively and stepwise in *Buffer* to obtain a solution containing nominally 1 mg/mL of anhydrous amoxicillin. Pass a portion of this solution through a suitable filter. [NOTE—Use this solution within 6 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 230 nm
Column: 4-mm × 25-cm;10-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of $C_{16}H_{19}N_3O_5S$ in the Amoxicillin for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of anhydrous amoxicillin in the *Sample solution* (mg/mL)
P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  For solids packaged in single-unit containers: Meets the requirements
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 5.0–7.5, in the suspension constituted as directed in the labeling
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amoxicillin RS

Amoxicillin Tablets

DEFINITION

Amoxicillin Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 45% potassium hydroxide TS to a pH of 5.0 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (1:24)
Standard solution: 1.2 mg/mL of USP Amoxicillin RS in *Buffer*. [NOTE—Use this solution within 6 h.]
Sample solution: Place NLT 5 Tablets in a high-speed glass blender jar containing *Buffer* sufficient to yield a concentration of 1 mg/mL of anhydrous amoxicillin. Blend for 4 ± 1 min, allow to stand for 5 min, and centrifuge a portion of the mixture. [NOTE—Where the volume of *Buffer* required would exceed 500 mL, place 5 Tablets in a volumetric flask of such capacity that when finally diluted to volume, a concentration of 1 mg of anhydrous amoxicillin per milliliter would be obtained. Add a volume of *Buffer* equivalent to three-fourths of the capacity of the volumetric flask, and sonicate for 5 min. Dilute with *Buffer* to volume, add a magnetic stirring bar, and stir for 30 min. Centrifuge a portion of this solution.]
Pass a portion of the clear supernatant through a suitable filter. [NOTE—Use this solution within 6 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4-mm × 25-cm; 10-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of amoxicillin from the *Sample solution*
r_S = peak response of amoxicillin from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)
P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Determine the amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) dissolved by using the following method.
Buffer: 27.2 g of monobasic potassium phosphate in 3 L of water. Adjust with 45% potassium hydroxide TS to a pH of 5.0 ± 0.1. Dilute with water to obtain 4 L of solution.
Mobile phase: Acetonitrile and *Buffer* (1:39)
Standard solution: 0.05 mg/mL of USP Amoxicillin RS in *Buffer*. [NOTE—Use this solution within 6 h.]
Sample solution: Pass a portion of the sample through a suitable filter of 0.5-µm pore size. Quantitatively dilute a volume of the filtrate with water to obtain an estimated concentration of 0.045 mg/mL of amoxicillin. Use this solution within 6 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Columns
Guard: 2-mm × 2-cm; packing L2
Analytical: 3.9-mm × 30-cm; packing L1
Column temperature: 40 ± 1°
Flow rate: 0.7 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 1.5%

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times P \times F \times 100$$

r_U = peak response of amoxicillin from the *Sample solution*
r_S = peak response of amoxicillin from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of the dissolution medium, 900 mL
D = dilution factor for the *Sample solution*
P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Tolerances: NLT 75% (Q) of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) is dissolved.
For products labeled as chewable Tablets: Proceed as directed above.
For chewable Tablets labeled to contain 200 or 400 mg
Time: 20 min
Tolerances: NLT 70% (Q) of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) is dissolved.
For chewable Tablets labeled to contain 125 or 250 mg
Time: 90 min
Tolerances: NLT 70% (Q) of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) is dissolved.
For veterinary products: Proceed as directed above, except use *Apparatus 2* at 100 rpm.

IMPURITIES

Change to read:

• **▲ORGANIC IMPURITIES**
Solution A: Dissolve 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 20% (w/v) solution of sodium hydroxide to a pH of 5.0 ± 0.1.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
25	94	6
40	84	16
50	84	16
51	100	0
60	100	0

Impurity stock solution: 0.15 mg/mL each of USP Amoxicillin Related Compound C RS and USP Amoxicillin Related Compound H RS in *Solution A* prepared as follows. Transfer a weighed amount of USP Amoxicillin Related Compound C RS and USP Amoxicillin Related Compound H RS to a suitable volumetric flask. Add acetonitrile to fill 10% of the flask volume and *Solution A* to fill 60% of the flask volume. Sonicate to dissolve and dilute with *Solution A* to volume.
System suitability solution: 1.5 mg/mL of USP Amoxicillin RS and 0.015 mg/mL each of USP Amoxicillin Related Compound C RS and USP Amoxicillin Related Compound H RS in *Solution A* prepared as follows. Transfer a weighed amount of USP Amoxicillin RS to a suitable volumetric flask. Add *Solution A* to fill 60% of the flask volume. Add an appropriate volume of *Impurity stock solution* to the volumetric flask. Sonicate to dissolve and dilute with *Solution A* to volume.
Standard solution: 0.017 mg/mL of USP Amoxicillin RS in *Solution A*. Sonicate if necessary to dissolve. Use this solution immediately after preparation.
Sample solution: Nominally 1.5 mg/mL of amoxicillin in *Solution A* from the powdered Tablets prepared as follows. Transfer powdered Tablets equivalent to 75 mg of amoxicillin into a 50-mL volumetric flask. Add *Solution A* to fill 60% of the final flask volume. Sonicate for 15 min and dilute with *Solution A* to volume. Pass through a suitable filter of 0.45-µm pore size. Use this solution immediately after preparation.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between amoxicillin related compound C and amoxicillin related compound H, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of amoxicillin from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)
P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)
F_1 = conversion factor, 0.001 mg/µg
F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

www.webofpharma.com

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound I[a, b] (D-hydroxyphenylglycine)	0.19	—	—
Amoxicillin related compound D[c, d] (amoxicillin open ring)	0.36, 0.47	0.8	2.0
Amoxicillin related compound A[a, e] (6-aminopenicillanic acid)	0.66	—	—
Amoxicillin related compound B[a, f] (L-amoxicillin)	0.82	—	—
Amoxicillin	1.0	1.0	—
Amoxicillin related compound E[d, g]	2.5, 3.32	1.0	5.0
Amoxicillin related compound G[a, h] (D-hydroxyphenylglycylamoxicillin)	3.03	—	—
Amoxicillin related compound C[i] (amoxicillin rearrangement product)	3.63, 3.84	1.0	1.0
Amoxicillin related compound H[a, j] (*N*-pivaloyl pHPG)	4.03	—	—
Amoxicillin related compound F[k] (pyrazine-2-ol)	4.12	1.1	1.0
Amoxicillin related compound K[d, l] (amoxicilloic acid dimers 1 and 2)	4.39, 4.75	0.64	1.0
6-APA amoxicillin amide[a, m]	6.24	—	—
Amoxicilloic amoxilloic acid dimers 1, 2, 3, and 4[d]	6.18, 6.40, 6.56	0.46	1.0
Amoxicillin related compound J[n] (amoxicillin open ring dimer)	7.02	0.64	2.5
N-Pivaloyl amoxicillin	7.96	—	—
Any individual unspecified degradation product	—	—	1.0
Total impurities	—	—	10.0

[a] These are process impurities that are controlled in the drug substance. They are listed here for reference only and are not to be reported.
[b] (*R*)-2-Amino-2-(4-hydroxyphenyl)acetic acid.
[c] (4*S*)-2-[[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.
[d] Some chromatographic systems may resolve the peaks from isomers, and the limit is for the sum of all the isomers.
[e] (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[f] (2*S*,5*R*,6*R*)-6-[(*S*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[g] (4*S*)-2-[[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid and (4*R*)-2-[[(*S*)-2-amino-2-(4-hydroxyphenyl)acetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.
[h] (2*S*,5*R*,6*R*)-6-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[i] (4*S*)-2-[5-(4-Hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid.
[j] (*R*)-2-(4-Hydroxyphenyl)-2-pivalamidoacetic acid.
[k] 3-(4-Hydroxyphenyl) pyrazin-2-ol.
[l] Oligomers of penicilloic acids of amoxicillin.
[m] (2*S*,5*R*,6*R*)-6-[[(2*S*,5*R*,6*R*)-6-[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[n] Co-oligomers of amoxicillin and penicilloic acids of amoxicillin.▲
(Postponed on 1-May-2018)

SPECIFIC TESTS

- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **Labeling:** Label chewable Tablets to indicate that they are to be chewed before swallowing. Tablets intended solely for veterinary use are so labeled.

Change to read:

- **USP Reference Standards** ⟨11⟩

USP Amoxicillin RS

▲USP Amoxicillin Related Compound C RS
(4*S*)-2-[5-(4-Hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid.
$C_{16}H_{19}N_3O_5S$ 365.40

USP Amoxicillin Related Compound H RS
(*R*)-2-(4-Hydroxyphenyl)-2-pivalamidoacetic acid.
$C_{13}H_{17}NO_4$ 251.28▲ (Postponed on 1-May-2018)

Amoxicillin Tablets for Oral Suspension

DEFINITION

Amoxicillin Tablets for Oral Suspension contain NLT 90.0% and NMT 110.0% of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$).

USP Monographs

www.webofpharma.com

IDENTIFICATION

• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩

Standard solution: 4 mg/mL of USP Amoxicillin RS in 0.1 N hydrochloric acid. Use within 10 min of preparation.

Sample solution: An aqueous dispersion of Tablets for Oral Suspension in 0.1 N hydrochloric acid containing 4 mg/mL of amoxicillin. Use within 10 min of preparation.

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL

Developing solvent system: Methanol, chloroform, pyridine, and water (90:80:1:30)

Spray reagent: 3 mg/mL of ninhydrin in alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter. Dry the plate with the aid of a current of warm air for 10 min. Spray lightly with *Spray reagent*, and dry at 110° for 15 min.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **Procedure**

Diluent: 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1.

Mobile phase: Acetonitrile and *Diluent* (1:24). Decrease the acetonitrile concentration to increase the retention time of amoxicillin.

Standard solution: 1.2 mg/mL of USP Amoxicillin RS in *Diluent*. Use this solution within 6 h.

Sample solution: Prepare a dispersion of 20 Tablets for Oral Suspension using a suitable aliquot of water. Dilute a portion of the dispersion with *Diluent* to obtain a solution containing 1.2 mg/mL of amoxicillin. Pass a portion of the solution through a filter of 1-µm or finer pore size. Use this solution within 6 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor: 1.1–2.8

Column efficiency: NLT 1700 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Disintegration** ⟨701⟩

Medium: Water at 20 ± 5°

Time: 3 min

Acceptance criteria: Meet the requirements

• **Dissolution** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 30 min

Buffer: 27.2 g of monobasic potassium phosphate in 3 L of water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1, and dilute with water to obtain 4 L of solution.

Mobile phase: Acetonitrile and *Buffer* (10:390). Pass through a filter of 0.5-µm or finer pore size.

Standard solution: 0.05 mg/mL of USP Amoxicillin RS in *Buffer*. Use this solution within 6 h.

Sample solution: Pass a portion of the sample through a filter of 0.5-µm or finer pore size. Dilute a suitable aliquot of the filtrate with water to obtain a concentration of 0.045 mg/mL of amoxicillin. Use this solution within 6 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Columns

Guard: 2-mm × 2-cm; packing L2

Analytical: 3.9-mm × 30-cm; packing L1

Column temperature: 40 ± 1°

Flow rate: 0.7 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor: 1.1–2.8

Column efficiency: NLT 1700 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times P \times F \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
V = volume of medium, 900 mL
D = dilution factor
P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)
F = conversion factor, 0.001 mg/µg
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

SPECIFIC TESTS

• **Dispersion Fineness:** Place 2 Tablets for Oral Suspension in 100 mL of water, and stir until completely dispersed. A

www.webofpharma.com

smooth dispersion that passes through a No. 25 sieve is obtained.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amoxicillin RS

Amoxicillin and Clavulanate Potassium for Oral Suspension

DEFINITION
Amoxicillin and Clavulanate Potassium for Oral Suspension contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) and the equivalent of NLT 90.0% and NMT 125.0% of the labeled amount of clavulanic acid ($C_8H_9NO_5$). It contains one or more suitable buffers, colors, flavors, preservatives, stabilizers, sweeteners, and suspending agents.

IDENTIFICATION
- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: 7.8 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 4.4 ± 0.1, and dilute with water to 1000 mL.

Mobile phase: Methanol and *Buffer* (1:19). Pass through a suitable filter.

Standard solution: 0.5 mg/mL of USP Amoxicillin RS and 0.2 mg/mL of USP Clavulanate Lithium RS in water

Sample solution: Nominally 0.5 mg/mL of amoxicillin in water, prepared as follows. Constitute Amoxicillin and Clavulanate Potassium for Oral Suspension with water using the volume specified in the labeling. Stir by mechanical means for 10 min, and filter. Use within 1 h.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4-mm × 30-cm; 3- to 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for clavulanic acid and amoxicillin are about 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.5 between the amoxicillin and clavulanic acid peaks
Tailing factor: NMT 1.5 for each analyte peak
Relative standard deviation: NMT 2.0% for each analyte peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) in the Amoxicillin and Clavulanate Potassium for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of amoxicillin from the *Sample solution*
r_S = peak response of amoxicillin from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)
P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Calculate the percentage of the labeled amount of clavulanic acid ($C_8H_9NO_5$) in the Amoxicillin and Clavulanate Potassium for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of clavulanic acid from the *Sample solution*
r_S = peak response of clavulanic acid from the *Standard solution*
C_S = concentration of USP Clavulanate Lithium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clavulanic acid in the *Sample solution* (mg/mL)
P = potency of clavulanic acid in USP Clavulanate Lithium RS (mg/mg)

Acceptance criteria: 90.0%–120.0% of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) and 90.0%–125.0% of the labeled amount of clavulanic acid ($C_8H_9NO_5$)

PERFORMANCE TESTS
- **DELIVERABLE VOLUME** ⟨698⟩
  For powder packaged in multiple-unit containers: Meets the requirements
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  For powder packaged in single-unit containers: Meets the requirements

SPECIFIC TESTS
- **PH** ⟨791⟩
  Sample solution: Constitute as directed in the labeling, and perform the test immediately after constitution. **Acceptance criteria:** 3.8–6.6
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amoxicillin RS
  USP Clavulanate Lithium RS

Amoxicillin and Clavulanate Potassium Tablets

DEFINITION
Amoxicillin and Clavulanate Potassium Tablets contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amounts of amoxicillin ($C_{16}H_{19}N_3O_5S$) and clavulanic acid ($C_8H_9NO_5$).

USP Monographs

www.webofpharma.com

IDENTIFICATION

• The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 7.8 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 4.4 ± 0.1, and dilute with water to 1000 mL.

Mobile phase: Methanol and *Buffer* (1:19). Pass through a suitable filter.

Standard solution: 0.5 mg/mL of USP Amoxicillin RS and 0.2 mg/mL of USP Clavulanate Lithium RS in water

Sample stock solution: Dissolve NLT 10 Tablets in water with the aid of mechanical stirring. Transfer to a suitable volumetric flask, and dilute with water to volume.

Sample solution: Dilute a suitable volume of the *Sample stock solution* with water to obtain a solution containing 0.5 mg/mL of amoxicillin. [NOTE—Use the *Sample solution* within 1 h.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4-mm × 30-cm; 3- to 10-µm packing L1

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for clavulanic acid and amoxicillin are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.5 between the amoxicillin and clavulanic acid peaks

Tailing factor: NMT 1.5 for each analyte peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{16}H_{19}N_3O_5S$ in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of amoxicillin from the *Sample solution*

r_S = peak response of amoxicillin from the *Standard solution*

C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)

P = potency of USP Amoxicillin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Calculate the percentage of $C_8H_9NO_5$ in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of clavulanic acid from the *Sample solution*

r_S = peak response of clavulanic acid from the *Standard solution*

C_S = concentration of USP Clavulanate Lithium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clavulanic acid in the *Sample solution* (mg/mL)

P = potency of clavulanic acid in USP Clavulanate Lithium RS (mg/mg)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩**:** Tablets labeled for veterinary use only; 30 min, simulated gastric fluid TS being substituted for water in the test

• **DISSOLUTION** ⟨711⟩

[NOTE—Tablets labeled for veterinary use only are exempt from this requirement.]

Test 1

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 30 min; or 45 min where the Tablets are labeled as chewable

Analysis: Determine the amount of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$ dissolved, using the *Analysis* set forth in the *Assay*, making any necessary volumetric adjustments.

Tolerances: NLT 85% (Q) of the labeled amount of $C_{16}H_{19}N_3O_5S$ and NLT 80% (Q) of the labeled amount of $C_8H_9NO_5$ are dissolved.

For Tablets labeled as chewable: NLT 80% (Q) of the labeled amounts of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$ is dissolved in 45 min.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Apparatus 2, and **Analysis:** Proceed as directed for *Test 1*.

Times: 45 min for amoxicillin, and 30 min for clavulanic acid

Tolerances: NLT 85% (Q) of the labeled amount of $C_{16}H_{19}N_3O_5S$ and NLT 80% (Q) of the labeled amount of $C_8H_9NO_5$ are dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:**

Tablet Label Claim Amoxicillin (mg/Tablet)	Acceptance Criteria, NMT (%)
≤250	7.5
>250 and ≤500	10.0
>500	11.0

For products labeled as chewable Tablets:

Tablet Label Claim Amoxicillin (mg/Tablet)	Acceptance Criteria, NMT (%)
≤125	6.0
>125	8.0

For Tablets labeled for veterinary use only: NMT 10.0%

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label chewable Tablets to include the word "chewable" in juxtaposition to the official name. The labeling indicates that chewable Tablets may be chewed before being swallowed or may be swallowed whole. Tablets intended for veterinary use only are so labeled.

www.webofpharma.com

When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amoxicillin RS
USP Clavulanate Lithium RS

Amoxicillin and Clavulanic Acid Extended-Release Tablets

DEFINITION
Amoxicillin and Clavulanic Acid Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of amoxicillin ($C_{16}H_{19}N_3O_5S$) and clavulanic acid ($C_8H_9NO_5$).

IDENTIFICATION
• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 6.9 g/L of monobasic sodium phosphate adjusted with phosphoric acid to a pH of 4.2
Mobile phase: Methanol and *Buffer* (5:95)
Standard solution: 1 mg/mL of USP Amoxicillin RS and 62.5 µg/mL of USP Clavulanate Lithium RS in water. Store the solution at 4°, and inject within 10 h.
Sample solution: Equivalent to 1 mg/mL of amoxicillin and 62.5 µg/mL of clavulanic acid from finely powdered Tablets (NLT 6) in water. Stir for about 60 min. Store the solution at 4°, and inject within 12 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 229 nm
Column: 8-mm × 10-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
Autosampler temperature: 4°
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between the amoxicillin and clavulanic acid peaks
Tailing factor: NMT 1.8 for the amoxicillin and clavulanic acid peaks
Relative standard deviation: NMT 1.0% for the amoxicillin and clavulanic acid peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = response of amoxicillin from the *Sample solution*
r_S = response of amoxicillin from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)
P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Calculate the percentage of the labeled amount of clavulanic acid ($C_8H_9NO_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = response of clavulanic acid from the *Sample solution*
r_S = response of clavulanic acid from the *Standard solution*
C_S = concentration of USP Clavulanate Lithium RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of clavulanic acid in the *Sample solution* (µg/mL)
P = potency of clavulanic acid in USP Clavulanate Lithium RS (mg/mg)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Water; 900 mL
Apparatus 2: 75 rpm
Times
Amoxicillin: 1, 3, and 5 h
Clavulanic acid: 1 h
Mobile phase, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: USP Amoxicillin RS and USP Clavulanate Lithium RS in *Medium* at known concentrations similar to those expected in the *Sample solution*
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amounts of amoxicillin ($C_{16}H_{19}N_3O_5S$) and clavulanic acid ($C_8H_9NO_5$) dissolved.
Tolerances
Amoxicillin: The percentage of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) dissolved at the times specified conforms to *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
1	50–65
3	65–85
5	NLT 85

Clavulanic acid: NLT 80% (*Q*) of the labeled amount of clavulanic acid ($C_8H_9NO_5$) is dissolved in 1 h.
Test 2
Medium: Water; 900 mL
Apparatus 2: 75 rpm
Times
Amoxicillin: 1, 3, and 5 h
Clavulanic acid: 45 min
Mobile phase, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: USP Amoxicillin RS and USP Clavulanate Lithium RS in *Medium* at known concentrations similar to those expected in the *Sample solution*
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.

Stimulants Monographs USP

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amounts of amoxicillin ($C_{16}H_{19}N_3O_5S$) and clavulanic acid ($C_8H_9NO_5$) dissolved.
Tolerances
Amoxicillin: The percentage of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) dissolved at the times specified conforms to *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
1	50–70
3	65–90
5	NLT 85

Clavulanic acid: NLT 85% (*Q*) of the labeled amount of clavulanic acid ($C_8H_9NO_5$) is dissolved in 45 min.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer: 13.8 g/L of monobasic sodium phosphate in water adjusted with phosphoric acid to a pH of 4.2
Solution A: Methanol and *Buffer* (1:199)
Solution B: Methanol and *Buffer* (10:90)
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
8	70	30
13	70	30
13.01	40	60
18	40	60
18.01	100	0
25	100	0
30	100	0

[NOTE—These gradient elution times are established on an HPLC system with a dwell volume of approximately 5 mL. The gradient elution times in *Table 3* can be adjusted as necessary to achieve the separation described.]
System suitability solution: 0.4 mg/mL of USP Amoxicillin RS and 30 µg/mL of USP Amoxicillin Related Compound D RS in water. Store the solution at 4°.
Standard solution: 0.4 mg/mL of USP Amoxicillin RS in water. Store the solution at 4°, and inject within 24 h.
Sample solution: 1 mg/mL of amoxicillin and 62.5 µg/mL of clavulanic acid from finely powdered Tablets (NLT 2) in water. Stir for about 60 min. Store the solution at 4°, and use within 24 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 5-cm; 3-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Autosampler temperature: 4°

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.25 between the amoxicillin and amoxicillin related compound D peaks at a relative retention time of 0.83, *System suitability solution*
Tailing factor: NMT 1.8 for the amoxicillin peak, *Standard solution*
Relative standard deviation: NMT 1.0% for the amoxicillin peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F_1 \times (1/F_2) \times 100$$

r_U = response of each impurity from the *Sample solution*
r_S = response of amoxicillin from the *Standard solution*
C_S = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)
P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)
F_1 = conversion factor, 0.001 mg/µg
F_2 = relative response factor (see *Table 4*)

Acceptance criteria: See *Table 4*. The reporting limit is 0.003%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound I (D-hydroxyphenylglycine)[a, b]	0.15	—	—
Amoxicillin related compound A (6-aminopenicillanic acid)[a, c]	0.30	—	—
Clavulanic acid	0.39	—	—
Amoxicillin related compound D (amoxicillin open ring)[a, d, e]	0.63	0.74	—
Amoxicillin related compound B (L-amoxicillin)[a, f]	0.78	—	—
Amoxicillin related compound D (amoxicillin open ring)[d, e]	0.83	0.74	2.5
Amoxicillin	1.0	—	—
Amoxicillin related compound G (D-hydroxyphenylglycylamoxicillin)[a, g]	2.57	—	—
Amoxicillin related compound E (amoxicillin penilloic derivatives)[a, h, i]	2.63 3.00	—	—
Amoxicillin related compound C (amoxicillin rearrangement product)[j]	3.22	1.1	2.5

USP Monographs

www.webofpharma.com

Table 4 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin open ring methyl ester[a,k]	3.38	—	—
Amoxicillin related compound J (amoxicillin open ring dimer)[l]	4.07	1.0	4.5
Any individual unspecified impurity	—	—	0.5

[a] These are synthetic process impurities, which are controlled in the drug substance. They are listed here for reference only and are not to be reported.
[b] (*R*)-2-Amino-2-(4-hydroxyphenyl)acetic acid.
[c] (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[d] The chromatographic system resolves two isomers of amoxicillin open ring.
[e] (4*S*)-2-{[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[f] (2*S*,5*R*,6*R*)-6-[(*S*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[g] (2*S*,5*R*,6*R*)-6-{(*R*)-2-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[h] The chromatographic system resolves two amoxicillin penilloic derivatives.
[i] (4*S*)-2-{[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[j] (4*S*)-2-[5-(4-Hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid.
[k] (4*S*)-2-{[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]methoxycarbonylmethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[l] (2*S*,5*R*,6*R*)-6-((2*R*)-2-{2-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido}-2-(4-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

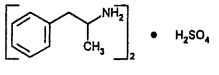
SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amoxicillin RS
  USP Amoxicillin Related Compound D RS
  (4*S*)-2-{[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
  $C_{16}H_{21}N_3O_6S$ 383.42
  USP Clavulanate Lithium RS

Amphetamine Sulfate



$(C_9H_{13}N)_2 \cdot H_2SO_4$ 368.49
Benzeneethanamine, α-methyl-, sulfate (2:1), (±)-;
(±)-α-Methylphenethylamine sulfate (2:1) [60-13-9].

DEFINITION

Amphetamine Sulfate contains NLT 98.0% and NMT 102.0% of $(C_9H_{13}N)_2 \cdot H_2SO_4$ on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩**:** Meets the requirements

Sample solution: 100 mg/mL

ASSAY

- **PROCEDURE**

Solution A: Add 5.0 mL of trifluoroacetic acid to 900 mL of water, adjust with ammonium hydroxide to a pH of 2.2 ± 0.1, and add 100 mL of acetonitrile.
Solution B: Use degassed acetonitrile.
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	65	35
20	0	100
22	0	100
23	100	0
30	100	0

Standard solution: 2.0 mg/mL of USP Dextroamphetamine Sulfate RS in *Solution A*
System suitability solution: Transfer 40 mL of the *Standard solution* to a 50-mL volumetric flask. Using a microliter syringe, add 1 µL each of USP Dextroamphetamine Related Compound A RS and USP Dextroamphetamine Related Compound B RS. Dilute with *Standard solution* to volume, and mix.
Sample solution: 2.0 mg/mL of Amphetamine Sulfate in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—Identify the peaks by the relative retention times in *Impurity Table 1* under *Organic Impurities*. Amphetamine and dextroamphetamine have exactly the same retention time.]
Suitability requirements
Resolution: NLT 3.0 between dextroamphetamine related compound A and dextroamphetamine related compound B, *System suitability solution*
Tailing factor: NMT 3.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the percentage of $(C_9H_{13}N)_2 \cdot H_2SO_4$ in the portion of Amphetamine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for amphetamine sulfate from the *Sample solution*
r_S = peak response for dextroamphetamine sulfate from the *Standard solution*
C_S = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Amphetamine Sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.2%

ORGANIC IMPURITIES

• **Procedure**

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Identify the impurities by the relative retention times in *Impurity Table 1*.]

Calculate the percentage of each impurity in the portion of Amphetamine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for dextroamphetamine from the *Standard solution*
C_S = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Amphetamine Sulfate in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cathinone	0.81	55.6	0.25
Amphetamine	1.0	1.0	—
Benzaldehyde	1.73	105.3	0.25
Dextroamphetamine related compound A	1.88	1.5	0.25
Dextroamphetamine related compound B	2.05	1.8	0.25
Individual unspecified impurity	—	1.0	0.1

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 2 h: it loses NMT 1.0% of its weight.

• **DEXTROAMPHETAMINE:** A solution (20 mg/mL) is optically inactive.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Dextroamphetamine Sulfate RS

USP Dextroamphetamine Related Compound A RS
1-Phenyl-2-propanol.
$C_9H_{12}O$ 136.20
[CAS-14898-87-4].

USP Dextroamphetamine Related Compound B RS
Phenyl acetone.
$C_9H_{10}O$ 134.18
[CAS-103-79-7].

Amphetamine Sulfate Tablets

DEFINITION

Amphetamine Sulfate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of amphetamine sulfate $[(C_9H_{13}N)_2 \cdot H_2SO_4]$.

IDENTIFICATION

• **A. MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩

Sample: Macerate a quantity of powdered Tablets, equivalent to 50 mg of amphetamine sulfate, with 10 mL of water for 30 min, and filter into a small flask. To the filtrate add 3 mL of 1 N sodium hydroxide. Cool to 10°–15°, add 1 mL of a mixture of absolute ether and benzoyl chloride (2:1), insert the stopper, and shake well for 3 min. Filter the precipitate, wash with 15 mL of cold water, and recrystallize twice from diluted alcohol. Dry the residue at 80° for 2 h.

Acceptance criteria: The crystals of the benzoyl derivative of amphetamine melt between 131° and 135°.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluted acetic acid: 14 mL of glacial acetic acid in 100 mL of water

Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 525 mL of water. Add 25 mL of diluted acetic acid and 450 mL of methanol. Adjust with glacial acetic acid to a pH of 3.3 ± 0.1. Pass through a 0.5-µm membrane filter.

Standard solution: 0.3 mg/mL of USP Dextroamphetamine Sulfate RS in 0.12 N phosphoric acid

Sample solution: Nominally 0.3 mg/mL of amphetamine sulfate from NLT 20 finely powdered Tablets prepared as follows. Transfer a suitable amount of the powdered tablets to a suitable volumetric flask. Add 80% of the flask volume of 0.12 N phosphoric acid, and sonicate for 15 min. Dilute with 0.12 N phosphoric acid to volume. Pass through a 0.5-µm membrane filter, discarding the first 20 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 2 mL/min. [NOTE—A 4.6-mm × 25-cm column; 5-µm packing L1 may be used with a flow rate of 1 mL/min.]

Injection size: 50 µL

System suitability

Sample: *Standard solution*

www.webofpharma.com

Suitability requirements
Tailing factor: NMT 3
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amphetamine sulfate $[(C_9H_{13}N)_2 \cdot H_2SO_4]$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amphetamine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: Water; 500 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: USP Dextroamphetamine Sulfate RS in *Medium* having a known concentration of USP Dextroamphetamine Sulfate RS similar to the concentration expected in the sample.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Diluted acetic acid: 14 mL of glacial acetic acid in 100 mL of water
Mobile phase: 1.1 g of sodium 1-heptanesulfonate in 575 mL of water. Add 25 mL of *Diluted acetic acid* and 400 mL of methanol. Adjust with glacial acetic acid to a pH of 3.3 ± 0.1.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection size: 500 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Calculate the percentage of the labeled amount of amphetamine sulfate $[(C_9H_{13}N)_2 \cdot H_2SO_4]$ dissolved:

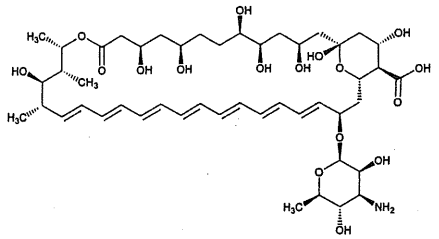
$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: NLT 75% (*Q*) of amphetamine sulfate $[(C_9H_{13}N)_2 \cdot H_2SO_4]$ is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dextroamphetamine Sulfate RS

Amphotericin B



$C_{47}H_{73}NO_{17}$ 924.08
Amphotericin B.
Amphotericin B.
[1*R*-(1*R**,3*S**,5*R**,6*R**,9*R**,11*R**,15*S**,16*R**,17*R**,18*S**,19*E*,21*E*,23*E*,25*E*,27*E*,29*E*,31*E*,33*R**,35*S**,36*R**,37*S**)]-33- [(3-Amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,-39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid [1397-89-3].

» Amphotericin B has a potency of not less than 750 µg of $C_{47}H_{73}NO_{17}$ per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store in a cold place.
Labeling—Label it to state whether it is intended for use in preparing dermatological and oral dosage forms or parenteral dosage forms.
USP Reference standards ⟨11⟩—
USP Amphotericin B RS
USP Nystatin RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Ultraviolet-Visible Spectroscopy:* ***197U***▲ (CN 1-May-2020)—
Spectral range 1: 240 to 320 nm.
Solution 1: prepared as directed for *Test preparation* in the *Limit of amphotericin A*, and compare its absorbance to that of the *Amphotericin B standard preparation*. An extra peak may occur at 304 nm in the spectrum of this solution.
Spectral range 2: 320 to 400 nm.
Solution 2: prepared as directed for *Test preparation* in the *Limit of amphotericin A* and then diluted with 9 volumes of methanol. Compare its absorbance to that of a similar dilution of the *Amphotericin B standard preparation*.
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.5%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid. [NOTE—Amphotericin B intended for use in preparing dermatological creams, lotions, and ointments, and oral suspensions and capsules, yields not more than 3.0%.]

Limit of amphotericin A—
Test preparation—Dissolve about 50 mg of Amphotericin B, accurately weighed, in 10.0 mL of dimethyl sulfoxide in a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

www.webofpharma.com

Nystatin standard preparation—Dissolve about 20 mg of USP Nystatin RS, accurately weighed, in 40.0 mL of dimethyl sulfoxide in a 200-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

Amphotericin B standard preparation—Dissolve about 50 mg of USP Amphotericin B RS, accurately weighed, in 10.0 mL of dimethyl sulfoxide in a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Prepare this solution fresh daily.

Procedure—Concomitantly determine the absorbances of the *Nystatin* and *Amphotericin B standard preparations* and the *Test preparation* in 1-cm cells at 304 nm and at 282 nm, with a suitable spectrophotometer, using a 1 in 62.5 solution of dimethyl sulfoxide in methanol as the blank. Calculate the percentage of amphotericin A taken by the formula:

$$25W_N[(A_{B_{282}} \times A_{U_{304}}) - (A_{B_{304}} \times A_{U_{282}})]/ [(A_{B_{282}} \times A_{N_{304}}) - (A_{B_{304}} \times A_{N_{282}})]W_U$$

in which W_N is the weight, in mg, of USP Nystatin RS taken, A_{B282} and A_{B304} are the absorbances of the *Amphotericin B standard preparation* at 282 nm and 304 nm, respectively, A_{N282} and A_{N304} are the absorbances of the *Nystatin standard preparation* at 282 nm and 304 nm, respectively, A_{U282} and A_{U304} are the absorbances of the *Test preparation* at 282 nm and 304 nm, respectively, and W_U is the weight, in mg, of the Amphotericin B taken: not more than 5%, calculated on the dried basis, is found. [NOTE—Amphotericin B intended for use in preparing dermatological creams, lotions, and ointments, and oral suspensions and capsules, contains not more than 15% of amphotericin A, calculated on the dried basis.]

Assay—Proceed with amphotericin B as directed under *Antibiotics—Microbial Assays* ⟨81⟩.

Amphotericin B Cream

» Amphotericin B Cream contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of Amphotericin B.

Packaging and storage—Preserve in collapsible tubes, or other well-closed containers.

USP Reference standards ⟨11⟩—
USP Amphotericin B RS

Minimum fill ⟨755⟩: meets the requirements.

Assay—Proceed as directed for amphotericin B under *Antibiotics—Microbial Assays* ⟨81⟩, blending a suitable accurately weighed portion of Cream in a high-speed blender with a sufficient accurately measured volume of dimethyl sulfoxide to give a convenient concentration. Quantitatively dilute an accurately measured volume of this solution with dimethyl sulfoxide to obtain a stock solution having a concentration of about 20 µg of amphotericin B per mL. Quantitatively dilute an accurately measured volume of this stock solution with *Buffer B.10* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Amphotericin B for Injection

» Amphotericin B for Injection is a sterile complex of Amphotericin B and deoxycholate sodium and one or more suitable buffers. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{47}H_{73}NO_{17}$.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, in a refrigerator and protected from light.

Labeling—Label it to indicate that it is intended for use by intravenous infusion to hospitalized patients only, and that the solution should be protected from light during administration.

USP Reference standards ⟨11⟩—
USP Amphotericin B RS

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 5.0 USP Endotoxin Units per mg of amphotericin B. For products used or labeled for intrathecal injection, it contains not more than 0.9 USP Endotoxin Unit per mg of amphotericin B.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed in the section *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 50 mg from each container being tested.

pH ⟨791⟩: between 7.2 and 8.0, in an aqueous solution containing 10 mg of amphotericin B per mL.

Loss on drying ⟨731⟩—Dry about 100 mg in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 8.0% of its weight.

Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—

Assay preparation 1 (where it is packaged as a single-dose container)—Constitute Amphotericin B for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively and stepwise with dimethyl sulfoxide to obtain a solution containing about 20 µg of amphotericin B per mL.

Assay preparation 2 (where the labeling states the quantity of amphotericin B in a given volume of constituted solution) —Constitute Amphotericin B for Injection as directed in the labeling. Withdraw an accurately measured volume of the resultant solution, using a suitable hypodermic needle and syringe, and dilute quantitatively and stepwise with dimethyl sulfoxide to obtain a solution containing about 20 µg of amphotericin B per mL.

Procedure—Proceed as directed for amphotericin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with *Buffer B.10* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

www.webofpharma.com

Amphotericin B Lotion

» Amphotericin B Lotion contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of amphotericin B.

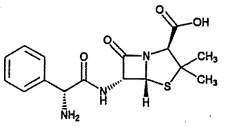
Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Amphotericin B RS
Minimum fill ⟨755⟩: meets the requirements.
pH ⟨791⟩: between 5.0 and 7.0.
Assay—Proceed as directed for amphotericin B under *Antibiotics—Microbial Assays* ⟨81⟩, quantitatively dissolving a suitable accurately measured volume of Lotion in sufficient dimethyl sulfoxide to give a convenient concentration. Quantitatively dilute an accurately measured volume of this solution with dimethyl sulfoxide to obtain a stock solution having a concentration of about 20 µg of amphotericin B per mL. Quantitatively dilute an accurately measured volume of this stock solution with *Buffer B.10* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Amphotericin B Ointment

» Amphotericin B Ointment is Amphotericin B in a suitable ointment base. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of amphotericin B.

Packaging and storage—Preserve in collapsible tubes, or other well-closed containers.
USP Reference standards ⟨11⟩—
USP Amphotericin B RS
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay—Proceed as directed for amphotericin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Ointment, equivalent to about 30 mg of amphotericin B, mixed with 10.0 mL of ether in a suitable glass-stoppered conical flask and allowed to stand, with intermittent shaking, for 1 hour. Add 20.0 mL of dimethyl sulfoxide and shake by mechanical means for 10 minutes. Dilute quantitatively and stepwise with dimethyl sulfoxide to a concentration of approximately 20 µg per mL. Quantitatively dilute an accurately measured volume of this stock solution with *Buffer B.10* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Ampicillin



$C_{16}H_{19}N_3O_4S$ (anhydrous) 349.41
4-Thia-1-azabicyclo[3.2.0]heptane-2 carboxylic acid, [6-(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-, [2*S*-[2α, 5α,6β(*S**)]]-;
(2*S*,5*R*,6*R*)-6-[(*R*)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid [69-53-4].
Trihydrate 403.46
[7177-48-2].

DEFINITION
Ampicillin is anhydrous or contains three molecules of water of hydration. It contains NLT 900 µg/mg and NMT 1050 µg/mg of ampicillin ($C_{16}H_{19}N_3O_4S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020): Except that where the specimen under test is the trihydrate, both it and the USP Ampicillin Trihydrate RS are undried.

ASSAY
- **PROCEDURE**

Solution A: 6.54 g/L of monobasic potassium phosphate and 0.34 g/L of dibasic potassium phosphate, adjusted with 1 N sodium hydroxide or 1 N phosphoric acid to a pH of 5.5 before final dilution
Solution B: Acetonitrile and *Solution A* (2:23)
Solution C: Acetonitrile and *Solution A* (3:7)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
6	100	0
15	0	100
16	0	100
18	100	0
20	100	0

Solution D: 46.3 g/L of monobasic potassium phosphate and 27.8 g/L of dibasic potassium phosphate, adjusted with 1 N sodium hydroxide or 1 N phosphoric acid to a pH of 6.5 before final dilution
System suitability solution: 0.5 mg/mL of USP Ampicillin RS and 0.1 mg/mL of USP Amoxicillin RS in acetonitrile, water, and *Solution D* (4:91:5) prepared as follows. Dissolve first in a mixture of acetonitrile, water, and *Solution D* (4:30:5), sonicating if necessary, and dilute with water to volume.
Standard solution: 0.5 mg/mL of USP Ampicillin RS in acetonitrile, water, and *Solution D* (4:91:5) prepared as follows. Dissolve first in a mixture of acetonitrile, water, and

www.webofpharma.com

Solution D (4:30:5), sonicating if necessary, and dilute with water to volume. Analyze immediately after preparation.

Sample solution: 0.5 mg/mL of Ampicillin in acetonitrile, water, and *Solution D* (4:91:5) prepared as follows. Dissolve first in a mixture of acetonitrile, water, and *Solution D* (4:30:5), sonicating if necessary, and dilute with water to volume. Analyze immediately after preparation.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 10 between ampicillin and amoxicillin, *System suitability solution*

Tailing factor: NMT 1.4 for ampicillin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of ampicillin ($C_{16}H_{19}N_3O_4S$) in each mg of Ampicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

C_U = concentration of Ampicillin in the *Sample solution* (mg/mL)

P = potency of USP Ampicillin RS (µg/mg)

Acceptance criteria: 900–1050 µg/mg on the anhydrous basis

IMPURITIES

• ORGANIC IMPURITIES, PROCEDURE 1

Organic Impurities, Procedure 1 is recommended when the impurity profile includes ampicillin thiazepine.

Solution A, Solution B, Solution C, Mobile phase, Solution D, System suitability solution, Sample solution, and **Chromatographic system:** Prepare as directed in the *Assay*.

Standard stock solution: Prepare as directed for the *Standard solution* in the *Assay*.

Standard solution: 0.005 mg/mL of ampicillin in *Solution D* and water (1:19) from *Standard stock solution*. Transfer an aliquot of the *Standard stock solution* to a suitable volumetric flask, add *Solution D*, using about 5% of the final volume, and dilute with water to volume. Analyze immediately after preparation.

Sensitivity solution: 0.5 µg/mL of ampicillin in *Solution D* and water (1:19) from the *Standard solution*. Transfer an aliquot of the *Standard solution* to a suitable volumetric flask, add *Solution D*, using about 5% of the final volume, and dilute with water to volume.

System suitability

Samples: *Sensitivity solution, System suitability solution,* and *Standard solution*

Suitability requirements

Signal-to-noise ratio: NLT 3, *Sensitivity solution*

Resolution: NLT 10 between ampicillin and amoxicillin, *System suitability solution*

Tailing factor: NMT 1.4 for ampicillin, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ampicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of ampicillin from the *Standard solution*

C_S = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

C_U = concentration of Ampicillin in the *Sample solution* (mg/mL)

P = potency of USP Ampicillin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine[a]	0.27	0.5
Amoxicillin related compound A (6-aminopenicillanic acid)[b]	0.31	0.5
Ampicilloic acid[c]	0.45	1.0
Ampicillin thiazepine analog[d]	0.65	0.3
Ampicillin	1.0	—
Ampicillin rearrangement product (isomer 1)[e]	1.8	0.4
Ampicillin rearrangement product (isomer 2)[e]	2.0	0.3
Ampicillin oligomer 2[f]	2.2	0.6
D-Phenylglycylampicillin[g]	2.5	0.8
Ampicillin oligomer 1 (dimer)[h]	2.6	1.0
Ampicillin oligomer 1 (trimer)[i]	2.9	0.4
Any individual unspecified impurity	—	0.25
Total impurities	—	3.0

[a] (*R*)-2-Amino-2-phenylacetic acid.

[b] (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[c] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido](carboxy)methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[d] (*S*)-6-[(*R*)-2-Amino-2-phenylacetamido]-2,2-dimethyl-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine-3-carboxylic acid.

[e] (4*S*)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.

[f] (4*S*)-2-{1-[(*R*)-2-Amino-2-phenylacetamido]-2-[(1*R*)-2-{carboxy[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino}-2-oxo-1-phenylethylamino]-2-oxoethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[g] (2*S*,5*R*,6*R*)-6-{(*R*)-2-[(*R*)-2-Amino-2-phenylacetamido]-2-phenylacetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[h] (2*S*,5*R*,6*R*)-6-[(2*R*)-2-{2-[(*R*)-2-Amino-2-phenylacetamido]-2-[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido}-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[i] (4*S*,4′*S*)-2,2′-{(1*R*,7*R*,13*R*)-1-Amino-14-[(2*S*,5*R*,6*R*)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-pentaoxo-1,7,13-triphenyl-3,6,9,12-tetraazatetradecane-4,10-diyl}bis(5,5-dimethylthiazolidine-4-carboxylic acid).

www.webofpharma.com

• **ORGANIC IMPURITIES, PROCEDURE 2, DIMETHYLANILINE** ⟨223⟩: Meets the requirements

Organic Impurities, Procedure 2 is recommended when dimethylaniline is used during the production of Ampicillin.

• **ORGANIC IMPURITIES, PROCEDURE 3**

Organic Impurities, Procedure 3 is recommended when the impurity profile includes phenylpyrazinol, pivaloyl phenylglycine, pivaloyl aminopenicillanic acid, diphenyldiketopiperazine, and open ring dimer.

Solution A: 4 g/L of monobasic sodium phosphate dihydrate adjusted with 1 N sodium hydroxide to a pH of 5.0

Solution B: Acetonitrile

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	98	2
20	90	10
40	85	15
50	80	20
55	75	25
60	75	25
62	98	2
70	98	2

Diluent: Acetonitrile and *Solution A* (2:98)

System suitability solution: 1.5 mg/mL of USP Ampicillin System Suitability Mixture RS in *Diluent*

Standard solution: 15 µg/mL of USP Ampicillin RS in *Diluent*

Sample solution: 1.5 mg/mL of Ampicillin in *Diluent*. Store the sample in the refrigerator, and discard after 60 min.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Autosampler temperature: 4°

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between the pivaloyl phenylglycine and diphenyldiketopiperazine peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ampicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of ampicillin from the *Standard solution*

C_S = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

C_U = concentration of Ampicillin in the *Sample solution* (mg/mL)

P = potency of ampicillin in USP Ampicillin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 4* and *Table 5*. The limits in *Table 5* are to be used only where Ampicillin is intended for use in preparing veterinary products.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine[a]	0.15	1.0
Amoxicillin related compound A (6-aminopenicillanic acid)[b]	0.21	1.0
Ampicilloic acid[c, d]	0.40 0.58	1.0
L-Ampicillin[e]	0.65	1.0
Ampicillin	1.0	—
Ampilloic acid[f, g]	1.16 1.40	1.0
Ampicillin rearrangement product[h, i]	1.25 1.48	1.0
Phenylpyrazinol[j]	1.75	1.0
Pivaloyl phenylglycine[k]	1.87	1.0
Diphenyldiketopiperazine[l]	1.94	1.0
Ampicillin oligomer 2[m]	2.08	1.0
D-Phenylglycylampicillin[n]	2.25	1.0
Pivaloyl aminopenicillanic acid[o]	2.54	1.0
Open ring dimer[p, q]	2.87 2.97 3.03	1.0
Ampicillin oligomer 1 (dimer)[r]	3.15	1.0
Ampicillinyl-D-phenylglycine[s]	3.86	1.0
Ampicillin oligomer 1 (trimer)[t]	4.19	1.0
Any individual unspecified impurity	—	0.10

www.webofpharma.com

Table 4 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	5.0

[a] (*R*)-2-Amino-2-phenylacetic acid.
[b] (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[c] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido](carboxy)methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[d] The system resolves the two isomers of ampicilloic acid. The sum of the two isomers is reported.
[e] (2*S*,5*R*,6*R*)-6-[(*S*)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[f] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[g] The system resolves the two isomers of ampilloic acid. The sum of the two isomers is reported.
[h] (4*S*)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.
[i] The system resolves the two isomers of ampicillin rearrangement product. The sum of the two isomers is reported.
[j] 3-Phenylpyrazin-2-ol.
[k] (*R*)-2-Phenyl-2-pivalamidoacetic acid.
[l] 3,6-Diphenylpiperazine-2,5-dione.
[m] (4*S*)-2-{1-[(*R*)-2-Amino-2-phenylacetamido]-2-[(1*R*)-2-{carboxy[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino}-2-oxo-1-phenylethylamino]-2-oxoethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[n] (2*S*,5*R*,6*R*)-6-{(*R*)-2-[(*R*)-2-Amino-2-phenylacetamido]-2-phenylacetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[o] (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-pivalamido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[p] (4*S*)-2-{1-[(*R*)-2-amino-2-phenylacetamido]-2-[(1*R*)-2-{[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino}-2-oxo-1-phenylethylamino]-2-oxoethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[q] The system may resolve the three isomers of open ring dimer. The sum of the three isomers is reported.
[r] (2*S*,5*R*,6*R*)-6-[(2*R*)-2-{2-[(*R*)-2-Amino-2-phenylacetamido]-2-[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido}-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[s] (*R*)-2-((2*S*,5*R*,6*R*)-6-((*R*)-2-Amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)-2-phenylacetic acid.
[t] (4*S*,4′*S*)-2,2′-{(1*R*,7*R*,13*R*)-1-Amino-14-[(2*S*,5*R*,6*R*)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-pentaoxo-1,7,13-triphenyl-3,6,9,12-tetraazatetradecane-4,10-diyl}bis(5,5-dimethylthiazolidine-4-carboxylic acid).

Where it is intended for use in preparing veterinary products:

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine[a]	0.15	2.0
Amoxicillin related compound A (6-aminopenicillanic acid)[b]	0.21	2.0
N-Formyl ampicilloic acid[c]	0.26	1.0
Ampicilloic acid[d, e]	0.40	2.0
	0.58	
L-Ampicillin[f]	0.65	2.0
Ampicillin	1.0	—
Ampilloic acid[g, h]	1.16	2.0
	1.40	
Ampicillin rearrangement product[i, j]	1.25	2.0
	1.48	
Phenylpyrazinol[k]	1.75	2.0

Table 5 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pivaloyl phenylglycine[l]	1.87	2.0
Diphenyldiketopiperazine[m]	1.94	2.0
Ampicillin oligomer 2[n]	2.08	2.0
D-Phenylglycylampicillin[o]	2.25	2.0
Pivaloyl aminopenicillanic acid[p]	2.54	2.0
Open ring dimer[q, r]	2.87	0.50
	2.97	0.50
	3.03	0.50
Ampicillin oligomer 1 (dimer)[s]	3.15	4.5
Ampicillinyl-D-phenylglycine[t]	3.86	2.0
Ampicillin oligomer 1 (trimer)[u]	4.19	2.0
Any individual unspecified impurity	—	0.5
Total impurities	—	5.0

[a] (*R*)-2-Amino-2-phenylacetic acid.
[b] (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[c] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido](carboxy)methyl}-3-formyl-5,5-dimethylthiazolidine-4-carboxylic acid.
[d] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido](carboxy)methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[e] The system resolves the two isomers of ampicilloic acid. The sum of the two isomers is reported.
[f] (2*S*,5*R*,6*R*)-6-[(*S*)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[g] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[h] The system resolves the two isomers of ampilloic acid. The sum of the two isomers is reported.
[i] (4*S*)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.
[j] The system resolves the two isomers of ampicillin rearrangement product. The sum of the two isomers is reported.
[k] 3-Phenylpyrazin-2-ol.
[l] (*R*)-2-Phenyl-2-pivalamidoacetic acid.
[m] 3,6-Diphenylpiperazine-2,5-dione.
[n] (4*S*)-2-{1-[(*R*)-2-Amino-2-phenylacetamido]-2-[(1*R*)-2-{carboxy[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino}-2-oxo-1-phenylethylamino]-2-oxoethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[o] (2*S*,5*R*,6*R*)-6-{(*R*)-2-[(*R*)-2-Amino-2-phenylacetamido]-2-phenylacetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[p] (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-pivalamido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[q] (4*S*)-2-{1-[(*R*)-2-amino-2-phenylacetamido]-2-[(1*R*)-2-{[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino}-2-oxo-1-phenylethylamino]-2-oxoethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[r] The system may resolve the three isomers of open ring dimer.
[s] (2*S*,5*R*,6*R*)-6-[(2*R*)-2-{2-[(*R*)-2-Amino-2-phenylacetamido]-2-[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido}-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[t] (*R*)-2-((2*S*,5*R*,6*R*)-6-((*R*)-2-Amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)-2-phenylacetic acid.
[u] (4*S*,4′*S*)-2,2′-{(1*R*,7*R*,13*R*)-1-Amino-14-[(2*S*,5*R*,6*R*)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-pentaoxo-1,7,13-triphenyl-3,6,9,12-tetraazatetradecane-4,10-diyl}bis(5,5-dimethylthiazolidine-4-carboxylic acid).

- **ORGANIC IMPURITIES, PROCEDURE 4**

Organic Impurities, Procedure 4 is recommended when the impurity profile includes ampilloyl aminopenicillanic acid and penicillanyl ampicillinamide.

www.webofpharma.com

Solution A: 3.4 g/L of dibasic sodium phosphate dodecahydrate and 1.4 g/L of monobasic potassium phosphate adjusted with phosphoric acid to a pH of 5.5
Solution B: Acetonitrile
Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	99	1
1.5	95	5
6.5	90	10
7.5	89	11
13.5	84	16
16.5	75	25
18	60	40
25	99	1

Standard solution: 30 µg/mL of USP Amoxicillin Related Compound A RS, 30 µg/mL of D-phenylglycine, and 25 µg/mL of USP Ampicillin RS in *Solution A*
Sample solution: 2.5 mg/mL of Ampicillin in *Solution A*. Store the *Sample solution* in the refrigerator, and use within 9 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.0-mm × 15-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1.3 mL/min
Injection volume: 5 µL
Autosampler temperature: 4°
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between D-phenylglycine and amoxicillin related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Ampicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of ampicillin from the *Standard solution*
C_S = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)
C_U = concentration of Ampicillin in the *Sample solution* (mg/mL)
P = potency of ampicillin in USP Ampicillin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 7*. Disregard any peak with an area less than 0.03 times the area of the ampicillin peak in the *System suitability solution*.

Table 7

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine[a]	0.21	0.5
Amoxicillin related compound A (6-aminopenicillanic acid)[b]	0.32	0.5
	0.46	1.0
Ampicilloic acid[c]	0.57	1.0
Ampicillin thiazepine analog[d, e]	0.72	—
L-Ampicillin[f]	0.84	0.5
Ampilloyl aminopenicillanic acid[g]	0.87	0.5
Ampicillin	1.00	—
	1.15	1.0
Ampilloic acid[h]	1.34	1.0
Ampicillin rearrangement product[i]	1.24	1.0
Pivaloyl phenylglycine[e, j]	1.47	—
Phenylpyrazinol[e, k]	1.84	—
Diphenyldiketopiperazine[e, l]	1.94	—
Pivaloyl aminopenicillanic acid[e, m]	1.95	—
D-Phenylglycylampicillin[n]	2.08	1.0
Ampicillin oligomer 1 (dimer)[o]	2.16	1.0
Penicillanyl ampicillinamide[p]	2.27	1.0
Ampicillinyl-D-phenylglycine[q]	2.64	1.0
Any individual unspecified impurity	—	1.0
Total impurities	—	5.0

[a] (*R*)-2-Amino-2-phenylacetic acid.
[b] (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[c] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido](carboxy)methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[d] (*S*)-6-[(*R*)-2-Amino-2-phenylacetamido]-2,2-dimethyl-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine-3-carboxylic acid.
[e] These impurities are listed for information only. They are not to be reported. They are not to be included in total impurities.
[f] (2*S*,5*R*,6*R*)-6-[(*S*)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[g] (2*S*,5*R*,6*R*)-6-{2-[(*R*)-2-Amino-2-phenylacetamido]-2-[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[h] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[i] (4*S*)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.
[j] (*R*)-2-Phenyl-2-pivalamidoacetic acid.
[k] 3-Phenylpyrazin-2-ol.
[l] 3,6-Diphenylpiperazine-2,5-dione.
[m] (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-pivalamido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[n] (2*S*,5*R*,6*R*)-6-{(*R*)-2-[(*R*)-2-Amino-2-phenylacetamido]-2-phenylacetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[o] (2*S*,5*R*,6*R*)-6-[(2*R*)-2-{2-[(*R*)-2-Amino-2-phenylacetamido]-2-[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido}-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[p] (2*S*,5*R*,6*R*)-6-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
[q] (*R*)-2-((2*S*,5*R*,6*R*)-6-((*R*)-2-Amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)-2-phenylacetic acid.

USP Monographs

www.webofpharma.com

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩
  Sample solution: Dissolve 6 g in 800 mL of *Fluid D* containing sufficient sterile penicillinase to inactivate the ampicillin, and swirl the vessel until dissolution is complete before filtering.
  Acceptance criteria: Where the label states that Ampicillin is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
- **pH** ⟨791⟩
  Sample solution: 10 mg/mL
  Acceptance criteria: 3.5–6.0
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.0% where it is labeled as Ampicillin (anhydrous); between 12.0% and 15.0% where it is labeled as Ampicillin (trihydrate)
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Ampicillin is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Unit/mg of ampicillin.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label to indicate whether it is anhydrous or is the trihydrate. Where the quantity of ampicillin is indicated in the labeling of any preparation containing Ampicillin, this shall be understood to be in terms of anhydrous ampicillin ($C_{16}H_{19}N_3O_4S$). Where it is intended for use in preparing injectable dosage forms, the label states that it is the trihydrate and that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
  If a test for *Organic Impurities* other than *Procedures 1* and *2* is used, then the labeling states with which *Organic Impurities* test the article complies. Where it is intended for use in preparing veterinary products, the label so states.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amoxicillin RS
  USP Amoxicillin Related Compound A RS
  (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
  $C_{16}H_{14}N_2O_2$ 266.29
  USP Ampicillin RS
  USP Ampicillin System Suitability Mixture RS
  This is a mixture which contains ampicillin, pivaloyl phenylglycine [(*R*)-2-phenyl-2-pivalamidoacetic acid; $C_{13}H_{17}NO_3$; 235.28], diphenyldiketopiperazine (3,6-diphenylpiperazine-2,5-dione; $C_{16}H_{14}N_2O_2$; 266.29), and other related compounds.
  USP Ampicillin Trihydrate RS

Ampicillin Boluses

» Ampicillin Boluses contain an amount of ampicillin (as the trihydrate) equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$).

Packaging and storage—Preserve in tight containers.
Labeling—Label the Boluses to indicate that they are for veterinary use only.

USP Reference standards ⟨11⟩—
USP Ampicillin RS
Identification—Powder 1 or more Boluses, and prepare a solution containing the equivalent of 10 mg of ampicillin per mL in a mixture of acetone and 0.1 N hydrochloric acid (4:1): the resulting solution responds to the *Identification* test under *Ampicillin Capsules.*
Uniformity of dosage units ⟨905⟩: meet the requirements.
Loss on drying ⟨731⟩: not more than 5.0%.

Assay—
Standard preparation—Prepare as directed for *Standard Preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩, using USP Ampicillin RS.
Assay preparation—Place not fewer than 5 Boluses in a high-speed glass blender jar containing an accurately measured volume of water, and blend for 4 ± 1 minutes. Dilute an accurately measured volume of this stock solution quantitatively and stepwise with water to obtain an *Assay preparation* containing about 1.25 mg of ampicillin per mL.
Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩. Calculate the quantity, in mg, of ampicillin ($C_{16}H_{19}N_3O_4S$) in each Bolus taken by the formula:

$$(T/D)(F/2000)(B - I)$$

in which T is the labeled quantity, in mg, of ampicillin in each Bolus; and D is the concentration, in mg per mL, of ampicillin in the *Assay preparation* on the basis of the labeled quantity in each Bolus and the extent of dilution.

Ampicillin Capsules

DEFINITION
Ampicillin Capsules contain an amount of ampicillin (anhydrous or as the trihydrate) equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**
  Diluent: Acetone and 0.1 N hydrochloric acid (4:1)
  Standard solution: 5 mg/mL of USP Ampicillin RS in *Diluent*
  Sample solution: 5 mg/mL of ampicillin in *Diluent* from the contents of Capsules
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 2 µL
  Developing solvent system: Acetone, toluene, glacial acetic acid, and water (650:100:25:100)
  Spray reagent: 3 mg/mL of ninhydrin in alcohol
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Apply the *Standard solution* and the *Sample solution* to the plate, and develop the chromatogram using the *Developing solvent system.* When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow to air-dry. Locate the spots on the plate by spraying lightly with *Spray reagent,* and dry at 90° for 15 min.
  Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution.*

www.webofpharma.com

ASSAY

• **PROCEDURE**

Standard solution: Prepare as directed for *Standard Preparation* in *Iodometric Assay—Antibiotics* ⟨425⟩, using USP Ampicillin RS.

Sample solution: Nominally 1.25 mg/mL of ampicillin prepared as follows. Place NLT 5 Capsules in a high-speed glass blender jar containing a suitable volume of water, and blend for 4 ± 1 min. Dilute a suitable aliquot with water.

Analysis: Proceed as directed for *Procedure* in *Iodometric Assay—Antibiotics* ⟨425⟩.

Calculate the percentage of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) in the portion of Capsules taken:

$$\text{Result} = (B - I) \times (F_1/2) \times (1/C_U) \times F_2 \times 100$$

B = volume of 0.01 N sodium thiosulfate consumed in the *Blank Determination* (mL)

I = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation and Titration* of the *Sample solution* (mL)

F_1 = factor as calculated in *Iodometric Assay—Antibiotics* ⟨425⟩

C_U = nominal concentration of ampicillin in the *Sample solution* (mg/mL)

F_2 = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: *L*/900 mg/mL of USP Ampicillin RS in water, where *L* is the labeled amount of ampicillin in mg/Capsule

Sample solution: Use a filtered portion of the solution under test.

Solution A: 1 in 1000 solution of polyoxyethylene (23) lauryl ether in water

Solution B: Dissolve 20 g of hydroxylamine hydrochloride in 5 mL of *Solution A*, and add water to make 1000 mL.

Buffer: 26 mg/mL of sodium hydroxide and 3.1 mg/mL of sodium acetate in water

Ferric nitrate solution: Suspend 233 g of ferric nitrate in about 600 mL of water, add 2.8 mL of sulfuric acid, stir until the ferric nitrate is dissolved, add 1 mL of polyoxyethylene (23) lauryl ether, dilute with water to 1000 mL, and mix.

Apparatus: Automatic analyzer consisting of (1) a liquid sampler, (2) a proportioning pump, (3) suitable spectrophotometers equipped with matched flow cells and analysis capability at 480 nm, (4) a means of recording spectrophotometric readings, and/or computer for data retrieval and calculation, and (5) a manifold consisting of the components illustrated in *Figure 1*.

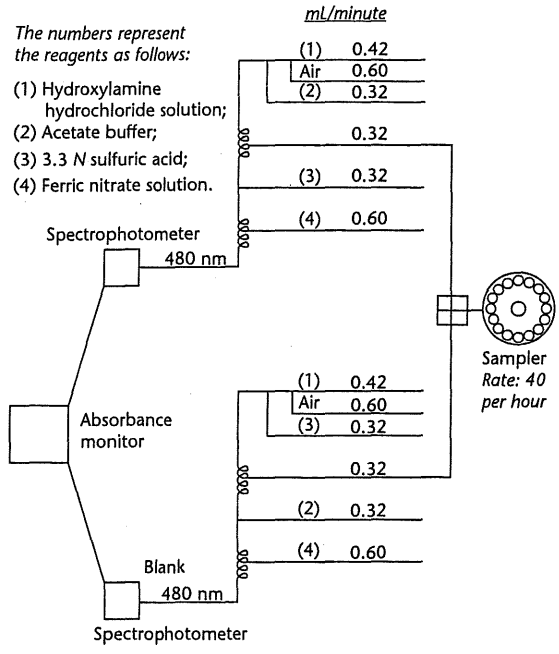


Figure 1

Analysis: With the sample line pumping water, the other lines pumping their respective reagents, and the spectrophotometer set at 480 nm, standardize the system until a steady absorbance baseline has been established. Transfer portions of the *Standard solution* and the *Sample solution* to sampler cups, and place in the sampler. Start the sampler, and conduct determinations of the *Standard solution* and the *Sample solution* typically at the rate of 40/h using a ratio of about 2:1 for sample and wash time.

Calculate the percentage of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times P \times F \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

P = potency of ampicillin in USP Ampicillin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

L = label claim (mg/Capsule)

Tolerances: NLT 75% (*Q*) of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 4.0% where the Capsules contain anhydrous ampicillin, or between 10.0% and 15.0% where the Capsules contain ampicillin trihydrate

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

www.webofpharma.com

- **LABELING:** Label the Capsules to indicate whether the ampicillin therein is in the anhydrous form or is the trihydrate.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ampicillin RS

Ampicillin for Injection

DEFINITION

Ampicillin for Injection contains an amount of Ampicillin Sodium equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$).

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, water, 1 M monobasic potassium phosphate, and 1 N acetic acid (80:909:10:1)

Diluent: Water, 1 M monobasic potassium phosphate, and 1 N acetic acid (989:10:1)

Standard solution: 1 mg/mL of USP Ampicillin RS in *Diluent*. Shake and sonicate, if necessary, to dissolve. Use this solution promptly after preparation.

System suitability solution: 0.12 mg/mL of caffeine in the *Standard solution*

Sample solution 1 (where it is represented as being in a single-dose container): 1 mg/mL of ampicillin in *Diluent*. Constitute Ampicillin for Injection in a volume of *Diluent*, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with *Diluent*. Use this solution promptly after preparation.

Sample solution 2 (where the label states the quantity of ampicillin in a given volume of constituted solution): 1 mg/mL of ampicillin in *Diluent*. Constitute 1 container of Ampicillin for Injection in a volume of *Diluent*, corresponding to the volume of solvent specified in the labeling. Dilute a suitable aliquot of the constituted solution with *Diluent*. Use this solution promptly after preparation.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Columns

Precolumn: 4-mm × 5-cm; 5- to 10-µm packing L1

Analytical: 4-mm × 30-cm; 5- to 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ampicillin and caffeine are 0.5 and 1.0, respectively, *System suitabilty solution*.]

Suitability requirements

Resolution: NLT 2.0 between caffeine and ampicillin, *System suitability solution*

Tailing factor: NMT 1.4, *Standard solution*

Capacity factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) in the container or in the volume of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

C_U = concentration of *Sample solution 1* or *Sample solution 2* (mg/mL)

P = potency of ampicillin in USP Ampicillin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Where the test for *Uniformity of Dosage Units* has been performed using the *Procedure for content uniformity*, use the average of these determinations as the *Assay* value.

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Analysis: Perform the *Assay* on individual containers using *Sample solution 1* or *Sample solution 2*, or both, as appropriate.

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements. Freeze-dried products are exempt from this requirement.
- **PH** ⟨791⟩

Sample solution: 10.0 mg/mL of ampicillin

Acceptance criteria: 8.0–10.0

- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.0%
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.15 USP Endotoxin Units/mg of ampicillin
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
- **OTHER REQUIREMENTS:** It meets the requirements of the tests for *Identification* in *Ampicillin Sodium*. It also meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*. Protect the constituted solution from freezing.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ampicillin RS
  USP Ampicillin Sodium RS

Ampicillin Soluble Powder

» Ampicillin Soluble Powder is a dry mixture of Ampicillin (as the trihydrate) and one or more suitable diluents and stabilizing agents. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$).

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate that it is for veterinary use only.

www.webofpharma.com

USP Reference standards ⟨11⟩—
USP Ampicillin RS
Identification—Dissolve a quantity of it in a mixture of acetone and 0.1 N hydrochloric acid (4:1) to obtain a solution containing 10 mg of ampicillin per mL: the resulting solution responds to the *Identification* test under *Ampicillin Capsules.*
pH ⟨791⟩: between 3.5 and 6.0, in an aqueous solution containing the equivalent of 20 mg of ampicillin per mL.
Water Determination, *Method I* ⟨921⟩: not more than 5.0%.

Assay—
Standard preparation—Prepare as directed for *Standard Preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩, using USP Ampicillin RS.
Assay preparation—Transfer an accurately weighed quantity of Soluble Powder, equivalent to about 125 mg of ampicillin, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.
Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩. Calculate the quantity, in mg, of ampicillin ($C_{16}H_{19}N_3O_4S$) in the portion of Soluble Powder taken by the formula:

$$(F/20)(B - I).$$

Ampicillin for Injectable Suspension

» Ampicillin for Injectable Suspension is a dry mixture of ampicillin trihydrate and one or more suitable buffers, preservatives, stabilizers, and suspending agents. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Ampicillin RS
Identification—Dissolve a quantity in a mixture of acetone and 0.1 N hydrochloric acid (4:1) to obtain a solution containing 5 mg of ampicillin per mL: the resulting solution responds to the *Identification* test under *Ampicillin Capsules.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.15 Endotoxin Unit per mg of ampicillin.
pH ⟨791⟩: between 5.0 and 7.0, in the suspension constituted as directed in the labeling.
Water Determination, *Method I* ⟨921⟩ **:** between 11.4% and 14.0%.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Antibiotic Solids, Bulks, and Blends* in the section *Membrane Filtration* under *Test for Sterility of the Product to be Examined,* except to use *Fluid D,* to which has been added sufficient sterile penicillinase to inactivate the ampicillin and to swirl the vessel until solution is complete before filtering. If it does not dissolve completely, proceed as directed for *Solids* in the section *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined,* except to use Fluid Thioglycollate Medium and Soybean–Casein Digest Medium containing sufficient penicillinase to inactivate the ampicillin in each vessel.

Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩, and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
Phosphate buffer solution—Accurately weigh 68 g of monobasic potassium phosphate, and transfer to a 500-mL volumetric flask. Dissolve in and dilute with water to volume.
Mobile phase—Prepare a suitable mixture of water, acetonitrile, *Phosphate buffer solution,* and glacial acetic acid (3600:360:40:4). Pass through a 0.45-µm nylon filter, and degas.
Standard preparation—Dissolve, with sonication, an accurately weighed quantity of USP Ampicillin RS in water to prepare a solution having 0.5 mg per mL. Pass through a 0.45-µm PTFE filter, discarding the first 3 mL of the filtrate.
Caffeine solution—Transfer about 30 mg of caffeine, accurately weighed, to a 50-mL volumetric flask. Add 25 mL of water, sonicate to dissolve, and dilute with water to volume. Pass through a 0.45-µm PTFE filter, discarding the first 3 mL of the filtrate.
System suitability solution—Prepare a solution of 1.0 mL of *Caffeine solution* and 9.0 mL of *Standard preparation,* and mix.
Assay preparation—Quantitatively dilute an accurately measured volume of Ampicillin for Injectable Suspension, constituted as directed in the labeling, with water to obtain a solution containing about 0.5 mg per mL. Pass through a 0.45-µm PTFE filter, discarding the first 3 mL of the filtrate.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm analytical column that contains 10-µm packing L1. The flow rate is about 2.0 mL per minute. The column temperature is maintained at 40°. Chromatograph the *System suitability solution* and the *Standard preparation,* and record the peak responses as directed for *Procedure:* the order of elution is ampicillin followed by caffeine; the resolution, *R,* between ampicillin and caffeine is greater than 2; the column efficiency is not less than 2000 theoretical plates for the ampicillin peak; the tailing factor is not greater than 1.4; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the quantity, in mg, of ampicillin ($C_{16}H_{19}N_3O_4S$) in each mL of the constituted solution of Ampicillin for Injectable Suspension taken by the formula:

$$CD(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Ampicillin RS in the *Standard preparation; D* is the dilution factor used in preparing the *Assay preparation;* and r_U and r_S are the average peak responses of the ampicillin peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ampicillin for Oral Suspension

DEFINITION
Ampicillin for Oral Suspension contains an amount of Ampicillin (anhydrous or as the trihydrate) equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$), when constituted as directed. It contains one or more suitable buffers, colors, flavors, preservatives, and sweetening ingredients.

USP Monographs

www.webofpharma.com

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Acetone and 0.1 N hydrochloric acid (4:1)

Standard solution: 5 mg/mL of USP Ampicillin RS in *Diluent*

Sample solution: Nominally 5 mg/mL of ampicillin in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 2 µL

Developing solvent system: Acetone, toluene, glacial acetic acid, and water (650:100:25:100)

Spray reagent: 3 mg/mL of ninhydrin in alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* to the plate, and develop the chromatogram in the *Developing solvent system*. When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow to air-dry. Locate the spots on the plate by spraying lightly with *Spray reagent*, and dry at 90° for 15 min.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Standard solution: Prepare as directed for *Standard preparation* in *Iodometric Assay—Antibiotics* ⟨425⟩, using USP Ampicillin RS.

Sample solution: Nominally 1.25 mg/mL of ampicillin prepared as follows. Dilute a suitable aliquot of Ampicillin for Oral Suspension, constituted as directed in the labeling, freshly mixed and free from air bubbles, with water.

Analysis: Proceed as directed for *Iodometric Assay—Antibiotics* ⟨425⟩, *Procedure*.

Calculate the percentage of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) in the portion of Ampicillin for Oral Suspension taken:

$$\text{Result} = (B - I) \times F \times (1/C_U) \times 100$$

B = volume of 0.01 N sodium thiosulfate consumed in the *Blank Determination* (mL)

I = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation and Titration* (mL)

F = factor as calculated in *Iodometric Assay—Antibiotics* ⟨425⟩

C_U = nominal concentration of ampicillin in the *Sample solution* (mg/ml)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For single-unit containers

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

• **PH** ⟨791⟩

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 5.0–7.5

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.5% where the solid for Oral Suspension contains anhydrous ampicillin or NMT 5.0% if it contains ampicillin trihydrate and the equivalent of 100 mg/mL of ampicillin when constituted as directed in the labeling

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label to indicate whether the ampicillin therein is in the anhydrous form or is the trihydrate.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ampicillin RS

Ampicillin Tablets

DEFINITION

Ampicillin Tablets contain an amount of Ampicillin (anhydrous form or trihydrate form) equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Acetone and 0.1 N hydrochloric acid (4:1)

Standard solution: 5 mg/mL of USP Ampicillin RS in *Diluent*

Sample solution: 5 mg/mL of ampicillin from powdered Tablets in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 2 µL

Developing solvent system: Acetone, toluene, glacial acetic acid, and water (650:100:25:100)

Spray reagent: 3 mg/mL of ninhydrin in alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* to the plate, and develop the chromatogram using the *Developing solvent system*. When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow to air-dry. Locate the spots on the plate by spraying lightly with the *Spray reagent*, and dry at 90° for 15 min.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Standard solution: Prepare as directed for *Standard Preparation* in *Iodometric Assay—Antibiotics* ⟨425⟩, using USP Ampicillin RS.

Sample solution: Place NLT 5 Tablets in a high-speed glass blender jar containing an accurately measured volume of water, and blend for 4 ± 1 min. Dilute a suitable aliquot with water to obtain a concentration of 1.25 mg/mL of ampicillin.

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed for *Procedure* in *Iodometric Assay—Antibiotics* ⟨425⟩.

Calculate the percentage of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) in the portion of Tablets taken:

$$\text{Result} = (B - I) \times (F_1/2) \times (1/C_U) \times F_2 \times 100$$

B = volume of 0.01 N sodium thiosulfate consumed in the *Blank Determination* (mL)

I = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation and Titration* of the *Sample solution* (mL)

www.webofpharma.com

F_1 = factor as calculated in *Iodometric Assay—Antibiotics* ⟨425⟩
C_U = nominal concentration of ampicillin in the *Sample solution* (mg/mL)
F_2 = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: *L*/900 mg/mL of USP Ampicillin RS in water, where *L* is the labeled amount of ampicillin in mg/Tablet
Sample solution: Use a filtered portion of the solution under test.
Solution A: 1 in 1000 solution of polyoxyethylene (23) lauryl ether in water
Solution B: Dissolve 20 g of hydroxylamine hydrochloride in 5 mL of *Solution A*, and add water to make 1000 mL.
Buffer: 26 mg/mL of sodium hydroxide and 3.1 mg/mL of sodium acetate in water
Ferric nitrate solution: Suspend 233 g of ferric nitrate in about 600 mL of water, add 2.8 mL of sulfuric acid, stir until the ferric nitrate is dissolved, add 1 mL of polyoxyethylene (23) lauryl ether, dilute with water to 1000 mL, and mix.
Apparatus: Automatic analyzer consisting of (1) a liquid sampler, (2) a proportioning pump, (3) suitable spectrophotometers equipped with matched flow cells and analysis capability at 480 nm, (4) a means of recording spectrophotometric readings, and/or computer for data retrieval and calculation, and (5) a manifold consisting of the components illustrated in *Figure 1*.

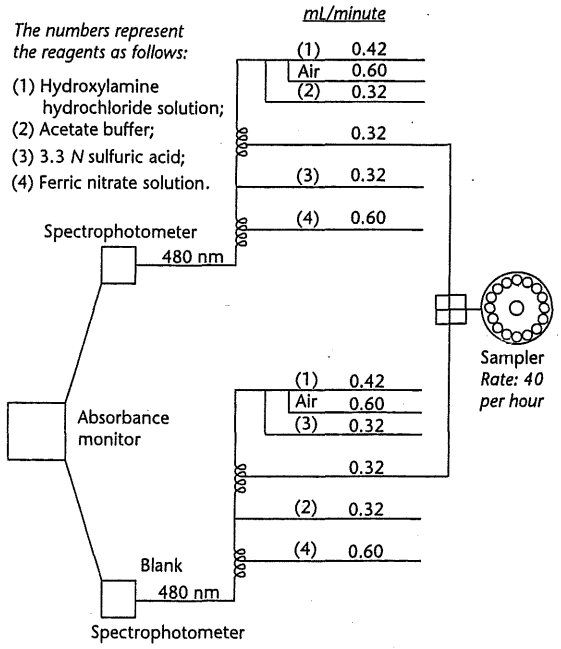


Figure 1

Analysis
Samples: *Standard solution* and *Sample solution*
With the sample line pumping water, the other lines pumping their respective reagents, and the spectrophotometer set at 480 nm, standardize the system until a steady absorbance baseline has been established. Transfer portions of the *Standard solution* and the *Sample solution* to sampler cups, and place in the sampler. Start the sampler, and conduct determinations of the *Standard solution* and the *Sample solution* typically at the rate of 40/h using a ratio of about 2:1 for sample and wash time.
Calculate the percentage of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times P \times F \times (1/L) \times 100$$

A_U = response of the *Sample solution*
A_S = response of the *Standard solution*
C_S = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)
V = volume of medium, 900 mL
P = potency of ampicillin in USP Ampicillin RS (µg/mg)
F = conversion factor, 0.001 mg/µg
L = label claim (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩

Type of Tablets	Form of Ampicillin	Limit (%)
Nonchewable	Anhydrous	NMT 4.0
Nonchewable	Trihydrate	9.5–12.0
Chewable	Anhydrous	NMT 3.0
Chewable	Trihydrate	NMT 5.0
Tablets labeled for veterinary use only	Trihydrate	NMT 13.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **LABELING:** Label the Tablets to indicate whether the ampicillin therein is in the anhydrous form or is the trihydrate. Label chewable Tablets to indicate that they are to be chewed before swallowing. Tablets intended for veterinary use only are so labeled.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ampicillin RS

Ampicillin and Probenecid for Oral Suspension

» Ampicillin and Probenecid for Oral Suspension contains an amount of Ampicillin (as the trihydrate) equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of probenecid

USP Monographs

www.webofpharma.com

($C_{13}H_{19}NO_4S$). It contains one or more suitable colors, flavors, and suspending agents.

Packaging and storage—Preserve in tight, unit-dose containers.
USP Reference standards ⟨11⟩—
USP Ampicillin RS
USP Probenecid RS

Uniformity of dosage units ⟨905⟩—
FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements for *Content Uniformity* with respect to ampicillin and probenecid.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 5.0 and 7.5, in the suspension constituted as directed in the labeling.
Water Determination, *Method I* ⟨921⟩: not more than 5.0%.

Assay for ampicillin—
Standard preparation—Prepare as directed for *Standard Preparation* under *Iodometric Assay—Antibiotics* ⟨425⟩, using USP Ampicillin RS.
Assay preparation—Constitute Ampicillin and Probenecid for Oral Suspension as directed in the labeling, and mix. Transfer the resulting suspension to a high-speed glass blender jar containing sufficient water to make 500.0 mL, and blend for about 10 minutes. Quantitatively dilute an accurately measured volume of this stock solution with water to obtain an *Assay preparation* containing about 1.25 mg of ampicillin per mL.
Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* ⟨425⟩. Calculate the quantity, in mg, of ampicillin ($C_{16}H_{19}N_3O_4S$) in the Ampicillin and Probenecid for Oral Suspension taken by the formula:

$$(L/D)(F/2000)(B - I)$$

in which L is the labeled quantity, in mg, of ampicillin in the Ampicillin and Probenecid for Oral Suspension; and D is the concentration, in mg per mL, of ampicillin in the *Assay preparation* on the basis of the labeled quantity in the Ampicillin and Probenecid for Oral Suspension and the extent of dilution.

Assay for probenecid—
Standard preparation—Dissolve an accurately weighed portion of USP Probenecid RS in sodium carbonate solution (1 in 100) to obtain a solution having a known concentration of about 1 mg per mL.
Assay preparation—Constitute Ampicillin and Probenecid for Oral Suspension as directed in the labeling, and mix. Quantitatively dilute the resulting suspension with sodium carbonate solution (1 in 100) to obtain a solution containing about 1 mg of probenecid per mL, mix, and filter.
Procedure—Transfer 2.0 mL of the clear *Assay preparation* to a 125-mL separator, and add 8.0 mL of 1.0 N hydrochloric acid. Extract this solution with four 20-mL portions of chloroform, filtering each extract through a glass wool pledget and 6 g of chloroform-washed anhydrous sodium sulfate into a 100-mL volumetric flask. Wash the pledget and the sodium sulfate with chloroform, collecting the washings in the 100-mL volumetric flask, dilute with chloroform to volume, and mix. Treat 2.0 mL of the *Standard preparation* in the same manner. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 257 nm, with a suitable spectrophotometer, using chloroform washed with sodium carbonate solution (1 in 100) as the blank. Calculate the quantity, in mg, of probenecid ($C_{13}H_{19}NO_4S$) in the Ampicillin and Probenecid for Oral Suspension taken by the formula:

$$C(L/D)(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Probenecid RS in the *Standard preparation; L* is the labeled quantity, in mg, of probenecid in the Ampicillin and Probenecid for Oral Suspension; D is the concentration, in mg per mL, of probenecid in the *Assay preparation* on the basis of the labeled quantity in the Ampicillin and Probenecid for Oral Suspension and the extent of dilution; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Ampicillin Sodium

$C_{16}H_{18}N_3NaO_4S$ 371.39
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, [6-(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-, monosodium salt, [2*S*-[2α,5α,6β(*S**)]]-;
Monosodium *D*-(−)-6-(2-amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [69-52-3].

DEFINITION
Ampicillin Sodium has a potency equivalent to NLT 845 µg and NMT 988 µg of ampicillin ($C_{16}H_{19}N_3O_4S$) per mg, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩

ASSAY
- **PROCEDURE**

Diluent: Water, 1 M monobasic potassium phosphate, and 1 N acetic acid (989:10:1)
Mobile phase: Acetonitrile, water, 1 M monobasic potassium phosphate, and 1 N acetic acid (80:909:10:1)
Standard solution: 1 mg/mL of USP Ampicillin RS in *Diluent* using shaking and sonication, if necessary, to dissolve. Use this solution promptly after preparation.
System suitability solution: 0.12 mg/mL of caffeine in *Standard solution*
Sample solution: [NOTE—Ampicillin Sodium is hygroscopic. Minimize exposure to the atmosphere, and weigh promptly.] Equivalent to 1 mg/mL of anhydrous ampicillin in *Diluent*. [NOTE—Use this solution promptly after preparation.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column
Pre-column: 4-mm × 5-cm; 5- to 10-µm packing L1
Analytical column: 4-mm × 30-cm; 5- to 10-µm packing L1
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*

USP Monographs

www.webofpharma.com

[NOTE—The relative retention times for ampicillin and caffeine are 0.5 and 1.0, respectively, *System suitability solution*.]

Suitability requirements

Resolution: NLT 2.0 between the caffeine and the ampicillin peaks, *System suitability solution*

Tailing factor: NMT 1.4, *Standard solution*

Capacity factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of $C_{16}H_{19}N_3O_4S$ in each mg of Ampicillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of Ampicillin Sodium in the *Sample solution* (mg/mL)

P = potency of USP Ampicillin RS (µg/mg)

Acceptance criteria: 845–988 µg/mg on the anhydrous basis

IMPURITIES

ORGANIC IMPURITIES

• **Procedure 1: Limit of Methylene Chloride**

Internal standard solution: 2.1 mg/mL of dioxane in dimethyl sulfoxide

Standard solution: 0.33 mg/mL of methylene chloride in *Internal standard solution*

Sample solution: 166.7 mg/mL of Ampicillin Sodium in *Internal standard solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 1.8-m × 4-mm glass column packed with a 10% phase G39 on unsilanized support S1A

Temperature

Column: 65°

Injector: 100°

Detector block: 260°

Carrier gas: Nitrogen

Flow rate: 60 mL/min

Injection size: 1 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for methylene chloride and dioxane are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4 between methylene chloride and dioxane

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylene chloride in the portion of Ampicillin Sodium taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of methylene chloride to dioxane from the *Sample solution*

R_S = peak response ratio of methylene chloride to dioxane from the *Standard solution*

C_S = concentration of methylene chloride in the *Standard solution* (mg/mL)

C_U = nominal concentration of Ampicillin Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2%.

• **Procedure 2: Dimethylaniline** ⟨223⟩: Meets the requirement

SPECIFIC TESTS

• **CRYSTALLINITY** ⟨695⟩: Meets the requirements. [NOTE—Ampicillin Sodium in the freeze-dried form is exempt from this requirement.]

• **PH** ⟨791⟩: 8.0–10.0

Sample solution: 10.0 mg/mL of ampicillin

• **WATER DETEMINATION,** *Method I* ⟨921⟩: NMT 2.0%

• **STERILITY TESTS** ⟨71⟩: Where the label states that Ampicillin Sodium is sterile, it meets the requirements.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Ampicillin Sodium is sterile or the label states that Ampicillin Sodium must be subjected to further processing during the processing of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Unit/mg of ampicillin.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ampicillin RS

USP Ampicillin Sodium RS

Ampicillin and Sulbactam for Injection

» Ampicillin and Sulbactam for Injection is a sterile, dry mixture of Ampicillin Sodium and Sulbactam Sodium. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of ampicillin ($C_{16}H_{19}N_3O_4S$) and sulbactam ($C_8H_{11}NO_5S$), the labeled amounts representing proportions of ampicillin to sulbactam of 2:1. It contains not less than 563 µg of ampicillin and 280 µg of sulbactam per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.

USP Reference standards ⟨11⟩—

USP Ampicillin RS

USP Sulbactam RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

Identification—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.17 USP Endotoxin Unit in a portion equivalent to 1 mg of a mixture of ampicillin and sulbactam (0.67 and 0.33 mg, respectively).

www.webofpharma.com

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 8.0 and 10.0, in a solution containing 10 mg of ampicillin and 5 mg of sulbactam per mL.
Water Determination, *Method I* ⟨921⟩ : not more than 2.0%.
Particulate Matter in Injections ⟨788⟩ : meets the requirements for small-volume injections.
Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
0.005 M Tetrabutylammonium hydroxide—Dilute 6.6 mL of a 40% solution of tetrabutylammonium hydroxide with water to obtain 1800 mL of solution. Adjust with 1 M phosphoric acid to a pH of 5.0 ± 0.1, dilute with water to 2000 mL, and mix.
Mobile phase—Prepare a filtered and degassed mixture of *0.005 M Tetrabutylammonium hydroxide* and acetonitrile (1650:350). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Quantitatively dissolve accurately weighed quantities of USP Ampicillin RS and USP Sulbactam RS in *Mobile phase* to obtain a solution having known concentrations of about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [NOTE—Inject this solution promptly.]
Resolution solution—Prepare a solution of USP Sulbactam RS in 0.01 N sodium hydroxide containing 0.3 mg per mL, and allow to stand for 30 minutes. Adjust with phosphoric acid to a pH of 5.0 ± 0.1. Transfer 5 mL of the solution to a 25-mL volumetric flask, add 4.25 mL of acetonitrile, dilute with *0.005 M Tetrabutylammonium hydroxide* to volume, and mix. Transfer 1 mL of this solution to a second 25-mL volumetric flask, add 15 mg of USP Ampicillin RS, dilute with *Mobile phase* to volume, and mix. [NOTE—Inject this solution promptly.]
Assay preparation 1—Mix the contents of a container of Ampicillin and Sulbactam for Injection. Quantitatively dissolve an accurately weighed portion of the powder in *Mobile phase* to obtain a solution having a concentration of about 1 mg of the powder per mL. [NOTE—Inject this solution promptly.]
Assay preparation 2 (where it is represented as being in a single-dose container)— Constitute a container of Ampicillin and Sulbactam for Injection with a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw the total withdrawable contents from the container, using a suitable hypodermic needle and syringe, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [NOTE—Inject this solution promptly.]
Assay preparation 3 (where the label states the quantities of ampicillin and sulbactam in a given volume of constituted solution)— Constitute a container of Ampicillin and Sulbactam for Injection with a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [NOTE—Inject this solution promptly.]
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 4-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution,* and record the responses as directed for *Procedure:* the relative retention times are about 0.7 for ampicillin and 1.0 for sulbactam alkaline degradation product; and the resolution, *R,* between ampicillin and sulbactam alkaline degradation product is not less than 4.0. Chromatograph the *Standard preparation,* and record the responses as directed for *Procedure:* the relative retention times are about 0.35 for ampicillin and 1.0 for sulbactam; the column efficiency determined from the sulbactam peak is not less than 3500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the appropriate *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in µg, of ampicillin ($C_{16}H_{19}N_3O_4S$) and of sulbactam ($C_8H_{11}NO_5S$) in the portion of Ampicillin and Sulbactam for Injection taken by the same formula:

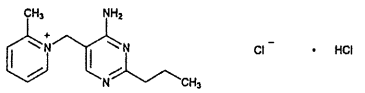
$$(C_S P/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation; P* is the assigned content, in µg per mg, of the appropriate USP Reference Standard; C_U is the concentration, in mg per mL, of Ampicillin and Sulbactam for Injection in *Assay preparation 1,* based on the weight, in mg, of powder removed from the container and the extent of dilution; and r_U and r_S are the peak areas for the appropriate analyte obtained from *Assay preparation 1* and the *Standard preparation,* respectively. Calculate the quantities of ampicillin ($C_{16}H_{19}N_3O_4S$) and of sulbactam ($C_8H_{11}NO_5S$) withdrawn from the container, or in the volume of constituted solution taken by the same formula:

$$(L/D)(C_S P)(r_U/r_S)$$

in which *L* is the labeled quantity, in mg, of ampicillin or sulbactam, as appropriate, in the container or in the volume of constituted solution taken; *D* is the concentration, in mg per mL, of ampicillin or sulbactam in *Assay preparation 2* or *Assay preparation 3,* on the basis of the labeled quantity, in mg, of ampicillin or sulbactam, as appropriate, in the container and the extent of dilution; r_U and r_S are the peak areas for the appropriate analyte obtained from *Assay preparation 2* or *Assay preparation 3* and the *Standard preparation,* respectively; and the other terms are as defined above.

Amprolium



$C_{14}H_{19}ClN_4 \cdot HCl$ 315.24
1-[(4-Amino-2-propyl-5-pyrimidinyl)methyl]-2-methylpyridinium chloride monohydrochloride;
1-[(4-Amino-2-propyl-5-pyrimidinyl)methyl]-2-picolinium chloride monohydrochloride [137-88-2].

DEFINITION
Amprolium contains NLT 97.0% and NMT 101.0% of amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$), calculated on the dried basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Previously dried
Acceptance criteria: Meets the requirements

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Analytical wavelength: 246 nm
Sample solution: 10 µg/mL in 0.1 N hydrochloric acid
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY

• **PROCEDURE**
Diluent: Methanol, acetonitrile, and water (45:5:50)
Mobile phase: 6 g of sodium 1-heptanesulfonate in 500 mL of water. Add 12 mL of glacial acetic acid, 2.0 mL of triethylamine, 450 mL of methanol, and 50 mL of acetonitrile. Pass through a suitable filter of 0.5-µm or finer pore size.
System suitability solution: 0.5 mg/mL of USP Amprolium RS and 0.2 mg/mL of 2-picoline in *Diluent*
Standard solution: 0.5 mg/mL of USP Amprolium RS in *Diluent*
Sample solution: 0.5 mg/mL of Amprolium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L13
Flow rate: 0.6 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 7 between amprolium and 2-picoline
Column efficiency: NLT 6500 theoretical plates from the amprolium peak
Tailing factor: NMT 2.3 for the amprolium peak
Relative standard deviation: NMT 1.0% for amprolium
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$) in the portion of Amprolium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amprolium RS in the *Standard solution* (mg/mL)
C_U = concentration of Amprolium in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–101.0% on the dried basis

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at a pressure not exceeding 5 mm of mercury at 100° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** Label it to indicate that it is for veterinary use only.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Amprolium RS

Amprolium Soluble Powder

DEFINITION

Amprolium Soluble Powder contains NLT 95.0% and NMT 105.0% of the labeled amount of amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 10 µg/mL (filtered) in 0.1 N hydrochloric acid
Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**
Diluent: Methanol, acetonitrile, and water (45:5:50)
Mobile phase: 6 g of sodium 1-heptanesulfonate in 500 mL of water. Add 12 mL of glacial acetic acid, 2.0 mL of triethylamine, 450 mL of methanol, and 50 mL of acetonitrile. Pass through a suitable filter of 0.5-µm or finer pore size.
System suitability solution: 0.5 mg/mL of USP Amprolium RS and 0.2 mg/mL of 2-picoline in *Diluent*
Standard solution: 0.5 mg/mL of USP Amprolium RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of amprolium in *Diluent*, prepared as follows. Transfer a portion of Soluble Powder, equivalent to 50 mg of amprolium, to a 100-mL volumetric flask, add 75 mL of *Diluent*, and sonicate for 10 min. Allow to cool to room temperature, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.5-µm or finer pore size, and use the clear filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L13
Flow rate: 0.6 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 7 between amprolium and 2-picoline
Column efficiency: NLT 6500 theoretical plates from the amprolium peak
Tailing factor: NMT 2.3 for the amprolium peak
Relative standard deviation: NMT 1.0% for amprolium
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$) in the portion of Soluble Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Amprolium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of amprolium in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amprolium RS

Amprolium Oral Solution

» Amprolium Oral Solution contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$).

Packaging and storage—Preserve in tight containers, protected from light. Store at a temperature between 5° and 30°, in a dry place.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Amprolium RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Ultraviolet-Visible Spectroscopy:* ***197U***▲ (CN 1-May-2020)—
Solution: 10 µg per mL, filtered.
Medium: 0.1 N hydrochloric acid.
pH ⟨791⟩**:** between 2.5 and 3.0.

Assay—
Mobile phase—To 4.5 g of sodium 1-hexanesulfonate add 1500 mL of water, 400 mL of methanol, and 100 mL of acetonitrile, mix, and allow to cool to room temperature. Adjust with phosphoric acid to a pH of 5.1, and pass through a filter having a 0.5-µm or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Amprolium RS in water to obtain a solution having a known concentration of about 0.5 mg per mL.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 960 mg of amprolium, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this stock solution to a second 100-mL volumetric flask, dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 268-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The column is maintained at a constant temperature of about 45°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for amprolium. Calculate the quantity, in mg, of amprolium ($C_{14}H_{19}ClN_4 \cdot HCl$) in each mL of the Oral Solution taken by the formula:

$$(2000C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Amprolium RS in the *Standard preparation; V* is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation;* and r_U and r_S are the amprolium peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Amyl Nitrite

$C_5H_{11}NO_2$ 117.15
Mixture of nitrous acid, 2-methylbutyl ester, and nitrous acid, 3-methylbutyl ester [8017-89-8; 110-46-3].

» Amyl Nitrite is a mixture of the nitrite esters of 3-methyl-1-butanol and 2-methyl-1-butanol. It contains not less than 85.0 percent and not more than 103.0 percent of $C_5H_{11}NO_2$.
[**CAUTION**—Amyl Nitrite is very flammable. Do not use where it may be ignited.]

Packaging and storage—Preserve in tight containers, and store in a cool place, protected from light.
USP Reference standards ⟨11⟩—
USP Benzyl Benzoate RS

Identification—
A: The NMR spectrum recorded as directed in the *Assay* exhibits, among other peaks, a doublet with a band centered at about 1 ppm and a multiplet with a band centered at about 4.8 ppm representing methyl protons and methylene protons alpha to the nitrite group, respectively, both relative to the tetramethylsilane singlet at 0 ppm.
B: To a few drops of it add a mixture of 1 mL of ferrous sulfate TS and 5 mL of 3 N hydrochloric acid: a greenish brown color is produced.
Specific gravity ⟨841⟩: between 0.870 and 0.876.
Acidity—To 0.30 mL in a glass-stoppered cylinder add a mixture of 0.60 mL of 0.1 N sodium hydroxide, 10 mL of water, and 1 drop of phenolphthalein TS, and invert the cylinder three times: the red tint of the water layer is still perceptible.
Limit of nonvolatile residue—Allow 10 mL to evaporate at room temperature in a tared evaporating dish, *in a well-ventilated hood,* and dry the residue at 105° for 1 hour: the weight of the residue does not exceed 2 mg (0.02%).
Content of total nitrites—Inject a portion of Amyl Nitrite of suitable volume, but not more than 2 µL, into a suitable gas chromatograph (see *Chromatography* ⟨621⟩) equipped with a thermal conductivity detector. Under typical conditions, the instrument contains a 3-mm × 2-m column packed with a methyl polysiloxane oil, 25% by weight on suitable calcined diatomite, the column is maintained at about 80°, the injection port and detector block are maintained about 10° above the temperature of the column, and helium is used as a carrier gas at a flow rate of about 60 mL per minute. From the area under the curve, calculate the percentage (a/a) of total nitrites, represented by the area under the main peak of

www.webofpharma.com

the chromatogram, in the Amyl Nitrite taken: not less than 97.0% is found.

Assay—

Solvent: carbon tetrachloride.

Internal standard—USP Benzyl Benzoate RS.

Procedure—Transfer 4 to 5 mEq of *Internal standard*, accurately weighed, to a semimicro sampling tube, add 2 to 3 mL of carbon tetrachloride, apply a sampling valve and septum,* thereby sealing the tube, and determine the weight of the sealed assembly. Open the valve, introduce about 500 µL of Amyl Nitrite with a syringe, close the valve, and determine the weight of the sealed assembly when it has attained constant weight. Shake the sampling tube and valve assembly, and transfer about 500 µL of the solution to a precision NMR tube as directed for *Absolute Method of Quantitation* under *Nuclear Magnetic Resonance* ⟨761⟩. With no spinning, or with the spinning adjusted so that the spinning side bands of neither the substance under assay nor the *Internal standard* interfere with the regions to be integrated, record as A_S the average area of the *Internal standard* singlet appearing at about 5.3 ppm, representing the methylene protons of benzyl benzoate, and record as A_U the average area of the multiplet with a band center at about 4.8 ppm, representing the alpha methylene protons of amyl nitrite, with reference to the tetramethylsilane singlet at 0 ppm. Calculate the quantity of $C_5H_{11}NO_2$ in the Amyl Nitrite taken, using 58.57 as the equivalent weight of amyl nitrite (EW_U) and 106.12 as that of benzyl benzoate (EW_S).

Amyl Nitrite Inhalant

» Amyl Nitrite Inhalant contains a mixture of the nitrite esters of 3-methyl-1-butanol and 2-methyl-1-butanol. It contains not less than 80.0 percent and not more than 105.0 percent of $C_5H_{11}NO_2$. It contains a suitable stabilizer.
[**CAUTION**—Amyl Nitrite Inhalant is very flammable. Do not use where it may be ignited.]

Packaging and storage—Preserve in tight, unit-dose glass containers, wrapped loosely in gauze or other suitable material, and store in a cool place, protected from light.

USP Reference standards ⟨11⟩—
USP Benzyl Benzoate RS

Specific gravity ⟨841⟩: between 0.870 and 0.880.

Content of total nitrites—Remove the gauze or other covering, place the glass container of Inhalant upright in a dry ice-acetone slurry, and cool for 10 minutes. Dry the container of Inhalant, place it in a pointed glass tube, and break the container with a glass rod. Proceed as directed for *Total nitrites* under *Amyl Nitrite:* not less than 95.0% is found.

Other requirements—It responds to the *Identification* tests and meets the requirements of the test for *Acidity*— under *Amyl Nitrite.*

Assay—

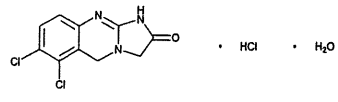
Solvent: carbon tetrachloride.

Internal standard—USP Benzyl Benzoate RS.

Procedure—Remove the gauze or other covering from 1 or more Inhalant ampuls containing a total of 300 to 400 µL of amyl nitrite. Weigh accurately the clean and dry intact glass ampul(s), and place the weighed specimen in a freezer for not less than 15 minutes. Transfer the chilled specimen to a glass-stoppered, 25-mL conical flask containing a solution of 4 to 5 mEq of *Internal standard*, accurately weighed, in 1 to 2 mL of carbon tetrachloride. Break the ampul(s) with a glass rod, and rinse any sample or glass fragments adhering to the glass rod with 1 mL of carbon tetrachloride into the main assay solution. Insert the stopper in the flask immediately, mix, and proceed as directed for *Absolute Method of Quantitation* under *Nuclear Magnetic Resonance* ⟨761⟩, beginning with "When dissolution has been completed." With no spinning, or with the spinning adjusted so that the spinning side bands of neither the substance under assay nor the *Internal standard* interfere with the regions to be integrated, record as A_S the average area of the *Internal standard* singlet appearing at about 5.3 ppm, representing the methylene protons of benzyl benzoate, and record as A_U the average area of the multiplet with a band center at about 4.8 ppm, representing the alpha methylene protons of amyl nitrite, with reference to the tetramethylsilane singlet at 0 ppm. Calculate the quantity of $C_5H_{11}NO_2$ in the Inhalant taken, using 58.57 as the equivalent weight of amyl nitrite (EW_U) and 106.12 as that of benzyl benzoate (EW_S). Rinse the flask containing the assay preparation with three 5-mL portions of ether, decanting each rinsing carefully to avoid loss of glass fragments, and evaporate any remaining ether with the aid of a current of dry air. Transfer the dry glass fragments to a tared watch glass, weigh, and subtract the weight of the glass fragments from that of the intact ampul(s) to obtain the weight of the Inhalant taken.

* Suitable sampling tubes, sampling valves, and septums are available, respectively, as catalog Nos. K-749000, K-749100, and K-749102 (50 septums) or K-749101 (100 septums), from Kontes Glass Company, Vineland, NJ 08360.

Anagrelide Hydrochloride



$C_{10}H_7Cl_2N_3O \cdot HCl \cdot H_2O$ 310.56
Anhydrous 292.55
[58579-51-4].
Imidazo[2,1-*b*]quinazolin-2(3*H*)-one, 6,7-dichloro-1,5-dihydro-, monohydrochloride, monohydrate;
6,7-Dichloro-1,5-dihydroimidazo[2,1-*b*]-quinazolin-2(3*H*)-one monohydrochloride, monohydrate [823178-43-4].

DEFINITION

Anagrelide Hydrochloride contains NLT 98.0% and NMT 102.0% of anagrelide hydrochloride ($C_{10}H_7Cl_2N_3O \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

www.webofpharma.com

ASSAY

• **PROCEDURE**

Use freshly prepared standard and sample solutions and inject within 2 h.

Solution A: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Solution A* (1:3)

Diluent: Acetonitrile and water (1:1)

Standard stock solution: 0.5 mg/mL of anagrelide hydrochloride in acetonitrile prepared as follows. Transfer USP Anagrelide Hydrochloride RS into a suitable volumetric flask, add a small amount of 2 N hydrochloric acid (3 drops per every 50 mL of the final volume) and acetonitrile equivalent to fill about 80% of the final volume. Sonicate to dissolve, and dilute with acetonitrile to volume.

Standard solution: 0.05 mg/mL of anagrelide hydrochloride in *Diluent* from *Standard stock solution*

Sample stock solution: Weigh Anagrelide Hydrochloride, equivalent to 25 mg of anhydrous salt, into a 50-mL volumetric flask, add 3 drops of 2 N hydrochloric acid and 40 mL of acetonitrile. Sonicate to dissolve, and dilute with acetonitrile to volume.

Sample solution: Transfer 5 mL of *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 4-µm packing L11

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of anagrelide hydrochloride ($C_{10}H_7Cl_2N_3O \cdot HCl$) in the portion of Anagrelide Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of anagrelide from the *Sample solution*

r_S = peak response of anagrelide from the *Standard solution*

C_S = concentration of USP Anagrelide Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Anagrelide Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**

Use freshly prepared standard and sample solutions and inject within 2 h.

Mobile phase: Proceed as directed in the *Assay*.

Diluent A: Use the *Diluent* as described in the *Assay*.

Diluent B: Acetonitrile and water (1:3)

Standard stock solution A: 0.05 mg/mL of USP Anagrelide Related Compound A RS in *Diluent A*

Standard stock solution B: 0.05 mg/mL of anagrelide related compound B in acetonitrile. Transfer USP Anagrelide Related Compound B RS into a suitable volumetric flask, add acetonitrile equivalent to fill about 50% of the final volume and a small amount of 2 N hydrochloric acid (3 drops per 200 mL of the final volume). Sonicate to dissolve, heat in the hot water bath if necessary, and dilute with acetonitrile to volume.

Standard stock solution C: 0.1 mg/mL of anagrelide hydrochloride in acetonitrile. Transfer USP Anagrelide Hydrochloride RS into a suitable volumetric flask, add acetonitrile equivalent to fill about 80% of the final volume and a small amount of 0.12 N hydrochloric acid (1 mL per 100 mL of the final volume). Sonicate to dissolve, and dilute with acetonitrile to volume.

System suitability solution: 0.25 µg/mL of each of anagrelide related compound A and anagrelide related compound B in *Mobile phase* from *Standard stock solution A* and *Standard stock solution B*

Standard solution: 0.05 µg/mL of anagrelide hydrochloride in *Mobile phase* from *Standard stock solution C*

Sample stock solution: Weigh Anagrelide Hydrochloride, equivalent to 25 mg of anhydrous salt, into a 50-mL volumetric flask. Add 45 mL of acetonitrile, sonicate, and swirl the flask until the preparation turns into a cloudy liquid. Add 1 drop of 0.12 N hydrochloric acid, swirl the flask until the liquid turns to clear, and dilute with acetonitrile to volume.

Sample solution: Transfer 5 mL of *Sample stock solution* into a 50-mL volumetric flask, and dilute with *Diluent B* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 4-µm packing L11

Autosampler temperature: 5°

Flow rate: 1.2 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between anagrelide related compound B and anagrelide related compound A, *System suitability solution*

Column efficiency: NLT 3000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Anagrelide Hydrochloride, on the anhydrous basis, taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of anagrelide from the *Standard solution*

C_S = concentration of USP Anagrelide Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Anagrelide Hydrochloride (anhydrous) in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria See *Table 1*. Disregard any impurity peak less than 0.05%.

USP Monographs

www.webofpharma.com

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Anagrelide related compound B[a]	0.40	0.43	0.3
Anagrelide related compound A[b]	0.55	0.37	0.15
Anagrelide open ring methyl ester (if present)[c]	0.80	0.51	0.25
Anagrelide	1.00	1.0	—
Anagrelide related compound C[d]	1.41	0.32	0.15
Anagrelide trichloro derivative[e]	2.44	1.0	0.15
Any unspecified impurity	—	1.0	0.1
Total impurities	—	—	1.0

[a] (2-Amino-5,6-dichloroquinazolin-3(4*H*)-yl)acetic acid.
[b] Ethyl 2-(6-amino-2,3-dichlorobenzylamino)acetate.
[c] Methyl 2-(5,6 dichloro-2-imino-1,2-dihydroquinazolin-3(4*H*)-yl)acetate.
[d] Ethyl 2-(5,6-dichloro-2-imino-1,2-dihydroquinazolin-3(4*H*)-yl)acetate hydrobromide.
[e] 6,7,8-Trichloro-3,5-dihydroimidazo[2,1-*b*]quinazolin-2(1*H*)-one.

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩: 4.5%–7.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a cold place.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Anagrelide Hydrochloride RS
  USP Anagrelide Related Compound A RS
  Ethyl 2-(6-amino-2,3-dichlorobenzylamino)acetate.
  $C_{11}H_{14}Cl_2N_2O_2$ 277.15
  USP Anagrelide Related Compound B RS
  (2-Amino-5,6-dichloroquinazolin-3(4*H*)-yl)acetic acid.
  $C_{10}H_9Cl_2N_3O_2$ 274.10

Anagrelide Capsules

DEFINITION
Anagrelide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of anagrelide ($C_{10}H_7Cl_2N_3O$).

IDENTIFICATION
- **A.** The UV absorption spectrum of the major peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: 1.0 g/L of sodium hexanesulfonate. Add 1.0 mL of phosphoric acid and filter.
Mobile phase: Acetonitrile and *Solution A* (7:13)
Diluent: Acetonitrile and water (1:1)
Standard stock solution: 0.25 mg/mL of USP Anagrelide Hydrochloride RS in acetonitrile. Initially add acetonitrile (about 80% of the volume of the flask) and a small quantity of 2 N hydrochloric acid (about 0.2 mL for every 100 mL of the final volume). Sonicate to dissolve, and dilute with acetonitrile to volume.
Standard solution: 0.01 mg/mL of anagrelide free base in *Diluent* from *Standard stock solution*
Sample solution: Nominally 0.01 mg/mL of anagrelide free base prepared from the contents of NLT 20 Capsules. Add *Diluent* (80% of the volume of the flask), sonicate for 10 min, and stir for 15 min. Further dilute with *Diluent* to volume, centrifuge for 15 min at 4000 rpm, and use the supernatant for analysis.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 4-µm packing L11
Column temperature: 60°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anagrelide ($C_{10}H_7Cl_2N_3O$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of anagrelide from the *Sample solution*
r_S = peak response of anagrelide from the *Standard solution*
C_S = concentration of anagrelide in the *Standard solution* (mg/mL)
C_U = nominal concentration of anagrelide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 15 min
Mobile phase: Prepare as directed in the *Assay*.
Standard solution: Transfer about 30.32 mg of USP Anagrelide Hydrochloride RS, equivalent to 25.00 mg of anagrelide, to a 100-mL volumetric flask. Add about 80 mL of acetonitrile and 3 drops of 2 N hydrochloric acid. Sonicate until dissolved. Dilute with acetonitrile to volume. Dilute this solution with *Medium* to obtain a final concentration of about (*L*/1000) mg/mL, where *L* is the label claim in mg/Capsule.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 274 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Sample cooler temperature: 5°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*

USP Monographs

www.webofpharma.com

Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anagrelide dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (V/L) \times 100$$

r_U = peak response of anagrelide from the *Sample solution*
r_S = peak response of anagrelide from the *Standard solution*
C_S = concentration of USP Anagrelide Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of anagrelide in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of anagrelide, 256.09
M_{r2} = molecular weight of anagrelide hydrochloride, 292.55
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of anagrelide is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer: 6.8 g/L of monobasic potassium phosphate. Adjust with diluted phosphoric acid to a pH of 3.50 ± 0.05. Mix well and filter.
Mobile phase: Acetonitrile and *Buffer* (27:73)
Diluent: Acetonitrile and water (7:13)
Related compound A stock solution: 10 µg/mL of USP Anagrelide Related Compound A RS in *Diluent*
Related compound C stock solution: 10 µg/mL of USP Anagrelide Related Compound C RS in *Diluent*
System suitability solution: 0.2 µg/mL each of USP Anagrelide Related Compound A RS and USP Anagrelide Related Compound C RS and 0.02 mg/mL of USP Anagrelide Hydrochloride RS prepared as follows. Initially dissolve USP Anagrelide Hydrochloride RS in *Diluent* (about 80% of the volume of the flask), sonicate for 10 min, and stir for 15 min. Add appropriate quantities of *Related compound A stock solution* and *Related compound C stock solution*, and dilute with *Diluent* to volume.
Standard stock solution: Prepare as directed in the *Assay*.
Standard solution: 0.10 µg/mL of anagrelide free base in *Diluent* from *Standard stock solution*
Sample solution: Nominally 0.02 mg/mL of anagrelide free base from NLT 20 Capsules. Initially add *Diluent* to about 80% of the volume of the flask, sonicate for 10 min, stir for about 15 min, and dilute with *Diluent* to volume. Centrifuge the solution at about 4000 rpm for 15 min, and use the supernatant for analysis.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 4-µm packing L11
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 30 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between anagrelide hydrochloride and anagrelide related compound C, and between anagrelide hydrochloride and anagrelide related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of anagrelide from the *Standard solution*
C_S = concentration of anagrelide in the *Standard solution* (mg/mL)
C_U = nominal concentration of anagrelide in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Anagrelide related compound A[a]	0.86	—	—
Anagrelide hydrochloride	1.0	—	—
Anagrelide related compound B[b]	0.3	0.34	1.0
Anagrelide related compound C[a]	1.15	—	—
Anagrelide trichloro derivative[c]	1.8–2.3	1.0	0.15
Any other individual impurity	—	—	0.2
Total impurities	—	—	1.5

[a] This is a process-related impurity and controlled in the drug substance.
[b] [2-Amino-5,6-dichloroquinazoline-3(4*H*)-yl]acetic acid.
[c] 6,7,8-Trichloro-3,5-dihydroimidazo[2,1-*b*]quinazolin-2(1*H*)-one.

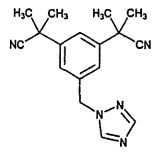
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Anagrelide Hydrochloride RS
USP Anagrelide Related Compound A RS
Ethyl 2-(6-amino-2,3-dichlorobenzylamino)acetate.
$C_{11}H_{14}Cl_2N_2O_2$ 277.15
USP Anagrelide Related Compound C RS
Ethyl 2-(5,6-dichloro-2-imino-1,2-dihydroquinazolin-3(4*H*)-yl)acetate hydrobromide.
$C_{12}H_{13}Cl_2N_3O_2 \cdot HBr$ 383.07

USP Monographs

www.webofpharma.com

Anastrozole



$C_{17}H_{19}N_5$ 293.37

1,3-Benzenediacetonitrile, α,α,α′,α′-tetramethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-;
α,α,α′,α′-Tetramethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-*m*-benzenediacetonitrile [120511-73-1].

DEFINITION

Anastrozole contains NLT 98.0% and NMT 102.0% of anastrozole ($C_{17}H_{19}N_5$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Acetonitrile, methanol, trifluoroacetic acid, and water (100: 300: 0.5: 600)
Solution B: Acetonitrile, methanol, trifluoroacetic acid, and water (150: 450: 0.5: 400)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
40	0	100
41	100	0
56	100	0

[NOTE—These gradient elution times are established on an HPLC system with a dwell time of approximately 0 min. The gradient elution times in the table can be adjusted by subtracting the dwell time to achieve the separation described.]

Standard solution: 0.5 mg/mL of USP Anastrozole RS prepared as follows. Transfer USP Anastrozole RS into a suitable volumetric flask. Dissolve in acetonitrile, using 40% of the final volume, and then dilute with *Solution A* to volume.
Sample solution: 0.5 mg/mL of Anastrozole prepared as follows. Transfer 25 mg of Anastrozole to a 50-mL volumetric flask, add 20 mL of acetonitrile to dissolve. Dilute with *Solution A* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 3.2-mm × 10-cm; 5-µm packing L42
Flow rate: 0.75 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: Between 0.9 and 1.4
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of anastrozole ($C_{17}H_{19}N_5$) in the portion of Anastrozole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of anastrozole from the *Sample solution*
r_S = peak area of anastrozole from the *Standard solution*
C_S = concentration of USP Anastrozole RS in the *Standard solution* (mg/mL)
C_U = concentration of Anastrozole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A, Solution B, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 0.2 mg/mL of USP Anastrozole RS prepared as follows. Dissolve in acetonitrile, using 40% of the final volume, and then dilute with *Solution A* to volume.
Standard solution: 0.02 mg/mL of USP Anastrozole RS in *Solution A* from the *Standard stock solution*
Sample solution: 2 mg/mL of Anastrozole prepared as follows. Transfer 50 mg of Anastrozole to a 25-mL volumetric flask. Add 10 mL of acetonitrile. Dissolve in and dilute with *Solution A* to volume.
Blank solution: Transfer 10 mL of acetonitrile into a 25-mL volumetric flask, and dilute with *Solution A* to volume.
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: Between 0.9 and 1.4
Relative standard deviation: NMT 5%
Analysis
Samples: *Standard solution, Sample solution,* and *Blank solution*. [NOTE—Adjust the peak areas for any interference from the *Blank solution*.]
Calculate the percentage of each individual impurity in the portion of Anastrozole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*
r_S = peak area of anastrozole from the *Standard solution*
C_S = concentration of USP Anastrozole RS in the *Standard solution* (mg/mL)
C_U = concentration of Anastrozole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity of less than 0.05%.

USP Monographs

www.webofpharma.com

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desmethyl anastrozole[a]	0.6	0.2
Anastrozole	1.0	—
Anastrozole dimer[b]	2.0	0.2
5-Bromomethyl anastrozole[c]	4.3	0.1
5-Dibromomethyl anastrozole[d]	5.4	0.1
Individual unspecified impurity	—	0.1
Total impurities	—	0.5

[a] 2-(3-(1-Cyanoethyl)-5-(1*H*-1,2,4-triazol-1-ylmethyl)phenyl)-2-methylpropionitrile.
[b] 2,3-Bis(3-(1-cyano-1-methylethyl)-5-(1*H*-1,2,4-triazol-1-ylmethyl)phenyl)-2-methylpropionitrile.
[c] 2,2′-(5-(Bromomethyl)-1,3-phenylene)bis(2-methylpropionitrile).
[d] 2,2′-(5-(Dibromomethyl)-1,3-phenylene)bis(2-methylpropionitrile).

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 0.3%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Anastrozole RS

Anastrozole Tablets

DEFINITION
Anastrozole Tablets contain NLT 90% and NMT 110% of the labeled amount of anastrozole ($C_{17}H_{19}N_5$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Transfer the finely ground Tablet powder containing 8 mg of anastrozole into a suitable container. Add 10 mL of diethyl ether and sonicate for 5 min. Aspirate the supernatant and pass through a nylon filter of 0.45-µm pore size into another suitable container containing 400 mg of potassium bromide. Evaporate the mixture to dryness under nitrogen. Further dry it under vacuum at 50° for 1 h. Add an additional 400 mg of potassium bromide for preparation of pellet and analysis.
Acceptance criteria: The spectrum obtained from the *Sample* shows bands at approximately 2235, 1606, 1500, 1359, 1205, 1137, 1013, and 875 cm^{-1}, similar to the spectrum from the Reference Standard similarly obtained.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (40:60)
Diluent: Acetonitrile and water (50:50)
Standard solution: 40 µg/mL of USP Anastrozole RS in *Diluent*. Sonication may be used to aid dissolution.
Sample solution: Nominally equivalent to 40 µg/mL of anastrozole in *Diluent*, prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask. Add 40% of the flask volume of water, and shake on a rotary shaker for 10 min to disintegrate the Tablets. Add 40% of the flask volume of acetonitrile, and sonicate for 15 min with intermittent shaking, maintaining the sonicator temperature at 25°. Dilute with *Diluent* to volume. Centrifuge a portion of the solution at 3500 rpm for 10 min, and use the clear solution for analysis.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anastrozole ($C_{17}H_{19}N_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Anastrozole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of anastrozole in the *Sample solution* (mg/mL)

Acceptance criteria: 90%–110%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Water; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 15 min
Mobile phase: Acetonitrile and water (40:60)
Diluent: Acetonitrile and water (50:50)
Standard stock solution: 0.2 mg/mL of USP Anastrozole RS in *Diluent*
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (*L*/1000) mg/mL, where *L* is the label claim in mg/Tablet.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard the first few mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anastrozole ($C_{17}H_{19}N_5$) dissolved:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of anastrozole ($C_{17}H_{19}N_5$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 1000 mL, deaerated
Apparatus 2: 50 rpm
Time: 15 min
Mobile phase: Acetonitrile, trifluoroacetic acid, and water (300:1:700)
Standard stock solution: 0.2 mg/mL of USP Anastrozole RS prepared as follows. Transfer USP Anastrozole RS into a suitable volumetric flask and add acetonitrile equivalent to 8% of the final volume. Sonicate to dissolve and dilute with water to volume.
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (*L*/1000) mg/mL, where *L* is the label claim in mg/Tablet.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard the first few mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 3.2-mm × 10-cm; 5-µm packing L42
Flow rate: 0.75 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.9–1.4
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anastrozole ($C_{17}H_{19}N_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of anastrozole ($C_{17}H_{19}N_5$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: Methanol, acetonitrile, trifluoroacetic acid, and water (200: 100: 0.7: 700)
Solution B: Methanol, acetonitrile, trifluoroacetic acid, and water (500: 250: 0.7: 250)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
25	100	0
25.1	0	100
30	0	100
31	100	0
40	100	0

Diluent: Acetonitrile, trifluoroacetic acid, and water (200: 0.8: 800)
System suitability stock solution: 0.5 mg/mL of USP Anastrozole RS and 0.3 mg/mL of ethyl 4-hydroxybenzoate in *Diluent* prepared as follows. Transfer USP Anastrozole RS and ethyl 4-hydroxybenzoate into a suitable volumetric flask and add *Diluent* equivalent to 50% of the final volume. Sonicate to dissolve and dilute with *Diluent* to volume.
System suitability solution: 10 µg/mL of USP Anastrozole RS and 6 µg/mL of ethyl 4-hydroxybenzoate in *Diluent* from the *System suitability stock solution*
Standard stock solution: 0.5 mg/mL of USP Anastrozole RS in *Diluent* prepared as follows. Transfer USP Anastrozole RS into a suitable volumetric flask and add *Diluent* equivalent to 50% of the final volume. Sonicate to dissolve and dilute with *Diluent* to volume.
Standard solution: 10 µg/mL of USP Anastrozole RS in *Diluent* from the *Standard stock solution*
Sample solution: Nominally equivalent to 1.0 mg/mL of anastrozole from NLT 25 finely powdered Tablets, prepared as follows. Transfer a weighed quantity of powdered Tablets, equivalent to 10 mg of anastrozole, to a suitable container and add 10.0 mL of *Diluent*. Sonicate for 30 min and allow to cool to room temperature. Pass through a suitable filter of 0.45-µm pore size, and discard the first few mL of the filtrate. If the filtrate is not clear, pass again through a suitable filter of 0.2-µm pore size, and discard the first few mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 3.2-mm × 10-cm; 5-µm packing L42
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Analysis time: 25 min
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for ethyl 4-hydroxybenzoate and anastrozole are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: Greater than 4 between the ethyl 4-hydroxybenzoate and anastrozole peaks
Tailing factor: 0.9–1.3 for the anastrozole peak
Relative standard deviation: NMT 5% for the anastrozole peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of anastrozole from the *Standard solution*
C_S = concentration of USP Anastrozole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of anastrozole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.1%.

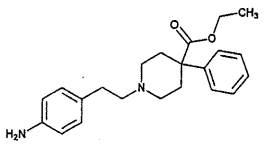
Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Anastrozole diamide[a]	0.11	0.5
Anastrozole monoacid monoamide[b]	0.26	0.5
Anastrozole monoamide mononitrile[c]	0.30	0.5
Desmethyl anastrozole[d]	0.51	—
Anastrozole diacid[e]	0.71	0.5
Anastrozole monoacid mononitrile[f]	0.87	0.5
Anastrozole	1.00	—
Any individual unspecified impurity	—	0.5
Total impurities	—	1.0

[a] 2,2′-{5-[(1*H*-1,2,4-Triazol-1-yl)methyl]-1,3-phenylene}bis(2-methylpropanamide).
[b] 2-{3-[(1*H*-1,2,4-Triazol-1-yl)methyl]-5-(1-amino-2-methyl-1-oxopropan-2-yl)phenyl}-2-methylpropanoic acid.
[c] 2-{3-[(1*H*-1,2,4-Triazol-1-yl)methyl]-5-(2-cyanopropan-2-yl)phenyl}-2-methylpropanamide.
[d] 2-(3-(1-Cyanoethyl)-5-(1*H*-1,2,4-triazol-1-ylmethyl)phenyl)-2-methylpropanenitrile. This process impurity is controlled in the drug substance monograph. It is included in the table for identification only, and it is not to be reported in the total impurities.
[e] 2,2′-{5-[(1*H*-1,2,4-Triazol-1-yl)methyl]-1,3-phenylene}bis(2-methylpropanoic acid).
[f] 2-{3-[(1*H*-1,2,4-Triazol-1-yl)methyl]-5-(2-cyanopropan-2-yl)phenyl}-2-methylpropanoic acid.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Anastrozole RS

Anileridine



$C_{22}H_{28}N_2O_2$ 352.47
4-Piperidinecarboxylic acid, 1-[2-(4-aminophenyl)ethyl]-4-phenyl-, ethyl ester;
Ethyl 1-(*p*-aminophenethyl)-4-phenylisonipecotate [144-14-9].

DEFINITION
Anileridine contains NLT 98.5% and NMT 101.0% of anileridine ($C_{22}H_{28}N_2O_2$), calculated on the anhydrous basis.

IDENTIFICATION
- **A.**
  Buffer solution: Dissolve 5.68 g of anhydrous dibasic sodium phosphate and 3.63 g of monobasic potassium phosphate in water to make 1000 mL: the pH is 7.0 ± 0.05.
  Sample stock solution: Dissolve 40 mg of Anileridine in 2.3 mL of 0.1 N hydrochloric acid in a 100-mL volumetric flask, and dilute with water to volume.
  Sample solution A: *Sample stock solution, Buffer solution,* and water (4:25:71)
  Sample solution B: *Sample stock solution, Buffer solution,* and water (20:25:55)
  Acceptance criteria: The UV absorption spectrum of *Sample solution A* exhibits a maximum at 234 ± 1 nm; and the UV absorption spectrum of *Sample solution B* exhibits a maximum at 285 ± 2 nm. The ratio $5A_{234}/A_{285}$ is 8.8.
- **B.**
  Sample solution: 0.2 mg/mL of Anileridine in 0.1 N hydrochloric acid
  Analysis: To 5 mL of the *Sample solution* add 2 mL of a solution of *p*-dimethylaminobenzaldehyde in alcohol (1 in 100).
  Acceptance criteria: A yellow color develops immediately.

ASSAY
- **PROCEDURE**
  Sample: 350 mg of Anileridine
  Titrimetric system
  Mode: Direct titration
  Titrant: 0.1 N perchloric acid VS
  Endpoint detection: Visual
  Analysis: Dissolve the *Sample* in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with *Titrant* to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 17.62 mg of anileridine ($C_{22}H_{28}N_2O_2$).
  Acceptance criteria: 98.5%–101.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Sample: 180 mg
  Analysis: Dissolve the *Sample* in a mixture of 1 mL of nitric acid and 40 mL of water.
  Acceptance criteria: NMT 400 ppm; the solution shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid.

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.

Anileridine Injection

DEFINITION
Anileridine Injection is a sterile solution of Anileridine in Water for Injection, prepared with the aid of Phosphoric Acid. It contains NLT 90.0% and NMT 115.0% of the labeled amount of anileridine ($C_{22}H_{28}N_2O_2$), as the phosphate.

USP Monographs

www.webofpharma.com

IDENTIFICATION

• **A.**

Sample solution: 0.25 mg/mL of anileridine from Injection diluted with water

Analysis: To 5 mL of the *Sample solution* add 2 mL of a 10-mg/mL solution of *p*-dimethylaminobenzaldehyde in alcohol.

Acceptance criteria: A yellow color develops immediately.

• **B.** A volume of Injection, diluted with water to a concentration of 0.025 mg/mL of anileridine, exhibits absorbance maxima at 234 ± 1 and 285 ± 2 nm.

ASSAY

• **PROCEDURE**

Standard solution: Prepare 250 µg/mL of USP Anileridine Hydrochloride RS in 0.1 N hydrochloric acid on the day of the assay. Each mg of anileridine hydrochloride is equivalent to 0.8286 mg of anileridine.

Sample solution: Nominally equivalent to 200 µg/mL of anileridine, from a volume of Injection diluted with 0.1 N hydrochloric acid

Blank: 0.1 N hydrochloric acid

Instrumental conditions

Analytical wavelength: 560 nm

Cell: 1 cm

Analysis

Samples: *Standard solution, Sample solution,* and *Blank*

Transfer 5.0 mL each of the *Standard solution, Sample solution,* and *Blank* to separate 200-mL volumetric flasks. To each flask add 25 mL of water, 5 mL of 1 N hydrochloric acid, and 5 mL of 1-mg/mL sodium nitrite solution. Allow to stand for 2 min, then add to each flask 5 mL of 5-mg/mL ammonium sulfamate solution. Allow to stand for 3 min, then add 5 mL of 1-mg/mL *N*-(1-naphthyl) ethylenediamine dihydrochloride solution. Allow to stand for 1 h, and dilute with water to volume. Use the reagent blank to set the instrument.

Calculate the percentage of anileridine ($C_{22}H_{28}N_2O_2$) in the volume of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Anileridine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of anileridine in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of anileridine, 352.48

M_{r2} = molecular weight of anileridine hydrochloride, 425.40

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 4.5–5.0

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 7.2 USP Endotoxin Units/mg of anileridine.

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Anileridine Hydrochloride RS

Anileridine Hydrochloride

$C_{22}H_{28}N_2O_2 \cdot 2HCl$ 425.39

4-Piperidinecarboxylic acid, 1-[2-(4-aminophenyl)ethyl]-4-phenyl-, ethyl ester, dihydrochloride;

Ethyl 1-(*p*-aminophenethyl)-4-phenylisonipecotate dihydrochloride [126-12-5].

DEFINITION

Anileridine Hydrochloride contains NLT 96.0% and NMT 102.0% of anileridine hydrochloride ($C_{22}H_{28}N_2O_2 \cdot 2HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.**

Buffer solution: 5.68 g/L of anhydrous dibasic sodium phosphate and 3.63 g/L of monobasic potassium phosphate in water: the pH, determined potentiometrically, is 7.0 ± 0.05.

Sample stock solution: 0.5 mg/mL of anileridine hydrochloride in water

Sample solution A: *Sample stock solution, pH 7.0 buffer solution,* and water (4:25:71)

Sample solution B: *Sample stock solution, pH 7.0 buffer solution,* and water (20:25:55)

Acceptance criteria: The UV absorption spectrum of *Sample solution A* exhibits a maximum at 234 ± 1 nm, and the UV absorption spectrum of *Sample solution B* exhibits a maximum at 285 ± 2 nm.

• **C.**

Sample solution: 0.2 mg/mL of Anileridine Hydrochloride

Analysis: To 5 mL of the *Sample solution* add 2 mL of a solution of 10 mg/mL of *p*-dimethylaminobenzaldehyde in alcohol.

Acceptance criteria: A yellow color develops immediately.

• **D. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

Sample: 10-mg/mL solution of Anileridine Hydrochloride

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Sample: 200 mg of Anileridine Hydrochloride

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 10 mL of glacial acetic acid by heating it on a steam bath. Cool immediately in a cold water bath, add 5 mL of mercuric acetate TS, 20 mL of acetone, and 0.5 mL of indicator solution (70 mg of α-naphtholbenzein, 10 mg of crystal violet, and 40 mg of quinaldine red in 100 mL of glacial acetic acid). Titrate with *Titrant* to a gray-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 21.27 mg of anileridine hydrochloride ($C_{22}H_{28}N_2O_2 \cdot 2HCl$).

Acceptance criteria: 96.0%–102.0% on the dried basis

OTHER COMPONENTS

• **CONTENT OF CHLORIDE**

Sample: 200 mg

Analysis: Dissolve the *Sample* in 50 mL of water in a glass-stoppered flask. Add 25.0 mL of 0.1 N silver nitrate VS, then add 5 mL of 2 N nitric acid and 5 mL of nitrobenzene, shake vigorously, and add 2 mL of ferric

USP Monographs

www.webofpharma.com

ammonium sulfate TS. Titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl.
Acceptance criteria: 16.0%–17.2%

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 50 mg/mL
Acceptance criteria: 2.5–3.0
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at a pressure below 5 mm of mercury at 100° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Anileridine Hydrochloride RS

Anileridine Hydrochloride Tablets

DEFINITION
Anileridine Hydrochloride Tablets contain an amount of anileridine hydrochloride ($C_{22}H_{28}N_2O_2 \cdot 2HCl$) equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of anileridine ($C_{22}H_{28}N_2O_2$).

IDENTIFICATION
• **A.**
Sample solution: Transfer an equivalent to 50 mg of anileridine, from finely powdered Tablets, to a 250-mL volumetric flask. Add 100 mL of water, and heat on a steam bath. Cool, dilute to volume, and filter.
Analysis: To 5 mL of the *Sample solution* add 2 mL of a 10-mg/mL solution of *p*-dimethylaminobenzaldehyde in alcohol.
Acceptance criteria: A yellow color develops immediately.
• **B.**
Buffer solution: 5.68 g/L of anhydrous dibasic sodium phosphate and 3.63 g/L of monobasic potassium phosphate in water: the pH, determined potentiometrically, is 7.0 ± 0.05.
Sample stock solution: Transfer an equivalent to 50 mg of anileridine, from finely powdered Tablets, to a 100-mL volumetric flask. Add 30 mL of water, and heat on a steam bath. Cool, dilute with water to volume, and filter.
Sample solution A: *Sample stock solution, Buffer solution,* and water (4:25:71)
Sample solution B: *Sample stock solution, Buffer solution,* and water (20:25:55)
Acceptance criteria: The UV absorption spectrum of *Sample solution A* exhibits a maximum at 234 ± 1 nm, and the UV absorption spectrum of *Sample solution B* exhibits a maximum at 285 ± 2 nm.

ASSAY
• **PROCEDURE**
Standard solution: 250 µg/mL of USP Anileridine Hydrochloride RS in 0.1 N hydrochloric acid. (Each mg of anileridine hydrochloride is equivalent to 0.8286 mg of anileridine.) Prepare on the day of the assay.
Sample solution: Transfer an equivalent to 50 mg of anileridine, from finely powdered Tablets (NLT 20), to a 250-mL volumetric flask. Add 25 mL of 1 N hydrochloric acid and 100 mL of water, and heat on a water bath. Cool, and dilute with water to volume. Filter the solution, discarding the first 25 mL of the filtrate.
Blank: 0.1 N hydrochloric acid
Instrumental conditions
Analytical wavelength: 560 nm
Cell: 1 cm
Analysis
Samples: *Standard solution, Sample solution,* and *Blank*
Transfer 5.0 mL each of the *Standard solution, Sample solution,* and *Blank* to separate 200-mL volumetric flasks. To each flask add 25 mL of water, 5 mL of 1 N hydrochloric acid, and 5 mL of 1-mg/mL sodium nitrite solution. Allow to stand for 2 min, then add to each flask 5 mL of 5-mg/mL ammonium sulfamate solution. Allow to stand for 3 min, then add 5 mL of 1-mg/mL *N*-(1-naphthyl)ethylenediamine dihydrochloride solution. Allow to stand for 1 h, and dilute with water to volume. Use the reagent blank to set the instrument.
Calculate the percentage of anileridine ($C_{22}H_{28}N_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the solution from the *Sample solution*
A_S = absorbance of the solution from the *Standard solution*
C_S = concentration of USP Anileridine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of anileridine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of anileridine, 352.48
M_{r2} = molecular weight of anileridine hydrochloride, 425.40

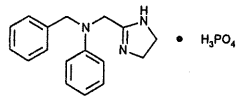
Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: USP Anileridine Hydrochloride RS of a known concentration, in *Medium*
Sample solution: Filtered portion of the solution under test
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of anileridine ($C_{22}H_{28}N_2O_2$) dissolved, using the *Analysis* set forth in the *Assay* and in comparison to the *Standard solution*.
Tolerances: NLT 65% (*Q*) of the labeled amount of anileridine ($C_{22}H_{28}N_2O_2$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Anileridine Hydrochloride RS

www.webofpharma.com

Antazoline Phosphate



$C_{17}H_{19}N_3 \cdot H_3PO_4$ 363.35

1*H*-Imidazole-2-methanamine, 4,5-dihydro-*N*-phenyl-*N*-(phenylmethyl)-, phosphate (1:1);
2-[(*N*-Benzylanilino)methyl]-2-imidazoline phosphate (1:1) [154-68-7].

DEFINITION

Antazoline Phosphate contains NLT 98.0% and NMT 102.0% of antazoline phosphate ($C_{17}H_{19}N_3 \cdot H_3PO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dilute 1.0 mL of formic acid with water to 1000 mL.
Mobile phase: Acetonitrile and *Solution A* (17:83)
Diluent: Acetonitrile and water (17:83)
Standard solution: 0.2 mg/mL of USP Antazoline Phosphate RS in *Diluent*
System suitability solution: 1 µg/mL of USP Antazoline Related Compound A RS in the *Standard solution*
Sample solution: 0.2 mg/mL of Antazoline Phosphate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L11
Temperatures
Column: 35°
Autosampler: 4°
Flow rate: 0.5 mL/min
Injection volume: 5 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times of antazoline and antazoline related compound A are 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 2.0 between antazoline and antazoline related compound A, *System suitability solution*
Tailing factor: NMT 1.5 for antazoline, *Standard solution*
Relative standard deviation: NMT 0.73% for antazoline, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of antazoline phosphate ($C_{17}H_{19}N_3 \cdot H_3PO_4$) in the portion of Antazoline Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Antazoline Phosphate RS in the *Standard solution* (mg/mL)
C_U = concentration of Antazoline Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: Dilute 1.0 mL of formic acid with water to 1000 mL.
Mobile phase: Acetonitrile and *Solution A* (17:83)
Diluent: Acetonitrile and water (17:83)
Standard solution A: 5 µg/mL of USP Antazoline Related Compound A RS and 1.0 mg/mL of USP Antazoline Phosphate RS in *Diluent*
Standard solution B: 1 µg/mL of USP Antazoline Phosphate RS in *Diluent*
Sample solution: 1.0 mg/mL of Antazoline Phosphate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L11
Temperatures
Column: 35°
Autosampler: 4°
Flow rate: 0.5 mL/min
Injection volume: 5 µL
Run time: NLT 4 times the retention time of antazoline
System suitability
Samples: *Standard solution A* and *Standard solution B*
[NOTE—The relative retention times of antazoline and antazoline related compound A are 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 2.0 between antazoline and antazoline related compound A peaks, *Standard solution A*
Relative standard deviation: NMT 5.0% for antazoline, *Standard solution B*
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Calculate the percentage of antazoline related compound A in the portion of Antazoline Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of antazoline related compound A from the *Sample solution*
r_S = peak response of antazoline related compound A from *Standard solution A*
C_S = concentration of USP Antazoline Related Compound A RS in *Standard solution A* (mg/mL)
C_U = concentration of Antazoline Phosphate in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Antazoline Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of antazoline phosphate from *Standard solution B*
C_S = concentration of USP Antazoline Phosphate RS in *Standard solution B* (mg/mL)

www.webofpharma.com

C_U = concentration of Antazoline Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

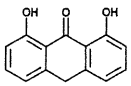
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Antazoline phosphate	1.0	—
Antazoline related compound A[a]	1.1	0.5
Any individual unspecified impurity	—	0.5
Total impurities	—	1.0

[a] *N*-(2-Aminoethyl)-2-[benzyl(phenyl)amino]acetamide.

SPECIFIC TESTS
- **pH** ⟨791⟩
  Sample: 20 mg/mL
  Acceptance criteria: 4.0–5.0
- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° for 4 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** ⟨11⟩
  USP Antazoline Phosphate RS
  USP Antazoline Related Compound A RS
  N-(2-Aminoethyl)-2-[benzyl(phenyl)amino]acetamide.
  $C_{17}H_{21}N_3O$ 283.38

Anthralin



$C_{14}H_{10}O_3$ 226.23
9(10*H*)-Anthracenone, 1,8-dihydroxy-;
1,8-Dihydroxy-9-anthrone [1143-38-0].

DEFINITION
Anthralin contains NLT 97.0% and NMT 102.0% of anthralin ($C_{14}H_{10}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Sample solution: 10 µg/mL in chloroform
  Acceptance criteria: Meets the requirements

ASSAY
- **Procedure**
  [Note—Use low-actinic glassware.]
  Mobile phase: *n*-Hexane, dichloromethane, and glacial acetic acid (82:12:6)
  Internal standard solution: 0.5 mg/mL of *o*-nitroaniline in *n*-hexane prepared as follows. First dissolve *o*-nitroaniline in a small quantity of dichloromethane, and then dilute with *n*-hexane.
  System suitability stock solution: 0.1 mg/mL of USP Anthralin RS and 0.2 mg/mL of danthron in dichloromethane
  System suitability solution: Transfer 5 mL of the *System suitability stock solution* into a 25-mL volumetric flask, add 5 mL of *n*-hexane, and dilute with *Mobile phase* to volume.
  Solvent blank solution: *Mobile phase*, *n*-hexane, and dichloromethane (3:1:1)
  Standard stock solution: 0.25 mg/mL of USP Anthralin RS in dichloromethane
  Standard solution: Transfer 5 mL each of *Standard stock solution* and *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Sample stock solution: 0.25 mg/mL of Anthralin in dichloromethane
  Sample solution: Transfer 5 mL each of *Sample stock solution* and *Internal standard solution* into a 25-mL volumetric flask, dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 354 nm
  Column: 4.6-mm × 25-cm; packing L3
  Flow rate: 2 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *System suitability solution*, *Solvent blank solution*, and *Standard solution*
  [Note—The relative retention times for anthralin, danthron, dianthrone, and *o*-nitroaniline are 1.0, 1.2, 1.7, and 2.3, respectively.]
  Suitability requirements
  Resolution: NLT 1.3 between anthralin and danthron, *System suitability solution*
  Tailing factor: NMT 1.5, *System suitability solution*
  Relative standard deviation: NMT 2.0% of the ratio of the peak responses, *Standard solution*
  Interference: No discernible signal is observed at the retention time of anthralin, *Solvent blank solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of anthralin ($C_{14}H_{10}O_3$) in the portion of Anthralin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of anthralin to *o*-nitroaniline from the *Sample solution*
R_S = peak response ratio of anthralin to *o*-nitroaniline from the *Standard solution*
C_S = concentration of USP Anthralin RS in the *Standard solution* (µg/mL)
C_U = concentration of Anthralin in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.1%
- **Chloride and Sulfate**, *Chloride* ⟨221⟩
  Sample: 1 g
  Analysis: To 15 mL of water add the *Sample*, mix, and filter. Acidify 5 mL of the filtrate with nitric acid, and add a few drops of silver nitrate TS.

USP Monographs

www.webofpharma.com

Acceptance criteria: No more opalescence is produced immediately than is present in a 5-mL portion of the filtrate to which nothing has been added.

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Sample: 5 mL of the untreated filtrate obtained in the test for *Chloride*
Analysis: To the *Sample* add 3 drops of 3 N hydrochloric acid and 5 drops of barium chloride TS.
Acceptance criteria:
No more turbidity is produced than is present in a 5-mL portion of the filtrate to which nothing has been added.

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩**:** 178°–181°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample over silica gel for 4 h.
Acceptance criteria: NMT 0.5%
• **ACIDITY OR ALKALINITY**
Analysis: Suspend a sample in water, and filter.
Acceptance criteria: The filtrate is neutral to litmus.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers in a cool place. Protect from light.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Anthralin RS

Anthralin Cream

DEFINITION
Anthralin Cream is Anthralin in an aqueous (oil-in-water) or oily (water-in-oil) cream vehicle. Cream labeled to contain more than 0.1% of anthralin contains NLT 90.0% and NMT 115.0% of the labeled amount of anthralin ($C_{14}H_{10}O_3$), and Cream labeled to contain 0.1% or less of anthralin contains NLT 90.0% and NMT 130.0% of the labeled amount of anthralin ($C_{14}H_{10}O_3$).

ASSAY
• **PROCEDURE**
[NOTE—Use low-actinic glassware.]
Mobile phase: *n*-Hexane, dichloromethane, and glacial acetic acid (82:12:6)
Internal standard solution: 0.5 mg/mL of *o*-nitroaniline in *n*-hexane prepared as follows. First dissolve *o*-nitroaniline in a small quantity of dichloromethane, and then dilute with *n*-hexane.
System suitability stock solution: 0.1 mg/mL of USP Anthralin RS and 0.2 mg/mL of danthron in dichloromethane
System suitability solution: Transfer 5 mL of the *System suitability stock solution* into a 25-mL volumetric flask, add 5 mL of *n*-hexane, and dilute with *Mobile phase* to volume.
Solvent blank solution: *Mobile phase*, *n*-hexane, and dichloromethane (3:1:1)
Standard stock solution: 0.25 mg/mL of USP Anthralin RS in dichloromethane
Standard solution: Transfer 2 mL each of *Standard stock solution* and *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample stock solution: Weigh 5 g of Cream into a 100-mL beaker. Add 20 mL of dichloromethane and 10 mL of glacial acetic acid, and stir to disperse the Cream. Transfer the contents of the beaker to a filter paper (Whatman No. 4, or equivalent) with the aid of dichloromethane, and filter into a 100-mL volumetric flask. Thoroughly wash the precipitate with dichloromethane, and allow the washings to drain into the flask. Dilute with dichloromethane to volume.
Sample solution: Transfer a volume of *Sample stock solution* equivalent to 0.5 mg of anthralin and 2 mL of *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 354 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution*, *Solvent blank solution*, and *Standard solution*
[NOTE—The relative retention times for anthralin, danthron, dianthrone, and *o*-nitroaniline are 1.0, 1.2, 1.7, and 2.3, respectively.]
Suitability requirements
Resolution: NLT 1.3 between anthralin and danthron, *System suitability solution*
Tailing factor: NMT 1.5, *System suitability solution*
Relative standard deviation: NMT 2.0% of the ratio of the peak responses, *Standard solution*
Interference: No discernible signal is observed at the retention time of anthralin, *Solvent blank solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of anthralin ($C_{14}H_{10}O_3$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of anthralin to *o*-nitroaniline from the *Sample solution*
R_S = peak response ratio of anthralin to *o*-nitroaniline peak from the *Standard solution*
C_S = concentration of USP Anthralin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of anthralin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–115.0% for Cream labeled to contain more than 0.1% of anthralin; 90.0%–130.0% for Cream labeled to contain 0.1% or less of anthralin

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, in a cool place. Protect from light.
• **LABELING:** Label it to indicate whether the cream vehicle is aqueous or oily.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Anthralin RS

Anthralin Ointment

DEFINITION
Anthralin Ointment is Anthralin in a petrolatum or other oleaginous vehicle. Ointment labeled to contain more than 0.1% of anthralin contains NLT 90.0% and NMT 115.0% of the labeled amount of anthralin ($C_{14}H_{10}O_3$), and Ointment labeled to contain 0.1% or less of anthralin contains NLT 90.0% and NMT 130.0% of the labeled amount of anthralin ($C_{14}H_{10}O_3$).

ASSAY
• **PROCEDURE**
[NOTE—Use low-actinic glassware.]

www.webofpharma.com

Mobile phase: *n*-Hexane, dichloromethane, and glacial acetic acid (82:12:6)
Internal standard solution: 0.5 mg/mL of *o*-nitroaniline in *n*-hexane prepared as follows. First dissolve *o*-nitroaniline in a small quantity of dichloromethane, and then dilute with *n*-hexane.
System suitability stock solution: 0.1 mg/mL of USP Anthralin RS and 0.2 mg/mL of danthron in dichloromethane
System suitability solution: Transfer 5 mL of the *System suitability stock solution* into a 25-mL volumetric flask, add 5 mL of *n*-hexane, and dilute with *Mobile phase* to volume.
Solvent blank solution: *Mobile phase*, *n*-hexane, and dichloromethane (3:1:1)
Standard stock solution: 0.25 mg/mL of USP Anthralin RS in dichloromethane
Standard solution: Transfer 2 mL each of *Standard stock solution* and *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample stock solution: Weigh 5 g of Ointment into a 100-mL beaker. Add 20 mL of dichloromethane and 10 mL of glacial acetic acid, and stir to disperse the Ointment. Transfer the contents of the beaker to a filter paper (Whatman No. 4, or equivalent) with the aid of dichloromethane, and filter into a 100-mL volumetric flask. Thoroughly wash the precipitate with dichloromethane, and allow the washings to drain into the flask. Dilute with dichloromethane to volume.
Sample solution: Transfer a volume of *Sample stock solution* equivalent to 0.5 mg of anthralin and 2 mL of *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 354 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution*, *Solvent blank solution*, and *Standard solution*
[NOTE—The relative retention times for anthralin, danthron, dianthrone, and *o*-nitroaniline are 1.0, 1.2, 1.7, and 2.3, respectively.]
Suitability requirements
Resolution: NLT 1.3 between anthralin and danthron, *System suitability solution*
Tailing factor: NMT 1.5, *System suitability solution*
Relative standard deviation: NMT 2.0% of the ratio of the peak responses, *Standard solution*
Interference: No discernible signal is observed at the retention time of anthralin, *Solvent blank solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of anthralin ($C_{14}H_{10}O_3$) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of anthralin to *o*-nitroaniline from the *Sample solution*
R_S = peak response ratio of anthralin to *o*-nitroaniline from the *Standard solution*
C_S = concentration of USP Anthralin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of anthralin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–115.0% for Ointment labeled to contain more than 0.1% of anthralin; 90.0%–130.0% for Ointment labeled to contain 0.1% or less of anthralin

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, in a cool place. Protect from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Anthralin RS

Anticoagulant Citrate Dextrose Solution

DEFINITION
Anticoagulant Citrate Dextrose Solution is a sterile solution of Citric Acid, Sodium Citrate, and Dextrose in Water for Injection. It contains no antimicrobial agents, and in each 1000 mL it contains:

	Solution A	Solution B
Total Citrate, expressed as anhydrous citric acid ($C_6H_8O_7$)	NLT 20.59 g	NLT 12.37 g
	NMT 22.75 g	NMT 13.67 g
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	NLT 23.28 g	NLT 13.96 g
	NMT 25.73 g	NMT 15.44 g
Sodium (Na)	NLT 4.90 g	NLT 2.94 g
	NMT 5.42 g	NMT 3.25 g

Prepare Anticoagulant Citrate Dextrose Solution as follows.

	Solution A	Solution B
Citric Acid (anhydrous)	7.3 g	4.4 g
Sodium Citrate (dihydrate)	22.0 g	13.2 g
Dextrose (monohydrate)	24.5 g	14.7 g
Water for Injection, a sufficient quantity to make	1000 mL	1000 mL

Dissolve the ingredients, and mix. Filter the solution until clear, place immediately in suitable containers, and sterilize.
If desired, 8 g and 4.8 g of monohydrated citric acid may be used instead of the indicated, respective amounts of anhydrous citric acid; 19.3 g and 11.6 g of anhydrous sodium citrate may be used instead of the indicated, respective amounts of dihydrated sodium citrate; and 22.3 g and 13.4 g of anhydrous dextrose may be used instead of the indicated, respective amounts of monohydrated dextrose.

IDENTIFICATION
- **A. DEXTROSE**
  Analysis: Add a few drops of solution (1 in 20) to 5 mL of hot alkaline cupric tartrate TS.
  Acceptance criteria: A copious red precipitate of cuprous oxide is formed.
- **B. IDENTIFICATION TESTS—GENERAL,** *Citrate* ⟨191⟩**:** Meets the requirements when concentrated to one-half its volume
- **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩**:** Meets the requirements when concentrated to one-half its volume

www.webofpharma.com

ASSAY

• **TOTAL CITRATE**

Mobile phase, Standard preparation 1, and Chromatographic system: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Sample solution: Pipet 5 mL of Solution into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Sample solution (for the assay of citric acid/citrate)*.

Analysis

Samples: *Standard preparation 1* and *Sample solution*

Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure*.

Calculate the quantity, in g, of anhydrous citric acid ($C_6H_8O_7$) in the volume of Solution taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times F \times D$$

r_U = peak area of citrate from the *Sample solution*
r_S = peak area of citrate from *Standard preparation 1*
C_S = concentration of citrate in *Standard preparation 1* (µg/mL)
M_{r1} = molecular weight of anhydrous citric acid, 192.12
M_{r2} = molecular weight of citrate ($C_6H_5O_7$), 189.10
F = conversion factor, 0.000001 g/µg
D = dilution factor

Acceptance criteria: Each 1000 mL should contain 20.59–22.75 g in Solution A and 12.37–13.67 g in Solution B of total citrate expressed as anhydrous citric acid.

• **SODIUM**

Solution A: Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, add water to volume, and mix. This solution contains 15 mEq/1000 mL of lithium.

Standard solution: Transfer 8.18 g of sodium chloride, previously dried at 105° for 2 h to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 140 mEq/1000 mL of sodium. Transfer 50 µL of this solution to a 10-mL volumetric flask, dilute with *Solution A* to volume, and mix.

Sample solution: Transfer 25 mL of Solution to a 50-mL volumetric flask, and dilute with water to volume. Transfer 50 µL of this solution to a 10-mL volumetric flask, dilute with *Solution A* to volume, and mix.

Analysis

Samples: *Standard solution* and *Sample solution*

Using a suitable flame photometer, adjusted to read zero with *Solution A*, concomitantly determine the sodium flame emission readings for the *Standard solution* and the *Sample solution* at the wavelength of maximum emission at 589 nm.

Calculate the quantity, in g, of sodium (Na) in 1000 mL of Solution taken:

$$\text{Result} = (r_U/r_S) \times (A_r/M_r) \times W \times F$$

r_U = sodium emission readings from the *Sample solution*
r_S = sodium emission readings from the *Standard solution*
A_r = atomic weight of sodium, 22.99
M_r = molecular weight of sodium chloride, 58.44
W = weight of sodium chloride taken to make the *Standard solution*, 8.18 g
F = conversion factor, 2

Acceptance criteria: Each 1000 mL should contain 4.90–5.42 g in Solution A and 2.94–3.25 g in Solution B of sodium.

• **DEXTROSE**

Analysis: Determine the angular rotation of Solution in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).

Where Solution is labeled to contain anhydrous dextrose, calculate the percentage, in g/100 mL, of anhydrous dextrose ($C_6H_{12}O_6$) in the portion of Solution taken:

$$\text{Result} = (100/F) \times A \times R$$

F = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
A = 100 mm divided by the length of the polarimeter tube (mm)
R = observed rotation (°)

Where Solution is labeled to contain dextrose monohydrate, calculate the percentage, in g/100 mL, of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Solution taken:

$$\text{Result} = (100/F) \times (M_{r1}/M_{r2}) \times A \times R$$

F = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
M_{r1} = molecular weight for dextrose monohydrate, 198.17
M_{r2} = molecular weight for anhydrous dextrose, 180.16
A = 100 mm divided by the length of the polarimeter tube (mm)
R = observed rotation (°)

Acceptance criteria: Each 1000 mL should contain 23.28–25.73 g in Solution A and 13.96–15.44 g in Solution B of dextrose monohydrate.

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩**:** A 10-mL portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.0035%).

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 4.5–5.5

• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 5.56 USP Endotoxin Units/mL.

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in single-dose containers of colorless, transparent Type I or Type II glass or of a suitable plastic material (see ▲*Medical Devices—Bacterial Endotoxin and Pyrogen Tests* ⟨161⟩).▲ (CN 1-May-2019)

• **LABELING:** Label to indicate the number of mL of Solution required per 100 mL of whole blood or the number of mL of Solution required per volume of whole blood to be collected.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Citric Acid RS

www.webofpharma.com

Anticoagulant Citrate Phosphate Dextrose Solution

DEFINITION

Anticoagulant Citrate Phosphate Dextrose Solution is a sterile solution of Citric Acid, Sodium Citrate, Monobasic Sodium Phosphate, and Dextrose in Water for Injection. It contains, in each 1000 mL, NLT 2.11 g and NMT 2.33 g of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$); NLT 24.22 g and NMT 26.78 g of dextrose ($C_6H_{12}O_6 \cdot H_2O$); NLT 19.16 g and NMT 21.18 g of total citrate, expressed as anhydrous citric acid ($C_6H_8O_7$); and NLT 6.21 g and NMT 6.86 g of Sodium (Na). It contains no antimicrobial agents.

Prepare Anticoagulant Citrate Phosphate Dextrose Solution as follows.

Citric Acid (anhydrous)	2.99 g
Sodium Citrate (dihydrate)	26.3 g
Monobasic Sodium Phosphate (monohydrate; $NaH_2PO_4 \cdot H_2O$)	2.22 g
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	25.5 g
Water for Injection, a sufficient quantity to make	1000 mL

Dissolve the ingredients, and mix. Filter the solution until clear, place immediately in suitable containers, and sterilize.

If desired, 3.27 g of monohydrated citric acid may be used instead of the indicated amount of anhydrous citric acid; 23.06 g of anhydrous sodium citrate may be used instead of the indicated amount of dihydrated sodium citrate; 1.93 g of anhydrous monobasic sodium phosphate may be used instead of the indicated amount of monohydrated monobasic sodium phosphate; and 23.2 g of anhydrous dextrose may be used instead of the indicated amount of monohydrated dextrose.

IDENTIFICATION

• **A. DEXTROSE**

Analysis: Add a few drops of solution (1 in 20) to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

• **B. IDENTIFICATION TESTS—GENERAL,** *Phosphate* ⟨191⟩**:** Meets the requirements

• **C. IDENTIFICATION TESTS—GENERAL,** *Citrate* ⟨191⟩**:** Meets the requirements when concentrated to one-half its volume

• **D. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩**:** Meets the requirements when concentrated to one-half its volume

ASSAY

• **TOTAL CITRATE AND TOTAL PHOSPHATE**

Mobile phase, Standard preparation 2, and **Chromatographic system:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Sample solution for total citrate: Pipet 10 mL of Solution into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Sample solution (for the assay of citric acid/citrate).*

Sample solution for total phosphate: Pipet 5 mL of Solution into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Sample solution (for the assay of phosphate).*

Analysis: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure.*

Calculate the quantity, in g, of anhydrous citric acid ($C_6H_8O_7$) in the volume of Solution taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times F \times D$$

r_U = peak area of citrate from the *Sample solution for total citrate*
r_S = peak area of citrate from *Standard preparation 2*
C_S = concentration of citrate in *Standard preparation 2* (µg/mL)
M_{r1} = molecular weight of anhydrous citric acid ($C_6H_8O_7$), 192.12
M_{r2} = molecular weight of citrate ($C_6H_5O_7$), 189.10
F = conversion factor, 0.000001 g/µg
D = dilution factor

Acceptance criteria: Each 1000 mL of Solution should contain 19.16 g–21.18 g of total citrate expressed as anhydrous citric acid.

Analysis: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure.*

Calculate the quantity of phosphate, in g, expressed as monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$), in the volume of Solution taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times F \times D$$

r_U = peak area of phosphate from the *Sample solution for total phosphate*
r_S = peak area of phosphate from *Standard preparation 2*
C_S = concentration of phosphate in *Standard preparation 2* (µg/mL)
M_{r1} = molecular weight of monobasic sodium phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$), 137.99
M_{r2} = molecular weight of phosphate (PO_4), 94.97
F = conversion factor, 0.000001 g/µg
D = dilution factor

Acceptance criteria: Each 1000 mL of Solution should contain 2.11–2.33 g of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$).

• **DEXTROSE**

Sample: 5.0 mL of Solution

Analysis: Tare a clean, medium-porosity filtering crucible containing several carborundum boiling chips or glass beads. Pipet 50 mL of freshly mixed alkaline cupric tartrate TS into a 400-mL beaker. Add the boiling chips or glass beads from the tared crucible, 45 mL of water, and 5.0 mL of Solution to the beaker. Heat the beaker and contents over a burner that has been adjusted to cause boiling of the solution to start in 3.5–4 min. Boil the solution for 2 min, accurately timed, and filter immediately through the tared crucible, taking care to transfer all of the boiling chips or glass beads to the crucible. Wash the precipitate with hot water and 10 mL of alcohol. Dry the crucible and contents at 110° to constant weight. Perform a blank determination, and correct the weight of the precipitate from the sample for any precipitate obtained in the blank.

Each mg of cuprous oxide precipitate of the substance under assay is equivalent to 0.496 mg of dextrose ($C_6H_{12}O_6 \cdot H_2O$).

Acceptance criteria: Each 1000 mL of Solution should contain 24.22–26.78 g of dextrose ($C_6H_{12}O_6 \cdot H_2O$).

• **SODIUM**

Solution A: Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, add water to volume, and mix. This solution contains 15 mEq/1000 mL of lithium.

Standard solution: Transfer 8.18 g of sodium chloride, previously dried at 105° for 2 h to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution

www.webofpharma.com

contains 140 mEq/1000 mL of sodium. Transfer 50 µL of this solution to a 10-mL volumetric flask, dilute with *Solution A* to volume, and mix.

Sample solution: Transfer 25 mL of Solution to a 50-mL volumetric flask, and dilute with water to volume. Transfer 50 µL of this solution to a 10-mL volumetric flask, dilute with *Solution A* to volume, and mix.

Analysis

Samples: *Standard solution* and *Sample solution*

Using a suitable flame photometer, adjusted to read zero with *Solution A*, concomitantly determine the sodium flame emission readings for the *Standard solution* and the *Sample solution* at the wavelength of maximum emission at 589 nm.

Calculate the quantity, in g, of sodium (Na) in 1000 mL of Solution taken:

$$\text{Result} = (r_U/r_S) \times (A_r/M_r) \times W \times F$$

r_U = sodium emission readings from the *Sample solution*
r_S = sodium emission readings from the *Standard solution*
A_r = atomic weight of sodium, 22.99
M_r = molecular weight of sodium chloride, 58.44
W = weight of sodium chloride taken to make the *Standard solution*, 8.18 g
F = conversion factor, 2

Acceptance criteria: Each 1000 mL of Solution should contain 6.21–6.86 g of sodium.

IMPURITIES

- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩**:** A 10-mL portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.0035%).

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 5.0–6.0
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 5.56 USP Endotoxin Units/mL.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in single-dose containers of colorless, transparent Type I or Type II glass, or of a suitable plastic material (see ▲*Medical Devices—Bacterial Endotoxin and Pyrogen Tests* ⟨161⟩).▲ (CN 1-May-2019)
- **LABELING:** Label it to indicate the number of mL of Solution required per 100 mL of whole blood or the number of mL of Solution required per volume of whole blood to be collected.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Citric Acid RS

Anticoagulant Citrate Phosphate Dextrose Adenine Solution

DEFINITION

Anticoagulant Citrate Phosphate Dextrose Adenine Solution is a sterile solution of Citric Acid, Sodium Citrate, Monobasic Sodium Phosphate, Dextrose, and Adenine in Water for Injection. It contains, in each 1000 mL, NLT 2.11 g and NMT 2.33 g of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$); NLT 30.30 g and NMT 33.50 g of dextrose ($C_6H_{12}O_6 \cdot H_2O$); NLT 19.16 g and NMT 21.18 g of total citrate, expressed as citric acid, anhydrous ($C_6H_8O_7$); NLT 6.21 g and NMT 6.86 g of sodium (Na); and NLT 0.247 g and NMT 0.303 g of adenine ($C_5H_5N_5$). It contains no antimicrobial agents.

Prepare Anticoagulant Citrate Phosphate Dextrose Adenine Solution as follows.

Citric Acid (anhydrous)	2.99 g
Sodium Citrate (dihydrate)	26.3 g
Monobasic Sodium Phosphate (monohydrate; $NaH_2PO_4 \cdot H_2O$)	2.22 g
Dextrose (monohydrate)	31.9 g
Adenine ($C_5H_5N_5$)	0.275 g
Water for Injection, a sufficient quantity to make	1000 mL

Dissolve the ingredients, and mix. Filter the solution until clear, place immediately in suitable containers, and sterilize.

If desired, 3.27 g of monohydrated citric acid may be used instead of the indicated amount of anhydrous citric acid; 23.06 g of anhydrous sodium citrate may be used instead of the indicated amount of dihydrated sodium citrate; 1.93 g of anhydrous monobasic sodium phosphate may be used instead of the indicated amount of monohydrated monobasic sodium phosphate; and 29.0 g of anhydrous dextrose may be used instead of the indicated amount of monohydrated dextrose.

ASSAY

- **TOTAL CITRATE AND TOTAL PHOSPHATE**

Mobile phase, Standard preparation 2, and **Chromatographic system:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Sample solution for total citrate: Pipet 10 mL of Solution into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Sample solution (for the assay of citric acid/citrate).*

Sample solution for total phosphate: Pipet 5 mL of Solution into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Sample solution (for the assay of citric acid/citrate).*

Analysis

Samples: *Standard preparation 2* and *Sample solution for total citrate*

Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure.*

Calculate the quantity, in g, of anhydrous citric acid ($C_6H_8O_7$) in the volume of Solution taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times F \times D$$

r_U = peak area of citrate from the *Sample solution for total citrate*
r_S = peak area of citrate from *Standard preparation 2*
C_S = concentration of citrate in *Standard preparation 2* (µg/mL)
M_{r1} = molecular weight of anhydrous citric acid, 192.12
M_{r2} = molecular weight of citrate ($C_6H_5O_7$), 189.10
F = conversion factor, 0.000001 g/µg
D = dilution factor

Acceptance criteria: Each 1000 mL of Solution should contain NLT 19.16 g and NMT 21.18 g of total citrate expressed as anhydrous citric acid ($C_6H_8O_7$).

www.webofpharma.com

Analysis
Samples: *Standard preparation 2* and *Sample solution for total phosphate*
Calculate the quantity of phosphate, in g, expressed as monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$), in the volume of Solution taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times F \times D$$

r_U = peak area of phosphate from the *Sample solution for total phosphate*
r_S = peak area of phosphate from *Standard preparation 2*
C_S = concentration of phosphate in *Standard preparation 2* (µg/mL)
M_{r1} = molecular weight of monobasic sodium phosphate monohydrate, 137.99
M_{r2} = molecular weight of phosphate (PO_4), 94.97
F = conversion factor, 0.000001 g/µg
D = dilution factor

Acceptance criteria: Each 1000 mL of Solution should contain 2.11–2.33 g of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$).

• **SODIUM**
Solution A: Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, add water to volume, and mix. This solution contains 15 mEq/1000 mL of lithium.
Standard solution: Transfer 8.18 g of sodium chloride, previously dried at 105° for 2 h to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 140 mEq/1000 mL of sodium. Transfer 50 µL of this solution to a 10-mL volumetric flask, dilute with *Solution A* to volume, and mix.
Sample solution: Transfer 25 mL of Solution to a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 50 µL of this solution to a 10-mL volumetric flask, dilute with *Solution A* to volume, and mix.
Analysis
Samples: *Standard solution* and *Sample solution*
Using a suitable flame photometer, adjusted to read zero with *Solution A*, concomitantly determine the sodium flame emission readings for the *Standard solution* and the *Sample solution* at the wavelength of maximum emission at 589 nm.
Calculate the quantity, in g, of sodium (Na) in 1000 mL of Solution taken:

$$\text{Result} = (r_U/r_S) \times (A_r/M_r) \times W \times F$$

r_U = sodium emission readings from the *Sample solution*
r_S = sodium emission readings from the *Standard solution*
A_r = atomic weight of sodium, 22.99
M_r = molecular weight of sodium chloride, 58.44
W = weight of sodium chloride taken to make the *Standard solution*, 8.18 g
F = conversion factor, 2

Acceptance criteria: Each 1000 mL of Solution should contain 6.21 g–6.86 g of sodium.

• **DEXTROSE**
Sample: 5 mL of Solution
Analysis: Tare a clean, medium-porosity filtering crucible containing several carborundum boiling chips or glass beads. Pipet 50 mL of freshly mixed alkaline cupric tartrate TS into a 400-mL beaker. Add the boiling chips or glass beads from the tared crucible, 45 mL of water, and 5.0 mL of Solution to the beaker. Heat the beaker and contents over a burner that has been adjusted to cause boiling of the solution to start in 3.5–4 min. Boil the solution for 2 min, accurately timed, and filter immediately through the tared crucible, taking care to transfer all of the boiling chips or glass beads to the crucible. Wash the precipitate with hot water and 10 mL of alcohol. Dry the crucible and contents at 110° to constant weight. Perform a blank determination, and make any necessary correction.
Each mg of cuprous oxide precipitate obtained is equivalent to 0.496 mg of dextrose ($C_6H_{12}O_6 \cdot H_2O$).
Acceptance criteria: Each 1000 mL of Solution should contain 30.30–33.50 g of dextrose ($C_6H_{12}O_6 \cdot H_2O$).

• **ADENINE**
Mobile phase: Dissolve 3.45 g of ammonium dihydrogen phosphate in 950 mL of water, add 10 mL of glacial acetic acid, dilute with water to 1000 mL, and mix. Pass the solution through a membrane filter with a 1-µm or finer pore size, and degas.
System suitability solution: 0.275 mg/mL of each USP Adenine RS and purine in dilute hydrochloric acid (1 in 120)
Standard solutions: Place quantities of USP Adenine RS in dilute hydrochloric acid (1 in 120) in three separate volumetric flasks, and dilute with the dilute hydrochloric acid solution to volume to obtain *Standard solutions* having known concentrations of 0.25, 0.275, and 0.30 mg of adenine per mL, respectively. Protect from light.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm stainless steel; packing L9
Flow rate: 2.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution* (NLT four injections)
Suitability requirements
Resolution: NLT 3.0 between adenine and purine
Relative standard deviation: NMT 2.5% for adenine peak and NMT 2.0% for the retention time of adenine peak
Analysis
Samples: *Standard solutions* and Solution
Plot the responses against the concentrations, in mg, of USP Adenine RS per mL of the *Standard solutions*.
Calculate the quantity, in mg, of adenine ($C_5H_5N_5$) in each mL of the Solution taken as the value read directly from the Standard curve corresponding to the response obtained from the portion of the Solution chromatographed.
Acceptance criteria: Each 1000 mL of Solution should contain 0.247–0.303 g of adenine ($C_5H_5N_5$).

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩**:** A 10-mL portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.0035%).

SPECIFIC TESTS

• **PH** ⟨791⟩**:** 5.0–6.0
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 5.56 USP Endotoxin Units/mL.
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

www.webofpharma.com

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, of colorless, transparent, Type I or Type II glass, or of a suitable plastic material (see ▲*Medical Devices—Bacterial Endotoxin and Pyrogen Tests* ⟨161⟩).▲ (CN 1-May-2019)
- **LABELING:** Label to indicate the number of mL of solution required per 100 mL of whole blood or the number of mL of solution required per volume of whole blood to be collected.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Adenine RS
  USP Citric Acid RS

Anticoagulant Sodium Citrate Solution

DEFINITION

Anticoagulant Sodium Citrate Solution is a sterile solution of Sodium Citrate in Water for Injection. It contains, in each 100 mL, NLT 3.80 g and NMT 4.20 g of sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$). It contains no antimicrobial agents.

Prepare Anticoagulant Sodium Citrate Solution as follows.

Sodium Citrate (dihydrate)	40 g
Water for Injection, a sufficient quantity to make	1000 mL

[NOTE—Anhydrous sodium citrate (35.1 g) may be used instead of the dihydrate.]

Dissolve the *Sodium Citrate* in sufficient *Water for Injection* to make 1000 mL, and filter until clear. Place the solution in suitable containers, and sterilize.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Citrate* ⟨191⟩**:** Meets the requirements when evaporated to a concentration of 1 in 20
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩**:** Meets the requirements when evaporated to a concentration of 1 in 20

ASSAY

- **PROCEDURE**

Mobile phase, Standard preparation 1, and Chromatographic system: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Sample solution: Pipet 10 mL of Solution into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Sample solution (for the assay of citric acid/citrate).*

Analysis

Samples: *Standard preparation 1* and *Sample solution*

Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure.*

Calculate the quantity, in g, of sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) in the volume of Solution taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times D \times F$$

r_U = peak area of citrate from the *Sample solution*
r_S = peak area of citrate from *Standard preparation 1*
C_S = concentration of citrate in *Standard preparation 1* (µg/mL)
M_{r1} = molecular weight of sodium citrate dihydrate, 294.10
M_{r2} = molecular weight of citrate ($C_6H_5O_7$), 189.10
D = dilution factor
F = conversion factor, 0.000001 g/µg

Acceptance criteria: In each 100 mL, 3.80–4.20 g of sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$)

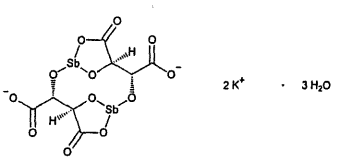
SPECIFIC TESTS

- **pH** ⟨791⟩**:** 6.4–7.5
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Contains NMT 5.56 USP Endotoxin Units/mL
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I or Type II glass.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Citric Acid RS

Antimony Potassium Tartrate



$C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$ 667.87

$C_8H_4K_2O_{12}Sb_2$ 613.82

Antimonate(2-), bis[µ-[2,3-dihydroxybutanedioato(4-)-$O^1,O^2:O^3,O^4$]]-di-, dipotassium, trihydrate, stereoisomer;
Dipotassium bis[µ-[L-(+)-tartrato(4-)]]diantimonate(2-) trihydrate [28300-74-5].
Anhydrous [11071-15-1].

DEFINITION

Antimony Potassium Tartrate contains NLT 99.0% and NMT 103.0% of antimony potassium tartrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$).

IDENTIFICATION

- **A.**

Sample: An appropriate quantity

Analysis: Heat the *Sample* to redness.

Acceptance criteria: It chars, emits an odor resembling that of burning sugar, and leaves a blackened residue. This residue has an alkaline reaction, and when a small fragment of it is held in a nonluminous flame, the flame is tinted violet.

- **B.**

Sample solution: Antimony Potassium Tartrate (1 in 20) in water acidified with hydrochloric acid

Analysis: Add hydrogen sulfide TS to the *Sample solution.*

Acceptance criteria: It yields an orange-red precipitate, which is soluble in ammonium sulfide TS and in 1 N sodium hydroxide.

- **C. IDENTIFICATION TESTS—GENERAL,** *Tartrate* ⟨191⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Sample: 500 mg

Analysis: Dissolve the *Sample* in 50 mL of water, add 5 g of potassium sodium tartrate, 2 g of sodium borate, and 3 mL of starch TS, and immediately titrate with 0.1 N iodine VS to the production of a persistent blue color. Each mL of 0.1

www.webofpharma.com

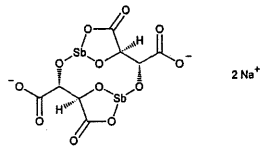
N iodine is equivalent to 16.70 mg of antimony potassium tartrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$).
Acceptance criteria: 99.0%–103.0%

IMPURITIES
- **ARSENIC**
  Sample solution: Dissolve 100 mg in 5 mL of hydrochloric acid. Add 10 mL of a recently prepared solution of 20 g of stannous chloride in 30 mL of hydrochloric acid.
  Blank: Add 5 mL of hydrochloric acid to 10 mL of a recently prepared solution of 20 g of stannous chloride in 30 mL of hydrochloric acid.
  Analysis: Transfer the *Sample solution* to a color-comparison tube, and allow to stand for 30 min.
  Acceptance criteria: NMT 0.015%; viewed downward over a white surface, the color of the solution appears no deeper than that of the *Blank* to which has been added 15 µg of arsenic.
- **LEAD** ⟨251⟩: NMT 20 ppm

SPECIFIC TESTS
- **COMPLETENESS OF SOLUTION** ⟨641⟩
  Sample: 750 mg
  Solvent: Water
  Acceptance criteria: Meets the requirements
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° to constant weight.
  Acceptance criteria: NMT 2.7%
- **ACIDITY OR ALKALINITY**
  Sample solution: Dissolve 1.0 g in 50 mL of carbon dioxide-free water.
  Analysis: Titrate the *Sample solution* with 0.010 N hydrochloric acid or 0.010 N sodium hydroxide to a pH of 4.5.
  Acceptance criteria: NMT 2.0 mL

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Antimony Sodium Tartrate



$C_8H_4Na_2O_{12}Sb_2$ 581.61
Antimonate(2-), bis[µ-[2,3-dihydroxybutanedioato(4-)-O^1,O^2:O^3,O^4]]di-, disodium, stereoisomer;
Disodium bis[µ-[L-(+)-tartrato(4-)]]diantimonate(2-) [34521-09-0].

DEFINITION
Antimony Sodium Tartrate contains NLT 98.0% and NMT 101.0% of antimony sodium tartrate ($C_8H_4Na_2O_{12}Sb_2$), calculated on the dried basis.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Antimony* ⟨191⟩, *Sodium* ⟨191⟩, and *Tartrate* ⟨191⟩

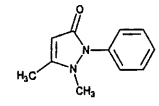
ASSAY
- **PROCEDURE**
  Sample: 500 mg
  Analysis: Dissolve the *Sample* in 50 mL of water, add 5 g of potassium sodium tartrate, 2 g of sodium borate, and 3 mL of starch TS, and immediately titrate with 0.1 N iodine VS to the production of a persistent blue color. Each mL of 0.1 N iodine is equivalent to 14.54 mg of antimony sodium tartrate ($C_8H_4Na_2O_{12}Sb_2$).
  Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES
- **ARSENIC,** *Method II* ⟨211⟩: NMT 8 ppm
- **LEAD** ⟨251⟩: NMT 20 ppm

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° to constant weight.
  Acceptance criteria: NMT 6.0%
- **ACIDITY OR ALKALINITY**
  Sample solution: Dissolve 1.0 g in 50 mL of carbon dioxide-free water.
  Analysis: Titrate the *Sample solution* with 0.010 N hydrochloric acid or 0.010 N sodium hydroxide to a pH of 4.5.
  Acceptance criteria: NMT 2.0 mL

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Antipyrine



$C_{11}H_{12}N_2O$ 188.23
1,2-Dihydro-1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one;
2,3-Dimethyl-1-phenyl-3-pyrazolin-5-one [60-80-0].

DEFINITION
Antipyrine contains NLT 98.0% and NMT 102.0% of antipyrine ($C_{11}H_{12}N_2O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 0.77 g/L of ammonium acetate in water. Adjust with diluted ammonium hydroxide to a pH of 7.0 and pass through a filter of 0.2-µm pore size.
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
1.0	75	25
5.0	20	80
5.01	75	25

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
8.0	75	25

System suitability solution: 0.12 mg/mL of USP Antipyrine RS and 0.12 µg/mL of USP Antipyrine Related Compound A RS in water
Standard solution: 0.12 mg/mL of USP Antipyrine RS in water
Sample solution: 0.12 mg/mL of Antipyrine in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 2.1-mm × 10-cm; 1.8-µm packing L1
Column temperature: 35°
Flow rate: 0.4 mL/min
Injection volume: 1 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between antipyrine and antipyrine related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of antipyrine ($C_{11}H_{12}N_2O$) in the portion of Antipyrine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Antipyrine RS in the *Standard solution* (mg/mL)
C_U = concentration of Antipyrine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.15%
• **Organic Impurities**
Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 1 L of water, add 2 mL of triethylamine, and adjust with 5 N sodium hydroxide solution to a pH of 7.0.
Mobile phase: Methanol and *Buffer* (43:100)
System suitability solution: 5 µg/mL each of USP Antipyrine RS and USP Antipyrine Related Compound A RS in *Mobile phase*
Standard solution: 0.5 µg/mL of USP Antipyrine RS and 0.25 µg/mL of USP Antipyrine Related Compound A RS in *Mobile phase*
Sample solution: 500 µg/mL of Antipyrine in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 6.0-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 3 times the retention time of antipyrine
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between antipyrine related compound A and antipyrine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of antipyrine related compound A in the portion of Antipyrine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of antipyrine related compound A from the *Sample solution*
r_S = peak response of antipyrine related compound A from the *Standard solution*
C_S = concentration of USP Antipyrine Related Compound A RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Antipyrine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*
r_S = peak response of antipyrine from the *Standard solution*
C_S = concentration of USP Antipyrine RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Antipyrine related compound A	0.8	0.05
Antipyrine	1.0	—
Individual unspecified impurity	—	0.05
Total impurities	—	0.1

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩
Analysis: Dry at 60° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Antipyrine RS
USP Antipyrine Related Compound A RS
3-Methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one.
$C_{10}H_{10}N_2O$ 174.20

Antipyrine and Benzocaine Otic Solution

DEFINITION

Antipyrine and Benzocaine Otic Solution is a solution of Antipyrine and Benzocaine in Glycerin. It contains NLT

www.webofpharma.com

90.0% and NMT 110.0% of the labeled amount of antipyrine ($C_{11}H_{12}N_2O$) and benzocaine ($C_9H_{11}NO_2$).

[NOTE—In the preparation of this Otic Solution, use Glycerin that has a low water content, in order that the Otic Solution may comply with the limit for *Water Determination*. This may be ensured by using Glycerin having a specific gravity of NLT 1.2607, corresponding to a concentration of 99.5%.]

IDENTIFICATION

- **A.** The UV spectrum of the antipyrine and benzocaine peaks of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 50 mM monobasic potassium phosphate and 0.2% triethylamine. Adjust with 10 N sodium hydroxide to a pH of 7.0.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
20	50	50
23	50	50
23.1	85	15
25	85	15

Diluent: Acetonitrile and water (1:1)

Standard solution: 0.014 mg/mL of USP Benzocaine RS and 0.054 mg/mL of USP Antipyrine RS in *Diluent*

Sample solution: Nominally 0.014 mg/mL of benzocaine and 0.054 mg/mL of antipyrine prepared as follows. Transfer a suitable quantity of Otic Solution to a suitable volumetric flask. Dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for the relative retention times for antipyrine and benzocaine.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of antipyrine ($C_{11}H_{12}N_2O$) and benzocaine ($C_9H_{11}NO_2$) in the portion of Otic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of antipyrine or benzocaine from the *Sample solution*

r_S = peak response of antipyrine or benzocaine from the *Standard solution*

C_S = concentration of USP Antipyrine RS or USP Benzocaine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of antipyrine or benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 0.1 mg/mL of USP Antipyrine RS and 0.01 mg/mL of USP Antipyrine Related Compound A RS in *Diluent*

Standard solution: 0.001 mg/mL each of USP Benzocaine RS and USP Ethyl 4-Nitrobenzoate RS, and 0.004 mg/mL of USP Antipyrine RS in *Diluent*

Sample solution: Nominally 3.2 mg/mL of antipyrine and 0.8 mg/mL of benzocaine prepared as follows. Transfer a suitable quantity of Otic Solution to a suitable volumetric flask. Dilute with *Diluent* to volume.

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between antipyrine related compound A and antipyrine, *System suitability solution*

Relative standard deviation: NMT 5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ethyl 4-nitrobenzoate in the portion of Otic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ethyl 4-nitrobenzoate from the *Sample solution*

r_S = peak response of ethyl 4-nitrobenzoate from the *Standard solution*

C_S = concentration of USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any individual, unspecified degradation product in the portion of Otic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual, unspecified degradation product from the *Sample solution*

r_S = peak response of benzocaine from the *Standard solution*

C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Antipyrine related compound A[a]	0.46	—

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Antipyrine	0.53	—
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate[b]	1.6	0.2
Any individual, unspecified degradation product	—	0.2
Total degradation products[a]	—	2.0

[a] Process impurities are controlled in the drug substance and are not to be reported here. They are not included in the calculation of total impurities.
[b] Specified impurity related to benzocaine.

• **LIMIT OF AMINOBENZOIC ACID**
Solution A: Glacial acetic acid and water (1:69)
Solution B: Methanol
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	90	10
10.1	15	85
13	15	85
13.1	90	10
16	90	10

Diluent: *Solution A* and *Solution B* (9:1)
Standard solution: 0.001 mg/mL of USP Aminobenzoic Acid RS in *Diluent*
Sample solution: Nominally 3.2 mg/mL of antipyrine and 0.8 mg/mL of benzocaine prepared as follows. Transfer a suitable quantity of Otic Solution to a suitable volumetric flask. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.0-mm × 15-cm; 3.5-µm packing L11
Flow rate: 0.4 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminobenzoic acid in the portion of Otic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aminobenzoic acid from the *Sample solution*
r_S = peak response of aminobenzoic acid from the *Standard solution*
C_S = concentration of USP Aminobenzoic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2%

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Aminobenzoic Acid RS
p-Aminobenzoic acid.
$C_7H_7NO_2$ 137.14
USP Antipyrine RS
USP Antipyrine Related Compound A RS
3-Methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one.
$C_{10}H_{10}N_2O$ 174.20
USP Benzocaine RS
USP Ethyl 4-Nitrobenzoate RS
Ethyl *p*-nitrobenzoate.
$C_9H_9NO_4$ 195.17

Antipyrine, Benzocaine, and Phenylephrine Hydrochloride Otic Solution

» Antipyrine, Benzocaine, and Phenylephrine Hydrochloride Otic Solution is a solution of Antipyrine, Benzocaine, and Phenylephrine Hydrochloride in a suitable nonaqueous solvent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of antipyrine ($C_{11}H_{12}N_2O$), benzocaine ($C_9H_{11}NO_2$), and phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Antipyrine RS
USP Benzocaine RS
USP Phenylephrine Hydrochloride RS
Identification—The retention times of the major peaks in the chromatograms of the *Assay preparations* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Assay—
Mobile phase—Mix 480 mL of acetonitrile, 3520 mL of a 0.005 M solution of sodium 1-heptanesulfonate in water, and 4 mL of phosphoric acid.
Standard preparation—Accurately weigh about 25 mg of USP Antipyrine RS, about 25 mg of USP Benzocaine RS, and about 25 mg of USP Phenylephrine Hydrochloride RS into a 250-mL volumetric flask. Add 5 mL of a 0.5 mg per mL solution of *p*-aminobenzoic acid in *Mobile phase*. Add 150 mL of *Mobile phase*, and mix to effect solution, sonicating if necessary. Dilute with *Mobile phase* to volume, and mix.
Assay preparation A—Transfer an accurately measured volume of Otic Solution, equivalent to about 100 mg of antipyrine, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

www.webofpharma.com

Assay preparation B—Transfer an accurately measured volume of Otic Solution, equivalent to about 100 mg of benzocaine, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation P—Transfer an accurately measured volume of Otic Solution, equivalent to about 5 mg of phenylephrine hydrochloride, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 272-nm detector and a 4.6-mm × 30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.19 for *p*-aminobenzoic acid, 0.26 for phenylephrine, 0.64 for antipyrine, and 1.0 for benzocaine; the resolution, *R*, between phenylephrine and aminobenzoic acid is not less than 1.5, and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 20 or 25 µL) of the *Standard preparation* and each of the *Assay preparations* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of antipyrine ($C_{11}H_{12}N_2O$) in each mL of the Otic Solution taken by the formula:

$$(C/V)(r_U/r_S)$$

in which C is the concentration, in µg per mL of USP Antipyrine RS in the *Standard preparation*; V is the volume, in mL, of Otic Solution taken; and r_U and r_S are the antipyrine peak responses obtained from *Assay preparation A* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of benzocaine ($C_9H_{11}NO_2$) in each mL of the Otic Solution taken by the formula:

$$(C/V)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Benzocaine RS in the *Standard preparation*; V is the volume, in mL, of Otic Solution taken; and r_U and r_S are the benzocaine peak responses obtained from *Assay preparation B* and the *Standard preparation*, respectively. Calculate the quantity, in µg of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) in each mL of the Otic Solution taken by the formula:

$$50(C/V)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Phenylephrine Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Otic Solution taken; and r_U and r_S are the phenylephrine peak responses obtained from *Assay preparation P* and the *Standard preparation*, respectively.

Antithrombin III Human

[9000-94-6].

DEFINITION

Antithrombin III Human is a glycoprotein, which is the major inhibitor of thrombin and other activated clotting factors, including factors IX, X, XI, and XII. It is obtained from human plasma of healthy donors who have been tested and shown to be free from detectable agents of infection transmissible by transfusion of blood or blood derivatives. The manufacturing steps are shown to remove or inactivate known agents of infection. If substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to an acceptable level and that any residues are such as not to compromise the safety of the preparation for patients. When reconstituted in the recommended volume of diluent, the potency is NLT 25 International Units (IU)/mL. It contains 80%–120% of the potency stated on the label.

IDENTIFICATION

• **A.** Meets the requirements of the *Assay*

ASSAY

• **PROCEDURE**

Solution A: Dissolve tris(hydroxymethyl)aminomethane, edetic acid, and sodium chloride in water to obtain a solution having concentrations of 0.050, 0.0075, and 0.175 M, respectively. Adjust with hydrochloric acid or sodium hydroxide solution to a pH of 8.4.

Solution B: 0.05% (w/v) of albumin human in *Solution A*

Solution C: 10 mg/mL of polybrene in *Solution B*

Solution D: Reconstitute thrombin bovine (factor IIa), and dilute with *Solution B* to obtain a solution having a concentration of 4 Thrombin IU/mL.

Solution E: Prepare a solution of chromogenic substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for thrombin (factor IIa) in *Solution C* to obtain a solution having a concentration of about 40.0 mM.

Solution F: Resuspend USP Heparin Sodium for Assays RS according to the USP Certificate and dilute to 3 USP Heparin Units/mL in *Solution A*.

Standard solutions: Prepare seven dilutions from USP Antithrombin III Human RS within the linear range of the assay in *Solution F* (for example, 1.7, 1.5, 1.2, 1.0, 0.8, 0.6, and 0.4 IU/mL).

Sample solutions: Prepare three or more dilutions in *Solution F* within the linear range of the assay.

Blank: *Solution A*

Analysis: [NOTE—The procedure also can be performed using alternative platforms.]

For each dilution of the *Standard solution* and *Sample solution*, at least duplicates should be tested. Label a suitable number of tubes depending on the number of replicates that will be tested. For example, if five blanks will be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, and T3 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, S4, S5, S6, and S7 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, S5, S6, S7, B2, T1, T2, T3, B3, T1, T2, T3, B4, S1, S2, S3, S4, S5, S6, S7, B5.]

Prewarm *Solution D* and *Solution E* at 37°. Pipet 50 µL each of the *Standard solutions*, *Sample solutions*, and *Blank* into suitable tubes placed in a water bath set at 37°. Add 350 µL of prewarmed *Solution D* to each tube, mix, and incubate for 1 min. Add 100 µL of prewarmed *Solution E* to each tube in the same order and mix. Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩). Calculate the change in absorbance/min. Plot standard concentrations against resulting absorbance values and determine potency by interpolating from the standard curve using mean sample absorbances.

USP Monographs

www.webofpharma.com

System suitability
Samples: *Standard solutions* and *Sample solutions*
The R^2 value of the standard curve is NLT 0.99. The initial and final blanks differ by NMT 10%. The absorbances of the three dilutions of the *Sample solution* must fall within the range of absorbances of the standard curve. The three dilutions of the *Sample solution* give potency estimates that differ by NMT 10%.
Acceptance criteria: 80%–120% of the potency stated on the label.

IMPURITIES

• **HEPARIN CONTENT**
Solution A: 9 g/L of sodium chloride
Sheep plasma substrate: Use sheep plasma suitable for the test procedure. If frozen, thaw at 37°.
APTT reagent: Use a suitable activated partial thromboplastin time (APTT) reagent containing phospholipid and a contact activator at a dilution giving a suitable blank recalcification time not exceeding 60 s.
Calcium chloride solution: 3.7 g/L of calcium chloride
System suitability solution: 5 IU/mL of heparin sodium for assays in USP Antithrombin III Human RS
Standard solutions: Make three or more dilutions of USP Heparin Sodium for Assays RS to known concentrations in USP Heparin Units/mL that are in the expected range of the sample (for example, 0.5–1.5 USP Heparin Units/mL).
Sample solutions: Make three or more dilutions of Antithrombin III Human in the range of the *Standard solution* dilutions.
Blank: *Solution A*
Analysis: [NOTE—The procedure also can be performed using alternative platforms.]
For each *System suitability solution*, *Standard solution*, and *Sample solution*, at least duplicates should be tested. Label a suitable number of tubes depending on the number of replicates that will be tested. For example, if five blanks will be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, and T3 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, and S3 each at least in duplicate for the dilutions of the *Standard solutions* and SS for the *System suitability solution*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, B2, SS, T1, T2, T3, B3, T1, T2, T3, SS, B4, S1, S2, S3, B5.] In the following order add 1.0 mL of thawed *Sheep plasma substrate* to 1.0 mL of the *Standard solution* dilutions or the *Sample solution* dilutions or the *System suitability solution*. After each addition, mix but do not allow bubbles to form. Transfer each tube to a water bath at 37°, allow to equilibrate at 37° for about 15 min, and add to each tube 1 mL of *APTT reagent* previously heated to 37°. After an appropriate time for the *APTT reagent* used, usually 2–5 min, add 1 mL of *Calcium chloride solution* previously heated to 37° and determine the clotting time. Plot standard concentrations against resulting clotting times and determine heparin content by interpolating from the standard curve using mean sample clotting times. For samples with clotting times longer than the lowest standard dilution, report the result as NMT the lowest *Standard solution* concentration.
System suitability
Samples: *Standard solutions* and *Sample solutions*
The R^2 value of the standard curve is NLT 0.99. The three dilutions of the *Sample solution* give heparin content estimates that differ by NMT 10%. The heparin content in the *System suitability solution* is in the range of 4.0–7.5 IU/mL.
Acceptance criteria: NMT 0.1 USP Heparin Unit/Antithrombin III IU

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*: Meets the requirements
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 3.0%
• **PYROGEN TEST** ⟨151⟩: Inject 50 USP Antithrombin III Units per kg of the rabbit's weight, calculated from the activity stated on the label: meets the requirements.
• **GENERAL SAFETY:** Meets the requirements for biologics in *Biological Reactivity Tests, In Vivo* ⟨88⟩, *Safety Tests—Biologicals*
• **OSMOLALITY AND OSMOLARITY** ⟨785⟩, *Osmolality*: Reconstitute with the diluent according to the manufacturer's instruction: NLT 240 mOsmol/kg for the solution.
• **pH** ⟨791⟩: Reconstitute with the diluent according to the manufacturer's instruction: 6.0–7.5.
• **MOLECULAR WEIGHT DISTRIBUTION**
Mobile phase: 0.05 M sodium phosphate (dibasic), 0.05 M sodium phosphate (monobasic), 0.4 M arginine monohydrochloride, and 0.05% sodium azide. Adjust with 1 N sodium hydroxide to a pH of 6. Degas and filter.
Solution A: 4–5 mg/mL of thyroglobulin in *Mobile phase*
Sample solution: 8–10 mg/mL of Antithrombin III Human
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Columns
Guard: 7.5 mm × 7.5 cm guard column containing packing L59
Analytical: 7.5 mm × 30 cm analytical column containing packing L59
Temperatures
Autosampler: 7°
Column: Ambient
Flow rate: 0.5 mL/min maintained constant to ±1%
Injection volume: 20 µL
System suitability
Sample: *Sample solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: 0.9–1.3
Analysis
Samples: *Solution A* and *Sample solution*
Acceptance criteria: Note the retention times of the major peak in the *Solution A* chromatogram. The relative peak area of the high-molecular weight peak eluting at about the same retention time as the major peak in the *Solution A* chromatogram, or earlier, is NMT 13%.
• **TOTAL PROTEIN CONTENT**
Solution A: 1000 mg/mL of trichloroacetic acid in water
Sample solution: 7.5 mg/mL of Antithrombin III Human in 0.15 M sodium chloride solution
Blank: 0.15 M solution of sodium chloride
Analysis: To each of 2.0 mL of the *Sample solution* and the *Blank* in suitable centrifuge tubes, add 1.5 mL of *Solution A*. Mix, allow to stand for at least 10 min, centrifuge for 5 min, and decant the supernatant. Resuspend the precipitates in 1.5 mL of *Solution A*, centrifuge for 5 min, decant the supernatant, and hold the tubes inverted on a filter paper to drain. Quantitatively transfer the residues with a minimum quantity of water to a micro-Kjeldahl flask, and determine the nitrogen content (see *Nitrogen Determination* ⟨461⟩, *Method II*). Multiply the result, corrected for the *Blank*, by 6.25 to calculate the quantity of protein.

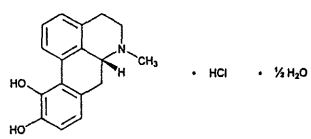
Stimuli Articles

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Use a Type I glass container with an appropriate stopper and seal. Store protected from light between 2° and 8°, excursions permitted up to 25°.
- **LABELING:** The labeling should state the content of antithrombin III in USP Antithrombin III Units. The diluent and the volume to be used to reconstitute the preparation are indicated.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Antithrombin III Human RS
  USP Heparin Sodium for Assays RS

Apomorphine Hydrochloride



$C_{17}H_{17}NO_2 \cdot HCl \cdot \frac{1}{2}H_2O$ 312.79

$C_{17}H_{17}NO_2 \cdot HCl$ 303.79

4*H*-Dibenzo[*de,g*]quinoline-10,11-diol, 5,6,6a,7-tetrahydro-6-methyl-, hydrochloride, hemihydrate, (*R*)-;
6aβ-Aporphine-10,11-diol hydrochloride hemihydrate [41372-20-7].
Anhydrous [314-19-2].

DEFINITION

Apomorphine Hydrochloride contains NLT 98.5% and NMT 101.5% of apomorphine hydrochloride ($C_{17}H_{17}NO_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
  Sample solution: 10 mg/mL of Apomorphine Hydrochloride in carbon dioxide-free water
  Analysis: To 2 mL of the *Sample solution* add 0.1 mL of nitric acid. Mix, filter, and use the filtrate.
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
  Sample solution: Dissolve 250 mg of Apomorphine Hydrochloride in a mixture of 5.0 mL of 0.01 N hydrochloric acid and 50 mL of alcohol.
  Analysis: Titrate the *Sample solution* with 0.1 N sodium hydroxide VS. Read the volume added between the first two points of inflexion. Each mL of 0.1 N sodium hydroxide is equivalent to 30.38 mg of apomorphine hydrochloride ($C_{17}H_{17}NO_2 \cdot HCl$).
  Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Diluent: Glacial acetic acid and water (1:99)
  Solution A: 1.1-g/L solution of sodium octanesulfonate, adjusted with diluted phosphoric acid (1:1) to a pH of 2.2
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
2	85	15
32	68	32
37	68	32

Return to original conditions and re-equilibrate the system.
System suitability solution: 0.25 mg/mL each of USP Apomorphine Hydrochloride RS and boldine in *Diluent*. [NOTE—Boldine is 2,9-dihydroxy-1,10-dimethoxyaporphine.]
Standard solution: 2.5 µg/mL of USP Apomorphine Hydrochloride RS in *Diluent*
Sensitivity solution: 0.14 µg/mL of USP Apomorphine Hydrochloride RS in *Diluent* from the *Standard solution*. [NOTE—The peak response of this solution is equivalent to that of a solution containing 1.25 µg/mL of morphine hydrochloride, taking into account the relative response factor of this impurity (see *Table 2*).]
Sample solution: 2.5 mg/mL of Apomorphine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 5-µm end-capped packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Sensitivity solution* [NOTE—The typical relative retention times for boldine and apomorphine are about 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.5 between boldine and apomorphine, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Apomorphine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of apomorphine from the *Standard solution*
C_S = concentration of USP Apomorphine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Apomorphine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Morphine	0.4	0.11	0.15

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Apomorphine	1.0	—	—
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.5

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL in *Diluent*
  Diluent: 2.06-g/L solution of hydrochloric acid in water
  Acceptance criteria: –48° to –52°, determined at 20°
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry a sample at 105° for 2 h.
  Acceptance criteria: 2.0%–4.2%
- **COLOR OF SOLUTION**
  Sample solution: Place 100 mg of Apomorphine Hydrochloride in a suitable test tube, add 10 mL of cold, oxygen-free water, and agitate gently until dissolved.
  Standard solution: Dissolve 5 mg of Apomorphine Hydrochloride in 100.0 mL of water. Transfer 1.0 mL of this solution to a test tube of the same size as that used for the *Sample solution*. Dilute with 6 mL of water, add 1 mL of a 50-mg/mL sodium bicarbonate solution, and then add 0.50 mL of iodine TS. Allow to stand for 30 s, add 0.60 mL of a 25-mg/mL sodium thiosulfate solution, and dilute with water to 10 mL.
  Acceptance criteria: The color of the *Sample solution*, observed promptly after the Apomorphine Hydrochloride has dissolved, is not more intense than that of a color of the *Standard solution*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Apomorphine Hydrochloride RS

Delete the following:

▲Apomorphine Hydrochloride Tablets

» Apomorphine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{17}NO_2 \cdot HCl \cdot \frac{1}{2}H_2O$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨ 11 ⟩ —
USP Apomorphine Hydrochloride RS

Color of solution—Dissolve a quantity of powdered Tablets, equivalent to 5 mg of apomorphine hydrochloride, in water to make 100.0 mL. Transfer 1.0 mL of the solution to a test tube, dilute with 6 mL of water, and, if necessary, filter through a small pledget of cotton. Add 1 mL of sodium bicarbonate solution (1 in 20), then add 0.50 mL of iodine TS. Allow to stand for 30 seconds, then add 0.60 mL of sodium thiosulfate solution (1 in 40), and dilute with water to 10 mL. This solution represents the color standard.

Place a quantity of powdered Tablets, equivalent to about 50 mg of apomorphine hydrochloride, in a test tube of suitably small size, add 10.0 mL of cold, oxygen-free water, insert the stopper in the test tube, and agitate gently until no more dissolves; if necessary, filter immediately through a small pledget of cotton. The color of the solution, observed promptly after preparation, is not more intense than that of the color standard. Use closely matched test tubes for the comparison.

Identification—To 5 mL of a filtered solution of Tablets, containing about 10 mg of apomorphine hydrochloride, add a slight excess of sodium bicarbonate solution (1 in 20): a white or greenish-white precipitate is formed. Add 3 drops of iodine TS, and shake vigorously: an emerald-green color is produced. Add 5 mL of ether, and, after vigorous shaking, allow the layers to separate: the ether is colored deep ruby-red while the water layer retains its green color.

Disintegration ⟨ 701 ⟩ : 15 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—Place 1 Tablet in a 500-mL volumetric flask containing 100 mL of 0.1 N hydrochloric acid, and shake for 15 minutes. Dilute with 0.1 N hydrochloric acid to volume, mix, and filter, discarding the first 20 mL of filtrate. Dilute a portion of the subsequent filtrate quantitatively and stepwise, if necessary, with 0.1 N hydrochloric acid to provide a solution containing approximately 12 µg of apomorphine hydrochloride per mL. Concomitantly determine the absorbances of this solution and of a solution of USP Apomorphine Hydrochloride RS in the same medium having a known concentration of about 12 µg of anhydrous apomorphine hydrochloride per mL, in 1-cm cells at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{17}H_{17}NO_2 \cdot HCl \cdot \frac{1}{2}H_2O$ in the Tablet taken by the formula:

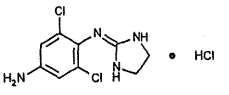
$$(312.80/303.79)(TC/D)(A_U/A_S)$$

in which 312.80 and 303.79 are the molecular weights of apomorphine hydrochloride hemihydrate and anhydrous apomorphine hydrochloride, respectively; T is the labeled quantity, in mg, of apomorphine hydrochloride in the Tablet; C is the concentration, in µg per mL, of anhydrous apomorphine hydrochloride in the Standard solution; D is the concentration, in µg per mL, of apomorphine hydrochloride in the solution from the Tablet, based upon the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—Weigh and finely powder not fewer than 20 Tablets. Dissolve an accurately weighed portion of the powder, equivalent to about 50 mg of apomorphine hydrochloride, in 25 mL of water in a separator, add 500 mg of sodium bicarbonate, and completely extract with successive small portions of ether. Combine the ether extracts in a separator, and wash them with three 5-mL portions of water. Shake the combined water washings with 10 mL of ether, and add this ether to the combined ether extracts. Extract the ether solutions with 20.0 mL of 0.02 N sulfuric acid VS, and wash with three 5-mL portions of water. Combine the acid extract and washings in a beaker, and warm on a steam bath to expel any residual ether. Cool, add methyl red TS, and titrate the excess acid with 0.02 N sodium hydroxide VS (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 0.02 N sulfuric acid is equivalent to 6.256 mg of $C_{17}H_{17}NO_2 \cdot HCl \cdot \frac{1}{2}H_2O$. ▲ (USP 1-May-2020)

www.webofpharma.com

Apraclonidine Hydrochloride



$C_9H_{10}Cl_2N_4 \cdot HCl$ 281.57

1,4-Benzenediamine, 2,6-dichloro-*N*[1]-2-imidazolidinylidene-, monohydrochloride.

2-[(4-Amino-2,6-dichlorophenyl)imino]imidazolidine monohydrochloride [73218-79-8].

» Apraclonidine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_9H_{10}Cl_2N_4 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Apraclonidine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: It responds to the tests for *Chloride* ⟨191⟩.

pH ⟨791⟩: between 5.0 and 6.6 in a solution (1 in 100).

Loss on drying ⟨731⟩—Dry it in vacuum at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—

Phosphate buffer—Transfer 6.8 mL of phosphoric acid to a 2000-mL volumetric flask, add about 1900 mL of water, and mix. Adjust with sodium hydroxide solution (1 in 2) to a pH of 3.0, dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, *Phosphate buffer*, and methanol (56:40:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare a solution in *Mobile phase* containing about 0.8 mg of USP Apraclonidine Hydrochloride RS per mL.

Test solution—Transfer about 20 mg of Apraclonidine Hydrochloride, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and an 8-mm × 100-mm column that contains packing L7. The flow rate is about 3 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the tailing factor for the apraclonidine peak is not more than 2.2, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject about 20 µL of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for the major peaks. [NOTE—Allow about five times the elution time of apraclonidine before making the next injection.] Calculate the percentage of each peak, other than the solvent peak and the apraclonidine peak, in the specimen of Apraclonidine Hydrochloride taken by the same formula:

$$100 r_i / r_t$$

in which r_i is the response of each peak other than the principal peak, and r_t is the sum of the responses of all of the peaks, excluding that of the solvent peak: not more than 1.0% for any individual impurity and not more than 2.0% total impurities are found.

Assay—Dissolve about 125 mg of Apraclonidine Hydrochloride, accurately weighed, in 40 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically from the second inflection point, using a calomel-glass electrode system (see *Titrimetry* ⟨541⟩). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 14.08 mg of $C_9H_{10}Cl_2N_4 \cdot HCl$.

Apraclonidine Ophthalmic Solution

» Apraclonidine Ophthalmic Solution is a sterile, aqueous solution of Apraclonidine Hydrochloride. It contains an amount of apraclonidine hydrochloride ($C_9H_{10}Cl_2N_4 \cdot HCl$) equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of apraclonidine ($C_9H_{10}Cl_2N_4$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Apraclonidine Hydrochloride RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the major peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: Apply 2 µL of Apraclonidine Ophthalmic Solution and 2 µL of a Standard solution of USP Apraclonidine Hydrochloride RS in methanol containing about 11.5 mg per mL to a suitable high performance thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.2-mm layer of chromatographic silica gel mixture, or equivalent. Allow the applications to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (74:22:4) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light. [NOTE—The apraclonidine spot should appear as a blue spot.] Spray the plate with fluorescamine solution, prepared by dissolving about 25 mg of fluorescamine in 25 mL of acetone. [NOTE—Avoid prolonged or repeated breathing of the aerosol from the fluorescamine spray. Also avoid prolonged or repeated contact with skin. Fluorescamine solution should be sprayed only in a hood.] Examine the plate under normal light and long-wavelength UV light. [NOTE—The apraclonidine spot should appear as a yellow spot under normal light and as a white spot under long-wavelength UV light.] The R_F value and appearance of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

www.webofpharma.com

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 4.4 and 7.8.

Assay—

Phosphate buffer—Prepare as directed in the test for *Chromatographic purity*— under *Apraclonidine Hydrochloride.*

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer*, acetonitrile, and methanol (68:30:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Apraclonidine Hydrochloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a *Stock standard solution* having a known concentration of about 0.23 mg per mL. Transfer 2.5 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 11.5 µg of USP Apraclonidine Hydrochloride RS per mL (equivalent to about 10 µg of apraclonidine per mL).

Resolution solution—Transfer about 1 mL of propiophenone to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 3.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 1.0 mL of this solution and 5.0 mL of the *Stock standard solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 20 mg of apraclonidine, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.5 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

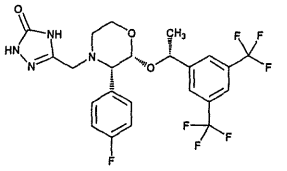
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and an 8-mm × 100-mm column that contains packing L7. The flow rate is about 3 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for apraclonidine and 1.0 for propiophenone; the column efficiency determined from the analyte peak is not less than 1000 theoretical plates; the tailing factor for the analyte peak is not more than 2.2; the resolution, *R*, between the analyte and propiophenone peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of apraclonidine ($C_9H_{10}Cl_2N_4$) in each mL of the Ophthalmic Solution taken by the formula:

$$(245.11/281.57)(2C/V)(r_U/r_S)$$

in which 245.11 and 281.57 are the molecular weights of apraclonidine and apraclonidine hydrochloride, respectively; C is the concentration, in µg per mL, of USP Apraclonidine Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Ophthalmic Solution taken; and r_U and r_S are the apraclonidine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Aprepitant



$C_{23}H_{21}F_7N_4O_3$ 534.43

3*H*-1,2,4-Triazol-3-one, 5-[[(2*R*,3*S*)-2-[(1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2-dihydro-;
3-[[(2*R*,3*S*)-3-(*p*-Fluorophenyl)-2-[[(α*R*)-α-methyl-3,5-bis(trifluoromethyl)benzyl]oxy]morpholino]methyl]-Δ²-1,2,4-triazolin-5-one;
3-[[(2*R*,3*S*)-2-[(*R*)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)morpholino]methyl]-1*H*-1,2,4-triazol-5(4*H*)-one;
5-[[(2*R*,3*S*)-2-[(*R*)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2-dihydro-3*H*-1,2,4-triazol-3-one [170729-80-3].

DEFINITION

Aprepitant contains NLT 98.0% and NMT 102.0% of aprepitant ($C_{23}H_{21}F_7N_4O_3$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Dilute phosphoric acid: Dilute 1 mL of phosphoric acid with water to 1 L.
Mobile phase: Acetonitrile and *Dilute phosphoric acid* (48:52)
Diluent: Acetonitrile and *Dilute phosphoric acid* (50:50)
Standard solution: 0.2 mg/mL of USP Aprepitant RS in *Diluent*; sonicate as needed
Sample solution: 0.2 mg/mL of Aprepitant in *Diluent*; sonicate as needed
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aprepitant ($C_{23}H_{21}F_7N_4O_3$) in the portion of Aprepitant taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%, on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Dilute phosphoric acid and **Diluent:** Proceed as directed in the *Assay*.
Solution A: *Dilute phosphoric acid*
Solution B: Acetonitrile
Mobile phase: See *Table 1*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	58	42
25	58	42
45	30	70
50	30	70

Return to original conditions and re-equilibrate the system.
System suitability solution: 2.0 mg/mL of USP Aprepitant RS and 0.003 mg/mL of USP Desfluoro Aprepitant RS in *Diluent*, using sonication as necessary to dissolve
Sensitivity solution: 0.001 mg/mL of USP Aprepitant RS in *Diluent*
Standard solution: 0.003 mg/mL of USP Aprepitant RS in *Diluent*, using sonication as necessary to dissolve
Sample solution: 2.0 mg/mL of Aprepitant in *Diluent*, using sonication as necessary to dissolve
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between the desfluoro aprepitant and aprepitant peaks, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Aprepitant taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for aprepitant from the *Standard solution*
C_S = concentration of USP Aprepitant RS in the *Standard solution* (mg/mL)
C_U = concentration of Aprepitant in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desfluoro aprepitant	0.85	0.15
Aprepitant	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.30

- **LIMIT OF *S,R,S*-ENANTIOMER** (if present)

[NOTE—Perform this test if this impurity is possible from the manufacturing process.]
Mobile phase: Hexane and dehydrated alcohol (90:10)
System suitability solution: 0.08 mg/mL of USP Aprepitant RS and 0.08 mg/mL of USP Aprepitant Related Compound B RS in *Mobile phase*
Sample solution: 0.5 mg/mL of Aprepitant in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L51
Column temperature: 30°
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: Greater than 2.0 between the enantiomer peaks. [NOTE—The elution order is the *S,R,S*-enantiomer followed by the aprepitant peak, which is the *R,S,R*-enantiomer.]
Analysis
Sample: *Sample solution*
Calculate the percentage of the *S,R,S*-enantiomer in the portion of Aprepitant taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of the *S,R,S*-enantiomer
r_T = sum of the peak responses of aprepitant and the *S,R,S*-enantiomer

Acceptance criteria: NMT 0.10% of the *S,R,S*-enantiomer

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method Ia* or *Ic* ⟨921⟩: NMT 0.5%
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL in methanol
Acceptance criteria: +66.0° to +71.0°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Aprepitant RS
USP Aprepitant Related Compound B RS
S,R,S-Enantiomer: 3-[[(2*S*,3*R*)-2-[(*S*)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl) morpholino]methyl]-1*H*-1,2,4-triazol-5(4*H*)-one.
$C_{23}H_{21}F_7N_4O_3$ 534.43

www.webofpharma.com

USP Desfluoro Aprepitant RS
5-[[(2*R*,3*S*)-2-[(*R*)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-phenylmorpholino]methyl]-2*H*-1,2,4-triazol-3(4*H*)-one.
$C_{23}H_{22}F_6N_4O_3$ 516.44

Aprepitant Capsules

DEFINITION
Aprepitant Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of aprepitant ($C_{23}H_{21}F_7N_4O_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Wavelength range: 200–400 nm
  Standard solution: 0.1 mg/mL of USP Aprepitant RS in methanol. Use sonication to dissolve.
  Sample solution: Transfer the contents of Capsules, equivalent to 100 mg of aprepitant, to a 100-mL volumetric flask, add about 75 mL of methanol, and sonicate for about 5 min with intermittent shaking. Cool, dilute with methanol to volume, further dilute with methanol to obtain a solution containing 0.1 mg/mL of aprepitant, and pass through a nylon filter of 0.45-µm pore size.
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Dilute phosphoric acid: Dilute 1 mL of phosphoric acid with water to 1 L.
  Mobile phase: Acetonitrile and *Dilute phosphoric acid* (45:55)
  Standard solution: 0.05 mg/mL of USP Aprepitant RS in *Mobile phase*. Use sonication as necessary to dissolve.
  Sample solution: Nominally 0.05 mg/mL of aprepitant in *Mobile phase*, prepared as follows. Mix the contents of NLT 20 Capsules, and transfer a portion of the contents, equivalent to 100 mg of aprepitant, to a 100-mL volumetric flask. Add about 75 mL of *Mobile phase* and sonicate for about 10 min with intermittent shaking. Cool, dilute to volume with *Mobile phase*, further dilute with *Mobile phase* to obtain a solution containing 0.05 mg/mL of aprepitant, and pass through a nylon filter of 0.45-µm pore size.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Column temperature: 40°
  Flow rate: 1.5 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*

  Calculate the percentage of the labeled amount of aprepitant ($C_{23}H_{21}F_7N_4O_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aprepitant RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aprepitant in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium: 2.2% sodium dodecyl sulfate in water; 900 mL
  Apparatus 2: 100 rpm, with sinkers. [NOTE—A suitable sinker is available from VanKel, www.chem.agilent.com, catalog number 12-3050. Proper placement of the Capsules is in the sinkers with the cap facing the fixed prong end.]
  Time: 20 min
  Dilute phosphoric acid: Dilute 1 mL of phosphoric acid with water to 1 L.
  Mobile phase: Acetonitrile and *Dilute phosphoric acid* (50:50)
  Standard solution: (*L*/900) mg/mL of USP Aprepitant RS in *Medium*, where *L* is the label claim in mg/Capsule. Dissolve first in a minimal amount of methanol (using NMT 2% of the final volume) prior to diluting with *Medium*.
  Sample solution: Pass a portion of the solution under test through a suitable filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 220 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L7
  Flow rate: 1.5 mL/min
  Injection volume: 50 µL for Capsules containing 40 mg/Capsule; 10 µL for all other strengths
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of aprepitant ($C_{23}H_{21}F_7N_4O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aprepitant RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

  Tolerances: NLT 80% (*Q*) of the labeled amount of aprepitant ($C_{23}H_{21}F_7N_4O_3$) is dissolved.
  Test 2: ▲If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.▲ (RB 1-May-2018)

www.webofpharma.com

Medium: 2.2% sodium dodecyl sulfate in water; 900 mL
Apparatus 2: 100 rpm, with wire helix sinkers or other suitable sinkers
Time: 30 min
Dilute phosphoric acid and **Mobile phase:** Proceed as directed in the *Assay*.
Standard solution: (*L*/900) mg/mL of USP Aprepitant RS in *Medium*, where *L* is the label claim in mg/Capsule. Dissolve first in a minimal amount of methanol (using NMT 2% of the final volume) prior to diluting with *Medium*.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system: Proceed as directed in the *Assay*, except use an autosampler temperature of 15°.
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aprepitant ($C_{23}H_{21}F_7N_4O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aprepitant RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (*Q*) of the labeled amount of aprepitant ($C_{23}H_{21}F_7N_4O_3$) is dissolved.
▲**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 2.2% sodium lauryl sulfate in water; 900 mL
Apparatus 2: 100 rpm, with suitable sinkers. Use peak vessels.
Time: 30 min
Dilute phosphoric acid: Prepare as directed in the *Assay*.
Mobile phase: *Dilute phosphoric acid* and acetonitrile (52:48)
Standard stock solution: 440 µg/mL of USP Aprepitant RS in *Mobile phase*. Sonication may be used to promote dissolution.
Standard solution: 44 µg/mL of USP Aprepitant RS from *Standard stock solution* in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.
Chromatographic system
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aprepitant ($C_{23}H_{21}F_7N_4O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aprepitant RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*
L = label claim (mg/Capsule)

Tolerances: NLT 75% (*Q*) of the labeled amount of aprepitant ($C_{23}H_{21}F_7N_4O_3$) is dissolved. ▲(RB 1-May-2018)
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Dilute phosphoric acid: Dilute 1 mL of phosphoric acid with water to 1 L.
Solution A: Acetonitrile and *Dilute phosphoric acid* (5:95)
Solution B: Acetonitrile and *Dilute phosphoric acid* (95:5)
Diluent: Acetonitrile and *Dilute phosphoric acid* (50:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
20	58	42
25	35	65
33	35	65

Return to original conditions and re-equilibrate the system for 10 min.
System suitability solution: 0.6 mg/mL of USP Aprepitant RS and 0.0012 mg/mL each of USP Desfluoro Aprepitant RS and USP Aprepitant Related Compound A RS in *Diluent*
Standard solution: 0.0012 mg/mL of USP Aprepitant RS in *Diluent*
Sample solution: Nominally 0.6 mg/mL of aprepitant, prepared as follows. Transfer the contents of Capsules, equivalent to 120 mg of aprepitant, to a 200-mL volumetric flask, add about 150 mL of *Diluent*, and sonicate for about 10 min with intermittent shaking. Cool, dilute with *Diluent* to volume, and pass through a nylon filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between the desfluoro aprepitant and aprepitant peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the percentage of any individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of aprepitant from the *Standard solution*
C_S = concentration of USP Aprepitant RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aprepitant in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desfluoro aprepitant	0.85	—[a]
Aprepitant	1.0	—
Aprepitant diastereomers (*R,R,R* and *R,S,S*)[b]	1.3	—[a]
Any other individual impurity	—	0.2
Total impurities	—	0.2

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.
[b] The diastereomers are not separated by this procedure and should be identified based on the retention time of aprepitant related compound A (*R,R,R*-diastereomer), which is a component of the *System suitability solution*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aprepitant RS
  USP Aprepitant Related Compound A RS
  R,R,R-Diastereomer: 3-[[(2*R*,3*R*)-2-[(*R*)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl) morpholino]methyl]-1*H*-1,2,4-triazol-5(4*H*)-one.
  $C_{23}H_{21}F_7N_4O_3$ 534.43
  USP Desfluoro Aprepitant RS
  5-[[(2*R*,3*S*)-2-[(*R*)-1-[3,5-Bis(trifluoromethyl)phenyl] ethoxy]-3-phenylmorpholino]methyl]-2*H*-1,2,4-triazol-3(4*H*)-one.
  $C_{23}H_{22}F_6N_4O_3$ 516.44

Aprotinin



$C_{284}H_{432}N_{84}O_{79}S_7$ 6511.44
Trypsin inhibitor, pancreatic basic;
L-Arginyl-L-prolyl-L-aspartyl-L-phenylalanyl-L-cysteinyl-L-leucyl-L-glutamyl-L-prolyl-L-prolyl-L-tyrosyl-L-threonylglycyl-L-prolyl-L-cysteinyl-L-lysyl-L-alanyl-L-arginyl-L-isoleucyl-L-isoleucyl-L-arginyl-L-tyrosyl-L-phenylalanyl-L-tyrosyl-L-asparaginyl-L-alanyl-L-lysyl-L-alanylglycyl-L-leucyl-L-cysteinyl-L-glutaminyl-L-threonyl-L-phenylalanyl-L-valyl-L-tyrosylglycylglycyl-L-cysteinyl-L-arginyl-L-alanyl-L-lysyl-L-arginyl-L-asparaginyl-L-asparaginyl-L-phenylalanyl-L-lysyl-L-seryl-L-alanyl-L-glutamyl-L-aspartyl-L-cysteinyl-L-methionyl-L-arginyl-L-threonyl-L-cysteinylglycylglycyl-L-alanine cyclic (5→55), (14→38), (30→51) tris(disulfide) [9087-70-1].

DEFINITION

Aprotinin is a polypeptide consisting of a chain of 58 amino acid residues, which inhibits stoichiometrically the activity of several proteolytic enzymes such as chymotrypsin, kallikrein, plasmin, and trypsin. Aprotinin is obtained from bovine tissues and purified by a suitable process, and is stored as a bulk solution or lyophilized powder. Its potency, calculated on the dried basis, is NLT 3 USP Aprotinin Units/mg. In addition, the method of manufacture is validated to result in NMT 0.2 µg of histamine per 3 USP Aprotinin Units using validated methods. The origin and sourcing of bovine material must be specified in compliance with FDA requirements. The manufacturing process is validated to demonstrate the clearance of potential infectious agents (i.e., viruses, TSE agents). One USP Aprotinin Unit is equivalent to 1800 Kallikrein Inhibition Units (K.I.U.).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
  Cupric chloride solution: 10 mg/mL of cupric chloride
  Sample solution: A solution of Aprotinin in water containing about 15 USP Aprotinin Units/mL
  Chromatographic system
  Developing solvent system: To a mixture of glacial acetic acid and water (5:4) add 100 g/L of sodium acetate.
  Spray reagent: Dissolve 0.1 g of ninhydrin in a mixture containing 6 mL of *Cupric chloride solution*, 21 mL of glacial acetic acid, and 70 mL of alcohol.
  Analysis: Proceed as directed in the chapter, except spray the plate with the *Spray reagent*, and heat at 60° to visualize the spots.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in *Limit of N-Pyroglutamyl-Aprotinin and Related Compounds*.

ASSAY

- **PROCEDURE**
  Buffer: Transfer about 0.93 g of boric acid into a 1000-mL volumetric flask, dissolve in 900 mL of water, adjust with 5 N sodium hydroxide to a pH of 8.0, and dilute with water to volume. Transfer 100 mL of this solution into a 1000-mL volumetric flask, and dilute with water to volume.
  Sample solution: A solution of Aprotinin in *Buffer* containing about 1.67 USP Aprotinin Units/mL (about 0.6 mg/mL)
  Trypsin solution: 4300 USP Trypsin Units/mL of USP Trypsin Crystallized RS in 0.001 N hydrochloric acid. Use a freshly prepared solution, and store in ice water.
  Trypsin and aprotinin solution: To 4.0 mL of the *Trypsin solution* add 1.0 mL of the *Sample solution*. Dilute immediately with *Buffer* to 40.0 mL. Allow to stand at room temperature for 10 min, then keep in ice water. Use within 6 h of preparation.
  Dilute trypsin solution: Dilute 0.5 mL of the *Trypsin solution* with *Buffer* to 10.0 mL. Allow to stand at room temperature for 10 min, then store in ice water.

USP Monographs

www.webofpharma.com

Substrate solution: 6.9 mg/mL of *N*-benzoyl-L-arginine ethyl ester hydrochloride in water. Use within 2 h.

Analysis: Mix 9.0 mL of *Buffer* and 1.0 mL of *Substrate solution* in a jacketed glass vessel with a capacity of about 30 mL and containing a stirring device. The lid of the reaction vessel should contain five holes to accommodate the electrodes, the tip of a buret, a tube for the admission of nitrogen, and the introduction of reactants. An automated or manual titration apparatus may be used. Adjust with 0.1 N sodium hydroxide VS to a pH of 8.0. Maintain an atmosphere of nitrogen within the vessel, and stir continuously. When the temperature has reached equilibrium at 25 ± 0.1°, add 1.0 mL of *Trypsin and aprotinin solution*, and start a timer. Maintain at a pH of 8.0 by the addition of 0.1 N sodium hydroxide VS, and record the volume added every 30 s. Continue the reaction for 6 min. Carry out a similar titration using 1.0 mL of the *Dilute trypsin solution*.

For the lyophilized powder, calculate the aprotinin activity in USP Aprotinin Units/mg:

$$\text{Result} = F_1 \times [(F_2 \times n_2 - n_1)/m]$$

F_1 = conversion factor, 4000
F_2 = difference in the amount of trypsin used in *Trypsin and aprotinin solution* and *Dilute trypsin solution*, 2
n_2 = volume of 0.1 N sodium hydroxide added per second, after adding *Dilute trypsin solution* (mL/s)
n_1 = volume of 0.1 N sodium hydroxide added per second, after adding *Trypsin and aprotinin solution* (mL/s)
m = quantity of Aprotinin used to prepare 1 mL of the *Sample solution* (mg)

For the concentrated solution, calculate the USP Aprotinin Units/mL:

$$\text{Result} = F_1 \times (F_2 \times n_2 - n_1) \times D$$

F_1 = conversion factor, 4000
F_2 = difference in the amount of trypsin used in *Trypsin and aprotinin solution* and *Dilute trypsin solution*, 2
n_2 = volume of 0.1 N sodium hydroxide added per second, after adding *Dilute trypsin solution* (mL/s)
n_1 = volume of 0.1 N sodium hydroxide added per second, after adding *Trypsin and aprotinin solution* (mL/s)
D = dilution factor of the concentrated solution used to prepare the *Sample solution*

Acceptance criteria: NLT 3 USP Aprotinin Units/mg on the dried basis

IMPURITIES

• LIMIT OF DES-ALA-APROTININ AND DES-ALA-DES-GLY-APROTININ

Buffer: Dissolve 8.21 g of monobasic potassium phosphate in 400 mL of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 500 mL.

System suitability solution: Dilute USP Aprotinin RS with water to obtain a solution containing about 4–7 USP Aprotinin Units/mL.

Sample solution: Dilute a concentrated solution of Aprotinin with water, or weigh out Aprotinin and dissolve in water to obtain about 4–7 USP Aprotinin Units/mL of Aprotinin.

Capillary rinse procedure: Rinse the capillary for NLT 1 min with NLT 10 total capillary volumes of 0.1 N sodium hydroxide, followed by at least 10 total capillary volumes of water and by at least 20 capillary volumes of *Buffer* between injections.

Electrophoretic system

Mode: CE

Detector: UV 214 nm

Capillary: 75-µm × 45- to 60-cm uncoated fused silica. Use *Buffer* as the electrolyte in both buffer reservoirs.

Capillary temperature: 25°

Injection procedure: Transfer a volume of the *Sample solution*, approximately 15 nL, into the anodic end of the capillary. Apply differential pressure of 3.5 kPa for 3 s either by vacuum or pressure.

Applied voltage: 0.2 kV/cm

Run time: 30 min

System suitability: The baseline is stable and shows little drift.

Sample: *System suitability solution*

Suitability requirements

Migration time: 19–25 min for aprotinin

Relative migration times: About 0.98 for des-Ala-des-Gly-aprotinin, 0.99 for des-Ala-aprotinin, and 1 for aprotinin

Resolution: NLT 0.8 between the des-Ala-des-Gly-aprotinin and des-Ala-aprotinin peaks, and NLT 0.5 between the des-Ala-aprotinin and aprotinin peaks

Tailing factor: NMT 3 for aprotinin (see *Chromatography* ⟨621⟩ for calculation)

Analysis

Sample: *Sample solution*

Calculate the percentage of des-Ala-des-Gly-aprotinin and des-Ala-aprotinin:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of des-Ala-des-Gly-aprotinin or des-Ala-aprotinin
r_T = sum of the peak responses of des-Ala-des-Gly-aprotinin, des-Ala-aprotinin, and aprotinin

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Migration Time	Acceptance Criteria, NMT (%)
des-Ala-des-Gly-aprotinin	0.98	8.0
des-Ala-aprotinin	0.99	7.5
Aprotinin	1	—

• LIMIT OF *N*-PYROGLUTAMYL-APROTININ AND RELATED COMPOUNDS

Solution A: 3.52 g/L of monobasic potassium phosphate and 7.26 g/L of dibasic sodium phosphate. Filter, and degas.

Solution B: 3.52 g/L of monobasic potassium phosphate, 7.26 g/L of dibasic sodium phosphate, and 66.07 g/L of ammonium sulfate. Filter, and degas.

Mobile phase: See *Table 2*.

USP Monographs

www.webofpharma.com

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	92	8
21	64	36
30	0	100
31	92	8
40	92	8

System suitability solution: USP Aprotinin System Suitability RS in *Solution A*, with 5 USP Aprotinin Units/mL
Sample solution: Aprotinin in *Solution A*, with 5 USP Aprotinin Units/mL
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 7.5-mm × 7.5-cm; packing L52
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 40 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Retention time: 17–20 min for aprotinin
Relative retention times: 0.9 and 1.0 for *N*-pyroglutamyl-aprotinin and aprotinin, respectively
Resolution: NLT 1.0 between *N*-pyroglutamyl-aprotinin and aprotinin
Tailing factor: NMT 2.0 for aprotinin
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity peak:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
N-Pyroglutamyl-aprotinin	0.9	1.0
Aprotinin	1.0	—
Any other impurity	—	0.5
Sum of all unknown impurities	—	1.0

• **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**
Mobile phase: Acetonitrile, glacial acetic acid, and water (1:1:3). Filter and degas.
System suitability solution: Aprotinin solution that contains about 5 USP Aprotinin Units/mL with about 2% aprotinin oligomers. [NOTE—This solution can be obtained by heating lyophilized aprotinin at 112° for about 2 h and dissolving the solid at the specified concentration in water.]
Sample solution: Aprotinin in water, with about 5 USP Aprotinin Units/mL
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Columns: Series of three 7.8-mm × 30-cm columns; packing L33
Flow rate: 1.0 mL/min
Injection volume: 100 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Retention time: 24.5–25.5 min for aprotinin
Relative retention times: 0.9 and 1.0 for the dimer and aprotinin, respectively
Resolution: NLT 1.3 between the dimer and aprotinin peaks
Tailing factor: NMT 2.5 for aprotinin
Analysis
Sample: *Sample solution*
Calculate the percentage of each oligomer peak:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each peak with a retention time less than that of the aprotinin monomer
r_T = sum of all the peak responses

Acceptance criteria: Sum of all the oligomer peak responses is NMT 1.0%.

SPECIFIC TESTS

• **ABSORBANCE**
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Sample solution: 3.0 USP Aprotinin Units/mL
Acceptance criteria: The *Sample solution* exhibits an absorption maximum at 277 nm. The absorbance at the maximum is NMT 0.80.
• **BIOLOGICAL REACTIVITY TESTS, IN VIVO,** *Safety Tests—Biologicals* ⟨88⟩
Sample solution: Prepare a solution of Aprotinin that contains 4 USP Aprotinin Units/mL using a sufficient quantity of Water for Injection.
Acceptance criteria: Meets the requirements
• **SPECIFIC ACTIVITY OF THE DRY RESIDUE**
This test should only be performed when product is a concentrated solution.
Sample solution: 25.0 mL of Aprotinin concentrated solution
Analysis: Evaporate the *Sample solution* to dryness in a water bath, dry the residue at 110° for 15 h, and weigh. From the weight of the residue and the activity determined in the *Assay*, calculate the number of USP Aprotinin Units/mg of dry residue.
Acceptance criteria: NLT 3.0 USP Aprotinin Units/mg of dried residue
• **LOSS ON DRYING** ⟨731⟩
This test should only be performed on the lyophilized powder.
Sample: 100 mg
Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at a pressure of NMT 5 mm of mercury at 60° for 3 h.
Acceptance criteria: NMT 6.0%
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩
Sample solution: 6 USP Aprotinin Units/mL
Acceptance criteria: NMT 0.14 USP Endotoxin Unit per USP Aprotinin Unit

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** For lyophilized powder, preserve in tight containers, and store in a cold place. Protect from light. For bulk solution, preserve in tight

www.webofpharma.com

containers at a temperature not exceeding 25°. Avoid freezing.

• **LABELING:** The labeling states the source of the material and the number of Kallikrein Inhibition Units/mg or the number of Kallikrein Inhibition Units/mL.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Aprotinin RS
USP Aprotinin System Suitability RS
USP Trypsin Crystallized RS

Aprotinin Injection

DEFINITION

Aprotinin Injection is a sterile solution of Aprotinin in Water for Injection that also contains sodium chloride. One USP Aprotinin Unit is equivalent to 1800 Kallikrein Inhibition Units (K.I.U.). It contains NLT 90.0% and NMT 110.0% of the potency stated on the label, expressed in K.I.U./mL.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Limit of N-Pyroglutamyl-Aprotinin and Related Compounds*.

• **B.** It meets the requirements in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Transfer about 0.93 g of boric acid into a 1000-mL volumetric flask, dissolve in 900 mL of water, adjust with 5 N sodium hydroxide to a pH of 8.0, and dilute with water to volume. Transfer 100 mL of this solution into a 1000-mL volumetric flask, and dilute with water to volume.

Sample solution: A solution of aprotinin in *Buffer* containing about 1.67 USP Aprotinin Units/mL (about 0.6 mg/mL)

Trypsin solution: 4300 USP Trypsin Units/mL of USP Trypsin Crystallized RS in 0.001 N hydrochloric acid.Use a freshly prepared solution, and store in ice water.

Trypsin and aprotinin solution: To 4.0 mL of the *Trypsin solution*, add 1.0 mL of the *Sample solution*. Dilute immediately with *Buffer* to 40.0 mL. Allow to stand at room temperature for 10 min, then keep in ice water. Use within 6 h of preparation.

Dilute trypsin solution: Dilute 0.5 mL of the *Trypsin solution* with *Buffer* to 10.0 mL. Allow to stand at room temperature for 10 min, then store in ice water.

Substrate solution: 6.9 mg/mL of *N*-benzoyl-L-arginine ethyl ester hydrochloride. Use within 2 h.

Analysis: Mix 9.0 mL of *Buffer* and 1.0 mL of *Substrate solution* in a jacketed-glass vessel with a capacity of about 30 mL and containing a stirring device. The lid of the reaction vessel should contain five holes to accommodate the electrodes, the tip of a buret, a tube for the admission of nitrogen, and the introduction of reactants. An automated or manual titration apparatus may be used. Adjust with 0.1 N sodium hydroxide VS to a pH of 8.0. Maintain an atmosphere of nitrogen within the vessel, and stir continuously. When the temperature has reached equilibrium at 25 ± 0.1°, add 1.0 mL of *Trypsin and aprotinin solution*, and start a timer. Maintain at a pH of 8.0 by the addition of 0.1 N sodium hydroxide VS, and record the volume added every 30 s. Continue the reaction for 6 min. Carry out a similar titration using 1.0 mL of the *Dilute trypsin solution*.

Calculate the potency in USP Aprotinin Units/mL:

$$\text{Result} = F_1 \times (F_2 \times n_2 - n_1) \times D$$

F_1 = conversion factor, 4000
F_2 = difference in the amount of trypsin used in *Trypsin and aprotinin solution* and *Dilute trypsin solution*, 2
n_2 = volume of 0.1 N sodium hydroxide added per second, after adding *Dilute trypsin solution* (mL/s)
n_1 = volume of 0.1 N sodium hydroxide added per second, after adding *Trypsin and aprotinin solution* (mL/s)
D = dilution factor used to prepare the *Sample solution*

Acceptance criteria: 90.0%–110.0% of the potency stated on the label, expressed in K.I.U./mL

OTHER COMPONENTS

• **CONTENT OF SODIUM CHLORIDE**

Sample: 5.0 mL of Injection

Analysis: Pipet the *Sample* into a beaker containing 50 mL of water. Add 10 mL of 25% nitric acid. Titrate with 0.1 N silver nitrate VS to a potentiometric endpoint, using a silver combination electrode. Perform a blank determination (see *Titrimetry* ⟨541⟩), and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride.

Acceptance criteria: 42.5–47.5 mg

IMPURITIES

• **LIMIT OF *N*-PYROGLUTAMYL-APROTININ AND RELATED COMPOUNDS**

Solution A: 3.52 g/L of monobasic potassium phosphate and 7.26 g/L of dibasic sodium phosphate. Filter, and degas.

Solution B: 3.52 g/L of monobasic potassium phosphate, 7.26 g/L of dibasic sodium phosphate, and 66.07 g/L of ammonium sulfate. Filter, and degas.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	92	8
21	64	36
30	0	100
31	92	8
40	92	8

System suitability solution: USP Aprotinin System Suitability RS in *Solution A* with 5 USP Aprotinin Units/mL

Sample solution: Aprotinin in *Solution A*, with 5 USP Aprotinin Units/mL

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 7.5-mm × 7.5-cm; packing L52
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 40 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Retention time: 17–20 min for aprotinin
Relative retention times: 0.9 and 1.0 for *N*-pyroglutamyl-aprotinin and aprotinin, respectively
Resolution: NLT 1.0 between *N*-pyroglutamyl-aprotinin and aprotinin

www.webofpharma.com

Tailing factor: NMT 2.0 for the aprotinin peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity peak:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
N-Pyroglutamyl-aprotinin	0.9	1.0
Aprotinin	1.0	—
Any other impurity	—	0.5
Sum of all unknown impurities	—	1.0

- **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**

Mobile phase: Acetonitrile, glacial acetic acid, and water (1:1:3). Filter, and degas.

System suitability solution: Aprotinin solution that contains about 5 USP Aprotinin Units/mL with about 2% aprotinin oligomers. [NOTE—This solution can be obtained by heating lyophilized aprotinin at 112° for about 2 h and dissolving the solid at the specified concentration in water.]

Sample solution: Aprotinin in water, with 5 USP Aprotinin Units/mL

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: Series of three 7.8-mm × 30-cm columns; packing L33

Flow rate: 1.0 mL/min

Injection volume: 100 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Retention time: 24.5–25.5 min for aprotinin

Relative retention times: 0.9 and 1.0 for the dimer and aprotinin, respectively

Resolution: NLT 1.3 between the dimer and aprotinin peaks

Tailing factor: NMT 2.5 for the aprotinin peak

Analysis

Sample: *System suitability solution*

Calculate the percentage of each oligomer peak:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each peak with a retention time less than that of the aprotinin monomer

r_T = sum of all the peak responses

Acceptance criteria: Sum of all the oligomer peak responses is NMT 1.5%.

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements
- **pH** ⟨791⟩: 4.5–6.5
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩: Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.14 USP Endotoxin Units per USP Aprotinin Unit.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers. Store at up to 25°, and avoid freezing.
- **USP REFERENCE STANDARDS** ⟨11⟩

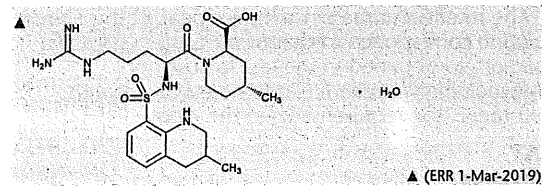
USP Aprotinin RS

USP Aprotinin System Suitability RS

USP Trypsin Crystallized RS

Change to read:

Argatroban



▲ (ERR 1-Mar-2019)

$C_{23}H_{36}N_6O_5S \cdot H_2O$ 526.65

2-Piperidinecarboxylic acid, 1-[(*S*)-5-[(aminoiminomethyl)amino]-1-oxo-2-{[(1,2,3,4-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino}pentyl]-4-methyl-, (2*R*,4*R*)-monohydrate;

(2*R*,4*R*)-4-Methyl-1-{N^2-[(1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]-L-arginyl}pipecolic acid, monohydrate [141396-28-3].

DEFINITION

Argatroban contains NLT 98.0% and NMT 102.0% of argatroban ($C_{23}H_{36}N_6O_5S$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—It is recommended to keep all solutions containing argatroban at about 4°.]

Solution A: 10 mM ammonium acetate and 5 mM sodium 1-heptanesulfonate

Solution B: Acetonitrile and methanol (500:300)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
20	60	40
35	50	50

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
50	20	80
60	20	80
60.1	60	40
72.1	60	40

Standard solution: 4 mg/mL of USP Argatroban RS in methanol
Sample solution: 4 mg/mL of Argatroban in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 259 nm
Column: 4.6-mm × 25-cm; 3-µm packing L1
Temperatures
Column: 50°
Autosampler: 4°
Flow rate: 0.6 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for both peaks
Relative standard deviation: NMT 1.0% for the sum of the peak responses of (*R*)-argatroban and (*S*)-argatroban
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of argatroban ($C_{23}H_{36}N_6O_5S$) in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses of (*R*)-argatroban and (*S*)-argatroban from the *Sample solution*
r_S = sum of the peak responses of (*R*)-argatroban and (*S*)-argatroban from the *Standard solution*
C_S = concentration of USP Argatroban RS in the *Standard solution* (mg/mL)
C_U = concentration of Argatroban in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%
- **Organic Impurities**

[Note—It is recommended to keep all solutions containing argatroban at about 4°.]
Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Sensitivity solution: 2 µg/mL of USP Argatroban RS in methanol
Standard solution: 4 µg/mL each of USP Argatroban RS, USP Argatroban Related Compound A RS, and USP Argatroban Related Compound B RS in methanol
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between (*R*)-argatroban and (*S*)-argatroban, *Standard solution*
Signal-to-noise ratio: NLT 10 for (*R*)-argatroban, *Sensitivity solution*
Relative standard deviation: NMT 5% for all peaks. For argatroban, use the sum of the peak responses of (*R*)-argatroban and (*S*)-argatroban, *Standard solution*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of argatroban related compound A and argatroban related compound B in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of argatroban related compound A or argatroban related compound B from the *Sample solution*
r_S = peak response of argatroban related compound A or argatroban related compound B from the *Standard solution*
C_S = concentration of USP Argatroban Related Compound A RS or USP Argatroban Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Argatroban in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = sum of the peak responses of (*R*)-argatroban and (*S*)-argatroban from the *Standard solution*
C_S = concentration of USP Argatroban RS in the *Standard solution* (mg/mL)
C_U = concentration of Argatroban in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Argatroban related compound A[a]	0.23	0.15
Argatroban related compound B[b]	0.39	0.15
(*R*)-Argatroban[c]	1.00	—
(*S*)-Argatroban[d]	1.03	—
Any unspecified impurity	—	0.10
Total impurities[e]	—	0.5

[a] (2*R*,4*R*)-1-[*N* ^{8}-Nitro-*N* ^{2}-(3-methylquinoline-8-sulfonyl)-L-arginyl]-4-methylpiperidine-2-carboxylic acid.
[b] Ethyl (4*R*)-1-[*N* ^{8}-nitro-L-arginyl]-4-methylpiperidine-2-carboxylate.
[c] (2*R*,4*R*)-4-Methyl-1-(*N* ^{2}-[((*R*)-1,2,3,4-tetrahydro-3-methyl-8-quinolyl) sulfonyl]-L-arginyl)pipecolic acid.
[d] (2*R*,4*R*)-4-Methyl-1-(*N* ^{2}-[((*S*)-1,2,3,4-tetrahydro-3-methyl-8-quinolyl) sulfonyl]-L-arginyl)pipecolic acid.
[e] Total impurities include specified and unspecified impurities and argatroban related compound C from the test for *Content of Argatroban Related Compound C*.

- **Content of Argatroban Related Compound C**

[Note—It is recommended to keep all solutions containing argatroban at about 4°.]
Buffer: 10 mM ammonium acetate and 5 mM sodium 1-heptanesulfonate
Solution A: Acetonitrile, dehydrated alcohol, and *Buffer* (80:240:680)

www.webofpharma.com

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Acetonitrile (%)
0	100	0
70	100	0
71	30	70
91	30	70
92	100	0
102	100	0

Sensitivity solution: 4 µg/mL of USP Argatroban Related Compound C RS in methanol
System suitability solution: 10 mg/mL of USP Argatroban RS and 0.1 mg/mL of USP Argatroban Related Compound C RS in methanol
Sample solution: 10 mg/mL of Argatroban in methanol
Chromatographic system: Proceed as directed in the *Assay*.
System suitability
Samples: *Sensitivity solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.4 between argatroban related compound C and (*R*)-argatroban, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 2.5% for argatroban related compound C, *System suitability solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of argatroban related compound C in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of argatroban related compound C from the *Sample solution*
r_T = total of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Argatroban related compound C[a]	0.94	0.15
(*R*)-Argatroban	1.00	—
(*S*)-Argatroban	1.07	—

[a] (2*R*,4*R*)-1-[*N* ^{8}-Amino-*N* ^{2}-(3-methyl-1,2,3,4-tetrahydroquinoline-8-sulfonyl)-L-arginyl]-4-methylpiperidine-2-carboxylic acid.

• **CONTENT OF STEREOISOMERS**
[NOTE—It is recommended to keep all solutions containing argatroban at about 4°.]
Mobile phase: Methanol and water (520:480)
Standard solution: 0.16 mg/mL of USP Argatroban RS in methanol
Sample solution: 0.16 mg/mL of Argatroban in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 259 nm
Column: 4.6-mm × 25-cm; 3-µm packing L1
Column temperature: 50°
Flow rate: 0.6 mL/min
Injection volume: 10 µL
Run time: NLT 1.4 times the retention time of (*R*)-argatroban
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between (*R*)-argatroban and (*S*)-argatroban
Relative standard deviation: NMT 2.0% for (*R*)-argatroban and (*S*)-argatroban
Analysis
Sample: *Sample solution*
Calculate the percentage of (*R*)-argatroban and (*S*)-argatroban in the portion of Argatroban taken:

$$\text{Result} = [(r_U \text{ or } r_S)/(r_U + r_S)] \times 100$$

r_U = peak response of (*R*)-argatroban from the *Sample solution*
r_S = peak response of (*S*)-argatroban from the *Sample solution*

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, (%)
(*R*)-Argatroban[a]	1.00	63–67
(*S*)-Argatroban[b]	1.06	33–37

[a] (2*R*,4*R*)-4-Methyl-1-{*N* ^{2}-[((*R*)-1,2,3,4-tetrahydro-3-methyl-8-quinolyl) sulfonyl]-L-arginyl}pipecolic acid.
[b] (2*R*,4*R*)-4-Methyl-1-{*N* ^{2}-[((*S*)-1,2,3,4-tetrahydro-3-methyl-8-quinolyl) sulfonyl]-L-arginyl}pipecolic acid.

SPECIFIC TESTS

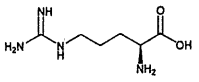
• **WATER DETERMINATION** ⟨921⟩, *Method Ia*: 3.0%–6.0%
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 2.0 USP Endotoxin Units/mg of argatroban
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count is NMT 10^2 cfu/g, and the total combined molds and yeasts count is NMT 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Argatroban RS
USP Argatroban Related Compound A RS
(2*R*,4*R*)-1-[*N*^{8}-Nitro-*N*^{2}-(3-methylquinoline-8-sulfonyl)-L-arginyl]-4-methylpiperidine-2-carboxylic acid.
$C_{23}H_{31}N_7O_7S$ 549.60
USP Argatroban Related Compound B RS
Ethyl (4*R*)-1-[*N*^{8}-nitro-L-arginyl]-4-methylpiperidine-2-carboxylate dihydrochloride.
$C_{15}H_{28}N_6O_5 \cdot 2HCl$ 445.34
USP Argatroban Related Compound C RS
(2*R*,4*R*)-1-[*N*^{8}-Amino-*N*^{2}-(3-methyl-1,2,3,4-tetrahydroquinoline-8-sulfonyl)-L-arginyl]-4-methylpiperidine-2-carboxylic acid.
$C_{23}H_{37}N_7O_5S$ 523.65

www.webofpharma.com

Arginine



$C_6H_{14}N_4O_2$ 174.20
L-Arginine [74-79-3].

DEFINITION
Arginine contains NLT 98.5% and NMT 101.5% of $C_6H_{14}N_4O_2$, as L-arginine, calculated on the dried basis.

IDENTIFICATION

Change to read:

▲• **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Sample: 80 mg of Arginine
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Blank: 3 mL of formic acid and 50 mL of glacial acetic acid
Analysis: Dissolve the *Sample* in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with Titrant. Calculate the percentage of $C_6H_{14}N_4O_2$ in the portion taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/W$$

V = *Sample* titrant volume (mL)
B = *Blank* titrant volume (mL)
N = titrant normality (mEq/mL)
F = equivalency factor: 87.10 mg/mEq
W = weight of *Sample* (mg)

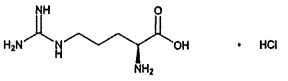
Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES
INORGANIC IMPURITIES
• **Residue on Ignition** ⟨281⟩: NMT 0.3%
• **Chloride and Sulfate,** *Chloride* ⟨221⟩: A 1.0-g portion shows no more chloride than corresponds to 0.70 mL of 0.020 N hydrochloric acid (0.05%).
• **Chloride and Sulfate,** *Sulfate* ⟨221⟩: A 1.0-g portion shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid (0.03%).
• **Iron** ⟨241⟩: NMT 30 ppm
ORGANIC IMPURITIES
• **Procedure**
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Standard solution: 0.05 mg/mL of USP L-Arginine RS in 0.1 N hydrochloric acid. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]
Sample solution: 10 mg/mL of Arginine in 2 N hydrochloric acid
System suitability solution: 0.4 mg/mL each of USP L-Arginine RS and USP L-Lysine Hydrochloride RS in 0.1 N hydrochloric acid
Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)
Application volume: 5 µL
Developing solvent system: Isopropyl alcohol and ammonium hydroxide (7:3)
Analysis
Samples: *Standard solution*, *Sample solution*, and *System suitability solution*
Proceed as directed under *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*. Dry the plate between 100° and 105° until the ammonia disappears completely. Spray with *Spray reagent*, and heat between 100° and 105° for about 15 min. Examine the plate under white light. The chromatogram obtained from the *System suitability solution* exhibits two clearly separated spots.
Acceptance criteria
Individual impurities: Any secondary spot from the *Sample solution* is not larger or more intense than the principal spot from the *Standard solution*, NMT 0.5%
Total impurities: NMT 2.0%

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩: +26.3° to +27.7°
Sample solution: 80 mg/mL in 6 N hydrochloric acid
• **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP L-Arginine RS
USP L-Lysine Hydrochloride RS

Arginine Hydrochloride



$C_6H_{14}N_4O_2 \cdot HCl$ 210.66
L-Arginine monohydrochloride;
L-(+)-Arginine monohydrochloride [1119-34-2].

DEFINITION
Arginine Hydrochloride contains NLT 98.5% and NMT 101.5% of arginine hydrochloride ($C_6H_{14}N_4O_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Sample: 100 mg of Arginine Hydrochloride
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Blank: 50 mL of glacial acetic acid and 3 mL of 98% formic acid. Add 6 mL of mercuric acetate TS.
Analysis: Dissolve the *Sample* in 3 mL of 98% formic acid and 50 mL of glacial acetic acid. Add 6 mL of mercuric acetate TS and titrate with the *Titrant*.
Calculate the percentage of arginine hydrochloride ($C_6H_{14}N_4O_2 \cdot HCl$) in the *Sample* taken:

www.webofpharma.com

Result = $[(V - B) \times N \times F \times 100]/W$

V = *Sample* titrant volume (mL)
B = *Blank* titrant volume (mL)
N = titrant normality (mEq/mL)
F = equivalency factor, 105.3 mg/mEq
W = weight of *Sample* (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩: A 1.6-g portion shows no more sulfate than corresponds to 0.50 mL of 0.020 N sulfuric acid (0.03%).
- **CHROMATOGRAPHIC PURITY**

System suitability solution: 0.4 mg/mL each of USP Arginine Hydrochloride RS and USP L-Lysine Hydrochloride RS in water

Standard solution: 0.05 mg/mL of USP Arginine Hydrochloride RS in water. [NOTE—This solution has a concentration equivalent to about 0.5% of that of the *Sample solution*.]

Sample solution: 10 mg/mL of Arginine Hydrochloride in water

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Isopropyl alcohol and ammonium hydroxide (70:30)
Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

Analysis
Samples: *System suitability solution*, *Standard solution*, and *Sample solution*
Proceed as directed in the chapter. Dry the plate between 100° and 105° until the ammonia disappears completely. Spray with *Spray reagent*, and heat between 100° and 105° for about 15 min. Examine the plate under white light. The *System suitability solution* exhibits two clearly separated spots.

Acceptance criteria: Any secondary spot from the *Sample solution* is not larger or more intense than the principal spot from the *Standard solution*.
Individual impurities: NMT 0.5%
Total impurities: NMT 2.0%

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩: +21.4° to +23.6° (t = 20°)

Sample solution: 80 mg/mL in 6 N hydrochloric acid

- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 2 h: it loses NMT 0.2% of its weight.
- **CHLORIDE CONTENT**

Sample: 350 mg of Arginine Hydrochloride
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Colorimetric
Analysis: Transfer the *Sample* to a porcelain casserole, and add 140 mL of water and 1 mL of dichlorofluorescein TS. Mix and titrate with the *Titrant* until the silver chloride flocculates and the mixture acquires a faint pink color. Calculate the percentage of chloride (Cl) in the *Sample* taken:

Result = $(V \times N \times F \times 100)/W$

V = *Sample* titrant volume (mL)
N = titrant normality (mEq/mL)
F = equivalency factor, 35.45 mg/mEq
W = weight of *Sample* (mg)

Acceptance criteria: 16.5%–17.1%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Arginine Hydrochloride RS
  USP L-Lysine Hydrochloride RS

Add the following:

▲Arginine Hydrochloride Compounded Oral Solution

DEFINITION

Arginine Hydrochloride Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of arginine hydrochloride ($C_6H_{14}N_4O_2 \cdot HCl$).

Prepare Arginine Hydrochloride Compounded Oral Solution 100 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Arginine Hydrochloride powder	10 g
Methylparaben	0.05 g
Propylparaben	0.025 g
Purified Water, a sufficient quantity to make	100 mL

In an appropriately sized container, add the *Methylparaben* and *Propylparaben* to about 80 mL of *Purified Water*. Stir until dissolved. [NOTE—May heat up to 50° to facilitate dissolution.] Dissolve the *Arginine Hydrochloride powder* in the previously prepared solution of *Methylparaben* and *Propylparaben*. Bring to final volume with *Purified Water*.

ASSAY

- **PROCEDURE**

Solution A: 20 mM ammonium acetate solution adjusted with glacial acetic acid to a pH of 6
Mobile phase: Acetonitrile and *Solution A* (22:78). Pass through a membrane filter of 0.22-µm pore size.
Standard solution: 0.5 mg/mL of arginine hydrochloride prepared from USP Arginine Hydrochloride RS in water
Sample solution: Transfer 1 mL of Oral Solution to a 200-mL volumetric flask, add approximately 160 mL of water, and vortex. Dilute with water to volume and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L10
Temperatures
Autosampler: 4°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 60 µL

Supplement Two

www.webofpharma.com

System suitability
Sample: *Standard solution*
[NOTE—The retention time for arginine hydrochloride is about 5.4 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of arginine hydrochloride ($C_6H_{14}N_4O_2 \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of arginine hydrochloride from the *Sample solution*
r_S = peak response of arginine hydrochloride from the *Standard solution*
C_S = concentration of USP Arginine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of arginine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 5.0–6.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator or at controlled room temperature
- **LABELING:** Label it to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Arginine Hydrochloride RS▲ (USP 1-May-2020)

Arginine Hydrochloride Injection

» Arginine Hydrochloride Injection is a sterile solution of Arginine Hydrochloride in Water for Injection. It contains not less than 9.5 percent and not more than 10.5 percent of $C_6H_{14}N_4O_2 \cdot HCl$. It contains no antimicrobial agents.
[NOTE—The chloride ion content of Arginine Hydrochloride Injection is approximately 475 mEq per L.]

Packaging and storage—Preserve in single-dose containers, preferably of Type II glass.
USP Reference standards ⟨11⟩—
USP Arginine Hydrochloride RS
Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

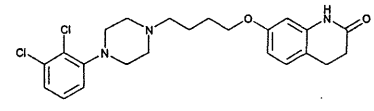
Identification—
A: Transfer 1 mL of the Injection to a 200-mL volumetric flask, and dilute with water to volume. To 1 mL of this dilution add 2 mL of a solution of 0.02% 8-hydroxyquinoline in 3 N sodium hydroxide, and add 1 mL of 0.1% *N*-bromosuccinimide solution: an orange color is produced.
B: It meets the requirements of the tests for *Chloride* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.01 USP Endotoxin Unit per mg of arginine hydrochloride.
pH ⟨791⟩: between 5.0 and 6.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Color reagent—Dissolve 28.0 g of potassium hydroxide and 2.0 g of potassium sodium tartrate in 100 mL of water. Cool, and add, in the order named, 100 mg of 2,4-dichloro-1-naphthol, 180 mL of alcohol, and 20.0 mL of 0.475% sodium hypochlorite solution. Mix by swirling, and allow to stand at room temperature for 1 hour before using. This *Color reagent* may be stored in a glass-stoppered bottle, in a refrigerator, for 2 months.
Standard preparation—Dissolve an accurately weighed quantity of USP Arginine Hydrochloride RS in water, and dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 40 µg per mL.
Assay preparation—Pipet into a 100-mL volumetric flask a volume of Injection, equivalent to 200 mg of arginine hydrochloride, add water to volume, and mix. Pipet 5 mL of this solution into a 250-mL volumetric flask, add water to volume, and mix.
Procedure—Transfer 2.0-mL portions of the *Assay preparation* and the *Standard preparation*, respectively, to separate flasks, and treat each as follows. Add 2.0 mL of potassium iodide solution (3 in 1000), mix, and allow to stand for 15 minutes. Add 6.0 mL of *Color reagent*, mix, and allow to stand for 15 minutes. Add 2.0 mL of sodium hypochlorite solution (19 in 10,000), mix, and allow to stand for 15 minutes. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 520 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_6H_{14}N_4O_2 \cdot HCl$ in each mL of the Injection taken by the formula:

$$5(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Arginine Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Aripiprazole



$C_{23}H_{27}Cl_2N_3O_2$ 448.39
2(1*H*)-Quinolinone, 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydro-;
7-[4-[4-(2,3-Dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydrocarbostyril [129722-12-9].

DEFINITION
Aripiprazole contains NLT 98.0% and NMT 102.0% of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$), calculated on the dried basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Protect the solutions from light.

Diluent: Acetonitrile, methanol, water, and acetic acid (30:10:60:1)

Solution A: Acetonitrile and 0.05% trifluoroacetic acid (10:90)

Solution B: Acetonitrile and 0.05% trifluoroacetic acid (90:10)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
2	80	20
10	65	35
20	10	90
25	10	90
26	80	20
35	80	20

[NOTE—The gradient was established on an HPLC system with a dwell volume of approximately 650 µL.]

System suitability solution: 1 µg/mL each of USP Aripiprazole RS and USP Aripiprazole Related Compound F RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Aripiprazole RS in *Diluent*

Sample solution: 0.1 mg/mL of Aripiprazole in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for aripiprazole and aripiprazole related compound F are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 2.0 between aripiprazole and aripiprazole related compound F, *System suitability solution*

Tailing factor: NMT 1.5 for aripiprazole, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) in the portion of Aripiprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Aripiprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Protect the solutions from light.

Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Aripiprazole taken:

$$\text{Result} = (r_i/r_U) \times (1/F) \times 100$$

r_i = peak response of each impurity from the *Sample solution*

r_U = peak response of Aripiprazole from the *Sample solution*

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Aripiprazole related compound G[a]	0.9	0.72	0.10
Aripiprazole	1.0	—	—
Aripiprazole related compound F[b, c]	1.1	1.0	0.10
Aripiprazole 4,4′-dimer[d]	1.3	1.0	0.10
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.50

[a] 7-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy}quinolin-2(1*H*)-one.

[b] 4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy) butyl]piperazine 1-oxide.

[c] If possible from the manufacturing process.

[d] 1,1′-(Ethane-1,1-diyl)bis(2,3-dichloro-4-{4-[3,4-dihydroquinolin-2(1*H*)-one-7-yloxybutyl]piperazin-1-yl}benzene).

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Aripiprazole RS

USP Aripiprazole Related Compound F RS

4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazine 1-oxide.

$C_{23}H_{27}Cl_2N_3O_3$ 464.38

www.webofpharma.com

Aripiprazole Tablets

DEFINITION

Aripiprazole Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Standard: Add 30 mL of ethyl acetate to 30 mg of USP Aripiprazole RS. Shake for 10 min, centrifuge for NLT 5 min, and pass the supernatant through a suitable membrane filter. To the filtrate add 15 mL of water, shake for 5 min, and centrifuge for NLT 10 min. Transfer 20 mL of the upper layer to a container and add anhydrous magnesium sulfate, as needed. Shake well, pass through a suitable membrane filter, and evaporate the ethyl acetate on a water bath under reduced pressure. Use the residue. [NOTE—A centrifuge speed of 2000 rpm may be suitable.]

Sample: Grind a suitable number of Tablets and transfer a suitable portion of the ground Tablets, equivalent to 30 mg of aripiprazole, to an appropriate container. Add 30 mL of ethyl acetate, shake for 10 min, centrifuge for NLT 5 min, and pass the supernatant through a suitable membrane filter. To the filtrate add 15 mL of water, shake for 5 min, and centrifuge for NLT 10 min. Transfer 20 mL of the upper layer to a container and add a suitable amount of anhydrous magnesium sulfate. Shake well, pass through a suitable membrane filter, and evaporate the ethyl acetate on a water bath under reduced pressure. Use the residue. [NOTE—A centrifuge speed of 2000 rpm may be suitable.]

Analysis

Samples: *Standard* and *Sample*

Acceptance criteria: Meet the requirements

• **B.** The retention time of the aripiprazole peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 2.8 g/L of anhydrous sodium sulfate in water

Mobile phase: Acetonitrile, methanol, *Solution A*, and glacial acetic acid (33:11:56:1)

Internal standard solution: 0.33 mg/mL of USP Propylparaben RS in *Mobile phase*

Standard stock solution: 1 mg/mL of USP Aripiprazole RS in *Mobile phase*

Standard solution: 0.2 mg/mL of USP Aripiprazole RS prepared as follows. Transfer 10.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.2 mg/mL of aripiprazole from Tablets prepared as follows. Powder NLT 20 Tablets and transfer a suitable portion of the powder to an appropriate volumetric flask. Add 40% of the final flask volume of *Mobile phase* and 20% of the final flask volume of *Internal standard solution*. Shake for 10 min, and dilute with *Mobile phase* to volume. Centrifuge, if necessary, and pass the supernatant through a suitable filter of NMT 0.5-µm pore size, discard the first 1 mL of filtrate, and use the subsequent filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 2 times the retention time of aripiprazole

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for aripiprazole and propylparaben are about 1.0 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 8 between aripiprazole and propylparaben

Tailing factor: NMT 1.7 for aripiprazole and for propylparaben

Relative standard deviation: NMT 2.0% for the peak response ratio of aripiprazole to propylparaben

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of aripiprazole to propylparaben from the *Sample solution*

R_S = peak response ratio of aripiprazole to propylparaben from the *Standard solution*

C_S = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of aripiprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

TEST 1

Medium: pH 1.2 hydrochloric acid buffer (Transfer 250 mL of 14.9 g/L of potassium chloride in water to a 1-L volumetric flask, add 425 mL of 0.2 N hydrochloric acid, and dilute with water to volume. Degas the resulting solution or pass the resulting solution through a filter under vacuum.), degassed; 900 mL

Apparatus 2: 60 rpm

Time: 30 min

Procedure: Determine the percentage of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) dissolved by using either the *Spectrometric procedure* or the *Chromatographic procedure* described below.

Spectrometric procedure

Standard stock solution: 1 mg/mL of USP Aripiprazole RS in alcohol

Standard solution: (*L*/900) mg/mL of USP Aripiprazole RS from *Standard stock solution* in *Medium*, where *L* is the label claim, in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter, discarding the first 5 mL of filtrate.

Instrumental conditions

Mode: UV

Analytical wavelengths: 249 and 325 nm

Cell length: 1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

USP Monographs

www.webofpharma.com

A_U = absorbance at 249 nm minus the absorbance at 325 nm of the *Sample solution*
A_S = absorbance at 249 nm minus the absorbance at 325 nm of the *Standard solution*
C_S = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Chromatographic procedure
Solution A: 2.8 g/L of anhydrous sodium sulfate
Solution B: 13.9 g/L of glacial acetic acid and 23.9 g/L of sodium acetate in water
Mobile phase: Acetonitrile, methanol, *Solution A*, and glacial acetic acid (40:10:50:1)
Diluent: *Solution B* and methanol (50:50)
Internal standard solution: 0.67 µg/mL of USP Propylparaben RS in *Diluent*
Standard stock solution A: 1 mg/mL of USP Aripiprazole RS in *Mobile phase*
Standard stock solution B: 0.002 mg/mL of USP Aripiprazole RS from *Standard stock solution A* in *Medium* passed through a suitable filter of NMT 0.5-µm pore size, discarding the first 6 mL of filtrate
Standard solution: 0.001 mg/mL of USP Aripiprazole RS from *Standard stock solution B* prepared by combining 5 mL of *Standard stock solution B* and 5 mL of *Internal standard solution*
Sample stock solution: Pass a portion of the solution under test through a suitable filter of NMT 0.5-µm pore size, discarding NLT the first 6 mL of filtrate.
Sample solution: Combine 2 mL of *Sample stock solution* with 2 mL of *Internal standard solution*.
Chromatographic system: Proceed as directed in the *Assay* except as follows.
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for aripiprazole and propylparaben are about 1.0 and 1.8, respectively.]
Suitability requirements
Resolution: NLT 10 between aripiprazole and propylparaben
Relative standard deviation: NMT 1.5% for the peak response ratio of aripiprazole to propylparaben
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) dissolved:

$$\text{Result} = (R_U/R_S) \times C_S \times V \times (1/L) \times 100$$

R_U = peak response ratio of aripiprazole to propylparaben from the *Sample solution*
R_S = peak response ratio of aripiprazole to propylparaben from the *Standard solution*
C_S = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) is dissolved.

TEST 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid VS; 900 mL
Apparatus 2: 60 rpm
Time: 15 min
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 1 N phosphoric acid TS to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (40:60)
Standard stock solution: 0.11 mg/mL of USP Aripiprazole RS in solution prepared as follows. Transfer a suitable amount of USP Aripiprazole RS to an appropriate volumetric flask. Add 2% of the flask volume of acetonitrile and 70% of the flask volume of *Medium*. Sonication may be used to promote dissolution. Dilute with *Medium* to volume.
Standard solution: (L/900) mg/mL of USP Aripiprazole RS from *Standard stock solution* in *Medium*, where L is the label claim, in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter, discarding NLT the first 5 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 5 µL
Run time: NLT 1.6 times the retention time of aripiprazole
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of aripiprazole from the *Sample solution*
r_S = peak response of aripiprazole from the *Standard solution*
C_S = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Protect solutions from light.
Buffer: 9.6 g/L of dibasic ammonium citrate, 1.6 g/L of citric acid, and 2.9 g/L of sodium dodecyl sulfate in water. Adjust with 11 g/L of dibasic ammonium citrate in water or 9.6 g/L of anhydrous citric acid in water to a pH of 4.7, if needed.
Mobile phase: Acetonitrile and *Buffer* (45:55)
Diluent: Acetonitrile, water, and glacial acetic acid (40:60:1)
System suitability solution: 0.5 mg/mL of USP Aripiprazole RS, and 0.0005 mg/mL each of USP Aripiprazole Related Compound F RS and USP Aripiprazole Related Compound G RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of aripiprazole from Tablets prepared as follows. Powder NLT 20 Tablets, transfer a suitable portion of the powder equivalent to NLT 4 mg of aripiprazole to an appropriate container, and add

www.webofpharma.com

a suitable volume of *Diluent*. Shake for 10 min and centrifuge, if necessary. Pass the supernatant through a suitable filter of NMT 0.5-µm pore size, discard the first 1 mL of filtrate, and use the subsequent filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of aripiprazole

System suitability
Sample: *System suitability solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 3 between aripiprazole related compound G and aripiprazole
Signal-to-noise ratio: NLT 10 for aripiprazole related compound F and aripiprazole related compound G

Analysis
Sample: *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*. Disregard peaks that are less than 0.1% of the aripiprazole peak.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aripiprazole related compound F	0.54	0.3
Aripiprazole related compound G	0.81	0.3
Aripiprazole	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	1.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** The labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aripiprazole RS
  USP Aripiprazole Related Compound F RS
  4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazine 1-oxide.
  $C_{23}H_{27}Cl_2N_3O_3$ 464.38
  USP Aripiprazole Related Compound G RS
  7-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy}quinolin-2(1*H*)-one.
  $C_{23}H_{25}Cl_2N_3O_2$ 446.37
  USP Propylparaben RS

Aripiprazole Orally Disintegrating Tablets

DEFINITION
Aripiprazole Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 2.84 g/L of sodium sulfate in water
  Buffer: 3.48 g/L of dibasic potassium phosphate adjusted with phosphoric acid to a pH of 8.2
  Mobile phase: Acetonitrile and *Buffer* (50:50)
  Diluent A: Acetonitrile, methanol, *Solution A*, and glacial acetic acid (33:11:56:1)
  Diluent B: Acetonitrile and 0.1 M hydrochloric acid (20:80)
  System suitability solution: 0.01 mg/mL each of USP Aripiprazole RS and USP Aripiprazole Related Compound G RS in *Diluent A*. Sonication and shaking may be used to aid in dissolution.
  Standard solution: 0.25 mg/mL of USP Aripiprazole RS in *Diluent B*. Sonication may be used to aid in dissolution.
  Sample solution: Nominally 0.2–0.3 mg/mL of aripiprazole from NLT 5 Orally Disintegrating Tablets prepared as follows. Transfer NLT 5 Orally Disintegrating Tablets to a suitable volumetric flask and dilute with *Diluent B* to NMT 75% of the final flask volume. Sonicate for 5 min and shake for 15 min. Dilute with *Diluent B* to volume. Pass the resulting solution through a suitable filter and use the filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 252 nm
  Column: 4.6-mm × 10-cm; 3.5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  Run time: NLT 1.4 times the retention time of aripiprazole
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times of aripiprazole related compound G and aripiprazole are 0.74 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 3.0 between aripiprazole related compound G and aripiprazole, *System suitability solution*
  Tailing factor: NMT 1.5, *Standard solution*
  Relative standard deviation: NMT 1.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) in the portion of Orally Disintegrating Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aripiprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩: NMT 60 s
- **DISSOLUTION** ⟨711⟩

Medium: pH 4.0 sodium acetate trihydrate buffer (3.0 g/L of sodium acetate prepared as follows. Transfer a suitable quantity of sodium acetate to a suitable container containing 90% of the final container volume of water. Adjust with glacial acetic acid to a pH of 4.0. Add water to the final volume.), degassed; 1000 mL
Apparatus 2: 75 rpm
Time: 30 min
Mobile phase: Acetonitrile and 0.025 M hydrochloric acid (40:60)
Standard solution: (*L*/1000) mg/mL of USP Aripiprazole RS in *Mobile phase* where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter, discarding the first few mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of aripiprazole
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: Water and trifluoroacetic acid (100: 0.05)
Solution B: Acetonitrile and trifluoroacetic acid (100: 0.05)
Solution C: 2.84 g/L of sodium sulfate in water
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20	70	30
40	42	58
50	10	90
55	10	90
56	90	10
60	90	10

[NOTE—The gradient was established on an HPLC system with a dwell volume of approximately 1.0 mL.]
Diluent: Acetonitrile, methanol, *Solution C*, and glacial acetic acid (33:11:56:1)
System suitability solution: 250 µg/mL of USP Aripiprazole RS, and 0.5 µg/mL each of USP Aripiprazole Related Compound F RS and USP Aripiprazole Related Compound G RS in *Diluent*. Sonication may be used to aid in dissolution.
Sample solution: Nominally 0.2–0.3 mg/mL of aripiprazole from NLT 5 Orally Disintegrating Tablets prepared as follows. Transfer NLT 5 Orally Disintegrating Tablets to a suitable volumetric flask. Add about 70% of the total volume of *Diluent*. Sonicate for 10 min and shake for 10 min. Dilute with *Diluent* to volume. Pass the resulting solution through a suitable filter and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between aripiprazole related compound G and aripiprazole; NLT 1.5 between aripiprazole and aripiprazole related compound F
Analysis
Sample: *Sample solution*
Calculate the total peak response for individual impurities and aripiprazole from the *Sample solution*:

$$\text{Result} = \Sigma[r_i \times (1/F)] + r_U$$

r_i = peak response of each degradation product from the *Sample solution*
F = relative response factor (see *Table 2*)
r_U = peak response of aripiprazole from the *Sample solution*

Calculate the percentage of each degradation product in the portion of Orally Disintegrating Tablets taken:

$$\text{Result} = (r_i/r_T) \times (1/F) \times 100$$

r_i = peak response of each degradation product from the *Sample solution*
r_T = total peak response for individual impurities and aripiprazole from the *Sample solution*
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

www.webofpharma.com

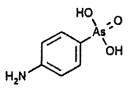
Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Aripiprazole related compound G	0.96	0.77	0.3
Aripiprazole	1.0	—	—
Aripiprazole related compound F	1.03	1.0	0.3
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aripiprazole RS
  USP Aripiprazole Related Compound F RS
  4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazine 1-oxide.
  $C_{23}H_{27}Cl_2N_3O_3$ 464.38
  USP Aripiprazole Related Compound G RS
  7-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy}quinolin-2(1*H*)-one.
  $C_{23}H_{25}Cl_2N_3O_2$ 446.37

Arsanilic Acid



$C_6H_8AsNO_3$ 217.05
p-Aminobenzenearsonic acid [98-50-0].

» Arsanilic Acid contains not less than 98.0 percent and not more than 102.0 percent of $C_6H_8AsNO_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Arsanilic Acid RS

Change to read:

Identification, ▲ ***Spectroscopic Identification Tests*** ⟨ 197 ⟩, *Infrared Spectroscopy:* ***197K***▲ (CN 1-May-2020)
Loss on drying ⟨731⟩—Dry it in vacuum at 80° for 4 hours: it loses not more than 0.5% of its weight.

Limit of *o*-arsanilic acid—
Mobile phase—Dissolve 4.04 g of monobasic potassium phosphate in 985 mL of water, add 2 mL of phosphoric acid, and mix. Add 10 mL of methanol, mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution—Transfer about 67 mg of *o*-arsanilic acid, accurately weighed, to a 100-mL volumetric flask, add about 65 mg of warm (about 70° to 80°) water, and shake or sonicate to dissolve. Allow to cool, dilute with water to volume, and mix. Dilute a portion of this solution quantitatively and stepwise with water to obtain a solution having a known concentration of about 0.0012 mg of *o*-arsanilic acid per mL.
Test solution—Transfer about 50 mg of Arsanilic Acid, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of warm water, and shake or sonicate to dissolve. Allow to cool, dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 242-nm detector and a 4.6-mm × 15-cm column that contains 5-µm base-deactivated packing L1 and is maintained at a constant temperature of about 30°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the capacity factor, *k'*, for the *o*-arsanilic acid peak is between 2.8 and 3.8; and the relative standard deviation for replicate injections is not more than 2.5%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the responses for the *o*-arsanilic acid peaks. Calculate the percentage of *o*-arsanilic acid in the portion of Arsanilic Acid taken by the formula:

$$5000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of *o*-arsanilic acid in the *Standard solution*; W is the weight, in mg, of Arsanilic Acid taken to prepare the *Test solution*; and r_U and r_S are the responses of the *o*-arsanilic acid peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.12% is found.

Limit of aniline—
Mobile phase—Dissolve 7.76 g of monobasic potassium phosphate in 950 mL of water, add 50 mL of methanol, mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution—Transfer about 176 mg of aniline, accurately weighed, to a 25-mL volumetric flask, add about 1 mL of methanol, swirl, then add about 15 mL of water, and shake to dissolve. Dilute with water to volume, and mix. Dilute a portion of this solution quantitatively and stepwise with water to obtain a solution having a known concentration of about 0.00045 mg of aniline per mL.
Test solution—Transfer about 50 mg of Arsanilic Acid, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of warm (about 70° to 80°) water, and shake or sonicate to dissolve. Allow to cool, dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 235-nm detector and a 4.6-mm × 15-cm column that contains 5-µm base-deactivated packing L1 and is maintained at a constant temperature of about 30°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the capacity factor, *k'*, for the aniline peak is between 2.3 and 3.3; and the relative standard deviation for replicate injections is not more than 3.0%.
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the responses for the aniline peaks. Calculate the percentage of aniline in the portion of Arsanilic Acid taken by the formula:

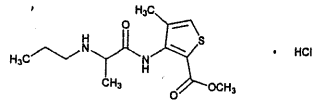
$$5000(C/W)(r_U/r_S)$$

www.webofpharma.com

in which C is the concentration, in mg per mL, of aniline in the *Standard solution*; *W* is the weight, in mg, of Arsanilic Acid taken to prepare the *Test solution*; and r_U and r_S are the responses of the aniline peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.045% is found.

Assay—Transfer about 125 mg of Arsanilic Acid, accurately weighed, to a 50-mL conical flask, and add 10.0 mL of a mixture of sulfuric acid, nitric acid, and perchloric acid (1000:50:50) and several glass beads. Digest on a hot plate for about 1 hour, increasing the temperature of the hot plate in steps until a ring of sulfuric acid rises into the neck of the flask. Allow to cool, to the colorless solution add about 400 mg of hydrazine sulfate, and heat the flask vigorously on a hot plate until a ring of sulfuric acid rises into the neck of the flask. Allow to cool, and wash down the rim, neck, and insides of the flask with about 1 mL of water. Heat the flask again until a ring of sulfuric acid rises into the neck of the flask. Allow to cool, and transfer the colorless solution, with the aid of about 80 mL of water, to a 125-mL conical flask. Add 10 mL of hydrochloric acid and several drops of 0.002 M potassium iodide, cool to between 0° and 5°, and titrate with 0.1 N potassium permanganate VS to a pale pink endpoint, maintaining the temperature between 0° and 5° during the titration. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N potassium permanganate is equivalent to 10.852 mg of $C_6H_8AsNO_3$.

Articaine Hydrochloride



$C_{13}H_{20}N_2O_3S \cdot HCl$ 320.84

2-Thiophenecarboxylic acid, 4-methyl-3-[[1-oxo-2-(propylamino)propyl]amino]-, methyl ester, monohydrochloride;

Methyl 4-methyl-3-[2-(propylamino)propionamido]-2-thiophenecarboxylate, monohydrochloride [23964-57-0].

DEFINITION

Articaine Hydrochloride contains NLT 98.5% and NMT 101.0% of $C_{13}H_{20}N_2O_3S \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

  Standard solution: 12 mg/mL of USP Articaine RS in methylene chloride. Transfer 20 µL of this solution onto a 300-mg disk.

  Sample solution: Dissolve 100 mg of Articaine Hydrochloride in 5 mL of water. Add 3 mL of a saturated solution of sodium bicarbonate, and shake twice with 2 mL of methylene chloride. Combine the methylene chloride layers, dilute with methylene chloride to 5.0 mL, and dry over anhydrous sodium sulphate. Transfer 20 µL of this solution onto a 300-mg disk.
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

ASSAY

- **PROCEDURE**

  Sample solution: 250 mg of Articaine Hydrochloride to a 250-mL conical flask. Add 5.0 mL of 0.01 M hydrochloric acid and 50 mL of alcohol. Stir to dissolve.

  Analysis: Titrate with 0.1 M sodium hydroxide VS, determining the endpoint potentiometrically, using a glass electrode. Calculate the volume of sodium hydroxide consumed by reading the volume added between the two points of inflection. Each mL of 0.1 M sodium hydroxide is equivalent to 32.08 mg of $C_{13}H_{20}N_2O_3S \cdot HCl$.

  Acceptance criteria: 98.5%–101.0% on the dried basis.

IMPURITIES

INORGANIC IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩

  Sample: 1 g

  Acceptance criteria: NMT 0.1%

ORGANIC IMPURITIES

- **Procedure**

Buffer solution: 2.02 g of sodium 1-heptanesulfonate and 4.08 g of potassium dihydrogen phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 2.0.

Mobile phase: Acetonitrile and *Buffer solution* (1:3)

Standard solution: 2 µg/mL of USP Articaine Related Compound A , and 1 µg/mL each of USP Articaine Related Compound E RS and USP Articaine Hydrochloride RS in *Mobile phase*. [NOTE—This solution is also used to determine the reporting threshold limit.]

Sample solution: 1.0 mg/mL of Articaine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 4.6-mm × 25-cm; packing L1

Temperature: 45°

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between articaine related compound A and articaine related compound E

Analysis

Samples: *Standard solution* and *Sample solution*. [NOTE—Run time is 5 times the retention time of articaine.]

Calculate the percentage of any articaine related compound A in the portion of Articaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response of articaine related compound A from the *Sample solution*

r_S = response of articaine related compound A from the *Standard solution*

C_S = concentration of USP Articaine Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Articaine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each individual impurity in the portion of Articaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response of each individual impurity from the *Sample solution*

www.webofpharma.com

r_S = response of articaine hydrochloride from the *Standard solution*
C_S = concentration of USP Articaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Articaine Hydrochloride in the *Sample solution* (mg/mL)

[NOTE—Disregard any peak below 0.05%.]
Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%. [NOTE—Excluding articaine related compound A.]

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Articaine acid[a]	0.6	0.1
Ethylarticaine[b]	0.7	0.1
Articaine related compound A[c]	0.8	0.2
Articaine related compound E[d]	0.86	0.1
Articaine acid-propionamide[e]	0.9	0.1
Articaine	1.0	—
Butylarticaine[f]	1.7	0.1
Dipropylarticaine[g]	2.1	0.1
3-Aminoarticaine[h]	2.6	0.1
Articaine isopropyl ester[i]	3.6	0.1
Bromo compound[j]	4.0	0.1
Any other individual impurity	—	0.10

[a] 4-Methyl-3-[[(2*RS*)-2-(propylamino)propanoyl]amino]thiophene-2-carboxylic acid.
[b] Methyl 3-[[(2*RS*)-2-(ethylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate.
[c] Methyl 4-methyl-3-[2-(propylamino)acetamido]thiophene-2-carboxylate.
[d] Methyl 3-[2-(isopropylamino)propanamido]-4-methylthiophene-2-carboxylate.
[e] 4-Methyl-*N*-propyl-3-[[(2*RS*)-2-(propylamino)propanoyl]amino]thiophene-2-carboxamide.
[f] Methyl 3-[[(2*RS*)-2-(butylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate.
[g] Methyl 3-[[(2*RS*)-2-(dipropylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate.
[h] Methyl 3-amino-4-methylthiophene-2-carboxylate.
[i] 1-Methylethyl 4-methyl-3-[[(2*RS*)-2-(propylamino)propanoyl]amino] thiophene-2-carboxylate.
[j] Methyl 3-[[(2*RS*)-2-bromopropanoyl]amino]-4-methylthiophene-2-carboxylate.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩: Dry at 105° for 5 h: it loses NMT 0.5% of its weight.
• **pH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 4.2–5.2

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Articaine RS
USP Articaine Hydrochloride RS
USP Articaine Related Compound A RS
Methyl 4-methyl-3-[2-(propylamino) acetamido] thiophene-2-carboxylate.
$C_{12}H_{18}N_2O_3S$ 270.35
USP Articaine Related Compound E RS
Methyl 3-[2-(isopropylamino) propanamido]-4-methylthiophene-2-carboxylate.
$C_{13}H_{20}N_2O_3S$ 284.37

Articaine Hydrochloride and Epinephrine Injection

DEFINITION
Articaine Hydrochloride and Epinephrine Injection is a sterile solution of Articaine Hydrochloride and Epinephrine, in Water for Injection, and contains NLT 95.0% and NMT 105.0% of the labeled amount of articaine hydrochloride ($C_{13}H_{20}N_2O_3S \cdot HCl$) and NLT 90.0% and NMT 115.0% of the labeled amount of epinephrine ($C_9H_{13}NO_3$).

IDENTIFICATION
• **A.** The retention times of the articaine and epinephrine peaks from the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assays* for *Articaine Hydrochloride* and *Epinephrine*, respectively.

ASSAY
• **ARTICAINE HYDROCHLORIDE**
Buffer: Glacial acetic acid and water (50:930). Adjust with 2 N sodium hydroxide to a pH of 3.4.
Mobile phase: Acetonitrile and *Buffer* (22:78)
Standard stock solution: 40 mg/mL of USP Articaine Hydrochloride RS in water
Standard solution: 0.8 mg/mL of USP Articaine Hydrochloride RS in *Mobile phase* from *Standard stock solution*
Sample solution: Equivalent to 0.8 mg/mL of articaine hydrochloride in *Mobile phase* from a portion of Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
Run time: 2.5 times the retention time of articaine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.2
Relative standard deviation: NMT 1.0%, from six injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of articaine hydrochloride ($C_{13}H_{20}N_2O_3S \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Articaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of articaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

USP Monographs

www.webofpharma.com

• **EPINEPHRINE**

Mobile phase: Mix 50 mL of glacial acetic acid and 930 mL of water. Adjust with 2 N sodium hydroxide to a pH of 3.4. In this solution, dissolve 1.2 g of sodium 1-heptanesulfonate, and add 1.0 mL of 0.1 M edetate disodium and 0.298 g of potassium chloride. Add 150 mL of methanol.

Diluent: 0.5 mg/mL potassium metabisulfite in water

System suitability solution: 22 µg/mL of epinephrine from USP Epinephrine Bitartrate RS and 20 µg/mL of norepinephrine from USP Norepinephrine Bitartrate RS in *Diluent*

Standard stock solution: 0.55 mg/mL of epinephrine from USP Epinephrine Bitartrate RS in *Diluent*

Standard solution: Dilute a suitable volume of the *Standard stock solution* with *Diluent* to obtain a final concentration of *L* mg/mL of epinephrine, where *L* is the label claim of epinephrine in the Injection.

Sample solution: Use the Injection directly.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Amperometric electrochemical

Reference electrode: Silver/silver chloride

Working electrode: Glassy carbon

Potential: +650 mV

Detector temperature: 28 ± 2°

Column: 4.0-mm × 25-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection size: 2 µL

Run time: 1.7 times the retention time of epinephrine

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for norepinephrine and epinephrine are 0.90 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between the norepinephrine and epinephrine peaks

Tailing factor: NMT 2.0 for the epinephrine peak

Relative standard deviation: NMT 1.0% for the epinephrine peak, from six injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of epinephrine ($C_9H_{13}NO_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of epinephrine in the *Standard solution* (mg/mL)

C_U = nominal concentration of epinephrine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

• **DELIVERABLE VOLUME** ⟨698⟩

For Articaine Hydrochloride and Epinephrine Injection packaged in single-dose containers: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES, LIMIT OF ARTICAINE RELATED COMPOUNDS**

Mobile phase, Standard stock solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay* for *Articaine Hydrochloride*.

Standard solution: 0.8 mg/mL of USP Articaine Hydrochloride RS from *Standard stock solution* and 40 µg/mL of USP Articaine Related Compound B RS in *Mobile phase*

System suitability

Sample: *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Tailing factor: NMT 2.2 for the articaine peak

Resolution: NLT 1.25 between the articaine related compound B and articaine peaks

Relative standard deviation: NMT 1.0% for the articaine peak

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Articaine related compounds elute at relative retention times of NMT 2.0 with respect to the articaine peak.]

Calculate the percentage of articaine related compounds and any other individual impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response of each individual impurity from the *Sample solution*

r_S = response of articaine from the *Standard solution*

C_S = concentration of articaine in the *Standard solution* (mg/mL)

C_U = nominal concentration of articaine in the *Sample solution* (mg/mL)

[NOTE—Disregard any peak below 0.05%.]

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Articaine related compound B	0.6	0.5
Articaine	1.0	—
Any other individual impurity	—	0.2
Total impurities	—	0.5

• **ORGANIC IMPURITIES, LIMIT OF EPINEPHRINE RELATED COMPOUNDS**

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* for *Epinephrine*.

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Epinephrine related compounds elute between relative retention times of 0.35 and 1.0, with respect to the epinephrine peak.]

Calculate the percentage of epinephrine related compounds and any other individual impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response of each individual impurity from the *Sample solution*

r_S = response of epinephrine from the *Standard solution*

C_S = concentration of epinephrine in the *Standard solution* (mg/mL)

www.webofpharma.com

C_U = nominal concentration of epinephrine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2.*

Table 2

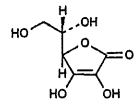
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Epinephrine sulfonate[a]	0.46	7.5
Specified impurity	0.52	8
Epinephrine	1.0	—
Any other individual impurity	—	1
Total impurities	—	10

[a] 1-(3,4-Dihydroxyphenyl)-2-(methylamino)ethanesulfonic acid.

SPECIFIC TESTS
- **pH** ⟨791⟩: 2.7–5.2
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 0.7 USP Endotoxin Unit/mg of articaine hydrochloride
- **Sterility Tests** ⟨71⟩: It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements
- **Other Requirements:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass. Store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Articaine Hydrochloride RS
  USP Articaine Related Compound B RS
  4-Methyl-3-{[2-(propylamino)propanoyl]amino} thiophene-2-carboxylic acid.
  $C_{12}H_{18}N_2O_3S$ 270.35
  USP Epinephrine Bitartrate RS
  USP Norepinephrine Bitartrate RS

Ascorbic Acid



$C_6H_8O_6$ 176.12
L-Ascorbic acid [50-81-7].

DEFINITION
Ascorbic Acid contains NLT 99.0% and NMT 100.5% of $C_6H_8O_6$.

IDENTIFICATION

Change to read:

- **A.** **▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** A 20-mg/mL solution reduces alkaline cupric tartrate TS slowly at room temperature but more readily upon heating.

ASSAY
- **Procedure**
  Sample: 400 mg of Ascorbic Acid
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N iodine VS
  Endpoint detection: Visual
  Blank: 100 mL of water and 25 mL of 2 N sulfuric acid. Add 3 mL of starch TS.
  Analysis: Dissolve the *Sample* in a mixture of 100 mL of water and 25 mL of 2 N sulfuric acid. Add 3 mL of starch TS, and titrate immediately with *Titrant* until a persistent violet-blue color is obtained.
  Calculate the percentage of ascorbic acid ($C_6H_8O_6$) in the portion of Ascorbic Acid taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/W$$

V = sample titrant volume (mL)
B = blank titrant volume (mL)
N = titrant normality (mEq/mL)
F = equivalency factor, 88.06 mg/mEq
W = weight of *Sample* (mg)

Acceptance criteria: 99.0%–100.5%

IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.1%

SPECIFIC TESTS
- **Optical Rotation,** *Specific Rotation* ⟨781S⟩
  Sample solution: 100 mg/mL in carbon dioxide-free water. Perform the test immediately after preparation of the *Sample solution.*
  Acceptance criteria: +20.5° to +21.5°

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩
  USP Ascorbic Acid RS

Ascorbic Acid Injection

DEFINITION
Ascorbic Acid Injection is a sterile solution, in Water for Injection, of Ascorbic Acid prepared with the aid of Sodium Hydroxide, Sodium Carbonate, or Sodium Bicarbonate. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ascorbic acid ($C_6H_8O_6$).

IDENTIFICATION
- **A.**
  Analysis: To a volume of Injection, equivalent to 40 mg of ascorbic acid, add 4 mL of 0.1 N hydrochloric acid, then add 4 drops of methylene blue TS, and warm to 40°.
  Acceptance criteria: The deep blue color becomes appreciably lighter or is completely discharged within 3 min.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* obtained as directed in the *Assay.*
- **C.** The Injection imparts an intense yellow color to a nonluminous flame.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Mobile phase: Dissolve 15.6 g of dibasic sodium phosphate and 12.2 g of monobasic potassium phosphate in 2000 mL of water, and adjust with phosphoric acid to a pH of 2.5 ± 0.05.

Standard solution: 0.5 mg/mL of USP Ascorbic Acid RS in *Mobile phase*. [NOTE—Refrigerate and store protected from light until use. The solution is stable for at least 24 h. Inject within 3 h after removal from the refrigerator.]

Sample solution: Dilute the Injection, if necessary, with *Mobile phase* to obtain a solution with a concentration of about 0.5 mg/mL. [NOTE—Refrigerate and store protected from light until use. The solution is stable for at least 24 h. Inject within 3 h after removal from the refrigerator.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 150-cm × 6-mm; packing L39

Flow rate: 0.6 mL/min

Injection volume: 4 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3500 theoretical plates

Tailing factor: NMT 1.6

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ascorbic acid ($C_6H_8O_6$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ascorbic Acid RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ascorbic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **LIMIT OF OXALATE**

Analysis: Dilute a volume of Injection, equivalent to 50 mg of ascorbic acid, with water to 5 mL. Add 0.2 mL of acetic acid and 0.5 mL of calcium chloride TS.

Acceptance criteria: No turbidity is produced in 1 min.

SPECIFIC TESTS

• **pH** ⟨791⟩: 5.5–7.0

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 1.2 USP Endotoxin Units/mg of ascorbic acid.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in light-resistant, single-dose containers, preferably of Type I or Type II glass.

• **LABELING:** In addition to meeting the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*, fused-seal containers of the Injection in concentrations of 250 mg/mL and greater are labeled to indicate that since pressure may develop on long storage, precautions should be taken to wrap the container in a protective covering while it is being opened.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ascorbic Acid RS

Ascorbic Acid Oral Solution

DEFINITION

Ascorbic Acid Oral Solution is a solution of Ascorbic Acid in a hydroxylic organic solvent or an aqueous mixture thereof. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ascorbic acid ($C_6H_8O_6$).

IDENTIFICATION

• **A.**

Sample solution: A volume of Oral Solution equivalent to 40 mg of ascorbic acid

Analysis: To the *Sample solution* add 4 mL of 0.1 N hydrochloric acid, then 4 drops of methylene blue TS, and warm to 40°.

Acceptance criteria: The deep blue color becomes appreciably lighter or is completely discharged within 3 min.

• **B.**

Sample solution: A volume of Oral Solution equivalent to 20 mg of ascorbic acid

Analysis: To the *Sample solution* add 15 mL of trichloroacetic acid solution (1 in 20). Add 200 mg of activated charcoal, shake the mixture vigorously for 1 min, and pass through a small fluted filter, returning the filtrate, if necessary, until clear. To 5 mL of the filtrate add 1 drop of pyrrole, agitate gently until dissolved, then heat in a bath at 50°.

Acceptance criteria: A blue color develops.

ASSAY

• **PROCEDURE**

Sample solution: Transfer a volume of Oral Solution equivalent to 50 mg of ascorbic acid, previously diluted with water if necessary, to a 100-mL volumetric flask. Add 20 mL of metaphosphoric–acetic acid TS, dilute with water to volume, and mix.

Blank: A mixture of 5.5 mL of metaphosphoric–acetic acid TS and 15 mL of water

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: Standard dichlorophenol–indophenol VS

Analysis: Transfer a volume of the *Sample solution*, equivalent to 2 mg of ascorbic acid, into a 50-mL conical flask. Add 5 mL of metaphosphoric–acetic acid TS, and titrate with *Titrant* until a rose-pink color persists for at least 5 s. Correct for the volume of the *Titrant* consumed by the *Blank*.

Calculate the percentage of ascorbic acid ($C_6H_8O_6$) in the portion of Oral Solution taken:

$$\text{Result} = \{[(V_S - V_B) \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample solution* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

F = concentration of *Titrant* in terms of its equivalent of ascorbic acid (mg/mL)

W = nominal amount of ascorbic acid taken for *Analysis* (mg)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **ALCOHOL DETERMINATION,** *Method I* ⟨611⟩ (if present): 90.0%–110.0% of the labeled content of alcohol (C_2H_5OH)

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label Oral Solution that contains alcohol to state the alcohol content.

Ascorbic Acid Compounded Oral Solution

DEFINITION
Ascorbic Acid Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of ascorbic acid ($C_6H_8O_6$).
Prepare Ascorbic Acid Compounded Oral Solution, 100 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Ascorbic Acid	10 g
Purified Water	50 mL
Ora-Sweet,[a] a sufficient quantity to make	100 mL

[a] Perrigo Laboratories, Allegan, MI.

Place the *Ascorbic Acid* into a suitable container. Dissolve the powder in the *Purified Water*. Transfer the contents to a calibrated container. Add sufficient *Ora-Sweet* to bring to final volume. Shake to mix well.

ASSAY
- **PROCEDURE**

Mobile phase: 50 mM monobasic sodium phosphate; adjusted with phosphoric acid to a pH of 2.5
Standard solution: 0.2 mg/mL of USP Ascorbic Acid RS in *Mobile phase*
Sample solution: Transfer 0.2 mL of Oral Solution into a 100-mL volumetric flask and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L96
Temperatures
Autosampler: 5°
Column: 10°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for ascorbic acid is about 4.4 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ascorbic acid ($C_6H_8O_6$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ascorbic acid from the *Sample solution*
r_S = peak response of ascorbic acid from the *Standard solution*
C_S = concentration of USP Ascorbic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ascorbic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 1.8–2.8

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant plastic containers. Store in a refrigerator or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator; or NMT 60 days after the date on which it was compounded when stored at controlled room temperature
- **LABELING:** Label it to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Ascorbic Acid RS

Ascorbic Acid Tablets

DEFINITION
Ascorbic Acid Tablets contain ascorbic acid in the form of ascorbic acid ($C_6H_8O_6$), sodium ascorbate ($C_6H_7NaO_6$), calcium ascorbate dihydrate ($C_{12}H_{14}CaO_{12} \cdot 2H_2O$), or their mixture in an amount equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ascorbic acid ($C_6H_8O_6$).

IDENTIFICATION
- **A.**

Sample solution: Triturate a quantity of finely powdered Tablets with diluted alcohol to make a solution of ascorbic acid with a concentration of 20 mg/mL, and filter.
Analysis: Add alkaline cupric tartrate TS to a portion of the *Sample solution*.
Acceptance criteria: The *Sample solution* reduces alkaline cupric tartrate TS slowly at room temperature but more readily upon heating.
- **B.**

Sample solution: Use the *Sample solution* from *Identification* test *A*.
Analysis: To 2 mL of the *Sample solution* add 4 drops of methylene blue TS, and warm to 40°.
Acceptance criteria: The deep blue color of methylene blue becomes appreciably lighter or is completely discharged within 3 min.
- **C.**

Sample solution: Use the *Sample solution* from *Identification* test *A*.
Analysis: To 1 mL of the *Sample solution* add 15 mL of trichloroacetic acid solution (1 in 20) and 200 mg of activated charcoal, shake the mixture vigorously for 1 min, and pass through a small fluted filter, returning the filtrate if necessary, until clear. To 5 mL of the filtrate add 1 drop of pyrrole, agitate gently until dissolved, and then heat in a bath at 50°.
Acceptance criteria: A blue color develops.

ASSAY
[NOTE—Where more than one assay procedure is given in the monograph, the requirements may be met by following any one of the specified procedures, the

USP Monographs

www.webofpharma.com

procedure used being stated in the labeling only if *Procedure 1* is not used.]

• **PROCEDURE 1**

Sample stock solution: Transfer NLT 20 Tablets to a 1000-mL volumetric flask containing 250 mL of metaphosphoric–acetic acids TS. Insert the stopper in the flask, and shake by mechanical means for 30 min or until the Tablets have disintegrated completely. Dilute with water to volume.

Sample solution: Transfer a portion of the *Sample stock solution* to a centrifuge tube, and centrifuge until a clear supernatant is obtained. Quantitatively dilute the clear supernatant with water, if necessary, to obtain a solution containing 0.5 mg/mL of ascorbic acid.

Blank: A mixture of 5.5 mL of metaphosphoric–acetic acids TS and 15 mL of water

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: Standard dichlorophenol–indophenol VS

Endpoint detection: Visual, a rose-pink color that persists for at least 5 s

Analysis: Transfer a volume of the *Sample solution*, equivalent to 2 mg of ascorbic acid, to a 50-mL conical flask. Add 5 mL of metaphosphoric–acetic acids TS, and titrate with *Titrant*. Correct for the volume of the *Titrant* consumed by the *Blank*.

Calculate the percentage of the labeled amount of ascorbic acid ($C_6H_8O_6$) in the portion of Tablets taken:

$$\text{Result} = [(V_S - V_B) \times F/W] \times 100$$

V_S = *Titrant* volume consumed by the *Sample solution* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
F = concentration of the *Titrant* in terms of the equivalent of ascorbic acid (mg/mL)
W = nominal weight of ascorbic acid taken for *Analysis* (mg)

Acceptance criteria: 90.0%–110.0%

• **PROCEDURE 2**

(See *Vitamin C Assay* ⟨580⟩, *Method II—Chromatographic Method*.)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Sample solution: Withdraw a portion of the solution under test, pass through a suitable filter, and use the pooled sample as the test specimen.

Analysis: Proceed as directed in the *Assay, Procedure 1* or *Procedure 2*, conducting the procedure without delay and making any necessary modifications.

Calculate the percentage of the labeled amount of ascorbic acid ($C_6H_8O_6$) dissolved:

For *Procedure 1*

$$\text{Result} = [(V_S - V_B) \times F \times (V_M/a)/L] \times 100$$

V_S = *Titrant* volume consumed by the *Sample solution* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
F = concentration of the *Titrant* in terms of the equivalent of ascorbic acid (mg/mL)
V_M = volume of *Medium*, 900 mL
a = volume of the aliquot taken for *Analysis*
L = label claim of ascorbic acid (mg/Tablet)

For *Procedure 2*

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak area of ascorbic acid from the *Sample solution*
r_S = peak area of ascorbic acid from the *Standard solution*
C_S = concentration of USP Ascorbic Acid RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of ascorbic acid ($C_6H_8O_6$) is dissolved.

• **DISINTEGRATION** ⟨701⟩

[NOTE—Meet this additional test if the label recommends to disintegrate the Tablets in the mouth before swallowing.]

Medium: Water

Time: NMT 5 min

Acceptance criteria: Meet the requirements

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

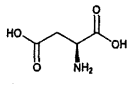
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **LABELING:** The label states the quantity of ascorbic acid in mg/Tablet, and the chemical form of ascorbic acid present in the Tablets. The labeling states with which assay procedure the product complies only if *Procedure 1* is not used. Tablets that are intended to be disintegrated in the mouth before swallowing are so labeled.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ascorbic Acid RS

Aspartic Acid



$C_4H_7NO_4$ 133.10

L-Aspartic acid [56-84-8].

DEFINITION

Aspartic Acid contains NLT 98.5% and NMT 101.5% of aspartic acid ($C_4H_7NO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Sample: 100 mg of Aspartic Acid

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N sodium hydroxide VS

Endpoint detection: Visual

USP Monographs

www.webofpharma.com

Blank: 50 mL of carbon dioxide-free water. Add 0.1 mL of bromothymol blue TS.

Analysis: Transfer the *Sample* to a 125-mL flask, and dissolve in 50 mL of carbon dioxide-free water. Heat slightly if necessary. Cool, add 0.1 mL of bromothymol blue TS, and titrate with *Titrant* until the color changes from yellow to blue. Perform the blank determination.

Calculate the percentage of aspartic acid ($C_4H_7NO_4$) in the portion of Aspartic Acid taken:

$$\text{Result} = [(V_S - V_B) \times N_A \times F \times 100]/W$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N_A = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 133.1 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*

Sample solution: Dissolve 0.7 g of Aspartic Acid in 10 mL of diluted nitric acid, and dilute with water to 15 mL.

Acceptance criteria: The *Sample solution* shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (NMT 0.02%).

- **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*

Sample solution: Dissolve 0.8 g of Aspartic Acid in 4 mL of hydrochloric acid, and dilute with water to 15 mL.

Acceptance criteria: The *Sample solution* shows no more sulfate than corresponds to 0.25 mL of 0.020 N sulfuric acid (NMT 0.03%).

- **IRON** ⟨241⟩: NMT 10 ppm
- **RELATED COMPOUNDS**

Mobile phase: 0.008 N sulfuric acid

System suitability solution: A mixture of 0.1 mg/mL of USP Fumaric Acid RS, 0.05 mg/mL of USP Maleic Acid RS, and 1.5 mg/mL of USP Malic Acid RS in water

Fumaric acid standard solution: 0.1 mg/mL of USP Fumaric Acid RS in water

Maleic acid standard solution: 0.05 mg/mL of USP Maleic Acid RS in water

Malic acid standard solution: 1.5 mg/mL of USP Malic Acid RS in water

Sample solution: Transfer 10 g of Aspartic Acid to a 100-mL volumetric flask, add 15–20 mL of 6 N hydrochloric acid, and mix to dissolve. Dilute with water to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 7.8-mm × 30-cm; 9-µm packing L17
Column temperature: 30°
Flow rate: 0.6 mL/min
Injection volume: 10 µL

System suitability
Sample: *System suitability solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between maleic acid and malic acid
Relative standard deviation: NMT 10.0% each for maleic acid, malic acid, and fumaric acid

Analysis
Samples: *Standard solutions* and *Sample solution*
[NOTE—A hydrochloric acid peak at around 6–7 min may be observed in the chromatogram of the *Sample solution*. Disregard this peak in the calculation of the impurity.]

Calculate the percentage of each specified acid in the portion of Aspartic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of maleic acid, malic acid, or fumaric acid from the *Sample solution*
r_S = peak response of maleic acid, malic acid, or fumaric acid from the corresponding *Standard solution*
C_S = concentration of USP Maleic Acid RS, USP Malic Acid RS, or USP Fumaric Acid RS in the corresponding *Standard solution* (mg/mL)
C_U = concentration of Aspartic Acid in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Aspartic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of fumaric acid from the *Fumaric acid standard solution*
C_S = concentration of USP Fumaric Acid RS in the *Fumaric acid standard solution* (mg/mL)
C_U = concentration of Aspartic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Maleic acid	0.5	0.05
Malic acid	0.6	0.20
Fumaric acid	1.0	0.10
Aspartic acid	Not observed	—
Any unspecified impurity	—	0.05
Total unspecified impurities	—	0.10

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 80 mg/mL in 6 N hydrochloric acid
Acceptance criteria: +24.0° to +26.0°, at 20°

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.5%

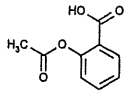
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Aspartic Acid RS
USP Fumaric Acid RS
USP Maleic Acid RS
USP Malic Acid RS

www.webofpharma.com

Aspirin



$C_9H_8O_4$ 180.16
Benzoic acid, 2-(acetyloxy)-.
Salicylic acid acetate [50-78-2].

» Aspirin contains not less than 99.5 percent and not more than 100.5 percent of $C_9H_8O_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Aspirin RS

Identification—
A: Heat it with water for several minutes, cool, and add 1 or 2 drops of ferric chloride TS: a violet-red color is produced.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Loss on drying ⟨731⟩—Dry it over silica gel for 5 hours: it loses not more than 0.5% of its weight.

Readily carbonizable substances ⟨271⟩—Dissolve 500 mg in 5 mL of sulfuric acid : the solution has no more color than *Matching Fluid Q*.

Residue on ignition ⟨281⟩: not more than 0.05%.

Substances insoluble in sodium carbonate TS—A solution of 500 mg in 10 mL of warm sodium carbonate TS is clear.

Chloride ⟨221⟩—Boil 1.5 g with 75 mL of water for 5 minutes, cool, add sufficient water to restore the original volume, and filter. A 25-mL portion of the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).

Sulfate —Dissolve 6.0 g in 37 mL of acetone, and add 3 mL of water. Titrate potentiometrically with 0.02 M lead perchlorate, prepared by dissolving 9.20 g of lead perchlorate in water to make 1000 mL of solution, using a pH meter capable of a minimum reproducibility of ±0.1 mV (see *pH* ⟨791⟩) and equipped with an electrode system consisting of a lead-specific electrode and a silver–silver chloride reference glass-sleeved electrode containing a solution of tetraethylammonium perchlorate in glacial acetic acid (1 in 44) (see *Titrimetry* ⟨541⟩): not more than 1.25 mL of 0.02 M lead perchlorate is consumed (0.04%). [NOTE—After use, rinse the lead-specific electrode with water, drain the reference electrode, flush with water, rinse with methanol, and allow to dry.]

Limit of free salicylic acid—Dissolve 2.5 g in sufficient alcohol to make 25.0 mL. To each of two matched color-comparison tubes add 48 mL of water and 1 mL of a freshly prepared, diluted ferric ammonium sulfate solution (prepared by adding 1 mL of 1 N hydrochloric acid to 2 mL of ferric ammonium sulfate TS and diluting with water to 100 mL). Into one tube pipet 1 mL of a standard solution of salicylic acid in water, containing 0.10 mg of salicylic acid per mL. Into the second tube pipet 1 mL of the 1 in 10 solution of Aspirin. Mix the contents of each tube: after 30 seconds, the color in the second tube is not more intense than that in the tube containing the salicylic acid (0.1%).

Assay—Place about 1.5 g of Aspirin, accurately weighed, in a flask, add 50.0 mL of 0.5 N sodium hydroxide VS, and boil the mixture gently for 10 minutes. Add phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 N sulfuric acid VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 0.5 N sodium hydroxide is equivalent to 45.04 mg of $C_9H_8O_4$.

Aspirin Boluses

DEFINITION
Aspirin Boluses contain NLT 90.0% and NMT 110.0% of the labeled amount of aspirin ($C_9H_8O_4$).

IDENTIFICATION
• **A. PROCEDURE**
Analysis: Crush 1 Bolus. Boil a portion of the powder, equivalent to 300 mg of aspirin, with 50 mL of water. Cool and add a drop of ferric chloride TS.
Acceptance criteria: A violet-red color is produced.
• **B.** The retention time of the aspirin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: 2 g/L of sodium 1-heptanesulfonate in a mixture of acetonitrile and water (15:85). Adjust with glacial acetic acid to a pH of 3.4.
Diluent: Acetonitrile and formic acid (99:1)
Standard solution: 0.4 mg/mL of USP Aspirin RS and 0.01 mg/mL of USP Salicylic Acid RS in *Diluent*
Sample stock solution: Nominally 4 mg/mL of aspirin prepared as follows. Finely powder NLT 10 Boluses. Transfer a portion of the powder to an appropriate volumetric flask and dilute with *Diluent* to volume. Stir the solution by mechanical means for 15 min.
Sample solution: Nominally 0.4 mg/mL of aspirin from *Sample stock solution* in *Diluent*. Pass a portion of this solution through a filter of 0.5-µm or finer pore size. Use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for salicylic acid and aspirin are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between salicylic acid and aspirin
Relative standard deviation: NMT 2.0% for aspirin
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Boluses taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aspirin from the *Sample solution*
r_S = peak response of aspirin from the *Standard solution*
C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)

USP Monographs

www.webofpharma.com

C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.5 M phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*), pH 7.4; 900 mL

Apparatus 2: 75 rpm

Time: 45 min

Diluent: Acetonitrile and formic acid (99:1)

Standard solution: USP Aspirin RS in *Diluent* at a suitable concentration. [NOTE—Prepare the solution at the time of use.]

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Diluent*, if necessary.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: The isosbestic point of aspirin and salicylic acid at 265 ± 2 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

D = dilution factor of the *Sample solution*, if necessary

L = label claim (mg/Bolus)

Tolerances: NLT 80% (Q) of the labeled amount of aspirin ($C_9H_8O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **LIMIT OF SALICYLIC ACID**

Mobile phase, Diluent, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Salicylic acid standard solution: 0.01 mg/mL of USP Salicylic Acid RS in *Diluent*

Analysis

Samples: *Sample solution* and *Salicylic acid standard solution*

Calculate the actual concentration (C), in mg/mL, of aspirin ($C_9H_8O_4$) in the *Sample solution* taken:

$$\text{Result} = C_U \times (F/100)$$

C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

F = percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Boluses taken, as determined in the *Assay*

Calculate the percentage of salicylic acid ($C_7H_6O_3$) in the portion of Boluses taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*

r_S = peak response of salicylic acid from the *Salicylic acid standard solution*

C_S = concentration of USP Salicylic Acid RS in the *Salicylic acid standard solution* (mg/mL)

C = actual concentration of aspirin in the *Sample solution*

Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label Boluses to indicate that they are for veterinary use only.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Aspirin RS

USP Salicylic Acid RS

Aspirin Capsules

» Aspirin Capsules contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of aspirin ($C_9H_8O_4$). [NOTE—Capsules that are enteric-coated or the contents of which are enteric-coated meet the requirements for *Aspirin Delayed-Release Capsules*.]

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Aspirin RS

Identification—

A: Heat about 100 mg of the Capsule contents with 10 mL of water for several minutes, cool, and add 1 drop of ferric chloride TS: a violet-red color is produced.

B: Shake a quantity of the contents of Capsules, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant and evaporate it to dryness. Dry the residue in vacuum at 60° for 1 hour: the residue responds to *Identification* — test *Identification*— under *Aspirin*.

Dissolution ⟨711⟩—

Medium: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of 4.50 ± 0.05; 500 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_9H_8O_4$ dissolved from UV absorbances at the wavelength of the isosbestic point of aspirin and salicylic acid at 265 ± 2 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same *Medium*. [NOTE—Prepare the Standard solution at the time of use. An amount of alcohol not to exceed 1% of the total volume of the Standard solution may be used to bring the Reference Standard into solution prior to dilution with *Medium*.]

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_9H_8O_4$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Limit of free salicylic acid—

Ferric chloride-urea reagent—Dissolve by swirling, without the aid of heat, 60 g of urea in a mixture of 8 mL of ferric chloride solution (6 in 10) and 42 mL of 0.05 N hydrochloric acid. Adjust the resulting solution, if necessary, with 6 N hydrochloric acid to a pH of 3.2.

www.webofpharma.com

Standard preparation—Transfer 75.0 mg of salicylic acid, previously dried over silica gel for 3 hours and accurately weighed, to a 100-mL volumetric flask, add chloroform to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with chloroform to volume, and mix. Transfer 10.0 mL of this last solution to a 50-mL volumetric flask containing 10 mL of methanol, 2 drops of hydrochloric acid, and 10 mL of a 1 in 10 solution of glacial acetic acid in ether, dilute with chloroform to volume, and mix.

Chromatographic column (see *Chromatography* ⟨621⟩)—Proceed as directed under *Column Partition Chromatography*, packing a chromatographic tube with two segments of packing material. The lower segment is a mixture of 1 g of *Solid Support* and 0.5 mL of 5 M phosphoric acid, and the upper segment is a mixture of 3 g of *Solid Support* and 2 mL of freshly prepared *Ferric chloride-urea reagent*.

Test preparation—Weigh accurately a portion of the contents of the Capsules, as determined by the *Assay*, equivalent to 100 mg of aspirin, mix with 10 mL of chloroform by stirring for 3 minutes, and then transfer to the chromatographic column with the aid of a few mL of chloroform. Pass 50 mL of chloroform through the column, rinse the tip of the chromatographic tube with chloroform, and discard the eluate. Prepare as a receiver a 50-mL volumetric flask containing 10 mL of methanol and 2 drops of hydrochloric acid, and elute any salicylic acid from the column by passing 10 mL of a 1 in 10 solution of glacial acetic acid in ether that has been recently saturated with water, followed by 30 mL of chloroform. Dilute the eluate with chloroform to volume, and mix.

Procedure—Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 306 nm, with a suitable spectrophotometer, using as the blank a solvent mixture of the same composition as that used for the *Standard preparation:* the absorbance of the *Test preparation* does not exceed that of the *Standard preparation* (0.75%, calculated on the labeled aspirin content).

Assay— [NOTE—In this assay use chloroform recently saturated with water.]

Standard preparation—Transfer about 50 mg of USP Aspirin RS, accurately weighed, to a 50-mL volumetric flask, add 0.5 mL of glacial acetic acid, add chloroform to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with a 1 in 100 solution of glacial acetic acid in chloroform to volume, and mix. The concentration of USP Aspirin RS is about 50 µg per mL.

Chromatographic column—Proceed as directed under *Column Partition Chromatography* (see *Chromatography* ⟨621⟩), packing a chromatographic tube with a mixture of 3 g of *Solid Support* and 2 mL of freshly prepared sodium bicarbonate solution (1 in 12).

Assay preparation—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to about 50 mg of aspirin, to a 50-mL volumetric flask containing 1 mL of a 1 in 50 solution of hydrochloric acid in methanol, add chloroform to volume, and mix. Transfer 5.0 mL of this solution to the column, wash with 5 mL and then with 25 mL of chloroform, and discard the washings. Elute into a 100-mL volumetric flask with about 10 mL of a 1 in 10 solution of glacial acetic acid in chloroform and then with about 85 mL of a 1 in 100 solution of glacial acetic acid in chloroform, dilute with the latter solvent to volume, and mix.

Procedure—Without delay, concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 280 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of aspirin ($C_9H_8O_4$) in the portion of Capsules taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Aspirin RS in the *Standard preparation;* and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation,* respectively.

Aspirin Delayed-Release Capsules

DEFINITION

Aspirin Delayed-Release Capsules contain NLT 93.0% and NMT 107.0% of the labeled amount of aspirin ($C_9H_8O_4$).

IDENTIFICATION

- **A.** The retention time of the aspirin peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay*.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

Sample: Shake a quantity of the contents of Capsules, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant and evaporate it to dryness. Dry the residue under vacuum at 60° for 1 h.

Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**

Mobile phase: 2 g/L of sodium 1-heptanesulfonate in a mixture of acetonitrile and water (15:85). Adjust with glacial acetic acid to a pH of 3.4.

Diluent: Acetonitrile and formic acid (99:1)

Standard solution: 0.5 mg/mL of USP Aspirin RS in *Diluent*

Sample stock solution: Nominally 5 mg/mL of aspirin prepared as follows. Remove, as completely as possible, the contents of NLT 20 Capsules. Mix the combined contents, and transfer a quantity equivalent to about 100 mg of aspirin to a suitable container. Add 20.0 mL of *Diluent* and about 10 glass beads. Shake vigorously for about 10 min, and centrifuge.

Sample solution: Nominally 0.5 mg/mL of aspirin in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

www.webofpharma.com

r_U = peak response of aspirin from the *Sample solution*
r_S = peak response of aspirin from the *Standard solution*
C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method A Procedure*
Apparatus 1: 100 rpm
Time: 90 min, for *Buffer stage*; 2 h, for *Acid stage*
Diluent: 0.1 N hydrochloric acid and 0.20 M tribasic sodium phosphate (3:1). Adjust, if necessary, with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8 ± 0.05.
Standard solution: A known concentration of USP Aspirin RS in a suitable medium
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with 0.1 N hydrochloric acid (for analyzing in the *Acid stage*) and with *Diluent* (for analyzing in the *Buffer stage*), if necessary.
Instrumental conditions
Mode: UV
Analytical wavelengths
Acid stage: 280 nm
Buffer stage: 265 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of aspirin ($C_9H_8O_4$) dissolved from UV absorbances at the isosbestic point of aspirin and salicylic acid (about 280 nm in the *Acid stage*, and about 265 nm in the *Buffer stage*).
Tolerances: The percentages of the labeled amount of aspirin ($C_9H_8O_4$) dissolved conform to *Dissolution* ⟨711⟩, *Acceptance Table 3* (*Acid stage*) and *Acceptance Table 4* (*Buffer stage*).

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **LIMIT OF FREE SALICYLIC ACID**
Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.015 mg/mL of USP Salicylic Acid RS and 0.5 mg/mL of USP Aspirin RS in *Diluent*
Standard solution: 0.015 mg/mL of USP Salicylic Acid RS in *Diluent*
Sample solution: Use the *Sample stock solution* prepared as directed in the *Assay*.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for salicylic acid and aspirin are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between salicylic acid and aspirin, *System suitability solution*
Relative standard deviation: NMT 4.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid ($C_7H_6O_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*
r_S = peak response of salicylic acid from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** The label indicates that the Capsules or the contents thereof are enteric-coated.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Aspirin RS
USP Salicylic Acid RS

Aspirin Suppositories

» Aspirin Suppositories contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ($C_9H_8O_4$).

Packaging and storage—Preserve in well-closed containers, in a cool place.
USP Reference standards ⟨11⟩—
USP Aspirin RS
Identification—Transfer a portion of the melted Suppositories obtained in the *Assay*, equivalent to about 1 g of aspirin, to a 125-mL conical flask. Add 20 mL of alcohol, and warm until completely disintegrated. Cool in an ice bath for 5 minutes, filter, and evaporate the filtrate to dryness: the residue responds to *Identification*— tests *A:* and *B:* under *Aspirin*.

Limit of free salicylic acid—
Ferric chloride-urea reagent—To a mixture of 8 mL of ferric chloride solution (6 in 10) and 42 mL of 0.05 N hydrochloric acid add 60 g of urea. Dissolve the urea by swirling and without the aid of heat, and adjust the resulting solution, if necessary, by the addition of 6 N hydrochloric acid to a pH of 3.2. Prepare on the day of use.
Procedure—Insert a small pledget of glass wool above the stem constriction of a 20- × 2.5-cm chromatographic tube, and uniformly pack with a mixture of about 1 g of chromatographic siliceous earth and 0.5 mL of 5 M phosphoric acid. Directly above this layer, pack a similar mixture of about 3 g of chromatographic siliceous earth and 2 mL of *Ferric chloride-urea reagent*. Transfer to a small beaker an accurately weighed portion of the cooled mass from the previously melted Suppositories obtained in the *Assay*, equivalent to 50 mg of aspirin, add 10 mL of chloroform, warm slightly, and stir until dissolved. With the aid of 5 mL of chloroform, transfer the mixture to the chromatographic adsorption column. Pass 50 mL of chloroform in several portions through the column, rinse the tip of the chromatographic tube with chloroform, and discard the eluate. If the purple zone reaches the bottom of the tube, discard the column, and repeat the test with a smaller quantity of melted Suppositories.
Elute the adsorbed salicylic acid into a 100-mL volumetric flask containing 20 mL of methanol and 0.2 mL of hydrochloric acid by passing two 10-mL portions of a 1 in 10 solution of glacial acetic acid in water-saturated ether, and then 30 mL of chloroform, through the column, and dilute the eluate with chloroform to volume. Dissolve a suitable, accurately weighed

www.webofpharma.com

quantity of salicylic acid in chloroform to obtain a Standard solution containing 150 µg of salicylic acid per mL. Pipet 5 mL of this solution into a 50-mL volumetric flask containing 10 mL of methanol, 0.1 mL of hydrochloric acid, and 10 mL of a 1 in 10 solution of glacial acetic acid in ether. Add chloroform to volume, and mix. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 306 nm, using as the blank a solvent mixture of the same composition as that of the Standard solution: the absorbance of the solution from the Suppositories does not exceed that of the Standard solution (3.0%).

Assay— [NOTE—In this assay, use chloroform that recently was saturated with water.]

Chromatographic column—Uniformly pack a chromatographic tube, as described in the test for *Limit of free salicylic acid* for *Procedure*, with a mixture of about 3 g of chromatographic siliceous earth and 2 mL of sodium bicarbonate solution (1 in 12) prepared on the day of use.

Standard preparation—Transfer about 50 mg of USP Aspirin RS, accurately weighed, to a 50-mL volumetric flask, add 0.5 mL of glacial acetic acid, and add chloroform to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add a 1 in 100 solution of glacial acetic acid in chloroform to volume, and mix.

Assay preparation—Tare a small dish and glass rod, place in the dish not fewer than 5 Suppositories, heat gently on a steam bath until melted, then stir, cool while stirring, and weigh. Transfer an accurately weighed portion of the mass, equivalent to about 50 mg of aspirin, to a 50-mL volumetric flask containing 1 mL of a 1 in 50 solution of hydrochloric acid in methanol, add 40 mL of chloroform, mix, and add chloroform to volume.

Procedure—Pipet 5 mL of the *Assay preparation* into the column, wash with 5 mL and then with 25 mL of chloroform, and discard the washings. Without delay, elute into a 100-mL volumetric flask with about 10 mL of a 1 in 10 solution of glacial acetic acid in chloroform, and then with about 85 mL of a 1 in 100 solution of glacial acetic acid in chloroform, dilute with the latter solvent to volume, and mix. Without delay, concomitantly determine the absorbances of the eluted *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 280 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of aspirin ($C_9H_8O_4$) in the portion of Suppositories taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Aspirin RS in the *Standard preparation*; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Aspirin Tablets

DEFINITION

Aspirin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of aspirin ($C_9H_8O_4$). Tablets of larger than 81-mg size contain no sweeteners or other flavors. [NOTE—Tablets that are enteric-coated meet the requirements for *Aspirin Delayed-Release Tablets*.]

IDENTIFICATION

- **A.** The retention time of the aspirin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)

Sample: Shake a quantity of finely powdered Tablets, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several min. Centrifuge the mixture. Pour off the clear supernatant, and evaporate it to dryness. Dry the residue under vacuum at 60° for 1 h.

Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**

Mobile phase: 2 g/L of sodium 1-heptanesulfonate in a mixture of acetonitrile and water (15:85). Adjust with glacial acetic acid to a pH of 3.4.

Diluent: Acetonitrile and formic acid (99:1)

Standard solution: 0.5 mg/mL of USP Aspirin RS in *Diluent*

Sample stock solution: Nominally 5 mg/mL of aspirin prepared as follows. Transfer a quantity, equivalent to about 100 mg of aspirin from NLT 20 finely powdered Tablets, to a suitable container. Add 20.0 mL of *Diluent* and 10 glass beads. Shake vigorously for about 10 min, and centrifuge.

Sample solution: Nominally 0.5 mg/mL of aspirin in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aspirin from the *Sample solution*

r_S = peak response of aspirin from the *Standard solution*

C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION ⟨711⟩**

Medium: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain a total of 1000 mL of solution with a pH of 4.50 ± 0.05; 500 mL

Apparatus 1: 50 rpm

Time: 30 min

Standard solution: A known concentration of USP Aspirin RS in *Medium*. Prepare the *Standard solution* at the time of use. [NOTE—A quantity of alcohol not to exceed 1% of the total volume of the *Standard solution* may be used to dissolve the Reference Standard prior to dilution with *Medium*.]

USP Monographs

www.webofpharma.com

Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: 265 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of aspirin ($C_9H_8O_4$) dissolved from UV absorbances at the isosbestic point of aspirin and salicylic acid at about 265 nm.
Tolerances: NLT 80% (*Q*) of the labeled amount of aspirin ($C_9H_8O_4$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Limit of Free Salicylic Acid**
Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.015 mg/mL of USP Salicylic Acid RS and 0.5 mg/mL of USP Aspirin RS in *Diluent*
Standard solution: 0.015 mg/mL of USP Salicylic Acid RS in *Diluent*
Sample solution: Use the *Sample stock solution* prepared as directed in the *Assay*.
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for salicylic acid and aspirin are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between salicylic acid and aspirin, *System suitability solution*
Relative standard deviation: NMT 4.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid ($C_7H_6O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*
r_S = peak response of salicylic acid from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.3%; for coated Tablets: NMT 3.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Preserve flavored or sweetened Tablets of 81-mg size or smaller in containers holding NMT 36 Tablets each.
• **USP Reference Standards** ⟨11⟩
USP Aspirin RS
USP Salicylic Acid RS

Buffered Aspirin Tablets

DEFINITION

Buffered Aspirin Tablets contain Aspirin and suitable buffering agents. Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of aspirin ($C_9H_8O_4$).

IDENTIFICATION

• **A.** The retention time of the aspirin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Shake a quantity of finely powdered Tablets, equivalent to about 500 mg of aspirin, with 10 mL of chloroform for several min. Centrifuge the mixture. Pour off the clear supernatant, and evaporate it to dryness.
Acceptance criteria: Meet the requirements

ASSAY

• **Procedure**
Mobile phase: 2 g/L of sodium 1-heptanesulfonate in a mixture of acetonitrile and water (15:85). Adjust with glacial acetic acid to a pH of 3.4.
Diluent: Acetonitrile and formic acid (99:1)
Standard solution: 0.5 mg/mL of USP Aspirin RS in *Diluent*
Sample stock solution: Nominally 5 mg/mL of aspirin prepared as follows. Transfer a quantity, equivalent to about 100 mg of aspirin from NLT 20 finely powdered Tablets, to a suitable container. Add 20.0 mL of *Diluent* and 10 glass beads. Shake vigorously for 10 min, and centrifuge.
Sample solution: Nominally 0.5 mg/mL of aspirin in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aspirin from the *Sample solution*
r_S = peak response of aspirin from the *Standard solution*
C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic

USP Monographs

www.webofpharma.com

acid with water to obtain a total of 1000 mL of solution with a pH of 4.50 ± 0.05; 500 mL
Apparatus 2: 75 rpm. [NOTE—Where the Tablet is composed of multiple layers, a stainless steel wire helix may be used, if needed, to hold the Tablet in proper orientation in the apparatus.]
Time: 30 min
Standard solution: A known concentration of USP Aspirin RS in *Medium*. Prepare the *Standard solution* at the time of use. [NOTE—A quantity of methanol not to exceed 1% of the total volume of the *Standard solution* may be used to dissolve the Reference Standard prior to dilution with *Medium*.]
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: 265 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of aspirin ($C_9H_8O_4$) dissolved from UV absorbances at the isosbestic point of aspirin and salicylic acid at about 265 nm.
Tolerances: NLT 80% (*Q*) of the labeled amount of aspirin ($C_9H_8O_4$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES
• **LIMIT OF FREE SALICYLIC ACID**
Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.015 mg/mL of USP Salicylic Acid RS and 0.5 mg/mL of USP Aspirin RS in *Diluent*
Standard solution: 0.015 mg/mL of USP Salicylic Acid RS in *Diluent*
Sample solution: Use the *Sample stock solution* prepared as directed in the *Assay*.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for salicylic acid and aspirin are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between salicylic acid and aspirin, *System suitability solution*
Relative standard deviation: NMT 4.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid ($C_7H_6O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*
r_S = peak response of salicylic acid from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0%

SPECIFIC TESTS
• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩**:** NLT 1.9 mEq of acid is consumed for each 325 mg of aspirin in the Tablets.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Aspirin RS
USP Salicylic Acid RS

Aspirin Delayed-Release Tablets

DEFINITION
Aspirin Delayed-Release Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of aspirin ($C_9H_8O_4$).

IDENTIFICATION
• **A.** The retention time of the aspirin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Shake a quantity of finely powdered Tablets, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant, and evaporate it to dryness. Dry the residue under vacuum at 60° for 1 h.
Acceptance criteria: Meet the requirements

ASSAY
• **PROCEDURE**
Mobile phase: 2 g/L of sodium 1-heptanesulfonate in a mixture of acetonitrile and water (15:85). Adjust with glacial acetic acid to a pH of 3.4.
Diluent: Acetonitrile and formic acid (99:1)
Standard solution: 0.5 mg/mL of USP Aspirin RS in *Diluent*
Sample stock solution: Nominally 5 mg/mL of aspirin prepared as follows. Transfer a quantity equivalent to about 100 mg of aspirin from NLT 20 finely powdered Tablets to a suitable container. Add 20.0 mL of *Diluent* and 10 glass beads. Shake vigorously for 10 min, and centrifuge.
Sample solution: Nominally 0.5 mg/mL of aspirin in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aspirin from the *Sample solution*
r_S = peak response of aspirin from the *Standard solution*
C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)

USP Monographs

www.webofpharma.com

C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure*

Apparatus 1: 100 rpm

Times

Acid stage: 2 h

Buffer stage: 90 min

Diluent: 0.1 N hydrochloric acid and 0.20 M tribasic sodium phosphate (3:1). Adjust, if necessary, with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8 ± 0.05.

Standard solution: USP Aspirin RS of a known concentration in 0.1 N hydrochloric acid (for analyzing the *Acid stage*) and in *Diluent* (for analyzing the *Buffer stage*)

Sample solution: Pass a portion of the solution under test through a suitable filter, diluted, if necessary, with 0.1 N hydrochloric acid (for analyzing the *Acid stage*) and with *Diluent* (for analyzing the *Buffer stage*).

Instrumental conditions

Mode: UV

Analytical wavelengths

Acid stage: 280 nm

Buffer stage: 265 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of aspirin ($C_9H_8O_4$) dissolved by determining UV absorbances at the isosbestic point of aspirin and salicylic acid (about 280 nm in the *Acid stage*, and about 265 nm in the *Buffer stage*).

Tolerances

Acid stage: NMT 10% (*Q*) of the labeled amount of aspirin ($C_9H_8O_4$) is dissolved.

Buffer stage: NLT 75% (*Q*) of the labeled amount of aspirin ($C_9H_8O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **LIMIT OF FREE SALICYLIC ACID**

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 0.015 mg/mL of USP Salicylic Acid RS and 0.5 mg/mL of USP Aspirin RS in *Diluent*

Standard solution: 0.015 mg/mL of USP Salicylic Acid RS in *Diluent*

Sample solution: Use the *Sample stock solution* from the *Assay*.

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for salicylic acid and aspirin are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between salicylic acid and aspirin, *System suitability solution*

Relative standard deviation: NMT 4.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of salicylic acid ($C_7H_6O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*

r_S = peak response of salicylic acid from the *Standard solution*

C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Aspirin RS

USP Salicylic Acid RS

Aspirin Effervescent Tablets for Oral Solution

DEFINITION

Aspirin Effervescent Tablets for Oral Solution contain Aspirin and an effervescent mixture of a suitable organic acid and an alkali metal bicarbonate and/or carbonate. Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of aspirin ($C_9H_8O_4$).

IDENTIFICATION

• **A.** The retention time of the aspirin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.**

Sample: ½ Tablet

Analysis: Add the *Sample* to 50 mL of water in a flask, and immediately stopper with a stopper fitted with tubing so that the evolved gas passes through calcium hydroxide TS.

Acceptance criteria: A white precipitate forms.

ASSAY

• **PROCEDURE**

Mobile phase: 2 g/L of sodium 1-heptanesulfonate in a mixture of acetonitrile and water (15:85). Adjust with glacial acetic acid to a pH of 3.4.

Diluent: Acetonitrile and formic acid (99:1)

Standard solution: 0.5 mg/mL of USP Aspirin RS in *Diluent*

Sample stock solution: Nominally 5 mg/mL of aspirin prepared as follows. Transfer a quantity, equivalent to about 100 mg of aspirin from NLT 20 finely powdered Tablets, to a suitable container. Add 20.0 mL of *Diluent* and 10 glass beads. Shake vigorously for 10 min, and centrifuge.

Sample solution: Nominally 0.5 mg/mL of aspirin in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion Tablets taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aspirin from the *Sample solution*
r_S = peak response of aspirin from the *Standard solution*
C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **SOLUTION TIME:** NMT 5 min for 2 Tablets completely dissolved in 180 mL of water at 17.5 ± 2.5°.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES
- **LIMIT OF FREE SALICYLIC ACID**

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.015 mg/mL of USP Salicylic Acid RS and 0.5 mg/mL of USP Aspirin RS in *Diluent*
Standard solution: 0.015 mg/mL of USP Salicylic Acid RS in *Diluent*
Sample solution: Use the *Sample stock solution* prepared as directed in the *Assay*.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for salicylic acid and aspirin are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between salicylic acid and aspirin, *System suitability solution*
Relative standard deviation: NMT 4.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid ($C_7H_6O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*
r_S = peak response of salicylic acid from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 8.0%

SPECIFIC TESTS
- **ACID-NEUTRALIZING CAPACITY** ⟨301⟩**:** NLT 5.0 mEq of acid is consumed by 1 Tablet.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Aspirin RS
USP Salicylic Acid RS

Aspirin Extended-Release Tablets

DEFINITION
Aspirin Extended-Release Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of aspirin ($C_9H_8O_4$).

IDENTIFICATION
- **A.** The retention time of the aspirin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Shake a quantity of finely powdered Tablets, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant, and evaporate it to dryness. Dry the residue under vacuum at 60° for 1 h.
Acceptance criteria: Meet the requirements

ASSAY
- **PROCEDURE**

Mobile phase: 2 g/L of sodium 1-heptanesulfonate in a mixture of acetonitrile and water (15:85). Adjust with glacial acetic acid to a pH of 3.4.
Diluent: Acetonitrile and formic acid (99:1)
Standard solution: 0.5 mg/mL of USP Aspirin RS in *Diluent*
Sample stock solution: Nominally 5 mg/mL of aspirin prepared as follows. Transfer a quantity, equivalent to about 100 mg of aspirin from NLT 20 finely powdered Tablets, to a suitable container. Add 20.0 mL of *Diluent* and 10 glass beads. Shake vigorously for about 10 min, and centrifuge.
Sample solution: Nominally 0.5 mg/mL of aspirin in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aspirin from the *Sample solution*
r_S = peak response of aspirin from the *Standard solution*
C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

www.webofpharma.com

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 60 rpm
Times: 1 and 4 h
Standard solution: A known concentration of USP Aspirin RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: 280 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of aspirin ($C_9H_8O_4$) dissolved from UV absorbances at the isosbestic point at about 280 nm.
Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
1	20–55
4	NLT 80

The percentages of the labeled amount of aspirin ($C_9H_8O_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 1000 mL
Apparatus 2: 30 rpm
Times: 1, 2, 4, and 8 h
Standard solution: A known concentration of USP Aspirin RS in *Medium*. Prepare the *Standard solution* at the time of use. [NOTE—A quantity of alcohol not to exceed 5% of the total volume of the *Standard solution* may be used to dissolve the USP Reference Standard prior to dilution with *Medium*.]
Sample solutions: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: 265 nm
Analysis
Samples: *Standard solution* and *Sample solutions*
Determine the percentage of the labeled amount of aspirin ($C_9H_8O_4$) dissolved from UV absorbances at the isosbestic point at about 265 nm.
Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
1	15–40
2	25–60
4	35–75
8	NLT 70

The percentages of the labeled amount of aspirin ($C_9H_8O_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **LIMIT OF FREE SALICYLIC ACID**

Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability solution: 0.015 mg/mL of USP Salicylic Acid RS and 0.5 mg/mL of USP Aspirin RS in *Diluent*
Standard solution: 0.015 mg/mL of USP Salicylic Acid RS in *Diluent*
Sample solution: Use the *Sample stock solution* prepared as directed in the *Assay*.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for salicylic acid and aspirin are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between salicylic acid and aspirin, *System suitability solution*
Relative standard deviation: NMT 4.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid ($C_7H_6O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*
r_S = peak response of salicylic acid from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The labeling indicates the *Dissolution* test with which the product complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Aspirin RS
USP Salicylic Acid RS

Aspirin, Alumina, and Magnesia Tablets

DEFINITION

Aspirin, Alumina, and Magnesia Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of aspirin ($C_9H_8O_4$), the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of aluminum hydroxide [$Al(OH)_3$], and NLT 90.0% and NMT 110.0% of the labeled amount of magnesium hydroxide [$Mg(OH)_2$].

IDENTIFICATION

- **A.** The retention time of the aspirin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Aspirin*.
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Magnesium*

Sample solution: To a 0.7-g portion of finely powdered Tablets, add 20 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution changes to deep yellow. Continue boiling for 2 min, and filter. Use the filtrate for analysis and use the precipitate in *Identification C*.
Acceptance criteria: Meet the requirements

USP Monographs

www.webofpharma.com

• **C. Identification Tests—General** ⟨191⟩, *Aluminum*
Sample solution: Wash the precipitate obtained in *Identification B* with a hot solution of ammonium chloride (1 in 50), and dissolve the precipitate in hydrochloric acid.
Acceptance criteria: Meet the requirements

ASSAY

• **Aspirin**
Mobile phase: Dissolve 225 mg of tetramethylammonium hydroxide pentahydrate and 200 mg of sodium 1-octanesulfonate in 700 mL of water. Add 150 mL of methanol, 150 mL of acetonitrile, and 1.0 mL of glacial acetic acid, and stir.
Diluent: To 2 g of anhydrous citric acid add 990 mL of acetonitrile, 990 mL of chloroform, and 20 mL of formic acid, and stir for about 30 min. Allow to settle, and use the decanted clear solution.
Internal standard solution: 2 mg/mL of phenacetin in *Diluent*
Salicylic acid stock solution: 1 mg/mL of USP Salicylic Acid RS in *Diluent*
Standard solution: 6.5 mg/mL of USP Aspirin RS and 0.2 mg/mL each of USP Salicylic Acid RS and phenacetin prepared as follows. Transfer, accurately weighed, about 325 mg of USP Aspirin RS to a 50-mL volumetric flask. Add 10.0 mL of *Salicylic acid stock solution* and 5.0 mL of *Internal standard solution*, dilute with *Diluent* to volume, and mix.
Sample solution: Nominally 6.5 mg/mL of aspirin prepared as follows. Transfer a quantity equivalent to about 325 mg of aspirin from NLT 20 finely powdered Tablets to a screw-capped, 120-mL bottle. Add 5.0 mL of *Internal standard solution* and 45.0 mL of *Diluent*, mix, and sonicate for 2–5 min. Centrifuge, and use the resultant clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[Note—The relative retention times for salicylic acid, aspirin, and phenacetin are about 0.3, 0.6, and 1.0, respectively. Record each chromatogram until the chloroform peak appears at the relative retention time of about 1.8.]
Suitability requirements
Resolution: NLT 2.0 between two adjacent peaks for salicylic acid, aspirin, and phenacetin
Tailing factor: NMT 2.0 for each peak
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of aspirin to phenacetin from the *Sample solution*
R_S = peak response ratio of aspirin to phenacetin from the *Standard solution*
C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

• **Aluminum Hydroxide**
Sample solution: Nominally 1.25 mg/mL of aluminum hydroxide prepared as follows. To a portion of NLT 20 powdered Tablets, equivalent to 250 mg of aluminum hydroxide in a 150-mL beaker, add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and transfer to a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the flask, dilute with water to volume, and mix.
Titrimetric system
Mode: Residual titration
Titrant: 0.05 M edetate disodium VS
Back titrant: 0.05 M zinc sulfate VS
Blank: Water, 50 mL
Endpoint detection: Visual
Analysis: To 50 mL of *Sample solution* add, in the order named and with continuous stirring, 25.0 mL of the *Titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate with *Back titrant* until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 50 mL of water for the *Sample solution*, and make any necessary corrections. Each mL of *Titrant* consumed is equivalent to 3.900 mg of aluminum hydroxide [$Al(OH)_3$].
Acceptance criteria: 90.0%–110.0%

• **Magnesium Hydroxide**
Indicator solution: Dissolve by mixing 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol.
Sample solution: Prepare as directed in the *Assay* for *Aluminum Hydroxide*.
Titrimetric system
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Blank: Water, 50 mL
Endpoint detection: Visual
Analysis: To a volume of *Sample solution*, equivalent to 80 mg of magnesium hydroxide, add 200 mL of water, 20 mL of triethanolamine, 50 mL of ammonia–ammonium chloride buffer TS, and 2 drops of *Indicator solution*. Cool the solution to between 3° and 4° by immersion in an ice bath, then remove, and titrate with *Titrant* until the color changes to pure blue. Perform a blank determination, substituting for the *Sample solution* a volume of water equal to the volume of *Sample solution* used, and make any necessary corrections. Each mL of *Titrant* is equivalent to 2.916 mg of magnesium hydroxide [$Mg(OH)_2$].
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate (trihydrate) and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution with a pH of 4.50 ± 0.05; 900 mL
Apparatus 2: 75 rpm
Time: 45 min
Standard solution: A known concentration of USP Aspirin RS in *Medium*. Prepare the *Standard solution* at the time of use. [Note—A quantity of methanol NMT 1% of the total volume of the *Standard solution* may be used to dissolve the Reference Standard into solution prior to dilution with *Medium*.]
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.

USP Monographs

www.webofpharma.com

Instrumental conditions
Mode: UV
Analytical wavelength: 265 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of aspirin ($C_9H_8O_4$) dissolved from UV absorbances at the isosbestic point of aspirin and salicylic acid at about 265 nm.
Tolerances: NLT 75% (*Q*) of the labeled amount of aspirin ($C_9H_8O_4$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩, *Weight Variation* and *Content Uniformity*: Meet the requirements for weight variation with respect to aluminum hydroxide and to magnesium hydroxide. Meet the requirements for content uniformity with respect to aspirin.

IMPURITIES

• **Limit of Free Salicylic Acid**
Mobile phase, Diluent, Internal standard solution, Salicylic acid stock solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay* for *Aspirin*.
System suitability solution: Transfer about 325 mg of USP Aspirin RS to a 50-mL volumetric flask. Add 10.0 mL of *Salicylic acid stock solution* and 5.0 mL of *Internal standard solution*, dilute with *Diluent* to volume, and mix.
Standard solution: 0.2 mg/mL of USP Salicylic Acid RS prepared as follows. Transfer 10.0 mL of *Salicylic acid stock solution* and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for salicylic acid, aspirin, and phenacetin are about 0.3, 0.6, and 1.0, respectively. Record each chromatogram until the chloroform peak appears at the relative retention time of about 1.8.]
Suitability requirements
Resolution: NLT 2.0 between two adjacent peaks for salicylic acid, aspirin, and phenacetin, *System suitability solution*
Tailing factor: NMT 2.0, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of salicylic acid to phenacetin from the *Sample solution*
R_S = peak response ratio of salicylic acid to phenacetin from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0%

SPECIFIC TESTS

• **Acid-Neutralizing Capacity** ⟨301⟩: NLT 1.9 mEq of acid is consumed for each 325 mg of aspirin in the Tablets.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Aspirin RS
USP Salicylic Acid RS

Aspirin, Alumina, and Magnesium Oxide Tablets

DEFINITION

Aspirin, Alumina, and Magnesium Oxide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of aspirin ($C_9H_8O_4$), the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of aluminum hydroxide [$Al(OH)_3$], and NLT 90.0% and NMT 110.0% of the labeled amount of magnesium oxide (MgO).

IDENTIFICATION

Sample: The *Sample* is prepared as follows. To a 0.7-g portion of finely powdered Tablets, add 20 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution changes to deep yellow. Continue boiling for 2 min, and filter. The filtrate is used in *Identification* test *B*, and the precipitate is used in *Identification* test *C*.

• **A.** The retention time of the aspirin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. Identification Tests—General,** *Magnesium* ⟨191⟩
Sample solution: *Sample* filtrate
Acceptance criteria: Meet the requirements

• **C. Identification Tests—General,** *Aluminum* ⟨191⟩
Sample solution: Wash the *Sample* precipitate with a hot solution of 20 mg/mL of ammonium chloride, and dissolve the precipitate in hydrochloric acid.
Acceptance criteria: Meet the requirements

• **D. Procedure**
Analysis: Where the Tablets are composed of two layers, scrape a small amount of each layer into separate test tubes. Add 2 mL of water and 2 drops of methyl red TS to each tube, and shake for 15 s.
Acceptance criteria: The solution from the aspirin-containing layer is red, and the solution from the buffer-containing layer is yellow.

ASSAY

• **Aspirin**
Mobile phase: Methanol, phosphoric acid, and water (30:3:70)
Diluent: Dehydrated alcohol and hydrochloric acid (2000:20)
Aspirin standard stock solution: 5 mg/mL of USP Aspirin RS in *Diluent* prepared by blending at high speed for 1.5 min
Aspirin standard solution: 0.25 mg/mL of USP Aspirin RS prepared immediately from the *Aspirin standard stock solution* in dehydrated alcohol. Use these solutions within 1 h.
Salicylic acid standard stock solution: 5 mg/mL of USP Salicylic Acid RS in dehydrated alcohol. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume.
Salicylic acid standard solution: 7.5 µg/mL of USP Salicylic Acid RS from the *Salicylic acid standard stock solution* in dehydrated alcohol
System suitability solution: Transfer 5.0 mL of the *Aspirin standard stock solution* to a 100-mL volumetric flask, add 5.0 mL of the *Salicylic acid standard stock solution*, and dilute with dehydrated alcohol to volume.
Sample solution: Transfer a counted number of Tablets, equivalent to 2500 mg of aspirin, to a 120-mL blender jar containing 100.0 mL of *Diluent*, and blend at high speed for 1.5 min. Immediately filter a portion of the mixture thus obtained, and transfer 1.0 mL of the filtrate to a 100-mL volumetric flask. Immediately dilute with dehydrated

USP Monographs

www.webofpharma.com

alcohol to volume. Promptly inject this *Sample solution* into the chromatograph as directed for *Analysis*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 3-cm; 5-µm packing L7

Flow rate: 3.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *Aspirin standard solution, Salicylic acid standard solution,* and *System suitability solution*

[NOTE—The relative retention times for aspirin and salicylic acid are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the aspirin peak and the salicylic acid peak, *System suitability solution*

Tailing factor: NMT 2.0 for the aspirin and salicylic acid peaks, *Aspirin standard solution* and *Salicylic acid standard solution*

Relative standard deviation: NMT 2.0% for the aspirin and salicylic acid peaks, *Aspirin standard solution* and *Salicylic acid standard solution*

Analysis

Samples: *Aspirin standard solution, Salicylic acid standard solution,* and *Sample solution*

Calculate the percentage of aspirin ($C_9H_8O_4$) in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aspirin from the *Sample solution*

r_S = peak response of aspirin from the *Standard solution*

C_S = concentration of USP Aspirin RS in the *Aspirin standard solution* (mg/mL)

C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

• **ALUMINUM HYDROXIDE**

Edetate disodium titrant: Dissolve 18.6 g of edetate disodium in water to make 1000 mL, and standardize the solution as follows. Weigh 2 g of aluminum wire, transfer to a 1000-mL volumetric flask, and add 50 mL of a mixture of hydrochloric acid and water (1:1). Swirl the flask to ensure contact of the aluminum and the acid, and allow the reaction to proceed until all of the aluminum has dissolved. Dilute with water to volume. Pipet 10 mL of this solution into a 250-mL beaker; add, in the order named and with continuous stirring, 25.0 mL of edetate disodium titrant and 20 mL of acetic acid–ammonium acetate buffer TS; and boil gently for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate with 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination, substituting 10 mL of water for the aluminum solution, and make any necessary correction.

Calculate the molarity of the solution taken:

$$\text{Result} = (W/A_r) \times V$$

W = weight of aluminum in the portion of the solution taken (mg)

A_r = atomic weight of aluminum, 26.98

V = volume of *Edetate disodium titrant* consumed (mL)

Sample solution: To a portion of the powdered Tablets (NLT 20) equivalent to 600 mg of aluminum hydroxide, add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and transfer to a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the flask, and add water to volume.

Analysis: To 20 mL of the *Sample solution* in a 250 mL-beaker, add 20 mL of water; then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of aluminum hydroxide [$Al(OH)_3$].

Acceptance criteria: 90.0%–110.0%

• **MAGNESIUM OXIDE**

Sample solution: Prepare as directed in the *Assay* for *Aluminum Hydroxide*.

Eriochrome black indicator solution: Dissolve 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol.

Titrant: 0.05 M edetate disodium VS

Analysis: To a volume of the *Sample solution* equivalent to 40 mg of magnesium oxide in a 400 mL beaker, add, while mixing, 20 mL of triethanolamine and 200 mL of water. Cool the solution for 10 min, while stirring, by immersion in an ice bath. Remove the beaker from the ice bath, and add 15 mL of ammonia–ammonium chloride buffer TS and 2 drops of *Eriochrome black indicator solution*. Titrate with *Titrant* to a blue endpoint, allowing 60 s between drops of *Titrant* as the endpoint is approached (after first color change is observed). The titration should be completed within 10 min after the addition of the buffer and indicator. If any precipitate is observed prior to titration, the solution should be discarded, and a new solution prepared. Perform a blank determination, substituting an equivalent volume of water for the volume of *Sample solution* used, and make any necessary correction. Each mL of *Titrant* consumed is equivalent to 2.015 mg of magnesium oxide (MgO).

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate (trihydrate) and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of 4.50 ± 0.05; 900 mL

Apparatus 1 (10-mesh screen): 100 rpm

Time: 45 min

Determine the amount of aspirin ($C_9H_8O_4$) dissolved, employing the following method.

Alkaline detergent solution: 30% solution of polyoxyethylene (23) lauryl ether and 1 N sodium hydroxide (0.5: 1000)

pH 4.3 buffer detergent: 12.9 g/L of citric acid monohydrate and 20.6 g/L of dibasic sodium phosphate heptahydrate in water. Add 0.5 mL of a 30% solution of polyoxyethylene (23) lauryl ether.

Standard solution: 0.45 mg/mL of USP Aspirin RS in *Medium*

Sample solution: Filtered portion of sample

Analysis: Use an automatic analyzer consisting of (1) a liquid sampler, (2) a proportioning pump, (3) a suitable fluorometer equipped with a 0.4-cm flow cell and suitable recording devices, and (4) a manifold consisting of the components illustrated in *Figure 1*.

www.webofpharma.com

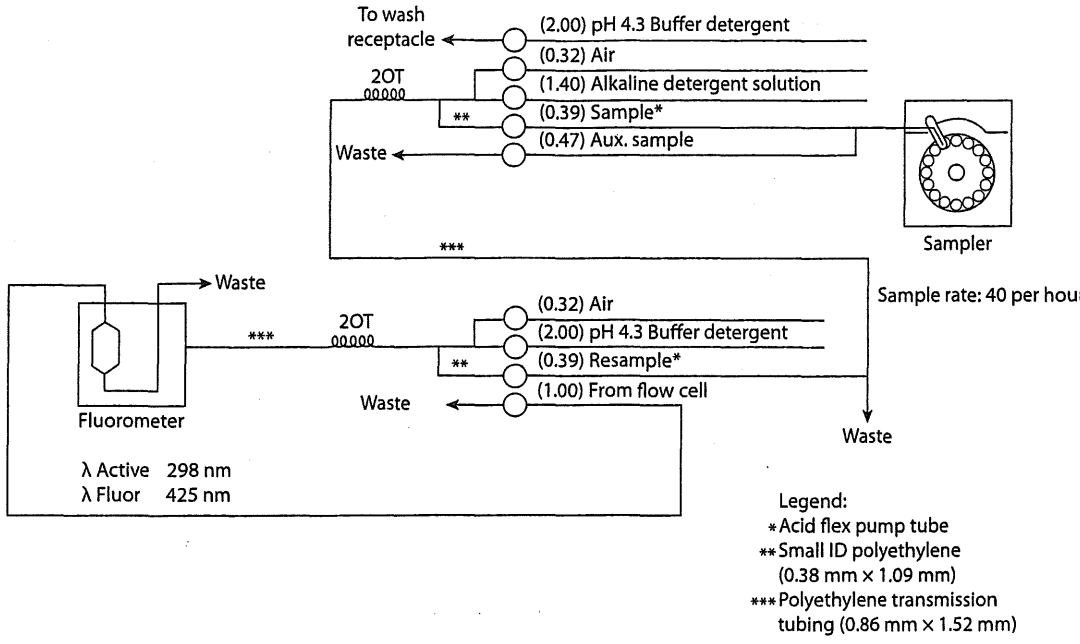


Figure 1 (Aspirin, Alumina, and Magnesium Oxide Tablets)

With the sample line pumping *pH 4.3 buffer detergent*, the other lines pumping their respective reagents, and the fluorometer set at an excitation wavelength of 298 nm and an emission wavelength of 425 nm, adjust the system until a steady fluorescence baseline has been achieved. Start the sampler, and conduct determinations at a rate of 40/h, using a ratio of 5:1 for sample and wash time. Record the fluorescence values of the *Standard solution* and the *Sample solution*.

Calculate the percentage of aspirin ($C_9H_8O_4$) dissolved:

$$\text{Result} = C_S \times (V/L) \times (F_U/F_S) \times 100$$

C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)
V = volume of medium, 900 mL
L = label claim (mg/Tablet)
F_U = fluorescence values of the solution under test
F_S = fluorescence values of the *Standard solution*

Tolerances: NLT 75% (*Q*) of the labeled amount of aspirin ($C_9H_8O_4$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium oxide, and for *Content Uniformity* with respect to aspirin

IMPURITIES

• **Organic Impurities Procedure: Limit of Free Salicylic Acid**

[Note—The results from the *Assay* for *Aspirin* may be used for this test when calculated as described in the *Analysis* section of this test.]

Mobile phase: Methanol, phosphoric acid, and water (30:3:70)

Diluent: Dehydrated alcohol and hydrochloric acid (2000:20)

Aspirin standard stock solution: 5 mg/mL of USP Aspirin RS in *Diluent* prepared by blending at high speed for 1.5 min

Aspirin standard solution: 0.25 mg/mL of USP Aspirin RS prepared immediately from *Aspirin standard stock solution* in dehydrated alcohol. Use these solutions within 1 h.

Salicylic acid standard stock solution: 5 mg/mL of USP Salicylic Acid RS in dehydrated alcohol. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Salicylic acid standard solution: 7.5 µg/mL of USP Salicylic Acid RS from *Salicylic acid standard stock solution* in dehydrated alcohol

System suitability solution: Transfer 5.0 mL of the *Aspirin standard stock solution* to a 100-mL volumetric flask, add 5.0 mL of the *Salicylic acid standard stock solution*, and dilute with dehydrated alcohol to volume.

Sample solution: Transfer a counted number of Tablets, equivalent to 2500 mg of aspirin, to a 120-mL blender jar containing 100.0 mL of *Diluent*, and blend at high speed for 1.5 min. Immediately filter a portion of the mixture thus obtained, and transfer 1.0 mL of the filtrate to a 100-mL volumetric flask. Immediately dilute with dehydrated alcohol to volume. Promptly inject this *Sample solution* into the chromatograph as directed for *Analysis*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 3-cm; 5-µm packing L7

Flow rate: 3.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *Aspirin standard solution, Salicylic acid standard solution,* and *System suitability solution*

[Note—The relative retention times for aspirin and salicylic acid are 0.7 and 1.0, respectively.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 2.0 between the aspirin and salicylic acid peaks, *System suitablity solution*
Tailing factor: NMT 2.0 for the aspirin and salicylic acid peaks, *Aspirin standard solution* and *Salicylic acid standard solution*
Relative standard deviation: NMT 2.0% for aspirin and salicylic acid peaks, *Aspirin standard solution* and *Salicylic acid standard solution*

Analysis
Samples: *Aspirin standard solution*, *Salicylic acid standard solution*, and *Sample solution*
Calculate the percentage of free salicylic acid in the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*
r_S = peak response from the *Salicylic acid standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Salicylic acid standard solution* (µg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0%

SPECIFIC TESTS
- **ACID-NEUTRALIZING CAPACITY** ⟨301⟩**:** NLT 1.9 mEq of acid is consumed for each 325 mg of aspirin in the Tablets.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aspirin RS
  USP Salicylic Acid RS

Aspirin, Caffeine, and Dihydrocodeine Bitartrate Capsules

» Aspirin, Caffeine, and Dihydrocodeine Bitartrate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aspirin ($C_9H_8O_4$), caffeine ($C_8H_{10}N_4O_2$), and dihydrocodeine bitartrate ($C_{18}H_{23}NO_3 \cdot C_4H_6O_6$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Aspirin RS
USP Caffeine RS
USP Dihydrocodeine Bitartrate RS
USP Salicylic Acid RS
Identification—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of 4.50 ± 0.05; 500 mL.
Apparatus 1: 50 rpm.
Time: 45 minutes.
Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay and limit of salicylic acid*.
Standard preparation—Prepare a solution in *Medium* containing known concentrations of about 0.002*A* mg of USP Aspirin RS, 0.002*C* mg of USP Caffeine RS, and 0.002*D* mg of USP Dihydrocodeine Bitartrate RS per mL, *A*, *C*, and *D* being the labeled amounts, in mg, of aspirin, caffeine, and dihydrocodeine bitartrate, respectively, in each Capsule.
Test preparation—Filter a portion of the solution under test.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of aspirin ($C_9H_8O_4$), caffeine ($C_8H_{10}N_4O_2$), and dihydrocodeine bitartrate ($C_{18}H_{23}NO_3 \cdot C_4H_6O_6$) dissolved by the same formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation;* and r_U and r_S are the peak responses of the relevant analyte obtained from the *Test preparation* and the *Standard preparation*, respectively.
Tolerances—Not less than 75% *(Q)* of the labeled amounts of $C_9H_8O_4$, $C_8H_{10}N_4O_2$, and $C_{18}H_{23}NO_3 \cdot C_4H_6O_6$ are dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay and limit of salicylic acid—
Mobile phase—Dissolve 1 g of sodium 1-pentanesulfonate and 2.3 g of monobasic ammonium phosphate in 850 mL of water. Add 150 mL of acetonitrile, mix, degas, and adjust with phosphoric acid to a pH of 2.5. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluent—Prepare a mixture of water and acetonitrile (53:46), and adjust with phosphoric acid to a pH of 2.5.
Standard preparation—Prepare a solution in *Diluent* containing known concentrations of about 0.001*A* mg of USP Aspirin RS, 0.001*C* mg of USP Caffeine RS, and 0.001*D* mg of USP Dihydrocodeine Bitartrate RS per mL, *A*, *C*, and *D* being the labeled amounts, in mg, of aspirin, caffeine, and dihydrocodeine bitartrate, respectively, in each Capsule. [NOTE—Use this solution within 3 hours.]
Standard salicylic acid preparation—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Diluent* to obtain a solution having a known concentration of about 0.005*A* µg per mL, *A* being the labeled amount, in mg, of aspirin per Capsule. [NOTE—Use this solution within 3 hours.]
Resolution solution—Prepare a solution in *Standard preparation* containing about 0.0001*A* mg of USP Salicylic Acid RS per mL, *A* being the labeled amount, in mg, of aspirin in each Capsule. [NOTE—Use this solution within 3 hours.]
Assay preparation—Transfer the contents of 10 Capsules to a 500-mL volumetric flask. Dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this mixture to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Centrifuge a portion of this mixture, and use the clear supernatant as the *Assay preparation*. [NOTE—Use this solution within 3 hours.]
Chromatographic system—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure:* the relative retention times are about 0.2 for caffeine, 0.3 for dihydrocodeine, 0.7 for aspirin, and 1.0 for salicylic acid; and the resolution, *R*, between the caffeine and dihydrocodeine peaks is not less than 2.5, between the dihydrocodeine and aspirin peaks is not less than 1.0, and between the aspirin and

USP Monographs

www.webofpharma.com

salicylic acid peaks is not less than 1.5. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0% for each analyte.

Procedure—Separately inject equal volumes (about 10 µL) of the *Assay preparation*, the *Standard preparation*, and the *Standard salicylic acid preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of aspirin ($C_9H_8O_4$), caffeine ($C_8H_{10}N_4O_2$), and dihydrocodeine bitartrate ($C_{18}H_{23}NO_3 \cdot C_4H_6O_6$) in each Capsule taken by the same formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation;* and r_U and r_S are the responses of the corresponding analyte peaks of the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentage of salicylic acid in the Capsules taken by the formula:

$$100(C/A)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Salicylic Acid RS in the *Standard salicylic acid preparation; A* is the labeled amount, in mg, of aspirin in each Capsule taken; and r_U and r_S are the salicylic acid peak responses obtained from the *Assay preparation* and the *Standard salicylic acid preparation*, respectively: not more than 3.0% is found.

Aspirin and Codeine Phosphate Tablets

» Aspirin and Codeine Phosphate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aspirin ($C_9H_8O_4$) and codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Aspirin RS
USP Codeine Phosphate RS
USP Salicylic Acid RS

Identification—Dissolve a suitable quantity of USP Aspirin RS in the *Solvent mixture* prepared as directed under *Assay for aspirin and codeine phosphate and limit of free salicylic acid* to obtain a *Standard aspirin solution* containing about 3.3 mg per mL. Dissolve a suitable quantity of USP Codeine Phosphate RS in the *Solvent mixture* to obtain a *Standard codeine phosphate solution* containing about 1 mg per mL. Chromatograph these solutions as directed for *Procedure* in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid.* The retention times of the major peaks in the chromatogram of the *Assay preparation,* obtained as directed in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid,* correspond to those in the chromatrograms of the *Standard aspirin solution* and the *Standard codeine phosphate solution,* respectively.

Dissolution ⟨711⟩—
Medium: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of 4.50 ± 0.05; 900 mL.

Apparatus 2: 75 rpm.
Time: 30 minutes.

Determine the amounts of aspirin ($C_9H_8O_4$) and codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) dissolved by employing the following method.

Mobile phase, Solvent mixture, and *Aspirin* and *codeine phosphate standard preparation*—Prepare as directed in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid.*

Internal standard solution—Dissolve phenacetin in methanol to obtain a solution having a concentration of about 0.07 mg per mL.

Standard solution A—Prepare a solution of USP Aspirin RS in *Solvent mixture* having an accurately known concentration of about 0.36 mg per mL.

Standard solution B—Transfer about 12 mg of USP Codeine Phosphate RS and 25 mg of USP Salicylic Acid RS, each accurately weighed, to a 50-mL volumetric flask, add 2.5 mL of methanol, and mix. Add *Medium* to volume, and mix. Pipet 10 mL of the resulting solution into a 100-mL volumetric flask, add *Medium* to volume, and mix.

Standard preparations A and *B*—Pipet 10 mL of *Standard solution A* and 10 mL of *Standard solution B* into separate containers, add 3.0 mL of the *Internal standard solution* to each container, and mix.

Test preparation—Withdraw a portion of the solution under test and filter, discarding the few mL of the filtrate. Pipet 10 mL of the filtrate and 3.0 mL of the *Internal standard solution* into a suitable container, and mix.

Chromatographic system—Proceed as directed for *Chromatographic system* in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid,* except to use only the *Aspirin and codeine phosphate preparation* for evaluation of the suitability of the system.

Procedure—Proceed as directed in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid,* except to inject about 50 µL of the *Standard preparations* and the *Test preparation.* The relative retention times are 0.3 for salicylic acid, 0.6 for aspirin, 0.8 for codeine phosphate, and 1.0 for phenacetin. Calculate the amount of codeine phosphate dissolved by comparison of the relative peak response ratios for the codeine phosphate peaks, obtained from *Standard preparation B* and the *Test preparation.* Calculate the percentage of aspirin dissolved by the formula:

$$[0.9C(R_U/R_S) + 0.9C'(R'_U/R'_S)(180.16/138.12)]/3.25$$

in which C is the concentration, in µg per mL, of USP Aspirin RS in *Standard solution A; R_U* and R_S are the peak response ratios for the aspirin component obtained from the *Test preparation* and *Standard preparation A,* respectively; C' is the concentration, in µg per mL, of USP Salicylic Acid RS in *Standard solution B; R'_U* and R'_S are the peak response ratios for the salicylic acid component obtained from the *Test preparation* and *Standard preparation B,* respectively; and 180.16 and 138.12 are the molecular weights of aspirin and salicylic acid, respectively.

Tolerances—Not less than 75% *(Q)* of the labeled amounts of aspirin ($C_9H_8O_4$) and codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements for *Content Uniformity* with respect to aspirin and codeine phosphate.

Assay for aspirin and codeine phosphate and limit of free salicylic acid—

Mobile phase—Dissolve 225 mg of tetramethylammonium hydroxide pentahydrate and 200 mg of sodium 1-octanesulfonate in 700 mL of water. Add 150 mL of

www.webofpharma.com

methanol, 150 mL of acetonitrile, and 1.0 mL of glacial acetic acid, and stir. Pass through a membrane filter, and degas. [NOTE—The amounts of sodium 1-octanesulfonate, methanol, and acetonitrile may be varied to obtain acceptable chromatography.]

Solvent mixture—To 15 g of anhydrous citric acid add 200 mL of methanol and 20 mL of glacial acetic acid, dilute with chloroform to 1000 mL, and mix until the citric acid is dissolved.

Internal standard solution—Dissolve phenacetin in *Solvent mixture* to obtain a solution having a concentration of about 2 mg per mL.

Salicylic acid stock standard solution—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Solvent mixture,* and quantitatively dilute with *Solvent mixture* to obtain a solution having a known concentration of about 1 mg per mL.

Salicylic acid standard preparation—Transfer 5.0 mL of *Salicylic acid stock standard solution* to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution,* dilute with *Solvent mixture* to volume, and mix.

Codeine phosphate stock standard solution—Transfer about 325*J* mg of USP Codeine Phosphate RS, accurately weighed, to a 25-mL volumetric flask, *J* being the ratio of the labeled amount, in mg, of codeine phosphate to the labeled amount, in mg, of aspirin per Tablet. Dissolve in and dilute with *Solvent mixture* to volume, and mix.

Aspirin and codeine phosphate standard preparation—Transfer about 65 mg of USP Aspirin RS, accurately weighed, to a 10-mL volumetric flask. Add 5.0 mL of *Codeine phosphate stock standard solution,* 1.0 mL of *Salicylic acid stock standard solution,* and 1.0 mL of *Internal standard solution,* dilute with *Solvent mixture* to volume, and mix.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a screw-capped, 120-mL bottle, add 5.0 mL of *Internal standard solution* and 45.0 mL of *Solvent mixture,* mix, and sonicate for 2 to 5 minutes. Centrifuge, and use a portion of the resultant clear solution as the *Assay preparation.* Use on the day prepared.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains 10-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph replicate injections of the *Salicylic acid standard preparation* and the *Aspirin and codeine phosphate standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times for salicylic acid, aspirin, codeine, and phenacetin are about 0.3, 0.5, 0.8, and 1.0, respectively; the resolution, *R,* between salicylic acid and aspirin, between aspirin and codeine, and between codeine and phenacetin is not less than 2.0; the tailing factor for each analyte peak is not more than 2.0; and the relative standard deviation of the ratios of the peak responses of salicylic acid, aspirin, and codeine to the peak response of phenacetin is not more than 3.0%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Salicylic acid standard preparation, Aspirin and codeine phosphate standard preparation,* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ($C_9H_8O_4$) in the portion of Tablets taken by the formula:

$$50C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Aspirin RS in the *Aspirin and codeine phosphate standard preparation;* and R_U and R_S are the ratios of the peak responses of aspirin and phenacetin obtained from the *Assay preparation* and the *Aspirin and codeine phosphate standard preparation,* respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$) in the portion of Tablets taken by the formula:

$$(406.37/397.37)(50C)(R_U/R_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively; *C* is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Aspirin and codeine phosphate standard preparation;* and R_U and R_S are the ratios of the peak responses of codeine phosphate and phenacetin obtained from the *Assay preparation* and the *Aspirin and codeine phosphate standard preparation,* respectively. Calculate the percentage of free salicylic acid in the Tablets taken by the formula:

$$5000(C/a)(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Salicylic acid standard preparation; a* is the quantity, in mg, of aspirin in the portion of powdered Tablets taken, based on the labeled amount; and R_U and R_S are the ratios of the peak responses of salicylic acid and phenacetin obtained from the *Assay preparation* and the *Salicylic acid standard preparation,* respectively: not more than 3.0% is found.

Aspirin, Codeine Phosphate, Alumina, and Magnesia Tablets

» Aspirin, Codeine Phosphate, Alumina, and Magnesia Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aspirin ($C_9H_8O_4$), codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$), aluminum hydroxide [$Al(OH)_3$], and magnesium hydroxide [$Mg(OH)_2$].

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Aspirin RS
USP Codeine Phosphate RS
USP Salicylic Acid RS

Identification—

A: Tablets respond to the *Identification*— test under *Aspirin and Codeine Phosphate Tablets.*

B: Tablets respond to the *IDENTIFICATION* tests under *Alumina and Magnesia Tablets.*

Dissolution ⟨711⟩—

Medium: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of 4.50 ± 0.05; 900 mL.

Apparatus 2: 75 rpm.

Time: 30 minutes.

Mobile phase, Internal standard solution, Solvent mixture, Aspirin and codeine phosphate standard preparation, Standard solution A, Standard solution B, Standard preparations A and B, Test preparation, Chromatographic system, and *Procedure*—

USP Monographs

www.webofpharma.com

Proceed as directed in the test for *Dissolution 711*— under *Aspirin and Codeine Phosphate Tablets*.

Tolerances—Not less than 75% *(Q)* of the labeled amounts of aspirin ($C_9H_8O_4$) and codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$) are dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements for *Content Uniformity* with respect to aspirin and codeine phosphate and for *Weight Variation* with respect to aluminum hydroxide and magnesium hydroxide.

Acid-neutralizing capacity ⟨301⟩: not less than 1.9 mEq per Tablet.

Assay for aspirin and codeine phosphate and limit of free salicylic acid—

Mobile phase, Solvent mixture, Salicylic acid stock standard solution, Salicylic acid standard preparation, Aspirin and *codeine phosphate standard preparation,* and *Chromatographic system*—Prepare as directed in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid* under *Aspirin and Codeine Phosphate Tablets*.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a screw-capped, 120-mL bottle, add 5.0 mL of *Internal standard solution* and 45.0 mL of *Solvent mixture*, mix, and sonicate for 2 to 5 minutes. Centrifuge, and use a portion of the resultant clear solution as the *Assay preparation*.

Procedure—Separately inject equal volumes (about 5 µL) of the *Salicylic acid standard preparation*, the *Aspirin and codeine phosphate standard preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times for salicylic acid, aspirin, codeine, and phenacetin are about 0.3, 0.5, 0.8, and 1.0, respectively. Calculate the quantity, in mg, of aspirin ($C_9H_8O_4$) in the portion of powdered Tablets taken by the formula:

$$50C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Aspirin RS in the *Aspirin and codeine phosphate standard preparation;* and R_U and R_S are the ratios of the peak responses of aspirin and phenacetin obtained from the *Assay preparation* and the *Aspirin and codeine phosphate standard preparation,* respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$), in the portion of powdered Tablets taken by the formula:

$$(406.37/397.37)(50C)(R_U/R_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively; C is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Aspirin and codeine phosphate standard preparation;* and R_U and R_S are the ratios of the peak responses of codeine phosphate and phenacetin obtained from the *Assay preparation* and the Aspirin and codeine phosphate *Standard preparation,* respectively. Calculate the percentage of free salicylic acid in the Tablets taken by the formula:

$$5000(C/a)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Salicylic acid standard preparation; a* is the quantity, in mg, of aspirin in the portion of Tablets taken, determined as directed above; and R_U and R_S are the ratios of the peak responses of salicylic acid and phenacetin obtained from the *Assay preparation* and the *Salicylic acid standard preparation,* respectively: not more than 3.0% is found.

Assay for aluminum hydroxide—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 600 mg of aluminum hydroxide, to a 150-mL beaker, add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, add water to volume, and mix.

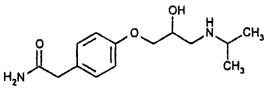
Procedure—Pipet 10 mL of *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid-ammonium acetate buffer TS. Add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M Edetate disodium titrant is equivalent to 3.900 mg of $Al(OH)_3$.

Assay for magnesium hydroxide—

Assay preparation—Prepare as directed in the *Assay for aluminum oxide*.

Procedure—Pipet a volume of *Assay preparation*, equivalent to about 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia-ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of $Mg(OH)_2$.

Atenolol



$C_{14}H_{22}N_2O_3$ 266.34

Benzeneacetamide, 4-[2-hydroxy-3-[(1-methylethyl) amino] propoxy]-;

2-[*p*-[2-Hydroxy-3-(isopropylamino)propoxy]-phenyl] acetamide [29122-68-7].

DEFINITION

Atenolol contains NLT 98.0% and NMT 102.0% of $C_{14}H_{22}N_2O_3$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

USP Monographs

www.webofpharma.com

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Sample solution: 20 µg/mL in methanol

ASSAY
• **PROCEDURE**
Mobile phase: 1.1 g of sodium 1-heptanesulfonate and 0.71 g of anhydrous dibasic sodium phosphate in 700 mL of water. Add 2 mL of dibutylamine, and adjust with 0.8 M phosphoric acid to a pH of 3.0. Add 300 mL of methanol, mix, and pass through a filter having a 0.5-µm or finer porosity. Degas this solution before use.
Standard solution: 0.01 mg/mL of USP Atenolol RS in *Mobile phase*
Sample solution: 0.01 mg/mL of Atenolol in *Mobile phase*. Sonicate for 5 min for complete dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 0.6 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_{22}N_2O_3$ in the portion of Atenolol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Atenolol RS in the *Standard solution* (mg/mL)
C_U = concentration of Atenolol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
INORGANIC IMPURITIES
• **Residue on Ignition** ⟨281⟩: NMT 0.2%
• **Chloride and Sulfate**, *Chloride* ⟨221⟩
Sample solution: Dissolve 1.0-g in 100 mL of 0.15 N nitric acid.
Acceptance criteria: Shows no more turbidity with 1 mL of silver nitrate TS than 1.4 mL of 0.020 N hydrochloric acid in 100 mL of 0.15 N nitric acid (0.1%)
ORGANIC IMPURITIES
• **Procedure**
Mobile phase: Prepare as directed in the *Assay*.
Sample solution 1: 0.1 mg/mL of Atenolol in *Mobile phase*
Sample solution 2: 0.5 µg/mL of Atenolol, from *Sample solution 1* in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*, except use the injection size listed below.
Injection size: 50 µL
Analysis
Samples: *Sample solution 1* and *Sample solution 2*
[NOTE—Chromatograph *Sample solution 1* for a period of time that is 6 times the retention time of the atenolol peak.]
Calculate the percentage of each impurity in *Sample solution 1*:

$$\text{Result} = 0.5(r_U/r_A)$$

r_U = peak response of any individual impurity in *Sample solution 1*
r_A = peak response of Atenolol in *Sample solution 2*

Acceptance criteria
Individual impurities: NMT 0.25%
Total impurities: NMT 0.5%

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE**, *Class I* ⟨741⟩: 152°–156.5°
• **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° to constant weight: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Atenolol RS

Atenolol Injection

» Atenolol Injection is a sterile solution of Atenolol in Water for Injection. It contains a suitable buffering agent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of atenolol ($C_{14}H_{22}N_2O_3$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, in a cool place or at controlled room temperature, protected from light. Avoid freezing.
USP Reference standards ⟨11⟩—
USP Atenolol RS

Identification—
A: The retention time of the main peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: *197U▲* (CN 1-May-2020)—
Solution: 10 µg of atenolol per mL.
Medium: methanol.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 33.3 USP Endotoxin Units per mg of atenolol.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
pH ⟨791⟩: between 5.5 and 6.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Assay—
Citric acid buffer—Transfer 2.5 g of citric acid to a 500-mL volumetric flask, add 400 mL of water, and swirl to dissolve. Adjust the solution with 2 N sodium hydroxide to a pH of 6.0, dilute with water to volume, and mix.

www.webofpharma.com

Mobile phase—Dissolve 930 mg of sodium octyl sulfate in 740 mL of water, add 8 mL of 3.6 N sulfuric acid, mix, and pass through a 1-µm or finer porosity filter. To the filtrate add 250 mL of acetonitrile, mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 50 mg of USP Atenolol RS to a 100-mL volumetric flask, add 80 mL of *Citric acid buffer*, and sonicate for about 30 seconds to achieve dissolution. Dilute with *Citric acid buffer* to volume, and mix. Transfer 4.0 mL of this solution to a 10-mL volumetric flask, dilute with *Citric acid buffer* to volume, and mix. This solution contains about 0.2 mg of USP Atenolol RS per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to 2 mg of atenolol, to a 10-mL volumetric flask, dilute with *Citric acid buffer* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.7 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{14}H_{22}N_2O_3$ in each mL of the Injection taken by the formula:

$$10(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Atenolol RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and r_U and r_S are the atenolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Atenolol Compounded Oral Solution

DEFINITION

Atenolol Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of atenolol ($C_{14}H_{22}N_2O_3$).

Prepare Atenolol Compounded Oral Solution at a 2-mg/mL concentration, for example, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Atenolol	200 mg
Glycerin	5 mL
Vehicle for Oral Suspension	45 mL
Vehicle for Oral Solution, Sugar Free, a sufficient quantity to make	100 mL

Calculate the quantity of each ingredient required for the total volume and atenolol strength to be prepared. Mix the *Atenolol*, previously pulverized, and *Glycerin* to form a smooth paste. Incorporate the *Vehicle for Oral Suspension* or an equal volume of *Vehicle for Oral Solution, Sugar Free*. [NOTE—The *Vehicle for Oral Suspension* may be omitted.] Incorporate sufficient *Vehicle for Oral Solution, Sugar Free* in increments to bring to volume, and mix well. [NOTE—Do not use a sucrose-containing vehicle for oral solution.] Package, and label.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in amber, tight containers, and store at controlled room temperature.
- **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded when stored at controlled room temperature
- **LABELING:** Label it to state that it is to be shaken well before use, and to state the *Beyond-Use Date*.

Atenolol Tablets

DEFINITION

Atenolol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of atenolol ($C_{14}H_{22}N_2O_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K▲** (CN 1-May-2020)

Sample: Mix a quantity of powdered Tablets, equivalent to 100 mg of atenolol, with 15 mL of methanol, heat the mixture to 50°, and shake for 5 min. Filter, and evaporate the filtrate on a water bath to dryness. Add 10 mL of 0.1 N hydrochloric acid to the residue, warm the solution, shake, and filter. To the filtrate add sufficient 1 N sodium hydroxide to make it alkaline, and extract the solution with 10 mL of chloroform, drying the chloroform extract over anhydrous sodium sulfate. Filter the dried chloroform solution, evaporate the filtrate on a water bath to dryness, and dry the residue at 105° for 1 h.

- **B.** The retention time of the atenolol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: 1.1 g of sodium 1-heptanesulfonate and 0.71 g of anhydrous dibasic sodium phosphate in 700 mL of water. Add 2 mL of dibutylamine, and adjust with 0.8 M phosphoric acid to a pH of 3.0. Add 300 mL of methanol, and pass through a filter having a 0.5-µm or finer porosity. Degas this solution before use.

Standard solution: 0.01 mg/mL of USP Atenolol RS in *Mobile phase*

Sample stock solution: Transfer 10 Tablets to a 1000-mL volumetric flask. Add 500 mL of *Mobile phase*, and sonicate for 15 min to disintegrate the Tablets. Dilute with *Mobile phase* to volume.

Sample solution: Centrifuge a portion of the *Sample stock solution*, and dilute a volume of the supernatant with *Mobile phase* to obtain a solution nominally containing 0.01 mg/mL of atenolol.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 226 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 0.6 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 5000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_{22}N_2O_3$ in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Atenolol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of atenolol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N acetate buffer, pH 4.6 (prepared by mixing 44.9 parts (v/v) of 0.1 N sodium acetate with 55.1 parts (v/v) of 0.1 N acetic acid solution, and adjust with either diluted sodium hydroxide or diluted acetic acid to a pH of 4.6); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Determine the amount of $C_{14}H_{22}N_2O_3$ dissolved by using the following method.
Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay* under Atenolol.
Standard solution: 0.01 mg/mL of USP Atenolol RS in *Mobile phase*
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter. Quantitatively dilute a measured volume of the filtrate with *Mobile phase* to obtain a solution estimated to contain about 0.01 mg/mL of atenolol.
Analysis: Proceed as directed in the *Assay*.
Calculate the percentage of $C_{14}H_{22}N_2O_3$ dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (100/L)$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Atenolol RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*
L = Tablet label claim (mg)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{14}H_{22}N_2O_3$ is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Atenolol RS

Atenolol Compounded Oral Suspension

DEFINITION
Atenolol Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of atenolol ($C_{14}H_{22}N_2O_3$).

Prepare Atenolol Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Atenolol powder	1 g
Vehicle: a 1:1 mixture of Ora-Plus[a] and Ora-Sweet SF[a], a sufficient quantity to make	100 mL

[a] Perrigo Pharmaceuticals, Allegan, MI.

Pour *Atenolol powder* into a suitable container. Wet the powder with a small amount of *Vehicle*, and triturate to make a smooth paste. Add *Vehicle* to make the contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the remainder of *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY
• **PROCEDURE**
Solution A: 25 mM sodium phosphate adjusted with phosphoric acid to a pH of 3.0
Solution B: Water adjusted with phosphoric acid to a pH of 3.0
Solution C: Methanol and *Solution B* (50:50)
Mobile phase: Acetonitrile and *Solution A* (15:85). Filter, and degas.
Standard stock solution: 10 mg/mL of USP Atenolol RS in *Solution C*. Mix well and sonicate for 3 min. Store at 2°–8°.
Standard solution: Transfer 2.0 mL of the *Standard stock solution* to a 500-mL volumetric flask, add 0.5 mL of *Solution C*, and dilute with *Solution B* to volume. Mix well. Centrifuge a portion of the resultant solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.
Sample solution: Shake each bottle of Oral Suspension thoroughly. Transfer 2.0 mL of Oral Suspension into a 500-mL volumetric flask, and add 0.5 mL of *Solution C*. Dilute with *Solution B* to volume. Mix well. Centrifuge a portion of the solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 227 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 30°
Autosampler: 5°
Flow rate: 0.7 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for atenolol is about 5.1 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of atenolol ($C_{14}H_{22}N_2O_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of atenolol from the *Sample solution*
r_S = peak response of atenolol from the *Standard solution*

www.webofpharma.com

C_S = concentration of atenolol in the *Standard solution* (mg/mL)
C_U = nominal concentration of atenolol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 6.4–7.4

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date.*
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or at controlled room temperature
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Atenolol RS

Atenolol Compounded Oral Suspension, Veterinary

DEFINITION
Atenolol Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of atenolol ($C_{14}H_{22}N_2O_3$). Prepare Atenolol Compounded Oral Suspension, Veterinary 25 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Atenolol powder	2.5 g
Vehicle: a 1:1 mixture of Ora-Plus[a] and Ora-Sweet SF[a], a sufficient quantity to make	100 mL

[a] Perrigo Pharmaceuticals, Allegan, MI.

Pour the *Atenolol powder* into a suitable container. Wet the powder with a small amount of *Vehicle*, and triturate to make a smooth paste. Add the *Vehicle* to make the contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY
• **PROCEDURE**
Solution A: 25 mM sodium phosphate adjusted with phosphoric acid to a pH of 3.0
Mobile phase: Acetonitrile and *Solution A* (15:85). Filter, and degas.
Solution B: Water adjusted with phosphoric acid to a pH of 3.0
Solution C: Methanol and *Solution B* (50:50)
Standard stock solution: 25 mg/mL of USP Atenolol RS in *Solution C*. Mix well, and sonicate for 3 min. Store at 2°–8°.
Standard solution: Transfer 2.0 mL of the *Standard stock solution* to a 1-L volumetric flask, and add 0.5 mL of *Solution C*. Dilute with *Solution B* to volume, and mix well. Centrifuge a portion of the resultant solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.
Sample solution: Shake thoroughly each bottle of Oral Suspension, Veterinary. Transfer 2.0 mL of Oral Suspension, Veterinary to a 1-L volumetric flask, and add 0.5 mL of *Solution C*. Dilute with *Solution B* to volume. Centrifuge a portion of the solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 227 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 30°
Autosampler: 5°
Flow rate: 0.7 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for atenolol is about 5.1 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of atenolol ($C_{14}H_{22}N_2O_3$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of atenolol from the *Sample solution*
r_S = peak response of atenolol from the *Standard solution*
C_S = concentration of atenolol in the *Standard solution* (mg/mL)
C_U = nominal concentration of atenolol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 9.1–10.1

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*. Label it to state that it is for veterinary use only.
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or controlled room temperature
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Atenolol RS

Atenolol and Chlorthalidone Tablets

» Atenolol and Chlorthalidone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of atenolol ($C_{14}H_{22}N_2O_3$) and chlorthalidone ($C_{14}H_{11}ClN_2O_4S$).

Packaging and storage—Preserve in well-closed containers.

www.webofpharma.com

USP Reference standards ⟨11⟩—
USP Atenolol RS
USP Chlorthalidone RS

Identification—

A: Shake a quantity of powdered Tablets, equivalent to about 50 mg of chlorthalidone, with 5 mL of methanol for 15 minutes, and filter. Apply 10 µL of this test solution, 10 µL of a Standard solution of USP Chlorthalidone RS in methanol containing 10 mg per mL, and 10 µL of a second Standard solution of USP Atenolol RS in methanol containing 10*J* mg per mL, *J* being the ratio of the labeled amount, in mg, of atenolol to the labeled amount, in mg, of chlorthalidone per Tablet to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-butyl alcohol and 1 N ammonium hydroxide (5:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and air-dry. Locate the spots on the plate by viewing under short-wavelength UV light: the principal spots obtained from the test solution correspond in R_F value, size, and intensity to those obtained from the respective Standard solutions.

B: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.

Determine the amounts of atenolol ($C_{14}H_{22}N_2O_3$) and chlorthalidone ($C_{14}H_{11}ClN_2O_4S$) dissolved by employing the following method.

Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay.*

Diluent—Prepare a mixture of 1000 mL of acetonitrile and 32 mL of 3.6 N sulfuric acid.

Standard solvent—Prepare a mixture of water and *Diluent* (750: 225).

Standard solution—Dissolve accurately weighed quantities of USP Atenolol RS and USP Chlorthalidone RS in *Standard solvent* to obtain a solution having known concentrations of about 0.00085*L* mg of USP Atenolol RS and 0.00085*L'* mg of USP Chlorthalidone RS per mL, *L* and *L'* being the labeled amounts, in mg, of atenolol and chlorthalidone, respectively, per Tablet.

Test solution—Mix 10.0 mL of the filtered solution under test and 3.0 mL of *Diluent.*

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of atenolol ($C_{14}H_{22}N_2O_3$) and chlorthalidone ($C_{14}H_{11}ClN_2O_4S$) dissolved by the same formula:

$$1170C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of the appropriate Reference Standard in the *Standard solution;* and r_U and r_S are the responses of the corresponding analyte obtained from the *Test solution* and the *Standard solution,* respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of atenolol ($C_{14}H_{22}N_2O_3$) is dissolved in 45 minutes, and not less than 70% *(Q)* of the labeled amount of chlorthalidone ($C_{14}H_{11}ClN_2O_4S$) is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Procedure for content uniformity—Proceed as directed in the *Assay,* except to prepare the *Assay preparation* as follows. Transfer 1 Tablet to a volumetric flask of such capacity that when filled to volume, a concentration of about 0.25 mg of chlorthalidone per mL is obtained. Add a mixture of water and acetonitrile (1:1) to about half the capacity of the flask, and shake by mechanical means for not less than 15 minutes to disintegrate the Tablet. Dilute with water to volume, and mix. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity, and use the filtrate as the *Assay preparation.* Calculate the quantities, in mg, of atenolol ($C_{14}H_{22}N_2O_3$) and chlorthalidone ($C_{14}H_{11}ClN_2O_4S$) in the Tablet taken by the formula:

$$CV(r_U/r_S)$$

in which *V* is the volume, in mL, of the volumetric flask used to prepare the *Assay preparation;* and the other terms are as defined in the *Assay.*

Assay—

Mobile phase—Prepare a mixture of 740 mL of water, 250 mL of acetonitrile, 8 mL of 3.6 N sulfuric acid, and 930 mg of sodium octyl sulfate. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve accurately weighed quantities of USP Atenolol RS and USP Chlorthalidone RS in a mixture of water and acetonitrile (3:1) to obtain a solution having known concentrations of about 0.25 mg of USP Chlorthalidone RS and 0.25*J* mg of USP Atenolol RS per mL, *J* being the ratio of the labeled amount, in mg, of atenolol to the labeled amount, in mg, of chlorthalidone per Tablet.

Assay preparation—Transfer 10 Tablets to a volumetric flask of such capacity that when filled to volume, a concentration of about 0.5 mg of chlorthalidone per mL is obtained. Add a mixture of water and acetonitrile (1:1) to about half the capacity of the flask, and shake by mechanical means for not less than 15 minutes to disintegrate the Tablets. Dilute with a mixture of water and acetonitrile (1:1) to volume, and mix. Pass a portion of this stock solution through a filter having a 0.5-µm or finer porosity. Transfer 25.0 mL of the clear filtrate to a 50-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.7 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for atenolol and 1.0 for chlorthalidone; the resolution, *R,* between the atenolol and chlorthalidone peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of atenolol ($C_{14}H_{22}N_2O_3$) and chlorthalidone ($C_{14}H_{11}ClN_2O_4S$) in each Tablet taken by the formula:

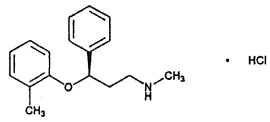
$$2C(V/10)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation; V* is the volume, in mL, of the volumetric flask used to prepare the stock solution for the *Assay preparation;* and r_U and r_S are the responses for the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation,* respectively.

www.webofpharma.com

[NOTE—If a trailing peak or shoulder is observed on the chlorthalidone peak with a relative retention time of not more than 1.1 in the chromatograms of both the *Standard preparation* and the *Assay preparation,* sum the areas for the chlorthalidone peak with the trailing peak or shoulder to report the peak responses for chlorthalidone.]

Atomoxetine Hydrochloride



$C_{17}H_{21}NO \cdot HCl$ 291.82

Benzenepropanamine, *N*-methyl-γ-(2-methylphenoxy)-, hydrochloride, (–);
(–)-*N*-Methyl-3-phenyl-3-(*o*-tolyloxy)propylamine hydrochloride [82248-59-7].

DEFINITION

Atomoxetine Hydrochloride contains NLT 98.0% and NMT 102.0% of atomoxetine hydrochloride ($C_{17}H_{21}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the atomoxetine *R*-isomer from the *System suitability solution,* as obtained in the test for *Organic Impurities, Procedure 2.*
- **C. IDENTIFICATION TESTS—GENERAL ⟨191⟩,** *Chloride*: Meets the requirements of test *A*

ASSAY

- **PROCEDURE**

Buffer: 2.9 g/L of phosphoric acid in water. Adjust with 5 M potassium hydroxide solution to a pH of 2.5. To 1 L of this solution add 5.9 g of octanesulfonic acid sodium salt monohydrate.

Mobile phase: *n*-Propanol and *Buffer* (27:73). [NOTE—The ratio of *n*-propanol in *Buffer* can be varied between 26:74 and 29:71 to meet system suitability requirements.]

System suitability solution: 0.1 mg/mL of USP Mandelic Acid RS, 0.15 mg/mL of USP Atomoxetine Related Compound A RS, and 0.25 mg/mL of USP Atomoxetine Hydrochloride RS in *Mobile phase*

Standard solution: 0.25 mg/mL of USP Atomoxetine Hydrochloride RS in *Mobile phase.* Sonication may be used to aid in dissolution.

Sample solution: 0.25 mg/mL of Atomoxetine Hydrochloride in *Mobile phase.* Sonication may be used to aid in dissolution.

Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability.*)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 1.3 times the retention time of atomoxetine

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* in *Organic Impurities, Procedure 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between mandelic acid and atomoxetine related compound A, *System suitability solution*
Tailing factor: NMT 1.5 for atomoxetine, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of atomoxetine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) in the portion of Atomoxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Atomoxetine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Atomoxetine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

[NOTE—It is required to perform *Organic Impurities, Procedure 1* and *Organic Impurities, Procedure 2.*]

Procedure 1

Buffer and **Mobile phase:** Prepare as directed in the *Assay.*

System suitability solution: 0.10 mg/mL of USP Mandelic Acid RS, 0.15 mg/mL of USP Atomoxetine Related Compound A RS, and 0.25 mg/mL of USP Atomoxetine Hydrochloride RS in *Mobile phase*

Standard solution: 0.0025 mg/mL of USP Atomoxetine Hydrochloride RS in *Mobile phase*

Sample solution: 2.5 mg/mL of Atomoxetine Hydrochloride in *Mobile phase*

Chromatographic system: Proceed as directed in the *Assay,* except for *Run time.*
Run time: 2.6 times the retention time of atomoxetine

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between mandelic acid and atomoxetine related compound A, *System suitability solution*
Tailing factor: NMT 1.5 for atomoxetine, *System suitability solution*
Relative standard deviation: NMT 5% from three injections, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Atomoxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of atomoxetine from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Atomoxetine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Atomoxetine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mandelic acid	0.20	0.10
Atomoxetine related compound A	0.27	0.10
Desmethyl atomoxetine[a]	0.73	0.3
Atomoxetine	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

[a] (*R*)-*N*-Methyl-3-phenoxy-3-phenylpropan-1-amine.

Procedure 2
Mobile phase: Isopropyl alcohol, diethylamine, trifluoroacetic acid, and *n*-hexane (150: 1.5: 2.0: 846.5)
System suitability solution: 3.5 mg/mL of USP Atomoxetine Hydrochloride RS, 17.5 µg/mL of USP Atomoxetine S-Isomer RS, and 3.5 µg/mL of USP Atomoxetine Related Compound B RS, prepared by first dissolving the Reference Standards in absolute alcohol, using 25% of the final volume. Dilute with *n*-hexane to volume.
Sample solution: 3.5 mg/mL of Atomoxetine Hydrochloride prepared by first dissolving it in absolute alcohol, using 25% of the final volume. Dilute with *n*-hexane to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 273 nm
Column: 4.6-mm × 25-cm; 5-µm packing L40
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 1.3 times the retention time of atomoxetine
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.75 between atomoxetine *S*-isomer and atomoxetine related compound B
Tailing factor: NMT 1.8 for atomoxetine
Analysis
Sample: *Sample solution*
Calculate the percentage of atomoxetine related compound B, atomoxetine related compound C, and atomoxetine *S*-isomer in the portion of Atomoxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of all the peak responses of atomoxetine related compound B, atomoxetine related compound C, atomoxetine *S*-isomer, and atomoxetine from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Atomoxetine *S*-isomer[a]	0.47	0.5
Atomoxetine related compound C[b]	0.52	0.1
Atomoxetine related compound B	0.56	0.1
Atomoxetine	1.0	—

[a] *N*-Methyl-3-phenyl-3-(*o*-tolyloxy)propan-1-amine.
[b] *N*-Methyl-3-phenyl-3-(*p*-tolyloxy)propan-1-amine.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Atomoxetine Hydrochloride RS
USP Atomoxetine Related Compound A RS
3-(Methylamino)-1-phenylpropan-1-ol.
$C_{10}H_{15}NO$ 165.23
USP Atomoxetine Related Compound B RS
N-Methyl-3-phenyl-3-(*m*-tolyloxy)propan-1-amine hydrochloride.
$C_{17}H_{21}NO \cdot HCl$ 291.82
USP Atomoxetine S-Isomer RS
(*S*)-*N*-Methyl-3-phenyl-3-(*o*-tolyloxy)propan-1-amine hydrochloride.
$C_{17}H_{21}NO \cdot HCl$ 291.82
USP Mandelic Acid RS
α-Hydroxyphenylacetic acid.
$C_8H_8O_3$ 152.15

Atomoxetine Capsules

DEFINITION
Atomoxetine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of atomoxetine ($C_{17}H_{21}NO$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K** or **197A**▲ (CN 1-May-2020)
Standard: 6 mg/mL of USP Atomoxetine Hydrochloride RS in methanol. Dry the solution to a dry powder under an air or nitrogen purge for a minimum of 3 h.
Sample: Shake the contents of a sufficient number of Capsules, equivalent to about 60 mg of atomoxetine, with 10 mL of methanol. Centrifuge at 4000 rpm for 5 min. Evaporate the solution to a dry powder with the aid of a current of air or stream of nitrogen.
Acceptance criteria: The IR spectrum exhibits main bands at (±2) wavenumbers (cm^{-1}) 2955, 2855, 1599–1604, 1492, 1048, 1023, and 1010.

www.webofpharma.com

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 5.8 g/L of monobasic potassium phosphate in water. To each liter of this solution add 3.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Buffer* (38:62)

System suitability solution: 0.1 mg/mL of atomoxetine (free base) from USP Atomoxetine Hydrochloride RS and 0.02 mg/mL of *o*-cresol in *Mobile phase*. Sonicate to aid in dissolution.

Standard solution: 0.1 mg/mL of atomoxetine (free base) from USP Atomoxetine Hydrochloride RS in *Mobile phase*. Sonicate to aid in dissolution.

Sample stock solution: From NLT 10 Capsules (including shells) prepared as follows. Add the intact Capsules to a suitable volumetric flask. Add *Mobile phase* to fill 65% of the final volume. Allow to stand for at least 10 min, then shake for 20 min. Dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.1 mg/mL of atomoxetine, prepared by diluting a suitable volume of *Sample stock solution* with *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 7.5-cm; 3.5-µm packing L7

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: 1.7 times the retention time of atomoxetine

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for atomoxetine and *o*-cresol are 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 3.5 between atomoxetine and *o*-cresol, *System suitability solution*

Tailing factor: NMT 2.0 for atomoxetine, *System suitability solution*

Relative standard deviation: NMT 1.0% for atomoxetine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of atomoxetine ($C_{17}H_{21}NO$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of atomoxetine in the *Standard solution* (mg/mL)
C_U = nominal concentration of atomoxetine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 1000 mL, deaerated

Apparatus 2: 50 rpm, with three-prong sinker

Time: 30 min

Buffer and **Mobile phase:** Prepare as directed in the *Assay*.

Standard stock solution: 0.1 mg/mL of atomoxetine (free base) from USP Atomoxetine Hydrochloride RS in *Medium*. Sonicate to aid in dissolution.

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (*L*/1000) mg/mL, where *L* is the Capsule label claim in mg.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.4%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of atomoxetine ($C_{17}H_{21}NO$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of atomoxetine in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium* (mL)

Tolerances: NLT 80% (*Q*) of the labeled amount of atomoxetine ($C_{17}H_{21}NO$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer: Dissolve 4.9 g of sodium 1-decanesulfonate and 6.9 g of monobasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 3.1.

Mobile phase: Acetonitrile and *Buffer* (41:59)

Sensitivity solution: 0.1 µg/mL of atomoxetine in *Mobile phase*

System suitability solution: 1 mg/mL of atomoxetine containing atomoxetine *N*-amide prepared as follows. Weigh equal amounts of USP Atomoxetine Hydrochloride RS and urea, and place in a volumetric flask. Add water to fill 10% of the final volume. Sonicate for 3 min. Place the flask in an 85° oven for 40 min. Allow the solution to cool to room temperature. Dilute with *Mobile phase* to volume. [NOTE—The oven temperature and time in the oven can be adjusted to give a suitable level of atomoxetine *N*-amide peak.]

Sample solution: 1 mg/mL of atomoxetine in *Mobile phase*, from the contents of NLT 5 Capsules prepared as follows. Transfer the Capsule contents to a suitable volumetric flask. Fill 50% of the final volume with *Mobile phase*. Swirl, and let stand for 15 min. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 2.3 times the retention time of atomoxetine

System suitability

Samples: *Sensitivity solution* and *System suitability solution*

[NOTE—See *Table 1* for the relative retention times.]

www.webofpharma.com

Suitability requirements
Resolution: NLT 2.6 between atomoxetine and atomoxetine *N*-amide, *System suitability solution*
Relative standard deviation: NMT 5%, *Sensitivity solution*

Analysis
Sample: *Sample solution*
Calculate the percentage of each individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*.

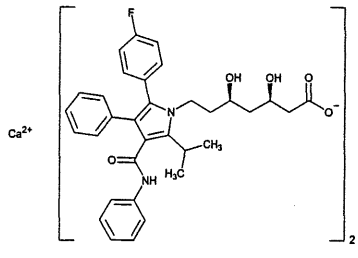
Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desmethyl atomoxetine[a]	0.76	0.3
Atomoxetine	1.0	—
Atomoxetine *N*-amide[b]	1.2	0.2
Any individual unspecified degradation product	—	0.2
Total impurities	—	1.0

[a] (*R*)-*N*-Methyl-3-phenoxy-3-phenylpropan-1-amine.
[b] (*R*)-1-Methyl-1-[3-phenyl-3-(*o*-tolyloxy)propyl]urea.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Atomoxetine Hydrochloride RS

Atorvastatin Calcium



$C_{66}H_{68}CaF_2N_4O_{10}$ 1155.36
1*H*-Pyrrole-1-heptanoic acid, 2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylami no)carbonyl]-, calcium salt (2:1), [*R*-(*R**,*R**)]-;
Calcium (β*R*,δ*R*)-2-(*p*-fluorophenyl)-β,δ-dihydroxy-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)pyrrole-1-heptanoate (1:2);
[(3*R*,5*R*)-7-[3-(Phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt]

Anhydrous [134523-03-8].
$C_{66}H_{68}CaF_2N_4O_{10} \cdot 3H_2O$ 1209.41
Trihydrate [344423-98-9].
$C_{66}H_{68}CaF_2N_4O_{10} \cdot C_3H_8O_2$
Propylene glycol solvate 1231.46

DEFINITION
Atorvastatin Calcium contains NLT 98.0% and NMT 102.0% of atorvastatin calcium ($C_{66}H_{68}CaF_2N_4O_{10}$), calculated on the anhydrous and solvent-free basis. If labeled as a propylene glycol solvate, it contains NLT 98.0% and NMT 102.0% of atorvastatin calcium ($C_{66}H_{68}CaF_2N_4O_{10}$), calculated on the anhydrous, propylene glycol-free, and solvent-free basis. It may contain a suitable antioxidant.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  [NOTE—If a difference appears in the IR spectra of the analyte and the standard, separately dissolve equal portions of the sample specimen and the USP Reference Standard in equal volumes of methanol, evaporate the solution to dryness in similar containers under identical conditions, and repeat the test on the residues.]
- **B. CALCIUM**
  Diluent: Methanol, water, and hydrochloric acid (75:25:2)
  Sample solution: 0.05 mg/mL of Atorvastatin Calcium in *Diluent*
  Blank: *Diluent*
  Analysis
  Samples: *Sample solution* and *Blank*
  Instrumental conditions
  (See *Atomic Absorption Spectroscopy* ⟨852⟩.)
  Mode: Atomic absorption spectrophotometry
  Analytical wavelength: Calcium emission line at 422.7 nm
  Flame: Air–acetylene
  Acceptance criteria: The *Sample solution* exhibits a significant absorption at the calcium emission line at 422.7 nm.

ASSAY
- **PROCEDURE**
  Buffer: 3.9 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 5.0 ± 0.1.
  Solution A: Acetonitrile, stabilizer-free tetrahydrofuran, and *Buffer* (21:12:67)
  Solution B: Acetonitrile, stabilizer-free tetrahydrofuran, and *Buffer* (61:12:27)
  Mobile phase: See *Table 1*. [NOTE—If necessary, adjust the *Mobile phase* by increasing or decreasing the percentage of acetonitrile or the pH of the ammonium acetate solution to achieve a retention time of 26–34 min for the atorvastatin peak.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	100	0
70	20	80
85	0	100
100	0	100

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
105	100	0
115	100	0

Diluent: *N,N*-dimethylformamide
System suitability solution: 0.05 mg/mL of USP Atorvastatin Calcium RS and 0.06 mg/mL of USP Atorvastatin Related Compound B RS in *Diluent*
Standard solution: 0.4 mg/mL of USP Atorvastatin Calcium RS in *Diluent*. [NOTE—Use sonication if necessary.]
Sample solution: 0.4 mg/mL of Atorvastatin Calcium in *Diluent*. [NOTE—Use sonication if necessary.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
[NOTE—If significant fronting of the peaks for atorvastatin related compound B and atorvastatin is observed, use the following diluent to prepare the *Sample solution*, the *Standard solution*, and the *System suitability solution*: acetonitrile, stabilizer-free tetrahydrofuran, and water (1:1:2).]
Mode: LC
Detector: UV 244 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between the peaks for atorvastatin related compound B and atorvastatin, *System suitability solution*
Tailing factor: NMT 1.6, *Standard solution*
Relative standard deviation: NMT 0.6%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of atorvastatin calcium ($C_{66}H_{68}CaF_2N_4O_{10}$) in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Atorvastatin Calcium RS in the *Standard solution* (mg/mL)
C_U = concentration of Atorvastatin Calcium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis. If labeled as a propylene glycol solvate, 98.0%–102.0% on the anhydrous, propylene glycol-free, and solvent-free basis.

OTHER COMPONENTS

• **CONTENT OF PROPYLENE GLYCOL** (if labeled as a propylene glycol solvate)
Diluent: Dimethylsulfoxide
Standard solution: 0.125 mg/mL of propylene glycol in *Diluent*
Sample solution: 2.5 mg/mL of Atorvastatin Calcium (as propylene glycol solvate) in *Diluent*. Use sonication as needed to achieve a complete dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 75-m; 3-µm coating of G43
Temperatures
Injection port: 230°
Detector: 250°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	0	100	1
100	10	140	5
140	30	225	3

Carrier gas: Helium
Flow rate: 6.0 mL/min
Injection volume: 1 µL
Injection type: Splitless, using a suitable inlet liner
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of propylene glycol in the portion of Atorvastatin Calcium as propylene glycol solvate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of propylene glycol from the *Sample solution*
r_S = peak response of propylene glycol from the *Standard solution*
C_S = concentration of propylene glycol in the *Standard solution* (mg/mL)
C_U = concentration of Atorvastatin Calcium (as propylene glycol solvate) in the *Sample solution* (mg/mL)

Acceptance criteria: 5.4%–7.3%

IMPURITIES

• **ORGANIC IMPURITIES, PROCEDURE 1:** [NOTE—On the basis of the synthetic route or of the solid state nature of the drug substance, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* may be suitable when atorvastatin lactone, atorvastatin epoxy tetrahydrofuran analog, and atorvastatin acetonide are possible related compounds, and it may be suitable for an amorphous form of the drug substance.]
Buffer, Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 1.5 µg/mL each of USP Atorvastatin Related Compound A RS, USP Atorvastatin Related Compound B RS, USP Atorvastatin Related Compound C RS, and USP Atorvastatin Related Compound D RS in *Diluent*
Sample solution: 1 mg/mL of Atorvastatin Calcium in *Diluent*. [NOTE—Use sonication if necessary.]
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between the peaks for atorvastatin related compound B and atorvastatin

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Chromatograph the *Standard solution,* and identify the components based on their relative retention times, given in *Table 3*.
Calculate the percentage of each of the atorvastatin related compounds A, B, C, and D in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the relevant atorvastatin related compound from the *Sample solution*
r_S = peak response of the relevant atorvastatin related compound from the *Standard solution*
C_S = concentration of the relevant atorvastatin related compound in the *Standard solution* (mg/mL)
C_U = concentration of Atorvastatin Calcium in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 3*. Disregard any peak observed in the blank; the reporting level for impurities is 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Atorvastatin related compound A[a]	0.8	0.3
Atorvastatin related compound B[b]	0.9	0.3
Atorvastatin	1.0	—
Atorvastatin related compound C[c]	1.2	0.3
Atorvastatin related compound D[d, e]	2.1	0.2
Any other individual impurity	—	0.1
Total impurities[f]	—	1.0

[a] Desfluoro impurity.
[b] 3*S*,5*R* Isomer.
[c] Difluoro impurity.
[d] Epoxide impurity.
[e] Atorvastatin related compound D may undergo a conversion to its cyclic hemiketal, which is a specified impurity listed in *Table 5* in *Organic Impurities, Procedure 2*, as "atorvastatin epoxy tetrahydrofuran analog". The cyclic hemiketal of atorvastatin related compound D elutes about 1–2 min before atorvastatin related compound D. Use the sum of the areas of the two peaks as a peak response for atorvastatin related compound D in the *Standard solution* and the *Sample solution*.
[f] This total does not include atorvastatin related compound E, as determined in the *Enantiomeric Purity* test.

• **ORGANIC IMPURITIES, PROCEDURE 2**
Buffer: pH 5.0 mixture of 0.045 M ammonium formate and 0.0045 M ammonium acetate solutions, prepared as follows. Weigh 2.84 g of ammonium formate and 0.35 g of ammonium acetate, and dissolve in 950 mL of water. Adjust with 20% formic acid to a pH of 5.0, and dilute with water to 1 L.
Solution A: Acetonitrile and *Buffer* (33:67)
Solution B: Acetonitrile
Solution C: Stabilizer-free tetrahydrofuran
Mobile phase: See *Table 4*. Return to original conditions, and re-equilibrate the system.

Table 4

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	91	0	9
15	91	6	3
20	82	16	2
25	82	16	2
50	32	66	2
55	32	66	2

Diluent: Acetonitrile, stabilizer-free tetrahydrofuran, and *Buffer* (60:5:35)
Peak identification solution: 0.5 mg/mL of USP Atorvastatin Calcium RS and 2.5 µg/mL each of USP Atorvastatin Related Compound A RS, USP Atorvastatin Related Compound B RS, USP Atorvastatin Related Compound H RS, and USP Atorvastatin Related Compound I RS in *Diluent*
Sample solution: 0.5 mg/mL of Atorvastatin Calcium in *Diluent*. Use sonication to dissolve. [NOTE—The solution is stable for 3 h at room temperature and for 24 h when stored at 2°–8°, protected from light.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 4-µm packing L11
Temperatures
Column: 40°
Autosampler: 4°
Flow rate: 1.1 mL/min
Injection volume: 15 µL
System suitability
Sample: *Peak identification solution*
Suitability requirements
Peak-to-valley ratio: NLT 2 between the peaks for atorvastatin related compound B and atorvastatin
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Sample solution*
r_T = sum of all the peak responses, each divided by the corresponding value of the relative response factor from *Table 5*
F = relative response factor for the impurity (see *Table 5*)

Acceptance criteria: See *Table 5*. Disregard any peak eluting before 2 min and any peak observed in the blank; the reporting level for impurities is 0.05%.

www.webofpharma.com

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Atorvastatin diamino[a]	0.58	0.74	0.15
Atorvastatin related compound A[b]	0.86	1.0	0.3
Atorvastatin related compound B[c]	0.94	1.0	0.3
Atorvastatin	1.0	—	—
Atorvastatin related compound C[d] (if present)	1.1	1.0	0.3
Atorvastatin 3-deoxy-hept-2-enoic acid[e]	1.45	1.0	0.10
Atorvastatin related compound H[f]	1.90	1.0	0.15
Atorvastatin epoxy tetrahydrofuran analog[g]	2.00	0.71	0.15
Atorvastatin ethyl ester[h]	2.08	1.0	0.15
Atorvastatin related compound D[i]	2.18	1.3	0.15
Atorvastatin related compound I[j]	2.75	1.0	0.15
Any other individual impurity	—	1.0	0.10
Total impurities[k]	—	—	1.0

[a] (3*R*,5*R*)-7-{(3*R*,5*R*)-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanamido}-3,5-dihydroxyheptanoic acid.
[b] Desfluoro impurity.
[c] 3*S*,5*R* Isomer.
[d] Difluoro impurity.
[e] (*S*,*E*)-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-5-hydroxyhept-2-enoic acid.
[f] Lactone impurity.
[g] 4-(4-Fluorophenyl)-2,4-dihydroxy-2-isopropyl-*N*,5-diphenyl-3,6-dioxabicyclo[3.1.0]hexane-1-carboxamide.
[h] (3*R*,5*R*)-Ethyl 7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl)-3,5-dihydroxyheptanoate.
[i] Epoxide impurity.
[j] Acetonide impurity.
[k] This total does not include atorvastatin related compound E, as determined in the *Enantiomeric Purity* test.

• **Enantiomeric Purity**
Mobile phase: Hexane, dehydrated alcohol, and trifluoroacetic acid (940:60:1)
System suitability stock solution: 5 mg/mL of USP Atorvastatin Calcium RS and 37.5 µg/mL of USP Atorvastatin Related Compound E RS in methanol. [Note—Atorvastatin related compound E is the 3*S*,5*S* enantiomer of atorvastatin.]
System suitability solution: Transfer 2.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, add 2.0 mL of dehydrated alcohol, and dilute with hexane to volume.
Sample solution: Transfer 10 mg of Atorvastatin Calcium to a 10-mL volumetric flask, dissolve in 2.0 mL of methanol, add 2.0 mL of dehydrated alcohol, and dilute with hexane to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 244 nm
Column: 4.6-mm × 25-cm; packing L51
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[Note—The elution order of the peaks is atorvastatin related compound E followed by atorvastatin.]
Resolution: NLT 2.0 between the peaks for atorvastatin related compound E and atorvastatin
Analysis
Sample: *Sample solution*
Calculate the percentage of atorvastatin related compound E in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for atorvastatin related compound E
r_T = sum of the peak responses for atorvastatin related compound E and atorvastatin

Acceptance criteria: NMT 0.3% of atorvastatin related compound E

SPECIFIC TESTS

• **Water Determination,** *Method Ia* ⟨921⟩**:** 3.5%–5.5% for the trihydrate form. If labeled as amorphous or as semicrystalline, NMT 6.0%. If labeled as a propylene glycol solvate, NMT 1.0%.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve the trihydrate form in well-closed containers, and store at room temperature. If labeled as amorphous or semicrystalline or as a propylene glycol solvate, store as per labeling instructions. Possible packaging and storage conditions could include the following: Preserve in well-closed containers protected from light and moisture, or in tight containers; store at room temperature, at controlled room temperature, or at 2°–8°; store under nitrogen atmosphere or packed with an oxygen absorber; and store under nitrogen atmosphere, packed with silica gel and an oxygen absorber.
• **Labeling:** Where it is an amorphous form, the label so indicates. Where it is a semicrystalline form, the label so indicates. Where it is a propylene glycol solvate form, the label so indicates. If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies. Label it to indicate the name and quantity of any added antioxidant.

Change to read:

• **USP Reference Standards** ⟨11⟩
USP Atorvastatin Calcium RS
USP Atorvastatin Related Compound A RS
▲Calcium (3*R*,5*R*)-7-[2-isopropyl-4,5-diphenyl-3-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoate (1:2);
Also known as▲ (ERR 1-May-2019) Desfluoro impurity, or (3*R*,5*R*)-7-[3-(phenylcarbamoyl)-2-isopropyl-4,5-diphenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoicacid, calcium salt.
$C_{66}H_{70}CaN_4O_{10}$ 1119.38
USP Atorvastatin Related Compound B RS
3*S*,5*R* Isomer, or (3*S*,5*R*)-7-[3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.
$C_{66}H_{68}CaF_2N_4O_{10}$ 1155.34

www.webofpharma.com

USP Atorvastatin Related Compound C RS
Difluoro impurity, or (3*R*,5*R*)-7-[3-(phenylcarbamoyl)-4,5-bis(4-fluorophenyl)-2-isopropyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.
$C_{66}H_{66}F_4N_4O_{10}$ 1191.34

USP Atorvastatin Related Compound D RS
Epoxide impurity, or 3-(4-fluorobenzoyl)-2-isobutyryl-3-phenyl-oxirane-2-carboxylic acid phenylamide.
$C_{26}H_{22}FNO_4$ 431.46

USP Atorvastatin Related Compound E RS
3*S*,5*S* Enantiomer, or (3*S*,5*S*)-7-[3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.
$C_{66}H_{68}CaF_2N_4O_{10}$ 1155.34

USP Atorvastatin Related Compound H RS (lactone impurity)
5-(4-Fluorophenyl)-1-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-2-isopropyl-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide.
$C_{33}H_{33}FN_2O_4$ 540.62

USP Atorvastatin Related Compound I RS (acetonide impurity)
tert-Butyl 2-((4*R*,6*R*)-6-{2-[2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]ethyl}-2,2-dimethyl-1,3-dioxan-4-yl)acetate.
$C_{40}H_{47}FN_2O_5$ 654.81

Atorvastatin Calcium Tablets

DEFINITION

Atorvastatin Calcium Tablets contain an amount of atorvastatin calcium $[(C_{33}H_{34}FN_2O_5)_2Ca]$, equivalent to NLT 94.5% and NMT 105.0% of the labeled amount of atorvastatin.

IDENTIFICATION

- **A.** The UV absorption spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.05 M ammonium citrate buffer pH 4.0 prepared as follows. Dissolve 9.62 g of anhydrous citric acid in 950 mL of water, adjust with ammonium hydroxide to a pH of 4.0, and dilute with water to 1000 mL.
Mobile phase: Acetonitrile, stabilizer-free tetrahydrofuran, and *Buffer* (27:20:53)
Solution A: Dissolve 9.62 g of anhydrous citric acid in 900 mL of water, adjust with ammonium hydroxide to a pH of 7.4, and dilute with water to 1000 mL.
Diluent: Acetonitrile and *Solution A* (1:1)
System suitability solution: 0.1 mg/mL of USP Atorvastatin Calcium RS and 0.01 mg/mL of USP Atorvastatin Related Compound H RS in *Diluent*. Shake mechanically for 30 min or until dissolved.
Standard solution: 0.1 mg/mL of USP Atorvastatin Calcium RS in *Diluent*. Shake mechanically for 15 min or until dissolved.
Sample stock solution: Prepare a known nominal concentration of atorvastatin by transferring NLT 10 Tablets to an appropriate volumetric flask. Add *Diluent* to about 50% of the final volume of the flask, and shake the mixture mechanically for 15 min or until dissolved. Dilute with *Diluent* to volume. Centrifuge or pass through a suitable filter of 0.45-µm pore size.
Sample solution: Nominally equivalent to 0.1 mg/mL of atorvastatin in *Diluent* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector
Assay: UV 244 nm
Identification A: Diode array; UV 200–400 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between atorvastatin and atorvastatin related compound H, *System suitability solution*
Tailing factor: NMT 1.5 for atorvastatin, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of atorvastatin ($C_{33}H_{35}FN_2O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M \times (M_{r1}/M_{r2})] \times 100$$

r_U = peak response of atorvastatin from the *Sample solution*
r_S = peak response of atorvastatin from the *Standard solution*
C_S = concentration of USP Atorvastatin Calcium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of atorvastatin in the *Sample solution* (mg/mL)
M = number of moles of atorvastatin per mole of atorvastatin calcium, 2
M_{r1} = molecular weight of atorvastatin, 558.64
M_{r2} = molecular weight of atorvastatin calcium, 1155.34

Acceptance criteria: 94.5%–105.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Test 1
Buffer: 0.05 M phosphate buffer prepared as follows. Dissolve 6.8 g of monobasic potassium phosphate in 900 mL of water. Adjust with 6 N sodium hydroxide to a pH of 6.8 and dilute with water to 1 L.
Medium: *Buffer*; 900 mL
Apparatus 2: 75 rpm
Time: 15 min
Diluent: Acetonitrile and water (50:50)
Standard stock solution: 1 mg/mL of USP Atorvastatin Calcium RS in *Diluent*. Shake mechanically for 10 min or until dissolved.
Standard solution: (*L*/900) mg/mL in *Medium* from *Standard stock solution*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter or centrifuge prior to analysis.

www.webofpharma.com

Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 244 nm
Cell: See *Table 1* or make appropriate dilutions of the solutions with *Medium* to be within the validated linearity range of the suitable spectrophotometer.

Table 1

Label Claim (mg/Tablet)	Cell (cm)
10	1.0
20 and 40	0.5
80	0.2

Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of atorvastatin ($C_{33}H_{35}FN_2O_5$) dissolved:

$$(A_U/A_S) \times C_S \times V \times D \times [M \times (M_{r1}/M_{r2})] \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Atorvastatin Calcium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*, if applicable
M = number of moles of atorvastatin per mole of atorvastatin calcium, 2
M_{r1} = molecular weight of atorvastatin, 558.64
M_{r2} = molecular weight of atorvastatin calcium, 1155.34
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of atorvastatin ($C_{33}H_{35}FN_2O_5$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*. *Dissolution Test 2* is suitable for products labeled to contain 80 mg of atorvastatin.
Medium and **Apparatus 2:** Proceed as directed in *Test 1*.
Time: 30 min
Diluent, Standard solution, Sample solution, Instrumental conditions, and **Blank:** Proceed as directed in *Test 1*.
Tolerances: NLT 85% (Q) of the labeled amount of atorvastatin ($C_{33}H_{35}FN_2O_5$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Buffer: Combine 250 mL of 0.2 M monobasic potassium phosphate, 112 mL of 0.2 N sodium hydroxide, and 638 mL of water. Adjust with either 0.02 N sodium hydroxide or phosphoric acid to a pH of 6.8.
Solution A: Acetonitrile, methanol, and 0.1% trifluoroacetic acid (5:5:90)
Solution B: Acetonitrile, methanol, and 0.1% trifluoroacetic acid (45:45:10)
Solution C: Dissolve 50 g of Tween 80 in 1 L of *Buffer*.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.00	30	70
0.69	30	70
0.74	0	100
2.73	0	100
2.77	30	70
5.00	30	70

Medium: *Solution C* and *Buffer* (6:94); 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Standard stock solution: 0.96 mg/mL of USP Atorvastatin Calcium RS in methanol
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (L/900) mg/mL, where L is the label claim in mg/Tablet.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 248 nm
Column: 2.1-mm × 5-cm; 2.6-µm packing L1
Column temperature: 40°
Flow rate: 0.7 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of atorvastatin ($C_{33}H_{35}FN_2O_5$) dissolved:

$$(r_U/r_S) \times C_S \times V \times [M \times (M_{r1}/M_{r2})] \times (1/L) \times 100$$

r_U = peak response of atorvastatin from the *Sample solution*
r_S = peak response of atorvastatin from the *Standard solution*
C_S = concentration of USP Atorvastatin Calcium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M = number of moles of atorvastatin per mole of atorvastatin calcium, 2
M_{r1} = molecular weight of atorvastatin, 558.64
M_{r2} = molecular weight of atorvastatin calcium, 1155.34
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of atorvastatin ($C_{33}H_{35}FN_2O_5$) is dissolved.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: Dissolve 6.8 g of monobasic potassium phosphate and 0.89 g of sodium hydroxide in 1 L of water. Adjust with either 1 N sodium hydroxide or phosphoric acid to a pH of 6.8; 900 mL.
Apparatus 2: 75 rpm
Time: 15 min

www.webofpharma.com

Buffer: Dissolve about 6.8 g of monobasic potassium phosphate in 1000 mL of water. Adjust with 0.5 N potassium hydroxide solution to a pH of 6.0.
Mobile phase: Acetonitrile and *Buffer* (55:45)
Standard stock solution: 0.225 mg/mL of atorvastatin from USP Atorvastatin Calcium RS prepared as follows. To a suitable amount of USP Atorvastatin Calcium RS, add 5% of total volume of methanol, sonicate to dissolve, and cool. Dilute with *Medium* to volume.
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (*L*/900) mg/mL, where *L* is the label claim in mg/Tablet.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 248 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of atorvastatin ($C_{33}H_{35}FN_2O_5$) dissolved:

$$(r_U/r_S) \times C_S \times V \times [M \times (M_{r1}/M_{r2})] \times (1/L) \times 100$$

r_U = peak response of atorvastatin from the *Sample solution*
r_S = peak response of atorvastatin from the *Standard solution*
C_S = concentration of USP Atorvastatin Calcium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M = number of moles of atorvastatin per mole of atorvastatin calcium, 2
M_{r1} = molecular weight of atorvastatin, 558.64
M_{r2} = molecular weight of atorvastatin calcium, 1155.34
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of atorvastatin ($C_{33}H_{35}FN_2O_5$) is dissolved.
▲**Test 5:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: Dissolve 6.8 g of monobasic potassium phosphate and 0.9 g of sodium hydroxide in 1 L of water. Adjust with either sodium hydroxide or phosphoric acid to a pH of 6.8; 900 mL.
Apparatus 2: 75 rpm
Time: 20 min
Buffer: Dissolve 10.5 g of citric acid in 1000 mL of water. Adjust with ammonium hydroxide to a pH of 4.0.
Mobile phase: Acetonitrile, tetrahydrofuran, and *Buffer* (50:10:40)
Diluent: Acetonitrile and water (50:50)
Standard stock solution: 0.925 mg/mL of USP Atorvastatin Calcium RS in *Diluent*. Sonicate to dissolve.
Standard solution: (*L*/900) mg/mL in *Medium* from *Standard stock solution*, where *L* is the label claim in mg/Tablet. Pass the solution through a suitable filter of 0.45-µm pore size and discard the first few milliliters of the filtrate.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first few milliliters of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 244 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 30°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
Run time: NLT 2 times the retention time of atorvastatin
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of atorvastatin ($C_{33}H_{35}FN_2O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times [M \times (M_{r1}/M_{r2})] \times (1/L) \times 100$$

r_U = peak response of atorvastatin from the *Sample solution*
r_S = peak response of atorvastatin from the *Standard solution*
C_S = concentration of USP Atorvastatin Calcium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M = number of moles of atorvastatin per mole of atorvastatin calcium, 2
M_{r1} = molecular weight of atorvastatin, 558.64
M_{r2} = molecular weight of atorvastatin calcium, 1155.34
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of atorvastatin ($C_{33}H_{35}FN_2O_5$) is dissolved. ▲(RB 1-Feb-2019)

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Rinse glassware with *Diluent* before preparing solutions containing atorvastatin calcium.
Buffer: 5.75 g/L of monobasic ammonium phosphate in water. Adjust with dilute acetic acid (10% v/v) or dilute ammonium hydroxide (10% v/v) to a pH of 4.3 ± 0.05.
Solution A: Acetonitrile and stabilizer-free tetrahydrofuran (925:75)
Solution B: *Solution A* and *Buffer* (42:58)
Solution C: Methanol, *Solution A*, and *Buffer* (60:20:20)
Diluent: *N,N*-Dimethylformamide
System suitability solution: 60 µg/mL of USP Atorvastatin Calcium RS, 50 µg/mL of USP Atorvastatin Related Compound B RS, 10 µg/mL of USP Atorvastatin Related Compound H RS, and 0.5 µg/mL of USP Atorvastatin Related Compound D RS in *Diluent*
Standard solution: 5 µg/mL of USP Atorvastatin Calcium RS in *Diluent*. Sonication may be necessary for complete dissolution.

www.webofpharma.com

Sample solution: Nominally equivalent to 1 mg/mL of atorvastatin, prepared as follows. Crush and finely powder NLT 20 Tablets. Transfer the amount of powder, equivalent to about 50 mg of atorvastatin, to a 50-mL volumetric flask. Add 30 mL of *Diluent* and shake mechanically for 15 min. Dilute with *Diluent* to volume and pass the solution through a suitable filter of 0.45-µm pore size, discarding the first few mL of the filtrate.

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution B (%)	Solution C (%)	Flow Rate (mL/min)
0	100	0	1.8
30	100	0	1.8
45	25	75	1.5
50	25	75	1.5
55	20	80	1.5
58	100	0	1.8
65	100	0	1.8

For the *Standard solution*, the run time is only 30 min. For the *System suitability solution* and *Sample solution*, the run time is 65 min.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 244 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Autosampler: 10°

Column: 30°

Flow rate: See *Table 3*.

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times of all peaks eluting before atorvastatin related compound H as given in *Table 4* are calculated with respect to the atorvastatin peak. The relative retention times for all peaks eluting after atorvastatin related compound H are calculated with respect to atorvastatin related compound H.]

Suitability requirements

Resolution: NLT 1.4 between atorvastatin related compound B and atorvastatin

Tailing factor: NMT 1.5 for the atorvastatin peak

Relative standard deviation: NMT 5% for the atorvastatin peak

Signal-to-noise ratio: NLT 10 for atorvastatin related compound D

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M \times (M_{r1}/M_{r2})] \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of atorvastatin from the *Standard solution*

C_S = concentration of USP Atorvastatin Calcium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of atorvastatin in the *Sample solution* (mg/mL)

M = number of moles of atorvastatin per mole of atorvastatin calcium, 2

M_{r1} = molecular weight of atorvastatin, 558.64

M_{r2} = molecular weight of atorvastatin calcium, 1155.34

F = relative response factor (see *Table 4*)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Atorvastatin amide[a, b]	0.44	—	—
Atorvastatin related compound A[b, c]	0.84	—	—
Atorvastatin pyrrolidone analog[d]	0.88	0.68	0.5
Atorvastatin related compound B[b, e]	0.94	—	—
Atorvastatin	1.00	—	—
Atorvastatin related compound C[b, f]	1.09	—	—
Atorvastatin pyrrolidone lactone[b, g]	1.62	—	—
Atorvastatin related compound H[h]	1.00	1.18	1.0
Atorvastatin epoxy pyrrolooxazin 6-hydroxy analog[i]	1.06	0.53	0.5
Atorvastatin methyl ester[b, j]	1.12	—	—
Atorvastatin epoxy pyrrolooxazin 7-hydroxy analog, if present[k]	1.14	0.53	0.5
Atorvastatin epoxy THF analog[l, m]	1.20	1.12	1.0
Atorvastatin related compound D[n]	1.27	1.12	0.5
Atorvastatin *tert*-butyl ester[b, o]	1.49	—	—
Any other unspecified degradation product	—	1.00	0.2

USP Monographs

www.webofpharma.com

Table 4 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total degradation products	—	—	4.0

[a] (3*R*,5*R*)-7-[(3*R*,5*R*)-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanamido]-3,5-dihydroxyheptanoic acid.

[b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

[c] (3*R*,5*R*)-7-[2-Isopropyl-4,5-diphenyl-3-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid.

[d] (3*R*,5*R*)-7-[5-(4-Fluorophenyl)-3-isopropyl-2-oxo-4-phenyl-3-(phenylcarbamoyl)-2,3-dihydro-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid.

[e] (3*S*,5*R*)-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid.

[f] (3*R*,5*R*)-7-[2,3-Bis(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid.

[g] 5-(4-Fluorophenyl)-1-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3-isopropyl-2-oxo-*N*,4-diphenyl-2,3-dihydro-1*H*-pyrrole-3-carboxamide.

[h] 5-(4-Fluorophenyl)-1-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-2-isopropyl-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide.

[i] 4-(6-(4-Fluorophenyl)-7,8-epoxy-6-hydroxy-8a-isopropyl-7-phenyl-8-(phenylcarbamoyl)hexahydro-2*H*-pyrrolo[2,1-*b*][1,3]oxazin-2-yl)-3-hydroxybutanoic acid.

[j] (3*R*,5*R*)-Methyl 7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl)-3,5-dihydroxyheptanoate.

[k] (3*R*)-4-(1b-(4-Fluorophenyl)-7-hydroxy-7-isopropyl-1a-phenyl-7a-(phenylcarbamoyl)hexahydro-1a*H*-oxireno[2′,3′:3,4]pyrrolo[2,1-*b*][1,3]oxazin-3-yl)-3-hydroxybutanoic acid.

[l] 4-(4-Fluorophenyl)-2,4-dihydroxy-2-isopropyl-*N*,5-diphenyl-3,6-dioxabicyclo[3.1.0]hexane-1-carboxamide.

[m] Atorvastatin related compound D can undergo transformation equilibrium to the atorvastatin epoxy THF analog. The equilibrium can be shifted under slightly acidic conditions and therefore some products could have a combined specification reported under atorvastatin related compound D.

[n] 3-(4-Fluorobenzoyl)-2-isobutyryl-*N*,3-diphenyloxirane-2-carboxamide.

[o] (3*R*,5*R*)-*tert*-Butyl 7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl)-3,5-dihydroxyheptanoate.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used, only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

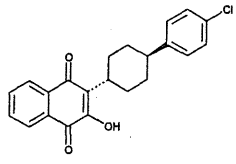
USP Atorvastatin Calcium RS

USP Atorvastatin Related Compound B RS
Calcium (3*S*,5*R*)-7-[2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoate (1:2).
$C_{66}H_{68}CaF_2N_4O_{10}$ 1155.34

USP Atorvastatin Related Compound D RS
3-(4-Fluorobenzoyl)-2-isobutyryl-*N*,3-diphenyloxirane-2-carboxamide.
$C_{26}H_{22}FNO_4$ 431.46

USP Atorvastatin Related Compound H RS
5-(4-Fluorophenyl)-1-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-2-isopropyl-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide.
$C_{33}H_{33}FN_2O_4$ 540.62

Atovaquone



$C_{22}H_{19}ClO_3$ 366.84

1,4-Naphthalenedione, 2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-, *trans*-;
2-[*trans*-4-(*p*-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone [95233-18-4].

DEFINITION

Atovaquone contains NLT 97.5% and NMT 101.5% of atovaquone ($C_{22}H_{19}ClO_3$), calculated on the anhydrous and organic solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, methanol, water, and phosphoric acid (525:175:300:5)
Diluent: Acetonitrile and water (80:20)
System suitability solution: 0.25 mg/mL of USP Atovaquone RS and 0.02 mg/mL of USP Atovaquone Related Compound A RS in *Diluent*. Store in a low-actinic glass container.
Standard solution: 0.25 mg/mL of USP Atovaquone RS in *Diluent*
Sample solution: 0.25 mg/mL of Atovaquone in *Diluent*. Use a low-actinic volumetric flask.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 3 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for atovaquone related compound A and atovaquone are about 0.85 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4 between atovaquone related compound A and atovaquone, *System suitability solution*
Column efficiency: NLT 9000 theoretical plates, *Standard solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of atovaquone ($C_{22}H_{19}ClO_3$) in the portion of Atovaquone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Atovaquone RS in the *Standard solution* (mg/mL)
C_U = concentration of Atovaquone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–101.5% on the anhydrous and organic solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **RELATED COMPOUNDS**

System suitability solution and **Sample solution:** Prepare as directed in the *Assay*.

Analysis

Samples: *System suitability solution* and *Sample solution*

Using the chromatograms of the *Sample solution* and the *System suitability solution*, calculate the percentage of atovaquone related compounds in the portion of Atovaquone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Indene isomer[a]	0.63	0.5
Atovaquone related compound A	0.85	1.0
Didehydroatovaquone[b]	0.89	0.3
Atovaquone	1.0	—
O-Methyl atovaquone[c]	1.8	0.5
Any other individual, unidentified impurity	—	0.2
Sum of all other individual impurities	—	1.0
Total impurities	—	1.5

[a] *trans*-2-[4-(4-Chlorophenyl)cyclohexyl]-1-oxo-1*H*-indene-3-carboxylic acid.
[b] (*RS*)-2-[4-(4-Chlorophenyl)cyclohex-3-enyl]-3-hydroxy-1,4-naphthoquinone.
[c] *trans*-2-[4-(4-Chlorophenyl)cyclohexyl]-3-methoxy-1,4-naphthoquinone.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Atovaquone RS
USP Atovaquone Related Compound A RS
cis-2-[4-(4-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone.
$C_{22}H_{19}ClO_3$ 366.84

Atovaquone Oral Suspension

DEFINITION

Atovaquone Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of atovaquone ($C_{22}H_{19}ClO_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Medium: Methanol and water (1:1)
Standard solution: Dilute 5 mL of *Standard solution* from the *Assay* with *Medium* to 50 mL.
Sample solution: Dilute 5 mL of *Sample solution* from the *Assay* with *Medium* to 50 mL.
Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, methanol, water, and phosphoric acid (480:160:360:5)
System suitability solution: 0.09 mg/mL of USP Atovaquone RS and 0.01 mg/mL of USP Atovaquone Related Compound A RS in 0.1 M methanolic sodium hydroxide. Store in a low-actinic glass container.
Standard stock solution: 3 mg/mL of USP Atovaquone RS in a low-actinic, appropriately sized volumetric flask. Add 20% water and 60% 0.1 M methanolic sodium hydroxide. Sonicate for 5 min or until the material has dissolved. Allow to cool, and dilute with 0.1 M methanolic sodium hydroxide to volume.
Standard solution: 0.09 mg/mL of USP Atovaquone RS from *Standard stock solution*. Transfer to an appropriately sized, low-actinic volumetric flask in a mixture of methanol and water (1:1). Minimize exposure of this solution to light.
Sample stock solution: Nominally 3 mg/mL from a known volume of well-mixed Oral Suspension NLT 750 mg of atovaquone prepared as follows. In an appropriately sized, low-actinic volumetric flask, add 20% volume of water, swirl for 5 min, add 60% volume of 0.1 M methanolic sodium hydroxide, and sonicate for 15 min. Allow to cool, and dilute with 0.1 M methanolic sodium hydroxide to volume. Immediately filter a 20-mL portion, discarding the first 5 mL of the filtrate.
Sample solution: 0.09 mg/mL of atovaquone from the clear filtrate of the *Sample stock solution*. Transfer to an appropriately sized, low-actinic volumetric flask, and dilute with a mixture of methanol and water (1:1) to volume. Minimize exposure of this solution to light.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 12.5-cm; packing L1
Flow rate: 3 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for atovaquone related compound A and atovaquone are 0.86 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

www.webofpharma.com

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of atovaquone ($C_{22}H_{19}ClO_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of atovaquone from the *Sample solution*

r_S = peak response of atovaquone from the *Standard solution*

C_S = concentration of USP Atovaquone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of atovaquone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements for oral suspension packaged in single-unit containers
- **DELIVERABLE VOLUME** ⟨698⟩: Meets the requirements for oral suspension packaged in multiple-unit containers

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *System suitability solution*, *Standard solution*, and *Sample solution*

Using the chromatograms of the *System suitability solution* and the *Sample solution*, calculate the percentage of atovaquone related compounds in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = individual peak response of an atovaquone related compound, if any, from the *Sample solution*

r_S = peak response of atovaquone from the *Standard solution*

C_S = concentration of USP Atovaquone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of Oral Suspension in the *Sample solution* (mg/mL)

F = relative response factor of an individual atovaquone related compound relative to the response of atovaquone (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Photodegradation peak[a]	0.3	—	—
Atovaquone impurity	0.65	1.08	0.5
Atovaquone related compound A	0.86	0.85	1.0
Atovaquone impurity	0.88	1.0	0.3
Atovaquone	1.0	1.0	—
Any other atovaquone related compound	—	1.0	0.2
Total impurities	—	—	2.0

[a] Disregard any peak having a relative retention time of 0.3, which is due to photodegradation during preparation of the *Sample solution*.

SPECIFIC TESTS

- **pH** ⟨791⟩: 3.5–7.0
- **SEDIMENTATION**

For oral suspension packaged in multiple-unit containers

Analysis: Transfer 50 mL of well-mixed Oral Suspension to a glass-stoppered graduated cylinder, and allow to stand for 16 h. Measure the volume, if any, of clear liquid observed in the cylinder.

Acceptance criteria: NMT 1 mL of clear liquid

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

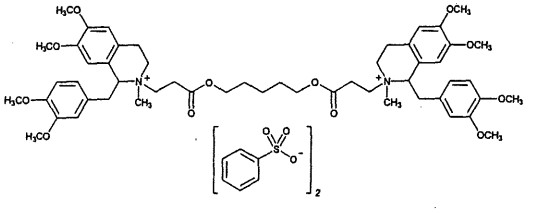
USP Atovaquone RS

USP Atovaquone Related Compound A RS

cis-2-[4-(4-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone.

$C_{22}H_{19}ClO_3$ 366.84

Atracurium Besylate



$C_{65}H_{82}N_2O_{18}S_2$ 1243.48

Isoquinolinium, 2,2′-[1,5-pentanediylbis[oxy(3-oxo-3,1-propanediyl)]]bis[1-[(3,4-dimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-, dibenzenesulfonate;

2-(2-Carboxyethyl)-1,2,3,4-tetrahydro-6,7-dimeth oxy-2-methyl-1-veratrylisoquinolinium benzenesulfonate, pentamethylene ester [64228-81-5].

DEFINITION

Atracurium Besylate contains NLT 96.0% and NMT 102.0% of $C_{65}H_{82}N_2O_{18}S_2$, calculated on the anhydrous basis. It contains NLT 5.0% and NMT 6.5% of the *trans-trans* isomer, NLT 34.5% and NMT 38.5% of the *cis-trans* isomer, and NLT 55.0% and NMT 60.0% of the *cis-cis* isomer.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention times of the three main isomeric peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: 10.2 g of monobasic potassium phosphate in a 1000-mL volumetric flask. Dissolve in 950 mL of water. While stirring, adjust with phosphoric acid to a pH of 3.1, and dilute with water to volume.

Solution A: Acetonitrile, methanol, and *Buffer* (20:5:75)

Solution B: Acetonitrile, methanol, and *Buffer* (20:30:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
15	40	60
25	40	60
30	0	100
45	0	100
50	80	20

Standard solution: 1 mg/mL of USP Atracurium Besylate RS in *Solution A*

Sample solution: 1 mg/mL of Atracurium Besylate in *Solution A*

Chromatographic system

(See *Chromatography*, ⟨621⟩ *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm base-deactivated packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—Refer to *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between the atracurium *trans-trans* isomer and the *cis-trans* isomer peaks; NLT 1.5 between the atracurium *cis-trans* isomer and the *cis-cis* isomer peaks

Relative standard deviation: NMT 2.0%, for the *cis-cis* isomer peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of atracurium besylate ($C_{65}H_{82}N_2O_{18}S_2$) in the portion of Atracurium Besylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer from the *Sample solution*

r_S = sum of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer from the *Standard solution*

C_S = concentration of USP Atracurium Besylate RS in the *Standard solution* (mg/mL)

C_U = concentration of Atracurium Besylate in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–102.0%, calculated on the anhydrous basis. It contains NLT 5.0% and NMT 6.5% of the *trans-trans* isomer, NLT 34.5% and NMT 38.5% of the *cis-trans* isomer, and NLT 55.0% and NMT 60.0% of the *cis-cis* isomer.

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

• **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, Chromatographic system, and **Sample solution:** Proceed as directed in the *Assay*.

Standard solution: 0.01 mg/mL of USP Atracurium Besylate RS in *Solution A*

System suitability solution: 1 mg/mL of USP Atracurium Besylate RS in *Solution A*

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between the atracurium *trans-trans* isomer and the *cis-trans* isomer peaks; NLT 1.5 between the atracurium *cis-trans* isomer and the *cis-cis* isomer peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms, and measure all of the peak responses, except the three main isomeric peaks.

Calculate the percentage of each impurity in the portion of Atracurium Besylate taken:

$$\text{Result} = (r_U/r_T) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_T = sum of peak responses due to the atracurium *cis-cis*, *trans-trans*, and *cis-trans* isomers from the *Standard solution*

C_S = concentration of USP Atracurium Besylate RS in the *Standard solution* (mg/mL)

C_U = concentration of Atracurium Besylate in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. [NOTE—Disregard any peak less than 0.05%.]

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity E[a]	0.2	1.0	1.5
Impurity F[b]	0.25	1.0	1.0
Impurity G (laudanosine)[c]	0.3	2.0	1.0
Impurity D	0.45[d] and 0.5[e]	1.0	1.5[p]
Atracurium *trans-trans* isomer	0.8	—	—
Atracurium *cis-trans* isomer	0.9	—	—
Atracurium *cis-cis* isomer	1.0	—	—
Impurity A	1.04[f] and 1.08[i]	1.0	1.5[p]
Impurity I	1.07[g] and 1.12[k]	1.0	1.0[p]
Impurity H	1.07[h] and 1.12[l]	1.0	1.0[p]
Impurity K[j]	1.09 and 1.12	1.0	1.0[p]
Impurity B[m]	1.15	1.0	0.1
Impurity C	1.2[n] and 1.3[o]	1.0	1.0[p]

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual impurity	—	1.0	0.1
Total impurities	—	—	3.5

[a] 3-[1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinio]propanoate.
[b] 1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium.
[c] 1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline.
[d] *trans* Isomer of 1-(3,4-dimethoxybenzyl)-2-[3-[(5-hydroxypentyl)oxy]-3-oxopropyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium.
[e] *cis* Isomer of 1-(3,4-dimethoxybenzyl)-2-[3-[(5-hydroxypentyl)oxy]-3-oxopropyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium.
[f] *cis-trans* Isomer of 1-(3,4-dimethoxybenzyl)-2-[13-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]-3,11-dioxo-4,10-dioxatridecyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium.
[g] *cis-trans* Isomer of 2,2′-[(3-methylpentane-1,5)-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].
[h] *cis-trans* Isomer of 2,2′-[hexane-1,6-diylbis[oxy(3-oxopropane-1,3-diyl)]] bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].
[i] *cis-cis* Isomer of 1-(3,4-dimethoxybenzyl)-2-[13-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]-3,11-dioxo-4,10-dioxatridecyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium.
[j] 2,2′-[(Hexane-1,5)-diylbis(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].
[k] *cis-cis* Isomer of 2,2′-[(3-methylpentane-1,5)-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].
[l] *cis-cis* Isomer of 2,2′-[hexane-1,6-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].
[m] Pentane-1,5-diyl bis[3-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanoate].
[n] *trans* Isomer of 1-(3,4-dimethoxybenzyl)-2-(3,11-dioxo-4,10-dioxatridec-12-enyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium benzenesulfonate.
[o] *cis* Isomer of 1-(3,4-dimethoxybenzyl)-2-(3,11-dioxo-4,10-dioxatridec-12-enyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium benzenesulfonate.
[p] Impurity consists of two isomers that are separated under these conditions; integrate both peaks for the impurity calculations.

• **Limit of Impurity J (Methyl Benzenesulfonate)**
Buffer, Solution A, and **Solution B:** Prepare as directed in the *Assay*.
Standard stock solution: 0.2 mg/mL of Impurity J (methyl benzenesulfonate) in acetonitrile
Standard solution: 1 µg/mL of Impurity J (methyl benzenesulfonate) in *Solution A* from *Standard stock solution*
Sample solution: 10 mg/mL of Atracurium Besylate in *Solution A*
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
15	75	25
25	75	25
30	55	45
38	0	100
45	0	100

Chromatographic system
Mode: LC
Detector: UV 217 nm
Column: 4.6-mm × 25-cm; 5-µm base-deactivated packing L1
Flow rate: 1 mL/min
Injection size: 100 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the responses for the Impurity J (methyl benzenesulfonate) peaks.
Acceptance criteria: NMT 0.01%, the peak response of the *Sample solution* being NMT that of the *Standard solution*

SPECIFIC TESTS
• **Water Determination,** *Method I* ⟨921⟩**:** NMT 5.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers, in a cold place. [Note—Atracurium Besylate is unstable at room temperature.]
• **USP Reference Standards** ⟨11⟩
USP Atracurium Besylate RS

Atracurium Besylate Injection

DEFINITION
Atracurium Besylate Injection is a sterile solution containing NLT 90.0% and NMT 115.0% of the labeled amount of atracurium besylate ($C_{65}H_{82}N_2O_{18}S_2$). It contains an amount of the *trans-trans* isomer equivalent to NLT 5.0% and NMT 6.5% of the labeled amount of atracurium besylate, an amount of the *cis-trans* isomer equivalent to NLT 34.5% and NMT 38.5% of the labeled amount of atracurium besylate, and an amount of the *cis-cis* isomer equivalent to NLT 55.0% and NMT 60.0% of the labeled amount of atracurium besylate.
[Note—The Injection is unstable at room temperature. Store all samples in the refrigerator. Analyze all preparations as soon as possible, or use a refrigerated injector.]

IDENTIFICATION
• **A.** The retention times of the peaks of the three atracurium besylate isomers from the *Sample solution* correspond to those from the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Buffer: 10.2 g of monobasic potassium phosphate in a 1000-mL volumetric flask. Dissolve in 950 mL of water. While stirring, adjust with phosphoric acid to a pH of 3.1, and dilute with water to volume.
Solution A: Acetonitrile, methanol, and *Buffer* (20:5:75)
Solution B: Acetonitrile, methanol, and *Buffer* (20:30:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
15	40	60
25	40	60
30	0	100

Add-in Software

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
45	0	100
50	80	20

Standard solution: 1 mg/mL of USP Atracurium Besylate RS in *Solution A*
Sample solution: Nominally equivalent to 1 mg/mL of atracurium besylate from Injection in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm base-deactivated packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—Refer to *Table 2* under *Organic Impurities* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between the atracurium *trans-trans* isomer and the *cis-trans* isomer peaks; NLT 1.5 between the atracurium *cis-trans* isomer and the *cis-cis* isomer peaks
Relative standard deviation: NMT 2.0%, for the *cis-cis* isomer peak
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the responses for the three atracurium besylate isomer peaks.
Calculate the percentage of the labeled amount of atracurium besylate ($C_{65}H_{82}N_2O_{18}S_2$) in each mL of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer from the *Sample solution*
r_S = sum of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer from the *Standard solution*
C_S = concentration of USP Atracurium Besylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of atracurium besylate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0% of the labeled amount of atracurium besylate ($C_{65}H_{82}N_2O_{18}S_2$). It contains NLT 5.0% and NMT 6.5% of the *trans-trans* isomer, NLT 34.5% and NMT 38.5% of the *cis-trans* isomer, and NLT 55.0% and NMT 60.0% of the *cis-cis* isomer.

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer, Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard stock solution: 1 mg/mL of USP Atracurium Besylate RS in *Solution A*
Standard solution: 0.02 mg/mL of USP Atracurium Besylate RS in *Solution A*, from *Standard stock solution*
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between the atracurium *trans-trans* isomer and the *cis-trans* isomer peaks; NLT 1.5 between the atracurium *cis-trans* isomer and the *cis-cis* isomer peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of *Sample solution* taken:

$$\text{Result} = (r_U/r_T) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Standard solution*
C_S = concentration of USP Atracurium Besylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of atracurium besylate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzenesulfonic acid[a]	0.08	—	—
Acidic compound	0.22	1.0	6.0
Impurity G (laudanosine)	0.29	2.0	3.0
cis- and *trans*-isomers of the hydroxy compound	0.44[b] and 0.50[c]	1.0	6.0[f]
Atracurium *trans-trans* isomer	0.8	—	—
Atracurium *cis-trans* isomer	0.9	—	—
Atracurium *cis-cis* isomer	1.0	—	—
cis- and *trans*-isomers of the monoacrylate	1.28[d] and 1.33[e]	1.0	3.0[f]
Any individual unspecified degradation product	—	1.0	0.1
Total impurities	—	—	15.0

[a] For identification purposes only.
[b] *trans* isomer of the hydroxy compound.
[c] *cis* isomer of the hydroxy compound.
[d] *trans* isomer of the monoacrylate.
[e] *cis* isomer of the monoacrylate.
[f] Impurity consists of two isomers that are separated under these conditions; integrate both peaks for the impurity calculations.

SPECIFIC TESTS

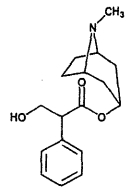
• **PH** ⟨791⟩: 3.00–3.65
• **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 5.56 USP Endotoxin Units/mg of atracurium besylate.
• **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩: Meets the requirements

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, in a refrigerator, and protect from freezing. Protect from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Atracurium Besylate RS

Atropine



$C_{17}H_{23}NO_3$ 289.37

Benzeneacetic acid, α-(hydroxymethyl)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, *endo*-(±)-;
1*αH*,5*αH*-Tropan-3α-ol (±)-tropate (ester) [51-55-8].

DEFINITION

Atropine contains NLT 99.0% and NMT 100.5% of atropine ($C_{17}H_{23}NO_3$), calculated on the anhydrous basis.
[**CAUTION**—Handle Atropine with exceptional care, because it is highly potent.]

IDENTIFICATION

- **A.**
  Standard: 36 mg of USP Atropine Sulfate RS
  Sample: 30 mg
  Analysis: Dissolve the *Standard* and *Sample* in individual 60-mL separators with the aid of 5-mL portions of water. To each separator add 1.5 mL of 1 N sodium hydroxide solution and 10 mL of chloroform. Shake for 1 min, allow the layers to separate, and pass the chloroform extracts through separate filters of 2 g of anhydrous granular sodium sulfate supported on pledgets of glass wool. Extract each aqueous layer with two additional 10-mL portions of chloroform, filtering and combining with the respective main extracts. Evaporate the chloroform solutions under reduced pressure to dryness, and dissolve each residue in 10 mL of carbon disulfide.
  Acceptance criteria: The IR absorption spectrum, determined in a 1-mm cell, of the solution of the *Sample* exhibits maxima only at the same wavelengths as that of the solution of the *Standard*.
- **B.**
  Sample solution: A solution (1 in 50) in 3 N hydrochloric acid
  Analysis: Add gold chloride TS to the *Sample solution*.
  Acceptance criteria: A lusterless precipitate is formed (distinction from hyoscyamine, which, similarly treated, yields a lustrous precipitate).

ASSAY

- **PROCEDURE**
  Sample: 400 mg of Atropine
  Analysis: Dissolve the *Sample* in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS to a green endpoint, using 1 drop of crystal violet TS. Perform a blank determination (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 28.94 mg of atropine ($C_{17}H_{23}NO_3$).
  Acceptance criteria: 99.0%–100.5% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF FOREIGN ALKALOIDS AND OTHER IMPURITIES**
  Standard solution: 24 mg/mL of USP Atropine Sulfate RS in methanol
  Sample solution A: 20 mg/mL of Atropine in methanol
  Sample solution B: 1 mg/mL of Atropine in methanol
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: 0.5-mm layer of chromatographic silica gel
  Application volume: See *Analysis*.
  Developing solvent system: Chloroform, acetone, and diethylamine (5:4:1)
  Spray reagent: Potassium iodoplatinate TS
  Analysis
  Samples: *Standard solution*, 5 µL; *Sample solution A*, 25 µL; *Sample solution B*, 1 µL
  Apply the *Samples* to the TLC plate. Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Allow the solvent to evaporate. Locate the spots on the plate by spraying with *Spray reagent*.
  Acceptance criteria: NMT 0.2%; the R_F value of the principal spot of each *Sample solution* corresponds to that of the *Standard solution*; no secondary spot of *Sample solution A* exhibits intensity equal to or greater than the principal spot of *Sample solution B*.
- **READILY CARBONIZABLE SUBSTANCES TEST** ⟨271⟩
  Sample solution: 200 mg in 5 mL of 2 N sulfuric acid
  Acceptance criteria: The solution has no more color than *Matching Fluid A*, and the solution is colored no more than light yellow upon the addition of 0.2 mL of nitric acid.

SPECIFIC TESTS

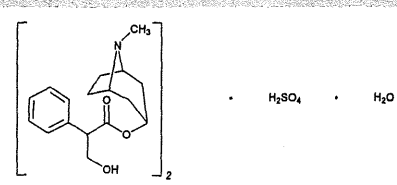
- **OPTICAL ROTATION,** *Angular Rotation* ⟨781⟩
  Sample solution: 1 g, previously dried at 105° for 1 h, in sufficient 50% alcohol (w/w) to obtain a volume of 20 mL at 25° (using a 200-mm tube)
  Acceptance criteria: −0.70° to +0.05° (limit of hyoscyamine)
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 114°–118°
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Atropine Sulfate RS

Atropine Sulfate

Change to read:



$(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ 694.83

www.webofpharma.com

Anhydrous ▲676.82▲ 2S (USP41)
Benzeneacetic acid, α-(hydroxymethyl)-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, *endo*-(±)-, sulfate (2:1) (salt), monohydrate;
1α*H*,5α*H*-Tropan-3-α-ol (±)-tropate (ester), sulfate (2:1) (salt) monohydrate [5908-99-6].
Anhydrous [55-48-1].

DEFINITION

Atropine Sulfate contains NLT 98.0% and NMT 102.0% of atropine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4]$, calculated on the anhydrous basis.
[**CAUTION**—Handle atropine sulfate with exceptional care, because it is highly potent.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197K or 197A▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sulfate*
  Sample solution: 50 mg/mL
  Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**
  Buffer: 1.8 g/L of monobasic potassium phosphate and 2.5 g/L of sodium 1-pentanesulfonate, adjusted with phosphoric acid to a pH of 2.5
  Diluent: Acetonitrile and *Buffer* (20:80)
  Solution A: Acetonitrile and *Buffer* (5:95)
  Solution B: Acetonitrile and *Buffer* (80:20)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	92	8
11	79	21
15	46	54
15.1	92	8
20	92	8

[NOTE—The gradient was established on an HPLC system with a dwell volume of approximately 0.8 mL.]
System suitability solution: 1 µg/mL of USP Hyoscyamine Related Compound A RS and 0.5 mg/mL of USP Atropine Sulfate RS in *Diluent*
Sensitivity solution: 0.25 µg/mL of USP Atropine Sulfate RS in *Diluent*
Standard solution: 0.5 mg/mL of USP Atropine Sulfate RS in *Diluent*
Sample solution: 0.5 mg/mL of Atropine Sulfate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 50°
Flow rate: 2 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, ▲and *Standard solution*▲ 2S (USP41)
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.4 between hyoscyamine related compound A and atropine, *System suitability solution*
Tailing factor: 0.8–1.8 for atropine, *System suitability solution*
Relative standard deviation: ▲NMT 0.73%, *Standard solution*▲ 2S (USP41)
Signal-to-noise ratio: NLT 10 for atropine, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of atropine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4]$ in the portion of Atropine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of atropine from the *Sample solution*
r_S = peak response of atropine from the *Standard solution*
C_S = concentration of USP Atropine Sulfate RS in the *Standard solution*▲(mg/mL)▲ 2S (USP41)
C_U = concentration of Atropine Sulfate in the *Sample solution*▲(mg/mL)▲ 2S (USP41)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

Change to read:

- **ORGANIC IMPURITIES**
  Buffer, Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Sensitivity solution, ▲Standard solution,▲ 2S (USP41) Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
  Analysis
  Sample: *Sample solution*
  Calculate the percentage of each impurity in the portion of Atropine Sulfate taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*
F = relative response factor for each impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. ▲The reporting threshold is 0.05%.▲ 2S (USP41)

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tropic acid[a]	0.56	2.1	0.2
7-Hydroxyhyoscyamine[b]	0.66	1.0	0.2
Scopolamine[c]	0.72	1.0	0.2

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
6-Hydroxyhyoscyamine[d]	0.75	1.0	0.2
Hyoscyamine related compound A	0.97	1.2	0.3
Atropine	1.0	1.0	—
Littorine[e]	1.13	1.2	0.2
Apoatropine[f]	1.60	2.0	0.2
Any individual, unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] 3-Hydroxy-2-phenylpropanoic acid; also known as (2*RS*)-3-Hydroxy-2-phenylpropanoic acid.
[b] (1*S*,3*R*,5*S*)-6-Hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (*S*)-3-hydroxy-2-phenylpropanoate; also known as (1*S*,3*R*,5*S*,6*RS*)-6-Hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate.
[c] (*S*)-(1*R*,2*R*,4*S*,5*S*,7*s*)-9-Methyl-3-oxa-9-azatricyclo[3.3.1.$0^{2,4}$]nonan-7-yl 3-hydroxy-2-phenylpropanoate; also known as (*S*)-(1*R*,2*R*,4*S*,5*S*,7*s*)-9-Methyl-3-oxa-9-azatricyclo[3.3.1.$0^{2,4}$]non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate.
[d] (1*R*,3*S*,5*R*)-6-Hydroxy-8-methyl-8-azabicyclo[3.2.1]octan-3-yl (*S*)-3-hydroxy-2-phenylpropanoate; also known as (1*R*,3*S*,5*R*,6*RS*)-6-Hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate.
[e] (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl 2-hydroxy-3-phenylpropanoate; also known as (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-2-hydroxy-3-phenylpropanoate.
[f] (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl 2-phenylacrylate; also known as (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl 2-phenylpropenoate.

SPECIFIC TESTS

Change to read:

- **OPTICAL ROTATION** ▲⟨781S⟩, *Procedures*, ▲2S (USP41) *Specific Rotation*

Sample solution: 0.1 g/mL of Atropine Sulfate in water
▲Temperature: 20°
Path length: 1.0 or 2.0 dm▲2S (USP41)
Acceptance criteria: Between –0.50° and +0.05°

- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 4.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Atropine Sulfate RS
  USP Hyoscyamine Related Compound A RS
  Norhyoscyamine sulfate; (1*R*,3*r*,5*S*)-8-Azabicyclo[3.2.1]octan-3-yl (*S*)-3-hydroxy-2-phenylpropanoate sulfate (2:1).
  $(C_{16}H_{21}NO_3)_2 \cdot H_2SO_4$ 648.77

Atropine Sulfate Injection

DEFINITION

Atropine Sulfate Injection is a sterile solution of Atropine Sulfate in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of atropine sulfate monohydrate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 4.1 g of anhydrous sodium acetate and 2.9 mL of glacial acetic acid in 1 L of water.
Mobile phase: Transfer 5.1 g of tetrabutylammonium hydrogen sulfate to a 1-L volumetric flask. Add 50 mL of acetonitrile, and dilute with *Buffer* to volume. Adjust with 5 N sodium hydroxide to a pH of 5.5.
System suitability solution: 0.5 µg/mL of *p*-hydroxybenzoic acid and 64 µg/mL of USP Atropine Sulfate RS in water
Standard solution: 80 µg/mL of USP Atropine Sulfate RS
Sample solution: Nominally equivalent to 80 µg/mL of atropine sulfate in water, from a volume of the Injection containing an amount equivalent to 2 mg of atropine sulfate
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 30-cm × 3.9-mm; packing L1
Flow rate: 2 mL/min
Injection volume: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times of atropine and *p*-hydroxybenzoic acid are 1.0 and 1.6, respectively.]
Suitability requirements
Resolution: NLT 2.2 between *p*-hydroxybenzoic acid and atropine, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of atropine sulfate monohydrate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ in the portion of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Atropine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of atropine sulfate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of atropine sulfate monohydrate, 694.85
M_{r2} = molecular weight of anhydrous atropine sulfate, 676.83

Acceptance criteria: 93.0%–107.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 3.0–6.5
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 55.6 USP Endotoxin Units/mg of atropine sulfate
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Atropine Sulfate RS

www.webofpharma.com

Atropine Sulfate Ophthalmic Ointment

DEFINITION

Atropine Sulfate Ophthalmic Ointment is Atropine Sulfate in a suitable ophthalmic ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of atropine sulfate monohydrate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$. It is sterile.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sulfate*

Sample solution: Transfer 5 g of Ophthalmic Ointment to a separator, dissolve in 50 mL of ether, and extract with 20 mL of water.

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: Stronger ammonia water and water (1:100), adjusted with perchloric acid to a pH of 3.0

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
3	95	5
14	75	25
19	60	40
21	60	40
22	95	5
25	95	5

Diluent: Acetonitrile and *Solution A* (25:75)

System suitability solution: 1.0 µg/mL each of USP Atropine Sulfate RS and littorine hydrochloride in *Diluent*

Standard solution: 0.1 mg/mL of USP Atropine Sulfate RS in *Diluent*

Sample stock solution: Nominally 0.5 mg/mL of atropine sulfate from a portion of Ophthalmic Ointment prepared as follows. Transfer 10 mg of atropine sulfate from a portion of Ophthalmic Ointment to a stoppered 250-mL flask, add 20 mL of *Diluent*, and shake at 40° for 60 min. Centrifuge and use the lower layer clear solution. [NOTE—The use of a centrifuge speed of 4000 rpm for 10 min may be suitable.]

Sample solution: Nominally 0.1 mg/mL of atropine sulfate from the *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

[NOTE—Rinse the system with water after each set of analysis.]

Mode: LC

Detector: UV 210 nm

Column: 2.1-mm × 10-cm; 1.8-µm packing L96

Column temperature: 30°

Flow rate: 0.4 mL/min

Injection volume: 3 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for atropine and littorine are 1.0 and 1.04, respectively.]

Suitability requirements

Resolution: NLT 2.0 between atropine and littorine, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of atropine sulfate monohydrate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of atropine from the *Sample solution*

r_S = peak response of atropine from the *Standard solution*

C_S = concentration of USP Atropine Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of atropine sulfate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of atropine sulfate monohydrate, 694.83

M_{r2} = molecular weight of anhydrous atropine sulfate, 676.82

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 1.0 µg/mL of USP Atropine Sulfate RS in *Diluent*

Sample solution: Nominally 500 µg/mL of atropine sulfate from a portion of Ophthalmic Ointment prepared as follows. Transfer a portion of Ophthalmic Ointment equivalent to 25 mg of atropine sulfate to a stoppered 250-mL flask, add 50 mL of *Diluent*, and shake at 40° for 60 min. Centrifuge and use the lower layer clear solution. [NOTE—The use of a centrifuge speed of 4000 rpm for 10 min may be suitable.]

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for atropine and littorine are 1.0 and 1.04, respectively.]

Suitability requirements

Resolution: NLT 2.0 between atropine and littorine, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified and any unspecified degradation product in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each specified and any unspecified degradation product from the *Sample solution*

r_S = peak response of atropine from the *Standard solution*

C_S = concentration of USP Atropine Sulfate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of atropine sulfate in the *Sample solution* (µg/mL)

www.webofpharma.com

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tropic acid[a]	0.35	2.1	0.2
Scopolamine[b]	0.74	1.0	0.2
Hyoscyamine related compound A[c]	0.92	1.0	0.3
Atropine	1.0	—	—
Apoatropine[d]	1.46	2.1	0.2
Any individual unspecified degradation product	—	1.0	0.2

[a] 3-Hydroxy-2-phenylpropanoic acid.
[b] (*S*)-(1*R*,2*R*,4*S*,5*S*,7*s*)-9-Methyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]nonan-7-yl 3-hydroxy-2-phenylpropanoate.
[c] (1*R*,3*r*,5*S*)-8-Azabicyclo[3.2.1]octan-3-yl (*S*)-3-hydroxy-2-phenylpropanoate.
[d] (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl 2-phenylacrylate.

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter.*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Atropine Sulfate RS

Atropine Sulfate Ophthalmic Solution

DEFINITION
Atropine Sulfate Ophthalmic Solution is a sterile, aqueous solution of Atropine Sulfate. It contains NLT 93.0% and NMT 107.0% of the labeled amount of atropine sulfate monohydrate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$. It may contain suitable stabilizers and antimicrobial agents.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sulfate*
  Sample solution: Evaporate to dryness a quantity of Ophthalmic Solution. Prepare a solution from the residue that contains the equivalent of 50 mg of atropine sulfate/mL.
  Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**
  Solution A: Stronger ammonia water and water (1:100), adjusted with perchloric acid to a pH of 3.0
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
3	95	5
14	75	25
19	60	40
21	60	40
22	95	5
25	95	5

Diluent: Acetonitrile and *Solution A* (25:75)
System suitability solution: 1.0 µg/mL each of USP Atropine Sulfate RS and littorine hydrochloride in *Diluent*
Standard solution: 0.1 mg/mL of USP Atropine Sulfate RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of atropine sulfate from a volume of Ophthalmic Solution in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
[NOTE—Rinse the system with water after each set of analysis.]
Mode: LC
Detector: UV 210 nm
Column: 2.1-mm × 10-cm; 1.8-µm packing L96
Column temperature: 30°
Flow rate: 0.4 mL/min
Injection volume: 3 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for atropine and littorine are 1.0 and 1.04, respectively.]
Suitability requirements
Resolution: NLT 2.0 between atropine and littorine, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of atropine sulfate monohydrate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of atropine from the *Sample solution*
r_S = peak response of atropine from the *Standard solution*
C_S = concentration of USP Atropine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of atropine sulfate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of atropine sulfate monohydrate, 694.83
M_{r2} = molecular weight of anhydrous atropine sulfate, 676.82

Acceptance criteria: 93.0%–107.0%

IMPURITIES
- **ORGANIC IMPURITIES**
  Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

www.webofpharma.com

Standard solution: 1.0 µg/mL of USP Atropine Sulfate RS in *Diluent*
Sample solution: Nominally 500 µg/mL of atropine sulfate from a volume of Ophthalmic Solution in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for atropine and littorine are 1.0 and 1.04, respectively.]
Suitability requirements
Resolution: NLT 2.0 between atropine and littorine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified and any unspecified degradation product in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each specified and any unspecified degradation product from the *Sample solution*
r_S = peak response of atropine from the *Standard solution*
C_S = concentration of USP Atropine Sulfate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of atropine sulfate in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tropic acid[a]	0.35	2.1	0.2
Scopolamine[b]	0.74	1.0	0.2
Hyoscyamine related compound A[c]	0.92	1.0	0.3
Atropine	1.0	—	—
Apoatropine[d]	1.46	2.1	0.2
Any individual unspecified degradation product	—	1.0	0.2

[a] 3-Hydroxy-2-phenylpropanoic acid.
[b] (*S*)-(1*R*,2*R*,4*S*,5*S*,7*s*)-9-Methyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]nonan-7-yl 3-hydroxy-2-phenylpropanoate.
[c] (1*R*,3*r*,5*S*)-8-Azabicyclo[3.2.1]octan-3-yl (*S*)-3-hydroxy-2-phenylpropanoate.
[d] (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl 2-phenylacrylate.

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.5–6.0
- **STERILITY TESTS** ⟨71⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Atropine Sulfate RS

Activated Attapulgite

» Activated Attapulgite is a highly heat-treated, processed, native magnesium aluminum silicate.

Packaging and storage—Preserve in well-closed containers.
Identification—Activated Attapulgite responds to the *Identification*— test for *Colloidal Activated Attapulgite*, the characteristic peak, however, being much less intense.
Loss on drying ⟨731⟩—Dry it at 105° to constant weight: it loses not more than 4.0% of its weight.
Loss on ignition ⟨733⟩—When ignited at 1000° for 1 hour, it loses between 4.0% and 12.0% of its weight.
Volatile matter—When ignited at 600° for 1 hour, it loses between 3.0% and 7.5% of its weight on the dried basis.
Powder fineness—Proceed as directed in the test for *Powder fineness*— under *Colloidal Activated Attapulgite*. The dry weight of the residue is not more than 0.10% of the weight of the specimen taken.
Acid-soluble matter—Boil 2.0 g with 100 mL of 0.2 N hydrochloric acid for 5 minutes, and cool. Add water to adjust the volume to 100 mL, and filter. Evaporate 50 mL of the filtrate so obtained to dryness, and ignite the residue at 600°: not more than 0.25 g is found (25%).
Other requirements—It meets the requirements of the tests for *Microbial enumeration tests 61 and Tests for specified microorganisms 62—, pH 791—, Carbonate—, Arsenic and Lead—*, and *Adsorptive capacity*— under *Colloidal Activated Attapulgite*.

Colloidal Activated Attapulgite

» Colloidal Activated Attapulgite is a purified native magnesium aluminum silicate.

Packaging and storage—Preserve in well-closed containers.
Identification—Add 2 g in small portions to 100 mL of water, with vigorous agitation. Allow to stand for at least 12 hours to ensure complete hydration. Place 2 mL of the resulting mixture on a suitable glass slide, and allow to air-dry at room temperature to produce a uniform film. Place the slide in a vacuum desiccator over a free surface of ethylene glycol. Evacuate the desiccator, and close the stopcock so that the ethylene glycol saturates the desiccator chamber. Allow to stand for 12 hours. Record the X-ray diffraction pattern (see *X-Ray Diffraction* ⟨941⟩), and calculate the *d* values: several peaks are observed; the characteristic peak corresponds to a *d* value between 10.3 and 10.7 Angstrom units.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the test for absence of *Escherichia coli*.
pH ⟨791⟩—Disperse 1.0 g in 10 mL of carbon dioxide-free water, and mix: the pH of the mixed dispersion so obtained is between 7.0 and 9.5.
Loss on drying ⟨731⟩—Dry it at 105° to constant weight: it loses between 5.0% and 17.0% of its weight.
Loss on ignition ⟨733⟩—When ignited at 1000° for 1 hour, it loses between 17.0% and 27.0% of its weight.
Volatile matter—When ignited at 600° for 1 hour, it loses between 7.5% and 12.5% of its weight on the dried basis.
Powder fineness—Add 50 g to 450 mL of water containing 5 g of sodium pyrophosphate, and stir for 10 minutes. Pour

www.webofpharma.com

the resulting dispersion slowly through a No. 325 standard sieve (see *Particle Size Distribution Estimation by Analytical Sieving* ⟨786⟩), and carefully wash the residue until clean. Dry the residue at 105° to constant weight: the dry weight of the residue so obtained is not more than 0.30% of the weight of the specimen taken.

Acid-soluble matter—Boil 2.0 g with 100 mL of 0.2 N hydrochloric acid for 5 minutes, and cool. Add water to adjust the volume to 100 mL, and filter. Evaporate 50 mL of the filtrate so obtained to dryness, and ignite the residue at 600°: not more than 0.15 g is found (15%).

Carbonate—Mix 1.0 g with 15 mL of 0.5 N sulfuric acid: no effervescence occurs.

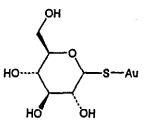
Arsenic and Lead—To 5.0 g add 50 mL of 1 N nitric acid, and boil for 30 minutes, adding 1 N nitric acid at times to maintain the volume. Filter into a 100-mL volumetric flask, wash the filter with water, and dilute the combined filtrate and washings with water to volume.

Arsenic—Determine the arsenic in the solution by atomic absorption spectrometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), using a graphite furnace to volatilize the arsenic, as directed by the manufacturer of the instrument used, and measuring the absorbance at 189.0 nm against a standard: not more than 2 ppm is found.

Lead—Determine the lead in the solution by atomic absorption spectrometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), using a graphite furnace to volatilize the lead, as directed by the manufacturer of the instrument used, and measuring the absorbance at 283.3 nm against a standard: not more than 0.001% is found.

Adsorptive capacity—To 10 mL of a 1 in 10 suspension of the specimen in water add 80 mL of methylene blue solution (1 in 1000), and shake. Add 10 mL of barium chloride solution (1 in 50), and shake. Allow to stand for 15 minutes. Transfer 40 mL of the supernatant to a 50-mL centrifuge tube, and centrifuge. To 5 mL of the clear supernatant add 495 mL of water, and mix: the color of the solution so obtained is not deeper than that of a solution containing 1.5 µg of methylene blue per mL.

Aurothioglucose



$C_6H_{11}AuO_5S$ 392.18

Gold, (1-thio-D-glucopyranosato)-.
(1-Thio-D-glucopyranosato)gold [12192-57-3].

» Aurothioglucose contains not less than 95.0 percent and not more than 105.0 percent of $C_6H_{11}AuO_5S$, calculated on the dried basis. It is stabilized by the addition of a small amount of Sodium Acetate.

Packaging and storage—Preserve in tight, light-resistant containers. Store at room temperature.

USP Reference standards ⟨11⟩—
USP Aurothioglucose RS

Identification—

A: Dissolve a suitable quantity in water to obtain a solution containing 4 mg per mL. Apply 10 µL of this solution and 10 µL of an aqueous Standard solution of USP Aurothioglucose RS containing 4 mg per mL to a suitable thin-layer chromatographic glass microfilament sheet (see *Chromatography* ⟨621⟩) impregnated with silicic acid and a suitable fluorescing substance. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-propyl alcohol, water, and ethyl acetate (3:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the sheet from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the R_F value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

B: To a portion of the filtrate obtained in the *Assay* add barium chloride TS: a heavy, white precipitate is formed.

Specific rotation ⟨781S⟩**:** between +65° and +75°.

Test solution: 10 mg per mL, in water.

Loss on drying ⟨731⟩—Dry it over phosphorus pentoxide for 24 hours: it loses not more than 1.0% of its weight.

Assay—Accurately weigh about 1 g of Aurothioglucose, and dissolve in 100 mL of water in a 300-mL Kjeldahl flask. Slowly add 10 mL of nitric acid, and when the reaction has subsided, boil the mixture for 5 minutes. Filter, wash well the separated gold with hot water, dry, and ignite to constant weight. The weight of the gold so obtained, multiplied by 1.991, represents the weight of $C_6H_{11}AuO_5S$ in the portion of Aurothioglucose taken.

Delete the following:

▲Aurothioglucose Injectable Suspension

» Aurothioglucose Injectable Suspension is a sterile suspension of Aurothioglucose in a suitable vegetable oil. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_6H_{11}AuO_5S$. It may contain suitable thickening agents.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass. Protect from light.

USP Reference standards ⟨ 11 ⟩—
USP Aurothioglucose RS

Identification—Transfer a volume of Injectable Suspension, equivalent to about 200 mg of aurothioglucose, to a centrifuge separator containing 20 mL of ethyl acetate and 50 mL of water. Shake the mixture thoroughly, and centrifuge until the liquid phases have been clearly separated. Withdraw the lower, aqueous phase, and filter, discarding the first 10 mL of the filtrate. Collect the filtrate in a glass-stoppered vessel, and proceed as directed in *Identification*— test *A:* under *Aurothioglucose*, beginning with "apply 10 µL of this solution."

Bacterial Endotoxins Test ⟨ 85 ⟩—It contains not more than 7.14 USP Endotoxin Units per mg of aurothioglucose.

Sterility Tests ⟨ 71 ⟩—It meets the requirements.

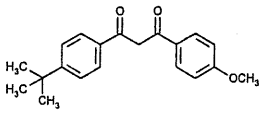
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—Transfer with a pipet, calibrated to contain rather than to deliver, an accurately measured volume of Injectable Suspension, equivalent to about 200 mg of aurothioglucose, to a beaker containing 400 mL of acetone. Wash the pipet into

www.webofpharma.com

the beaker with a small quantity of acetone, mix, allow the solids to settle, and decant the supernatant through a filter. Wash the solids with another 400-mL portion of acetone, and repeat the decantation. Transfer the solids to the filter with the aid of acetone, then transfer the filter and its contents to a short-necked, 300-mL Kjeldahl flask, add 5 mL of water, and proceed as directed in the *Assay*—under *Gold Sodium Thiomalate*, beginning with "add 20 mL of nitric acid." The weight of gold so obtained, multiplied by 1.991, represents the weight of $C_6H_{11}AuO_5S$ in the portion of Injectable Suspension taken. ▲ (USP 1-May-2020)

Avobenzone



$C_{20}H_{22}O_3$ 310.40

1,3-Propanedione, 1-[4-(1,1-dimethylethyl)phenyl]-3-(4-methoxyphenyl)-;
1-(*p*-*tert*-Butylphenyl)-3-(*p*-methoxyphenyl)-1,3-propanedione [70356-09-1].

DEFINITION

Avobenzone contains NLT 95.0% and NMT 105.0% of avobenzone ($C_{20}H_{22}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Analytical wavelength: 360 nm
Sample solution: 5 µg/mL in alcohol
Acceptance criteria: Absorptivities do not differ by more than 3.0%.

ASSAY

• **PROCEDURE**

Standard solution: 50 mg/mL of USP Avobenzone RS in acetone
Sample solution: 50 mg/mL of Avobenzone in acetone
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 25-m fused silica capillary coated with phase G1
Temperatures
Injection port: 200°
Detector: 280°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
200	4	280	—

Carrier gas: Helium
Injection volume: 1 µL
Split ratio: 50:1
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of avobenzone ($C_{20}H_{22}O_3$) in the portion of Avobenzone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Avobenzone RS in the *Standard solution* (mg/mL)
C_U = concentration of Avobenzone in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the dried basis

IMPURITIES

• **ORGANIC IMPURITIES**

Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Avobenzone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria
Each individual impurity: NMT 3.0%
Total impurities: NMT 4.5%

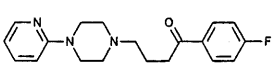
SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample under vacuum at 70° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Avobenzone RS

Azaperone



$C_{19}H_{22}FN_3O$ 327.40

1-Butanone, 1-(4-fluorophenyl)-4-[4-(2-pyridinyl)-1-piperazinyl]-.
4′-Fluoro-4-[4-(2-pyridyl)-1-piperazinyl]butyrophenone [1649-18-9].

www.webofpharma.com

» Azaperone contains not less than 98.0 percent and not more than 102.0 percent of $C_{19}H_{22}FN_3O$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, protected from light. Store at room temperature.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Azaperone RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197K***▲ (CN 1-May-2020) previously dried.
Melting range ⟨741⟩: between 92° and 95°.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chromatographic purity—Dissolve an accurately weighed quantity of USP Azaperone RS in a mixture of acetone and methylene chloride (5:1) to obtain a solution having a concentration of 0.50 mg per mL *(Standard solution A).* Quantitatively dilute a volume of *Standard solution A* with the same solvent mixture to obtain a solution having a concentration of 0.25 mg per mL *(Standard solution B).* Prepare a test solution by dissolving an accurately weighed quantity of Azaperone in a mixture of acetone and methylene chloride (5:1) to obtain a solution containing 50 mg per mL. Separately apply 1 µL each of *Standard solution A, Standard solution B,* and the test solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.2-mm layer of chromatographic silica gel mixture with chemically bonded amino groups, and allow the spots to dry. Develop the chromatograms in a solvent system consisting of a mixture of cyclohexane, acetone, and methanol (65:30:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, and allow the plate to air-dry. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots, other than any spot at the origin, observed in the chromatogram of the test solution with those of the principal spots in the chromatograms of *Standard solution A* and *Standard solution B:* the sum of the intensities of the secondary spots obtained from the test solution corresponds to not more than the intensity of the principal spot in the chromatogram of *Standard solution A* (1.0%).
Assay—Dissolve about 120 mg of Azaperone, accurately weighed, in 50 mL of a mixture of methyl ethyl ketone and glacial acetic acid (7:1). Add 3 drops of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 16.37 mg of $C_{19}H_{22}FN_3O$.

Azaperone Injection

» Azaperone Injection is a sterile solution of Azaperone in Water for Injection, prepared with the aid of Tartaric Acid. It may contain a suitable preservative and a stabilizing agent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{19}H_{22}FN_3O$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Azaperone RS
Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for azaperone, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation,* obtained as directed in the *Assay.*
pH ⟨791⟩: between 4.0 and 5.6.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase—Prepare a filtered and degassed mixture containing 6 volumes of acetonitrile and 4 volumes of 0.01 M dibasic potassium phosphate, and adjust by the addition of dilute phosphoric acid (1 in 10) to a pH of 7.8 ± 0.1. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Prepare a solution of benzophenone in methanol containing about 0.5 mg per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Azaperone RS in methanol, and dilute quantitatively with methanol to obtain a solution having a known concentration of about 0.5 mg per mL. Combine 2.5 mL of this solution with 2.5 mL of *Internal standard solution,* dilute quantitatively with methanol to 10.0 mL, and mix.
Assay preparation—Dilute an accurately measured volume of Injection quantitatively with methanol to obtain a solution containing about 0.5 mg of azaperone per mL. Combine 2.5 mL of this solution with 2.5 mL of *Internal standard solution,* dilute quantitatively with methanol to 10.0 mL, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with 243-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the azaperone and internal standard peaks is not less than 2.7; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of azaperone ($C_{19}H_{22}FN_3O$) in each mL of the Injection taken by the formula:

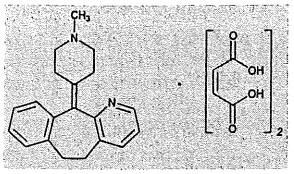
$$(C)(L/D)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Azaperone RS in the *Standard preparation; L* is the labeled quantity, in mg, of azaperone in each mL of the Injection; D is the concentration, in mg per mL, of azaperone in the *Assay preparation,* based on the volume of Injection taken and the extent of dilution; and R_U and R_S are the ratios of the azaperone peak to the benzophenone peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

www.webofpharma.com

Delete the following:

▲Azatadine Maleate



$C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$ 522.55

5H-Benzo[5,6]cyclohepta[1,2-*b*]pyridine, 6,11-dihydro-11-(1-methyl-4-piperidinylidene)-, (*Z*)-2-butenedioate (1:2); 6,11-Dihydro-11-(1-methyl-4-piperidylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine maleate (1:2) [3978-86-7].

DEFINITION

Azatadine Maleate contains NLT 98.0% and NMT 102.0% of azatadine maleate ($C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

• **A. Infrared Absorption** ⟨197M⟩

• **B. Ultraviolet Absorption** ⟨197U⟩

Medium: 0.25 N hydrochloric acid in methanol

Sample solution: 40 µg/mL in *Medium*

Acceptance criteria: Meets the requirements

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Solution A: 3.854 g/L of ammonium acetate in water. Adjust with 25% ammonium hydroxide solution to a pH of 7.6 and pass through a suitable filter of 0.2-µm pore size.

Solution B: Acetonitrile and methanol (20:80)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
7.0	70	30
12.0	60	40
14.0	50	50
16.0	30	70
18.0	30	70
18.1	80	20
20.0	80	20

Standard solution: 0.5 mg/mL of USP Azatadine Maleate RS in water

Sample solution: 0.5 mg/mL of Azatadine Maleate in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 237 nm

Column: 4.6-mm × 15-cm; 5-µm packing L11

Column temperature: 45°

Flow rate: 1.2 mL/min

Injection volume: 3 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of azatadine maleate ($C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$) in the portion of Azatadine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of azatadine from the *Sample solution*

r_S = peak response of azatadine from the *Standard solution*

C_S = concentration of USP Azatadine Maleate RS in the *Standard solution* (mg/mL)

C_U = concentration of Azatadine Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

• **Organic Impurities**

Solution A, Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 1.0 mg/mL of USP Azatadine Maleate RS in water

Standard solution: 0.001 mg/mL of USP Azatadine Maleate RS in water from the *Standard stock solution*

Sample solution: 1.0 mg/mL of Azatadine Maleate in water

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified impurity in the portion of Azatadine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*

r_S = peak response of azatadine from the *Standard solution*

C_S = concentration of USP Azatadine Maleate RS in the *Standard solution* (mg/mL)

C_U = concentration of Azatadine Maleate in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurity: NMT 0.10%

Total impurities: NMT 2.0%. Disregard any impurity peak less than 0.05%.

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩

Analysis: Dry under vacuum at 60° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards** ⟨11⟩

USP Azatadine Maleate RS▲ (USP 1-May-2020)

www.webofpharma.com

Delete the following:

▲Azatadine Maleate Tablets

» Azatadine Maleate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$.

Packaging and storage—Preserve in well-closed containers.

USP Reference Standards ⟨ 11 ⟩ —
USP Azatadine Maleate RS

Identification—Transfer 15.0 mL of the *Standard preparation* and 15.0 mL of the *Assay preparation*, respectively, prepared as directed in the *Assay*, to separate 50-mL centrifuge tubes fitted with glass stoppers. To each centrifuge tube add 10.0 mL of 1.0 N sodium hydroxide and 20 mL of solvent hexane, insert the stoppers, rotate the centrifuge tubes for about 15 minutes, and centrifuge. Transfer the solvent hexane extracts (upper phase) from each centrifuge tube to separate 50-mL conical flasks fitted with glass stoppers. Evaporate the solvent hexane extracts on a steam bath under a stream of nitrogen to dryness, pipet 1 mL of solvent hexane into each flask, insert the stoppers, and mix by use of a vortex mixer (or equivalent) until the residues have dissolved. Use these solutions as the Standard solution and the test solution, respectively. Apply separately 100 µL each of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of toluene, isopropyl alcohol, and diethylamine (10:10:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Examine the plate under short-wavelength UV light: the R_F value and intensity of the principal spot in the chromatogram of the test solution correspond to those obtained from the chromatogram of the Standard solution.

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 500 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 283 nm on filtered portions of the solution under test, diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Azatadine Maleate RS in the same *Medium*.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$ is dissolved in 30 minutes.

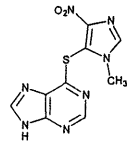
Uniformity of Dosage Units ⟨ 905 ⟩ : meet the requirements.

Assay—
Standard preparation—Dissolve an accurately weighed quantity of USP Azatadine Maleate RS in 0.1 N hydrochloric acid, and dilute quantitatively, and stepwise if necessary, with 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 0.06 mg per mL.
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1.5 mg of azatadine maleate, to a 50-mL flask fitted with a glass stopper. Add 25.0 mL of 0.1 N hydrochloric acid, insert the stopper, and shake the mixture by mechanical means for about 30 minutes. Filter the mixture into a suitable glass-stoppered vessel, discarding the first 5 mL of the filtrate.
Procedure—Separately transfer 15.0 mL of the *Standard preparation*, 15.0 mL of the *Assay preparation*, and 15.0 mL of 0.1 N hydrochloric acid to provide the reagent blank to three 50-mL centrifuge tubes fitted with glass stoppers. To each centrifuge tube add 10.0 mL of 1.0 N sodium hydroxide and 20 mL of solvent hexane, insert the stoppers, rotate the centrifuge tubes for about 15 minutes, and centrifuge until the supernatants (solvent hexane phase) are clear. With the aid of separate syringes, transfer the supernatants to separate 50-mL centrifuge tubes fitted with glass stoppers. Rinse each syringe with 10 mL of solvent hexane, and add the rinse to the aqueous phase from which the respective supernatant was removed. Insert the stoppers, rotate each tube for about 10 minutes, and centrifuge. Transfer each supernatant to the respective supernatant previously collected. Pipet 15 mL of 0.1 N hydrochloric acid into each centrifuge tube containing the combined supernatants, insert the stoppers, rotate each tube for about 15 minutes, and centrifuge. Remove and discard the supernatants. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 283 nm, with a suitable spectrophotometer zeroed with 0.1 N hydrochloric acid, using the prepared reagent blank. Calculate the quantity, in mg, of $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$ in the portion of Tablets taken by the formula:

$$25C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Azatadine Maleate RS in the *Standard preparation;* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively. ▲ (USP 1-May-2020)

Azathioprine



$C_9H_7N_7O_2S$ 277.26
1*H*-Purine, 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-; 6-[(1-Methyl-4-nitroimidazol-5-yl)thio]purine [446-86-6].

DEFINITION
Azathioprine contains NLT 98.0% and NMT 102.0% of azathioprine ($C_9H_7N_7O_2S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: 1.6 g/L of sodium 1-heptanesulfonate in water

Mobile phase: Methanol and *Solution A* (30:70). Adjust with 1 N hydrochloric acid to a pH of 3.5 ± 0.1.

Standard stock solution: 0.5 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS to a suitable volumetric flask. Add 25% of the flask volume of methanol and 1% of ammonium hydroxide to the flask. Swirl, and sonicate for 2 min or until dissolved. Dilute with methanol to volume.

Standard solution: 0.1 mg/mL of USP Azathioprine RS in water from the *Standard stock solution*

Sample solution: 0.1 mg/mL of Azathioprine prepared as follows. Transfer 50 mg of sample to a 100-mL volumetric flask. Add 25 mL of methanol and 1.0 mL of ammonium hydroxide to the flask, swirl, and sonicate for 2 min or until dissolved. Dilute with methanol to volume. Transfer 10.0 mL of this solution into a 50-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1.8 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of azathioprine ($C_9H_7N_7O_2S$) in the portion of Azathioprine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of azathioprine from the *Sample solution*

r_S = peak response of azathioprine from the *Standard solution*

C_S = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL)

C_U = concentration of Azathioprine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**

Buffer: 2.76 g/L of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.5.

Solution A: Methanol and *Buffer* (5:95)

Solution B: Methanol and *Buffer* (60:40)

Mobile phase: See *Table 1*. Return to original conditions, and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
15	0	100
20	0	100

Diluent: 0.8 g/L of sodium hydroxide in water

System suitability stock solution A: 0.2 mg/mL each of USP Azathioprine Related Compound A RS and USP Mercaptopurine RS prepared as follows. Transfer USP Azathioprine Related Compound A RS and USP Mercaptopurine RS to a suitable volumetric flask. Add 35% of the flask volume of *Diluent*, and dilute with *Buffer* to volume.

System suitability stock solution B: 0.1 mg/mL each of USP Azathioprine Related Compound G RS and USP Azathioprine RS prepared as follows. Transfer USP Azathioprine Related Compound G RS and USP Azathioprine RS to a suitable volumetric flask. Add 35% of the flask volume of *Diluent*, and dilute with *Buffer* to volume.

System suitability solution: 0.002 mg/mL each of USP Azathioprine Related Compound A RS, USP Mercaptopurine RS, USP Azathioprine Related Compound G RS, and USP Azathioprine RS prepared as follows. Transfer 1 mL of *System suitability stock solution A* and 2 mL of *System suitability stock solution B* to a 100-mL volumetric flask. Add 35 mL of *Diluent*, and dilute with *Buffer* to volume.

Standard stock solution: 0.1 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS to a suitable volumetric flask. Add 35% of the flask volume of *Diluent*, and dilute with *Buffer* to volume.

Standard solution: 0.1 µg/mL of USP Azathioprine RS in *Buffer* from *Standard stock solution*

Sample solution: 0.1 mg/mL of Azathioprine prepared as follows. Transfer Azathioprine to a suitable volumetric flask. Add 35% of the flask volume with *Diluent*, and dilute with *Buffer* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; 5-µm packing L11

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2 between azathioprine related compound A and mercaptopurine; NLT 2 between azathioprine related compound G and azathioprine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Azathioprine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of azathioprine from the *Standard solution*

C_S = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL)

C_U = concentration of Azathioprine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

USP Monographs

www.webofpharma.com

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azathioprine related compound A[a]	0.3	0.15
Mercaptopurine[b]	0.4	0.15
Azathioprine related compound G[c]	0.97	0.10
Azathioprine	1.0	—
Any other unspecified impurity	—	0.10
Total impurities	—	0.5

[a] 1-Methyl-4-nitro-1*H*-imidazol-5-amine.
[b] 9*H*-Purine-6-thiol.
[c] 6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9*H*-purin-2-amine.

SPECIFIC TESTS
- **Loss on Drying** ⟨731⟩
  Analysis: Dry under vacuum at 105° for 5 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩
  USP Azathioprine RS
  USP Azathioprine Related Compound A RS
  1-Methyl-4-nitro-1*H*-imidazol-5-amine.
  $C_4H_6N_4O_2$ 142.12
  USP Azathioprine Related Compound G RS
  6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9*H*-purin-2-amine.
  $C_9H_8N_8O_2S$ 292.28
  USP Mercaptopurine RS

Azathioprine Compounded Oral Suspension

DEFINITION
Azathioprine Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of azathioprine ($C_9H_7N_7O_2S$).
Prepare Azathioprine Compounded Oral Suspension 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Azathioprine	5 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, (regular or sugar-free), *NF* and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

If using tablets, comminute them to a fine powder in a suitable mortar, or add *Azathioprine* powder to the mortar. Add about 10 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.
[**Caution**—Avoid skin contact or inhalation of azathioprine by using protective gloves and a fume hood or surgical mask.]

ASSAY
- **Procedure**
  Mobile phase: Dissolve 1.1 g of sodium-1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust with 1 N hydrochloric acid to a pH of 3.5.
  Standard solution: Transfer 25 mg of USP Azathioprine RS to a 50-mL volumetric flask. Add 15 mL of methanol and 0.5 mL of ammonium hydroxide to the flask, swirl, and sonicate for 2 min. Dilute with methanol to volume. Transfer 10 mL of this solution to a 50-mL volumetric flask, and dilute with water to volume.
  Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at −70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 2 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  [Note—The retention time for the azathioprine peak is about 4 min.]
  Suitability requirements
  Relative standard deviation: NMT 1.3% for replicate injections
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of azathioprine ($C_9H_7N_7O_2S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of azathioprine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.8–4.8

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store at room temperature, or in a refrigerator.
- **Beyond-Use Date:** NMT 60 days after the day on which it was compounded when stored at room temperature, or in a refrigerator
- **Labeling:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP Reference Standards** ⟨11⟩
  USP Azathioprine RS

USP Monographs

www.webofpharma.com

Azathioprine Tablets

DEFINITION

Azathioprine Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of azathioprine ($C_9H_7N_7O_2S$).

IDENTIFICATION

Change to read:

• **A.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

Add the following:

▲• **B.** The UV spectrum of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the *Assay*.▲ (USP 1-May-2020)

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust the solution with 1 N hydrochloric acid to a pH of 3.5. ▲▲ (USP 1-May-2020)

Standard stock solution: 0.5 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS to a suitable volumetric flask. Add methanol equivalent to 30% of the flask volume and ammonium hydroxide equivalent to 1% of the flask volume, swirl, and sonicate for 2 min. Dilute with methanol to volume.

Standard solution: 0.1 mg/mL of USP Azathioprine RS in water prepared ▲from the *Standard stock solution*▲ (USP 1-May-2020)

Sample stock solution: Nominally 0.5 mg/mL of azathioprine prepared as follows. ▲Finely powder NLT 20 Tablets and transfer a portion of the powder to a suitable volumetric flask. Add methanol equivalent to 25% of the flask volume and ammonium hydroxide equivalent to 1% of the flask volume, swirl, and sonicate for 2 min. Dilute with methanol to volume. Allow the excipients to settle.▲ (USP 1-May-2020)

Sample solution: Nominally 0.1 mg/mL of azathioprine ▲from the *Sample stock solution* in water. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.▲ (USP 1-May-2020)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2020)

Column: 4-mm × 30-cm; ▲10-µm▲ (USP 1-May-2020) packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

▲▲ (USP 1-May-2020)

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of azathioprine ($C_9H_7N_7O_2S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of azathioprine from the *Sample solution*

r_S = peak response of azathioprine from the *Standard solution*

C_S = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of azathioprine in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Azathioprine RS in *Medium*

Sample solutions: ▲Pass portions of the solution under test through a suitable▲ (USP 1-May-2020) filter and dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 280 nm

▲Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the percentage of the labeled amount of azathioprine ($C_9H_7N_7O_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of azathioprine in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

D = dilution factor for the *Sample solution*, if applicable

L = label claim (mg/Tablet)▲ (USP 1-May-2020)

Tolerances: NLT 75% (Q) of the labeled amount of azathioprine ($C_9H_7N_7O_2S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**

Protect the solutions from light.

Mobile phase: Dissolve 1.2 g of sodium 1-heptanesulfonate in 750 mL of water, and add 250 mL of methanol. Adjust the solution with 1 N hydrochloric acid to a pH of 3.0.

System suitability stock solution: 0.25 mg/mL each of USP Mercaptopurine RS, USP Azathioprine Related Compound A RS, and chloromethylnitroimidazole in methanol

System suitability solution: 0.5 mg/mL of USP Azathioprine RS and 0.005 mg/mL each of USP Mercaptopurine RS, USP Azathioprine Related Compound

www.webofpharma.com

A RS, and chloromethylnitroimidazole prepared as follows. Transfer a suitable amount of USP Azathioprine RS to a suitable volumetric flask. Add methanol equivalent to 20% of the flask volume and sonicate to dissolve. Add a suitable amount of the *System suitability stock solution* and dilute with *Mobile phase* to volume.

Standard stock solution: 0.25 mg/mL of USP Azathioprine RS prepared as follows. Transfer a suitable amount of USP Azathioprine RS to a suitable volumetric flask. Add methanol equivalent to 75% of the flask volume, sonicate to dissolve, and dilute with methanol to volume.

Standard solution: 0.005 mg/mL of USP Azathioprine RS in *Mobile phase* from the *Standard stock solution*

Sensitivity solution: 0.0005 mg/mL of USP Azathioprine RS in *Mobile phase* from the *Standard solution*

Sample solution: Nominally 0.5 mg/mL of azathioprine prepared as follows. Transfer a portion of the powder, from NLT 20 powdered Tablets, to a suitable volumetric flask. Add 20% of the flask volume of methanol, sonicate for 20 min with intermittent shaking, and dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*)
Mode: LC
Detector: UV 220 nm
Column: 3.9 mm × 30 cm; 10-µm packing L1
Column temperature: 30°
Flow rate: 1.1 mL/min
Injection volume: 25 µL

System suitability
Samples: *System suitability solution, Standard solution,* and *Sensitivity solution*

Suitability requirements
Resolution: NLT 1.5 between azathioprine related compound A and mercaptopurine; NLT 2.0 between chloromethylnitroimidazole and azathioprine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of azathioprine from the *Standard solution*
C_S = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of azathioprine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azathioprine related compound A[a]	0.47	—
Mercaptopurine	0.52	0.5
Dipurinyl sulfide[a,b]	0.75	—
Chloromethylnitroimidazole[a,c]	0.86	—
Azathioprine	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	2.0

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total degradation products for the drug product.
[b] Di(9*H*-purin-6-yl)sulfide.
[c] 5-Chloro-1-methyl-4-nitro-1*H*-imidazole. ▲(USP 1-May-2020)

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Protect from light. ▲Store at controlled room temperature.▲ (USP 1-May-2020)

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Azathioprine RS
  ▲USP Azathioprine Related Compound A RS
  1-Methyl-4-nitro-1*H*-imidazol-5-amine.
  $C_4H_6N_4O_2$ 142.12
  USP Mercaptopurine RS
  9*H*-Purine-6-thiol monohydrate.
  $C_5H_4N_4S \cdot H_2O$ 170.19▲ (USP 1-May-2020)

Azathioprine Sodium for Injection

DEFINITION
Azathioprine Sodium for Injection is a sterile solid prepared by the freeze-drying of an aqueous solution of Azathioprine and Sodium Hydroxide. It contains NLT 93.0% and NMT 107.0% of the labeled amount of azathioprine ($C_9H_7N_7O_2S$).

IDENTIFICATION
- **A.** The principal spot from the *Sample solution* shows the same R_F value as that obtained from *Standard solution A* in the test for *Limit of Mercaptopurine*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 1.6 g/L of sodium 1-heptanesulfonate in water
  Mobile phase: Methanol and *Solution A* (30:70). Adjust with 1 N hydrochloric acid to a pH of 3.5 ± 0.1.
  Standard stock solution: 0.5 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS to a suitable volumetric flask. Add 25% of the flask volume of methanol and 1% of ammonium hydroxide to the flask, swirl, and sonicate for 2 min or until dissolved. Dilute with methanol to volume.

www.webofpharma.com

Standard solution: 0.1 mg/mL of USP Azathioprine RS in water from the *Standard stock solution*
Sample solution: Nominally equivalent to 0.1 mg/mL of azathioprine in water from Azathioprine Sodium for Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1.8 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of azathioprine ($C_9H_7N_7O_2S$) in the portion of Azathioprine Sodium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of azathioprine from the *Sample solution*
r_S = peak response of azathioprine from the *Standard solution*
C_S = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of azathioprine in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

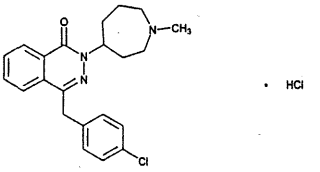
PERFORMANCE TESTS
• **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements

IMPURITIES
• **Limit of Mercaptopurine**
Standard solution A: 10 mg/mL of USP Azathioprine RS in dimethylformamide
Standard solution B: 100 µg/mL of USP Mercaptopurine RS in dimethylformamide
Sample solution: 10 mg/mL of Azathioprine Sodium for Injection in dimethylformamide
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of microcrystalline cellulose
Application volume: 5 µL for *Standard solution A* and the *Sample solution*, and 15 µL for *Standard solution B*
Developing solvent system: Butyl alcohol saturated with 5 N ammonium hydroxide
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Proceed as directed in the chapter. Apply the *Samples* at points 2 cm from the bottom edge of a TLC plate. Allow the spots to dry, and develop the chromatogram in a suitable chamber until the solvent front has moved 15 cm from the point of application. Remove the plate, air-dry, and locate the spots by viewing under short- and long-wavelength UV light.
Acceptance criteria: 3.0%; any spot from the *Sample solution*, other than the principal spot, is not more intense than the spot from *Standard solution B*.

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: The contents of one container dissolved in 10 mL of water
Acceptance criteria: 9.8–11.0
• **Bacterial Endotoxins Test** ⟨85⟩: It contains NMT 1.0 USP Endotoxin Unit/mg of azathioprine.
• **Water Determination** ⟨921⟩, *Method I*: NMT 7.0%, when the *Test Preparation* is prepared as directed for a hygroscopic specimen
• **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Azathioprine RS
USP Mercaptopurine RS

Azelastine Hydrochloride



$C_{22}H_{24}ClN_3O \cdot HCl$ 418.36
1(2*H*)-Phthalazinone, 4-[(4-chlorophenyl)methyl]-2-(hexahydro-1-methyl-1*H*-azepin-4-yl), monohydrochloride; 4-(*p*-Chlorobenzyl)-2-(hexahydro-1-methyl-1*H*-azepin-4-yl)-1(2*H*)-phthalazinone monohydrochloride [79307-93-0].

DEFINITION
Azelastine Hydrochloride contains NLT 99.0% and NMT 101.0% of azelastine hydrochloride ($C_{22}H_{24}ClN_3O \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Identification solution*, as obtained in the test for *Organic Impurities*.
• **C. Identification Tests—General** ⟨191⟩, *Chloride*: Meets the requirements

ASSAY
• **Procedure**
Sample: 300 mg
Blank: 5 mL of anhydrous formic acid and 30 mL of acetic anhydride
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Analysis: In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the endpoint has been reached.

USP Monographs

www.webofpharma.com

Dissolve the *Sample* in 5 mL of anhydrous formic acid, and add 30 mL of acetic anhydride. Titrate with *Titrant*. Calculate the percentage of azelastine hydrochloride ($C_{22}H_{24}ClN_3O \cdot HCl$) in the portion of Azelastine Hydrochloride taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 41.84 mg/mEq
W = weight of the *Sample* (mg)

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.2%
- **Organic Impurities**

Dilute phosphoric acid: 115 g/L of phosphoric acid in water

Buffer: 2.92 g/L of octanesulfonic acid sodium salt and 0.92 g/L of monobasic potassium phosphate in water. Adjust with *Dilute phosphoric acid* to a pH of 3.0–3.1.

Mobile phase: Acetonitrile and *Buffer* (260:740)

Diluent: Acetonitrile and water (45:55)

Identification solution: 2.5 mg/mL of USP Azelastine Hydrochloride RS in *Diluent*. [Note—This solution is used for *Identification* test *B*.]

System suitability stock solution: 0.5 mg/mL each of USP Azelastine Related Compound B RS, USP Azelastine Related Compound D RS, and USP Azelastine Related Compound E RS in acetonitrile

System suitability solution: 50 µg/mL each of USP Azelastine Related Compound B RS, USP Azelastine Related Compound D RS, and USP Azelastine Related Compound E RS from the *System suitability stock solution* and 2.5 mg/mL of USP Azelastine Hydrochloride RS in *Diluent*

Standard stock solution: 50 µg/mL of USP Azelastine Hydrochloride RS in acetonitrile

Standard solution: 2.5 µg/mL of USP Azelastine Hydrochloride RS in *Diluent*

Sample solution: 2.5 mg/mL of Azelastine Hydrochloride in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 10-µm packing L10
Column temperature: 30°
Flow rate: 2 mL/min
Injection volume: 10 µL
Run time: 2.4 times the retention time of azelastine

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between azelastine related compound B and azelastine related compound D; NLT 1.5 between azelastine related compound D and azelastine; NLT 1.5 between azelastine and azelastine related compound E, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Identification solution*, *Standard solution*, and *Sample solution*
Calculate the percentage of each impurity in the portion of Azelastine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of azelastine from the *Standard solution*
C_S = concentration of USP Azelastine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Azelastine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. [Note—Disregard peaks that are less than 0.05% of the azelastine peak.]

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzohydrazide	0.2	0.38	0.1
Azelastine related compound B[a]	0.3	0.22	0.1
Chlorophenylacetylbenzoic acid[b]	0.4	0.45	0.1
Azelastine related compound D[c]	0.6	1.2	0.1
Azelastine	1.0	1.0	—
Azelastine related compound E[d]	1.4	0.48	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.2

[a] *N'*-(1-Methylazepan-4-yl)benzohydrazide; also known as 1-Benzoyl-2-[(4*RS*)-1-methylhexahydro-1*H*-azepin-4-yl]diazane.
[b] 2-[2-(4-Chlorophenyl)acetyl]benzoic acid.
[c] 4-(4-Chlorobenzyl)phthalazin-1(2*H*)-one.
[d] 3-(4-Chlorobenzylidene)isobenzofuran-1(3*H*)-one.

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩

Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 1.0%

- **Acidity or Alkalinity**

Sample solution: 10 mg/mL of Azelastine Hydrochloride in water
Analysis: Add 0.2 mL of bromothymol blue TS to 10 mL of the *Sample solution*.
Acceptance criteria: NMT 0.1 mL of 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is required to produce a color change.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. Protect from light and moisture. Store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩

USP Azelastine Hydrochloride RS
USP Azelastine Related Compound B RS
N'-(1-Methylazepan-4-yl)benzohydrazide.
$C_{14}H_{21}N_3O$ 247.34
USP Azelastine Related Compound D RS
4-(4-Chlorobenzyl)phthalazin-1(2*H*)-one.
$C_{15}H_{11}ClN_2O$ 270.71
USP Azelastine Related Compound E RS
3-(4-Chlorobenzylidene)isobenzofuran-1(3*H*)-one.
$C_{15}H_9ClO_2$ 256.68

Stimuli Article

www.webofpharma.com

Add the following:

▲Azelastine Hydrochloride Ophthalmic Solution

DEFINITION

Azelastine Hydrochloride Ophthalmic Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of azelastine hydrochloride ($C_{22}H_{24}ClN_3O \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer 1: 2.88 g/L of octanesulfonic acid sodium salt and 0.91 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Buffer 2: 0.87 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.5.
Solution A: Acetonitrile and *Buffer 1* (25:75)
Solution B: Acetonitrile
Mobile phase: See *Table 1*. Return to the original conditions, and re-equilibrate the system for NLT 5 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	85	15
35	65	35
45	45	55
60	45	55

Diluent: Acetonitrile and *Buffer 2* (25:75)
System suitability solution: 2.5 µg/mL each of USP Azelastine Hydrochloride RS and USP Azelastine Related Compound F RS in *Diluent*
Standard solution: 0.08 mg/mL of USP Azelastine Hydrochloride RS in *Diluent*
Sample stock solution: Combine the contents of NLT 5 containers of Ophthalmic Solution.
Sample solution: Nominally 0.08 mg/mL of azelastine hydrochloride from the *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L10
Column temperature: 30°
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between azelastine and azelastine related compound F, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of azelastine hydrochloride ($C_{22}H_{24}ClN_3O \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Azelastine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of azelastine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Standard solution, Sample stock solution, and Chromatographic system: Proceed as directed in the *Assay*.
Sensitivity solution: 0.0005 mg/mL of USP Azelastine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of azelastine hydrochloride (use the *Sample stock solution*)
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between azelastine and azelastine related compound F, *System suitability solution*.
[NOTE—If azelastine related compound F splits into two peaks, use the first peak for the determination of *Resolution*.]
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Azelastine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of azelastine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzohydrazide[a]	0.12	—	—
Azelastine related compound B[a, b]	0.17	—	—

www.webofpharma.com

Table 2 *(continued)*

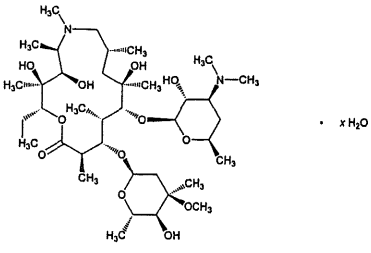
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chlorophenylacetylbenzoic acid[a, c]	0.65	—	—
Azelastine related compound D[d]	0.7	1.20	0.5
Azelastine	1.0	—	—
Azelastine related compound F[e]	1.08	0.71	1.5
Azelastine related compound E[a, f]	1.68	—	—
Any individual degradation product	—	1.0	0.5
Total degradation products	—	—	1.5

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total degradation products.
[b] *N'*-(1-Methylazepan-4-yl)benzohydrazide hydrochloride; also known as 1-Benzoyl-2-[(4*RS*)-1-methylhexahydro-1*H*-azepin-4-yl]diazane.
[c] 2-[2-(4-Chlorophenyl)acetyl]benzoic acid.
[d] 4-(4-Chlorobenzyl)phthalazin-1(2*H*)-one.
[e] If the peak splits into two, identify both the peaks and quantify together.
[f] 3-(4-Chlorobenzylidene)isobenzofuran-1(3*H*)-one.

SPECIFIC TESTS
- **pH** ⟨791⟩: 5.0–6.5
- **Osmolality and Osmolarity** ⟨785⟩: 265–375 mOsmol/kg
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **Other Requirements:** It meets the requirements in *Ophthalmic Products—Quality Tests* ⟨771⟩.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Azelastine Hydrochloride RS
  USP Azelastine Related Compound F RS
  4-[4-(4-Chlorobenzyl)-1-oxophthalazin-2(1*H*)-yl]-1-methylazepane 1-oxide.
  $C_{22}H_{24}ClN_3O_2$ 397.90▲ (USP 1-Aug-2019)

Azithromycin



$\cdot x H_2O$

$C_{38}H_{72}N_2O_{12}$	748.98
$C_{38}H_{72}N_2O_{12} \cdot H_2O$	767.00
$C_{38}H_{72}N_2O_{12} \cdot 2H_2O$	785.02

1-Oxa-6-azacyclopentadecan-15-one, 13-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-, [2*R*-(2*R**,3*S**,4*R**,5*R**,8*R**,10*R**,11*R**,12*S**,13*S**,14*R**)];
(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one;
9-Deoxo-9a-aza-9a-methyl-9a-homoerythromycin A
Anhydrous [83905-01-5].
Monohydrate [121470-24-4].
Dihydrate [117772-70-0].

DEFINITION
Azithromycin is anhydrous or contains 1 or 2 molecules of water of hydration. It contains the equivalent of NLT 945 µg/mg and NMT 1030 µg/mg of azithromycin ($C_{38}H_{72}N_2O_{12}$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020): If a difference appears in the IR spectra of the analyte and the Standard, dissolve equal portions of the test specimen and the USP Reference Standard in equal volumes of methanol. Evaporate the solutions to dryness on a water bath, and dry at 80° for 30 min under vacuum. Perform the test on the residues.
- **B.** The retention time of the azithromycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**

Solution A: 10 M potassium hydroxide
Solution B: 6.7 g/L of dibasic potassium phosphate, adjusted with *Solution A* to a pH of 11.0
Solution C: 6.7 g/L of dibasic potassium phosphate, adjusted with phosphoric acid to a pH of 8.0
Mobile phase: Acetonitrile and *Solution B* (60:40)
Diluent: Acetonitrile and *Solution C* (60:40)
System suitability solution: 0.5 mg/mL each of USP Azithromycin RS and USP Azaerythromycin A RS prepared as follows. Dissolve USP Azithromycin RS and USP Azaerythromycin A RS first in acetonitrile, using 5% of the final volume, and then dilute with *Diluent* to volume.
Standard solution: 0.53 mg/mL of USP Azithromycin RS prepared as follows. Dissolve USP Azithromycin RS first in acetonitrile, using 2% of the final volume, and then dilute with *Diluent* to volume.
Sample solution: 0.53 mg/mL of Azithromycin prepared as follows. Dissolve Azithromycin first in acetonitrile, using 2% of the final volume, and then dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L67
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*

USP Monographs

www.webofpharma.com

[NOTE—The relative retention times for azaerythromycin A and azithromycin are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between azaerythromycin A and azithromycin, *System suitability solution*

Tailing factor: 0.8–1.5 for azithromycin, *Standard solution*

Relative standard deviation: NMT 1.10% for azithromycin, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of azithromycin ($C_{38}H_{72}N_2O_{12}$) in each milligram of Azithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Azithromycin RS in the *Standard solution*
C_U = concentration of Azithromycin in the *Sample solution*
P = potency of USP Azithromycin RS (µg/mg of azithromycin)

Acceptance criteria: 945–1030 µg/mg on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%, moistening the charred residue with 2 mL of nitric acid and 5 drops of sulfuric acid

Delete the following:

▲• **ORGANIC IMPURITIES, PROCEDURE 1**

Use *Organic Impurities, Procedure 1* when the impurity profile includes erythromycin A oxime and erythromycin A iminoether.

Use water that has a resistivity of NLT 18 Mohm-cm.

Solution A: 20 mM dibasic potassium phosphate

Mobile phase: Acetonitrile and *Solution A* (250:750). Adjust with 5 M potassium hydroxide to a pH of 10.55 ± 0.05.

Standard stock solution: 45 µg/mL of USP Desosaminylazithromycin RS, 105 µg/mL of USP *N*-Demethylazithromycin RS, 150 µg/mL of USP Azaerythromycin A RS, and 160 µg/mL of USP Azithromycin RS in acetonitrile. Sonicate as necessary to dissolve.

Standard solution: 0.9 µg/mL of USP Desosaminylazithromycin RS, 2.1 µg/mL of USP *N*-Demethylazithromycin RS, 3.0 µg/mL of USP Azaerythromycin A RS, and 3.2 µg/mL of USP Azithromycin RS from the *Standard stock solution* in *Mobile phase*

Sample solution: 0.33 mg/mL of Azithromycin prepared as follows. Transfer a suitable amount of Azithromycin to a suitable volumetric flask. Add acetonitrile, using 5% of the final volume, and sonicate as necessary to dissolve. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Amperometric electrochemical

Detector type: Dual glassy carbon electrodes

Detector mode: Oxidative screen mode

Detector settings

Electrode 1: +0.70V

Electrode 2: +0.82V

Column: 4.6-mm × 15-cm; 3-µm packing L49

Temperatures

Detector preheater: 28°

Autosampler: 5°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between azithromycin and azaerythromycin A

Tailing factor: NMT 2.0 for azithromycin; NMT 2.5 for *N*-demethylazithromycin

Relative standard deviation: NMT 10.0% for azithromycin, azaerythromycin A, *N*-demethylazithromycin, and desosaminylazithromycin

Analysis

Samples: *Standard solution* and *Sample solution*

Record the *Sample solution* chromatograms for NLT 3.3 times the retention time of the azithromycin peak.

Calculate the percentages of desosaminylazithromycin, *N*-demethylazithromycin, and azaerythromycin A in the portion of Azithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*
r_S = peak area of the relevant analyte from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
F = conversion factor, 0.001 mg/µg

Calculate the percentages of other related substances in the portion of Azithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak area of each additional impurity from the *Sample solution*
r_S = peak area of the azithromycin peak from the *Standard solution*
C_S = concentration of USP Azithromycin RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Erythromycin A iminoether[a]	0.19	0.5
Desosaminylazithromycin[b]	0.29	0.3
Erythromycin A oxime[c]	0.37	0.5
N-Demethylazithromycin[d]	0.49	0.7
Azaerythromycin A[e]	0.80	1.0
Azithromycin	1.0	—
3-Deoxyazithromycin (azithromycin B)[f]	2.33	1.0

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	3.0

[a] (3*R*,4*R*,5*S*,6*R*,9*R*,10*S*,11*S*,12*R*,13*S*,15*R*,*Z*)-12-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-6-ethyl-4,5-dihydroxy-10-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-3,5,9,11,13,15-hexamethyl-7,16-dioxa-2-azabicyclo[11.2.1]hexadec-1-en-8-one.

[b] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[c] (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*,*E*)-6-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-14-ethyl-7,12,13-trihydroxy-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-10-(hydroxyimino)-3,5,7,9,11,13-hexamethyloxacyclotetradecan-2-one.

[d] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[e] 9-Deoxo-9a-aza-9a-homoerythromycin A; 6-Demethylazithromycin.

[f] (2*R*,3*R*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one. ▲ (USP 1-May-2019)

Change to read:

- **ORGANIC IMPURITIES**

▲▲ (USP 1-May-2019)

Solution A: 1.8 mg/mL of anhydrous dibasic sodium phosphate in water. Adjust with 1 N sodium hydroxide or 10% phosphoric acid to a pH of 8.9.

Solution B: Acetonitrile and methanol (3:1)

Solution C: 1.73 mg/mL of monobasic ammonium phosphate. Adjust with ammonia TS to a pH of 10.0 ± 0.05.

Solution D: Methanol, acetonitrile, and *Solution C* (7:6:7)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	50	50
25	45	55
30	40	60
80	25	75
81	50	50
93	50	50

System suitability solution: 0.0165 mg/mL of USP Azithromycin Related Compound F RS and 0.027 mg/mL of USP Desosaminylazithromycin RS in *Solution D*

Standard solution: 86 µg/mL of USP Azithromycin RS in *Solution D*

Sample solution: 8.6 mg/mL of Azithromycin in *Solution D*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Peak-to-valley ratio: NLT 1.4, *System suitability solution*

Calculate the peak-to-valley ratio:

$$\text{Result} = H_P/H_V$$

H_P = height above the baseline of the desosaminylazithromycin peak

H_V = height above the baseline of the lowest point of the curve separating the desosaminylazithromycin and azithromycin related compound F peaks

Tailing factor: 0.8–1.5, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Azithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F_1 \times (100/F_2)$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of azithromycin from the *Standard solution*

C_S = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

C_U = concentration of Azithromycin in the *Sample solution* (mg/mL)

P = potency of USP Azithromycin RS (µg/mg of azithromycin)

F_1 = conversion factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks eluting before azithromycin *N*-oxide and after 3-deoxyazithromycin (azithromycin B). Disregard peaks with a response less than 0.1 times the response of the azithromycin peak in the *Standard solution* (0.1%).

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 20 mg/mL in dehydrated alcohol

Acceptance criteria: −45° to −49°, at 20°

- **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements, except where it is labeled as amorphous, most of the particles do not exhibit birefringence and extinction positions
- **pH** ⟨791⟩

Sample stock solution: 4 mg/mL in methanol

Sample solution: 2 mg/mL obtained by mixing equal volumes of *Sample stock solution* and water

Acceptance criteria: 9.0–11.0

- **WATER DETERMINATION** ⟨921⟩, *Method I*

Where it is labeled as anhydrous: NMT 2.0%

Where it is labeled as the dihydrate: 4.0%–5.0%

Where it is labeled as the monohydrate: 1.8%–4.0%, except that it may be 4.0%–6.5% when the requirements of the *Loss on Drying* test are met

- **LOSS ON DRYING:** Where it is labeled as Azithromycin monohydrate and has a water content of 4.0%–6.5% (see *Thermal Analysis* ⟨891⟩)

[NOTE—The quantity taken for this procedure may be adjusted, if necessary, for instrument sensitivity.]

Analysis: Determine the percentage of volatile substances by thermogravimetric analysis in an appropriately calibrated instrument, using about 10 mg of Azithromycin. Heat the specimen at the rate of 10°/min between ambient temperature and 150° in an atmosphere of nitrogen at a constant flow rate of about 35 mL/min. From the thermogram plot the derivatives of the loss on drying

Supplement No. 1

www.webofpharma.com

Table 2 (Azithromycin)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Azithromycin *N*-oxide[a]	0.29	0.43	0.5
3′-(*N*,*N*-Didemethyl)-3′-*N*-formylazithromycin[b,▲c▲] (USP 1-May-2019)	0.37	1.7	0.5
▲Erythromycin A iminoether[d]	0.42	1.0	0.5▲ (USP 1-May-2019)
3′-(*N*,*N*-Didemethyl)azithromycin(aminoazithromycin)[e]	0.43	1.0	0.5
Azithromycin related compound F[c,f]	0.51	3.8	0.5
Desosaminylazithromycin[g]	0.54	1.0	0.3
3′-*N*-{[4-(Acetylamino)phenyl]sulfonyl}-3′,3′-didemethylazithromycin[h]	0.55	12	0.15
N-Demethylazithromycin[i]	0.61	1.0	0.7
▲Erythromycin A oxime[j]	0.64	1.0	0.5▲ (USP 1-May-2019)
Azithromycin C (3″-*O*-demethylazithromycin)[k]	0.73	1.0	0.5
3′-De(dimethylamino)-3′-oxoazithromycin[l]	0.76	1.5	0.5
3′-*N*-{[4-(Acetylamino)phenyl]sulfonyl}-3′-demethylazithromycin[m]	0.79	10	0.5
Azaerythromycin A[n]	0.83	1.0	0.5
Azithromycin impurity P[o]	0.92	1.0	0.2
Azithromycin	1.0	—	—
2-Desethyl-2-propylazithromycin[p]	1.23	1.0	0.5
3′-*N*-Demethyl-3′-*N*-[(4-methylphenyl)sulfonyl]azithromycin[q]	1.26	5	0.5
3-Deoxyazithromycin (azithromycin B)[r]	1.31	1.0	1.0
Any individual, unidentified impurity	—	1.0	0.2
Total impurities	—	—	3.0

[a] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylazinoyl)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[b] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[c] The system may resolve two rotamers. ▲▲ (USP 1-May-2019) The ▲limit is for the▲ (USP 1-May-2019) sum of the two rotamers. ▲▲ (USP 1-May-2019)

[d] (3*R*,4*R*,5*S*,6*R*,9*R*,10*S*,11*S*,12*R*,13*S*,15*R*,*Z*)-12-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-6-ethyl-4,5-dihydroxy-10-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-3,5,9,11,13,15-hexamethyl-7,16-dioxa-2-azabicyclo[11.2.1]hexadec-1-en-8-one.

[e] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[f] 3′-*N*-Demethyl-3′-*N*-formylazithromycin; (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[g] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[h] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-acetamidophenylsulfonyl)amino]-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[i] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[j] (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*,*E*)-6-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-14-ethyl-7,12,13-trihydroxy-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-10-(hydroxyimino)-3,5,7,9,11,13-hexamethyloxacyclotetradecan-2-one.

[k] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[l] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[m] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-acetamidophenylsulfonyl)-*N*-methylamino]-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[n] 9-Deoxo-9a-aza-9a-homoerythromycin A; 6-Demethylazithromycin.

[o] Specified unidentified impurity.

[p] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-propyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[q] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-methylphenylsulfonyl)-*N*-methylamino]-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[r] (2*R*,3*R*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

USP Monographs

www.webofpharma.com

(percentage loss/min), and identify the inflection points of the two weight loss steps at about 70° and 130°.
Acceptance criteria: NMT 4.5% between ambient temperature and the inflection point at about 70°, and 1.8%–2.6% between the inflection point at about 70° and the inflection point at about 130°.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.

Change to read:

- **LABELING:** Label it to indicate whether it is anhydrous, the monohydrate, or the dihydrate. The amorphous form is so labeled. Where the quantity of azithromycin is indicated in the labeling of any preparation containing Azithromycin, this shall be understood to be in terms of anhydrous azithromycin ($C_{38}H_{72}N_2O_{12}$). ▲▲ (USP 1-May-2019)

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Azaerythromycin A RS
  9-Deoxo-9a-aza-9a-homoerythromycin A; 6-Demethylazithromycin.
  $C_{37}H_{70}N_2O_{12}$ 734.96
  USP Azithromycin RS
  USP Azithromycin Related Compound F RS
  3′-*N*-Demethyl-3′-*N*-formylazithromycin; (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl) formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl] oxy]-1-oxa-6-azacyclopentadecan-15-one.
  $C_{38}H_{70}N_2O_{13}$ 762.97
  ▲▲ (USP 1-May-2019)
  USP Desosaminylazithromycin RS
  (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
  $C_{30}H_{58}N_2O_9$ 590.79

Azithromycin Capsules

DEFINITION
Azithromycin Capsules contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$).

IDENTIFICATION
- **A.** The retention time of the azithromycin peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **PROCEDURE**
  [NOTE—Use water that has a resistivity of NLT 18 Mohm-cm.]
  Mobile phase: Dissolve 5.8 g of monobasic potassium phosphate in 2130 mL of water, and add 870 mL of acetonitrile. Adjust with about 6 mL of 10 N potassium hydroxide to a pH of 11.0 ± 0.1, and pass through a suitable filter.
  Standard stock solution: 0.165 mg/mL of USP Azithromycin RS in acetonitrile. Swirl, and sonicate as necessary.
  Standard solution: 3.3 µg/mL of USP Azithromycin RS from the *Standard stock solution* in *Mobile phase*
  System suitability stock solution: 0.16 mg/mL of USP Azaerythromycin A RS in acetonitrile and *Mobile phase* (1:9). Dissolve first in acetonitrile, using 10% of the final volume. Swirl, and sonicate to dissolve. Dilute with *Mobile phase* to volume.
  System suitability solution: 3.2 µg/mL of azaerythromycin A from the *System suitability stock solution* and 3.3 µg/mL of azithromycin from the *Standard stock solution* in *Mobile phase*
  Sample stock solution: Remove, as completely as possible, the contents of NLT 20 Capsules. Prepare a 1-mg/mL solution of anhydrous azithromycin in acetonitrile. Dissolve a portion of the mixed Capsule contents first in 70% of the final volume of acetonitrile, and shake by mechanical means for 30 min. Dilute with acetonitrile to volume. Place 40 mL of the resulting suspension in a centrifuge tube, and centrifuge. Use the supernatant to prepare the *Sample solution.*
  Sample solution: 3.2 µg/mL of azithromycin from the *Sample stock solution* in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: Amperometric electrochemical detector
  Electrode: Dual glassy carbon electrodes
  Mode: Oxidative screen mode
  Electrode 1: +0.70 ± 0.05 V
  Electrode 2: +0.82 ± 0.05 V
  Background current: 85 ± 15 nanoampheres
  Columns
  Guard: 4.6-mm × 5-cm; 5-µm packing L29
  Analytical: 4.6-mm × 15-cm; 5-µm packing L29 or 3-µm packing L49 without the guard column
  Flow rate: 1.5 mL/min
  Injection size: 50 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  [NOTE—The relative retention times for azaerythromycin A and azithromycin with the L29 column are 0.7 and 1.0, respectively; the relative retention times for azaerythromycin A and azithromycin with the L49 column are 0.8 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2.5 between azaerythromycin A and azithromycin, *System suitability solution*
  Column efficiency: NLT 1000 theoretical plates, *Standard solution*
  Tailing factor: 0.9–1.5, *Standard solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Azithromycin RS in the *Standard solution* (µg/mL)

www.webofpharma.com

C_U = nominal concentration of azithromycin in the *Sample solution* (µg/mL)
P = potency of azithromycin in USP Azithromycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

[NOTE—Use water that has a resistivity of NLT 18 Mohm-cm.]

Medium: pH 6.0 sodium phosphate buffer (Prepare 6 L of 0.1 M dibasic sodium phosphate. Adjust with about 40 mL of hydrochloric acid to a pH of 6.0 ± 0.05, and add 600 mg of trypsin); 900 mL
Apparatus 2: 100 rpm
Time: 45 min
Mobile phase, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard stock solution: 0.3 mg/mL of USP Azithromycin RS in *Medium*. Sonicate briefly to dissolve.
Standard solution: 3.84 µg/mL of azithromycin from the *Standard stock solution* in *Mobile phase*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.5-µm or finer pore size. Transfer 2.0 mL of the filtrate to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume. Transfer 4.0 mL of this solution to a second 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amount of azithromycin ($C_{38}H_{72}N_2O_{12}$) dissolved using the procedure in the *Assay*, making any necessary modifications.
Calculate the percentage of azithromycin ($C_{38}H_{72}N_2O_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 5.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Where packaged in unit-of-use containers, each container contains six 250-mg Capsules, and the label indicates the intended sequential day of use for each Capsule.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Azaerythromycin A RS
USP Azithromycin RS

Azithromycin for Injection

DEFINITION

Azithromycin for Injection is a sterile, dry mixture of azithromycin and a suitable stabilizing agent. It contains NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

▲• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A**▲ (CN 1-May-2020)
Standard: 25 mg/mL of USP Azithromycin RS in acetonitrile. Pass the solution through a suitable filter, and remove the solvent by natural evaporation.
Sample: Equivalent to 25 mg/mL of azithromycin from Azithromycin for Injection in acetonitrile. Pass the solution through a suitable filter, and remove the solvent by natural evaporation.
Analysis: Examine the spectra of the *Standard* and the *Sample* in the range between 3800 and 650 cm^{-1}.
Acceptance criteria: The *Sample* exhibits bands at about 900, 995, 1165, 1376, 1456, 1725, and 2936 cm^{-1} similar to the spectrum from the *Standard* similarly obtained.▲ (USP 1-Aug-2019)

ASSAY

• **PROCEDURE**
Buffer: 6.7 mg/mL of dibasic potassium phosphate in water
Mobile phase: Acetonitrile and *Buffer* (52:48). Adjust with 10 N potassium hydroxide to a pH of 11.0 ± 0.1.
Diluent: Acetonitrile and water (52:48)
System suitability solution: 1 mg/mL each of USP Azaerythromycin A RS and USP Azithromycin RS in a mixture of acetonitrile and water (52:48). Dissolve first in acetonitrile, and then dilute with water to volume.
Standard solution: 1 mg/mL of USP Azithromycin RS in a mixture of acetonitrile and water (52:48). Dissolve first in acetonitrile, and dilute with water to volume.
Sample solution: Nominally equivalent to 1 mg/mL of azithromycin from Azithromycin for Injection in *Diluent* prepared as follows. Reconstitute 3 vials individually as directed in the labeling. Mix the contents of all the reconstituted vials. Dilute a portion of the mixture with *Diluent*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Columns
Guard: 4.6-mm × 1-cm; 5-µm packing L67
Analytical: 4.6-mm × 15-cm; 5-µm packing L67
Temperatures
Autosampler: 15°
Column: 40°
Flow rate: 1 mL/min
Injection volume: 15 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for azaerythromycin A and azithromycin are 0.68 and 1.0, respectively.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 2.5 between azaerythromycin A and azithromycin, *System suitability solution*
Tailing factor: NLT 0.9 and NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$) in the portion of Azithromycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of azithromycin in the *Sample solution* (mg/mL)
P = potency of USP Azithromycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Uniformity of Dosage Units** ⟨905⟩**:** Meets the requirements

IMPURITIES
[Note—The test for *Limit of Azithromycin N-Oxide, Desosaminylazithromycin, and N-Demethylazithromycin* does not quantify aminoazithromycin, formamido analog, methylformamido analog, and 3′-de(dimethylamino)-3′-oxoazithromycin. If these impurities are part of the impurity profile, the *Limit of Aminoazithromycin, Formamido Analog, Methylformamido Analog, and 3′-De(dimethylamino)-3′-oxoazithromycin* test is recommended in addition to the test for *Limit of Azithromycin N-Oxide, Desosaminylazithromycin, and N-Demethylazithromycin.*]

Change to read:

• **Limit of Azithromycin *N*-Oxide, Desosaminylazithromycin, and *N*-Demethylazithromycin**
Buffer: 3.5 g/L of dibasic potassium phosphate
Mobile phase: Acetonitrile and *Buffer* (23:77). Adjust with 5 N potassium hydroxide to a pH of 10.55 ± 0.05.
Standard stock solution: 0.05 mg/mL of USP Azithromycin *N*-oxide RS, 45 µg/mL of USP Desosaminylazithromycin RS, and 160 µg/mL each of USP *N*-Demethylazithromycin RS and USP Azithromycin RS in acetonitrile. Sonicate if necessary to dissolve.
Standard solution: 0.001 mg/mL of azithromycin *N*-oxide, 0.9 µg/mL of desosaminylazithromycin, and 3.2 µg/mL each of *N*-demethylazithromycin and azithromycin from *Standard stock solution* in *Mobile phase*
Sample solution: Nominally equivalent to 0.3 mg/mL of azithromycin in *Mobile phase* from Azithromycin for Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: Amperometric electrochemical
Electrodes: Dual series glassy carbon
Mode: Oxidative screen
Electrode 1: +0.70 ± 0.05 V
Electrode 2: +0.82 ± 0.05 V
▲▲ (USP 1-Aug-2019)
Column: 4.6-mm × 15-cm; 3-µm packing L49
Autosampler temperature: 5°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
[Note—See *Table 1* for relative retention times.]
Suitability requirements
Tailing factor: NMT 2.0 for azithromycin and NMT 2.6 for *N*-demethylazithromycin
Relative standard deviation: NMT 10.0% for azithromycin *N*-oxide, desosaminylazithromycin, *N*-demethylazithromycin, and azithromycin
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified impurity in the portion of Azithromycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of each specified impurity from the *Sample solution*
r_S = peak response of each specified impurity from the *Standard solution*
C_S = concentration of the relevant impurity Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of azithromycin in the *Sample solution* (mg/mL)
P = potency of the relevant Reference Standard (mg/mg)

Acceptance criteria: See *Table 1*. The reporting ▲threshold▲ (USP 1-Aug-2019) is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azithromycin *N*-oxide[a]	0.17	1.0
Desosaminylazithromycin[b]	0.27	0.3
Erythromycin A oxime[c, d]	0.35	—
N-Demethylazithromycin[e]	0.50	1.0
Azaerythromycin A[c, f]	0.85	—
Azithromycin	1.00	—

[a] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylazinoyl)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
[b] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
[c] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.
[d] (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*,*E*)-6-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-14-ethyl-7,12,13-trihydroxy-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-10-(hydroxyimino)-3,5,7,9,11,13-hexamethyloxacyclotetradecan-2-one.
[e] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
[f] 9-Deoxo-9a-aza-9a-homoerythromycin A.

www.webofpharma.com

Change to read:

- **LIMIT OF AMINOAZITHROMYCIN, FORMAMIDO ANALOG, METHYLFORMAMIDO ANALOG, AND 3′-DE(DIMETHYLAMINO)-3′-OXOAZITHROMYCIN** (if present)

Buffer: 3.5 g/L of dibasic potassium phosphate in water
Mobile phase: Acetonitrile and *Buffer* (46:54). Adjust with 10 N potassium hydroxide to a pH of 11.0 ± 0.1.
Diluent: Acetonitrile and water (46:54)
Standard stock solution: 0.09 mg/mL of USP Desosaminylazithromycin RS, 0.21 mg/mL of USP *N*-Demethylazithromycin RS, and 0.30 mg/mL of USP Azithromycin RS in acetonitrile
Standard solution: 0.0018 mg/mL of desosaminylazithromycin, 0.0042 mg/mL of *N*-demethylazithromycin, and 0.006 mg/mL of azithromycin in *Diluent*
Sample solution: Nominally equivalent to 0.6 mg/mL of azithromycin from Azithromycin for Injection in *Diluent*. Reconstitute 3 vials individually, as directed in the labeling. Mix the contents of all the reconstituted vials. Dilute a portion of the mixture with *Diluent*. The *Sample solution* must be injected immediately after preparation.
Blank: Use the *Diluent*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Amperometric electrochemical
Electrodes: Dual series glassy carbon
Mode: Oxidative screen
Electrode 1: +0.70 ± 0.05 V
Electrode 2: +0.82 ± 0.05 V
▲▲ (USP 1-Aug-2019) ,
Columns
Guard: 4.6-mm × 1-cm; 5-µm packing L67
Analytical: 4.6-mm × 25-cm; 5-µm packing L67
Temperatures
Autosampler: 15°
Column: 40°
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between desosaminylazithromycin and *N*-demethylazithromycin
Tailing factor: NMT 1.5 for azithromycin
Relative standard deviation: NMT 5% for azithromycin
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Disregard any peaks corresponding to those obtained from the *Blank*.
Calculate the percentage of each impurity in the portion of Azithromycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of azithromycin from the *Standard solution*
C_S = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of azithromycin in the *Sample solution* (mg/mL)
P = potency of USP Azithromycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Erythromycin A iminoether[a, b]	0.20	—
3′-(*N*,*N*-Didemethyl)azithromycin (aminoazithromycin)[c] + 3′-(*N*,*N*-didemethyl)-3′-*N*-formylazithromycin (formamido analog)[d]	0.25	1.0
Azithromycin F[a, e]	0.30	—
Desosaminylazithromycin[f, g]	0.31	—
3′-*N*-Demethyl-3′-*N*-formylazithromycin (methylformamido analog)[h]	0.32	1.0
N-Demethylazithromycin[f, i]	0.35	—
Erythromycin A oxime[a, j]	0.42	—
Azaerythromycin A[a, k]	0.63	—
3′-De(dimethylamino)-3′-oxoazithromycin[l]	0.72	1.0
3′-*N*-Demethyl-3′-*N*-[(4-methylphenyl)sulfonyl]-azithromycin[a, m]	0.85	—
Azithromycin	1.00	—
Azithromycin B (3-deoxyazithromycin)[a, n]	1.64	—
Any other unspecified impurity	—	0.2

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities[o]	—	3.0

[a] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.
[b] (3*R*,4*R*,5*S*,6*R*,9*R*,10*S*,11*S*,12*R*,13*S*,15*R*,*Z*)-12-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-6-ethyl-4,5-dihydroxy-10-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-3,5,9,11,13,15-hexamethyl-7,16-dioxa-2-azabicyclo[11.2.1]hexadec-1-en-8-one.
[c] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
[d] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
[e] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12-hexamethyl-14-hydroxymethyl-11-[[3-dimethylamino-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
[f] These impurities are controlled using the *Limit of Azithromycin N-Oxide, Desosaminylazithromycin, and N-Demethylazithromycin* test. They are listed here for information only.
[g] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
[h] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
[i] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
[j] (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*,*E*)-6-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-14-ethyl-7,12,13-trihydroxy-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-10-(hydroxyimino)-3,5,7,9,11,13-hexamethyloxacyclotetradecan-2-one.
[k] 9-Deoxo-9a-aza-9a-homoerythromycin A.
[l] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
[m] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-acetamidophenylsulfonyl)-*N*-methylamino]-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
[n] (2*R*,3*R*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
[o] Total impurities include desosaminylazithromycin and *N*-demethylazithromycin.

SPECIFIC TESTS

Change to read:

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲Meets the requirements▲ (USP 1-Aug-2019)

Change to read:

- **STERILITY TESTS** ⟨71⟩: ▲Meets the requirements▲ (USP 1-Aug-2019)
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements
- **PH** ⟨791⟩: 6.4–6.8, determined in a solution constituted as directed in the labeling
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 2.0%
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described under *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for Constitution*. Store at controlled room temperature.
- **LABELING:** It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Azaerythromycin A RS
9-Deoxo-9a-aza-9a-homoerythromycin A.
$C_{37}H_{70}N_2O_{12}$ 734.96

USP Azithromycin RS

USP Azithromycin *N*-oxide RS
(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylazinoyl)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
$C_{38}H_{72}N_2O_{13}$ 764.98

USP *N*-Demethylazithromycin RS
(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
$C_{37}H_{70}N_2O_{12}$ 734.96

USP Desosaminylazithromycin RS
(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
$C_{30}H_{58}N_2O_9$ 590.79

Azithromycin for Oral Suspension

DEFINITION

Azithromycin for Oral Suspension is a dry mixture of Azithromycin and one or more buffers, sweeteners, diluents, anticaking agents, and flavors. It contains NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$).

IDENTIFICATION

- **A.** The retention time of the azithromycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

▲[NOTE—Solutions containing azithromycin are stable up to 12 h at 10°.]

Solution A: Dissolve 8.7 g of dipotassium hydrogen phosphate anhydrous in 1000 mL of water and adjust with potassium hydroxide or dilute orthophosphoric acid to a pH of 8.2.

Solution B: Acetonitrile

Mobile phase: *Solution A* and *Solution B* (30:70)

Diluent: Acetonitrile, methanol, and water (40:40:20)

Standard solution: 0.6 mg/mL of USP Azithromycin RS in *Diluent*. Sonicate in cool water to dissolve as needed.

www.webofpharma.com

Sample solution: Nominally 0.6 mg/mL of azithromycin in *Diluent* prepared as follows. Transfer an accurately measured portion of the constituted suspension to a suitable volumetric flask. Add *Diluent* equal to 50% of the volume of the flask, and sonicate for 20 min with shaking in cool water. Dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 30°
Flow rate: 2 mL/min
Injection volume: 50 µL
Run time: NLT 2 times the retention time of azithromycin

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$) in the portion of Azithromycin for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of azithromycin from the *Sample solution*
r_S = peak response of azithromycin from the *Standard solution*
C_S = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of azithromycin in the *Sample solution* (mg/mL)
P = potency of USP Azithromycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg ▲(USP 1-May-2019)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Deliverable Volume** ⟨698⟩: Meets the requirements
• **Uniformity of Dosage Units** ⟨905⟩
For single-unit containers: Meets the requirements

Add the following:

▲• **Dissolution** ⟨711⟩
[Note—Solutions containing azithromycin are stable up to 12 h at 10°.]
Medium: Sodium phosphate buffer at a pH of 6.0; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Solution A: Dissolve 8.7 g of dipotassium hydrogen phosphate anhydrous in 1000 mL of water and adjust with potassium hydroxide or dilute orthophosphoric acid to a pH of 8.2.
Solution B: Acetonitrile
Mobile phase: *Solution A* and *Solution B* (35:65)
Standard stock solution: 0.55 mg/mL of USP Azithromycin RS prepared as follows. Transfer an accurately weighed amount of USP Azithromycin RS to a suitable volumetric flask. Add acetonitrile to fill 5% of the volume of the flask and sonicate in cool water for 5 min to dissolve completely. Dilute with *Medium* to volume.
Standard solution
For Azithromycin for Oral Suspension labeled to contain 100 mg/5 mL: 0.11 mg/mL of USP Azithromycin RS in *Medium* from *Standard stock solution*
For Azithromycin for Oral Suspension labeled to contain 200 mg/5 mL: 0.22 mg/mL of USP Azithromycin RS in *Medium* from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 50°
Flow rate: 2 mL/min
Injection volume: 100 µL
Run time: NLT 2 times the retention time of azithromycin

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage (*Q*) of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times (d/W) \times V \times 100$$

r_U = peak response of azithromycin from the *Sample solution*
r_S = peak response of azithromycin from the *Standard solution*
C_S = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)
L = label claim of Azithromycin for Oral Suspension (mg/5 mL)
D = dilution factor, necessary only if the *Sample solution* requires dilution (mL/mL)
d = density of the *Sample solution* (g/mL)
W = weight of Azithromycin for Oral Suspension taken (g)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$) is dissolved. ▲(USP 1-May-2019)

IMPURITIES

Add the following:

▲• **Organic Impurities**
Solution A: Dissolve 1.8 g of disodium hydrogen phosphate dihydrate in 1000 mL of water and adjust with dilute phosphoric acid to a pH of 8.9.
Solution B: Acetonitrile and methanol (75:25)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	50	50

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
25	45	55
30	40	60
80	25	75
81	50	50
90	50	50

Buffer: Dissolve 1.73 g of ammonium dihydrogen phosphate in 1000 mL of water and adjust with ammonia solution to a pH of 10.0 ± 0.05.
Diluent: *Buffer*, methanol, and acetonitrile (35:35:30)
System suitability solution: 0.015 mg/mL of USP Azithromycin Related Compound F RS and 0.025 mg/mL of USP Desosaminylazithromycin RS in *Diluent*
Standard solution: 0.04 mg/mL of USP Azithromycin RS in *Diluent*. Sonicate in cool water to dissolve as needed.
Sample solution: Nominally 4.0-mg/mL solution of azithromycin in *Diluent* prepared as follows. Transfer a portion of the constituted suspension, equivalent to about 400.0 mg of azithromycin, to a 100-mL volumetric flask. Add 70 mL of *Diluent* and sonicate in cool water for about 15 min. Dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 60°
Flow rate: 0.9 mL/min
Injection volume: 100 µL
Run time: NLT 2 times the retention time of azithromycin
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between desosaminylazithromycin and azithromycin related compound F, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Azithromycin for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F_1 \times (1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of azithromycin from the *Standard solution*
C_S = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of azithromycin in the *Sample solution* (mg/mL)
P = potency of USP Azithromycin RS (µg/mg)
F_1 = conversion factor, 0.001 mg/µg
F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peaks at relative retention times before 0.29 and after 1.31.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Azithromycin *N*-oxide[a]	0.29	0.43	0.50
3′-(*N*,*N*-Didemethyl)-3′-*N*-formylazithromycin[b]	0.37	1.7	0.50
3′-(*N*,*N*-Didemethyl)azithromycin(aminoazithromycin)[c]	0.43	1.0	0.50
Azithromycin related compound F[d]	0.51	3.8	0.50
Desosaminylazithromycin[e]	0.54	1.0	0.30
N-Demethylazithromycin[f]	0.61	1.0	0.50
Azithromycin C (3″-*O*-demethylazithromycin)[g, h]	0.73	—	—
3′-De(dimethylamino)-3′-oxoazithromycin[i]	0.76	1.5	0.50
Azaerythromycin A[h, j]	0.83	—	—
Specified unidentified impurity[h, k]	0.92	—	—
Azithromycin	1.0	—	—
2-Desethyl-2-propylazithromycin[h, l]	1.23	—	—
3′-*N*-Demethyl-3′-*N*-[(4-methylphenyl)sulfonyl]azithromycin[h, m]	1.26	—	—
3-Deoxyazithromycin (azithromycin B)[h, n]	1.31	—	—
Any individual unspecified degradation product	—	1.0	0.20

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total degradation products	—	—	3.5

[a] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylazinoyl)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[b] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[c] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[d] 3′-(*N*-Demethyl)-3′-*N*-formylazithromycin; (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[e] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[f] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[g] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[h] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only. The unspecified impurities and total impurities limits do not include these impurities.

[i] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[j] 9-Deoxo-9a-aza-9a-homoerythromycin A.

[k] Specified unidentified impurity.

[l] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-propyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one dihydrate.

[m] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-methylphenylsulfonyl)-*N*-methylamino]-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[n] (2*R*,3*R*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one. ▲(USP 1-May-2019)

SPECIFIC TESTS

- **pH** ⟨791⟩
  - **For a solid packaged in single-unit containers**
    - **Sample:** The suspension constituted as directed in the labeling
    - **Acceptance criteria:** 9.0–11.0
  - **For a solid packaged in multiple-unit containers**
    - **Sample:** The suspension constituted as directed in the labeling
    - **Acceptance criteria:** 8.5–11.0

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Change to read:

- **USP Reference Standards** ⟨11⟩

▲▲(USP 1-May-2019)

USP Azithromycin RS

▲USP Azithromycin Related Compound F RS
3′-(*N*-Demethyl)-3′-*N*-formylazithromycin; (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
$C_{38}H_{70}N_2O_{13}$ 762.97

USP Desosaminylazithromycin RS
(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
$C_{30}H_{58}N_2O_9$ 590.79 ▲(USP 1-May-2019)

Azithromycin Tablets

DEFINITION

Azithromycin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

▲• **B.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197A**▲ (CN 1-May-2020)

Standard solution: 25 mg/mL of USP Azithromycin RS in acetonitrile. Pass the solution through a suitable filter, and remove the solvent by natural evaporation.

Sample solution: Equivalent to 25 mg/mL of azithromycin from Tablets in acetonitrile. Pass the solution through a suitable filter, and remove the solvent by natural evaporation.

Acceptance criteria: Meet the requirements▲ (USP 1-Dec-2019)

ASSAY

- **Procedure**

Buffer: Dissolve 4.6 g of monobasic potassium phosphate anhydrous in 900 mL of water. Adjust with 1 N sodium hydroxide to a pH of 7.5, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (65:35)

Standard solution: 1 mg/mL of USP Azithromycin RS in *Mobile phase*. Sonicate and shake as needed to dissolve.

Sample solution: Nominally 1 mg/mL of azithromycin in *Mobile phase* from NLT 20 Tablets, finely powdered. Sonicate and shake as needed to dissolve.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 2 mL/min
Injection volume: 100 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of azithromycin from the *Sample solution*
r_S = peak response of azithromycin from the *Standard solution*
C_S = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of azithromycin in the *Sample solution* (mg/mL)
P = potency of USP Azithromycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩
Medium: pH 6.0 phosphate buffer; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Solution A: 4.4 mg/mL of dibasic potassium phosphate and 0.5 mg/mL of sodium 1-octanesulfonate; adjusted with phosphoric acid to a pH of 8.20 ± 0.05
Mobile phase: Acetonitrile, methanol, and *Solution A* (9:3:8)
Diluent: 17.5 mg/mL of dibasic potassium phosphate. Adjust with phosphoric acid to a pH of 8.00 ± 0.05. Prepare a mixture of this solution and acetonitrile (80:20).
Standard stock solution: Dissolve USP Azithromycin RS in *Medium* to obtain a solution with a known concentration of about (L/1000) mg/mL, where L is the label claim in mg/Tablet.
Standard solution: Dilute the *Standard stock solution* with *Diluent* to obtain a solution with a known concentration of about (L/2000) mg/mL, where L is the label claim in mg/Tablet.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute a portion of the filtrate with *Diluent* to obtain a solution with a theoretical concentration of about (L/2000) mg/mL, where L is the label claim in mg/Tablet, assuming complete dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times {}^{\blacktriangle}D_{\blacktriangle\,(\text{USP 1-Dec-2019})} \times 100$$

r_U = peak response of azithromycin from the *Sample solution*
r_S = peak response of azithromycin from the *Standard solution*
C_S = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL
${}^{\blacktriangle}D$ = dilution factor for the *Sample solution*, if necessary▲ (USP 1-Dec-2019)

Tolerances: NLT 80% (Q) of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**
Protect all solutions containing azithromycin from light. Refrigerate the *Standard solution* and the *Sample solution* after preparation and during analysis, using a refrigerated autosampler set at 4°. The solutions must be analyzed within 24 h of preparation.
Solution A: Water and ammonium hydroxide (2000: 1.2). The pH of this solution is about 10.5.
Solution B: Acetonitrile, methanol, and ammonium hydroxide (1800: 200: 1.2)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	54	46
20	54	46
35	10	90
35.1	54	46
▲50.1▲ (USP 1-Dec-2019)	54	46

Buffer: 1.7 g/L of monobasic ammonium phosphate in water. Adjust with ammonium hydroxide to a pH of 10 ▲± 0.05.▲ (USP 1-Dec-2019)
Diluent A: Methanol, acetonitrile, and *Buffer* (350:300:350). ▲ [Note—*Diluent A* is stable for 24 h after mixing the organic and buffer phases.]▲ (USP 1-Dec-2019)
Diluent B: Methanol and *Buffer* (1:1)
System suitability stock solution: 0.1 mg/mL each of USP Desosaminylazithromycin RS, USP Azithromycin Related Compound F RS, and ▲USP *N*-Demethylazithromycin RS▲ (USP 1-Dec-2019) in acetonitrile
System suitability solution: 0.028 mg/mL each of USP Desosaminylazithromycin RS, USP Azithromycin Related Compound F RS, and ▲USP *N*-Demethylazithromycin RS▲ (USP 1-Dec-2019) from the *System suitability stock solution* in *Diluent A*
▲**Peak identification solution:** 0.004 mg/mL each of USP Desosaminylazithromycin RS, USP Azithromycin Related Compound F RS, and USP *N*-Demethylazithromycin RS from the *System suitability solution* in *Diluent A*▲ (USP 1-Dec-2019)
Standard stock solution: 0.4 mg/mL of USP Azithromycin RS in acetonitrile. Sonicate and shake as needed to dissolve.
Standard solution: 0.02 mg/mL of azithromycin from the *Standard stock solution* in *Diluent A*

www.webofpharma.com

Sensitivity solution: 0.004 mg/mL of azithromycin from the *Standard solution* in *Diluent A*

Sample stock solution: Nominally 14.3 mg/mL of azithromycin prepared as follows. Transfer nominally 1430 mg of azithromycin, from finely powdered Tablets (NLT 20), to a 100-mL volumetric flask. Add 75 mL of acetonitrile, and sonicate for NLT 15 min. Shake by mechanical means for NLT 15 min. Allow the solution to equilibrate to room temperature, dilute with acetonitrile to volume, and mix.

Sample solution: Nominally 4 mg/mL of azithromycin prepared as follows. Centrifuge an aliquot of the *Sample stock solution* for NLT 15 min. Transfer 7.0 mL of the supernatant to a 25-mL volumetric flask, and dilute with *Diluent B* to volume.

Blank: *Diluent A*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Temperatures

Autosampler: 4°

Column: 50°

Flow rate: 1.2 mL/min

Injection volume: 100 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.0 between desosaminylazithromycin and azithromycin related compound F, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: ▲*Standard solution*,▲ (USP 1-Dec-2019) *Sample solution*, and *Blank*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F_1 \times (1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of azithromycin from the *Standard solution*

C_S = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of azithromycin in the *Sample solution* (mg/mL)

P = potency of USP Azithromycin RS (µg/mg)

F_1 = conversion factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%. Disregard any peaks in the *Sample solution* that correspond to peaks in the *Blank*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Azithromycin *N*-oxide[a]	0.20	▲0.46▲ (USP 1-Dec-2019)	1.0
3′-(*N*,*N*-Didemethyl)-3′-*N*-formylazithromycin[b,▲c▲] (USP 1-Dec-2019)	0.29		
	0.30	1.7	1.0
3′-(*N*,*N*-Didemethyl)azithromycin (aminoazithromycin)[d]	0.34	▲0.44▲ (USP 1-Dec-2019)	0.5
Azithromycin related compound F▲[c]▲ (USP 1-Dec-2019)[e]	▲0.40▲ (USP 1-Dec-2019)		
	▲0.46▲ (USP 1-Dec-2019)	▲5.5▲ (USP 1-Dec-2019)	1.0
Desosaminylazithromycin[f]	▲0.47▲ (USP 1-Dec-2019)	1.1	0.5
N-Demethylazithromycin[g]	0.50	▲0.47▲ (USP 1-Dec-2019)	0.7
3′-De(dimethylamino)-3′-oxoazithromycin[h]	0.87	▲1.7▲ (USP 1-Dec-2019)	1.0
Azaerythromycin A[i,j]	0.94	—	—
Azithromycin	1.0	—	—
2-Desethyl-2-propylazithromycin[i,k]	1.10	—	—
3′-*N*-Demethyl-3′-*N*-[(4-methylphenyl)sulfonyl] azithromycin[i,l]	1.11	—	—
3-Deoxyazithromycin (azithromycin B)[i,m]	1.14	—	—
Any individual unspecified impurity[i]	—	1.0	0.2

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities[l]	—	—	5.0

[a] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylazinoyl)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[b] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[c] The system may resolve two rotamers. The limit is for the sum of the two rotamers.

[d] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[e] 3′-(*N*-Demethyl)-3′-*N*-formylazithromycin; (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[f] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[g] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[h] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[i] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only. The unspecified impurities and total impurities limits do not include these impurities.

[j] 9-Deoxo-9a-aza-9a-homoerythromycin A.

[k] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-propyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one dihydrate.

[l] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-methylphenylsulfonyl)-*N*-methylamino]-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[m] (2*R*,3*R*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

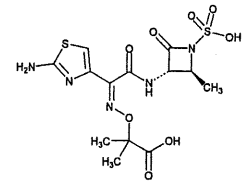
USP Azithromycin RS

USP Azithromycin Related Compound F RS
3′-(*N*-Demethyl)-3′-*N*-formylazithromycin;
(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
$C_{38}H_{70}N_2O_{13}$ 762.97

▲USP *N*-Demethylazithromycin RS
(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
$C_{37}H_{70}N_2O_{12}$ 734.96 ▲(USP 1-Dec-2019)

USP Desosaminylazithromycin RS
(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
$C_{30}H_{58}N_2O_9$ 590.79

Aztreonam



$C_{13}H_{17}N_5O_8S_2$ 435.43

Propanoic acid, 2-[[[1-(2-amino-4-thiazolyl)-2-[(2-methyl-4-oxo-1-sulfo-3-azetidinyl)amino]-2-oxoethylidene]amino]oxy]-2-methyl-, [2*S*-[2α,3β(*Z*)]]-;
(*Z*)-2-({[(2-Amino-4-thiazolyl){[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl}methylene]amino}oxy)-2-methylpropionic acid [78110-38-0].

DEFINITION

Aztreonam, which may be anhydrous or hydrated, contains NLT 92.0% and NMT 105.0% of aztreonam ($C_{13}H_{17}N_5O_8S_2$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020): If a difference appears in the IR spectra of the analyte and the standard, dissolve equal portions of the test specimen and the Reference Standard in equal volumes of methanol. [NOTE—To achieve a complete dissolution, it is suggested to use about 25 mL of methanol for each 50 mg of material, and stir the mixture for 40 min at room temperature.]
Evaporate the solutions to dryness under vacuum, and dry at 40° for 4 h under vacuum. Perform the test on the residues.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Store the *System suitability solution*, *Standard solution*, and *Sample solution* at 5°, and protect from light to prevent isomerization of aztreonam *Z*-isomer to aztreonam *E*-isomer.]

Buffer: 6.8 mg/mL of monobasic potassium phosphate in water. Adjust with 1 M phosphoric acid to a pH of 3.0.

www.webofpharma.com

Mobile phase: Methanol and *Buffer* (1:4)
System suitability solution: 1 mg/mL of USP Aztreonam RS and 1 mg/mL of USP Aztreonam E-Isomer RS in *Mobile phase*
Standard solution: 1 mg/mL of USP Aztreonam RS in *Mobile phase*
Sample solution: 1 mg/mL of Aztreonam in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for aztreonam and aztreonam *E*-isomer are 1.0 and 1.8, respectively.]
Suitability requirements
Resolution: NLT 2.0 between aztreonam and aztreonam *E*-isomer, *System suitability solution*
Tailing factor: NMT 2 for aztreonam, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aztreonam ($C_{13}H_{17}N_5O_8S_2$) in the portion of Aztreonam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)
C_U = concentration of Aztreonam in the *Sample solution* (mg/mL)
P = potency of USP Aztreonam RS (µg/mg)
F = unit conversion factor, 0.001 mg/µg

Acceptance criteria: 92.0%–105.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid
- **ORGANIC IMPURITIES**

[NOTE—Store the *System suitability solution*, *Standard solution*, and *Sample solution* at 5°, and protect from light to prevent isomerization of aztreonam *Z*-isomer to aztreonam *E*-isomer.]
Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Aztreonam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of aztreonam from the *Standard solution*
C_S = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)
C_U = concentration of Aztreonam in the *Sample solution* (mg/mL)
P = potency of USP Aztreonam RS (µg/mg)
F = unit conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Open-ring aztreonam[a] and open-ring desulfated aztreonam[b,c]	0.55	1.0
Aztreonam (*Z*-isomer)	1.0	—
Desulfated aztreonam[d]	1.6	1.5
Aztreonam *E*-isomer[e]	1.8	0.5
Aztreonam ethyl ester[f]	3.9	1.5
Any individual unspecified impurity	—	0.1
Total impurities	—	3.0

[a] (2*S*,3*S*)-2-[(*Z*)-2-[2-Aminothiazol-4-yl]-2-[2-carboxypropan-2-yloxyimino] acetamido}-3-(sulfoamino)butanoic acid.
[b] (2*S*,3*S*)-3-Amino-2-[(*Z*)-2-[2-aminothiazol-4-yl]-2-[2-carboxypropan-2-yloxyimino]acetamido}butanoic acid.
[c] Open-ring aztreonam and open-ring desulfated aztreonam coelute. The limit is for the sum of these two impurities.
[d] (*Z*)-2-({[(2-Amino-4-thiazolyl){[(2*S*,3*S*)-2-methyl-4-oxo-3-azetidinyl] carbamoyl}methylene]amino}oxy)-2-methylpropionic acid.
[e] (*E*)-2-({[(2-Amino-4-thiazolyl){[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfo-3-azetidinyl] carbamoyl}methylene]amino}oxy)-2-methylpropionic acid.
[f] Ethyl (*Z*)-2-({[(2-amino-4-thiazolyl){[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl}methylene]amino}oxy)-2-methylpropionate.

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Where the label states that Aztreonam is sterile, it meets the requirements using *Fluid A*, to which 23.4 g of sterile arginine has been added to each 1000 mL.
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 2.0%; if labeled as the hydrated form: 12.0%–18.0%. [NOTE—The term "hydrated form" refers to the α-form of Aztreonam, which is not a stoichiometric hydrate.]
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Aztreonam is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.17 USP Endotoxin Units/mg of aztreonam.
- **LIMIT OF ALCOHOL**

[NOTE—This test is to be performed if alcohol is used while manufacturing Aztreonam.]
Standard solution: 0.004 mL/mL of alcohol from USP Alcohol Determination—Alcohol RS and 0.004 mL/mL of acetonitrile from USP Alcohol Determination—Acetonitrile RS in dimethylformamide. [NOTE—The *Standard solution* contains 0.4% alcohol and 0.4% acetonitrile.]
Sample solution: 80 mg/mL of Aztreonam and 0.004 mL/mL of acetonitrile in dimethylformamide. [NOTE—Dissolve Aztreonam in dimethylformamide using 20% of the final volume. Add a suitable aliquot of USP Alcohol Determination—Acetonitrile RS, and dilute with dimethylformamide to volume. The concentration of acetonitrile in the *Sample solution* is 0.4%.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m; phase G43

www.webofpharma.com

Film thickness: 3.0 µm
Temperatures
Injector: 210°
Detector: 280°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	5
50	10	200	4

Carrier gas: Helium
Linear velocity: 35 cm/s
Injection mode: Split
Injection volume: 0.5 µL
Injection type: Split ratio, 5:1
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for alcohol and acetonitrile are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 2.0 between alcohol and acetonitrile
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of alcohol in the portion of Aztreonam taken:

$$\text{Result} = (R_U/R_S) \times [C_S \times (D/C_U)] \times F \times 100$$

R_U = peak response ratio of alcohol to acetonitrile from the *Sample solution*
R_S = peak response ratio of alcohol to acetonitrile from the *Standard solution*
C_S = concentration of alcohol in the *Standard solution* (mL/mL)
D = density of alcohol (g/mL)
C_U = concentration of Aztreonam in the *Sample solution* (mg/mL)
F = unit conversion factor, 1000 mg/g

Acceptance criteria: NMT 4%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Where it is the hydrated form, the label so indicates.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Alcohol Determination—Acetonitrile RS
C_2H_3N 41.05
USP Alcohol Determination—Alcohol RS
C_2H_5OH 46.07
USP Aztreonam RS
USP Aztreonam E-Isomer RS
(*E*)-2-({[(2-Amino-4-thiazolyl){[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl}methylene]amino}oxy)-2-methylpropionic acid.
$C_{13}H_{17}N_5O_8S_2$ 435.43

Aztreonam Injection

DEFINITION

Aztreonam Injection is a sterile solution of Aztreonam and Arginine and a suitable osmolality-adjusting substance in Water for Injection. It contains NLT 90.0% and NMT 120.0% of the labeled amount of aztreonam ($C_{13}H_{17}N_5O_8S_2$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**
Buffer: 1.15 g/L of monobasic ammonium phosphate in water. Before final dilution, adjust with phosphoric acid to a pH of 2.0 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (75:25)
System suitability solution: 0.2 mg/mL each of USP Aztreonam RS and USP Open Ring Aztreonam RS in *Mobile phase*
Standard solution: 0.2 mg/mL each of USP Aztreonam RS and USP L-Arginine RS in *Mobile phase*
Sample solution: Nominally 0.2 mg/mL of aztreonam from Injection in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 206 nm
Column: 4-mm × 25-cm; 5- to 10-µm packing L20
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for aztreonam and open ring aztreonam are 0.8 and 1.0, respectively. The relative retention times for aztreonam and arginine are 0.3 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between aztreonam and open ring aztreonam
Tailing factor: NMT 2.0 for the aztreonam peak
Relative standard deviation: NMT 2.0% for the aztreonam peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of aztreonam ($C_{13}H_{17}N_5O_8S_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of aztreonam from the *Sample solution*
r_S = peak response of aztreonam from the *Standard solution*
C_S = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aztreonam in the *Sample solution* (mg/mL)
P = potency of aztreonam in USP Aztreonam RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.25 USP Endotoxin Unit/mg of aztreonam

www.webofpharma.com

- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **pH** ⟨791⟩: 4.5–7.5
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements for small-volume injections.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.* Maintain in the frozen state.
- **LABELING:** It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.* The label states that the Injection is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP L-Arginine RS
  USP Aztreonam RS
  USP Open Ring Aztreonam RS
  (2*S*,3*S*)-2-{(*Z*)-2-[2-Aminothiazol-4-yl]-2-[2-carboxypropan-2-yloxyimino]acetamido}-3-(sulfoamino)butanoic acid.
  $C_{13}H_{19}N_5O_9S_2$ 453.45

Aztreonam for Injection

DEFINITION

Aztreonam for Injection is a dry mixture of sterile Aztreonam and Arginine. It contains NLT 90.0% and NMT 105.0% of aztreonam ($C_{13}H_{17}N_5O_8S_2$), calculated on the anhydrous and arginine-free basis. Each container contains NLT 90.0% and NMT 120.0% of the labeled amount of aztreonam ($C_{13}H_{17}N_5O_8S_2$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer: 1.15 g/L of monobasic ammonium phosphate in water. Before final dilution, adjust with phosphoric acid to a pH of 2.0 ± 0.1.

Mobile phase: Acetonitrile and *Buffer* (75:25)

System suitability solution: 0.2 mg/mL each of USP Aztreonam RS and USP Open Ring Aztreonam RS in *Mobile phase*

Standard solution: 0.2 mg/mL each of USP Aztreonam RS and USP L-Arginine RS in *Mobile phase*

Sample solution 1: Nominally 0.2 mg/mL of aztreonam in *Mobile phase* from Aztreonam for Injection prepared as follows. Weigh one container of Aztreonam for Injection, transfer the contents to a suitable container, and dilute with *Mobile phase* to the appropriate volume. Weigh the empty container, and calculate the weight, in mg, of Aztreonam for Injection used.

Sample solution 2: Nominally 0.2 mg/mL of aztreonam from Aztreonam for Injection constituted as directed below and diluted with *Mobile phase.*

Where the vial has a capacity of less than 100 mL, constitute with water using the volume of solvent specified in the labeling.

Where the vial capacity is ≥100 mL, constitute with 10 mL of water and dilute the entire withdrawable contents of the container with *Mobile phase* to obtain the final concentration.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 206 nm
Column: 4-mm × 25-cm; 5- to 10-µm packing L20
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for aztreonam and open ring aztreonam are about 0.8 and 1.0, respectively. The relative retention times for aztreonam and arginine are 0.3 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between aztreonam and open ring aztreonam
Tailing factor: NMT 2.0 for the aztreonam peak
Relative standard deviation: NMT 2.0% for the aztreonam peak

Analysis
Samples: *Standard solution, Sample solution 1,* and *Sample solution 2*
Calculate the percentage of the labeled amount of aztreonam ($C_{13}H_{17}N_5O_8S_2$) in the portion of Aztreonam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response for aztreonam from *Sample solution 1*
r_S = peak response for aztreonam from the *Standard solution*
C_S = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Aztreonam for Injection in *Sample solution 1* (mg/mL), corrected for water and arginine content (see *Content of Arginine*)
P = potency of aztreonam in USP Aztreonam RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–105.0% on the anhydrous and arginine-free basis

Calculate the percentage of the labeled amount of aztreonam ($C_{13}H_{17}N_5O_8S_2$) in each container of Aztreonam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response for aztreonam from *Sample solution 2*
r_S = peak response for aztreonam from the *Standard solution*
C_S = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aztreonam in *Sample solution 2* (mg/mL)
P = potency of aztreonam in USP Aztreonam RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

www.webofpharma.com

OTHER COMPONENTS

• **CONTENT OF ARGININE**

Use the result of this test to calculate, on the anhydrous and arginine-free basis, the *Assay* result from *Sample solution 1*, obtained as directed in the *Assay*.

Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution 1, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution 1*

Calculate the percentage of arginine ($C_6H_{14}N_4O_2$) in the portion of Aztreonam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for arginine from *Sample solution 1*

r_S = peak response for arginine from the *Standard solution*

C_S = concentration of USP L-Arginine RS in the *Standard solution* (mg/mL)

C_U = concentration of Aztreonam for Injection in *Sample solution 1* (mg/mL)

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

SPECIFIC TESTS

• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.17 USP Endotoxin Unit/mg of aztreonam.

• **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*

• **pH** ⟨791⟩

Sample solution: 100 mg/mL of aztreonam

Acceptance criteria: 4.5–7.5

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.0%

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** It meets the requirements for small-volume injections.

• **OTHER REQUIREMENTS:** It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*

• **USP REFERENCE STANDARDS** ⟨11⟩

USP L-Arginine RS

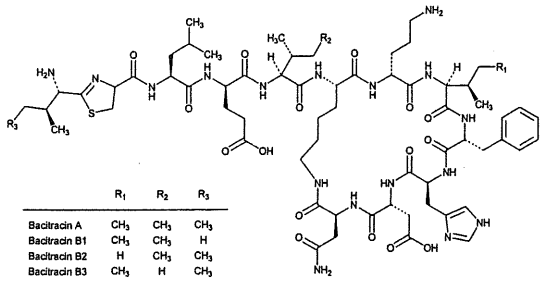
USP Aztreonam RS

USP Open Ring Aztreonam RS

(2*S*,3*S*)-2-{(*Z*)-2-[2-Aminothiazol-4-yl]-2-[2-carboxypropan-2-yloxyimino]acetamido}-3-(sulfoamino)butanoic acid.

$C_{13}H_{19}N_5O_9S_2$ 453.45

Bacitracin



	R_1	R_2	R_3
Bacitracin A	CH_3	CH_3	CH_3
Bacitracin B1	CH_3	CH_3	H
Bacitracin B2	H	CH_3	CH_3
Bacitracin B3	CH_3	H	CH_3

Bacitracin [1405-87-4].

DEFINITION

Bacitracin is a mixture of polypeptides produced by the growth of an organism of the *licheniformis* group of *Bacillus subtilis* (Fam. Bacillacaea), the main components being bacitracins A, B1, B2, and B3. It has a potency of NLT 65 Bacitracin units/mg, calculated on the dried basis.

IDENTIFICATION

• **A.** Meets the requirements of the test for *Composition of Bacitracin*

Change to read:

• **B.**

Sample: 0.2 g

Analysis: Ignite the *Sample*. Allow to cool. Dissolve the residue in 0.1 mL of dilute hydrochloric acid. Add 5 mL of water and 0.2 mL of ▲sodium hydroxide TS 3.▲ 2S (USP41)

Acceptance criteria: No white precipitate is formed.

ASSAY

• **PROCEDURE**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Analysis: Proceed as directed in the chapter.

Acceptance criteria: NLT 65 Bacitracin units/mg on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.5%

SPECIFIC TESTS

• **COMPOSITION OF BACITRACIN**

Diluent: 40 g/L of edetate disodium in water adjusted with 8 N sodium hydroxide to a pH of 7.0

Solution A: 34.8 g/L of dibasic potassium phosphate in water

Solution B: 27.2 g/L of monobasic potassium phosphate in water

Solution C: *Solution A* and *Solution B* (2:9). The pH of the mixture is about 6.

Solution D: 0.1 mM edetate disodium in a mixture of *Solution C* and water (1:3)

Solution E: Methanol and acetonitrile (27:2)

Mobile phase: *Solution D* and *Solution E* (37:63)

System suitability solution: 2 mg/mL of USP Bacitracin Zinc RS in *Diluent*

Reporting threshold solution: 0.01 mg/mL of USP Bacitracin Zinc RS from *System suitability solution* in water

Peak identification solution: 2 mg/mL of USP Bacitracin Zinc RS in *Diluent*. Heat in a boiling water bath for 30 min, and cool to room temperature.

Sample solution: 2 mg/mL of Bacitracin in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

www.webofpharma.com

Detector: UV 254 and 300 nm. Quantitative analysis is performed at 254 nm; 300 nm is only used to identify the location of bacitracin F.
Column: 4.6-mm × 25-cm; end-capped 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL

System suitability
Samples: *System suitability solution* and *Peak identification solution*
Analyze the *Peak identification solution* at 300 nm. Identify bacitracin F, a known impurity, using the relative retention time provided in *Table 1*. Analyze the *System suitability solution* at 254 nm. Identify the peaks of the most active components of bacitracin (bacitracins A, B1, B2, and B3), early-eluting peptides (those eluting before the bacitracin B1 peak), and the impurity (bacitracin F) using the relative retention times in *Table 1*.

Table 1

Name	Nature of Component	Relative Retention Time
Bacitracin C1	Early-eluting peptides	0.5
Bacitracin C2		0.6
Bacitracin C3		0.6
Bacitracin B1	Active bacitracin	0.7
Bacitracin B2		0.7
Bacitracin B3		0.8
Bacitracin A		1.0
Bacitracin F	Impurity	2.4

Suitability requirements
Peak-to-valley ratio: NLT 1.2, *System suitability solution*
The *Peak-to-valley ratio* is calculated as follows:

$$\text{Result} = H_P/H_V$$

H_P = height above the baseline of the peak due to bacitracin B1
H_V = height above the baseline of the lowest point of the curve separating the bacitracin B1 peak from the bacitracin B2 peak

Analysis
Samples: *Diluent, Reporting threshold solution,* and *Sample solution*

Content of bacitracin A
Calculate the percentage of bacitracin A in the portion of Bacitracin taken:

$$\text{Result} = (r_A/r_T) \times 100$$

r_A = peak area of bacitracin A from the *Sample solution*
r_T = sum of all peak areas above the reporting threshold from the *Sample solution*

Content of active bacitracin
Calculate the percentage of active bacitracin (bacitracin A, B1, B2, and B3) in the portion of Bacitracin taken:

$$\text{Result} = [(r_A + r_{B1} + r_{B2} + r_{B3})/r_T] \times 100$$

r_A = peak area of bacitracin A from the *Sample solution*
r_{B1} = peak area of bacitracin B1 from the *Sample solution*
r_{B2} = peak area of bacitracin B2 from the *Sample solution*
r_{B3} = peak area of bacitracin B3 from the *Sample solution*
r_T = sum of all peak areas above the reporting threshold from the *Sample solution*

Limit of early-eluting peptides
Calculate the percentage of early-eluting peptides (peaks eluting before bacitracin B1) in the portion of Bacitracin taken:

$$\text{Result} = (r_P/r_T) \times 100$$

r_P = sum of peak areas for all peaks before bacitracin B1 from the *Sample solution*
r_T = sum of all peak areas above the reporting threshold from the *Sample solution*

Limit of bacitracin F
Calculate the percentage of bacitracin F in the portion of Bacitracin taken:

$$\text{Result} = (r_F/r_A) \times 100$$

r_F = peak area of bacitracin F from the *Sample solution*
r_A = peak area of bacitracin A from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peaks from the *Sample solution* that are observed in the *Diluent* chromatogram. Disregard any peaks from the *Sample solution* that have a peak area less than bacitracin A in the *Reporting threshold solution*.

Table 2

Name	Acceptance Criteria, (%)
Content of bacitracin A	NLT 40.0
Content of active bacitracin	NLT 70.0
Limit of early-eluting peptides	NMT 20.0
Limit of bacitracin F	NMT 6.0

- **pH** ⟨791⟩
Sample solution: 10,000 Bacitracin units/mL in water
Acceptance criteria: 5.5–7.5
- **Loss on Drying** ⟨731⟩
Sample: 100 mg
Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.
Acceptance criteria: NMT 5.0%
- **Sterility Tests** ⟨71⟩**:** Where the label states that the Bacitracin is sterile, it meets the requirements.

Change to read:

- **Bacterial Endotoxins Test** ⟨85⟩**:** ▲The level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Bacitracin is used can be met. Where the label states that Bacitracin must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Bacitracin is used can be met.▲ 2S (USP41)

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store below 8°.

Change to read:

- **LABELING:** Where it is packaged for prescription compounding, label it to indicate that it is not sterile and that the potency cannot be assured for longer than 60 days after opening, and to state the number of Bacitracin Units/mg. ▲Where Bacitracin must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled.▲ 2S (USP41)

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bacitracin Zinc RS
  ▲▲ (CN 1-May-2018)

Bacitracin for Injection

DEFINITION

Bacitracin for Injection has a potency of NLT 50 Bacitracin Units/mg. It contains NLT 90.0% and NMT 115.0% of the labeled amount of bacitracin.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201BNP⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution 1: Nominally 100 Bacitracin Units/mL, prepared as follows. Constitute one container of Bacitracin for Injection as directed in the labeling. Using a suitable hypodermic needle and syringe, withdraw the contents of the container, and dilute with *Buffer B.1* (see the chapter) to a suitable volume.

Sample solution 2 (where the label states the number of Bacitracin Units in a given volume of constituted solution): Nominally 100 Bacitracin Units/mL, prepared as follows. Constitute one container of Bacitracin for Injection as directed in the labeling. Dilute a suitable aliquot of the constituted solution with *Buffer B.1* (see the chapter) to a suitable final volume.

Analysis

Samples: *Sample solution 1* or *Sample solution 2*

Proceed as directed in the chapter. Add sufficient 0.01 N hydrochloric acid to the *Sample solution* so that the amount of hydrochloric acid in the *Test Dilution* is the same as in the median level of the standard. Dilute with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩

Analysis: Moisten the charred residue with 2 mL of nitric acid and 5 drops of sulfuric acid.

Acceptance criteria: NMT 3.0%

SPECIFIC TESTS

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
- **pH** ⟨791⟩

Sample solution: A solution containing 10,000 Bacitracin Units/mL

Acceptance criteria: 5.5–7.5

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry 100 mg in a capillary-stoppered bottle under vacuum at a pressure of NMT 5 mm of mercury at 60° for 3 h.

Acceptance criteria: NMT 5.0%

- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.01 USP Endotoxin Unit/Bacitracin Unit.
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, and store in a cool place.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bacitracin Zinc RS

Bacitracin Ointment

DEFINITION

Bacitracin Ointment is Bacitracin in an anhydrous ointment base. It contains NLT 90.0% and NMT 140.0% of the labeled amount of bacitracin. It may contain a suitable anesthetic.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201BNP⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution: Use a portion of Ointment shaken with about 50 mL of ether in a separator and extracted with four 20-mL portions of *Buffer B.1* (see the chapter). Combine the buffer extracts, and dilute with *Buffer B.1* to a suitable volume.

Analysis: Proceed as directed in the chapter. Add sufficient 0.01 N hydrochloric acid to a suitable aliquot of the *Sample solution* so that the amount of hydrochloric acid in the *Test Dilution* is the same as in the median level of the standard. Dilute with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–140.0%

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩

Analysis: Use 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.

Acceptance criteria: NMT 0.5%

- **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers containing NMT 60 g, unless labeled solely for hospital use, preferably at controlled room temperature.

www.webofpharma.com

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bacitracin Zinc RS

Bacitracin Ophthalmic Ointment

DEFINITION
Bacitracin Ophthalmic Ointment is a sterile preparation of Bacitracin in an anhydrous ointment base. It contains NLT 90.0% and NMT 140.0% of the labeled amount of bacitracin.

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201BNP⟩**:** Meets the requirements

ASSAY
- **PROCEDURE**
  (See *Antibiotics—Microbial Assays* ⟨81⟩.)
  Sample solution: Use a portion of Ophthalmic Ointment shaken with about 50 mL of ether in a separator and extracted with four 20-mL portions of *Buffer B.1* (see the chapter). Combine the buffer extracts, and dilute with *Buffer B.1* to a suitable volume.
  Analysis: Proceed as directed in the chapter. Add sufficient 0.01 N hydrochloric acid to a suitable aliquot of the *Sample solution* so that the amount of hydrochloric acid in the *Test Dilution* is the same as in the median level of the standard. Dilute with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.
  Acceptance criteria: 90.0%–140.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter.*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bacitracin Zinc RS

Soluble Bacitracin Methylenedisalicylate

DEFINITION
Soluble Bacitracin Methylenedisalicylate is a mixture of Bacitracin Methylenedisalicylate and Sodium Bicarbonate. It has a potency of NLT 8 Bacitracin Units/mg, calculated on the dried basis.

ASSAY
- **ANTIBIOTICS—MICROBIAL ASSAYS** ⟨81⟩
  Diluent: 20 g/L of sodium bicarbonate
  Sample stock solution: Transfer a suitable amount of Soluble Bacitracin Methylenedisalicylate to a high-speed glass blender jar, add 99.0 mL of *Diluent* and 1.0 mL of polysorbate 80, and blend for 3 min.
  Test dilution: To a suitable aliquot of the *Sample stock solution*, add a suitable volume of 0.01 N hydrochloric acid, and dilute with *Buffer B.1* to obtain a concentration of bacitracin assumed to be equal to the median dose level of the Standard. [NOTE—The amount of hydrochloric acid in the *Test dilution* should be the same as that in the median dose level of the Standard.]
  Analysis: Proceed as directed for Bacitracin in *Antibiotics—Microbial Assays* ⟨81⟩.
  Acceptance criteria: NLT 8 Bacitracin Units/mg on the dried basis

SPECIFIC TESTS
- **PH** ⟨791⟩**:** 8.0–9.5, in a 25 mg/mL solution
- **LOSS ON DRYING** ⟨731⟩**:** Dry 100 mg in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 8.5% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bacitracin Zinc RS

Bacitracin Methylenedisalicylate Soluble Powder

DEFINITION
Bacitracin Methylenedisalicylate Soluble Powder contains NLT 90.0% and NMT 120.0% of the labeled amount of bacitracin.

ASSAY
- **ANTIBIOTICS—MICROBIAL ASSAYS** ⟨81⟩
  Diluent: 20 g/L of sodium bicarbonate
  Sample stock solution: Transfer a suitable amount of Bacitracin Methylenedisalicylate Soluble Powder to a high-speed glass blender jar, add 99.0 mL of *Diluent* and 1.0 mL of polysorbate 80, and blend for 3 min.
  Test dilution: To a suitable aliquot of the *Sample stock solution*, add a suitable volume of 0.01 N hydrochloric acid and dilute with *Buffer B.1* to obtain a concentration of bacitracin assumed to be equal to the median dose level of the Standard. [NOTE—The amount of hydrochloric acid in the *Test dilution* should be the same as that in the median dose level of the Standard.]
  Analysis: Proceed as directed for Bacitracin in *Antibiotics—Microbial Assays* ⟨81⟩.
  Acceptance criteria: 90.0%—120.0%

SPECIFIC TESTS
- **PH** ⟨791⟩**:** 8.0–9.5 in a 50 mg/mL solution
- **LOSS ON DRYING** ⟨731⟩**:** Dry 100 mg in a capillary-stoppered bottle in a vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 8.5% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is for veterinary use only. Label it to state the content of bacitracin in terms of grams per pound, each gram of bacitracin being equivalent to 42,000 Bacitracin Units.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bacitracin Zinc RS

www.webofpharma.com

Bacitracin and Polymyxin B Sulfate Topical Aerosol

DEFINITION

Bacitracin and Polymyxin B Sulfate Topical Aerosol is a suspension of Bacitracin and Polymyxin B Sulfate in a suitable vehicle, packaged in a pressurized container with a suitable inert propellant. It contains NLT 90.0% and NMT 130.0% of the labeled amounts of bacitracin and polymyxin B. It may contain a suitable local anesthetic.

Prepare the specimen for the following tests and assays as follows. Maintain the container in the inverted position throughout this procedure. Store the container in a freezer at –70° for 16–24 h. Remove the container from the freezer, promptly puncture the container, and allow the propellant to volatilize. Open the container, and mix the contents.

IDENTIFICATION

- **A. Thin-Layer Chromatographic Identification Test** ⟨201BNP⟩

Sample: Prepare as directed above.

Analysis: Test as directed in the section *For Creams, Lotions, and Ointments* in the chapter.

Acceptance criteria: Meets the requirements

ASSAY

- **Bacitracin**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution: Use a portion of the contents of one container, containing nominally 500 USP Bacitracin Units, prepared as directed above. Transfer to a suitable separator containing 50 mL of ether, and extract with three 25-mL portions of *Buffer B.1* (see the chapter). Combine the buffer extracts in a 100-mL volumetric flask, dilute with *Buffer B.1* to volume, and mix.

Analysis: Proceed as directed in the chapter. Add sufficient 0.01 N hydrochloric acid to this solution so that the amount of hydrochloric acid in the *Test Dilution* is the same as in the median level of the standard. Dilute with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–130.0%

- **Polymyxin B**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution: Use a portion of the contents of one container, containing nominally 5000 USP Polymyxin B Units, prepared as directed above. Transfer to a suitable separator containing 50 mL of ether, and extract with three 25-mL portions of *Buffer B.6* (see the chapter). Combine the buffer extracts in a 100-mL volumetric flask, dilute with *Buffer B.6* to volume, and mix.

Analysis: Proceed as directed in the chapter. Dilute a suitable aliquot of the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a polymyxin B concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–130.0%

SPECIFIC TESTS

- **Water Determination,** *Method I* ⟨921⟩

Analysis: Use a portion of the contents of one container, prepared as directed above, and 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.

Acceptance criteria: NMT 0.5%

- **Other Requirements:** It meets the requirements for *Topical Aerosols* ⟨603⟩, in the sections *Pressure Test, Minimum Fill,* and *Leakage Test.*

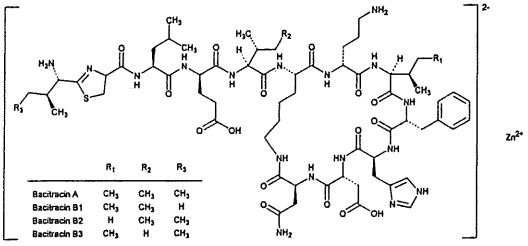
ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in pressurized containers, and avoid exposure to excessive heat.
- **USP Reference Standards** ⟨11⟩

USP Bacitracin Zinc RS

USP Polymyxin B Sulfate RS

Bacitracin Zinc



Bacitracins, zinc complex;
Bacitracin zinc complex [1405-89-6].

DEFINITION

Bacitracin Zinc is the zinc complex of bacitracin, which consists of a mixture of antimicrobial polypeptides, the main components being bacitracins A, B1, B2, and B3. It has a potency of NLT 65 Bacitracin Units/mg, calculated on the dried basis. It contains NLT 4.0% and NMT 6.0% of zinc (Zn), calculated on the dried basis.

IDENTIFICATION

- **A.** Meets the requirements of the test for *Composition of Bacitracin*
- **B.** Meets the requirements of the test for *Zinc Content*

ASSAY

- **Procedure**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Analysis: Proceed as directed in the chapter.

Acceptance criteria: NLT 65 Bacitracin Units/mg on the dried basis

SPECIFIC TESTS

- **Composition of Bacitracin**

Diluent: 40 g/L of edetate disodium in water adjusted with 8 N sodium hydroxide to a pH of 7.0

Solution A: 34.8 g/L of dibasic potassium phosphate in water

Solution B: 27.2 g/L of monobasic potassium phosphate in water

Solution C: *Solution B* and *Solution A* (9:2). The pH of the mixture is about 6.

Solution D: 0.1 mM edetate disodium in a mixture of *Solution C* and water (1:3)

Solution E: Methanol and acetonitrile (27:2)

Mobile phase: *Solution E* and *Solution D* (63:37)

System suitability solution: 2 mg/mL of USP Bacitracin Zinc RS in *Diluent*

Reporting threshold solution: 0.01 mg/mL of USP Bacitracin Zinc RS from *System suitability solution* in water

Peak identification solution: 2 mg/mL of USP Bacitracin Zinc RS in *Diluent.* Heat in a boiling water bath for 30 min, and cool to room temperature.

Sample solution: 2 mg/mL of Bacitracin Zinc in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

www.webofpharma.com

Detector: UV 254 nm and 300 nm. Quantitative analysis is performed at 254 nm; 300 nm is only used to identify the location of bacitracin F.
Column: 4.6-mm × 25-cm; end-capped 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
Run time: NLT 3 times the retention time of bacitracin A

System suitability
Samples: *System suitability solution* and *Peak identification solution*
Analyze the *Peak identification solution* at 300 nm. Identify bacitracin F, a known impurity, using the relative retention time provided in *Table 1*. Analyze the *System suitability solution* at 254 nm. Identify the peaks of the most active components of bacitracin (bacitracins A, B1, B2, and B3), early eluting peptides (those eluting before the peak due to bacitracin B1), and the impurity (bacitracin F) using the relative retention time values in *Table 1*.

Table 1

Name	Nature of Component	Relative Retention Time
Bacitracin C1	Early eluting peptides	0.5
Bacitracin C2		0.6
Bacitracin C3		0.6
Bacitracin B1	Active bacitracin	0.7
Bacitracin B2		0.7
Bacitracin B3		0.8
Bacitracin A		1.0
Bacitracin F	Impurity	2.4

Suitability requirements
Peak-to-valley ratio: NLT 1.2
The *Peak-to-valley ratio* is calculated as follows:

$$\text{Result} = H_P/H_V$$

H_P = height above the baseline of the peak due to bacitracin B1
H_V = height above the baseline of the lowest point of the curve separating the bacitracin B1 peak from the bacitracin B2 peak

Analysis
Samples: *Diluent, Reporting threshold solution,* and *Sample solution*
Quantitative analysis is performed at 254 nm.

Content of bacitracin A
Calculate the percentage of bacitracin A in the portion of Bacitracin Zinc taken:

$$\text{Result} = (r_A/r_T) \times 100$$

r_A = peak area of bacitracin A from the *Sample solution*
r_T = sum of all peak areas above the reporting threshold from the *Sample solution*

Content of active bacitracin
Calculate the percentage of active bacitracin (bacitracin A, B1, B2, and B3) in the portion of Bacitracin Zinc taken:

$$\text{Result} = [(r_A + r_{B1} + r_{B2} + r_{B3})/r_T] \times 100$$

r_A = peak area of bacitracin A from the *Sample solution*
r_{B1} = peak area of bacitracin B1 from the *Sample solution*
r_{B2} = peak area of bacitracin B2 from the *Sample solution*
r_{B3} = peak area of bacitracin B3 from the *Sample solution*
r_T = sum of all peak areas above the reporting threshold from the *Sample solution*

Limit of early eluting peptides
Calculate the percentage of early eluting peptides (peaks eluting before bacitracin B1) in the portion of Bacitracin Zinc taken:

$$\text{Result} = (r_P/r_T) \times 100$$

r_P = sum of peak areas for all peaks before bacitracin B1 from the *Sample solution*
r_T = sum of all peak areas above the reporting threshold from the *Sample solution*

Limit of bacitracin F
Calculate the percentage of bacitracin F in the portion of Bacitracin Zinc taken:

$$\text{Result} = (r_F/r_A) \times 100$$

r_F = peak area of bacitracin F from the *Sample solution*
r_A = peak area of bacitracin A from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peaks from the *Sample solution* that are observed in the *Diluent* chromatogram. Disregard any peaks from the *Sample solution* having a peak area less than bacitracin A in the *Reporting threshold solution*.

Table 2

	Acceptance Criteria (%)
Content of bacitracin A	NLT 40.0
Content of active bacitracin	NLT 70.0
Limit of early eluting peptides	NMT 20.0
Limit of bacitracin F	NMT 6.0

• **ZINC CONTENT**
[NOTE—The *Standard solutions* and the *Sample solution* may be quantitatively diluted with 1 mM hydrochloric acid, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]
Standard stock solution: 10 mg/mL of zinc from zinc oxide in 1 N hydrochloric acid. Prepare as follows. Transfer a suitable amount of zinc oxide to a suitable volumetric flask, add 1 N hydrochloric acid using 32% of the final volume, warm to dissolve, cool and dilute with water to volume.
Standard solutions: 0.5, 1.5, and 2.5 µg/mL of zinc from *Standard stock solution* in 0.001 N hydrochloric acid
Sample stock solution: 2 mg/mL of Bacitracin Zinc in 0.01 N hydrochloric acid
Sample solution: 0.02 mg/mL of Bacitracin Zinc from *Sample stock solution* in 0.001 N hydrochloric acid
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 213.8 nm
Lamp: Zinc hollow-cathode
Flame: Air–acetylene

USP Monographs

www.webofpharma.com

Blank: 0.001 N hydrochloric acid
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of zinc, and draw the straight line best fitting the three plotted points. From the graph, determine the concentration, in µg/mL, of zinc in the *Sample solution*.
Calculate the percentage of zinc in the portion of Bacitracin Zinc taken:

$$\text{Result} = C \times D \times (V/W) \times F \times 100$$

C = concentration of zinc in the *Sample solution* obtained from the curve (µg/mL)
D = dilution factor for the *Sample solution*, 100 mL/mL
V = volume of *Sample stock solution* (mL)
W = weight of Bacitracin Zinc used to prepare the *Sample stock solution* (mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 4.0%–6.0% on the dried basis
• **pH** ⟨791⟩
Sample solution: A saturated solution in water containing about 100 mg/mL
Acceptance criteria: 6.0–7.5
• **Loss on Drying** ⟨731⟩
Sample: 100 mg
Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.
Acceptance criteria: NMT 5.0%
• **Sterility Tests** ⟨71⟩**:** Where the label states that it is sterile, it meets the requirements of the chapter. If the membrane filtration test is used, add 20 g of edetate disodium to each L of *Fluid A*.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers, and store below 25°.
• **Labeling:** Label it to indicate that it is to be used in the manufacture of nonparenteral drugs only. Where it is packaged for prescription compounding, label it to indicate that it is not sterile and that the potency cannot be assured for longer than 60 days after opening, and to state the number of Bacitracin Units/mg. Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.
• **USP Reference Standards** ⟨11⟩
USP Bacitracin Zinc RS

Bacitracin Zinc Ointment

DEFINITION
Bacitracin Zinc Ointment is Bacitracin Zinc in an anhydrous ointment base. It contains NLT 90.0% and NMT 140.0% of the labeled amount of bacitracin.

IDENTIFICATION
• **A. Thin-Layer Chromatographic Identification Test** ⟨201BNP⟩**:** Meets the requirements

ASSAY
• **Procedure**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Standard solution: Proceed as directed in the chapter. To each *Test Dilution* of the standard add sufficient hydrochloric acid to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of Ointment.
Sample solution: Use a portion of Ointment shaken with about 50 mL of ether in a separator and extracted with four 20-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard. Add sufficient hydrochloric acid to each *Test Dilution* of the standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of the sample.
Acceptance criteria: 90.0%–140.0%

SPECIFIC TESTS
• **Water Determination,** *Method I* ⟨921⟩
Analysis: Use 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.
Acceptance criteria: NMT 0.5%
• **Minimum Fill** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers containing NMT 60 g, unless labeled solely for hospital use, preferably at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Bacitracin Zinc RS

Bacitracin Zinc Soluble Powder

» Bacitracin Zinc Soluble Powder is a mixture of Bacitracin Zinc and zinc proteinates. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of bacitracin.

Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate that it is for veterinary use only. Label it to state the content of bacitracin in terms of grams per pound, each gram of bacitracin being equivalent to 42,000 Bacitracin Units.
USP Reference standards ⟨11⟩—
USP Bacitracin Zinc RS
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.
Zinc content—Using Powder, proceed as directed for *Zinc Content* under *Bacitracin Zinc*. Calculate the zinc content, in g, in relation to each 42,000 Bacitracin Units in the specimen taken by the formula:

$$280{,}000C/WA$$

in which A is the bacitracin content of the specimen, in Bacitracin Units per g, and the other terms are as defined therein: it contains not more than 2.0 g for each 42,000 Bacitracin Units.
Assay—Dissolve an accurately weighed quantity of Powder quantitatively in 0.01 N hydrochloric acid to obtain a stock solution containing about 100 Bacitracin Units per mL. Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this stock solution diluted quantitatively and stepwise with *Buffer B.1* to obtain a *Test Dilution* having a concentration assumed to be equal to

www.webofpharma.com

the median dose level of the Standard. In preparing each test dilution of the Standard, add additional hydrochloric acid to each to obtain the same concentration of hydrochloric acid as in the *Test Dilution*.

Bacitracin Zinc and Polymyxin B Sulfate Ointment

DEFINITION
Bacitracin Zinc and Polymyxin B Sulfate Ointment contains the equivalent of NLT 90.0% and NMT 130.0% of the labeled amounts of bacitracin and polymyxin B. It may contain a suitable local anesthetic.

IDENTIFICATION
- **A. Thin-Layer Chromatographic Identification Test** ⟨201BNP⟩: Meets the requirements

ASSAY
- **Bacitracin**

(See *Antibiotics—Microbial Assays* ⟨81⟩).

Standard solution: Proceed as directed in the chapter. To each *Test Dilution* of the standard add sufficient hydrochloric acid to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of Ointment.

Sample solution: Shake a portion of Ointment with about 50 mL of ether in a separator, and extract with four 20-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard. Add sufficient hydrochloric acid to each *Test Dilution* of the standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of the sample.

Acceptance criteria: 90.0%–130.0%

- **Polymyxin B**

(See *Antibiotics—Microbial Assays* ⟨81⟩).

Sample solution: Shake a portion of Ointment with about 50 mL of ether in a separator, and extract with four 20-mL portions of *Buffer B.6* (see the chapter). Combine the buffer extracts, and dilute with *Buffer B.6* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a polymyxin B concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–130.0%

SPECIFIC TESTS
- **Water Determination,** *Method I* ⟨921⟩

Analysis: Use 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.

Acceptance criteria: NMT 0.5%

- **Minimum Fill** ⟨755⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
- **USP Reference Standards** ⟨11⟩

USP Bacitracin Zinc RS
USP Polymyxin B Sulfate RS

Bacitracin Zinc and Polymyxin B Sulfate Ophthalmic Ointment

DEFINITION
Bacitracin Zinc and Polymyxin B Sulfate Ophthalmic Ointment contains the equivalent of NLT 90.0% and NMT 130.0% of the labeled amounts of bacitracin and polymyxin B.

IDENTIFICATION
- **A. Thin-Layer Chromatographic Identification Test** ⟨201BNP⟩: Meets the requirements

ASSAY
- **Bacitracin**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Standard solution: Proceed as directed in the chapter. To each *Test Dilution* of the standard add sufficient hydrochloric acid to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of Ophthalmic Ointment.

Sample solution: Use a portion of Ophthalmic Ointment shaken with about 50 mL of ether in a separator and extracted with four 20-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–130.0%

- **Polymyxin B**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution: Shake a portion of Ophthalmic Ointment with about 50 mL of ether in a separator, and extract with four 20-mL portions of *Buffer B.6* (see the chapter). Combine the buffer extracts, and dilute with *Buffer B.6* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a polymyxin B concentration that is nominally equivalent to the median level of the standard.

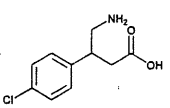
Acceptance criteria: 90.0%–130.0%

SPECIFIC TESTS
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **Other Requirements:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩

USP Bacitracin Zinc RS
USP Polymyxin B Sulfate RS

Baclofen



$C_{10}H_{12}ClNO_2$ 213.66

USP Monographs

www.webofpharma.com

Butanoic acid, 4-amino-3-(4-chlorophenyl)-;
β-(Aminomethyl)-*p*-chlorohydrocinnamic acid [1134-47-0].

DEFINITION

Baclofen contains NLT 98.0% and NMT 102.0% of baclofen ($C_{10}H_{12}ClNO_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 1.38 g of potassium dihydrogen phosphate and 1.74 g of sodium-1-pentanesulfonate in 1 L of water. Adjust with dilute phosphoric acid to a pH of 3.0.
Solution B: Acetonitrile and methanol (1:1)
Diluent: *Solution A* and *Solution B* (65:35)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	65	35
5	65	35
15	45	55
25	45	55
27	65	35
30	65	35

Standard solution: 0.2 mg/mL of USP Baclofen RS in *Diluent*
Sample solution: 0.2 mg/mL of Baclofen in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25.0-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of baclofen ($C_{10}H_{12}ClNO_2$) in the portion of the Baclofen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Baclofen RS in the *Standard solution* (mg/mL)
C_U = concentration of Baclofen in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.3%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Diluent, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.0015 mg/mL of USP Baclofen RS and 0.003 mg/mL of USP Baclofen Related Compound A RS in *Diluent*
Sample solution: 0.3 mg/mL of Baclofen in *Diluent*
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Tailing factor: NMT 1.5 for baclofen
Relative standard deviation: NMT 5.0% for both baclofen and baclofen related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of baclofen related compound A in the portion of the Baclofen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of baclofen related compound A from the *Sample solution*
r_S = peak response of baclofen related compound A from the *Standard solution*
C_S = concentration of USP Baclofen Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Baclofen in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of the Baclofen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of baclofen from the *Standard solution*
C_S = concentration of USP Baclofen RS in the *Standard solution* (mg/mL)
C_U = concentration of Baclofen in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Baclofen	1.0	—
Baclofen related compound A	2.3	1.0
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 3.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Baclofen RS

USP Monographs

www.webofpharma.com

USP Baclofen Related Compound A RS
4-(4-Chlorophenyl)-2-pyrrolidinone.
$C_{10}H_{10}ClNO$ 195.65

Baclofen Compounded Oral Suspension

DEFINITION

Baclofen Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of baclofen ($C_{10}H_{12}ClNO_2$).

Prepare Baclofen Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Baclofen	500 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

If using *Baclofen Tablets*, place the Tablets in a suitable mortar and comminute to a fine powder, or add *Baclofen* powder. Add 5 mL of the *Vehicle* to wet the powder, and triturate the powder to form a fine paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer, stepwise and quantitatively, the contents of the mortar to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and 0.05 M monobasic sodium phosphate (20:80). Adjust with phosphoric acid to a pH of 3.5.

Standard solution: 5 µg/mL of USP Baclofen RS in water

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 0.5 mL of Oral Suspension from each bottle to a 500-mL volumetric flask, dilute with water to volume to obtain a concentration of 5 µg/mL, and pass through a 0.22-µm polyvinylidene fluoride (PVDF) filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time of baclofen is about 5.5 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of baclofen ($C_{10}H_{12}ClNO_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Baclofen RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of baclofen in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 4.2–5.2

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator.
- **BEYOND-USE DATE:** NMT 35 days after the day on which it was compounded when stored in a refrigerator
- **LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Baclofen RS

Baclofen Tablets

DEFINITION

Baclofen Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of baclofen ($C_{10}H_{12}ClNO_2$).

IDENTIFICATION

- **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 1.4 g/L of monobasic sodium phosphate and 1.7 g/L of sodium 1-pentanesulfonate in water. Adjust with 1.5 M phosphoric acid TS to a pH of 3.0.

Solution B: Acetonitrile and methanol (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	65	35
5	65	35
15	45	55
25	45	55
27	65	35
35	65	35

Diluent: *Solution A* and *Solution B* (65:35)

Standard solution: 200 µg/mL of USP Baclofen RS in *Diluent*

Sample solution: Nominally 200 µg/mL of baclofen prepared as follows. Finely powder NLT 20 Tablets and transfer a portion of the powder to an appropriate volumetric flask. Add *Diluent* to about 80% of the flask volume, sonicate for 10 min, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume. Centrifuge a portion of this solution and use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 225 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 0.8 mL/min

Injection volume: 10 µL

www.webofpharma.com

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of baclofen ($C_{10}H_{12}ClNO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Baclofen RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of baclofen in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms, Procedure for a pooled sample for immediate-release dosage forms*
Medium: 0.01 N hydrochloric acid; 500 mL for Tablets containing NMT 10 mg of baclofen; 1000 mL for Tablets containing more than 10 mg of baclofen
Apparatus 2: 50 rpm
Time: 30 min
Solution A: 62.7 g/L of sodium 1-pentanesulfonate in water
Mobile phase: Methanol, 0.3 N acetic acid, and *Solution A* (44:55:2)
Standard solution: USP Baclofen RS in *Medium*
Sample solution: Use a portion of the solution under test.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 0.6 mL/min
Injection volume: 190 µL
Run time: NLT 2 times the retention time of the baclofen peak
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of baclofen ($C_{10}H_{12}ClNO_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Baclofen RS in the *Standard solution* (mg/mL)
V = volume of *Medium*; 500 or 1000 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of baclofen ($C_{10}H_{12}ClNO_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A, Solution B, Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.4 µg/mL of USP Baclofen RS and 8 µg/mL of USP Baclofen Related Compound A RS in *Diluent*
System suitability
Sample: *Standard solution*
[Note—See *Table 2* for the relative retention times.]
Suitability requirements
Tailing factor: NMT 1.5 for baclofen
Relative standard deviation: NMT 5.0% each for baclofen and baclofen related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of baclofen related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of baclofen related compound A from the *Sample solution*
r_S = peak response of baclofen related compound A from the *Standard solution*
C_S = concentration of USP Baclofen Related Compound A RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of baclofen in the *Sample solution* (µg/mL)

Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*
r_S = peak response of baclofen from the *Standard solution*
C_S = concentration of USP Baclofen RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of baclofen in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Baclofen	1.0	—
Baclofen related compound A	3.0	4.0
Any individual degradation product	—	0.2
Total degradation products	—	1.0[a]

[a] Baclofen related compound A is not included in the total degradation products.

ADDITIONAL REQUIREMENTS

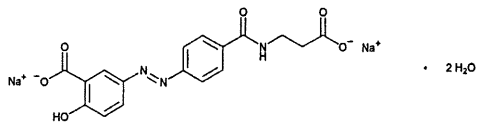
• **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Baclofen RS

USP Monographs

www.webofpharma.com

USP Baclofen Related Compound A RS
4-(4-Chlorophenyl)-2-pyrrolidinone.
$C_{10}H_{10}ClNO$ 195.65

Balsalazide Disodium



$C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$ 437.31
Benzoic acid, 5-[[4-[[(2-carboxyethyl)amino]carbonyl] phenyl]azo]-2-hydroxy-, disodium salt, dihydrate, (*E*)-; (*E*)-5-[[*p*-[(2-Carboxyethyl)carbamoyl]phenyl]azo]salicylic acid, disodium salt, dihydrate [150399-21-6].

DEFINITION
Balsalazide Disodium contains NLT 98.0% and NMT 102.0% of $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$, calculated on the as-is basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 10 µg/mL in water

- **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩

ASSAY
- **PROCEDURE**

Sample: 219 mg
Analysis: Add 80 mL of glacial acetic acid to the *Sample*, sonicate to dissolve, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 21.87 mg of $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$.
Acceptance criteria: 98.0%–102.0% on the as-is basis

IMPURITIES
ORGANIC IMPURITIES
[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* is recommended when impurities 1, 2, and 3 listed in *Impurity Table 2* may be present.]

- **Procedure 1**

Solution A: Dissolve 2.7 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 10% potassium hydroxide solution to a pH of 6.00±0.1.
Diluent: Use *Solution A*.
Solution B: Use acetonitrile.
Standard solution: 0.5 µg/mL of USP Balsalazide Disodium RS, 0.5 µg/mL of USP Balsalazide Related Compound A RS, 0.5 µg/mL of USP Balsalazide Related Compound B RS, and 0.5 µg/mL of USP Salicylic Acid RS in *Diluent*. If needed, a small amount of acetonitrile may be added to facilitate dissolution. [NOTE—USP Balsalazide Related Compound A RS is the disodium salt of (*E*)-5-[(*p*-carboxyphenyl)azo]-2-salicylic acid. Use the correction factor stated on the label of the USP Reference Standard to calculate the concentration, as appropriate.]
Sample solution: 1 mg/mL of Balsalazide Disodium in *Diluent*
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
40	75	25
47	75	25
55	50	50
60	50	50
60.1	90	10
70	90	10

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 238 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection size: 30 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 5 between balsalazide and balsalazide related compound B
Relative standard deviation: NMT 5% for each peak
Tailing factor: NMT 1.8 for the balsalazide peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Balsalazide Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each individual impurity from the *Sample solution*
r_S = peak response for the corresponding impurity from the *Standard solution*. [NOTE—For unspecified impurities, r_S is the peak response for the balsalazide peak from the *Standard solution*.]
C_S = concentration of the corresponding impurity in the *Standard solution* (mg/mL). [NOTE—For unspecified impurities, C_S is the concentration of balsalazide disodium in the *Standard solution*.]
C_U = concentration of Balsalazide Disodium in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Reporting level for impurities: 0.03%
Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid	0.37	0.05
Balsalazide related compound A[a]	0.70	0.05
Balsalazide	1.00	—

USP Monographs

www.webofpharma.com

Impurity Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Balsalazide related compound B[b]	1.2	0.05
Any other individual unspecified impurity	—	0.05

[a] (*E*)-5-[(*p*-Carboxyphenyl)azo]-2-salicylic acid.
[b] (*E*)-5-({*m*-[(2-Carboxyethyl)carbamoyl]phenyl}azo)-2-salicylic acid.

• **Procedure 2**

Solution A: Prepare 50 mM monobasic potassium phosphate buffer as follows: Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 2 N potassium hydroxide solution to a pH of 6.8–7.0. [NOTE—To ensure proper identification of impurity 1, the pH must be maintained between 6.8 and 7.0.]
Solution B: Acetonitrile, methanol and *Solution A* (5:1:14)
Solution C: Acetonitrile, methanol and *Solution A* (9:1:10)
Diluent: Use *Solution B*.
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	100	0	0
37	0	100	0
60	0	0	100
75	100	0	0
85	100	0	0

Standard solution: 0.075 mg/mL of USP Balsalazide Disodium RS in *Diluent*. [NOTE—Use sonication to dissolve.]
Sensitivity solution: 0.375 µg/mL in *Diluent*, from *Standard solution*
System suitability solution: 1.5 mg/mL of USP Balsalazide Disodium RS and 1.5 µg/mL of USP Balsalazide Related Compound A RS in *Diluent*
Sample solution: 1.5 mg/mL of Balsalazide Disodium in *Diluent*. [NOTE—Use sonication to dissolve.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 25°–27°. [NOTE—To ensure proper identification of impurity 1, the column temperature must be maintained between 25° and 27°.]
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *Standard solution*, *Sensitivity solution*, and *System suitability solution*
Suitability requirements
Resolution: NLT 8.5 between balsalazide related compound A and balsalazide, from the *System suitability solution*
Tailing factor: NMT 3.4 for the balsalazide peak, from the *System suitability solution*
Signal-to-noise ratio: NLT 10, from the *Sensitivity solution*
Relative standard deviation: NMT 2.0%, from the *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Balsalazide Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each individual impurity from the *Sample solution*
r_S = peak response for balsalazide from the *Standard solution*
C_S = concentration of USP Balsalazide Disodium RS in the *Standard solution*
C_U = concentration of Balsalazide Disodium in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 2*)

Acceptance criteria
Individual impurities: See *Impurity Table 2*.
Reporting level for impurities: 0.03%
Total impurities: NMT 0.50%

Impurity Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-(4-Aminobenzoyl)-β-alanine[a]	0.29	1.8	0.05
Salicylic acid	0.55	1.4	0.05
Balsalazide related compound A[b]	0.88	1.4	0.05
Impurity 1[c]	0.91	1.9	0.05
Impurity 2[d]	0.92	1.4	0.05
Impurity 3[e]	0.94	0.83	0.05
Balsalazide	1.00		—
Impurity 4[f]	1.35	2.1	0.05
Impurity 5[g]	1.77	0.91	0.05
Any other individual unspecified impurity	—	—	0.05

[a] This impurity may be present as two peaks. Use the sum of the two peaks to determine compliance.
[b] (*E*)-5-[(*p*-Carboxyphenyl)azo]-2-salicylic acid.
[c] (*E,E*)-3,5-di-[4-(2-Carboxyethylcarbamoyl) phenylazo]-salicylic acid.
[d] (*E*)-3-[4-(2-Carboxyethylcarbamoyl) phenylazo]-salicylic acid.
[e] (*E,E*)-5-({2-[4-(2-Carboxyethylcarbamoyl)phenylazo]-4-[2-carboxyethylcarbamoyl]}phenylazo)-salicylic acid.
[f] (*E*)-2-[4-(2-Carboxyethylcarbamoyl)phenoxy]-5-{[4-(2-carboxyethylcarbamoyl)]phenylazo}-benzoic acid
[g] (*E*)-2-Hydroxy-5-[[4-[[(3-isopropoxy-3-oxopropyl)amino]carbonyl]phenyl]azo]benzoic acid

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** 7.8%–9.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **LABELING:** If a test for *Organic Impurities* other than *Test 1* is used, the labeling states the test with which the article complies.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Balsalazide Disodium RS
USP Balsalazide Related Compound A RS
(*E*)-5-[(*p*-Carboxyphenyl)azo]-2-salicylic acid, disodium salt.
$C_{14}H_8N_2O_5Na_2$ 330.12

USP Monographs

www.webofpharma.com

USP Balsalazide Related Compound B RS
(*E*)-5-({*m*-[(2-Carboxyethyl)carbamoyl]phenyl}azo)-2-salicylic acid.
$C_{17}H_{15}N_3O_6$ 357.17
USP Salicylic Acid RS

Balsalazide Disodium Capsules

DEFINITION
Balsalazide Disodium Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of balsalazide disodium ($C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Using a 0.2-cm cell, record the UV spectrum of the *Sample solution*, obtained in the *Assay*, in the range of 200–400 nm: it exhibits maxima at about 261 nm and 357 nm.

ASSAY
• **PROCEDURE**
Buffer: Add 5 mL of triethylamine to 1000 mL of water, and adjust with phosphoric acid to a pH of 6.00 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (1:4)
Diluent: Water
Standard solution: 60 µg/mL of USP Balsalazide Disodium RS in *Diluent*. [NOTE—Use sonication as necessary.]
Sample stock solution: Transfer an equivalent to 150 mg of balsalazide disodium, from the Capsules contents, to a 100-mL volumetric flask, add 70 mL of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume.
Sample solution: 60 µg/mL of balsalazide disodium, from the *Sample stock solution*, in *Diluent*. Pass a portion of this solution through a suitable filter, discarding the first 3 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 360 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 10,000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Sample: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$ in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Balsalazide Disodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of balsalazide disodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
ORGANIC IMPURITIES
• **Procedure**
Buffer: Dissolve 2.7 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 10% potassium hydroxide solution to a pH of 6.00 ± 0.1.
Diluent: Water
Solution A: *Buffer*
Solution B: Acetonitrile
Sample solution: Transfer an amount of finely crushed powder equivalent to 100 mg of balsalazide disodium to a 100-mL volumetric flask, add 70 mL of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass a portion of this solution through a PVDF filter of 0.45-µm pore size.
Standard solution: 1.0 µg/mL of USP Balsalazide Disodium RS in *Diluent*
System suitability solution: 1.0 µg/mL of USP Balsalazide Disodium RS, 1.5 µg/mL of USP Balsalazide Related Compound A RS, 0.5 µg/mL of USP Balsalazide Related Compound B RS, and 0.5 µg/mL of USP Salicylic Acid RS in *Diluent*
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
40	75	25
47	75	25
55	50	50
60	50	50
60.1	90	10
70	90	10

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 238 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection size: 30 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 5 between balsalazide and balsalazide related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Tailing factor: NMT 1.5, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each individual impurity from the *Sample solution*
r_S = peak response for the balsalazide peak from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Balsalazide Disodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of balsalazide disodium in the *Sample solution*, based on the label claim (mg/mL)
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Reporting level for impurities: 0.05%
Total impurities: NMT 1.0%. [NOTE—When reporting results for *Individual impurities* and *Total impurities*, disregard peaks corresponding to salicylic acid and balsalazide related compound B, as these impurities are controlled in the drug substance only.]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Salicylic acid	0.37	—	—
Balsalazide related compound A[a]	0.70	1.3	0.15
Balsalazide	1.00	—	—
Balsalazide related compound B[b]	1.2	—	—
Any other individual unspecified impurity	—	1.0	0.10

[a] (*E*)-5-[(*p*-Carboxyphenyl)azo]-2-salicylic acid.
[b] (*E*)-5-({*m*-[(2-Carboxyethyl)carbamoyl]phenyl}azo)-2-salicylic acid.

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: pH 6.8 phosphate buffer; 900 mL
Apparatus 2: 50 rpm, with stainless steel wire helix sinkers
Time: 30 min
Detector: UV 357 nm, with background correction at 590 nm
Path length: 0.02-cm flow cell
Blank: *Medium*
Standard solution: 0.83 mg/mL of USP Balsalazide Disodium RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 20-µm pore size.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$ dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (100/L)$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Balsalazide Disodium RS in the *Standard solution* (mg/mL)
V = volume of *Medium* (mL), 900
L = Capsule label claim (mg)

Tolerances: NLT 70% (Q) of the labeled amount of $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$ is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Balsalazide Disodium RS
USP Balsalazide Related Compound A RS
(*E*)-5-[(*p*-Carboxyphenyl)azo]-2-salicylic acid, disodium salt.
$C_{14}H_8N_2O_5Na_2$ 330.12
USP Balsalazide Related Compound B RS
(*E*)-5-({*m*-[(2-Carboxyethyl)carbamoyl]phenyl}azo)-2-salicylic acid.
$C_{17}H_{15}N_3O_6$ 357.17
USP Salicylic Acid RS

Adhesive Bandage

» Adhesive Bandage consists of a compress of four layers of Type I Absorbent Gauze, or other suitable material, affixed to a film or fabric coated with a pressure-sensitive adhesive substance. It is sterile. The compress may contain a suitable antimicrobial agent and may contain one or more suitable colors. The adhesive surface is protected by a suitable removable covering.

Packaging and storage—Package Adhesive Bandage that does not exceed 15 cm (6 inches) in width individually in such manner that sterility is maintained until the individual package is opened. Package individual packages in a second protective container.
Labeling—The label of the second protective container bears a statement that the contents may not be sterile if the individual package has been damaged or previously opened, and it bears the names of any added antimicrobial agents. Each individual package is labeled to indicate the dimensions of the compress and the name of the manufacturer, packer, or distributor, and each protective container indicates also the address of the manufacturer, packer, or distributor.
Sterility Tests ⟨71⟩: meets the requirements.

Gauze Bandage

» Gauze Bandage is Type I Absorbent Gauze. Its length is not less than 98.0 percent of that declared on the label, and its average width is not more than 1.6 mm less than the declared width. It contains no dye or other additives.

Packaging and storage—Gauze Bandage that has been rendered sterile is so packaged that the sterility of the contents of the package is maintained until the package is opened for use.
Labeling—The width and length of the Bandage, the number of pieces contained, and the name of the manufacturer, packer, or distributor, are stated on the package. The designation "non-sterilized" or "not sterilized" appears prominently on the package unless the Gauze Bandage has been rendered sterile, in which case it may be labeled to indicate that it is sterile and that the contents may not be sterile if the package bears evidence of damage or if the package has been previously opened.

USP Monographs

www.webofpharma.com

[NOTE—Before determining the thread count, dimensions, and weight, hold the Bandage, unrolled, for not less than 4 hours in a standard atmosphere of 65 ± 2% relative humidity at 21 ± 1.1° C (70 ± 2°F).]

Thread count—Count the number of warp and filling threads of it in areas of 1.27 cm (½ inch) square at 5 points evenly spread along the center line of the Bandage, no point being within 30.5 cm (12 inches) of either end of the Bandage, and calculate the average number of threads per 2.54 cm (1 inch) in each direction. A variation of not more than 3 threads per inch is allowed in either warp or filling, provided that the combined variations do not exceed 5 threads per square inch.

Width—Measure its width at each of the 5 points selected for the determination of the thread count: the average of 5 measurements is not more than 1.6 mm ($^1/_{16}$ inch) less than the labeled width of the Bandage.

Length—Measure the length of the unrolled Gauze Bandage, smoothed without tension, along the center line of the Gauze Bandage: the length is not less than 98.0% of the labeled length of the Bandage.

Weight—Weigh the entire Bandage: the calculated weight in g per 0.894 square meter (1 linear yard *Type I gauze*), using the measurements obtained as described in the two paragraphs just preceding, is not less than 39.2 g.

Absorbency—Hold a rolled Gauze Bandage horizontal to and almost in contact with the surface of water at 25°, and allow it to drop lightly upon the water: complete submersion takes place in not more than 30 seconds.

Sterility Tests ⟨71⟩: Gauze Bandage that has been rendered sterile meets the requirements.

Other requirements—It meets the requirements of the tests for *Ignited residue, Acid or alkali,* and *Dextrin or starch, in water extract, Residue on ignition, Fatty matter,* and *Alcohol-soluble dyes* under *Absorbent Gauze*

Barium Hydroxide Lime

DEFINITION

Barium Hydroxide Lime is a mixture of barium hydroxide octahydrate and Calcium Hydroxide. It may also contain Potassium Hydroxide and may contain an indicator that is inert toward anesthetic gases such as Ether, Cyclopropane, and Nitrous Oxide and that changes color when the Barium Hydroxide Lime no longer can absorb carbon dioxide.

[CAUTION—Because Barium Hydroxide Lime contains a soluble form of barium, it is toxic if swallowed.]

IDENTIFICATION

- **A.**

Analysis: Place a granule of it on a piece of moistened red litmus paper.

Acceptance criteria: The paper turns blue immediately.

- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Barium*

Sample solution: 100 mg/mL in 6 N acetic acid

Acceptance criteria: Meets the requirements

- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Calcium*

Sample solution: 100 mg/mL in 6 N acetic acid

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING** ⟨786⟩

Sample: 100 g

Analysis: Screen the *Sample* for 5 min as directed in the chapter, using a mechanical shaker.

Acceptance criteria: It passes completely through a No. 2 standard-mesh sieve, and NMT 2.0% passes through a No. 40 standard-mesh sieve. NMT 7.0% is retained on the coarse-mesh sieve, and NMT 15.0% passes through the fine-mesh sieve designated on the label.

- **LOSS ON DRYING** ⟨731⟩

Sample: 10 g

Analysis: Weigh the *Sample* in a tared weighing bottle, and dry at 105° for 2 h.

Acceptance criteria: 11.0%–16.0%

- **HARDNESS**

Sample: 200 g

Analysis: Screen the *Sample* on a mechanical sieve shaker (see ⟨786⟩) with a frequency of oscillation of 285 ± 3 cycles/min, for 3 min, to remove granules coarser than 4-mesh and finer than 8-mesh. Weigh 50 g of the granules retained on the screen, and place them in a hardness pan that has a diameter of 200 mm and a concave brass bottom. The bottom of the pan is 7.9 mm thick at the circumference and 3.2 mm thick at the center and has an inside spherical radius of curvature of 109 cm. Add 15 steel balls of 7.9-mm diameter, and shake on a mechanical sieve shaker for 30 min. Remove the steel balls, brush the contents of the hardness pan onto a sieve of the fine-mesh size designated on the label, shake for 3 min on the mechanical sieve shaker, and weigh.

Acceptance criteria: The percentage of Barium Hydroxide Lime retained on the screen is NLT 75.0% and represents the hardness.

- **CARBON DIOXIDE ABSORBENCY**

Analysis: Fill the lower transverse section of a U-shaped drying tube of about 15-mm internal diameter and 15-cm height with loosely packed glass wool. Place in one arm of the tube about 5 g of anhydrous calcium chloride, and weigh the tube and the contents. Into the other arm of the tube, place 9.5–10.5 g of Barium Hydroxide Lime, and again weigh. Insert stoppers in the open arms of the U-tube, and connect the side tube of the arm filled with Barium Hydroxide Lime to a calcium chloride drying tube, which in turn is connected to a suitable supply source of carbon dioxide. Pass the carbon dioxide through the U-tube at a rate of 75 mL/min for 20 min, timed. Disconnect the U-tube, cool to room temperature, remove the stoppers, and weigh.

Acceptance criteria: The increase in weight is NLT 19.0% of the weight of Barium Hydroxide Lime used for the test.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** If an indicator has been added, the name and color change of such indicator are stated on the container label. The container label indicates also the mesh size in terms of standard-mesh sieve sizes (see *Powder Fineness* ⟨811⟩).

Barium Sulfate

$BaSO_4$ 233.39

Sulfuric acid, barium salt (1:1);
Barium sulfate (1:1) [7727-43-7].

DEFINITION

Barium Sulfate contains NLT 97.5% and NMT 100.5% of barium sulfate ($BaSO_4$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩

Sample solution: Mix 0.5 g of Barium Sulfate with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is

www.webofpharma.com

complete, treat the resulting fused mass with hot water, and filter.
Acceptance criteria: The filtrate, acidified with hydrochloric acid, meets the requirements.
• **B. IDENTIFICATION TESTS—GENERAL,** *Barium* ⟨191⟩
Sample solution: Dissolve a portion of the well-washed residue from *Identification* test *A* in 6 N acetic acid.
Acceptance criteria: The solution meets the requirements.

ASSAY
• **PROCEDURE**
Sample: 0.58–0.62 g, weighed in a tared platinum crucible
Analysis: Add 10 g of anhydrous sodium carbonate to the crucible, and mix by rotating the crucible. Fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper. Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.] Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ($BaSO_4$).
Acceptance criteria: 97.5%–100.5%

IMPURITIES
• **LIMIT OF SULFIDE**
Sample solution: Transfer 10 g to a 500-mL conical flask. Add 100 mL of 0.3 N hydrochloric acid.
Control solution: 100 mL of 0.3 N hydrochloric acid containing 5 µg of sulfide in a 500-mL conical flask
Analysis: Cover the mouth of both conical flasks with a circle of filter paper that has been moistened at the area over the mouth of the flask with 0.15 mL of lead acetate TS, the paper being held in place with a string tied around the neck of the flask. Boil each mixture gently for 10 min, taking care to avoid spattering the paper.
Acceptance criteria: NMT 0.5 µg/g; any darkening of the paper by the *Sample solution* is not greater than that produced by the similarly treated *Control solution.*
• **LIMIT OF ACID-SOLUBLE SUBSTANCES**
Sample solution: Cool the mixture obtained in the test for *Limit of Sulfide,* add water to restore approximately the original volume, and filter it through paper that previously has been washed with a mixture of 10 mL of 3 N hydrochloric acid and 90 mL of water, returning the first portions, if necessary, to obtain a clear filtrate.
Analysis: Evaporate 50 mL of the filtrate on a steam bath to dryness, and add 2 drops of hydrochloric acid and 10 mL of hot water. Filter again through acid-washed paper, prepared as directed above. Wash the filter with 10 mL of hot water, and evaporate the combined filtrate and washings in a tared dish on a steam bath to dryness. Dry the residue at 105° for 1 h.
Acceptance criteria: NMT 0.3%; the residue weighs NMT 15 mg.
• **LIMIT OF SOLUBLE BARIUM SALTS**
Sample: The residue obtained in the test for *Limit of Acid-Soluble Substances*
Control: 10 mL of water containing 0.5 mL of 2 N sulfuric acid and 50 µg of barium
Analysis: Treat the *Sample* with 10 mL of water, pass the solution through a filter previously washed with 100 mL of 0.3 N hydrochloric acid, and add 0.5 mL of 2 N sulfuric acid.
Acceptance criteria: NMT 0.001%; any turbidity formed in the *Sample* within 30 min is NMT that produced in the similarly treated *Control.*

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 3.5–10.0, in a 10% (w/w) aqueous suspension

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Barium Sulfate Paste

DEFINITION
Barium Sulfate Paste is a semisolid formulation of finely divided particles of Barium Sulfate in a suitable base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of barium sulfate ($BaSO_4$). It may contain one or more suitable colors, flavors, suspending or dispersing agents, and preservatives.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩
Sample: Ignite a quantity of Paste equivalent to 0.5 g of barium sulfate to constant weight.
Analysis: Mix 0.5 g of the ignited *Sample* with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter. Proceed as directed in the chapter.
Acceptance criteria: The filtrate, acidified with hydrochloric acid, meets the requirements.
• **B. IDENTIFICATION TESTS—GENERAL,** *Barium* ⟨191⟩
Sample solution: Dissolve a portion of the well-washed residue from *Identification* test *A* in 6 N acetic acid.
Acceptance criteria: The solution meets the requirements.

ASSAY
• **PROCEDURE**
Sample: Barium Sulfate Paste, equivalent to 0.60 g of barium sulfate, weighed in a tared platinum crucible
Analysis: Ignite the *Sample* over a low flame until any organic matter is thoroughly carbonized. Cool, cautiously add 0.5 mL of nitric acid and 0.5 mL of sulfuric acid, and continue the ignition over a low flame until the residue becomes gray in color, then ignite over the full heat of a blast burner. Allow the contents of the crucible to cool to room temperature.
[NOTE—If the specimen contains a silicate, such as bentonite, proceed as follows. Add 10 mL of water

USP Monographs
www.webofpharma.com

and 1 mL of sulfuric acid to the residue in the crucible, mix, and add 10 mL of hydrofluoric acid. Heat gently over a low flame until fumes of sulfur trioxide appear. Add 5 mL more of hydrofluoric acid, heat again over a low flame to the appearance of dense fumes, and continue heating until the sulfuric acid has been completely volatilized. Allow the contents of the crucible to cool.]

[NOTE—If the specimen does not contain a silicate, omit the treatment of the specimen with hydrofluoric and sulfuric acids.]

Add to the treated or untreated specimen in the platinum crucible 10 g of anhydrous sodium carbonate, fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ($BaSO_4$).

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩

For products labeled for oral administration only: The total aerobic microbial count does not exceed 10^2 cfu/g. The total combined molds and yeasts count does not exceed 10^1 cfu/g. It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.* The total enterobacterial count does not exceed 10^1 cfu/g.

For products labeled for oral administration and rectal administration: The total aerobic microbial count does not exceed 10^2 cfu/g. The total combined molds and yeasts count does not exceed 10^1 cfu/g. It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.* The total enterobacterial count does not exceed 10^1 cfu/g.

For products labeled for rectal administration only: The total aerobic microbial count does not exceed 10^3 cfu/g. The total combined molds and yeasts count does not exceed 10^2 cfu/g. It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.* The total enterobacterial count does not exceed 10^1 cfu/g.

- **pH** ⟨791⟩: 3.0–10.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from freezing and from excessive heat.

Barium Sulfate Suspension

DEFINITION

Barium Sulfate Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of barium sulfate ($BaSO_4$). It contains suitable dispersing and/or suspending agents so that when mixed as directed in the labeling, it yields a uniformly dispersed suspension. It may contain one or more suitable colors, flavors, fluidizing agents, and preservatives.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩

Sample: Shake the Suspension, and transfer a volume equivalent to 0.5 g of barium sulfate to a suitable container. Ignite to constant weight.

Analysis: Mix 0.5 g of the ignited *Sample* with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter. Proceed as directed in the chapter.

Acceptance criteria: The filtrate, acidified with hydrochloric acid, meets the requirements.

- **B. IDENTIFICATION TESTS—GENERAL,** *Barium* ⟨191⟩

Sample solution: Dissolve a portion of the well-washed residue from *Identification* test *A* in 6 N acetic acid.

Acceptance criteria: The solution meets the requirements.

ASSAY

- **PROCEDURE**

Sample: A volume of Suspension, previously well shaken in its original container, equivalent to 0.60 g of barium sulfate, in a tared platinum crucible

Analysis: Ignite over a low flame until any organic matter is thoroughly carbonized. Cool, cautiously add 0.5 mL of nitric acid and 0.5 mL of sulfuric acid, and continue the ignition over a low flame until the residue becomes gray in color, then ignite over the full heat of a blast burner. Allow the contents of the crucible to cool to room temperature.

[NOTE—If the specimen contains a silicate, such as bentonite, proceed as follows. Add 10 mL of water and 1 mL of sulfuric acid to the residue in the crucible, mix, and add 10 mL of hydrofluoric acid. Heat gently over a low flame until fumes of sulfur trioxide appear. Add 5 mL more of hydrofluoric acid, heat again over a low flame to the appearance of dense fumes, and continue heating until the sulfuric acid has been completely volatilized. Allow the contents of the crucible to cool.]

[NOTE—If the specimen does not contain a silicate, omit the treatment of the specimen with hydrofluoric and sulfuric acids.]

Add to the treated or untreated specimen in the platinum crucible 10 g of anhydrous sodium carbonate, fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in

USP Monographs

www.webofpharma.com

the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ($BaSO_4$).

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total bacterial count does not exceed 10^2 cfu/mL, the total combined molds and yeasts count does not exceed 10^1 cfu/mL, and it meets the requirements of the tests for absence of *Salmonella* species, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.
- **pH** ⟨791⟩**:** 3.5–10.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.

Barium Sulfate for Suspension

DEFINITION

Barium Sulfate for Suspension is a dry mixture of Barium Sulfate and one or more suitable dispersing and/or suspending agents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of barium sulfate ($BaSO_4$). It may contain one or more suitable colors, flavors, fluidizing agents, and preservatives.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩

Sample: Ignite 1g to constant weight.

Analysis: Mix 0.5 g of the ignited *Sample* with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter.

Acceptance criteria: The filtrate, acidified with hydrochloric acid, meets the requirements.

- **B. IDENTIFICATION TESTS—GENERAL,** *Barium* ⟨191⟩

Sample solution: Dissolve a portion of the well-washed residue from *Identification* test *A* in 6 N acetic acid.

Acceptance criteria: The solution meets the requirements.

ASSAY

- **PROCEDURE**

Sample: Barium Sulfate for Suspension, equivalent to 0.60 g of barium sulfate, weighed in a tared platinum crucible

Analysis: Ignite over a low flame until any organic matter is thoroughly carbonized. Cool, cautiously add 0.5 mL of nitric acid and 0.5 mL of sulfuric acid, and continue the ignition over a low flame until the residue becomes gray in color, then ignite over the full heat of a blast burner. Allow the contents of the crucible to cool to room temperature.

[NOTE—If the specimen contains a silicate, such as bentonite, proceed as follows. Add 10 mL of water and 1 mL of sulfuric acid to the residue in the crucible, mix, and add 10 mL of hydrofluoric acid. Heat gently over a low flame until fumes of sulfur trioxide appear. Add 5 mL more of hydrofluoric acid, heat again over a low flame to the appearance of dense fumes, and continue heating until the sulfuric acid has been completely volatilized. Allow the contents of the crucible to cool.]

[NOTE—If the specimen does not contain a silicate, omit the treatment of the specimen with hydrofluoric and sulfuric acids.]

Add to the treated or untreated specimen in the platinum crucible 10 g of anhydrous sodium carbonate, fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ($BaSO_4$).

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.

www.webofpharma.com

Acceptance criteria: NMT 1.0%

• **pH** ⟨791⟩**:** 3.5–10.0, in a 60% (w/w) aqueous suspension, or constituted for its intended use as directed in the labeling

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

Barium Sulfate Tablets

DEFINITION

Barium Sulfate Tablets are flat-sided disks between 11.5 mm and 13.5 mm in diameter and contain NLT 90.0% and NMT 110.0% of the labeled amount of barium sulfate ($BaSO_4$).

IDENTIFICATION

• **A. Identification Tests—General,** *Sulfate* ⟨191⟩

Sample: A portion of powdered Tablets equivalent to 0.6 g of barium sulfate

Analysis: Mix the *Sample* with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter. Proceed as directed in the chapter.

Acceptance criteria: The filtrate, acidified with hydrochloric acid, meets the requirements.

• **B. Identification Tests—General,** *Barium* ⟨191⟩

Sample solution: Dissolve a portion of the well-washed residue from *Identification* test *A* in 6 N acetic acid.

Acceptance criteria: The solution meets the requirements.

ASSAY

• **Procedure**

Sample: A portion of powdered Tablets, equivalent to 0.6 g of barium sulfate, weighed in a tared platinum crucible

Analysis: Add 10 g of anhydrous sodium carbonate to the crucible, and mix by rotating the crucible. Fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper. Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [Note—The solution may be slightly hazy.] Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ($BaSO_4$).

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

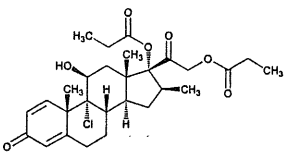
• **Disintegration** ⟨701⟩**:** NLT 10 min and NMT 30 min

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

Beclomethasone Dipropionate



$C_{28}H_{37}ClO_7 \cdot H_2O$ 539.07

$C_{28}H_{37}ClO_7$ 521.04

Pregna-1,4-diene-3,20-dione, 9-chloro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, (11β,16β)-;
9-Chloro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [5534-09-8].

DEFINITION

Beclomethasone Dipropionate is anhydrous or contains one molecule of water of hydration. It contains NLT 97.0% and NMT 103.0% of beclomethasone dipropionate ($C_{28}H_{37}ClO_7$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

ASSAY

• **Procedure**

Mobile phase: Acetonitrile and water (60:40) such that the retention times for beclomethasone dipropionate and testosterone propionate are approximately 6 and 10 min, respectively

Internal standard solution: 1.2 mg/mL of USP Testosterone Propionate RS in methanol

Standard stock solution: 1.4 mg/mL of USP Beclomethasone Dipropionate RS in methanol

Standard solution: 0.7 mg/mL of USP Beclomethasone Dipropionate RS and 0.6 mg/mL of USP Testosterone Propionate RS prepared as follows. Transfer 4.0 mL of *Standard stock solution* to a suitable vial, and add 4.0 mL of *Internal standard solution*.

Sample stock solution: 1.4 mg/mL of Beclomethasone Dipropionate in methanol

Sample solution: 0.7 mg/mL of Beclomethasone Dipropionate and 0.6 mg/mL of USP Testosterone Propionate RS prepared as follows. Transfer 4.0 mL of *Sample stock solution* to a suitable vial, and add 4.0 mL of *Internal standard solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

USP Monographs

www.webofpharma.com

Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Injection volume: 5–25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0% for five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of beclomethasone dipropionate ($C_{28}H_{37}ClO_7$) in the portion of Beclomethasone Dipropionate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio of beclomethasone dipropionate to the internal standard from the *Sample solution*
R_S = peak height ratio of beclomethasone dipropionate to the internal standard from the *Standard solution*
C_S = concentration of USP Beclomethasone Dipropionate RS in the *Standard solution* (mg/mL)
C_U = concentration of Beclomethasone Dipropionate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in dioxane
Acceptance criteria: +88° to +94°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 3 h.
Acceptance criteria: NMT 0.5% for the anhydrous form; 2.8%–3.8% for the monohydrate form

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Beclomethasone Dipropionate RS
USP Testosterone Propionate RS

Beclomethasone Dipropionate Compounded Oral Solution

DEFINITION
Beclomethasone Dipropionate Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of beclomethasone dipropionate ($C_{28}H_{37}ClO_7$).
Prepare Beclomethasone Dipropionate Compounded Oral Solution 0.5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Beclomethasone Dipropionate powder	50 mg
Corn Oil, *NF*, a sufficient quantity to make	100 mL

Pour the *Beclomethasone Dipropionate powder* into a suitable container. Wet the powder with a small amount of *Corn Oil* and triturate to make a smooth paste. Add the *Corn Oil* to make the contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the *Corn Oil*. Add sufficient *Corn Oil* to bring to final volume. Place on a shaker until dissolved. [NOTE—May take up to 24 h to dissolve.]

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (65:35)
Standard stock solution: 0.5 mg/mL of beclomethasone dipropionate prepared from USP Beclomethasone Dipropionate RS in ethanol. Sonicate and mix well.
Standard solution: 0.02 mg/mL of beclomethasone dipropionate prepared from *Standard stock solution* and ethanol
Sample solution: Transfer 1.0 mL of Oral Solution to a 25-mL volumetric flask, add approximately 20 mL of ethanol, vortex for 30 s, and warm under running water until dissolved. Dilute with ethanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV-Vis 240 nm
Column: 2.0-mm × 10-cm; 2.5-µm packing L1
Column temperature: 35°
Flow rate: 0.35 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for beclomethasone dipropionate is about 3.2 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of beclomethasone dipropionate ($C_{28}H_{37}ClO_7$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of beclomethasone dipropionate from the *Sample solution*
r_S = peak response of beclomethasone dipropionate from the *Standard solution*
C_S = concentration of USP Beclomethasone Dipropionate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of beclomethasone dipropionate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant, plastic containers. Store at controlled room temperature.
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature.
• **LABELING:** Label it to be well-shaken before use, and to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Beclomethasone Dipropionate RS

USP Monographs

www.webofpharma.com

Belladonna Leaf

DEFINITION

Belladonna Leaf consists of the dried leaf and flowering or fruiting top of *Atropa belladonna* L. or of its variety *acuminata* Royle ex Lindley (Fam. Solanaceae). Belladonna Leaf yields NLT 0.35% of the alkaloids of belladonna leaf.

ASSAY

• **PROCEDURE**

Phosphate buffer: 34.8 g of dibasic potassium phosphate in 900 mL of water. Adjust to a pH of 9.5 by the addition of 3 N hydrochloric acid or sodium hydroxide, with mixing.

Diluent: Dilute sulfuric acid (1 in 350)

Internal standard solution: 0.8 mg/mL of USP Homatropine Hydrobromide RS in *Diluent*. [NOTE—Prepare fresh on the day of use.]

Standard stock solution A: 1.0 mg/mL of USP Scopolamine Hydrobromide RS in *Diluent*

Standard stock solution B: Dissolve 20 mg of USP Atropine Sulfate RS in 25 mL of *Diluent* in a 50-mL volumetric flask, add 2.0 mL of *Standard stock solution A*, and mix. Add *Diluent* to volume. [NOTE—Prepare fresh on the day of use.]

Standard solutions: Pipet into three separate 60-mL separators 1.0-, 2.0-, and 3.0-mL portions, respectively, of *Standard stock solution A*, and add 9.0, 8.0, and 7.0 mL, respectively, of *Diluent*. Add 1.0 mL of *Internal standard solution*, then add 15 mL of chloroform, shake vigorously, allow the layers to separate, and discard the chloroform layer.

Sample solution: Moisten 10 g, previously reduced to a moderately coarse powder, with a mixture of 8 mL of ammonium hydroxide, 10 mL of alcohol, and 20 mL of ether, and extract the alkaloids by either *Method I* or *Method II* as follows. If necessary, reduce the volume of the extract to 100 mL by evaporation on a steam bath.

Extraction blank: Place 10 mL of *Diluent* in a 60-mL separator. Prepare as directed under *Standard solutions*, beginning with "then add 15 mL of chloroform". The blank chromatogram contains no significant interferences at the locus of atropine, scopolamine, or homatropine.

Method I: Place the moistened drug in a continuous-extraction thimble, and allow maceration to proceed overnight, then extract with ether for 3 h, or longer if necessary, to effect complete extraction.

Method II: Place the moistened drug in a small percolator, and allow maceration to proceed overnight. Percolate slowly with a mixture of three volumes of ether and one volume of chloroform. Continue the percolation until the residue from 3–4 mL of percolate last passed, when dissolved in dilute sulfuric acid (1 in 70) and treated with mercuric iodide TS, shows NMT a faint turbidity.

Transfer the extract from *Method I* or *Method II* to a separator with the aid of ether. Extract with five 15-mL portions of dilute sulfuric acid (1 in 70), filtering each portion drawn off into a 100-mL volumetric flask. Wash the filter with dilute sulfuric acid (1 in 70), and collect the washings in the flask. Add dilute sulfuric acid (1 in 70) to volume, and mix. Dilute 20.0 mL of the resulting solution with the same dilute acid to 100.0 mL. Pipet 10 mL of this solution into a 60-mL separator. Add 1.0 mL of *Internal standard solution*, then add 15 mL of chloroform, shake vigorously, allow the layers to separate, and discard the chloroform layer. [NOTE—If emulsions are formed, a mixed solvent consisting of chloroform-isopropyl alcohol (10:3) may be substituted for chloroform throughout the extraction procedure.]

Add another 15 mL of chloroform, and extract again, discarding the chloroform phase. Add 15 mL of *Phosphate buffer* and sufficient 1 N sodium hydroxide to yield a final pH between 9.0 and 9.5. Add 15 mL of chloroform, shake vigorously, and allow the layers to separate. Filter the organic phase through 10 g of anhydrous sodium sulfate (see *Reagents, Indicators, and Solutions—Sodium Sulfate, Anhydrous, Suitability for Alkaloid Assays*), previously washed with chloroform and supported in a funnel with a small pledget of glass wool, into a suitable container. Extract again with two 15-mL portions of chloroform, again collecting the clarified organic phase. Wash the sodium sulfate and the tip of the funnel with 5 mL of chloroform. Evaporate the combined organic phases under reduced pressure at a temperature below 45°, add 1 mL of chloroform, and mix to dissolve the alkaloids, taking care to wet the sides of the container.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 1.2-m × 4-mm glass; packed with 3% G3 on S1AB. [NOTE—The column may be cured and conditioned as specified under *Chromatography* ⟨621⟩, *General Procedures, Gas Chromatography*.]

Temperatures

Injection port: 240°

Detector: 240°

Column: 215°

Carrier gas: Dry helium

Flow rate: 65 mL/min

Injection volume: 5 µL

System suitability

Sample: *Sample solution*

Suitability requirements

Resolution: NLT 3.0 between atropine and homatropine peaks

Tailing factor: NMT 2.0 for atropine peak

Relative standard deviation: The analytical system is suitable for conducting this *Assay* if the relative standard deviation for the ratio, R_A, is NMT 2.0%, for the atropine peak in repeated injections.

Analysis

Samples: *Standard solutions* and *Sample solution*

Measure the areas, a_A, a_H, and a_S, of the atropine, homatropine, and scopolamine peaks, respectively, in each chromatogram of *Standard solution*, and calculate the ratios A_A and A_S:

$$A_A = a_A/a_H$$

$$A_S = a_S/a_H$$

Plot the standard curves of the values of R_A and R_S against the amounts, in milligrams, of atropine and scopolamine in the solutions. (The ratio of the molecular weight of atropine to that of anhydrous atropine sulfate is 0.8551, and the ratio of the molecular weight of scopolamine to that of anhydrous scopolamine hydrobromide is 0.7894.) Inject a portion of the *Sample solution* into the chromatograph, measure the peak areas, and calculate the area ratios, as with the *Standard solutions*. Record from the standard curves the quantities, in milligrams, of atropine and scopolamine in the weight of the specimen taken. Add the quantity, in milligrams, of atropine and scopolamine, and multiply by 50 to obtain the weight, in milligrams, of alkaloids in the portion of Belladonna Leaf taken.

www.webofpharma.com

Acceptance criteria: NLT 0.35% of the alkaloids of belladonna leaf

CONTAMINANTS

- **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Acid-Insoluble Ash*: NMT 3.0%
- **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Pesticide Residue Analysis*: Meets the requirements
- **BELLADONNA STEMS:** The proportion of belladonna stems over 10 mm in diameter does not exceed 3.0%.

SPECIFIC TESTS

- **BOTANICAL CHARACTERISTICS**

Macroscopic: Usually partly matted together, crumpled or broken leaves, together with some smaller stems and a number of flowers and fruits. The leaves are thin and brittle, mostly light green to moderate olive-green. The lamina is mostly 5–25 cm in length and 4–12 cm in width and possesses an ovate-lanceolate to broadly ovate outline, an acute to acuminate apex, an entire margin, an acute to somewhat decurrent base and slightly hairy surface, the hairs being more abundant along the veins; when broken transversely, it shows numerous light-colored dots (crystal cells) visible with a lens. The petiole is slender and usually up to 4 cm in length. The flowers possess a campanulate corolla with five small, reflexed lobes, purplish to yellowish purple, becoming faded to brown or dusky yellow or yellow; a green, five-lobed calyx; five epipetalous stamens; and a superior, bilocular ovary with numerous ovules. The fruit is subglobular, dark yellow to yellowish brown to dusky red or black, up to 12 mm in width, and sometimes subtended by the persistent calyx and containing numerous flattened, somewhat reniform seeds, the latter up to 2 mm in width. The stems are more or less flattened and hollow and finely hairy when young.

Microscopic

Leaf: The epidermis of the lamina possesses wavy anticlinal walls and a distinctly striated cuticle. Stomata are more numerous in the lower epidermis and are surrounded by three or four neighboring cells, one of which is smaller than the others. The nonglandular hairs are uniseriate and up to six-celled. Short club-shaped glandular hairs with a one-celled stalk and multicellular head and long glandular hairs with a uniseriate stalk and unicellular head occur on both epidermises. The mesophyll consists of a single layer of palisade parenchyma beneath which occurs spongy parenchyma, the latter with scattered cells filled with microcrystals. The midrib contains an arc of bicollateral bundles, collenchyma beneath upper epidermis, and scattered parenchyma cells with microcrystals.

Stem: The stem shows an epidermis with striated cuticle and few hairs; a distinct endodermis; small strands of long, thin-walled, slightly lignified pericyclic fibers; and a circle of bicollateral bundles. The parenchyma of the cortex and pith is interspersed with crystal cells.

Flower: The calyx possesses numerous glandular hairs with uniseriate stalks and one- to three-celled glandular heads. The corolla shows a papillose inner epidermis and an outer epidermis with glandular hairs similar to those of the calyx. The pollen grains, when mounted in chloral hydrate solution, are subspherical, 40 µm in diameter, tricolpate, having three germinal furrows and rows of pits between the ridges on the exine.

Fruit: The epicarp exhibits polygonal epidermal cells with a striated cuticle and stomata. The mesocarp consists of large pulp cells some of which contain rosette aggregate crystals of calcium oxalate.

Seed: The seed is characterized by an epidermis of large, wavy-walled cells with prominent ridges over the anticlinal walls.

Powdered Belladonna Leaf: Light olive-brown to moderate olive-green in color. The following are among the elements of identification: the separate microcrystals, the dark gray crystal cells, the cuticular striping of the epidermal cells, the vessels with ellipsoidal bordered pits, the fibers of the stem, and occasional hairs and pollen grains. Rosette aggregates of calcium oxalate and fragments of the seed occur when the drug contains belladonna fruits. Examine Belladonna Leaf for hairs having a papillose cuticle and for raphides of calcium oxalate: their presence indicates adulteration.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers and avoid long exposure to direct sunlight. Preserve powdered Belladonna Leaf in light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Atropine Sulfate RS
  USP Homatropine Hydrobromide RS
  USP Scopolamine Hydrobromide RS

Belladonna Extract

» Belladonna Extract contains, in each 100 g, not less than 1.15 g and not more than 1.35 g of the alkaloids of belladonna leaf.

PILULAR BELLADONNA EXTRACT

Prepare the extract by percolating 1000 g of Belladonna Leaf, using a mixture of 3 volumes of alcohol and 1 volume of water as the menstruum. Macerate the drug for 16 hours, and then percolate it at a moderate rate. Evaporate the percolate under reduced pressure and at a temperature not exceeding 60° to a pilular consistency, and adjust the remaining extract, after assaying, by dilution with liquid glucose so that the finished Extract will contain, in each 100 g, 1.25 g of the alkaloids of belladonna leaf.

POWDERED BELLADONNA EXTRACT

Prepare the extract by percolating 1000 g of Belladonna Leaf, using alcohol as the menstruum. Macerate the drug for 16 hours, and then percolate it slowly. Evaporate the percolate under reduced pressure and at a temperature not exceeding 60° to a soft extract, add 50 g of dry starch, and continue the evaporation, at the same temperature, until the product is dry. Powder the residue. The extract may be deprived of its fat by treating either the soft extract first obtained, or the dry and powdered extract, as directed under *Extracts* (see *Pharmaceutical Dosage Forms* ⟨1151⟩). Assay the powdered residue, and add sufficient starch, previously dried at 100°, to obtain a finished Extract containing 1.25 g of the alkaloids of belladonna leaf in each 100 g. Mix the powders, and pass the Extract through a fine sieve.

USP Monographs

www.webofpharma.com

Packaging and storage—Preserve in tight containers, at a temperature not exceeding 30°.
USP Reference standards ⟨11⟩—
USP Atropine Sulfate RS
USP Homatropine Hydrobromide RS
USP Scopolamine Hydrobromide RS

Assay—
pH 9.5 Phosphate buffer—Dissolve 34.8 g of dibasic potassium phosphate in 900 mL of water, and adjust to a pH of 9.5, determined electrometrically, by the addition of 3 N hydrochloric acid or sodium hydroxide, with mixing.
Internal standard solution—Dissolve about 40 mg of USP Homatropine Hydrobromide RS, accurately weighed, in about 25 mL of dilute sulfuric acid (1 in 350) in a 50-mL volumetric flask, add the same dilute acid to volume, and mix. Prepare fresh on the day of use.
Standard preparation—Dissolve about 10 mg of USP Scopolamine Hydrobromide RS, accurately weighed, in about 5 mL of dilute sulfuric acid (1 in 350) in a 10-mL volumetric flask, add the same dilute acid to volume, and mix (*Solution A*). Dissolve about 20 mg of USP Atropine Sulfate RS, accurately weighed, in about 25 mL of dilute sulfuric acid (1 in 350) in a 50-mL volumetric flask, add 2.0 mL of *Solution A*, and mix. Add dilute sulfuric acid (1 in 350) to volume, and mix. Prepare fresh on the day of use.
Extraction blank—Place about 10 mL of dilute sulfuric acid (1 in 350) in a 60-mL separator. Proceed as directed under *Assay preparation*, beginning with "then add 15 mL of chloroform." The blank chromatogram contains no significant interferences at the locus of atropine, scopolamine, or homatropine.
Assay preparation—Weigh accurately about 0.5 g of Extract, transfer to a 125-mL conical flask, and add 40 mL of dilute sulfuric acid (1 in 350). Heat to a temperature not above 45°, and stir to hasten solution. Filter the solution through filter paper into a 100-mL volumetric flask. Wash the flask and the filter with two 20-mL portions of warmed dilute sulfuric acid (1 in 350), and collect the washings in the 100-mL volumetric flask. Add dilute sulfuric acid (1 in 350) to volume, and mix.
Pipet 10 mL of this solution into a 60-mL separator. To the separator add 1.0 mL of *Internal standard solution*, then add 15 mL of chloroform, shake vigorously, allow the layers to separate, and discard the chloroform layer. (If emulsions are formed, a *mixed solvent* consisting of chloroform and isopropyl alcohol (10:3) may be substituted for chloroform throughout the extraction procedure.) Add another 15 mL of chloroform, and extract again, discarding the chloroform phase. Add 15 mL of *pH 9.5 Phosphate buffer* and sufficient 1 N sodium hydroxide to yield a final pH between 9.0 and 9.5. Add 15 mL of chloroform, shake vigorously, and allow the layers to separate. Filter the organic phase through 10 g of anhydrous sodium sulfate (see *Suitability for alkaloid assays* under *Sodium Sulfate, Anhydrous*, in the section *Reagents, Indicators, and Solutions*), previously washed with chloroform and supported in a funnel with a small pledget of glass wool, into a suitable container. Extract again with two 15-mL portions of chloroform, again collecting the clarified organic phase. Wash the sodium sulfate and the tip of the funnel with 5 mL of chloroform. Evaporate the combined organic phases under reduced pressure, at a temperature below 45°, add 1 mL of chloroform, and mix to dissolve the alkaloids, taking care to wet the sides of the container.
Standard curve—Prepare three *Standard solutions* as follows. Pipet into three separate 60-mL separators 1.0-, 2.0-, and 3.0-mL portions, respectively, of *Standard preparation*, and add 9.0, 8.0, and 7.0 mL, respectively, of dilute sulfuric acid (1 in 350). Proceed as directed under *Assay preparation*, beginning with "add 1.0 mL of *Internal standard solution*."
Chromatographic system—Under typical condition, the instrument contains a 1.2-m × 4-mm glass column packed with 3% G3 on S1AB. The column may be cured and conditioned as specified under *Gas Chromatography* (see *Chromatography* ⟨621⟩). The column is maintained at a temperature of about 215°, and the injection port and detector block at about 240°, and dry helium is used as a carrier gas at a flow rate of about 65 mL per minute.
System suitability—Chromatograph six to ten injections of the *Assay preparation*, and record peak areas as directed for *Procedure*. The analytical system is suitable for conducting this assay if the relative standard deviation for the ratio, R_A, calculated by the formula:

$$100 \times (\text{standard deviation/mean ratio})$$

does not exceed 2.0%; the resolution, R, between a_H and a_A is not less than 3; and the tailing factor (the sum of the distances from peak center to the leading edge and to the tailing edge divided by twice the distance from peak center to the leading edge), measured at 5% of the peak height of a_A, does not exceed 2.0.
Procedure—Inject a portion (about 5 µL) of each *Standard solution* into a suitable gas chromatograph equipped with a flame-ionization detector. Measure the areas, a_A, a_H, and a_S, of the atropine, homatropine, and scopolamine peaks, respectively, in each chromatogram, and calculate the ratios A_A and A_S by the formulas:

$$a_A/a_H \text{ and } a_S/a_H.$$

Plot the *Standard curves* of the values of R_A and R_S against the amounts, in mg, of atropine and scopolamine in the solutions. (The ratio of the molecular weight of atropine to that of anhydrous atropine sulfate is 0.8551, and the ratio of the molecular weight of scopolamine to that of anhydrous scopolamine hydrobromide is 0.7894.) Inject a portion of the *Assay preparation* into the chromatograph, obtain the chromatogram area ratios, measure the peak areas, and calculate the area ratios, as with the *Standard solutions*. Record from the *Standard curve* the quantities, in mg, of atropine and scopolamine in the volume of specimen taken. Add the quantity, in mg, of atropine and scopolamine, and multiply by 10 to obtain the weight, in mg, of alkaloids in the portion of Extract taken.

Belladonna Extract Tablets

» Belladonna Extract Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of the alkaloids of belladonna leaf.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Atropine Sulfate RS
USP Homatropine Hydrobromide RS
USP Scopolamine Hydrobromide RS
Identification—Macerate a quantity of powdered Tablets, equivalent to about 5 mg of the alkaloids of belladonna extract, with 20 mL of water, and transfer to a separator. Render the solution alkaline with 6 N ammonium hydroxide, and extract the alkaloids with 50 mL of chloroform. Filter the chloroform layer, divide it into two equal portions, and

www.webofpharma.com

evaporate to dryness: the residue responds to the following tests.

A: To one portion of the dry residue add 2 drops of nitric acid, evaporate on a steam bath to dryness, and add a few drops of alcoholic potassium hydroxide TS: a violet color is produced.

B: Dissolve the other portion of the residue in 1 mL of dilute hydrochloric acid (1 in 120), and add gold chloride TS, dropwise with shaking, until a definite precipitate separates. Slowly heat until the precipitate dissolves, and allow the solution to cool: a lusterless precipitate is produced.

Disintegration ⟨701⟩: 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

pH 9.5 Phosphate buffer, Internal standard solution, Standard preparation, Extraction blank, Standard curve, Chromatographic system, and *System suitability*—Proceed as directed in the *Assay* under *Belladonna Extract*

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 600 µg of atropine and scopolamine, to a 60-mL separator, add 10.0 mL of dilute sulfuric acid (1 in 350), and sonicate to dissolve as much as possible of the specimen. Proceed as directed for *Assay preparation* in the *Assay* under *Belladonna Leaf*, beginning with "add 1.0 mL of *Internal standard solution.*"

Procedure—Proceed as directed for *Procedure*— in the *Assay* — under *Belladonna Extract.* Record from the *Standard curves* the quantities, in mg, of atropine and scopolamine in the weight of specimen taken.

Belladonna Tincture

» Belladonna Tincture yields, from each 100 mL, not less than 27 mg and not more than 33 mg of the alkaloids of belladonna leaf.

Belladonna Leaf, in moderately coarse powder	100 g
To make about	1000 mL

Prepare a tincture by *Process P* as modified for assayed *Tinctures* (see *Pharmaceutical Dosage Forms* ⟨1151⟩), using a mixture of 3 volumes of alcohol and 1 volume of water as the menstruum. Finally adjust the Tincture to contain, in each 100 mL, 30 mg of the alkaloids of belladonna leaf.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

USP Reference standards ⟨11⟩—
USP Atropine Sulfate RS
USP Homatropine Hydrobromide RS
USP Scopolamine Hydrobromide RS

Alcohol Determination, *Method II* ⟨611⟩: between 65.0% and 70.0% of C_2H_5OH, determined by the gas-liquid chromatographic procedure, acetone being used as the internal standard.

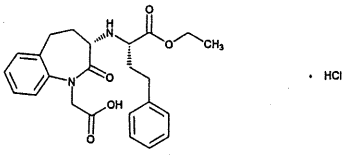
Assay—

pH 9.5 Phosphate buffer , Internal standard solution, Standard preparation, Extraction blank, Standard curve, Chromatographic system, and *System suitability*—Proceed as directed in the *Assay* under *Belladonna Extract.*

Assay preparation—Proceed with Tincture as directed in the *Assay* under *Belladonna Leaf* but pipet 2 mL of Tincture (in place of "10 mL of this solution") into a 60-mL separator containing 10 mL of dilute sulfuric acid (1 in 350).

Procedure—Proceed as directed in the *Assay* under *Belladonna Leaf.* Record from the *Standard curve* the quantities, in mg, of atropine and scopolamine in the specimen. Add the quantity, in mg, of atropine and scopolamine, and multiply by 50 to obtain the weight, in mg, of alkaloids per 100 mL.

Benazepril Hydrochloride



$C_{24}H_{28}N_2O_5 \cdot HCl$ 460.95

1*H*-1-Benzazepine-1-acetic acid, 3-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-, monohydrochloride, [*S*-(*R**,*R**)]-.

(3*S*)-3-[[(1*S*)-1-Carboxy-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepine-1-acetic acid, 3-ethyl ester, monohydrochloride [86541-74-4].

» Benazepril Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{24}H_{28}N_2O_5 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, and store at a temperature below 30°, preferably between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Benazepril Hydrochloride RS
USP Benazepril Related Compound A RS
(3*R*) 3-[[(1*R*) 1-(Ethoxycarbonyl)-3-phenylpropyl] amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepine-1-acetic acid, monohydrochloride.
$C_{24}H_{28}N_2O_5 \cdot HCl$ 460.95
USP Benazepril Related Compound B RS
(3*S*) 3-[[(1*R*) 1-(Ethoxycarbonyl)-3-phenylpropyl] amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepine-1-acetic acid, monohydrochloride.
$C_{24}H_{28}N_2O_5 \cdot HCl$ 460.95
USP Benazepril Related Compound C RS
3-(1-Carboxy-3-phenyl-1(*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid.
$C_{22}H_{24}N_2O_5$ 396.44
USP Benazepril Related Compound D RS
(3-(1-Ethoxycarbonyl-3-cyclohexyl-(1*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine)-1-acetic acid, monohydrochloride.
$C_{24}H_{34}N_2O_5 \cdot HCl$ 467.00
USP Benazepril Related Compound E RS
3-Amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid.

USP Monographs

www.webofpharma.com

USP Benazepril Related Compound F RS
tert-Butyl-3-amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid.
USP Benazepril Related Compound G RS
(3-(1-Ethoxycarbonyl-3-phenyl-(1*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine)-1-acetic acid ethyl ester.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

C: It responds to the test for *Chloride* ⟨191⟩.

Absorbance of solution—The absorbance of a 1 in 100 solution of it in methanol, determined in a 1-cm cell at 420 nm, is not more than 0.015, methanol being used as the blank.

Absorptivity—

Test preparation—Dissolve an accurately weighed quantity of Benazepril Hydrochloride in methanol, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.025 mg per mL.

Procedure—Proceed as directed under *Ultraviolet-Visible Spectroscopy* ⟨857⟩, and measure the absorbance at 238 nm: the absorptivity is between 21.0 and 23.2.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 1.5% of its weight.

Residue on ignition ⟨281⟩—Ignite at 600°. Not more than 0.1% residue is found.

Related compounds—

TEST 1 (FOR BENAZEPRIL RELATED COMPOUND A)—

pH 6.0 Phosphate buffer—Dissolve 9.66 g of monobasic potassium phosphate and 2.68 g of dibasic sodium phosphate, heptahydrate in about 900 mL of water, and dilute with water to 1000 mL.

Mobile phase—Prepare a filtered and degassed mixture of *pH 6.0 Phosphate buffer* and methanol (80:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Dissolve accurately weighed quantities of USP Benazepril Hydrochloride RS and USP Benazepril Related Compound A RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 1.0 mg per mL and 0.005 mg per mL, respectively.

Standard stock solution—Dissolve an accurately weighed quantity of USP Benazepril Related Compound A RS in *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

Standard solution—Dilute a suitable portion of *Standard stock solution,* accurately measured, with *Mobile phase* to obtain a solution having a known concentration of about 5 µg per mL.

Dilute standard solution—Dilute a suitable portion of *Standard stock solution,* accurately measured, with *Mobile phase* to obtain a solution having a known concentration of about 1 µg per mL.

Test solution—Transfer about 50 mg of Benazepril Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4.0-mm × 10-cm column that contains packing L41. The flow rate is about 0.9 mL per minute. The column temperature is maintained at 30°. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between benazepril hydrochloride and benazepril related compound A is not less than 2.0. Chromatograph the *Dilute standard solution,* and record the peak responses as directed for *Procedure:* the signal-to-noise ratio is not less than 10:1. Chromatograph the *Standard solution:* the relative standard deviation for replicate injections determined from the benazepril related compound A peak is not more than 10%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the area for the benazepril related compound A peak. Calculate the percentage of benazepril related compound A in the portion of Benazepril Hydrochloride taken by the formula:

$$100(C_S/C_T)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Benazepril Related Compound A RS in the *Standard solution;* C_T is the concentration, in mg per mL, of Benazepril Hydrochloride in the *Test solution;* r_U is the peak response for benazepril related compound A obtained from the *Test solution;* and r_S is the peak response for benazepril related compound A obtained from the *Standard solution:* The limit of benazepril related compound A is given in the table below.

Benazepril Related Compound	Relative Retention Time	Limit (%)
A[1]	2.3	0.1

[1] ((3*R*)-3-[[(1*R*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepine-1-acetic acid, monohydrochloride

TEST 2 (FOR BENAZEPRIL RELATED COMPOUNDS B, C, D, E, F, AND G)—

Tetrabutylammonium bromide solution, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay.*

Standard solution—Dissolve accurately weighed quantities of USP Benazepril Hydrochloride RS, USP Benazepril Related Compound B RS, USP Benazepril Related Compound C RS, USP Benazepril Related Compound D RS, USP Benazepril Related Compound E RS, USP Benazepril Related Compound F RS, and USP Benazepril Related Compound G RS in *Mobile phase* to obtain a solution having known concentrations of about 1 µg of USP Benazepril Hydrochloride RS per mL and 10 µg of each related compound per mL.

Test solution—Transfer about 50 mg of Benazepril Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Calculate the percentage of benazepril related compounds in the portion of Benazepril Hydrochloride taken by the formula:

$$100(C_S/C_T)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of the relevant USP Reference Standard in the *Standard solution;* C_T is the concentration, in mg per mL, of benazepril hydrochloride in the *Test solution;* r_U is the peak response for the relevant benazepril related compound obtained from the *Test solution;* and r_S is the peak response for the relevant benazepril related

www.webofpharma.com

compound obtained from the *Standard solution* (see *Table 1* for values).

Table 1

Benazepril Related Compound	Relative Retention Time	Limit (%)
E[1]	0.4	0.2
F[2]	0.5	0.2
C[3]	0.6	0.3
B[4]	1.5	0.5
D[5]	1.7	0.2
G[6]	2.0	0.2

[1] 3-Amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid
[2] *t*-Butyl-3-amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid
[3] 3-(1-Carboxy-3-phenyl-(1*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine)-1-acetic acid
[4] Mixture of diastereoisomers (3-(1-ethoxycarbonyl-3-phenyl-(1*R*)-propyl) amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine)-1-acetic acid and (3-(1-ethoxycarbonyl-3-phenyl-(1*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*R*)-benzazepine)-1-acetic acid
[5] 3-(1-Ethoxycarbonyl-3-cyclohexyl-(1*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine)-1-acetic acid monohydrochloride
[6] 3-(1-Ethoxycarbonyl-3-phenyl-(1*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine)-1-acetic acid ethyl ester

In addition to not exceeding the limits for benazepril related compounds in *Table 1* not more than 0.1% of any other single impurity is found; [NOTE—For calculating any other single unspecified impurity, C_S is the concentration of the USP Benazepril Hydrochloride RS in the *Standard solution.*] and not more than 2.0% of total impurities (excluding benazepril related compound A from *Test 1*) is found.

Assay—

Tetrabutylammonium bromide solution—Dissolve 0.81 g of tetrabutylammonium bromide in 360 mL of water containing 0.2 mL of glacial acetic acid.

Mobile phase—Prepare a filtered and degassed mixture of methanol and *Tetrabutylammonium bromide solution* (64:36). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve accurately weighed quantities of USP Benazepril Hydrochloride RS and USP Benazepril Related Compound B RS in *Mobile phase* to obtain a solution having known concentrations of about 0.4 mg of each per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Benazepril Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer about 10.0 mL of the *Test solution* (from either *Test 1* or *Test 2*), prepared as directed in the test for *Related compounds,* to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 3-cm guard column that contains packing L1 connected to a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between benazepril hydrochloride and benazepril related compound B is not less than 1.7; and the relative standard deviation for replicate injections determined from benazepril hydrochloride and benazepril related compound B is not more than 2.0% for each.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in mg, of $C_{24}H_{28}N_2O_5 \cdot HCl$ in the portion of Benazepril Hydrochloride taken by the formula:

$$250C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Benazepril Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Benazepril Hydrochloride Compounded Oral Suspension, Veterinary

DEFINITION

Benazepril Hydrochloride Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of benazepril hydrochloride ($C_{24}H_{28}N_2O_5 \cdot HCl$).

Prepare Benazepril Hydrochloride Compounded Oral Suspension, Veterinary, 5 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Benazepril Hydrochloride powder	500 mg
Vehicle: a 1:1 mixture of Ora-Plus[a] and Ora-Sweet[a], a sufficient quantity to make	100 mL

[a] Perrigo Pharmaceuticals, Allegan, MI.

Pour the *Benazepril Hydrochloride powder* into a suitable container. Wet the powder with a small amount of *Vehicle,* and triturate to make a smooth paste. Add the *Vehicle* to make the contents pourable. Transfer the contents, stepwise and quantitatively, to a calibrated container using the remainder of the *Vehicle.* Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: 25 mM sodium phosphate adjusted with phosphoric acid to a pH of 3.0. Pass through a nylon filter of 0.45-µm pore size.

Mobile phase: Acetonitrile and *Solution A* (40:60)

Diluent: Water adjusted with phosphoric acid to a pH of 3.0

Standard stock solution: 5 mg/mL of USP Benazepril Hydrochloride RS in *Diluent.* Sonicate for 3 min. Mix well, and store at 2°–8°.

Standard solution: 0.01 mg/mL of benazepril hydrochloride prepared with *Standard stock solution* and *Diluent.* Centrifuge for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

Sample solution: Shake thoroughly each bottle of Oral Suspension, Veterinary. Transfer 2.0 mL of the Oral Suspension, Veterinary into a 1-L volumetric flask, and dilute with *Diluent* to volume. Mix well. Centrifuge for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

www.webofpharma.com

Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 30°
Autosampler: 5°
Flow rate: 1.2 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for benazepril hydrochloride is about 6.5 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benazepril hydrochloride ($C_{24}H_{28}N_2O_5 \cdot HCl$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benazepril hydrochloride from the *Sample solution*
r_S = peak response of benazepril hydrochloride from the *Standard solution*
C_S = concentration of benazepril hydrochloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of benazepril hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 3.8–4.8

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
• **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date:* Label it to state that it is for veterinary use only.
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or at controlled room temperature
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benazepril Hydrochloride RS

Benazepril Hydrochloride Tablets

DEFINITION
Benazepril Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of benazepril hydrochloride ($C_{24}H_{28}N_2O_5 \cdot HCl$).

IDENTIFICATION

Delete the following:

▲• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Sample solution: Equivalent to 1.0 mg/mL of benazepril hydrochloride from powdered Tablets (NLT 20) in methanol. [NOTE—Shake by mechanical means for 15 min. Dilute with methanol to volume, mix, and centrifuge. Pass an aliquot of the supernatant through a suitable filter, discarding the first 6 mL of the filtrate.]
Application volume: 20 µL
Developing solvent system: Ethyl acetate, methanol, and ammonium hydroxide (80:20:15)▲ (USP 1-Aug-2019)

Add the following:

▲• **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**
Solution A: Dissolve 0.81 g of tetrabutylammonium bromide in 360 mL of water containing 0.2 mL of acetic acid, glacial.
Mobile phase: Methanol and *Solution A* (64:36)
System suitability solution: 0.4 mg/mL each of USP Benazepril Hydrochloride RS and USP Benazepril Related Compound B RS in *Mobile phase*
Standard solution: 0.2 mg/mL of USP Benazepril Hydrochloride RS in *Mobile phase*
Sample solution: ▲Nominally 0.2 mg/mL of benazepril hydrochloride in *Mobile phase* prepared as follows.▲ (USP 1-Aug-2019) Transfer a portion from NLT 20 finely powdered Tablets, equivalent to 50 mg of benazepril hydrochloride, to a 250-mL volumetric flask. Add ▲▲ (USP 1-Aug-2019) 150 mL of *Mobile phase*, and shake by mechanical means for 30 min. Dilute with *Mobile phase* to volume, mix, and centrifuge. Pass an aliquot of the supernatant through a suitable filter, discarding the first 6 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. ▲For *Identification A*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Aug-2019)
Columns
Guard: 4.6-mm × 3-cm; ▲7-µm▲ (USP 1-Aug-2019) packing ▲L7▲ (USP 1-Aug-2019)
Analytical: 3.9-mm × 30-cm; ▲10-µm▲ (USP 1-Aug-2019) packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL
▲Run time: NLT 3 times the retention time of benazepril▲ (USP 1-Aug-2019)
System suitability
Sample: *System suitability solution*
▲[NOTE—The relative retention times for benazepril and benazepril related compound B peaks are about 1.0 and 1.5, respectively.]▲ (USP 1-Aug-2019)
Suitability requirements
Resolution: NLT ▲2.0▲ (USP 1-Aug-2019) between benazepril ▲▲ (USP 1-Aug-2019) and benazepril related compound B peaks
Relative standard deviation: NMT 2.0% for both benazepril ▲▲ (USP 1-Aug-2019) and benazepril related compound B peaks
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of ▲the labeled amount of▲ (USP 1-Aug-2019) benazepril hydrochloride ($C_{24}H_{28}N_2O_5 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benazepril from the *Sample solution*
r_S = peak response of benazepril from the *Standard solution*
C_S = concentration of USP Benazepril Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benazepril hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Test 1

Medium: Water; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
Solution A, Mobile phase, System suitability solution, ▲▲ (USP 1-Aug-2019) and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: ▲0.02 µg/mL of▲ (USP 1-Aug-2019) USP Benazepril Hydrochloride RS▲▲ (USP 1-Aug-2019) in *Medium*
Sample solution: ▲Pass a portion of the solution under test through a suitable filter. [NOTE—The amount of benazepril injected should not exceed 125% of the quantity of the *Standard solution* (1.5 µg).]
Chromatographic system: Proceed as directed in the *Assay*, except for the *Injection volume*.▲ (USP 1-Aug-2019)
Injection volume: ▲25 µL for *System suitability solution*;▲ (USP 1-Aug-2019) 60 µL ▲for *Standard solution* and *Sample solution*▲ (USP 1-Aug-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲the labeled amount of▲ (USP 1-Aug-2019) benazepril hydrochloride ($C_{24}H_{28}N_2O_5 \cdot HCl$) dissolved:

$$\text{▲Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of benazepril from the *Sample solution*
r_S = peak response of benazepril from the *Standard solution*
C_S = concentration of USP Benazepril Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL▲ (USP 1-Aug-2019)

Tolerances: NLT 80% (Q) of the labeled amount of benazepril hydrochloride ($C_{24}H_{28}N_2O_5 \cdot HCl$) is dissolved.

Test 2: If the product complies to this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium, Apparatus ▲2,▲ (USP 1-Aug-2019) **Standard solution, Sample solution, Chromatographic system,** and **Analysis:** Proceed as directed in *Dissolution Test 1*.
Solution A, Mobile phase, System suitability solution, and **System suitability:** Proceed as directed in the *Assay*.
Time: 45 min

Tolerances: NLT 70% (Q) of the labeled amount of benazepril hydrochloride ($C_{24}H_{28}N_2O_5 \cdot HCl$) is dissolved.

Change to read:

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements
▲▲ (USP 1-Aug-2019)

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Solution A, Mobile phase, System suitability solution, Sample solution, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 0.006 mg/mL of USP Benazepril Related Compound C RS in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*, ▲except for the *Injection volume*.▲ (USP 1-Aug-2019)
Injection volume: ▲25 µL for *System suitability solution*;▲ (USP 1-Aug-2019) 80 µL ▲for *Standard solution* and *Sample solution*▲ (USP 1-Aug-2019)
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of benazepril related compound C in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benazepril related compound C from the *Sample solution*
r_S = peak response of benazepril related compound C from the *Standard solution*
C_S = concentration of USP Benazepril Related Compound C RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benazepril hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the responses of all the peaks including benazepril related compound C from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	▲Relative Retention Time▲ (USP 1-Aug-2019)	Acceptance Criteria, NMT (%)
Benazepril related compound C	▲0.6▲ (USP 1-Aug-2019)	3.0
Benazepril	▲1.0▲ (USP 1-Aug-2019)	—
▲Benazepril related compound B[a, b]	1.5	—▲ (USP 1-Aug-2019)
Any unspecified impurity	—	▲0.2▲ (USP 1-Aug-2019)

www.webofpharma.com

Table 1 *(continued)*

Name	▲Relative Retention Time▲ (USP 1-Aug-2019)	Acceptance Criteria, NMT (%)
Total impurities[c]	—	2.0

[a] 2-[(*SR*)-3-{[(*RS*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino}-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepin-1-yl]acetic acid.
[b] For identification only.
[c] Excluding benazepril related compound C.

ADDITIONAL REQUIREMENTS

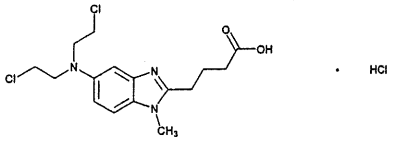
Change to read:

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. ▲Store at controlled room temperature.▲ (USP 1-Aug-2019)
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benazepril Hydrochloride RS
  USP Benazepril Related Compound B RS
  ▲(3*S*)-3-[[(1*R*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepine-1-acetic acid, monohydrochloride;
  Also known as 2-[(*SR*)-3-{[(*RS*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino}-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepin-1-yl]acetic acid hydrochloride.▲ (USP 1-Aug-2019)
  $C_{24}H_{28}N_2O_5 \cdot HCl$ 460.95
  USP Benazepril Related Compound C RS
  ▲(3*S*)-3-[[(1*S*)-1-Carboxy-3-phenylpropyl]amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benazepine]-1-acetic acid;
  Also known as (*S*)-2-{[(*S*)-1-(Carboxymethyl)-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepin-3-yl]amino}-4-phenylbutanoic acid.▲ (USP 1-Aug-2019)
  $C_{22}H_{24}N_2O_5$ 396.44

Bendamustine Hydrochloride



$C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$ 394.72
1*H*-Benzimidazole-2-butanoic acid, 5-[bis(2-chloroethyl) amino]-1-methyl-, monohydrochloride;
4-{5-[Bis(2-chloroethyl)amino]-1-methyl-1*H*-benzimidazole-2-yl}butanoic acid monohydrochloride [3543-75-7].
Bendamustine (free base)
$C_{16}H_{21}Cl_2N_3O_2$ 358.26
[16506-27-7].
Monohydrate
$C_{16}H_{21}Cl_2N_3O_2 \cdot HCl \cdot H_2O$ 412.74
[1374784-02-7].

DEFINITION

Bendamustine Hydrochloride is anhydrous or contains one molecule of hydration. The anhydrous form contains NLT 98.0% and NMT 102.0% of bendamustine hydrochloride ($C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$), calculated on the as-is basis. The monohydrate form contains NLT 98.0% and NMT 102.0% of bendamustine hydrochloride ($C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*

ASSAY

- **PROCEDURE**

Solution A: 0.1% (v/v) trifluoroacetic acid in water
Solution B: 0.1% (v/v) trifluoroacetic acid in acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93	7
5	93	7
13	73	27
16	73	27
25	43	57
26	10	90
31	10	90
40	93	7
45	93	7

Diluent: 1-Methyl-2-pyrrolidone and *Solution A* (1:1)
Standard solution: 4.2 mg/mL of USP Bendamustine Hydrochloride RS in *Diluent*
Sample solution: 4.2 mg/mL of Bendamustine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L60
Temperatures
Autosampler: 2°–8°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 2 µL
Analysis time: 25 min
System suitability
[NOTE—The slower syringe draw rate and higher detector sampling rate can be applied in order to improve the precision.]
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of bendamustine hydrochloride ($C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$) in the portion of Bendamustine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bendamustine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Bendamustine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the as-is basis for the anhydrous form; 98.0%–102.0% on the anhydrous and solvent-free basis for the monohydrate form

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

Change to read:

• **Organic Impurities**
Mobile phase, Diluent, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 4.2 mg/mL of USP Bendamustine Hydrochloride RS, and 0.02 mg/mL each of USP Bendamustine Related Compound A RS, USP Bendamustine Related Compound C RS, USP Bendamustine Related Compound D RS, USP Bendamustine Related Compound E RS, USP Bendamustine Related Compound G RS, USP Bendamustine Related Compound H RS, and USP Bendamustine Related Compound I RS in *Diluent*
Sensitivity solution: 2 µg/mL of USP Bendamustine Hydrochloride RS in *Diluent*, from the *Standard solution*
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 5 between the bendamustine related compound G and bendamustine peaks; NLT 4 between the bendamustine related compound H and bendamustine related compound I peaks, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Bendamustine Hydrochloride taken:

$$\text{Result} = (r_U/\{\Sigma[r_U \times (1/F)] + r_S\}) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
F = relative response factor for each impurity (see *Table 2*)
r_S = peak area of bendamustine from the *Sample solution*

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bendamustine related compound A	0.25	0.76	0.25
Bendamustine related compound C	0.60	0.83	0.20
Bendamustine related compound D	0.69	0.93	▲0.15▲ (RB 1-Mar-2019)
Bendamustine related compound E	0.73	1.2	0.45
Bendamustine related compound G	0.90	3.1	0.35
Bendamustine	1.0	—	—
Bendamustine related compound H	1.15	0.98	0.30
Bendamustine related compound I	1.20	1.1	0.40
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

SPECIFIC TESTS

• **Water Determination** ⟨921⟩, *Method I, Method Ia*: NMT 1.0% for the anhydrous form; 3.0%–5.5% for the monohydrate form
• **Bacterial Endotoxins Test** ⟨85⟩: Meets the requirements
• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total aerobic microbial count is NMT 10^3 cfu/g. The total combined molds and yeasts count is NMT 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.
• **USP Reference Standards** ⟨11⟩
USP Bendamustine Hydrochloride RS
USP Bendamustine Related Compound A RS
4-{5-[Bis(2-hydroxyethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoic acid.
$C_{16}H_{23}N_3O_4$ 321.38
USP Bendamustine Related Compound C RS
Ethyl 4-{5-[bis(2-hydroxyethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoate.
$C_{18}H_{27}N_3O_4$ 349.43
USP Bendamustine Related Compound D RS
4-{5-[(2-Chloroethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoic acid.
$C_{14}H_{18}ClN_3O_2$ 295.77
USP Bendamustine Related Compound E RS
4-{5-[(2-Chloroethyl)(2-hydroxyethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoic acid.
$C_{16}H_{22}ClN_3O_3$ 339.82
USP Bendamustine Related Compound G RS
4-[6-(2-Chloroethyl)-3,6,7,8-tetrahydro-3-methylimidazo[4,5-*h*][1,4]benzothiazin-2-yl]butanoic acid.
$C_{16}H_{20}ClN_3O_2S$ 353.86
USP Bendamustine Related Compound H RS
4-[5-({2-[(4-{5-[Bis(2-chloroethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoyl)oxy]ethyl}(2-chloroethyl)amino)-1-methyl-1*H*-benzimidazol-2-yl]butanoic acid.
$C_{32}H_{41}Cl_3N_6O_4$ 680.07

www.webofpharma.com

USP Bendamustine Related Compound I RS
Ethyl 4-{5-[bis(2-chloroethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoate.
$C_{18}H_{25}Cl_2N_3O_2$ 386.32

Add the following:

▲Bendamustine Hydrochloride for Injection

DEFINITION
Bendamustine Hydrochloride for Injection is a sterile, lyophilized mixture of Bendamustine Hydrochloride and Mannitol. It contains NLT 90.0% and NMT 110.0% of the labeled amount of bendamustine hydrochloride ($C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: Nominally equivalent to 5 µg/mL of bendamustine hydrochloride, from Bendamustine Hydrochloride for Injection, in water
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Solution A: 0.1% (v/v) trifluoroacetic acid in water
Solution B: 0.1% (v/v) trifluoroacetic acid in acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93	7
5	93	7
13	73	27
16	73	27
25	43	57
26	10	90
31	10	90
40	93	7
45	93	7

Diluent: 1-Methyl-2-pyrrolidone and *Solution A* (1:1)
Standard solution: 4.2 mg/mL of USP Bendamustine Hydrochloride RS in *Diluent*
Sample solution: Nominally equivalent to 4.2 mg/mL of bendamustine hydrochloride in *Diluent*, from Bendamustine Hydrochloride for Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L60
Temperatures
Autosampler: 2°–8°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 2 µL
Analysis time: 25 min
System suitability
Sample: *Standard solution*
Suitability requirements
[NOTE—The slower syringe draw rate and higher detector sampling rate can be applied in order to improve the precision.]
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bendamustine hydrochloride ($C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$) in the portion of Bendamustine Hydrochloride for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bendamustine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bendamustine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase, Diluent, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability solution: 4.2 mg/mL of USP Bendamustine Hydrochloride RS, and 0.02 mg/mL each of USP Bendamustine Related Compound A RS, USP Bendamustine Related Compound B RS, USP Bendamustine Related Compound C RS, USP Bendamustine Related Compound D RS, USP Bendamustine Related Compound E RS, USP Bendamustine Related Compound F RS, USP Bendamustine Related Compound G RS, USP Bendamustine Related Compound H RS, and USP Bendamustine Related Compound I RS in *Diluent*
Sensitivity solution: 2 µg/mL of USP Bendamustine Hydrochloride RS in *Diluent*, from the *Standard solution*
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 5 between the bendamustine related compound G and bendamustine peaks; NLT 4 between the bendamustine related compound H and bendamustine related compound I peaks, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Bendamustine Hydrochloride for Injection taken:

www.webofpharma.com

$$\text{Result} = (r_U/\{\Sigma[r_U \times (1/F)] + r_S\}) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
F = relative response factor for each impurity (see *Table 2*)
r_S = peak area of bendamustine from the *Sample solution*

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bendamustine related compound A	0.25	0.76	0.3
Bendamustine related compound B	0.57	0.84	0.2
Bendamustine related compound C[a]	0.60	0.83	—
Bendamustine related compound D	0.69	0.93	0.6
Bendamustine related compound E	0.73	1.2	1.5
Bendamustine related compound F	0.88	0.61	0.5
Bendamustine related compound G[a]	0.90	3.1	—
Bendamustine	1.0	—	—
Bendamustine related compound H	1.15	0.98	0.9
Bendamustine related compound I[a]	1.20	1.1	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	3.5

[a] This process impurity is controlled in the drug substance monograph. It is included in the table for identification only, and it is not to be reported in the total impurities.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Meets the requirements
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **pH** ⟨791⟩: 2.5–3.5 in a constituted solution prepared as directed in the labeling
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Store at controlled room temperature.
- **LABELING** ⟨7⟩: Meets the requirements
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Bendamustine Hydrochloride RS

USP Bendamustine Related Compound A RS
4-{5-[Bis(2-hydroxyethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoic acid.
$C_{16}H_{23}N_3O_4$ 321.38

USP Bendamustine Related Compound B RS
4-(1-Methyl-5-morpholino-1*H*-benzimidazol-2-yl) butanoic acid.
$C_{16}H_{21}N_3O_3$ 303.36

USP Bendamustine Related Compound C RS
Ethyl 4-{5-[bis(2-hydroxyethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoate.
$C_{18}H_{27}N_3O_4$ 349.43

USP Bendamustine Related Compound D RS
4-{5-[(2-Chloroethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoic acid.
$C_{14}H_{18}ClN_3O_2$ 295.77

USP Bendamustine Related Compound E RS
4-{5-[(2-Chloroethyl)(2-hydroxyethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoic acid.
$C_{16}H_{22}ClN_3O_3$ 339.82

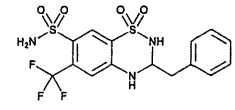
USP Bendamustine Related Compound F RS
Mannitol-1-yl 4-{5-[bis(2-chloroethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoate.
$C_{22}H_{33}Cl_2N_3O_7$ 522.42

USP Bendamustine Related Compound G RS
4-[6-(2-Chloroethyl)-3,6,7,8-tetrahydro-3-methylimidazo[4,5-*h*][1,4]benzothiazin-2-yl]butanoic acid.
$C_{16}H_{20}ClN_3O_2S$ 353.86

USP Bendamustine Related Compound H RS
4-[5-({2-[(4-{5-[Bis(2-chloroethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoyl)oxy]ethyl}(2-chloroethyl) amino)-1-methyl-1*H*-benzimidazol-2-yl]butanoic acid.
$C_{32}H_{41}Cl_3N_6O_4$ 680.07

USP Bendamustine Related Compound I RS
Ethyl 4-{5-[bis(2-chloroethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoate.
$C_{18}H_{25}Cl_2N_3O_2$ 386.32▲ 2S (USP41)

Bendroflumethiazide



$C_{15}H_{14}F_3N_3O_4S_2$ 421.41

2*H*-1,2,4-Benzothiadiazine-7-sulfonamide, 3,4-dihydro-3-(phenylmethyl)-6-(trifluoromethyl)-, 1,1-dioxide, (±)-; (±)-3-Benzyl-3,4-dihydro-6-(trifluoromethyl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [73-48-3].

DEFINITION

Bendroflumethiazide contains NLT 98.0% and NMT 102.0% of bendroflumethiazide ($C_{15}H_{14}F_3N_3O_4S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Use low-actinic glassware for the *Standard solution* and the *Sample solution*, and use the solutions within 30 min of preparation.

Diluent: Methanol and water (80:20)

Mobile phase: Dissolve 5.62 g of sodium chloride and 1.97 g of anhydrous sodium sulfate in 1000 mL of water in a 2-L volumetric flask. Add 4.0 mL of glacial acetic acid and 800 mL of methanol, and dilute with water to volume.

Standard solution: 0.05 mg/mL of USP Bendroflumethiazide RS in *Diluent*

Sample solution: 0.05 mg/mL of Bendroflumethiazide in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 15-cm; 5-µm packing L11

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Run time: NLT 2 times the retention time of bendroflumethiazide

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of bendroflumethiazide ($C_{15}H_{14}F_3N_3O_4S_2$) in the portion of Bendroflumethiazide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of bendroflumethiazide from the *Sample solution*

r_S = peak response of bendroflumethiazide from the *Standard solution*

C_S = concentration of USP Bendroflumethiazide RS in the *Standard solution* (mg/mL)

C_U = concentration of Bendroflumethiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%

• **SELENIUM** ⟨291⟩

Sample: 100 mg of Bendroflumethiazide and 100 mg of magnesium oxide

Acceptance criteria: The absorbance from the *Test Solution* is NMT one-half that from the *Standard Solution* (NMT 30 ppm).

• **LIMIT OF 2,4-DISULFAMYL-5-TRIFLUOROMETHYLANILINE**

Use low-actinic glassware for the *Standard solution* and the *Sample solution*.

Mobile phase: Dissolve 5.62 g of sodium chloride and 1.97 g of anhydrous sodium sulfate in 1000 mL of water in a 2-L volumetric flask. Add 4.0 mL of glacial acetic acid and 800 mL of methanol, and dilute with water to volume.

Standard solution: 0.75 µg/mL of USP 2,4-Disulfamyl-5-trifluoromethylaniline RS in methanol

Sample solution: 50 µg/mL of Bendroflumethiazide in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 30-cm; packing L11

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.4 between the methanol and 2,4-disulfamyl-5-trifluoromethylaniline peaks

Relative standard deviation: NMT 3.0% for 5 replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 2,4-disulfamyl-5-trifluoromethylaniline in the portion of Bendroflumethiazide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 2,4-disulfamyl-5-trifluoromethylaniline from the *Sample solution*

r_S = peak response of 2,4-disulfamyl-5-trifluoromethylaniline from the *Standard solution*

C_S = concentration of USP 2,4-Disulfamyl-5-trifluoromethylaniline RS in the *Standard solution* (µg/mL)

C_U = concentration of Bendroflumethiazide in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 1.5%

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Bendroflumethiazide RS

USP 2,4-Disulfamyl-5-trifluoromethylaniline RS

4-Amino-6-(trifluoromethyl)-benzene-1,3-disulfonamide.

$C_7H_8F_3N_3O_4S_2$ 319.29

Bendroflumethiazide Tablets

DEFINITION

Bendroflumethiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bendroflumethiazide ($C_{15}H_{14}F_3N_3O_4S_2$).

IDENTIFICATION

• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

[NOTE—Use low-actinic glassware for the *Sample solution* and the *Standard solution*.]

Mobile phase: Dissolve 5.62 g of sodium chloride and 1.97 g of anhydrous sodium sulfate in 1000 mL of water in a 2-L volumetric flask. Add 4.0 mL of glacial acetic acid and 800 mL of methanol, and dilute with water to volume.

Standard solution: 50 µg/mL of USP Bendroflumethiazide RS in methanol

www.webofpharma.com

Sample solution: Nominally equivalent to 50 µg/mL from finely powdered Tablets (NLT 20). Initially add methanol (70% of the volume of the flask) and sonicate for 15 min with occasional shaking. Dilute further with methanol to the required concentration, and centrifuge for 15 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 30-cm; packing L11
Temperature: 35 ± 5°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0% for five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{15}H_{14}F_3N_3O_4S_2$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bendroflumethiazide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of bendroflumethiazide in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

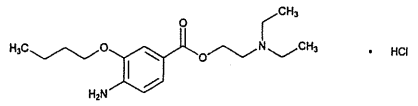
PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
[Note—Protect solutions from light throughout this test.]
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Detector: UV 271 nm
Sample solution: Sample per *Dissolution* ⟨711⟩.
Standard solution: Prepare a stock solution of USP Bendroflumethiazide RS in an appropriate organic solvent, and dilute this solution with *Medium* to obtain a final concentration similar to the one expected in the *Sample solution*.
Analysis: Determine the amount of $C_{15}H_{14}F_3N_3O_4S_2$ dissolved by using UV absorption on filtered portions of the *Sample solution*, suitably diluted with water, if necessary, in comparison with a *Standard solution* having a known concentration of USP Bendroflumethiazide RS.
Tolerances: NLT 75% (Q) of the labeled amount of $C_{15}H_{14}F_3N_3O_4S_2$ is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Bendroflumethiazide RS

Benoxinate Hydrochloride



$C_{17}H_{28}N_2O_3 \cdot HCl$ 344.88
Benzoic acid, 4-amino-3-butoxy-, 2-(diethylamino)ethyl ester, monohydrochloride;
2-(Diethylamino)ethyl 4-amino-3-butoxybenzoate monohydrochloride [5987-82-6].

DEFINITION

Benoxinate Hydrochloride contains NLT 98.5% and NMT 101.5% of benoxinate hydrochloride ($C_{17}H_{28}N_2O_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Previously dried
Acceptance criteria: Meets the requirements

Change to read:

• **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 15 µg/mL in water
Acceptance criteria: Meets the requirements
• **C. Identification Tests—General,** *Chloride* ⟨191⟩
Sample solution: 10 mg/mL
Acceptance criteria: Meets the requirements

ASSAY

• **Procedure**
Sample solution: Dissolve 250 mg of Benoxinate Hydrochloride in a mixture of 20 mL of glacial acetic acid and 20 mL of acetic anhydride.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Analysis: Titrate, perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 34.49 mg of benoxinate hydrochloride ($C_{17}H_{28}N_2O_3 \cdot HCl$).
Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.2%
• **Ordinary Impurities** ⟨466⟩
Standard solution: Methanol
Sample solution: Methanol
Eluant: A mixture of chloroform, cyclohexane, and diethylamine (5:4:1)
Visualization: 12

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 5.0–6.0
• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample at 105° for 3 h.
Acceptance criteria: NMT 1.0%

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benoxinate Hydrochloride RS

Benoxinate Hydrochloride Ophthalmic Solution

DEFINITION
Benoxinate Hydrochloride Ophthalmic Solution is a sterile solution of Benoxinate Hydrochloride in water. It contains NLT 95.0% and NMT 105.0% of the labeled amount of benoxinate hydrochloride ($C_{17}H_{28}N_2O_3 \cdot HCl$).

IDENTIFICATION
- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩
  Sample solution: Nominally 2 mg/mL of benoxinate hydrochloride, prepared as follows. Dilute a volume of solution, equivalent to 50 mg of benoxinate hydrochloride, with 0.01 N hydrochloric acid to 25 mL.
  Analysis: Proceed as directed in the chapter, beginning with "Transfer the liquid to a separator".
  Acceptance criteria: The solution meets the requirements.

ASSAY
- **PROCEDURE**
  Standard solution: 400 µg/mL of USP Benoxinate Hydrochloride RS in 0.1 N hydrochloric acid
  Sample solution: Nominally 400 µg/mL of benoxinate hydrochloride, prepared as follows. Transfer a volume of Ophthalmic Solution, equivalent to 20 mg of benoxinate hydrochloride, to a separator containing 15 mL of water. Add 1 mL of ammonium hydroxide, and extract with five 20-mL portions of ether. Wash the combined ether extracts with 10 mL of water, extract the water washing with 10 mL of ether, and add this ether extract to the main extract. Extract the ether solution with three 5-mL portions of 0.1 N hydrochloric acid, collect the acid extracts in a 50-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.
  Instrumental conditions
  Mode: UV
  Analytical wavelength: About 308 nm
  Cell: 1 cm
  Blank: 0.1 N hydrochloric acid
  Analysis
  Samples: *Standard solution*, *Sample solution*, and *Blank*
  Transfer 5.0 mL of each sample to separate 200-mL volumetric flasks. Dilute the contents of each flask with water to volume. Concomitantly determine the absorbances of the solutions, using the *Blank* to set the instrument.
  Calculate the percentage of the labeled amount of benoxinate hydrochloride ($C_{17}H_{28}N_2O_3 \cdot HCl$) in each mL of the Ophthalmic Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

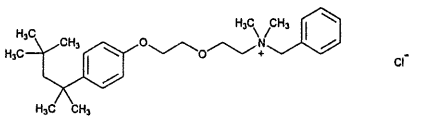
A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Benoxinate Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of benoxinate hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **pH** ⟨791⟩**:** 3.0–6.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benoxinate Hydrochloride RS

Benzethonium Chloride



$C_{27}H_{42}ClNO_2$ 448.08
Benzenemethanaminium, *N*,*N*-dimethyl-*N*-[2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]-, chloride;
Benzyldimethyl[2-[2-[*p*-(1,1,3,3-tetramethylbutyl)phenoxy] ethoxy]ethyl]ammonium chloride [121-54-0].

DEFINITION
Benzethonium Chloride contains NLT 97.0% and NMT 103.0% of benzethonium chloride ($C_{27}H_{42}ClNO_2$), calculated on the dried basis.

IDENTIFICATION
- **A.**
  Sample solution: 10 mg/mL
  Analysis: Add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS to 1 mL of the *Sample solution*.
  Acceptance criteria: A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Buffer: Dilute 20 mL of triethylamine with water to 1000 mL, and adjust with phosphoric acid to a pH of 3.0.
  Mobile phase: Acetonitrile and *Buffer* (42:58)
  Diluent: Acetonitrile and water (42:58)
  System suitability solution: 0.15 mg/mL each of USP Benzethonium Chloride RS and USP Methylbenzethonium Chloride RS in *Diluent*
  Standard solution: 0.15 mg/mL of USP Benzethonium Chloride RS in *Diluent*
  Sample solution: 0.15 mg/mL of Benzethonium Chloride in *Diluent*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 225 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L7
  Column temperature: 40°
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  Run time: 1.5 times the retention time of the methylbenzethonium peak

USP Monographs

www.webofpharma.com

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for benzethonium and methylbenzethonium are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 7.0 between the benzethonium and methylbenzethonium peaks
Tailing factor: NMT 2.0 for the benzethonium peak
Relative standard deviation: NMT 1.0% for the benzethonium peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of benzethonium chloride ($C_{27}H_{42}ClNO_2$) in the portion of Benzethonium Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzethonium from the *Sample solution*
r_S = peak response of benzethonium from the *Standard solution*
C_S = concentration of USP Benzethonium Chloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Benzethonium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

• **ORGANIC IMPURITIES**
Buffer, Mobile phase, Diluent, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1 µg/mL of USP Benzethonium Chloride RS in *Diluent*
Sample solution: 1 mg/mL of Benzethonium Chloride in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements: Proceed as directed in the *Assay*, except for *Relative standard deviation*.
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Benzethonium Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of benzethonium from the *Standard solution*
C_S = concentration of USP Benzethonium Chloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Benzethonium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria
Total impurities: ▲NMT▲ (ERR 1-Feb-2019) 1.0%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benzethonium Chloride RS
USP Methylbenzethonium Chloride RS

Benzethonium Chloride Concentrate

DEFINITION
Benzethonium Chloride Concentrate contains NLT 94.0% and NMT 106.0% of the labeled amount of benzethonium chloride ($C_{27}H_{42}ClNO_2$).

IDENTIFICATION
• **A.**
Sample: Evaporate a volume of the Concentrate, equivalent to 200 mg of benzethonium chloride, on a steam bath.
Analysis: To the residue add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS.
Acceptance criteria: A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.
• **B.**
Sample: Evaporate a volume of the Concentrate, equivalent to 200 mg of benzethonium chloride, on a steam bath.
Analysis: To the residue add 0.1 g of potassium nitrate, and heat on a steam bath for 3 min. Cautiously dilute the solution with water to 10 mL, add 0.5 g of granulated zinc, and warm the mixture for 10 min. Cool. Add 0.2 g of sodium nitrite to 1 mL of the clear liquid, and add this mixture to 20 mg of naphthol dipotassium disulfonate or naphthol disodium disulfonate in 1 mL of ammonium hydroxide.
Acceptance criteria: The solution turns orange-red, and a brown precipitate may be formed.

ASSAY
• **PROCEDURE**
Sample solution: Equivalent to 200 mg of benzethonium chloride from a volume of Concentrate, in a glass-stoppered flask
Analysis: Add 0.4 mL of bromophenol blue solution (1 in 2000), 10 mL of chloroform, and 1 mL of 1 N sodium hydroxide. Titrate with 0.02 M sodium tetraphenylboron VS until the blue color disappears from the chloroform layer. Add the last portions of the sodium tetraphenylboron solution dropwise, agitating vigorously after each addition. Each mL of 0.02 M sodium tetraphenylboron is equivalent to 8.962 mg of benzethonium chloride ($C_{27}H_{42}ClNO_2$).
Acceptance criteria: 94.0%–106.0% of the labeled amount of benzethonium chloride

IMPURITIES
• **LIMIT OF NITRITES**
Sample: One drop of Concentrate on a spot plate
Analysis: To the *Sample* add one drop each of glacial acetic acid, sulfanilic acid in acetic acid solution (1 in 100), and 1-naphthylamine-acetic acid solution (prepared by boiling 30 mg of 1-naphthylamine in 70 mL of water, decanting the colorless solution from the blue-violet residue, and mixing with 30 mL of glacial acetic acid).

www.webofpharma.com

Acceptance criteria: No red color develops in the resulting solution within 10 min.

SPECIFIC TESTS

- **OXIDIZING SUBSTANCES**

Sample: 5 mL

Analysis: To the *Sample* add 0.5 mL of potassium iodide TS and a few drops of 3 N hydrochloric acid.

Acceptance criteria: The solution does not acquire a yellow color.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
- **LABELING:** The label states that this article is not intended for direct administration to humans or animals.

Benzethonium Chloride Topical Solution

DEFINITION

Benzethonium Chloride Topical Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of benzethonium chloride ($C_{27}H_{42}ClNO_2$).

IDENTIFICATION

- **A.**

Sample solution: Evaporate a volume of Topical Solution, equivalent to 200 mg of benzethonium chloride, on a steam bath.

Analysis: To the residue add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS.

Acceptance criteria: A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.

- **B.**

Sample solution: Evaporate a volume of Topical Solution, equivalent to 200 mg of benzethonium chloride, on a steam bath.

Analysis: To the residue add 0.1 g of potassium nitrate, and heat on a steam bath for 3 min. Cautiously dilute the solution with water to 10 mL, add 0.5 g of granulated zinc, and warm the mixture for 10 min. Cool. Add 0.2 g of sodium nitrite to 1 mL of the clear liquid, and add this mixture to 20 mg of naphthol dipotassium disulfonate or naphthol disodium disulfonate in 1 mL of ammonium hydroxide.

Acceptance criteria: The solution turns orange-red, and a brown precipitate may be formed.

ASSAY

- **PROCEDURE**

Sample solution: Equivalent to 200 mg of benzethonium chloride from a volume of Topical Solution, in a glass-stoppered flask

Analysis: Add 0.4 mL of bromophenol blue solution (1 in 2000), 10 mL of chloroform, and 1 mL of 1 N sodium hydroxide. Titrate with 0.02 M sodium tetraphenylboron VS until the blue color disappears from the chloroform layer. Add the last portions of the sodium tetraphenylboron solution dropwise, agitating vigorously after each addition. Each mL of 0.02 M sodium tetraphenylboron is equivalent to 8.962 mg of benzethonium chloride ($C_{27}H_{42}ClNO_2$).

Acceptance criteria: 95.0%–105.0% of the labeled amount of benzethonium chloride

IMPURITIES

- **ORGANIC IMPURITIES, LIMIT OF NITRITES**

Sample: One drop of Topical Solution on a spot plate

Analysis: To the *Sample* add one drop each of glacial acetic acid, sulfanilic acid in acetic acid (1 in 100), and 1-naphthylamine–acetic acid solution (prepared by boiling 30 mg of 1-naphthylamine in 70 mL of water, decanting the colorless solution from the blue-violet residue, and mixing with 30 mL of glacial acetic acid).

Acceptance criteria: No red color develops in the resulting solution within 10 min.

SPECIFIC TESTS

- **OXIDIZING SUBSTANCES**

Sample: 5 mL

Analysis: To the *Sample* add 0.5 mL of potassium iodide TS and a few drops of 3 N hydrochloric acid.

Acceptance criteria: The solution does not acquire a yellow color.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Benzethonium Chloride Tincture

DEFINITION

Benzethonium Chloride Tincture contains, in each 100 mL, NLT 190 mg and NMT 210 mg of benzethonium chloride ($C_{27}H_{42}ClNO_2$).

Prepare Benzethonium Chloride Tincture 2 mg/mL as follows.

Benzethonium Chloride	2 g
Alcohol	685 mL
Acetone	100 mL
Purified Water, a sufficient quantity to make	1000 mL

Dissolve the *Benzethonium Chloride* in a mixture of *Alcohol* and *Acetone*. Add sufficient *Purified Water* to make 1000 mL. [NOTE—Benzethonium Chloride Tincture may be colored by the addition of any suitable color or combination of colors certified by the FDA for use in drugs.]

IDENTIFICATION

- **A. PROCEDURE**

Sample: 50 mL

Analysis: To the residue obtained by evaporating the *Sample* on a steam bath, add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS.

Acceptance criteria: A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.

- **B. PROCEDURE**

Sample: 50 mL

Analysis: Evaporate the *Sample* on a steam bath.

Acceptance criteria: The residue obtained forms precipitates with 2 N nitric acid and with mercuric chloride TS, both of which dissolve upon the addition of alcohol.

ASSAY

- **PROCEDURE**

Sample: 50 mL

Analysis: Transfer the *Sample* to a 150-mL beaker, and add, with continuous stirring, 10 mL of 25 mg/mL of sodium tetraphenylboron solution. Cover, and allow to stand for 16

www.webofpharma.com

h. Decant the supernatant into a tared sintered-glass crucible, applying vacuum filtration. Suspend the precipitate in 20 mL of water. Transfer the precipitate to the crucible, washing well with water. Dry the precipitate and the crucible at 105° for 1 h, cool, and weigh. The weight of the precipitate so obtained, multiplied by 0.6122, represents its equivalent of benzethonium chloride ($C_{27}H_{42}ClNO_2$).

Acceptance criteria: 190–210 mg

OTHER COMPONENTS

• **ALCOHOL AND ACETONE CONTENT**

Standard solution A (alcohol low standard solution): Add 5.0 mL of methanol and 11.0 mL of dehydrated alcohol to a 100-mL volumetric flask, and dilute with water to volume.

Standard solution B (alcohol high standard solution): Add 5.0 mL of methanol and 14.0 mL of dehydrated alcohol to a 100-mL volumetric flask, and dilute with water to volume.

Standard solution C (acetone low standard solution): Add 5.0 mL of methanol and 1.7 mL of acetone to a 100-mL volumetric flask, and dilute with water to volume.

Standard solution D (acetone high standard solution): Add 5.0 mL of methanol and 2.2 mL of acetone to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 20 mL of Tincture to a 100-mL volumetric flask, add 5.0 mL of methanol as the internal standard, and dilute with water to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 120-cm × 4-mm packed with a suitable type of support, such as 80- to 100-mesh S3
Carrier gas: Dry helium
Temperature
Injector port: 240°
Detector block: 240°
Column: 120°
Flow rate: 90 mL/min
Injection size: 0.8 µL

Analysis
Samples: *Standard solutions A, B, C,* and *D,* and *Sample solution*
From the respective chromatograms obtained as described previously, calculate the ratios of peak areas for alcohol to internal standard and for acetone to internal standard. Calculate the percentage of alcohol and of acetone in the portion of Tincture taken:

$$\text{Result} = [A(Y - Z) + B(Z - X)]/(Y - X)$$

A = percentage of alcohol, or of acetone, in *Standard solution A* and *Standard solution C,* respectively
Y = ratio of the alcohol peak areas, or the acetone peak areas, to the internal standard peak areas for *Standard solution B* and *Standard solution D,* respectively
Z = ratio of the alcohol peak areas, or the acetone peak areas, to the internal standard peak areas for the *Sample solution*
B = percentage of alcohol, or of acetone, in *Standard solution B* and *Standard solution D,* respectively
X = ratio of the alcohol peak areas, or the acetone peak areas, to the internal standard peak areas for *Standard solution A* and *Standard solution C,* respectively

Acceptance criteria
Alcohol (C_2H_5OH): 62.0%–68.0%
Acetone (C_3H_6O): 9.0%–11.0%

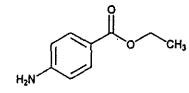
SPECIFIC TESTS

• **SPECIFIC GRAVITY** ⟨841⟩: 0.868–0.876

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers.

Benzocaine



$C_9H_{11}NO_2$ 165.19
Benzoic acid, 4-amino-, ethyl ester;
Ethyl *p*-aminobenzoate [94-09-7].

DEFINITION
Benzocaine, dried over phosphorus pentoxide for 3 h, contains NLT 98.0% and NMT 102.0% of benzocaine ($C_9H_{11}NO_2$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Previously dried over phosphorus pentoxide for 3 h
Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Solution A: Acetic acid, triethylamine, and water (20:1:980). The pH should be between 2.95 and 3.0 (adjust as needed).
Mobile phase: Methanol and *Solution A* (40:60)
Standard solution: 0.024 mg/mL of USP Benzocaine RS in *Mobile phase*
Sample solution: 0.024 mg/mL of Benzocaine in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 2.0-mm × 15-cm; 5-µm packing L11
Flow rate: 0.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of benzocaine ($C_9H_{11}NO_2$) in the portion of Benzocaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

Stage 6 Harmonization

www.webofpharma.com

C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = concentration of Benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the previously dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE**

Analysis: To a solution of 200 mg in 5 mL of alcohol, previously acidified with a few drops of diluted nitric acid, add a few drops of silver nitrate TS.
Acceptance criteria: No turbidity is produced immediately.

- **ORGANIC IMPURITIES**

Solution A: Add 1.0 mL of trifluoroacetic acid in 1 L of water.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

Diluent: *Solution A* and *Solution B* (1:1)
Standard stock solution: 0.1 mg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-nitrobenzoate RS in *Diluent*. Sonicate for 2–5 min to dissolve before diluting to final volume.
Standard solution: 1 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-nitrobenzoate RS in *Diluent* from the *Standard stock solution*
Sample solution: 1 mg/mL of Benzocaine in *Diluent*. Sonicate for 2–5 min to assist in dissolution as needed before diluting to final volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 10 from any two peaks
Relative standard deviation: NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Benzocaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*
r_S = peak response of corresponding reference standard from the *Standard solution*
C_S = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-nitrobenzoate RS in the *Standard solution* (mg/mL)
C_U = concentration of Benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity in the portion of Benzocaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = concentration of Benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.29	0.10
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.10
Any other unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry over phosphorus pentoxide for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Aminobenzoic Acid RS
Benzoic acid, 4-amino.
$C_7H_7NO_2$ 137.14
USP Benzocaine RS
USP Ethyl 4-nitrobenzoate RS
Benzoic acid, 4-nitro-, ethyl ester.
$C_9H_9NO_4$ 195.17

Benzocaine Topical Aerosol

DEFINITION
Benzocaine Topical Aerosol is a solution of Benzocaine in a pressurized container. It contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ($C_9H_{11}NO_2$).

IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

Diluent: *Solution A* and *Solution B* (1:1)

System suitability solution: 1 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Benzocaine RS in *Diluent*

Sample composite: Spray a portion of Topical Aerosol into a beaker or glass tube, and heat on a steam bath or a heating module at 100° for a few min to expel residual propellant. Use the resulting benzocaine solution.

Sample solution: Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a known quantity of benzocaine solution from a portion of *Sample composite* to an appropriate volumetric flask, dissolve, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm. For *Identification* test *A*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 6 between aminobenzoic acid and benzocaine; NLT 6 between benzocaine and ethyl 4-nitrobenzoate, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzocaine ($C_9H_{11}NO_2$) in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzocaine from the *Sample solution*

r_S = peak response of benzocaine from the *Standard solution*

C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Diluent: *Solution A* and *Solution B* (1:1)

Standard solution: 1 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*

Sample composite: Spray a portion of the Topical Aerosol into a beaker or a glass tube, and heat on a steam bath or a heating module at 100° for a few min to expel residual propellant. Use the resulting benzocaine solution.

Sample solution: Nominally 0.5 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer 50 mg of benzocaine from a portion of *Sample composite* to a 100-mL volumetric flask, dissolve, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 6 between aminobenzoic acid and benzocaine; NLT 6 between benzocaine and ethyl 4-nitrobenzoate

Relative standard deviation: NMT 3% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*

r_S = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Standard solution*

C_S = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual, unspecified impurity in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual, unspecified impurity from the *Sample solution*

r_S = peak response of benzocaine from the *Standard solution*

C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

www.webofpharma.com

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.3	0.20
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.20
Any other individual, unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS
- **TOPICAL AEROSOLS,** *Pressure Test* ⟨603⟩**:** Meets the requirements
- **LEAK RATE** ⟨604⟩**:** Meets the requirements
- **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in pressurized containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aminobenzoic Acid RS
  Benzoic acid, 4-amino.
  $C_7H_7NO_2$ 137.14
  USP Benzocaine RS
  USP Ethyl 4-Nitrobenzoate RS
  Benzoic acid, 4-nitro-, ethyl ester.
  $C_9H_9NO_4$ 195.17

Benzocaine Cream

DEFINITION
Benzocaine Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ($C_9H_{11}NO_2$) in a suitable cream base.

IDENTIFICATION
- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

Diluent: *Solution A* and *Solution B* (1:1)
System suitability solution: 1 µg/mL of USP Benzocaine RS and 2 µg/mL each of USP Aminobenzoic Acid RS and USP Ethyl 4-nitrobenzoate RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Benzocaine RS in *Diluent*. Sonicate to dissolve, if necessary.
Sample solution: Nominally equivalent to 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a portion of Cream, nominally equivalent to 10 mg of benzocaine, into a 100-mL volumetric flask, and dissolve it in about 2% of the final volume of tetrahydrofuran. Dilute with *Diluent* to volume, and pass through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. For *Identification* test *A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 10 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzocaine ($C_9H_{11}NO_2$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzocaine from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

IMPURITIES
- **ORGANIC IMPURITIES**

Solution A: 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L
Solution B: Acetonitrile
Mobile phase: See *Table 1* in the *Assay*.
Diluent: *Solution A* and *Solution B* (1:1)
Standard solution: 1 µg/mL of USP Benzocaine RS and 2 µg/mL each of USP Aminobenzoic Acid RS and USP Ethyl 4-nitrobenzoate RS in *Diluent*
Sample solution: Nominally equivalent to 1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a portion of Cream, nominally equivalent to 50 mg of benzocaine, into a volumetric flask, and dissolve in 10% of the final volume of tetrahydrofuran, with the aid of sonication as needed. Dilute with *Diluent* to volume, and pass through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7

www.webofpharma.com

Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 10 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate
Relative standard deviation: NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*
r_S = peak response of the corresponding Reference Standard from the *Standard solution*
C_S = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-nitrobenzoate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified impurity from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.29	0.20
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.20
Any other individual unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and avoid prolonged exposure to temperatures exceeding 30°.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Aminobenzoic Acid RS
Benzoic acid, 4-amino.
$C_7H_7NO_2$ 137.14
USP Benzocaine RS
USP Ethyl 4-nitrobenzoate RS
Benzoic acid, 4-nitro-, ethyl ester.
$C_9H_9NO_4$ 195.17

Benzocaine Gel

DEFINITION
Benzocaine Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ($C_9H_{11}NO_2$).

IDENTIFICATION
• **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

Diluent: *Solution A* and *Solution B* (1:1)
System suitability solution: 1 µg/mL of USP Benzocaine RS and 2 µg/mL each of USP Aminobenzoic Acid RS and USP Ethyl 4-nitrobenzoate RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Benzocaine RS in *Diluent*. Sonicate to dissolve, if necessary.
Sample solution: Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a portion of Gel, equivalent to 10 mg of benzocaine, into a 100-mL volumetric flask and dissolve it in *Diluent*. Pass through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. For *Identification* test *A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 10 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*

www.webofpharma.com

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzocaine ($C_9H_{11}NO_2$) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzocaine from the *Sample solution*

r_S = peak response of benzocaine from the *Standard solution*

C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Minimum Fill** ⟨755⟩**:** Meets the requirements

IMPURITIES

• **Organic Impurities**

Solution A: 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L

Solution B: Acetonitrile

Mobile phase: See *Table 1* in the *Assay*.

Diluent: *Solution A* and *Solution B* (1:1)

Standard solution: 1 µg/mL of USP Benzocaine RS and 2 µg/mL each of USP Aminobenzoic Acid RS and USP Ethyl 4-nitrobenzoate RS in *Diluent*

Sample solution: Nominally 1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a portion of Gel, equivalent to 50 mg of benzocaine, into a 50-mL volumetric flask, and dissolve in *Diluent*. Pass through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 10 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate

Relative standard deviation: NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*

r_S = peak response of the corresponding Reference Standard from the *Standard solution*

C_S = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-nitrobenzoate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified impurity from the *Sample solution*

r_S = peak response of benzocaine from the *Standard solution*

C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.29	0.20
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.20
Any other individual unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards** ⟨11⟩

USP Aminobenzoic Acid RS

Benzoic acid, 4-amino.

$C_7H_7NO_2$ 137.14

USP Benzocaine RS

USP Ethyl 4-nitrobenzoate RS

Benzoic acid, 4-nitro-, ethyl ester.

$C_9H_9NO_4$ 195.17

Benzocaine Lozenges

DEFINITION

Benzocaine Lozenges contain NLT 85.0% and NMT 120.0% of the labeled amount of benzocaine ($C_9H_{11}NO_2$).

IDENTIFICATION

• **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: 1.0 M monobasic potassium phosphate, adjusted with phosphoric acid to a pH of 3.0

Mobile phase: Acetonitrile, water, and *Buffer* (250:700:50)

Diluent A: 0.1 N hydrochloric acid

Diluent B: Acetonitrile and water (1:1)

Standard solution A: 0.01 mg/mL of USP Benzocaine RS in *Diluent A*

Standard solution B: 0.01 mg/mL of USP Benzocaine RS in *Diluent B*

Sample stock solution A: Transfer the equivalent of 40 mg of benzocaine from powdered Lozenges (NLT 20) to a 200-mL volumetric flask. Add 150 mL of *Diluent A*, and stir for NLT 2 h. Dilute with *Diluent A* to volume.

Sample stock solution B: Transfer the equivalent of 40 mg of benzocaine from powdered Lozenges (NLT 20) to a 200-mL volumetric flask. Add 150 mL of *Diluent B*, and stir for NLT 30 min. Dilute with *Diluent B* to volume.

Sample solution A: Nominally 0.01 mg/mL of benzocaine in *Diluent A* from *Sample stock solution A*

Sample solution B: Nominally 0.01 mg/mL of benzocaine in *Diluent B* from *Sample stock solution B*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm. For *Identification* test *A*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Sample solution A*, and *Sample solution B*

Calculate the percentage of the total labeled amount of benzocaine ($C_9H_{11}NO_2$) in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from *Sample solution A*

r_S = peak response from *Standard solution A*

C_S = concentration of USP Benzocaine RS in *Standard solution A* (mg/mL)

C_U = nominal concentration of benzocaine in *Sample solution A* (mg/mL)

Calculate the percentage of free benzocaine ($C_9H_{11}NO_2$) in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from *Sample solution B*

r_S = peak response from *Standard solution B*

C_S = concentration of USP Benzocaine RS in *Standard solution B* (mg/mL)

C_U = nominal concentration of benzocaine in *Sample solution B* (mg/mL)

Acceptance criteria

Total labeled amount of benzocaine: 85.0%–120.0%

Free benzocaine: 85.0%–120.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: Dissolve 9.1 g of monobasic potassium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
10	45	55
10.1	60	40
13	60	40

Diluent: Acetonitrile and water (10:90)

Standard stock solution: 0.03 mg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*. Sonicate for 2–5 min to dissolve before diluting to final volume.

Standard solution: 0.3 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent* from the *Standard stock solution*

Sample solution: Nominally 150 µg/mL of benzocaine in *Diluent* prepared as follows. Transfer 10 Lozenges to an appropriate volumetric flask to obtain a nominal benzocaine concentration of 0.15 mg/mL. Dissolve Lozenges in *Diluent* and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 10 between benzocaine and aminobenzoic acid; NLT 10 between ethyl 4-nitrobenzoate and benzocaine

Relative standard deviation: NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*

r_S = peak response of the corresponding Reference Standard from the *Standard solution*

C_S = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of benzocaine in the *Sample solution* (µg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.46	0.2
Benzocaine	1.00	—
Ethyl 4-nitrobenzoate	1.86	0.2
Any unspecified degradation product	—	0.1
Total degradation products	—	2.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aminobenzoic Acid RS
  Benzoic acid, 4-amino.
  $C_7H_7NO_2$ 137.14
  USP Benzocaine RS
  USP Ethyl 4-Nitrobenzoate RS
  Benzoic acid, 4-nitro-, ethyl ester.
  $C_9H_9NO_4$ 195.17

Benzocaine Ointment

DEFINITION

Benzocaine Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ($C_9H_{11}NO_2$) in a suitable ointment base.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.1% Trifluoroacetic acid, prepared by adding 1.0 mL of trifluoroacetic acid to 1 L of water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

Diluent: *Solution A* and *Solution B* (1:1)
Standard solution: 0.1 mg/mL of USP Benzocaine RS in *Diluent*. Sonication may be needed to aid in the dissolution.
Sample solution: Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows
Ointments having water-soluble bases: Transfer a portion of Ointment, equivalent to 10 mg of benzocaine, into a volumetric flask, and dissolve in *Diluent*.
Ointments having water-insoluble bases: Transfer a portion of Ointment, equivalent to 10 mg of benzocaine, into a volumetric flask, and dissolve in tetrahydrofuran, using about 2% of the final volume, then dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. For *Identification* test *B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzocaine ($C_9H_{11}NO_2$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzocaine from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: 0.1% Trifluoroacetic acid, prepared by adding 1.0 mL of trifluoroacetic acid to 1 L of water
Solution B: Acetonitrile
Mobile phase: See *Table 1* in the *Assay*.
Diluent: *Solution A* and *Solution B* (1:1)
System suitability solution: 0.2 mg/mL of USP Benzocaine RS and 0.01 mg/mL each of USP Aminobenzoic Acid RS and USP Ethyl 4-Nitrobenzoate RS in *Diluent*
Standard solution: 1 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*
Sample solution: Nominally 1 mg/mL of benzocaine in *Diluent* prepared as follows

USP Monographs

www.webofpharma.com

Ointments having water-soluble bases: Transfer a portion of Ointment, equivalent to 50 mg of benzocaine, into a volumetric flask, and dissolve in *Diluent*.
Ointments having water-insoluble bases: Transfer a portion of Ointment, equivalent to 50 mg of benzocaine, into a volumetric flask, and dissolve in about 10% of final volume of tetrahydrofuran, then dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 10 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate, *System suitability solution*
Relative standard deviation: NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*
r_S = peak response of the corresponding Reference Standard from the *Standard solution*
C_S = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for any other individual unspecified impurity from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.29	0.10
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.10
Any other individual unspecified impurity	—	0.10
Total impurities	—	1.0

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and store at room temperature at 15°–25°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Aminobenzoic Acid RS
Benzoic acid, 4-amino.
$C_7H_7NO_2$ 137.14
USP Benzocaine RS
USP Ethyl 4-Nitrobenzoate RS
Benzoic acid, 4-nitro-, ethyl ester.
$C_9H_9NO_4$ 195.17

Benzocaine Otic Solution

DEFINITION
Benzocaine Otic Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ($C_9H_{11}NO_2$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

Diluent: *Solution A* and *Solution B* (1:1)
System suitability solution: 1 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*. Sonicate to dissolve, if necessary.
Standard solution: 0.1 mg/mL of USP Benzocaine RS in *Diluent*. Sonicate to dissolve, if necessary.

www.webofpharma.com

Sample solution: Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a portion of Otic Solution, equivalent to 10 mg of benzocaine, into a 100-mL volumetric flask and dissolve it in *Diluent*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. For *Identification* test *B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 6 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzocaine ($C_9H_{11}NO_2$) in the portion of Otic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzocaine from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*. Sonicate to dissolve, if necessary.
Sample solution: Nominally 0.5 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a portion of Otic Solution, equivalent to 50 mg of benzocaine, into a 100-mL volumetric flask, dissolve, and dilute with *Diluent* to volume.

System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 6 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate
Relative standard deviation: NMT 3.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Otic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*
r_S = peak response of the corresponding Reference Standard from the *Standard solution*
C_S = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Otic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified degradation product from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.3	0.20
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.20
Any individual unspecified degradation product	—	0.20
Total impurities	—	1.0

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli* and for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The total aerobic microbial count is less than 10^2 cfu/mL.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Aminobenzoic Acid RS
Benzoic acid, 4-amino.
$C_7H_7NO_2$ 137.14
USP Benzocaine RS
USP Ethyl 4-Nitrobenzoate RS
Benzoic acid, 4-nitro-, ethyl ester.
$C_9H_9NO_4$ 195.17

Benzocaine Topical Solution

DEFINITION

Benzocaine Topical Solution is a solution of Benzocaine in a suitable solvent. It contains NLT 90.0% and NMT 110.0% of

www.webofpharma.com

the labeled amount of benzocaine ($C_9H_{11}NO_2$). It contains a suitable antimicrobial agent.

IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

Diluent: *Solution A* and *Solution B* (1:1)
System suitability solution: 1 µg/mL of USP Benzocaine RS and 2 µg/mL each of USP Aminobenzoic Acid RS and USP Ethyl 4-Nitrobenzoate RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Benzocaine RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer 10 mg of benzocaine from a portion of Topical Solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size as needed, discarding the first 2–3 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. For *Identification* test *A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for aminobenzoic acid, benzocaine, and ethyl 4-nitrobenzoate are 0.3, 1.0, and 2.1, respectively.]
Suitability requirements
Resolution: NLT 6 between aminobenzoic acid and benzocaine; and between benzocaine and ethyl 4-nitrobenzoate, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzocaine ($C_9H_{11}NO_2$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzocaine from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L
Solution B: Acetonitrile
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	85	15
34	55	45
35	85	15
38	85	15

Diluent: *Solution A* and *Solution B* (1:1)
Standard solution: 1 µg/mL of USP Benzocaine RS and 2 µg/mL each USP Aminobenzoic Acid RS and USP Ethyl 4-Nitrobenzoate RS in *Diluent*
Sample solution: Nominally 1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer 50 mg of benzocaine from a portion of Topical Solution to a volumetric flask and dissolve with aid of sonication as needed, then dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size as needed, discarding the first 2–3 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 3* for relative retention times.]
Suitability requirements
Resolution: NLT 6 between aminobenzoic acid and benzocaine; and between benzocaine and ethyl 4-nitrobenzoate
Relative standard deviation: NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*
r_S = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Standard solution*
C_S = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

www.webofpharma.com

Calculate the percentage of any other individual unspecified impurity in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified impurity from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard peaks less than 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.27	0.20
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.5	0.20
Any other individual unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and avoid prolonged exposure to temperatures exceeding 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aminobenzoic Acid RS
  Benzoic acid, 4-amino.
  $C_7H_7NO_2$ 137.14
  USP Benzocaine RS
  USP Ethyl 4-Nitrobenzoate RS
  Benzoic acid, 4-nitro-, ethyl ester.
  $C_9H_9NO_4$ 195.17

Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Aerosol

DEFINITION

Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Aerosol is Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution packaged in a pressurized container with a suitable inert propellant. It contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ($C_9H_{11}NO_2$), butamben ($C_{11}H_{15}NO_2$), and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peaks of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.1% formic acid in water
Solution B: 0.1% formic acid in acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	91	9
2.5	50	50
3.9	50	50
4	91	9
5	91	9

Diluent: Acetonitrile and water (10:90)
Standard stock solution A: 1750 µg/mL of USP Benzocaine RS prepared as follows. Transfer a suitable amount of USP Benzocaine RS to a suitable volumetric flask and dissolve in 10% of the total volume of acetonitrile. Dilute with water to volume.
Standard stock solution B: 250 µg/mL each of USP Butamben RS and USP Tetracaine Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Butamben RS and USP Tetracaine Hydrochloride RS to a suitable volumetric flask and dissolve in 10% of the total volume of acetonitrile. Dilute with water to volume.
Standard solution: 175 µg/mL of USP Benzocaine RS from *Standard stock solution A* and 25 µg/mL each of USP Butamben RS and USP Tetracaine Hydrochloride RS from *Standard stock solution B* diluted in *Diluent*
Sample solution: Nominally 175 µg/mL of benzocaine and 25 µg/mL each of butamben and tetracaine hydrochloride, prepared as follows. Accurately weigh about 125 mg of the evaporated sample into a 100-mL volumetric flask. Dissolve in 50 mL of methanol and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 2.1-mm × 5-cm; 1.7-µm packing L1
Flow rate: 0.6 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzocaine, tetracaine, and butamben are about 0.71, 0.74, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2 between benzocaine and tetracaine
Relative standard deviation: NMT 2.0% for each of the three analyte peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzocaine ($C_9H_{11}NO_2$), butamben ($C_{11}H_{15}NO_2$), and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

www.webofpharma.com

r_U = peak response of the corresponding analyte from the *Sample solution*
r_S = peak response of the corresponding analyte from the *Standard solution*
C_S = concentration of the corresponding Reference Standard in the *Standard solution* (µg/mL)
C_U = nominal concentration of the corresponding analyte in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 10 µg/mL each of USP Benzocaine RS, USP Tetracaine Hydrochloride RS, USP Butamben RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*

Standard solution: 3.4 µg/mL each of USP Benzocaine RS and USP Ethyl 4-Nitrobenzoate RS and 1 µg/mL of USP Tetracaine Hydrochloride RS in *Diluent*

Sample solution: Nominally 1.68 mg/mL of benzocaine, 0.24 mg/mL of butamben, and 0.24 mg/mL of tetracaine prepared as follows. Accurately weigh about 600 mg of evaporated sample into a 50-mL volumetric flask, dissolve with 25 mL of methanol, and dilute with *Diluent* to volume. [NOTE—Sonication for about 1 min may be necessary.]

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2 between butamben and ethyl 4-nitrobenzoate; NLT 2 between benzocaine and tetracaine, *System suitability solution*

Relative standard deviation: NMT 5.0% for each of the analyte peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 4-aminobenzoic acid in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of 4-aminobenzoic acid from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Calculate the percentage of ethyl 4-nitrobenzoate in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ethyl 4-nitrobenzoate from the *Sample solution*
r_S = peak response of ethyl 4-nitrobenzoate from the *Standard solution*
C_S = concentration of USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (µg/mL)

Calculate the percentage of tetracaine related compound B and any individual unspecified degradation product in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of tetracaine related compound B or any individual unspecified degradation product from the *Sample solution*
r_S = peak response of tetracaine from the *Standard solution*
C_S = concentration of USP Tetracaine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of tetracaine hydrochloride in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-Aminobenzoic acid	0.15	1.3	0.3
Benzocaine	0.70	—	—
Tetracaine	0.74	—	—
Tetracaine related compound B[a]	0.93	0.6	0.4
Butamben	1.0	—	—
Ethyl 4-nitrobenzoate	1.04	—	0.2
Any individual unspecified degradation product	—	1.0	0.4
Total degradation products	—	—	2.0

[a] 4-(Butylamino)benzoic acid.

SPECIFIC TESTS

• **TOPICAL AEROSOLS** ⟨603⟩, *Pressure Test*: Meets the requirements
• **LEAK RATE** ⟨604⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in pressurized containers, and avoid exposure to excessive heat.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benzocaine RS
USP Butamben RS
USP Ethyl 4-Nitrobenzoate RS
Ethyl *p*-nitrobenzoate.
$C_9H_9NO_4$ 195.17
USP Tetracaine Hydrochloride RS

Benzocaine, Butamben, and Tetracaine Hydrochloride Gel

DEFINITION

Benzocaine, Butamben, and Tetracaine Hydrochloride Gel is Benzocaine, Butamben, and Tetracaine Hydrochloride in a suitable gel base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ($C_9H_{11}NO_2$),

www.webofpharma.com

butamben ($C_{11}H_{15}NO_2$), and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peaks of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.1% formic acid in water
Solution B: 0.1% formic acid in acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	91	9
2.5	50	50
3.9	50	50
4	91	9
5	91	9

Diluent: Acetonitrile and water (20:80)
Standard solution: 175 µg/mL of USP Benzocaine RS and 25 µg/mL each of USP Butamben RS and USP Tetracaine Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Benzocaine RS, USP Butamben RS, and USP Tetracaine Hydrochloride RS to a suitable volumetric flask and dissolve in 20% of the total volume of acetonitrile. Dilute with water to volume.
Sample solution: Nominally 175 µg/mL of benzocaine and 25 µg/mL each of butamben and tetracaine hydrochloride, prepared as follows. Transfer a suitable quantity of Gel into a suitable volumetric flask and dissolve in 20% of the total volume of acetonitrile. [NOTE—Sonication for about 1 min may be necessary.] Dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 2.1-mm × 5-cm; 1.7-µm packing L1
Flow rate: 0.6 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2 between benzocaine and tetracaine
Relative standard deviation: NMT 2.0% for each of the three analyte peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzocaine ($C_9H_{11}NO_2$), butamben ($C_{11}H_{15}NO_2$), and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*
r_S = peak response of the corresponding analyte from the *Standard solution*
C_S = concentration of the corresponding Reference Standard in the *Standard solution* (µg/mL)
C_U = nominal concentration of the corresponding analyte in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 10 µg/mL each of USP Benzocaine RS, USP Tetracaine Hydrochloride RS, USP Butamben RS, and USP Ethyl 4-Nitrobenzoate RS prepared as follows. Transfer a suitable amount of USP Benzocaine RS, USP Tetracaine Hydrochloride RS, USP Butamben RS, and USP Ethyl 4-Nitrobenzoate RS to a suitable volumetric flask and dissolve in 20% of the total volume of acetonitrile. Dilute with water to volume.
Standard solution: 3.4 µg/mL each of USP Benzocaine RS and USP Ethyl 4-Nitrobenzoate RS and 1 µg/mL of USP Tetracaine Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Benzocaine RS, USP Tetracaine Hydrochloride RS, and USP Ethyl 4-Nitrobenzoate RS to a suitable volumetric flask and dissolve in 20% of the total volume of acetonitrile. Dilute with water to volume.
Sample solution: Nominally 1.68 mg/mL of benzocaine, 0.24 mg/mL of butamben, and 0.24 mg/mL of tetracaine prepared as follows. Transfer a suitable quantity of Gel into a suitable volumetric flask and dissolve in 20% of the total volume of acetonitrile. Dilute with water to volume. [NOTE—Sonication for about 1 min may be necessary.]
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2 between butamben and ethyl 4-nitrobenzoate; NLT 2 between benzocaine and tetracaine, *System suitability solution*
Relative standard deviation: NMT 5.0% for each of the analyte peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 4-aminobenzoic acid in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of 4-aminobenzoic acid from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Calculate the percentage of ethyl 4-nitrobenzoate in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ethyl 4-nitrobenzoate from the *Sample solution*

USP Monographs

www.webofpharma.com

r_S = peak response of ethyl 4-nitrobenzoate from the *Standard solution*
C_S = concentration of USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (µg/mL)

Calculate the percentage of tetracaine related compound B and any individual unspecified degradation product in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of tetracaine related compound B or any individual unspecified degradation product from the *Sample solution*
r_S = peak response of tetracaine from the *Standard solution*
C_S = concentration of USP Tetracaine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of tetracaine hydrochloride in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-Aminobenzoic acid	0.23	1.3	0.3
Benzocaine	1.0	—	—
Tetracaine	1.07	—	—
Tetracaine related compound B[a]	1.34	0.6	0.4
Butamben	1.45	—	—
Ethyl 4-nitrobenzoate	1.50	—	0.2
Any individual unspecified degradation product	—	1.0	0.4
Total degradation products	—	—	2.0

[a] 4-(Butylamino)benzoic acid.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 10^2 cfu/g. The total combined molds and yeasts count is NMT 10^1 cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers in a cool dry place and avoid freezing.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzocaine RS
  USP Butamben RS
  USP Ethyl 4-Nitrobenzoate RS
  Ethyl *p*-nitrobenzoate.
  $C_9H_9NO_4$ 195.17
  USP Tetracaine Hydrochloride RS

Benzocaine, Butamben, and Tetracaine Hydrochloride Ointment

DEFINITION

Benzocaine, Butamben, and Tetracaine Hydrochloride Ointment is Benzocaine, Butamben, and Tetracaine Hydrochloride in a suitable ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of benzocaine ($C_9H_{11}NO_2$), butamben ($C_{11}H_{15}NO_2$), and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: Methanol and water (60:40)

Mobile phase: Methanol, water, and 0.25 M sodium 1-heptanesulfonate (500:500:20)

Standard solution: Transfer 140 mg of USP Benzocaine RS to a 100-mL volumetric flask with the aid of 25 mL of methanol, and swirl. Transfer 140*J* mg of USP Butamben RS to the same volumetric flask with the aid of 25 mL of water, *J* being the ratio of the labeled amount, as a percentage, of butamben to the labeled amount, as a percentage, of benzocaine in the Ointment. Transfer 140*J′* mg of USP Tetracaine Hydrochloride RS to the same volumetric flask with the aid of 25 mL of water, *J′* being the ratio of the labeled amount, as a percentage, of tetracaine hydrochloride to the labeled amount, as a percentage, of benzocaine in the Ointment. Sonicate for about 1 min, and dilute with *Diluent* to volume.

Sample stock solution: Nominally 14 mg/mL of benzocaine, prepared as follows. Transfer a portion of Ointment, equivalent to 1400 mg of benzocaine, to a 100-mL volumetric flask. Add 75 mL of methanol, and mix. Sonicate for about 1 min, and dilute with methanol to volume.

Sample solution: Nominally 1.4 mg/mL of benzocaine in *Diluent* from the *Sample stock solution*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 313 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzocaine, butamben, and tetracaine are about 0.3, 0.8, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2 between benzocaine and butamben, and between butamben and tetracaine
Relative standard deviation: NMT 2.0% for each of the three analyte peaks

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of benzocaine ($C_9H_{11}NO_2$), butamben ($C_{11}H_{15}NO_2$), and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak response of the corresponding analyte from the *Sample solution*
r_S = peak response of the corresponding analyte from the *Standard solution*
C_S = concentration of the corresponding Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benzocaine RS
USP Butamben RS
USP Tetracaine Hydrochloride RS

Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution

DEFINITION
Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ($C_9H_{11}NO_2$), butamben ($C_{11}H_{15}NO_2$), and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$).

IDENTIFICATION
• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peaks of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 0.1% formic acid in water
Solution B: 0.1% formic acid in acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	91	9
2.5	50	50
3.9	50	50
4	91	9
5	91	9

Diluent: Acetonitrile and water (10:90)
Standard stock solution A: 1750 µg/mL of USP Benzocaine RS prepared as follows. Transfer a suitable amount of USP Benzocaine RS to a suitable volumetric flask and dissolve in 80% of the total volume of *Diluent*. [NOTE—Sonication for about 5 min may be necessary.] Dilute with *Diluent* to volume.
Standard stock solution B: 125 µg/mL each of USP Butamben RS and USP Tetracaine Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Butamben RS and USP Tetracaine Hydrochloride RS to a suitable volumetric flask and dissolve in 80% of the total volume of *Diluent*. [NOTE—Sonication for about 10 min may be necessary.] Dilute with *Diluent* to volume.
Standard solution: 175 µg/mL of USP Benzocaine RS from *Standard stock solution A* and 25 µg/mL each of USP Butamben RS and USP Tetracaine Hydrochloride RS from *Standard stock solution B* diluted in *Diluent*
Sample solution: Nominally 175 µg/mL of benzocaine and 25 µg/mL each of butamben and tetracaine hydrochloride, prepared as follows. Transfer a suitable portion of Topical Solution to a suitable volumetric flask and dissolve in 80% of the total volume of *Diluent*. [NOTE—Sonication for about 10 min may be necessary.] Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 2.1-mm × 5-cm; 1.7-µm packing L1
Flow rate: 0.6 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for benzocaine, tetracaine, and butamben are about 0.71, 0.74, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between benzocaine and tetracaine
Relative standard deviation: NMT 2.0% for each of the three analyte peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzocaine ($C_9H_{11}NO_2$), butamben ($C_{11}H_{15}NO_2$), and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*
r_S = peak response of the corresponding analyte from the *Standard solution*
C_S = concentration of the corresponding Reference Standard in the *Standard solution* (µg/mL)
C_U = nominal concentration of the corresponding analyte in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 10 µg/mL each of USP Benzocaine RS, USP Tetracaine Hydrochloride RS, USP Butamben RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*
Standard solution: 3.4 µg/mL each of USP Benzocaine RS and USP Ethyl 4-Nitrobenzoate RS and 1 µg/mL of USP Tetracaine Hydrochloride RS in *Diluent*
Sample solution: Nominally 1.68 mg/mL of benzocaine, 0.24 mg/mL of butamben, and 0.24 mg/mL of tetracaine prepared as follows. Accurately weigh a suitable quantity of Topical Solution into a suitable volumetric flask, dissolve with 20% of the total volume of acetonitrile, and dilute with

www.webofpharma.com

water to volume. [NOTE—Sonication for about 1 min may be necessary.]

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2 between butamben and ethyl 4-nitrobenzoate; NLT 2 between benzocaine and tetracaine, *System suitability solution*

Relative standard deviation: NMT 5.0% for each of the analyte peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 4-aminobenzoic acid in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of 4-aminobenzoic acid from the *Sample solution*

r_S = peak response of benzocaine from the *Standard solution*

C_S = concentration of USP Benzocaine RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of benzocaine in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2*)

Calculate the percentage of ethyl 4-nitrobenzoate in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ethyl 4-nitrobenzoate from the *Sample solution*

r_S = peak response of ethyl 4-nitrobenzoate from the *Standard solution*

C_S = concentration of USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of benzocaine in the *Sample solution* (µg/mL)

Calculate the percentage of tetracaine related compound B and any individual unspecified degradation product in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of tetracaine related compound B or any individual unspecified degradation product from the *Sample solution*

r_S = peak response of tetracaine from the *Standard solution*

C_S = concentration of USP Tetracaine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of tetracaine hydrochloride in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-Aminobenzoic acid	0.15	1.3	0.3
Benzocaine	0.70	—	—
Tetracaine	0.74	—	—
Tetracaine related compound B[a]	0.93	0.6	0.4
Butamben	1.0	—	—
Ethyl 4-nitrobenzoate	1.04	—	0.2
Any individual unspecified degradation product	—	1.0	0.4
Total degradation products	—	—	2.0

[a] 4-(Butylamino)benzoic acid.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzocaine RS
  USP Butamben RS
  USP Ethyl 4-Nitrobenzoate RS
  Ethyl *p*-nitrobenzoate.
  $C_9H_9NO_4$ 195.17
  USP Tetracaine Hydrochloride RS

Benzocaine and Menthol Topical Aerosol

DEFINITION

Benzocaine and Menthol Topical Aerosol is a solution of Benzocaine and Menthol with suitable propellants in a pressurized container. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of benzocaine ($C_9H_{11}NO_2$) and menthol ($C_{10}H_{20}O$).

IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak for benzocaine of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.** The retention time of the major peak for menthol of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **BENZOCAINE**

Solution A: Dilute 1.0 mL of trifluoroacetic acid with water to 1 L.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
15	72	28
18	50	50
18.1	90	10

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
20	90	10

Diluent: *Solution A* and *Solution B* (1:1)
Standard solution: 0.1 mg/mL of USP Benzocaine RS in *Diluent*. Sonicate for 2–5 min to dissolve before diluting to final volume.
Sample solution: Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Spray the contents of the Topical Aerosol into a flask with a stopper. Heat and stir the sprayed Topical Aerosol at 100° for 30 min in an oil bath to obtain a viscous liquid sample. Cool the sample to room temperature. Transfer an amount of Topical Aerosol equivalent to 10 mg of benzocaine to a 100-mL volumetric flask. Dissolve the sample in *Diluent* and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. For *Identification* test *A*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzocaine ($C_9H_{11}NO_2$) in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

• **MENTHOL**
Internal standard solution: 1 mg/mL of decanol in isopropyl alcohol
Standard stock solution: 1 mg/mL of USP Menthol RS in isopropyl alcohol
Standard solution: 0.5 mg/mL each of USP Menthol RS and decanol in isopropyl alcohol from *Internal standard solution* and *Standard stock solution*
Sample solution: Nominally 0.5 mg/mL of menthol prepared as follows. Spray the contents of the Topical Aerosol into a flask. Transfer an amount of Topical Aerosol equivalent to 5 mg of menthol to a 10-mL volumetric flask and add 5.0 mL of *Internal standard solution*. Dilute with isopropyl alcohol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m; fused-silica capillary column bonded with a 1.0-µm film of phase G16
Carrier gas: Hydrogen
Temperatures
Injection port: 250°
Detector: 250°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Hold Time at 130° (min)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	7.5	35	240	1.0

Flow rate: 10 mL/min
Injection volume: 1 µL
Injection type: Split, split ratio 10:1
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for menthol and decanol are 1.0 and 1.6, respectively.]
Suitability requirements
Resolution: NLT 2.5 between the menthol and decanol peaks
Relative standard deviation: NMT 1.0% of the ratio of the peak response of menthol to that of decanol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of menthol ($C_{10}H_{20}O$) in the portion of Topical Aerosol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of menthol to decanol from the *Sample solution*
R_S = peak response ratio of menthol to decanol from the *Standard solution*
C_S = concentration of USP Menthol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of menthol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: Dilute 1.0 mL of trifluoroacetic acid with water to 1 L.
Solution B: Acetonitrile
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

Diluent: *Solution A* and *Solution B* (1:1)
Standard stock solution: 0.1 mg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*. Sonicate for about 5 min to dissolve before diluting to final volume.
Standard solution: 0.001 mg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl

www.webofpharma.com

4-Nitrobenzoate RS in *Diluent* from the *Standard stock solution*

Sample solution: Nominally 0.5 mg/mL of benzocaine in *Diluent* prepared as follows. Spray the contents of the Topical Aerosol into a flask. Heat and stir the sprayed Topical Aerosol at 100° for 30 min in an oil bath to obtain a viscous liquid sample. Cool the sample to room temperature. Transfer an amount of Topical Aerosol equivalent to 50 mg of benzocaine to a 100-mL volumetric flask. Dissolve the sample in *Diluent* and dilute with *Diluent* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*
r_S = peak response of the corresponding Reference Standard from the *Standard solution*
C_S = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of benzocaine from the *Standard solution*
C_S = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 4*. Disregard peaks less than 0.05%.

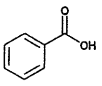
Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.3	0.2
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.2
Any other unspecified degradation product	—	0.1
Total degradation products	—	2.0

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **PRESSURE TEST:** It meets the requirements in *Topical Aerosols* ⟨603⟩.
- **LEAKAGE TEST:** It meets the requirements in *Leak Rate* ⟨604⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, pressurized containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aminobenzoic Acid RS
  Benzoic acid, 4-amino.
  $C_7H_7NO_2$ 137.14
  USP Benzocaine RS
  USP Ethyl 4-Nitrobenzoate RS
  Benzoic acid, 4-nitro-, ethyl ester.
  $C_9H_9NO_4$ 195.17
  USP Menthol RS

Benzoic Acid



$C_7H_6O_2$ 122.12
Benzoic acid [65-85-0].

DEFINITION
Benzoic Acid contains NLT 99.5% and NMT 100.5% of benzoic acid ($C_7H_6O_2$), calculated on the anhydrous basis.

IDENTIFICATION
- **A.**
  Sample solution: Prepare a saturated solution of Benzoic Acid in water, and filter twice.
  Analysis 1: To one portion of the filtrate add ferric chloride TS.
  Acceptance criteria 1: A salmon-colored precipitate is formed.
  Analysis 2: To a separate 10-mL portion of the filtrate add 1 mL of 7 N sulfuric acid, and cool the mixture.
  Acceptance criteria 2: A white precipitate forms in 10 min. This precipitate is soluble in ether.

ASSAY
- **PROCEDURE**
  Sample: 500 mg of Benzoic Acid
  Analysis: Dissolve the *Sample* in 25 mL of diluted alcohol that previously has been neutralized with 0.1 N sodium hydroxide. Add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS to a pink color. Each mL of 0.1 N sodium hydroxide is equivalent to 12.21 mg of benzoic acid ($C_7H_6O_2$).
  Acceptance criteria: 99.5%–100.5% on the anhydrous basis

USP Monographs

www.webofpharma.com

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.05%

SPECIFIC TESTS

• **CONGEALING TEMPERATURE** ⟨651⟩: 121°–123°

• **WATER DETERMINATION,** *Method I* ⟨921⟩

Sample solution: A 1-in-2 solution of methanol in pyridine is used as the solvent.

Acceptance criteria: NMT 0.7%

• **READILY CARBONIZABLE SUBSTANCES TEST** ⟨271⟩

Sample solution: 500 mg in 5 mL of sulfuric acid

Acceptance criteria: The solution has no more color than *Matching Fluid Q*.

• **READILY OXIDIZABLE SUBSTANCES**

Sample solution: Add 1.5 mL of sulfuric acid to 100 mL of water. Heat to boiling, and add 0.1 N potassium permanganate, dropwise, until the pink color persists for 30 s. Dissolve 1.00 g of Benzoic Acid in the hot solution.

Analysis: Titrate with 0.1 N potassium permanganate VS to a pink color that persists for 15 s.

Acceptance criteria: NMT 0.50 mL of 0.10 N potassium permanganate is consumed.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Benzoic and Salicylic Acids Ointment

DEFINITION

Benzoic and Salicylic Acids Ointment is Benzoic Acid and Salicylic Acid, present in a ratio of 2:1, in a suitable ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of benzoic acid ($C_7H_6O_2$) and salicylic acid ($C_7H_6O_3$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Mixture of chloroform and methanol (1:1)

Standard solution A: 2.4 mg/mL of USP Benzoic Acid RS in *Diluent*

Standard solution B: 1.2 mg/mL of USP Salicylic Acid RS in *Diluent*

Sample solution: Equivalent to 60 mg of benzoic acid and 30 mg of salicylic acid from Ointment, in 25 mL of *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL of each solution at separate points 2.5 cm from the bottom edge of a 20- × 20-cm thin-layer chromatographic plate

Developing solvent system: Chloroform, acetone, isopropyl alcohol, methanol, and ammonium hydroxide (30:30:15:15:10)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, mark the solvent front, and allow the solvent to evaporate. View the chromatogram under short-wavelength (254 nm) UV radiation.

Acceptance criteria: The two major fluorescent spots from the *Sample solution* correspond in color and in R_F value to those from *Standard solution A* and *Standard solution B*.

ASSAY

• **PROCEDURE**

Ferric chloride–urea reagent: On the day of use, dissolve, without heating, 18 g of urea in a mixture of 2.5 mL of ferric chloride solution (6 in 10) and 12.5 mL of 0.05 N hydrochloric acid.

Column A: Insert a small pledget of glass wool above the stem constriction of a 20- × 2.5-cm chromatographic tube. Mix 1 g of chromatographic siliceous earth with 0.5 mL of dilute phosphoric acid (3 in 10) to form a uniform, fluffy mixture; transfer to the chromatographic tube; and pack evenly over the glass wool, exerting gentle pressure. Similarly, mix 4 g of chromatographic siliceous earth with 3 mL of *Ferric chloride–urea reagent*, and pack uniformly over the first layer. Cover the column with a pad of glass wool.

Column B: Insert a small pledget of glass wool above the stem constriction of a second 20- × 2.5-cm chromatographic tube. Mix 4 g of chromatographic siliceous earth with 2 mL of sodium bicarbonate solution (1 in 12), prepared just before use, to a uniform, fluffy mixture; and pack evenly over the glass wool. Cover the column with a pad of glass wool.

Diluent: Glacial acetic acid in chloroform (3 in 100)

Standard solution A: 20 µg/mL of USP Salicylic Acid RS in *Diluent*

Standard solution B: 40 µg/mL of USP Benzoic Acid RS in *Diluent*

Sample solution: Transfer an amount of the Ointment, equivalent to 100 mg of benzoic acid and 50 mg of salicylic acid, to a 250-mL volumetric flask, and dissolve in 150 mL of chloroform by warming on a steam bath. Cool. Dilute with chloroform to volume to obtain a solution having a nominal concentration of 200 µg/mL of salicylic acid and 400 µg/mL of benzoic acid.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Mount *Column A* directly over *Column B*, then pipet 10 mL of *Sample solution* onto *Column A*, and allow it to pass into the column. Wash the columns with two 40-mL portions of chloroform, allowing the first portion to recede to the top of each column before adding the second portion. Discard the eluates, and separate the columns.

Salicylic acid content: Elute *Column A* with 95 mL of *Diluent*, collecting the eluate in a 100-mL volumetric flask. Dilute the contents of the flask with *Diluent* to volume, and mix. Concomitantly determine the absorbances of the eluate and *Standard solution A* in 1-cm cells at the wavelength of maximum absorbance at 311 nm, with a suitable spectrophotometer, using *Diluent* as the blank. Calculate the percentage of the labeled amount of salicylic acid ($C_7H_6O_3$) in the portion of Ointment taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times F \times 100$$

A_U = absorbance of the diluted eluate from *Column A*

A_S = absorbance of *Standard solution A*

C_S = concentration of USP Salicylic Acid RS in *Standard solution A* (µg/mL)

C_U = nominal concentration of the salicylic acid in the *Sample solution* (µg/mL)

F = sample dilution factor, 10

Acceptance criteria: 90.0%–110.0%

Benzoic acid content: Elute *Column B* with 95 mL of *Diluent*, collecting the eluate in a 100-mL volumetric flask. Dilute the contents of the flask with *Diluent* to volume,

USP Monographs

www.webofpharma.com

and mix. Concomitantly determine the absorbances of eluate and *Standard solution B* in 1-cm cells at the wavelength of maximum absorbance at 275 nm, with a suitable spectrophotometer, using *Diluent* as the blank. Calculate the percentage of the labeled amount of benzoic acid ($C_7H_6O_2$) in the portion of Ointment taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times F \times 100$$

A_U = absorbance of the diluted eluate from *Column B*
A_S = absorbance of *Standard solution B*
C_S = concentration of USP Benzoic Acid RS in *Standard solution B* (µg/mL)
C_U = nominal concentration of benzoic acid in the *Sample solution* (µg/mL)
F = sample dilution factor, 10

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Minimum Fill** ⟨755⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers, and avoid exposure to temperatures exceeding 30°.
• **Labeling:** Label Ointment to indicate the concentrations of Benzoic Acid and Salicylic Acid and to indicate whether the ointment base is water-soluble or water-insoluble.
• **USP Reference Standards** ⟨11⟩
USP Benzoic Acid RS
USP Salicylic Acid RS

Benzoin

DEFINITION
Benzoin is the balsamic resin obtained from *Styrax benzoin* Dryand. or *Styrax paralleloneurus* Perkins, known in commerce as Sumatra Benzoin, or from *Styrax tonkinensis* (Pierre) Craib ex Hartwich, or other species of the Section *Anthostyrax* of the genus *Styrax*, known in commerce as Siam Benzoin (Fam. Styraceae).
Sumatra Benzoin yields NLT 75.0% of alcohol-soluble extractive, and Siam Benzoin yields NLT 90.0% of alcohol-soluble extractive.

IDENTIFICATION
• **A.** A solution in alcohol becomes milky upon the addition of water, and the mixture is acid to litmus paper.
• **B. Identification of Articles of Botanical Origin** ⟨563⟩
Analysis: Heat a few fragments in a test tube.
Acceptance criteria: Sumatra Benzoin evolves a sublimate consisting of plates and small, rod-like crystals of cinnamic acid and its esters that strongly polarize light. Siam Benzoin evolves a sublimate directly above the melted mass, consisting of numerous long, rod-shaped crystals of benzoic acid that do not strongly polarize light.

ASSAY
• **Procedure**
Sample: Place 2 g of Benzoin in a tared extraction thimble, and insert the thimble in a continuous-extraction apparatus. Place 100 mg of sodium hydroxide in the receiving flask of the apparatus, and extract the Benzoin with alcohol for 5 h, or until completely extracted. Dry the extraction thimble containing the insoluble residue at 105° for 2 h.
Analysis: On a separate portion of Benzoin, determine the water content as directed for *Water Determination* ⟨921⟩, *Method II*. Calculate the weight of water in the quantity of the Benzoin taken for the *Assay*, and subtract it from the original weight of the Benzoin taken. The difference between this result and the weight of the residue in the extraction thimble represents the alcohol-soluble extractive.
Acceptance criteria: The alcohol-soluble extractive is NLT 75.0% for Sumatra Benzoin and NLT 90.0% for Siam Benzoin.

OTHER COMPONENTS
• **Content of Benzoic Acid**
Analysis: Treat 1 g of powdered Benzoin with 15 mL of warm carbon disulfide. Filter through a small pledget of cotton, wash the cotton with an additional 5 mL of carbon disulfide, and allow the filtrate to evaporate spontaneously.
Acceptance criteria: The weight of the residue is NLT 6.0% of the weight of Benzoin taken for Sumatra Benzoin and NLT 12.0% for Siam Benzoin. This residue meets the requirements for *Identification Tests—General* ⟨191⟩, *Benzoate*.

IMPURITIES
Inorganic Impurities
• **Articles of Botanical Origin,** *Acid-Insoluble Ash* ⟨561⟩: NMT 1.0% in Sumatra Benzoin; NMT 0.5% in Siam Benzoin
Organic Impurities
• **Procedure: Articles of Botanical Origin,** *Foreign Organic Matter* ⟨561⟩: NMT 1.0% in Siam Benzoin

SPECIFIC TESTS
• **Botanic Characteristics**
Sumatra Benzoin: Blocks or lumps of varying size, made up of tears, compacted together, with a reddish brown, reddish gray, or grayish brown resinous mass; the tears are externally yellowish or rusty brown, milky white on fresh fracture; hard and brittle at ordinary temperatures, but softened by heat.
Siam Benzoin: Pebble-like tears of variable size and shape, compressed, yellowish brown to rusty brown externally, milky white on fracture, separate or very slightly agglutinated; hard and brittle at ordinary temperatures, but softened by heat.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **Labeling:** Label it to indicate whether it is Sumatra Benzoin or Siam Benzoin.

Compound Benzoin Tincture

DEFINITION
Prepare Compound Benzoin Tincture as follows.

Benzoin, in moderately coarse powder	100 g
Aloe, in moderately coarse powder	20 g
Storax	80 g
Tolu Balsam	40 g
Alcohol, a sufficient quantity to make	1000 mL

Macerate the ingredients with 750 mL of *Alcohol* in a container that can be closed, and put in a warm place. Agitate it frequently during 3 days or until the soluble matter is

www.webofpharma.com

dissolved. Transfer the mixture to a filter. When most of the liquid has drained away, wash the residue on the filter with a sufficient quantity of *Alcohol*, combining the filtrates to produce 1000 mL of Tincture, and mix.

OTHER COMPONENTS

- **ALCOHOL DETERMINATION** ⟨611⟩, *Method II*: 74.0%–80.0% of alcohol (C_2H_5OH), the dilution made with methanol instead of with water, to approximately 2% alcohol

SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩: 0.870–0.885
- **LIMIT OF NONVOLATILE RESIDUE**

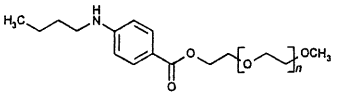
Analysis: Evaporate 3 mL of Tincture in a suitable tared dish on a steam bath, and dry the residue at 100° for 2 h.

Acceptance criteria: The weight of the residue is 525–675 mg.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.
- **LABELING:** Label it to indicate that it is flammable.

Benzonatate



$C_{30}H_{53}NO_{11}$ (av.) 603.74 (average)

Benzoic acid, 4-(butylamino)-, 2,5,8,11,14,17,20,23,26-nonaoxaoctacos-28-yl ester.

2,5,8,11,14,17,20,23,26-Nonaoxaoctacosan-28-yl *p*-(butylamino)benzoate [104-31-4].

» Benzonatate contains not less than 95.0 percent and not more than 105.0 percent of $C_{30}H_{53}NO_{11}$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Benzonatate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197F*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 15 µg per mL.
Medium: water.

Refractive index ⟨831⟩: between 1.509 and 1.511 at 20°.

Water Determination, *Method I* ⟨921⟩: not more than 0.3%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chloride ⟨221⟩—Mix 20 mL of a solution (1 in 10) with 20 mL of water and 1 mL of nitric acid, shake for 1 hour, and allow to stand for 1 hour. Pass through a filter having a porosity of 0.2 µm, and to the filtrate add 1 mL of silver nitrate TS. Dilute with water to 50 mL, mix, and allow to stand protected from light for 10 minutes: the turbidity does not exceed that produced by 0.10 mL of 0.020 N hydrochloric acid (0.0035%).

Sulfate ⟨221⟩—Mix 5 mL of a solution (1 in 20) with 5 mL of water and 1 mL of 3 N hydrochloric acid, shake for 1 hour, and allow to stand for 1 hour. Pass through a filter having a porosity of 0.2 µm, and to the filtrate add 1 mL of barium chloride TS. Mix, and allow to stand for 10 minutes: the turbidity does not exceed that produced by 0.10 mL of 0.020 N sulfuric acid (0.04%).

Assay—Weigh accurately about 5 g of Benzonatate, and reflux with 25.0 mL of 0.5 N sodium hydroxide VS for 1 hour. Cool, add 25 mL of water and 10 drops of bromothymol blue TS, and titrate the excess alkali with 0.5 N hydrochloric acid VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 0.5 N sodium hydroxide is equivalent to 301.5 mg of $C_{30}H_{53}NO_{11}$.

Benzonatate Capsules

DEFINITION

Benzonatate Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of benzonatate [$C_{30}H_{53}NO_{11}$ (av.)].

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

Sample: The contents of Capsules

Acceptance criteria: Meets the requirements. If a difference is observed, or if excipients are present, use an amount of the contents of Capsules equivalent to about 100 mg of benzonatate. Mix with 25 mL of 0.01 N hydrochloric acid, and proceed as directed in *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Transfer the liquid to a separator".

Change to read:

- **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: Nominally 15 µg/mL of benzonatate from the contents of Capsules

Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**

Standard solution: 500 µg/mL of USP Benzonatate RS

Sample stock solution: Nominally 5 mg/mL of benzonatate in chloroform, prepared as follows. Mix a number of Capsules, equivalent to about 500 mg of benzonatate, with 40 mL of chloroform in a suitable high-speed blender, and dilute with chloroform to 100.0 mL.

Sample solution: Nominally 500 µg/mL of benzonatate prepared as follows. Transfer 10.0 mL of *Sample stock solution* into a 100-mL volumetric flask. Evaporate the chloroform on a steam bath with the aid of a current of air. Dissolve the residue in water and dilute with water to volume.

Instrumental conditions

Mode: Vis

Analytical wavelength: 500 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

USP Monographs

www.webofpharma.com

Transfer 4.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to separate test tubes. To each tube add in succession 1.0 mL of 1 M hydroxylamine hydrochloride and 1.0 mL of 3.5 N sodium hydroxide, mixing after each addition. Allow to stand for 10 min, accurately timed, then add 1.0 mL of 3.5 N hydrochloric acid, mix, add 1.0 mL of an 80-mg/mL ferric chloride solution, and mix. Allow to stand for 30 min, accurately timed. Gently swirl the tubes for 1 min to remove any gas bubbles present, then concomitantly determine the absorbances of the solutions.

Calculate the percentage of the labeled amount of benzonatate [$C_{30}H_{53}NO_{11}$ (av.)] in the Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Benzonatate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of benzonatate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

▲Test 1▲ (RB 1-Jul-2018)

Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Mobile phase: Acetonitrile and 0.04 M monobasic potassium phosphate (75:25)
Standard solution: 0.1 mg/mL of USP Benzonatate RS. Sonicate to dissolve, if needed.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 310 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzonatate [$C_{30}H_{53}NO_{11}$ (av.)] dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times 1/L \times 100$$

r_U = peak response of benzonatate from the *Sample solution*
r_S = peak response of benzonatate from the *Standard solution*
C_S = concentration of USP Benzonatate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of benzonatate [$C_{30}H_{53}NO_{11}$ (av.)] is dissolved.

▲Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Tier 1
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: 0.022 mg/mL of USP Benzonatate RS in *Medium*. Sonicate to dissolve, if needed.
Sample solution: Withdraw a portion of the solution under test, dilute with *Medium* to a concentration of about 0.022 mg/mL, and pass through a suitable filter of 0.45-µm or finer pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 310 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzonatate [$C_{30}H_{53}NO_{11}$ (av.)] dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times 1/L \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Benzonatate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*
L = label claim (mg/Capsule)

Tolerances: NLT 75% (Q) of the labeled amount of benzonatate [$C_{30}H_{53}NO_{11}$ (av.)] is dissolved. If this tolerance cannot be met because of the presence of cross-linking in the gelatin Capsules, proceed to *Tier 2*.

Tier 2: Perform this test only if the *Tolerances* in *Tier 1* cannot be met because of the presence of cross-linking in the gelatin Capsules.
Medium: Simulated gastric fluid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Solution A: Dissolve about 5.44 g of monobasic potassium phosphate in 1000 mL of water.
Mobile phase: Acetonitrile and *Solution A* (75:25)
Standard solution: 0.022 mg/mL of USP Benzonatate RS in *Medium*. Sonicate to dissolve, if needed.
Sample solution: Withdraw a portion of the solution under test, dilute with *Medium* to a concentration of about 0.022 mg/mL, and pass through a suitable filter of 0.45-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: UV
Detector: UV 310 nm
Columns
Guard: 4.0-mm × 4.0-mm; 5-µm packing L1
Analytical: 4.0-mm × 25-cm; 7-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the percentage of the labeled amount of benzonatate [$C_{30}H_{53}NO_{11}$ (av.)] dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times 1/L \times 100$$

r_U = peak response of benzonatate from the *Sample solution*
r_S = peak response of benzonatate from the *Standard solution*
C_S = concentration of USP Benzonatate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*
L = label claim (mg/Capsule)

Tolerances: NLT 75% (Q) of the labeled amount of benzonatate [$C_{30}H_{53}NO_{11}$ (av.)] is dissolved. ▲ (RB 1-Jul-2018)

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

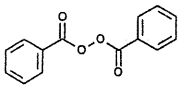
• **Packaging and Storage:** Preserve in tight, light-resistant containers.

Add the following:

▲• **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. ▲ (RB 1-Jul-2018)

• **USP Reference Standards** ⟨11⟩
USP Benzonatate RS

Hydrous Benzoyl Peroxide



$C_{14}H_{10}O_4$ (anhydrous) 242.23
Peroxide, dibenzoyl;
Benzoyl peroxide [94-36-0].

DEFINITION

Hydrous Benzoyl Peroxide contains NLT 90.0% and NMT 110.0% of the labeled amount of $C_{14}H_{10}O_4$. It contains a minimum of 20% of water for the purpose of reducing flammability and shock sensitivity.
[**Caution**—Hydrous Benzoyl Peroxide may explode at temperatures higher than 60° or cause fires in the presence of reducing substances. Store it in the original container, treated to reduce static charges.]

IDENTIFICATION

• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Standard solution: 10 mg/mL of Hydrous Benzoyl Peroxide, previously subjected to the *Assay*, in methanol
Sample solution: 10 mg/mL of benzoyl peroxide in methanol
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Toluene, dichloromethane, and glacial acetic acid (50:2:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Place the plate in a developing chamber containing and equilibrated with the *Developing solvent system*. Develop the chromatogram until the solvent front has moved three-fourths of the length of the plate. Remove the plate, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

• **B.** The *Sample solution* in the test for *Organic Impurities* exhibits a major peak for benzoyl peroxide, the retention time of which corresponds to that exhibited by the *Standard solution*.

ASSAY

• **Procedure**
Sample: 300 mg of previously mixed Hydrous Benzoyl Peroxide in a conical flask fitted with a ground-glass stopper. Weigh again to obtain the weight of the *Sample*.
Analysis: Add 30 mL of glacial acetic acid, previously sparged with carbon dioxide for NLT 2 min just before use, and swirl the flask gently to dissolve. Add 5 mL of potassium iodide solution (1 in 2), and mix. Allow the solution to stand for 1 min. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS. As the endpoint is approached, add 1 drop of starch iodide paste TS, or equivalent, and continue the titration to the discharge of the blue color. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N sodium thiosulfate is equivalent to 12.11 mg of $C_{14}H_{10}O_4$.
Acceptance criteria: 90.0%–110.0% of the labeled amount

IMPURITIES

Organic Impurities

• **Procedure**
Solution A: Prepare a mixture of acetonitrile and glacial acetic acid (1000:1).
Solution B: Prepare a mixture of water and glacial acetic acid (1000:1).
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	18	82
20	60	40
30	60	40

System suitability solution: 100 µg/mL of benzoic acid and 60 µg/mL of methylparaben in acetonitrile
Standard solution: Dissolve a quantity of Hydrous Benzoyl Peroxide, previously subjected to the *Assay*, in acetonitrile to obtain a solution containing 0.32 mg/mL.
Sample solution: 0.32 mg/mL of benzoyl peroxide in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.2 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between benzoic acid and methylparaben

USP Monographs

www.webofpharma.com

Tailing factors: NMT 2.0 for the benzoic acid and methylparaben peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the area, as a percentage, of each peak in the chromatogram of the *Sample solution*:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for any individual peak other than the principal peak in the *Sample solution*

r_T = sum of the peak responses of all the individual peaks including the principal peak in the *Sample solution*

Acceptance criteria: The area of any individual peak other than the principal peak is NMT 1.5% of the total area. The sum of the areas of all peaks other than the principal peak is NMT 2.0% of the total area.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in the original container, at room temperature. [NOTE—Do not transfer Hydrous Benzoyl Peroxide to metal or glass containers fitted with friction tops. Do not return unused material to its original container, but destroy it by treatment with sodium hydroxide solution (1 in 10) until addition of a crystal of potassium iodide results in no release of free iodine.]

Benzoyl Peroxide Gel

DEFINITION

Benzoyl Peroxide Gel is benzoyl peroxide in a suitable gel base. It contains NLT 90.0% and NMT 125.0% of the labeled amount of benzoyl peroxide ($C_{14}H_{10}O_4$).

IDENTIFICATION

A. The retention time of the major peak for benzoyl peroxide of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile in water (5 in 10)

Internal standard solution: 3.6 mg/mL of ethyl benzoate in acetonitrile

Standard stock solution: 0.8 mg/mL of benzoyl peroxide prepared as follows. Transfer a suitable quantity of benzoyl peroxide, recently subjected to the *Assay* in Hydrous Benzoyl Peroxide, into a weighed conical flask fitted with a glass stopper. Weigh again to obtain the weight of the specimen, and quantitatively dissolve in acetonitrile.

Standard solution: 0.32 mg/mL of benzoyl peroxide prepared as follows. Mix 10 mL of *Standard stock solution* and 5 mL of *Internal standard solution*, and dilute with acetonitrile to 25 mL.

Sample stock solution: Transfer an equivalent to 40 mg of benzoyl peroxide from Gel into a 50-mL volumetric flask, add 40 mL of acetonitrile, and shake until the material is thoroughly dispersed. Sonicate the mixture for 5 min, dilute with acetonitrile to volume, mix, and filter.

Sample solution: 10 mL of *Sample stock solution* and 5 mL of *Internal standard solution*; dilute with acetonitrile to 25 mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution* (three replicate injections)

[NOTE—The retention times for ethyl benzoate and benzoyl peroxide are 7 and 14 min, respectively.]

Suitability requirements

Resolution: NLT 2.0 between ethyl benzoate and benzoyl peroxide

Tailing factors: NMT 2.0 for the ethyl benzoate and benzoyl peroxide peaks

Peak response ratios: The lowest and highest peak response ratios (R_S) agree within 2.0%.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzoyl peroxide ($C_{14}H_{10}O_4$) in the portion of Gel taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of benzoyl peroxide to ethyl benzoate from the *Sample solution*

R_S = peak response ratio of benzoyl peroxide to ethyl benzoate from the *Standard solution*

C_S = concentration of benzoyl peroxide in the *Standard solution* (mg/mL)

C_U = nominal concentration of benzoyl peroxide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–125.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: Acetonitrile and glacial acetic acid (1000:1)

Solution B: Water and glacial acetic acid (1000:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	18	82
20	60	40
30	60	40

System suitability solution: 100 µg/mL of benzoic acid and 60 µg/mL of methylparaben in acetonitrile

Standard solution A: 500 µg/mL of benzoic acid in acetonitrile

Standard solution B: 20 µg/mL of ethyl benzoate in acetonitrile

Standard solution C: 20 µg/mL of benzaldehyde in acetonitrile

Standard solution D: Equivalent to 40 µg/mL of anhydrous benzoyl peroxide in acetonitrile, prepared from hydrous benzoyl peroxide, which has been analyzed as follows. Place 300 mg of previously mixed hydrous benzoyl peroxide in a conical flask fitted with a ground-glass stopper. Weigh again to obtain the weight of the sample. Add 30 mL of glacial acetic acid, previously sparged with carbon dioxide for NLT 2 min just before use, and swirl the flask gently to dissolve. Add 5 mL of potassium iodide solution (1 in 2), and mix. Allow the solution to stand for 1 min. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS. As the endpoint is approached, add 1 drop of starch iodide paste TS, or equivalent, and continue the titration to the discharge of the blue color. Perform a blank determination, and make any necessary correction (see

www.webofpharma.com

Titrimetry ⟨541⟩). Each mL of 0.1 N sodium thiosulfate is equivalent to 12.11 mg of anhydrous benzoyl peroxide ($C_{14}H_{10}O_4$).

Sample solution: Transfer an amount of Gel equivalent to 100 mg of benzoyl peroxide to a 50-mL volumetric flask, and add 25 mL of acetonitrile. Shake vigorously to disperse the specimen, sonicate for 5 min, dilute with acetonitrile to volume, and filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between benzoic acid and methylparaben
Tailing factor: NMT 2.0 for the benzoic acid and methylparaben peaks

Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The responses of any peaks from the *Sample solution* corresponding to benzoic acid, ethyl benzoate, and benzaldehyde are NMT those of the main peaks from *Standard solution A* (25%), *Standard solution B* (1%), and *Standard solution C* (1%), respectively. The response of any other impurity peak from the *Sample solution*—other than the main benzoyl peroxide peak, any benzoic acid, ethyl benzoate, benzaldehyde, methylparaben, or propylparaben peak, and any solvent peak—is NMT that from *Standard solution D* (2%); and the sum of the responses of all the impurity peaks—other than those of benzoic acid, ethyl benzoate, and benzaldehyde—is NMT that from *Standard solution D* (2%).

SPECIFIC TESTS
• **pH** ⟨791⟩: 2.8–6.6

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.

Benzoyl Peroxide Lotion

DEFINITION
Benzoyl Peroxide Lotion is benzoyl peroxide in a suitable lotion base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of benzoyl peroxide ($C_{14}H_{10}O_4$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: Acetonitrile in water (5 in 10)
Internal standard solution: 3.6 mg/mL of ethyl benzoate in acetonitrile
Standard stock solution: Transfer a suitable quantity of benzoyl peroxide, recently subjected to the *Assay* under *Hydrous Benzoyl Peroxide*, in a weighed conical flask fitted with a glass stopper. Weigh again to obtain the weight of the specimen, and quantitatively dissolve in acetonitrile to obtain a solution containing 0.8 mg/mL.
Standard solution: 10 mL of *Standard stock solution* and 5 mL of *Internal standard solution*. Dilute with acetonitrile to 25 mL. This *Standard solution* contains 0.32 mg/mL of benzoyl peroxide.
Sample stock solution: Transfer the equivalent to 40 mg of benzoyl peroxide from Lotion in a 50-mL volumetric flask, and add 40 mL of acetonitrile. Shake vigorously until the material is thoroughly dispersed. Sonicate the mixture for 5 min, dilute with acetonitrile to volume, mix, and filter.
Sample solution: 10 mL of *Sample stock solution* and 5 mL of *Internal standard solution*. Dilute with acetonitrile to 25 mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution* (three replicate injections)
[NOTE—The retention times for ethyl benzoate and benzoyl peroxide are 7 and 14 min, respectively.]
Suitability requirements
Resolution: NLT 2.0 between ethyl benzoate and benzoyl peroxide
Tailing factor: NMT 2.0 for the ethyl benzoate and benzoyl peroxide peaks
Peak response ratios: The lowest and highest peak response ratios (R_S) agree within 2.0%.

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzoyl peroxide ($C_{14}H_{10}O_4$) in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of benzoyl peroxide to ethyl benzoate from the *Sample solution*
R_S = peak response ratio of benzoyl peroxide to ethyl benzoate from the *Standard solution*
C_S = concentration of benzoyl peroxide in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzoyl peroxide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **Organic Impurities**
Solution A: Acetonitrile and glacial acetic acid (1000:1)
Solution B: Glacial acetic acid and water (1:1000)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	18	82
20	60	40
30	60	40

System suitability solution: 100 µg/mL of benzoic acid and 60 µg/mL of methylparaben in acetonitrile
Standard solution A: 500 µg/mL of benzoic acid in acetonitrile
Standard solution B: 20 µg/mL of ethyl benzoate in acetonitrile

USP Monographs

www.webofpharma.com

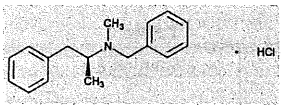
Standard solution C: 20 µg/mL of benzaldehyde in acetonitrile
Standard solution D: Prepare a solution of hydrous benzoyl peroxide, previously subjected to the *Assay* under *Hydrous Benzoyl Peroxide*, in acetonitrile containing the equivalent of 40 µg/mL of anhydrous benzoyl peroxide.
Sample solution: Equivalent to 100 mg of benzoyl peroxide from Lotion. In a 50-mL volumetric flask add 25 mL of acetonitrile, and shake vigorously to disperse the specimen. Sonicate for 5 min, dilute with acetonitrile to volume, mix, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between benzoic acid and methylparaben
Tailing factor: NMT 2.0 for the benzoic acid and methylparaben peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The responses of any peaks from the *Sample solution* corresponding to benzoic acid, ethyl benzoate, and benzaldehyde are NMT those of the main peaks from *Standard solution A* (25%), *Standard solution B* (1%), and *Standard solution C* (1%), respectively. The response of any other impurity peak from the *Sample solution*, other than the main benzoyl peroxide peak, any benzoic acid, ethyl benzoate, benzaldehyde, methylparaben, or propylparaben peak, and any solvent peak, is NMT that from *Standard solution D* (2%); and the sum of the responses of all the impurity peaks, other than those of benzoic acid, ethyl benzoate, and benzaldehyde, is NMT that from *Standard solution D* (2%).

SPECIFIC TESTS
• **pH** ⟨791⟩: 2.8–6.6

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.

Add the following:

▲Benzphetamine Hydrochloride



$C_{17}H_{21}N \cdot HCl$ 275.82
(+)-*N*-Benzyl-*N*, α-dimethylphenethylamine hydrochloride; (*S*)-*N*-Benzyl-*N*-methyl-1-phenylpropan-2-amine hydrochloride [5411-22-3].

DEFINITION
Benzphetamine Hydrochloride contains NLT 98.0% and NMT 102.0% of benzphetamine hydrochloride ($C_{17}H_{21}N \cdot HCl$), calculated on the dried basis.

IDENTIFICATION
Change to read:
• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
• **B.** The retention time of the benzphetamine peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the *Enantiomeric Purity* test.
• **C. Identification Tests—General** ⟨191⟩, *Chemical Identification Tests, Chloride*: Meets the requirements

ASSAY
• **Procedure**
Buffer: 0.01 M monobasic sodium phosphate in water
Solution A: Acetonitrile and *Buffer* (10:90)
Solution B: Acetonitrile and water (70:30)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
30	70	30
35	60	40
40	10	90
45	0	100
47	100	0
55	100	0

Diluent: Methanol and water (50:50)
Standard solution: 0.1 mg/mL of USP Benzphetamine Hydrochloride RS in *Diluent*
Sample solution: 0.1 mg/mL of Benzphetamine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 207 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of benzphetamine hydrochloride ($C_{17}H_{21}N \cdot HCl$) in the portion of Benzphetamine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzphetamine from the *Sample solution*
r_S = peak response of benzphetamine from the *Standard solution*
C_S = concentration of USP Benzphetamine Hydrochloride RS in the *Standard solution* (mg/mL)

www.webofpharma.com

C_U = concentration of Benzphetamine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

• **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, and **Diluent:** Prepare as directed in the *Assay*.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
30	70	30
35	60	40
45	55	45
60	15	85
62	100	0
70	100	0

System suitability solution: 1 mg/mL of USP Benzphetamine Hydrochloride RS and 0.001 mg/mL each of USP Benzphetamine Related Compound E RS and USP Benzphetamine Related Compound F RS in *Diluent*

Sensitivity solution: 0.0001 mg/mL of USP Benzphetamine Hydrochloride RS in *Diluent*

Standard solution: 0.001 mg/mL of USP Benzphetamine Hydrochloride RS in *Diluent*

Sample solution: 1 mg/mL of Benzphetamine Hydrochloride in *Diluent*

Chromatographic system: Proceed as directed in the *Assay*, except for *Injection volume*.

Injection volume: 20 µL

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between benzphetamine related compound E and benzphetamine; NLT 2.0 between benzphetamine related compound F and benzphetamine, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Benzphetamine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of benzphetamine from the *Standard solution*

C_S = concentration of USP Benzphetamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Benzphetamine Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor of each individual impurity (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Pseudoephedrine hydrochloride	0.22	0.62	0.10
Methamphetamine hydrochloride	0.37	0.65	0.10
Benzyl alcohol	0.57	1.18	0.10
Benzphetamine related compound E	0.87	0.93	0.10
Benzphetamine hydrochloride	1.00	1.00	—
Benzphetamine related compound F	1.11	0.50	0.10
Any other individual impurity	—	1.00	0.10
Total impurities[a]	—	—	0.5

[a] Benzphetamine related compound A monitored in the *Enantiomeric Purity* test is not included in the total impurities.

• **LIMIT OF BENZYL CHLORIDE**

Buffer: 0.01 M monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (50:50)

Diluent: Acetonitrile and water (50:50)

Standard solution: 0.001 mg/mL of USP Benzyl Chloride RS in *Diluent*

Sample solution: 10 mg/mL of Benzphetamine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 207 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

Run time: NLT 1.4 times the retention time of benzyl chloride

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of benzyl chloride in the portion of Benzphetamine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzyl chloride from the *Sample solution*

r_S = peak response of benzyl chloride from the *Standard solution*

C_S = concentration of USP Benzyl Chloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Benzphetamine Hydrochloride in the *Sample solution* (mg/mL)

www.webofpharma.com

Acceptance criteria: NMT 0.010%

• **ENANTIOMERIC PURITY**

Mobile phase: Methanol and diethylamine (100: 0.1)

System suitability solution: 0.1 mg/mL each of USP Benzphetamine Hydrochloride RS and USP Benzphetamine Related Compound A RS in *Mobile phase*

Standard solution: 0.0015 mg/mL of USP Benzphetamine Related Compound A RS in *Mobile phase*

Sample solution: 1 mg/mL of Benzphetamine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 207 nm

Column: 4.6-mm × 25-cm; 5-µm packing L80

Flow rate: 0.8 mL/min

Injection volume: 35 µL

Run time: NLT 1.9 times the retention time of benzphetamine

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between benzphetamine and benzphetamine related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of benzphetamine related compound A in the portion of Benzphetamine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzphetamine related compound A from the *Sample solution*

r_S = peak response of benzphetamine related compound A from the *Standard solution*

C_S = concentration of USP Benzphetamine Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Benzphetamine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzphetamine	1.00	—
Benzphetamine related compound A	1.16	0.15

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 1.0%

• **pH** ⟨791⟩

Sample solution: 20 mg/mL of Benzphetamine Hydrochloride in water

Acceptance criteria: 4.0–6.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Benzphetamine Hydrochloride RS

USP Benzphetamine Related Compound A RS

(*R*)-*N*-Benzyl-*N*-methyl-1-phenylpropan-2-amine hydrochloride.

$C_{17}H_{21}N \cdot HCl$ 275.82

USP Benzphetamine Related Compound E RS

(1*S*,2*S*)-2-[Benzyl(methyl)amino]-1-phenylpropan-1-ol hydrochloride.

$C_{17}H_{21}NO \cdot HCl$ 291.82

USP Benzphetamine Related Compound F RS

(1*S*,2*S*)-2-[Benzyl(methyl)amino]-1-cyclohexylpropan-1-ol hydrochloride.

$C_{17}H_{27}NO \cdot HCl$ 297.87

USP Benzyl Chloride RS

(Chloromethyl)benzene.

C_7H_7Cl 126.58▲ (USP 1-Aug-2019)

Add the following:

▲Benzphetamine Hydrochloride Tablets

DEFINITION

Benzphetamine Hydrochloride Tablets contain NLT 93.0% and NMT 105.0% of the labeled amount of benzphetamine hydrochloride ($C_{17}H_{21}N \cdot HCl$).

IDENTIFICATION

• **A.** The retention time of the benzphetamine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.** The UV spectrum of the benzphetamine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 2.76 g of monobasic sodium phosphate dihydrate in 1000 mL of water. Add 1.0 mL of triethylamine and adjust with phosphoric acid to a pH of 4.5.

Solution A: Acetonitrile and *Buffer* (10:90)

Solution B: Acetonitrile and water (80:20)

Mobile phase: *Solution A* and *Solution B* (65:35)

Diluent: Methanol and water (50:50)

Standard solution: 0.2 mg/mL of USP Benzphetamine Hydrochloride RS in *Diluent*. Sonicate to dissolve if necessary.

Sample solution: Nominally 0.2 mg/mL of benzphetamine hydrochloride in *Diluent*, prepared as follows. Transfer an adequate amount of benzphetamine hydrochloride from NLT 20 finely powdered Tablets to a suitable volumetric flask. Add about 60% of the final volume of *Diluent* and shake vigorously to disperse the Tablet powder. Sonicate for an additional NLT 60 min with intermediate shaking. Cool to room temperature and dilute with *Diluent* to volume. Pass a portion through a suitable filter with a 0.45-µm pore size. Discard the first few milliliters of filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 207 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L7

Temperatures

Autosampler: 15°

www.webofpharma.com

Column: 40°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Run time: NLT 2.1 times the retention time of benzphetamine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzphetamine hydrochloride ($C_{17}H_{21}N \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzphetamine from the *Sample solution*
r_S = peak response of benzphetamine from the *Standard solution*
C_S = concentration of USP Benzphetamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzphetamine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Use plastic vials for analysis.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer: Dissolve 2.76 g of monobasic sodium phosphate dihydrate in 1000 mL of water. Add 0.5 mL of triethylamine and adjust with phosphoric acid to a pH of 6.0.
Mobile phase: Acetonitrile and *Buffer* (70:30)
Standard stock solution: 0.55 mg/mL of USP Benzphetamine Hydrochloride RS, prepared as follows. Transfer an adequate amount of USP Benzphetamine Hydrochloride RS to a suitable volumetric flask. Add 5% of the final volume of acetonitrile and sonicate to dissolve. Dilute with *Medium* to volume.
Standard solution: 0.055 mg/mL of USP Benzphetamine Hydrochloride RS in *Medium* from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 207 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 25°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Run time: NLT 1.8 times the retention time of benzphetamine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzphetamine hydrochloride ($C_{17}H_{21}N \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of benzphetamine from the *Sample solution*
r_S = peak response of benzphetamine from the *Standard solution*
C_S = concentration of USP Benzphetamine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium* (mL), 900
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of benzphetamine hydrochloride ($C_{17}H_{21}N \cdot HCl$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A: 1.38 g of monobasic sodium phosphate dihydrate in 1000 mL of water
Solution B: Acetonitrile and water (80:20)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	75	25
10	60	40
15	40	60
20	15	85
22	15	85
23	80	20
30	80	20

Diluent: Methanol and water (50:50)
System suitability solution: 0.002 mg/mL each of USP Benzphetamine Hydrochloride RS, USP Benzphetamine Related Compound E RS, and USP Methamphetamine Hydrochloride RS in *Diluent*. Sonicate to dissolve if necessary.
Sensitivity solution: 0.0001 mg/mL of USP Benzphetamine Hydrochloride RS in *Diluent*
Standard solution: 0.002 mg/mL of USP Benzphetamine Hydrochloride RS in *Diluent*. Sonicate to dissolve if necessary.
Sample solution: Nominally 1 mg/mL of benzphetamine hydrochloride in *Diluent*, prepared as follows. Transfer an adequate amount of benzphetamine hydrochloride from NLT 20 finely powdered Tablets to a suitable volumetric flask. Add about 40% of the final volume of *Diluent* and shake vigorously to disperse the Tablet powder. Sonicate for an additional NLT 60 min with intermediate shaking. Cool to room temperature and dilute with *Diluent* to volume. Pass a portion through a suitable filter with a 0.45-µm pore size. Discard the first few milliliters of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 207 nm

www.webofpharma.com

Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 15°
Column: 40°
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between benzphetamine related compound E and benzphetamine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of benzphetamine from the *Standard solution*
C_S = concentration of USP Benzphetamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzphetamine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor of each individual impurity (see *Table 2*)

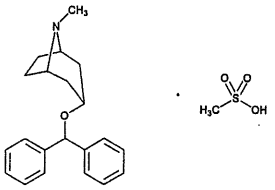
Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Methamphetamine hydrochloride	0.35	0.68	0.10
Benzphetamine related compound E	0.90	0.93	0.10
Benzphetamine hydrochloride	1.00	1.00	—
Any unspecified impurity	—	1.00	0.10
Total impurities	—	—	1.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benzphetamine Hydrochloride RS
USP Benzphetamine Related Compound E RS
(1*S*,2*S*)-2-[Benzyl(methyl)amino]-1-phenylpropan-1-ol hydrochloride.
$C_{17}H_{21}NO \cdot HCl$ 291.82
USP Methamphetamine Hydrochloride RS
(*S*)-*N*-Methyl-1-phenylpropan-2-amine hydrochloride.
$C_{10}H_{15}N \cdot HCl$ 185.69▲ (USP 1-Aug-2019)

Benztropine Mesylate



$C_{21}H_{25}NO \cdot CH_4O_3S$ 403.53
8-Azabicyclo[3.2.1]octane, 3-(diphenylmethoxy)-*N*-methyl-, *endo*-, methanesulfonate;
3α-(Diphenylmethoxy)-1α*H*,5α*H*-tropane methanesulfonate [132-17-2].

DEFINITION
Benztropine Mesylate contains NLT 98.0% and NMT 100.5% of benztropine mesylate ($C_{21}H_{25}NO \cdot CH_4O_3S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Sample: 60 mg of Benztropine Mesylate
Analysis: Dissolve the *Sample* in 25 mL of water, add 5 mL of sodium carbonate TS, and extract with four 10-mL portions of chloroform. Wash the combined chloroform extracts with about 10 mL of water, and extract the wash solution with 5 mL of chloroform. Filter the combined chloroform extracts through a tightly packed pledget of cotton, and wash the cotton with about 5 mL of chloroform. Add methyl red TS, and titrate the chloroform solution with 0.01 N perchloric acid in dioxane VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.01 N perchloric acid is equivalent to 4.035 mg of benztropine mesylate ($C_{21}H_{25}NO \cdot CH_4O_3S$).
Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 141°–148°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 2 h.
Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benztropine Mesylate RS

Benztropine Mesylate Injection

DEFINITION
Benztropine Mesylate Injection is a sterile solution of Benztropine Mesylate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of benztropine mesylate ($C_{21}H_{25}NO \cdot CH_4O_3S$).

www.webofpharma.com

IDENTIFICATION

• **A.**

Standard stock solution: 0.2 mg/mL of USP Benztropine Mesylate RS

Standard solution: In a separator containing the *Standard stock solution* add 2 mL of 1 N sodium hydroxide. Extract with three 10-mL portions of chloroform, collecting the chloroform extracts to a 50-mL beaker. Evaporate the chloroform extracts with the aid of gentle heat and a current of air to dryness, and dissolve the residue in 1 mL of chloroform.

Sample stock solution: Dilute a volume of Injection, equivalent to 10 mg of benztropine mesylate, in a separator to 50 mL with water (0.2 mg/mL).

Sample solution: In a separator containing the *Sample stock solution* add 2 mL of 1 N sodium hydroxide. Extract with three 10-mL portions of chloroform, collecting the chloroform extracts to a 50-mL beaker. Evaporate the chloroform extracts with the aid of gentle heat and a current of air to dryness, and dissolve the residue in 1 mL of chloroform.

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 1 µL

Developing solvent system: Chloroform, methanol, and a 1-in-4 solution of ammonium hydroxide (40:10:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the applications to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with potassium iodoplatinate TS.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Buffer: Transfer 0.83 mL of octylamine to a 1-L volumetric flask, dilute with water to volume, and adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (65:35)

Standard solution: 1 mg/mL of USP Benztropine Mesylate RS

Sample solution: Nominally 1 mg/mL of benztropine mesylate from the volume of Injection

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 259 nm

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 1.3 mL/min adjusted, as needed, to obtain a retention time of 7 min for benztropine mesylate

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of labeled amount of benztropine mesylate ($C_{21}H_{25}NO \cdot CH_4O_3S$) in each mL of the Injection:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Benztropine Mesylate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of benztropine mesylate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 55.6 USP Endotoxin Units/mg of benztropine mesylate

• **PH** ⟨791⟩**:** 5.0–8.0

• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Benztropine Mesylate RS

Benztropine Mesylate Tablets

DEFINITION

Benztropine Mesylate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of benztropine mesylate ($C_{21}H_{25}NO \cdot CH_4O_3S$).

IDENTIFICATION

• **A.**

Standard stock solution: 0.2 mg/mL of USP Benztropine Mesylate RS

Standard solution: In a separator containing the *Standard stock solution* add 2 mL of 1 N sodium hydroxide. Extract with three 10-mL portions of chloroform, collecting the chloroform extracts to a 50-mL beaker. Evaporate the chloroform extracts with the aid of gentle heat and a current of air to dryness, and dissolve the residue in 1 mL of chloroform.

Sample stock solution: Dissolve a portion of finely powdered Tablets, equivalent to 10 mg of benztropine mesylate, in 50 mL of water, shake by mechanical means for 30 min, and filter into a separator (0.2 mg/mL).

Sample solution: In a separator containing the *Sample stock solution* add 2 mL of 1 N sodium hydroxide. Extract with three 10-mL portions of chloroform, collecting the chloroform extracts to a 50-mL beaker. Evaporate the chloroform extracts with the aid of gentle heat and a current of air to dryness, and dissolve the residue in 1 mL of chloroform.

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 1 µL

Developing solvent system: Chloroform, methanol, and a 1-in-4 solution of ammonium hydroxide (40:10:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the applications to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with potassium iodoplatinate TS.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: Transfer 0.83 mL of octylamine to a 1-L volumetric flask, dilute with water to volume, and adjust with phosphoric acid to a pH of 3.0.

Diluent: Isopropyl alcohol, water, and phosphoric acid (40: 60: 0.1)

Mobile phase: Acetonitrile and *Buffer* (45:55)

Standard solution: 0.04 mg/mL of USP Benztropine Mesylate RS in *Diluent*

Sample solution: Nominally 0.04 mg/mL of benztropine mesylate from a suitable amount of powdered Tablets in *Diluent* prepared as follows. Add a suitable amount of fine powder from NLT 20 Tablets to a portion of *Diluent* corresponding to 60% of the final volume. Mix by mechanical means for NLT 60 min, and dilute with *Diluent* to volume. Centrifuge a portion of this mixture, and filter the supernatant layer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 259 nm

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 0.7 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 4.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benztropine mesylate ($C_{21}H_{25}NO \cdot CH_4O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Benztropine Mesylate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of benztropine mesylate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Determine the amount of benztropine mesylate ($C_{21}H_{25}NO \cdot CH_4O_3S$) dissolved by using the following method.

Buffer: Transfer 0.83 mL of octylamine to a 1-L volumetric flask, dilute to volume, and adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (65:35)

Standard solution: USP Benztropine Mesylate RS in *Medium*. Dilute to obtain a solution having a known concentration similar to that of the *Sample solution*.

Sample solution: Use a filtered portion of the solution under test from the dissolution vessel.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 2 mL/min

Injection volume: 300 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benztropine mesylate ($C_{21}H_{25}NO \cdot CH_4O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Benztropine Mesylate RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim (mg/Tablet)

Acceptance criteria: NLT 80% (Q) of the labeled amount of benztropine mesylate ($C_{21}H_{25}NO \cdot CH_4O_3S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

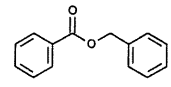
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Benztropine Mesylate RS

Benzyl Benzoate



$C_{14}H_{12}O_2$ 212.24

Benzoic acid, phenylmethyl ester;
Benzyl benzoate [120-51-4].

DEFINITION

Benzyl Benzoate contains NLT 99.0% and NMT 100.5% of $C_{14}H_{12}O_2$.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Sample: 2 g of Benzyl Benzoate

Analysis: Transfer the *Sample* to a conical flask fitted with a reflux condenser. Add 50.0 mL of 0.5 N alcoholic potassium hydroxide VS, and boil gently for 1 h. Cool, add phenolphthalein TS, and titrate with 0.5 N hydrochloric acid VS. Perform a blank determination (see *Titrimetry* ⟨541⟩). Each mL of 0.5 N alcoholic potassium hydroxide is equivalent to 106.1 mg of Benzyl Benzoate ($C_{14}H_{12}O_2$).

Acceptance criteria: 99.0%–100.5%

IMPURITIES

• **LIMIT OF ALDEHYDES**

Sample solution: Transfer 10.0 g to a 125-mL conical flask containing 50 mL of alcohol and 5 mL of hydroxylamine

www.webofpharma.com

hydrochloride solution (3.5 in 100), mix, and allow to stand for 10 min.

Analysis: Add 1 mL of bromophenol blue TS, and titrate with 0.1 N sodium hydroxide VS to a light green endpoint. Perform a blank determination, and match the color of the endpoint with that of the titrated *Sample solution*.

Acceptance criteria: The net volume of 0.1 N sodium hydroxide consumed does not exceed 0.50 mL (0.05% as benzaldehyde).

SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩: 1.116–1.120
- **CONGEALING TEMPERATURE** ⟨651⟩: Congelation may be brought about by the addition of a fragment of previously congealed Benzyl Benzoate when the temperature has reached the expected congealing temperature.

Acceptance criteria: NLT 18.0°

- **REFRACTIVE INDEX** ⟨831⟩: 1.568–1.570 at 20°
- **ACIDITY:** Add 2 drops of phenolphthalein TS to 25 mL of alcohol, and add 0.020 N sodium hydroxide until a pink color is produced. Add 5.0 g of Benzyl Benzoate, and titrate with 0.020 N sodium hydroxide.

Acceptance criteria: NMT 1.5 mL of 0.020 N sodium hydroxide is required to restore the pink color.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, well-filled, light-resistant containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzyl Benzoate RS

Benzyl Benzoate Lotion

DEFINITION

Benzyl Benzoate Lotion contains NLT 26.0% and NMT 30.0% (w/w) of benzyl benzoate ($C_{14}H_{12}O_2$).

Benzyl Benzoate	250 mL
Triethanolamine	5 g
Oleic Acid	20 g
Purified Water	750 mL
To make about	1000 mL

Mix the *Triethanolamine* with the *Oleic Acid*, add the *Benzyl Benzoate*, and mix. Transfer the mixture to a suitable container of 2000-mL capacity, add 250 mL of *Purified Water*, and shake the mixture thoroughly. Add the remaining *Purified Water*, and again shake thoroughly.

ASSAY

- **PROCEDURE**

Sample: 5 g of Lotion, accurately weighed, in a conical flask

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Residual titration

Titrant: 0.1 N sodium hydroxide VS

Back-titrant: 0.5 N hydrochloric acid VS

Endpoint detection: Visual

Analysis: To the *Sample* add 25 mL of alcohol and 2 drops of phenolphthalein TS. Cool the solution to 15°, and titrate quickly with *Titrant* to a slight pink color. Add 50.0 mL of 0.5 N alcoholic potassium hydroxide VS, connect the flask to a reflux condenser, and boil gently for 1 h. Cool. Promptly add phenolphthalein TS and titrate with *Back-titrant*. Perform a blank determination. Each mL of 0.5 N alcoholic potassium hydroxide is equivalent to 106.1 mg of benzyl benzoate ($C_{14}H_{12}O_2$).

Acceptance criteria: 26.0%–30.0% (w/w)

SPECIFIC TESTS

- **pH** ⟨791⟩: 8.5–9.2

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight containers.

Benzylpenicilloyl Polylysine Concentrate

» Benzylpenicilloyl Polylysine Concentrate has a molar concentration of benzylpenicilloyl moiety ($C_{16}H_{19}N_2O_5S$) of not less than 0.0125 M and not more than 0.020 M. It contains one or more suitable buffers.

Packaging and storage—Preserve in tight containers.

Labeling—The label states that this article is not intended for direct administration to humans or animals.

USP Reference standards ⟨11⟩—
USP L-Lysine Hydrochloride RS

pH ⟨791⟩: between 6.5 and 8.5, the undiluted Concentrate being used.

Limit of penicillenate and penamaldate—Transfer 1 mL of Concentrate to a 50-mL volumetric flask, dilute with *Saline phosphate buffer*, prepared as directed in the *Assay*, to volume, and mix. Using a suitable spectrophotometer and using *Saline phosphate buffer* as a blank, determine the absorbances at the wavelengths of maximum absorption at about 322 nm and 282 nm. Calculate the molar concentration of penicillenate taken by the formula:

$$50A_{322}/26{,}600b$$

in which A_{322} is the absorbance at 322 nm, 26,600 is the molar absorptivity of the penicillenate moiety at pH 7.6, and b is the length of the cell, in cm: not more than 0.00020 M is found. Calculate the molar concentration of penamaldate taken by the formula:

$$50A_{282}/22{,}325b$$

in which A_{282} is the absorbance at 282 nm, 22,325 is the molar absorptivity of the penamaldate moiety at pH 7.6, and b is the length of the cell, in cm: not more than 0.00060 M is found.

Benzylpenicilloyl substitution—

Citrate buffer—Dissolve 19.69 g of sodium citrate dihydrate, 0.1 mL of pentachlorophenol, and 5 mL of 2,2′-thiodiethanol in 900 mL of 0.2 N hydrochloric acid, adjust with hydrochloric acid to a pH of 2.2, dilute with water to 1000 mL, and mix.

Ninhydrin reagent—Dissolve 18 g of ninhydrin and 0.7 g of hydrindantin in 675 mL of dimethyl sulfoxide, add 225 mL of 4 M lithium acetate solution previously adjusted with glacial acetic acid to a pH of 5.2, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP L-Lysine Hydrochloride RS in *Citrate buffer* to obtain a solution having a known concentration of about 91 µg per mL (5×10^{-4} M).

Test preparation—Transfer 1.0 mL of Concentrate to a 10-mL volumetric flask, dilute with water to volume, and mix.

USP Monographs

www.webofpharma.com

Transfer 1.0 mL of this solution to an ampul, add 1.5 mL of 6 N hydrochloric acid, and seal the ampul under nitrogen. Heat the ampul at 110° for 22 hours. Transfer the contents of the ampul to a round-bottom, 50-mL flask, and dry by vacuum rotary evaporation. Dissolve the residue three times, using 5-mL portions of water, evaporating to dryness after each dissolution. Dissolve the residue in 10 mL of *Citrate buffer.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 1.75-mm × 50-cm column that contains a packing of 8-µm 8% cross-linked sulfonated divinylbenzene polystyrene cation-exchange resin. The column effluent is mixed continuously with flowing *Ninhydrin reagent,* and the flowing mixture is heated at 130° for 1.5 minutes in a reaction coil. The absorbance of the reaction mixture is measured continuously by a 570-nm detector. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 1800 theoretical plates, and the relative standard deviation for replicate injections is not more than 4.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention time is about 57 minutes for L-lysine. Calculate the molar concentration of lysine in the Concentrate taken by the formula:

$$(0.1C/182.65)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP L-Lysine Hydrochloride RS in the *Standard preparation;* 182.65 is the molecular weight of anhydrous lysine hydrochloride; and r_U and r_S are the peak responses obtained from the *Test preparation* and the *Standard preparation,* respectively. Calculate the percentage of benzylpenicilloyl substitution taken by the formula:

$$100(B/L)$$

in which B is the molar concentration of benzylpenicilloyl moiety in the Concentrate, as determined in the *Assay;* and L is the molar concentration of lysine in the Concentrate: not less than 50% and not more than 70% is found.

Assay—

Saline phosphate buffer—Dissolve 9 g of sodium chloride and 1.38 g of monobasic sodium phosphate in 900 mL of water, adjust with 5 N sodium hydroxide or phosphoric acid to a pH of 7.6, dilute with water to 1000 mL, and mix.

Mercuric chloride solution—Dissolve 35 mg of mercuric chloride in 500 mL of water, and mix.

Assay preparation—Transfer 1.0 mL of Concentrate to a 500-mL volumetric flask, dilute with *Saline phosphate buffer* to volume, and mix.

Procedure—Transfer 3.0 mL of *Assay preparation* to a spectrophotometric cell. Using a suitable spectrophotometer and using *Saline phosphate buffer* as the blank, determine the initial absorbance at the wavelength of maximum absorbance at about 282 nm. Add 0.02 mL of *Mercuric chloride solution* to the *Assay preparation* in the spectrophotometric cell, mix, and determine the absorbance at the same wavelength after 1 and 3 minutes. Repeat the addition of 0.02-mL portions of *Mercuric chloride solution* until a maximum absorbance reading is obtained. Calculate the molar concentration of benzylpenicilloyl moiety in the Concentrate taken by the formula:

$$500\{[A_m(3 + 0.02n)/3] - A_i\}/22{,}325b$$

in which A_m is the highest absorbance observed; A_i is the initial absorbance, n is the number of 0.02-mL portions of *Mercuric chloride solution* added to the *Assay preparation* to obtain the maximum absorbance; 22,325 is the molar absorptivity of the penamaldate formed by the reaction of benzylpenicilloyl with mercuric chloride at pH 7.6; and b is the length of the cell, in cm: between 0.0125 M and 0.020 M is found.

Benzylpenicilloyl Polylysine Injection

» Benzylpenicilloyl Polylysine Injection has a molar concentration of benzylpenicilloyl moiety ($C_{16}N_2H_{19}O_5S$) of not less than 5.4×10^{-5} M and not more than 7.0×10^{-5} *M.* It contains one or more suitable buffers.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, in a refrigerator.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 5833.0 USP Endotoxin Units per mL.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 6.5 and 8.5.

Assay—

Saline phosphate buffer and *Mercuric chloride solution*—Prepare as directed in the *Assay*— under *Benzylpenicilloyl Polylysine Concentrate.*

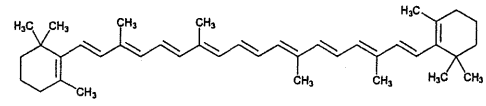
Assay preparation—Combine the contents of a sufficient number of containers to obtain not less than 3 mL of Injection. Transfer 3.0 mL of Injection to a 10-mL volumetric flask, dilute with *Saline phosphate buffer* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Benzylpenicilloyl Polylysine Concentrate.* Calculate the molar concentration of benzylpenicilloyl moiety in the Injection taken by the formula:

$$(10/3)\{[A_m(3 + 0.02n)/3] - A_i\}/22{,}325b$$

in which the terms are as defined therein.

Beta Carotene



$C_{40}H_{56}$ 536.87

β,β-Carotene;
all-*trans*-β-Carotene;
(all-*E*)-1,1′-(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethylcyclohexene] [7235-40-7].

DEFINITION

Beta Carotene contains NLT 96.0% and NMT 101.0% of total carotenoids calculated as beta carotene ($C_{40}H_{56}$). It contains NLT 95% of all-*trans*-beta carotene in the total carotenoids content.

www.webofpharma.com

IDENTIFICATION

• **A.**

Sample solution: Prepare as directed in the *Sample solution* in the test for *Content of Total Carotenoids.*

Analysis: Record the UV-Vis spectrum from 300–600 nm.

Acceptance criteria: The *Sample solution* shows a shoulder at about 427 nm, an absorption maximum at about 455 nm, and another maximum at about 483 nm. The absorbance ratio A_{455}/A_{483} is between 1.14 and 1.18.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Content of Beta Carotene.*

COMPOSITION

• **CONTENT OF TOTAL CAROTENOIDS**

[NOTE—Use low-actinic glassware.]

Sample stock solution: 0.1 mg/mL of Beta Carotene in tetrahydrofuran

Sample solution: Transfer 3.0 mL of *Sample stock solution* to a 100-mL volumetric flask, and dilute with cyclohexane to volume.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Analytical wavelength: 456 nm

Cell path: 1 cm

Blank: Cyclohexane

Analysis

Sample: *Sample solution*

Calculate the percentage of total carotenoids (*T*) as beta carotene ($C_{40}H_{56}$):

$$T = A/(F \times C)$$

A = absorbance of the *Sample solution*

F = 2505, coefficient of extinction ($E^{1\%}$) of pure all-*trans*-beta carotene in cyclohexane (100 mL · g^{-1} · cm^{-1})

C = concentration of the *Sample solution* (g/mL)

Acceptance criteria: 96.0%–101.0% of total carotenoids as beta carotene ($C_{40}H_{56}$)

• **CONTENT OF BETA CAROTENE**

[NOTE—Use low-actinic glassware.]

Mobile phase: Transfer 50 mg of butylated hydroxytoluene to a 1-L volumetric flask, and dissolve with 20 mL of 2-propanol. Add 0.2 mL of *N*-ethyldiisopropylamine, 25 mL of 0.2% ammonium acetate solution, 455 mL of acetonitrile, and about 450 mL of methanol. Allow the solution to reach room temperature, and dilute with methanol to volume.

Diluent: 50 µg/mL of butylated hydroxytoluene in alcohol

System suitability solution: Transfer 20 mg of USP Beta Carotene System Suitability RS to a 50-mL volumetric flask. Add 1 mL of water and 4 mL of tetrahydrofuran, and sonicate for 5 min. Dilute with *Diluent* to volume, and sonicate for 5 min. Cool to room temperature, pass the suspension through a membrane filter of 0.45-µm pore size, and use the clear filtrate.

Standard solution: 10 µg/mL of USP Beta Carotene RS in tetrahydrofuran and *Diluent* (1:9). Dissolve an appropriate amount of USP Beta Carotene RS in a volumetric flask first with tetrahydrofuran, using 10% of the volume of the flask, then dilute with *Diluent* to volume.

Sample solution: Dilute the freshly prepared *Sample stock solution* as prepared in the test for *Content of Total Carotenoids* (1 in 10) with *Diluent.*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 448 nm

Column: 4.6-mm × 25-cm; 5-µm packing L68

Column temperature: 30°

Flow rate: 0.6 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

The approximate relative retention times of the components in the *System suitability solution* are listed in *Table 1.*

Table 1

Analyte	Relative Retention Time	Relative Response Factor
all-*trans*-Alpha carotene	0.93	1.0
all-*trans*-Beta carotene	1.00	1.0
9-*cis*-Beta carotene	1.07	1.0
13-*cis*-Beta carotene	1.17	1.2
15-*cis*-Beta carotene	1.21	1.4

Suitability requirements

Chromatogram similarity: The chromatogram from the *System suitability solution* is similar to the reference chromatogram provided with the lot of USP Beta Carotene System Suitability RS being used.

Resolution: NLT 1.5 between all-*trans*-beta carotene and all-*trans*-alpha carotene; NLT 1.2 between all-*trans*-beta carotene and 9-*cis*-beta carotene, *System suitability solution*

Tailing factor: NMT 2.0 for the all-*trans*-beta carotene peak, *Standard solution*

Relative standard deviation: NMT 2.0% for the all-*trans*-beta carotene peak from replicate injections, *Standard solution*

Analysis

Sample: *Sample solution*

Record the chromatograms, and identify the peaks of the relevant analytes of the *Sample solution* by comparing with those of the *System suitability solution.* Measure the peak area responses.

Calculate the percentage of all-*trans*-beta carotene relative to total carotenoids in the sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of all-*trans*-beta carotene from the *Sample solution*

r_T = [(peak area of all-*trans*-alpha carotene × 1.0) + (peak area of all-*trans*-beta carotene) + (peak area of 9-*cis*-beta carotene) + (peak area of 13-*cis*-beta carotene × 1.2) + (peak area of 15-*cis*-beta carotene × 1.4) + (sum of peak areas of other *cis*-isomers of beta carotene)] from the *Sample solution*

Acceptance criteria: NLT 95% of all-*trans*-beta carotene in the total carotenoids content

• **ALPHA CAROTENE AND OTHER RELATED COMPOUNDS**

Mobile phase, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the test for *Content of Beta Carotene.*

Analysis

Sample: *Sample solution*

Calculate the percentage of alpha carotene and other individual related compounds relative to total carotenoids in the portion of the *Sample* taken:

USP Monographs

www.webofpharma.com

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = (peak area of all-*trans*-alpha carotene × 1.0) or (peak area response of other individual related compounds × appropriate relative response factor, *Table 1*) in the *Sample solution*

r_T = [(peak area of all-*trans*-alpha carotene × 1.0) + (peak area of all-*trans*-beta carotene) + (peak area of 9-*cis*-beta carotene) + (peak area of 13-*cis*-beta carotene × 1.2) + (peak area of 15-*cis*-beta carotene × 1.4) + (sum of peak areas of other *cis*-isomers of beta carotene)] from the *Sample solution*

Acceptance criteria
Alpha carotene: NMT 1.0%
Total related compounds (including alpha carotene): NMT 5%

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%, 2 g of specimen being used

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum over phosphorus pentoxide at 40° for 4 h.
Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Beta Carotene RS
(all-*E*)-1,1′-(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18- diyl) bis[2,6,6-trimethylcyclohexene].
USP Beta Carotene System Suitability RS

Beta Carotene Capsules

DEFINITION

Change to read:

Beta Carotene Capsules contain NLT ▲90.0%▲ (USP 1-May-2019) and NMT 125.0% of the labeled amount of total beta carotene ($C_{40}H_{56}$).
▲▲ (USP 1-May-2019)

IDENTIFICATION
• **A.**
Sample solution: Dilute the *Sample stock solution* from the test for *Content of Total Beta Carotene* with cyclohexane to a final concentration of 1–5 µg/mL of beta carotene. Pass through a membrane filter of 0.45-µm pore size.
Analysis: Record the UV-Vis spectrum from 300 to 600 nm.
Acceptance criteria: The *Sample solution* shows a shoulder at about 427 nm, an absorption maximum at about 455 nm, and another maximum at about 483 nm. The absorbance ratio A_{455}/A_{483} is between 1.14 and 1.18.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Content of Total Beta Carotene*.

ASSAY

Change to read:

• **CONTENT OF TOTAL BETA CAROTENE**
[NOTE—Use low-actinic glassware.]
Mobile phase: Transfer 50 mg of butylated hydroxytoluene into a 1-L volumetric flask, and dissolve with 20 mL of 2-propanol. Add 0.2 mL of *N*-ethyldiisopropylamine, 25 mL of 0.2% ammonium acetate solution, 455 mL of acetonitrile, and about 450 mL of methanol. Allow the solution to reach room temperature, and dilute with methanol to volume.
Diluent: 50 mg/L of butylated hydroxytoluene in alcohol
System suitability solution: Transfer 20 mg of USP Beta Carotene System Suitability RS to a 50-mL volumetric flask. Add 1 mL of water and 4 mL of tetrahydrofuran, and sonicate for 5 min. Dilute with *Diluent* to volume, and sonicate for 5 min. Cool to room temperature, pass through a membrane filter of 0.45-µm pore size, and use the clear filtrate.
Standard stock solution: 60 µg/mL of USP Beta Carotene RS in tetrahydrofuran. [NOTE—The USP Beta Carotene RS is subjected to the spectrophotometric purity test at the time of analysis; see the determination of the concentration of *Standard solution A* below.]
Standard solution A: Transfer 5.0 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 5.0 mL of tetrahydrofuran, and dilute with *Diluent* to volume. Determine the concentration of *Standard solution A* according to the *Analysis* of *Standard solution B*. [NOTE—The concentration of *Standard solution B* equals the concentration of *Standard solution A*.]
Standard solution B: Transfer 5.0 mL of the *Standard stock solution* into a 100-mL volumetric flask, and dilute with cyclohexane to volume. Prepare in triplicate.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Analytical wavelength: ▲456 nm▲ (USP 1-May-2019)
Cell: 1 cm
Blank: Cyclohexane
Analysis
Sample: *Standard solution B*
Calculate the concentration of total beta carotene (µg/mL) as *all-trans*-beta carotene ($C_{40}H_{56}$) in *Standard solution B*:

$$\text{Result} = (A_U/a) \times F$$

A_U = average absorbance of the three preparations of *Standard solution B*
a = absorptivity of pure *all-trans*-beta carotene in cyclohexane▲▲ (USP 1-May-2019), 250
F = conversion factor, 1000 µg/mg

Sample stock solution: Randomly select a number of Capsules, equivalent to 10–50 mg of beta carotene, with a total weight not exceeding 5 g. For powder-containing Capsules, empty the shell, and transfer shell and contents into a 250-mL volumetric flask. For Capsules containing liquid formulations, place the Capsules directly into a 250-mL volumetric flask. Add 250 mg of butylated hydroxytoluene, 0.5 mL of alkaline protease R, and 15 mL of water. Swirl the flask gently to wet the entire contents. Sonicate the flask at 50° for 30 min, and swirl the flask every 10 min. Add 100 mL of alcohol to the warm suspension, and shake vigorously. Add 110 mL of methylene chloride, and shake vigorously again. Disperse any clumps with a homogenizer, and rinse the homogenizer probe with 15 mL of methylene chloride into the flask. Allow the solution

Stimuli Articles

www.webofpharma.com

to stand in the dark until it reaches room temperature (about 2 h), dilute with methylene chloride to volume, shake vigorously, and allow the solids to settle.

Sample solution: Dilute a volume of the *Sample stock solution* with a *Diluent*–methylene chloride mixture (1:1) so that the final concentration of beta carotene is 1–5 µg/mL. Pass through a membrane filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 448 nm

Column: 4.6-mm × 25-cm; 5-µm packing L68

Column temperature: 30°

Flow rate: 0.6 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution A*

[NOTE—The approximate relative retention times of the components in the *System suitability solution* are listed in *Table 1*.]

Table 1

Name	Relative Retention Time	Relative Response Factor
all-trans-Alpha carotene	0.93	▲1.05▲ (USP 1-May-2019)
all-trans-Beta carotene	1.00	1
9-*cis*-Beta carotene	1.07	1
13-*cis*-Beta carotene	1.17	1.2
15-*cis*-Beta carotene	1.21	1.4

Suitability requirements

Chromatogram similarity: The chromatogram from the *System suitability solution* is similar to the reference chromatogram provided with the lot of USP Beta Carotene System Suitability RS being used.

Resolution: NLT ▲1.2▲ (USP 1-May-2019) between *all-trans*-beta carotene and *all-trans*-alpha carotene and between *all-trans*-beta carotene and 9-*cis*-beta carotene, *System suitability solution*

Tailing factor: NMT 2.0 for the *all-trans*-beta carotene peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the *all-trans*-beta carotene peak from replicate injections, *Standard solution A*

Analysis

Samples: *Standard solution A* and *Sample solution*

Identify the peaks of the relevant analytes of the *Sample solution* by comparing with those of the *System suitability solution*. Measure the peak area responses.

Calculate the percentage of the labeled amount of total beta carotene in the portion of Capsules taken:

$$\text{Result} = (\Sigma r_U / r_S) \times (C_S / C_U) \times 100$$

r_U = (peak area of *all-trans*-beta carotene) + (peak area of 9-*cis*-beta carotene) + (peak area of 13-*cis*-beta carotene × 1.2) + (peak area of 15-*cis*-beta carotene × 1.4) from the *Sample solution*

r_S = peak area of *all-trans*-beta carotene from *Standard solution A*

C_S = concentration of *all-trans*-beta carotene in *Standard solution A* as determined above (µg/mL)

C_U = nominal concentration of total beta carotene in the *Sample solution* (µg/mL)

Calculate the percentage of *all-trans*-beta carotene in the portion of Capsules taken:

$$\text{Result} = (r_{all\text{-}trans} / \Sigma r_U) \times 100$$

$r_{all\text{-}trans}$ = peak area of *all-trans*-beta carotene from the *Sample solution*

r_U = (peak area of *all-trans*-beta carotene) + (peak area of 9-*cis*-beta carotene) + (peak area of 13-*cis*-beta carotene × 1.2) + (peak area of 15-*cis*-beta carotene × 1.4) from the *Sample solution*

Acceptance criteria: ▲90.0%▲ (USP 1-May-2019)–125.0% of the labeled amount of total beta carotene ($C_{40}H_{56}$)

▲▲ (USP 1-May-2019)

SPECIFIC TESTS

Change to read:

- **ALPHA CAROTENE AND OTHER RELATED COMPOUNDS**

Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the test for *Content of Total Beta Carotene*.

Analysis

Sample: *Sample solution*

Calculate the percentage of alpha carotene and other individual related compounds relative to total beta carotene in the portion of Capsules taken:

$$\text{Result} = (r_U / r_T) \times 100$$

r_U = peak area of alpha carotene or other individual related compounds

r_T = sum of the areas of all the peaks

Acceptance criteria

Alpha carotene: NMT 1.0%

Total related compounds (including alpha carotene): NMT ▲5%▲ (USP 1-May-2019)

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Change to read:

- **LABELING:** The label states the name and content of any carriers and antioxidants added to the formulation and the content of total carotenoids as beta carotene. ▲The label states the percentages of *cis*- and *all-trans*-isomers in the total beta carotene at the time of product manufacture and release.▲ (USP 1-May-2019)
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Beta Carotene RS

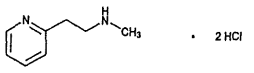
(*all-E*)-1,1′-(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18- diyl) bis[2,6,6-trimethylcyclohexene].

$C_{40}H_{56}$ 536.87

USP Beta Carotene System Suitability RS

www.webofpharma.com

Betahistine Hydrochloride



$C_8H_{12}N_2 \cdot 2HCl$ 209.12

2-Pyridineethanamine, *N*-methyl-, dihydrochloride;
2-[2-(Methylamino)ethyl]pyridine, dihydrochloride [5579-84-0].

DEFINITION

Betahistine Hydrochloride contains NLT 99.0% and NMT 101.0% of betahistine hydrochloride ($C_8H_{12}N_2 \cdot 2HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020) Meets the requirements.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*: Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: 0.69 mg/mL of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 4.7.
Mobile phase: Acetonitrile and *Solution A* containing 2.88 mg/mL of sodium lauryl sulfate (7:13)
Standard solution: 0.38 mg/mL of USP Betahistine Hydrochloride RS in *Mobile phase*
Sample solution: 0.38 mg/mL of Betahistine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.0-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 0.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of betahistine hydrochloride ($C_8H_{12}N_2 \cdot 2HCl$) in the portion of Betahistine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of betahistine from the *Sample solution*
r_S = peak response of betahistine from the *Standard solution*
C_S = concentration of USP Betahistine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Betahistine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Betahistine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 1/F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*
F = relative response factor of each impurity (see *Table 1*); 1.0 for all other peaks

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-(2-Hydroxyethyl) pyridine[a]	0.3	1.5	0.2
2-Vinylpyridine[a]	0.4	2.0	0.2
N-Methyl-*N*,*N*-bis(2-pyridin-2-yl-ethyl) amine[b]	2.4	1.1	0.2
Any unspecified impurity	—	—	0.10
Total impurities	—	—	0.5

[a] This impurity is a hydrochloride salt.
[b] This impurity exists as a trihydrochloride.

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample: 100 mg/mL of Betahistine Hydrochloride in water
Acceptance criteria: 2.0–3.0

- **LOSS ON DRYING** ⟨731⟩

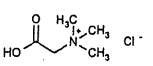
Analysis: Dry between 100° and 105° to constant weight.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Betahistine Hydrochloride RS

Betaine Hydrochloride



$C_5H_{11}NO_2 \cdot HCl$ 153.61

Methanaminium, 1-carboxy-*N*, *N*, *N*-trimethyl-, chloride.
Betaine hydrochloride.
(Carboxymethyl)trimethylammonium chloride [590-46-5].

» Betaine Hydrochloride contains not less than 98.0 percent and not more than 100.5 percent of $C_5H_{11}NO_2 \cdot HCl$, calculated on the anhydrous basis.

www.webofpharma.com

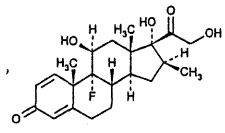
Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Betaine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: A solution (1 in 20) responds to the tests for *Chloride* ⟨191⟩.
pH ⟨791⟩: between 0.8 and 1.2, in a solution (1 in 4).
Water Determination, *Method I* ⟨921⟩: not more than 0.5%.
Residue on ignition ⟨281⟩: not more than 0.1%.
Assay—Transfer about 400 mg of Betaine Hydrochloride, accurately weighed, to a conical flask, add 50 mL of glacial acetic acid, and heat gently with swirling until solution is complete. Add 25 mL of mercuric acetate TS, cool, add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 15.36 mg of $C_5H_{11}NO_2 \cdot HCl$.

Betamethasone



$C_{22}H_{29}FO_5$ 392.46
Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17,21-trihydroxy-16-methyl-, (11β,16β)-.
9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione [378-44-9].

» Betamethasone contains not less than 97.0 percent and not more than 103.0 percent of $C_{22}H_{29}FO_5$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers. Store between 2° and 30°.
USP Reference standards ⟨11⟩—
USP Betamethasone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy*▲ (CN 1-May-2020).
B: Thin-Layer Chromatographic Identification Test ⟨201⟩—
Test solution—Prepare a solution of Betamethasone in dehydrated alcohol containing 0.5 mg per mL.
Developing solvent system: a mixture of chloroform and diethylamine (2:1).
Procedure—Proceed as directed in the chapter, except to locate the spots by lightly spraying with dilute sulfuric acid (1 in 2) and heating on a hot plate or under a lamp until spots appear.
Specific rotation ⟨781S⟩: between +118° and +126°, calculated on the dried basis.
Test solution: 5 mg per mL, in methanol.
Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%, a platinum crucible being used.

Ordinary impurities ⟨466⟩—
Test solution: methanol.
Standard solution: methanol.
Application volume: 10 µL.
Eluant: a mixture of toluene, acetone, methyl ethyl ketone, and formic acid (55:20:20:5), in a nonequilibrated chamber.
Visualization: 5.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (63:37). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Prepare a solution of propylparaben in alcohol having a known concentration of about 0.25 mg per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Betamethasone RS in alcohol to obtain a solution having a known concentration of about 0.2 mg per mL. Transfer 10.0 mL of this solution to a suitable vial, and add 10.0 mL of *Internal standard solution,* to obtain a *Standard preparation* having known concentrations of about 0.1 mg of betamethasone and about 0.125 mg of propylparaben per mL.
Assay preparation—Using about 80 mg of Betameth asone, accurately weighed, prepare as directed for *Standard preparation.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for betamethasone and 1.4 for propylparaben; the resolution, *R,* between betamethasone and propylparaben is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{22}H_{29}FO_5$ in the portion of Betamethasone taken by the formula:

$$800C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Betamethasone RS in the *Standard preparation;* and R_U and R_S are the peak height ratios of the betamethasone peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Betamethasone Cream

DEFINITION
Betamethasone Cream contains NLT 90.0% and NMT 115.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$) in a suitable cream base.

IDENTIFICATION
• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Standard solution: 1 mg/mL of USP Betamethasone RS in dehydrated alcohol

www.webofpharma.com

Sample solution: Nominally 1 mg/mL of betamethasone prepared by concentrating 10 mL of the *Sample solution* from the *Assay* on a steam bath to 1 mL
Chromatographic system
Developing solvent system: Chloroform and diethylamine (2:1)
Spray reagent: Methanol, sulfuric acid, and nitric acid (10:10:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in the chapter, except spray the plate with the *Spray reagent*, and heat at 105° for 10 min.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (37:63)
Internal standard solution: 0.25 mg/mL of propylparaben in alcohol
Standard stock solution: 0.2 mg/mL of USP Betamethasone RS in alcohol
Standard solution: 0.1 mg/mL of USP Betamethasone RS prepared by combining 10.0 mL of the *Internal standard solution* and 10.0 mL of the *Standard stock solution*
Sample solution: Nominally 0.1 mg/mL of betamethasone prepared as follows. To a portion of Cream, nominally equivalent to 2 mg of betamethasone, add 10.0 mL of *Internal standard solution* and 10.0 mL of alcohol. Mix by rotation for 20 min. Centrifuge at 2500 rpm for 10 min. Transfer a portion of the supernatant to a suitable vial.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for betamethasone and propylparaben are 1.0 and 1.4, respectively.]
Suitability requirements
Resolution: NLT 3.0 between betamethasone and propylparaben
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio of the betamethasone peak to the internal standard peak from the *Sample solution*
R_S = peak height ratio of the betamethasone peak to the internal standard peak from the *Standard solution*
C_S = concentration of USP Betamethasone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Betamethasone RS

Betamethasone Oral Solution

DEFINITION
Betamethasone Oral Solution contains NLT 90.0% and NMT 115.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$).

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Diluent: Chloroform and methanol (1:1)
Standard solution: 1 mg/mL of USP Betamethasone RS in alcohol
Sample solution: Place a volume of Oral Solution, equivalent to about 1 mg of betamethasone, in a centrifuge tube. Add 15 mL of 0.1 N hydrochloric acid and 20 mL of ethyl acetate. Shake the tube for about 1 min. Centrifuge to separate the phases. Transfer the upper phase (ethyl acetate) to a suitable container. Evaporate to dryness on a steam bath under a gentle stream of nitrogen.
Allow to cool to room temperature. Dissolve the residue in about 0.5 mL of *Diluent* by using a vortex mixer. Transfer the solution to a 2-mL volumetric flask with small portions of *Diluent*. Dilute with *Diluent* to volume, and mix.
Evaporate 1 mL of the resulting solution on a steam bath just to dryness, and dissolve the residue in 0.5 mL of alcohol.
Chromatographic system
Application volume: 10 µL
Developing solvent system: Chloroform and diethylamine (2:1)
Spray reagent: Dilute sulfuric acid (1 in 2)
Analysis: Proceed as directed in the chapter. Locate the spots by lightly spraying with *Spray reagent*, and heat on a hot plate or under a lamp until spots appear.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Protect all standard and sample solutions from light.
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.9.
Solution A: Acetonitrile and *Buffer* (25:75)
Solution B: Acetonitrile and *Buffer* (45:55)
Diluent: Dehydrated alcohol and water (2:3)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
25.0	0	100
25.1	100	0
35.0	100	0

Standard stock solution: 0.12 mg/mL of USP Betamethasone RS prepared as follows. Transfer a quantity of USP Betamethasone RS to a suitable container, and dilute, using sonication, with dehydrated alcohol to obtain a solution containing 0.3 mg/mL. Quantitatively dilute an aliquot of this solution with water to obtain a 0.12-mg/mL solution of betamethasone.

Standard solution: 0.048 mg/mL of USP Betamethasone RS in *Diluent* from *Standard stock solution*

Beclomethasone solution: 0.12 mg/mL of beclomethasone prepared as follows. Transfer a quantity of beclomethasone to a suitable container, and dilute, using sonication, with dehydrated alcohol to obtain a solution containing 0.3 mg/mL. Quantitatively dilute an aliquot of this solution with water to obtain a 0.12 mg/mL solution of beclomethasone.

System suitability solution: 0.048 mg/mL each of USP Betamethasone RS and beclomethasone in *Diluent*, prepared from the *Standard stock solution* and *Beclomethasone solution*

Sample solution: Nominally 0.048 mg/mL of betamethasone prepared as follows. Transfer a measured volume of Oral Solution, containing a known amount of betamethasone, to a suitable volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 4-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 50 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for betamethasone and beclomethasone are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 4.0 between betamethasone and beclomethasone, *System suitability solution*
Tailing factor: NMT 1.5 for betamethasone, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Betamethasone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

IMPURITIES

• ORGANIC IMPURITIES
Protect all sample and standard solutions from light.
Buffer, Solution A, Solution B, Diluent, Mobile phase, Standard stock solution, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.48 µg/mL of USP Betamethasone RS in *Diluent* from the *Standard stock solution*
Sensitivity solution: 0.024 µg/mL of USP Betamethasone RS in *Diluent* from the *Standard solution*
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
[NOTE—The relative retention times for betamethasone and beclomethasone are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 4.0 between betamethasone and beclomethasone, *System suitability solution*
Relative standard deviation: NMT 10% for betamethasone, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each related compound in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for individual related compounds from the *Sample solution*
r_S = peak response for betamethasone from the *Standard solution*
C_S = concentration of USP Betamethasone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Betamethasone	1.0	—
9,11-Expoxy-17α,21-dihydroxy-16β-methylpregna-1,4 diene-3,20-dione	1.25	1.3
17α,21-Dihydroxy-16β-methylpregna-1,4,11-triene-3,20-dione	1.33	0.7

SPECIFIC TESTS

• MICROBIAL ENUMERATION TESTS ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the test for the absence of *Escherichia coli*. The total aerobic microbial count is NMT 10^2 cfu/mL, and the total combined molds and yeasts count is NMT 10^1 cfu/mL.
• pH ⟨791⟩**:** 2.8–3.6
• DELIVERABLE VOLUME ⟨698⟩**:** Meets the requirements for oral solution packaged in multiple-unit containers

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Store at controlled room temperature, protected from light. Preserve in tight containers.
• USP REFERENCE STANDARDS ⟨11⟩
USP Betamethasone RS

www.webofpharma.com

Betamethasone Acetate

$C_{24}H_{31}FO_6$ 434.50
Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(acetyloxy)-, (11β,16β)-.
9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 21-acetate [987-24-6].

» Betamethasone Acetate contains not less than 97.0 percent and not more than 103.0 percent of $C_{24}H_{31}FO_6$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers. Store between 2° and 30°.
USP Reference standards ⟨11⟩—
USP Betamethasone Acetate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).
B: Thin-Layer Chromatographic Identification Test ⟨201⟩—
Test solution: 0.5 mg per mL in dehydrated alcohol.
Developing solvent system: a mixture of chloroform and diethylamine (2:1).
Procedure—Proceed as directed in the chapter. Locate the spots on the plate by lightly spraying with 10% sulfuric acid in alcohol and heating on a hot plate or under a lamp until spots appear.
Specific rotation ⟨781S⟩: between +120° and +128°.
Test solution: 10 mg per mL, in dioxane.
Water Determination, *Method I* ⟨921⟩ : not more than 4.0%.
Residue on ignition ⟨281⟩: not more than 0.2%, a platinum crucible being used.

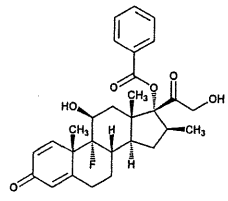
Ordinary impurities ⟨466⟩—
Test solution: methanol.
Standard solution: methanol.
Application volume: 10 µL.
Eluant: a mixture of toluene and isopropyl alcohol (90:10), in a nonequilibrated chamber.
Visualization: 5.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (800: 700: 1.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Transfer about 35 mg of progesterone to a 50-mL volumetric flask, add *Mobile phase* to volume, and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Betamethasone Acetate RS in *Mobile phase,* and quantitatively dilute with *Mobile phase* to obtain a solution containing about 0.5 mg per mL. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution,* dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.1 mg of USP Betamethasone Acetate RS per mL.
Assay preparation—Transfer about 50 mg of Betameth asone Acetate, accurately weighed, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution,* dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 3 for progesterone and 1.0 for betamethasone acetate; the resolution, *R,* between the analyte and internal standard peaks is not less than 2; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{24}H_{31}FO_6$ in the portion of Betamethasone Acetate taken by the formula:

$$500C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Betamethasone Acetate RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Betamethasone Benzoate



$C_{29}H_{33}FO_6$ 496.57
Pregna-1,4-diene-3,20-dione, 17-(benzoyloxy)-9-fluoro-11,21-dihydroxy-16-methyl-, (11β,16β)-;
9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-benzoate [22298-29-9].

DEFINITION
Betamethasone Benzoate contains NLT 98.0% and NMT 102.0% of betamethasone benzoate ($C_{29}H_{33}FO_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (60:40)
Internal standard solution: 0.6 mg/mL of betamethasone dipropionate in methanol
Standard stock solution: 0.6 mg/mL of USP Betamethasone Benzoate RS in methanol
Standard solution: 0.2 mg/mL of USP Betamethasone Benzoate RS prepared by mixing 5.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution*
Sample stock solution: 0.6 mg/mL of Betamethasone Benzoate in methanol
Sample solution: 0.2 mg/mL of Betamethasone Benzoate prepared by mixing 5.0 mL of *Sample stock solution* and 10.0 mL of *Internal standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)

www.webofpharma.com

Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for betamethasone benzoate and betamethasone dipropionate are 1.0 and 1.4, respectively.]
Suitability requirements
Resolution: NLT 3 between betamethasone benzoate and the internal standard
Relative standard deviation: NMT 2.0% from three replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of betamethasone benzoate ($C_{29}H_{33}FO_6$) in the portion of Betamethasone Benzoate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the betamethasone benzoate peak to the internal standard peak from the *Sample solution*
R_S = peak response ratio of the betamethasone benzoate peak to the internal standard peak from the *Standard solution*
C_S = concentration of USP Betamethasone Benzoate RS in the *Standard solution* (mg/mL)
C_U = concentration of Betamethasone Benzoate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RELATED STEROIDS**
Standard solution A: 5 mg/mL of USP Betamethasone Benzoate RS in methanol
Standard solution B: 100 µg/mL of USP Betamethasone Benzoate RS from *Standard solution A* in methanol
Sample solution: 20.0 mg/mL of Betamethasone Benzoate in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Toluene, acetone, and methanol (75:25:4)
Analysis
Samples: *Standard solutions* and *Sample solution*
Proceed as directed in the chapter. Examine the plate under short-wavelength UV light.
Acceptance criteria: The principal spot from the *Sample solution* corresponds in R_F value to that of *Standard solution A*; and the *Sample solution* shows NMT 3 additional spots, the intensity and size of which do not exceed those of the spot from *Standard solution B*.

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 40 mg/mL, in dioxane
Acceptance criteria: +60° to +66°
• **LOSS ON DRYING** ⟨731⟩
Sample: 200 mg
Analysis: Dry the *Sample* at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 2° and 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Betamethasone Benzoate RS

Betamethasone Benzoate Gel

DEFINITION

Betamethasone Benzoate Gel contains an amount of betamethasone benzoate ($C_{29}H_{33}FO_6$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone benzoate ($C_{29}H_{33}FO_6$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Mobile phase: Methanol, acetonitrile, and water (23:9:18)
Internal standard solution: 250 µg/mL of USP Methyltestosterone RS in methanol
Standard stock solution: 0.5 mg/mL of USP Betamethasone Benzoate RS in methanol
Standard solution: 0.05 mg/mL of USP Betamethasone Benzoate RS prepared as follows. Transfer 5.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, and dilute with methanol to volume.
Sample solution: 0.05 mg/mL of betamethasone benzoate in methanol prepared as follows. Transfer a portion of Gel, nominally equivalent to 0.5 mg of betamethasone benzoate, into a 125-mL separatory funnel. Add 20 mL of water and 2 mL of saturated sodium acetate solution, shake to disperse the Gel, and add 2.0 mL of *Internal standard solution*. Extract this solution with one 50-mL portion of chloroform, followed by three 40-mL portions of chloroform. Discard the aqueous layer. Wash the chloroform extract with 10 mL of water, allow to stand for 10 min, then pass through chloroform-wetted glass fiber filter paper and anhydrous sodium sulfate into a suitable container. Evaporate to dryness under a vacuum at 30°. Dissolve the residue in 10 mL of methanol.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 236 nm
Column: 3.9-mm × 30-cm; packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for methyltestosterone and betamethasone benzoate are about 1.0 and 1.33, respectively.]
Suitability requirements
Resolution: NLT 3.0 between methyltestosterone and betamethasone benzoate
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of betamethasone benzoate ($C_{29}H_{33}FO_6$) in the portion of Gel taken:

www.webofpharma.com

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the betamethasone benzoate peak to the internal standard peak from the *Sample solution*
R_S = peak response ratio of the betamethasone benzoate peak to the internal standard peak from the *Standard solution*
C_S = concentration of USP Betamethasone Benzoate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of betamethasone benzoate in the *Sample solution* (mg/mL)

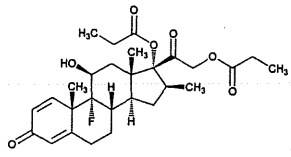
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Minimum Fill** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS
• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°. Protect from freezing.
• **USP Reference Standards** ⟨11⟩
USP Betamethasone Benzoate RS
USP Methyltestosterone RS

Betamethasone Dipropionate



$C_{28}H_{37}FO_7$ 504.59
Pregna-1,4-diene-3,20-dione, 9-fluoro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, (11β,16β);
9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [5593-20-4].

DEFINITION
Betamethasone Dipropionate contains NLT 97.0% and NMT 103.0% of betamethasone dipropionate ($C_{28}H_{37}FO_7$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
• **B. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Sample solution: 1 mg/mL, in chloroform
Chromatographic system
Developing solvent system: Chloroform and acetone (7:1)
Acceptance criteria: Meets the requirements

ASSAY
• **Procedure**
Mobile phase: Acetonitrile and water (1 in 2) such that the retention times for betamethasone dipropionate and beclomethasone dipropionate are 14 and 18 min, respectively. Degas by sonicating for 5–10 min. Do not leave *Mobile phase* in the column overnight, but flush the system after use with water for 15 min, followed by methanol for 15 min.
Diluent: Acetic acid and methanol (1 in 1000)
Internal standard solution: 0.9 mg/mL of USP Beclomethasone Dipropionate RS in *Diluent*
Standard stock solution: 0.6 mg/mL of USP Betamethasone Dipropionate RS in *Diluent*
Standard solution: 0.3 mg/mL of USP Betamethasone Dipropionate RS and 0.45 mg/mL of USP Beclomethasone Dipropionate RS, prepared by combining 5.0 mL each of the *Internal standard solution* and the *Standard stock solution*
Sample stock solution: 0.6 mg/mL of Betamethasone Dipropionate in *Diluent*
Sample solution: 0.3 mg/mL of Betamethasone Dipropionate, prepared by combining 5.0 mL each of the *Internal standard solution* and the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 or 240 nm
Column: 4-mm × 30-cm; packing L1
Injection volume: 5–25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Peak area ratios: The lowest and highest peak area ratios of three successive injections agree within 2.0%.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of betamethasone dipropionate ($C_{28}H_{37}FO_7$) in the portion of Betamethasone Dipropionate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio of betamethasone dipropionate to the internal standard from the *Sample solution*
R_S = peak height ratio of betamethasone dipropionate to the internal standard from the *Standard solution*
C_S = concentration of USP Betamethasone Dipropionate RS in the *Standard solution* (mg/mL)
C_U = concentration of Betamethasone Dipropionate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES
• **Residue on Ignition** ⟨281⟩**:** NMT 0.2%, using a platinum crucible
• **Organic Impurities**
Mobile phase: Acetonitrile and water (65:35)
System suitability solution: 0.05 mg/mL of USP Betamethasone Dipropionate RS and 0.05 mg/mL of USP Betamethasone Valerate RS in *Mobile phase*
Sample solution: 0.3 mg/mL of Betamethasone Dipropionate in *Mobile phase.* Shake until dissolved.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; packing L1

www.webofpharma.com

Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 4.0 between betamethasone valerate and betamethasone dipropionate
Column efficiency: NLT 8000 theoretical plates
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Betamethasone Dipropionate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of the responses for all the peaks

Acceptance criteria
Individual impurities: NMT 1.0%
Total impurities: NMT 2.0%

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL, in dioxane
Acceptance criteria: +63° to +70°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Beclomethasone Dipropionate RS
USP Betamethasone Dipropionate RS
USP Betamethasone Valerate RS

Betamethasone Dipropionate Cream

DEFINITION
Betamethasone Dipropionate Cream contains an amount of betamethasone dipropionate ($C_{28}H_{37}FO_7$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$), in a suitable cream base.

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 150 µg/mL of USP Betamethasone Dipropionate RS in chloroform
Sample solution: Nominally equivalent to 150 µg/mL of betamethasone dipropionate, prepared as follows. Transfer 1.5 g of Cream to a glass-stoppered, 50-mL centrifuge tube. Add 15 mL of a methanol–hydrochloric acid solution prepared by mixing 1 volume of dilute hydrochloric acid (1 in 120) with 4 volumes of methanol. Shake to obtain a homogeneous mixture. Add 30 mL of solvent hexane, mix for 10 min, and centrifuge. Using a syringe, transfer the lower aqueous phase to a second centrifuge tube, and add 20 mL of water. Extract this aqueous mixture with chloroform by shaking, centrifuging, and removing the lower, chloroform phase with a syringe. Evaporate the chloroform on a steam bath with the aid of a stream of nitrogen to dryness, cool, and dissolve the residue in chloroform.
Chromatographic system
Application volume: 40 µL
Developing solvent system: Chloroform and acetone (7:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in the chapter.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (1 in 2) such that the retention times for betamethasone dipropionate and beclomethasone dipropionate are 14 and 18 min, respectively. Degas by sonicating for 5–10 min. Do not leave the *Mobile phase* in the column overnight, but flush the system after use with water for 15 min, followed by methanol for 15 min.
Diluent: Acetic acid in methanol (1 in 1000)
Internal standard solution: 0.45 mg/mL of USP Beclomethasone Dipropionate RS in *Diluent*
Standard stock solution: 0.2 mg/mL of USP Betamethasone Dipropionate RS in *Diluent*
Standard solution: 0.133 mg/mL of USP Betamethasone Dipropionate RS and 0.15 mg/mL of USP Beclomethasone Dipropionate RS prepared by combining 10.0 mL of the *Standard stock solution* and 5.0 mL of the *Internal standard solution*
Sample solution: Nominally equivalent to 0.1 mg/mL of betamethasone, prepared as follows. Transfer a portion of Cream, equivalent to 2 mg of betamethasone dipropionate (1.6 mg of betamethasone), into a capped 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution* and 10.0 mL of *Diluent*. Heat in a water bath at 60°, shaking intermittently, until the Cream melts. Remove from the bath, and shake vigorously until the Cream has resolidified. Repeat the heating and shaking. Freeze in an ice–methanol bath for 15 min, and centrifuge at 2500 rpm for 5 min. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 or 240 nm
Column: 4-mm × 30-cm; packing L1
Injection volume: 5–25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Peak area ratios: The lowest and highest peak area ratios of three successive injections agree within 2.0%.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak height ratio of betamethasone dipropionate to the internal standard from the *Sample solution*
R_S = peak height ratio of betamethasone dipropionate to the internal standard from the *Standard solution*
C_S = concentration of USP Betamethasone Dipropionate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of betamethasone, 392.46
M_{r2} = molecular weight of betamethasone dipropionate, 504.59

www.webofpharma.com

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers. Store at 25°, excursions permitted between 15° and 30°. Protect from freezing.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Beclomethasone Dipropionate RS
  USP Betamethasone Dipropionate RS

Betamethasone Dipropionate Lotion

DEFINITION
Betamethasone Dipropionate Lotion contains an amount of betamethasone dipropionate ($C_{28}H_{37}FO_7$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$), in a suitable lotion base.

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 150 µg/mL of USP Betamethasone Dipropionate RS in chloroform
Sample solution: Nominally 150 µg/mL of betamethasone dipropionate, prepared as follows. Transfer a portion of Lotion, equivalent to 0.6 mg of betamethasone dipropionate, to a 50-mL vial; add 10 mL of 0.1 N hydrochloric acid; then add 4 mL of chloroform. Disperse on a vortex mixer for about 1 min, shake vigorously for 10 min, and centrifuge at 2000 rpm for about 5 min. Transfer the chloroform layer to a suitable vial.
Chromatographic system
Application volume: 40 µL
Developing solvent system: Chloroform and acetone (7:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in the chapter.
Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile and water (1 in 2) such that the retention times for betamethasone dipropionate and beclomethasone dipropionate are 14 and 18 min, respectively. Degas by sonicating for 5–10 min. Do not leave the *Mobile phase* in the column overnight, but flush the system after use with water for 15 min, followed by methanol for 15 min.
Internal standard solution: 0.9 mg/mL of USP Beclomethasone Dipropionate RS in chloroform
Standard stock solution A: 0.6 mg/mL of USP Betamethasone Dipropionate RS in chloroform
Standard stock solution B: 0.3 mg/mL of betamethasone dipropionate and 0.45 mg/mL of beclomethasone dipropionate, prepared by combining 5.0 mL each of *Internal standard solution* and *Standard stock solution A*
Standard solution: To 10.0 mL of 0.1 N hydrochloric acid in a capped 5-mL centrifuge tube, add 4.0 mL of *Standard stock solution B*. Cap the tube, and shake vigorously for about 2 min, or disperse on a vortex mixer for about 1 min. Centrifuge at 2500 rpm for about 3 min. Transfer the chloroform phase to a suitable vial. Evaporate the chloroform under a stream of nitrogen at a slightly elevated temperature to dryness. Cool the vial to room temperature, add 4.0 mL of methanol, and swirl to dissolve the residue.
Sample solution: Nominally 0.23 mg/mL of betamethasone, prepared as follows. Transfer a portion of Lotion, equivalent to 1.2 mg of betamethasone dipropionate (0.93 mg of betamethasone), into a capped 50-mL centrifuge tube. Add 10.0 mL of 0.1 N hydrochloric acid, shake to disperse, then add 2.0 mL of *Internal standard solution* and 2.0 mL of chloroform. Cap, and shake vigorously for about 2 min, or disperse on a vortex mixer for about 1 min. Centrifuge at 2500 rpm for about 3 min. Transfer the chloroform phase to a suitable vial. Evaporate the chloroform under a stream of nitrogen at a slightly elevated temperature to dryness. Cool the vial to room temperature, add 4.0 mL of methanol, and swirl to dissolve the residue.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 or 240 nm
Column: 4-mm × 30-cm; packing L1
Injection volume: 5–25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Peak area ratios: The lowest and highest peak area ratios of three successive injections agree within 2.0%.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak height ratio of betamethasone dipropionate to the internal standard from the *Sample solution*
R_S = peak height ratio of betamethasone dipropionate to the internal standard from the *Standard solution*
C_S = concentration of USP Betamethasone Dipropionate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of betamethasone, 392.46
M_{r2} = molecular weight of betamethasone dipropionate, 504.59

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°. Protect from light and freezing.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Beclomethasone Dipropionate RS
  USP Betamethasone Dipropionate RS

Betamethasone Dipropionate Ointment

DEFINITION
Betamethasone Dipropionate Ointment contains an amount of betamethasone dipropionate ($C_{28}H_{37}FO_7$) equivalent to

USP Monographs

www.webofpharma.com

NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$), in a suitable ointment base.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 150 µg/mL of USP Betamethasone Dipropionate RS in chloroform

Sample solution: Nominally 150 µg/mL of betamethasone dipropionate, prepared as follows. Transfer 1.5 g of Ointment to a glass-stoppered, 50-mL centrifuge tube. Add 15 mL of methanol–hydrochloric acid solution prepared by mixing 1 volume of dilute hydrochloric acid (1 in 120) with 4 volumes of methanol. Shake to obtain a homogeneous mixture. Add 30 mL of solvent hexane, mix for 10 min, and centrifuge. Using a syringe, transfer the lower aqueous phase to a second centrifuge tube, and add 20 mL of water. Extract this aqueous mixture with chloroform by shaking, centrifuging, and removing the lower, chloroform phase with a syringe. Evaporate the chloroform on a steam bath with the aid of a stream of nitrogen to dryness, cool, and dissolve the residue in chloroform.

Chromatographic system

Application volume: 40 µL

Developing solvent system: Chloroform and acetone (7:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter.

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (1 in 2) such that the retention times for betamethasone dipropionate and beclomethasone dipropionate are 14 and 18 min, respectively. Degas by sonicating for 5–10 min. Do not leave the *Mobile phase* in the column overnight, but flush the system after use with water for 15 min, followed by methanol for 15 min.

Diluent: Acetic acid and alcohol (1 in 1000)

Internal standard solution: 0.45 mg/mL of USP Beclomethasone Dipropionate RS in *Diluent*

Standard stock solution: 0.2 mg/mL of USP Betamethasone Dipropionate RS in *Diluent*

Standard solution: 0.133 mg/mL of USP Betamethasone Dipropionate RS and 0.15 mg/mL of USP Beclomethasone Dipropionate RS prepared by combining 10.0 mL of the *Standard stock solution* and 5.0 mL of the *Internal standard solution*

Sample solution: Nominally equivalent to 0.1 mg/mL of betamethasone, prepared as follows. Transfer a portion of Ointment, equivalent to 2 mg of betamethasone dipropionate (1.6 mg of betamethasone), into a capped 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution* and 10.0 mL of *Diluent*. Heat in a water bath at 70°, shaking intermittently, until the sample melts. Remove from the bath, and shake vigorously until the Ointment has solidified. Repeat the heating and shaking. Freeze in an ice–methanol bath for 15 min, and centrifuge at 2500 rpm for 5 min. Use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 or 240 nm

Column: 4-mm × 30-cm; packing L1

Injection volume: 5–25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Peak area ratios: The lowest and highest peak area ratios of three successive injections agree within 2.0%.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak height ratio of betamethasone dipropionate to the internal standard from the *Sample solution*

R_S = peak height ratio of betamethasone dipropionate to the internal standard from the *Standard solution*

C_S = concentration of USP Betamethasone Dipropionate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of betamethasone from the *Sample solution* (mg/mL)

M_{r1} = molecular weight of betamethasone, 392.46

M_{r2} = molecular weight of betamethasone dipropionate, 504.59

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

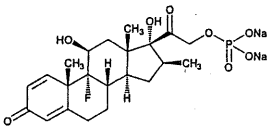
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers. Store at 25°, with excursions permitted between 15° and 30°. Protect from freezing.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Beclomethasone Dipropionate RS

USP Betamethasone Dipropionate RS

Betamethasone Sodium Phosphate



$C_{22}H_{28}FNa_2O_8P$ 516.40

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(phosphonooxy)-, disodium salt, (11β,16β)-; 9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 21-(disodium phosphate) [151-73-5].

DEFINITION

Betamethasone Sodium Phosphate contains NLT 97.0% and NMT 103.0% of betamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 1 mg/mL of USP Betamethasone Sodium Phosphate RS in methanol

USP Monographs

www.webofpharma.com

Sample solution: 1 mg/mL of Betamethasone Sodium Phosphate in methanol
Chromatographic system
Application volume: 10 µL
Developing solvent system: 500 mL of butyl alcohol and 200 mL of dilute hydrochloric acid (1 in 12). Place in a separatory funnel, and mix. Use the organic layer as the developing solvent.
Spray reagent: Sulfuric acid, methanol, and nitric acid (10:10:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in the chapter, except to spray the plate with *Spray reagent*, and heat at 105° for 10 min.
Acceptance criteria: Meets the requirements
• **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩ and *Phosphate* ⟨191⟩
Analysis: Ignite it at 800° (see *Residue on Ignition* ⟨281⟩).
Acceptance criteria: The residue meets the requirements for sodium and phosphate.

ASSAY

• **PROCEDURE**
Mobile phase: Methanol and 0.07 M anhydrous monobasic potassium phosphate (3:2)
Diluent: Methanol and water (3:2)
Standard solution: 0.17 mg/mL of USP Betamethasone Sodium Phosphate RS in *Diluent*
Sample solution: 0.17 mg/mL of Betamethasone Sodium Phosphate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of betamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$) in the portion of Betamethasone Sodium Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Betamethasone Sodium Phosphate RS in the *Standard solution* (mg/mL)
C_U = concentration of Betamethasone Sodium Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

• **LIMIT OF PHOSPHATE IONS**
Standard phosphate solution and **Phosphate reagent A:** Prepare as directed in *Phosphate in Reagents* (see *Reagents, Indicators, and Solutions—General Tests for Reagents*).
Phosphate reagent B: Dissolve 350 mg of *p*-methylaminophenol sulfate in 50 mL of water. Add 20 g of sodium metabisulfite, mix to dissolve, and dilute with water to 100 mL.
Standard solution: Dilute 5.0 mL of *Standard phosphate solution* in a mixture of 10 mL of water and 5 mL of 2 N sulfuric acid contained in a 25-mL volumetric flask, by warming if necessary. Add 1 mL each of *Phosphate reagent A* and *Phosphate reagent B*, dilute with water to 25.0 mL, mix, and allow to stand at room temperature for 30 min.
Sample solution: Dissolve 50 mg of Betamethasone Sodium Phosphate in a mixture of 10 mL of water and 5 mL of 2 N sulfuric acid contained in a 25-mL volumetric flask, by warming if necessary. Add 1 mL each of *Phosphate reagent A* and *Phosphate reagent B*, dilute with water to 25.0 mL, mix, and allow to stand at room temperature for 30 min.
Instrumental conditions
Mode: Vis
Analytical wavelength: Maximum absorbance at about 730 nm
Cell: 1 cm
Blank: Water
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution*. The limit is 1.0% of phosphate (PO_4).
• **LIMIT OF FREE BETAMETHASONE**
Sample stock solution: 1.0 mg/mL of Betamethasone Sodium Phosphate in water, prepared as follows. Dissolve 25.0 mg of Betamethasone Sodium Phosphate in water to make 25.0 mL.
Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a separator, and extract with three 25-mL portions of chloroform. Filter each extract through a chloroform-saturated cotton pledget, combining the filtrates in a conical flask. Evaporate the chloroform on a steam bath to dryness with the aid of a current of air, and dissolve the residue in methanol to make 25.0 mL.
Blank solution: Transfer 5.0 mL of water to a separator. Proceed as directed in *Sample solution*, beginning with "extract with three 25-mL portions of chloroform".
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 239 nm
Cell: 1 cm
Blank: *Blank solution*
Analysis
Sample: *Sample solution*
Calculate the quantity, in mg, of free betamethasone in the portion of Betamethasone Sodium Phosphate taken:

$$\text{Result} = A \times 3.125$$

A = absorbance of the *Sample solution*

Acceptance criteria: NMT 0.25 mg (1.0%)

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL
Acceptance criteria: +99° to +105°
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 10.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Betamethasone Sodium Phosphate RS

USP Monographs

www.webofpharma.com

Betamethasone Sodium Phosphate Injection

» Betamethasone Sodium Phosphate Injection is a sterile solution of Betamethasone Sodium Phosphate in Water for Injection. It contains an amount of betamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Betamethasone Sodium Phosphate RS
Identification—Dilute the Injection with methanol, if necessary, to obtain a solution containing about 2 mg of betamethasone sodium phosphate per mL. Separately apply 10 µL of this test solution and 10 µL of a solution of USP Betamethasone Sodium Phosphate RS in methanol containing 2 mg per mL to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with chromatographic silica gel mixture. Develop the chromatogram in an equilibrated chamber containing *n*-butyl alcohol previously shaken with 1 N hydrochloric acid, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, air-dry, then spray with a mixture of sulfuric acid, methanol, and nitric acid (10:10:1). Heat the plate at 105° for 10 minutes: the R_F value of the principal spot from the test solution corresponds to that obtained from the Standard solution.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 29.2 USP Endotoxin Units per mg of betamethasone.
pH ⟨791⟩: between 8.0 and 9.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of methanol and 0.05 M monobasic potassium phosphate (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Transfer about 100 mg of butylparaben to a 100-mL volumetric flask, add methanol to volume, and mix.
Standard preparation—Using an accurately weighed quantity of USP Betamethasone Sodium Phosphate RS, prepare a solution in water containing 4 mg per mL. Transfer 3.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.5 mg of USP Betamethasone Sodium Phosphate RS per mL.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 9 mg of betamethasone, to a 25-mL volumetric flask. Add 5.0 mL of the *Internal standard solution*, dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the analyte and internal standard peaks is not less than 5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 2.4 for butylparaben and 1.0 for betamethasone sodium phosphate. Calculate the quantity, in mg, of $C_{22}H_{29}FO_5$ in each mL of the Injection taken by the formula:

$$(392.47/516.41)(25C/V)(R_U/R_S)$$

in which 392.47 and 516.41 are the molecular weights of betamethasone and betamethasone sodium phosphate, respectively; *C* is the concentration, in mg per mL, of USP Betamethasone Sodium Phosphate RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Betamethasone Sodium Phosphate and Betamethasone Acetate Injectable Suspension

» Betamethasone Sodium Phosphate and Betamethasone Acetate Injectable Suspension is a sterile preparation of Betamethasone Sodium Phosphate in solution and Betamethasone Acetate in suspension in Water for Injection. It contains an amount of betamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$) equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$), and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of betamethasone acetate ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Betamethasone Acetate RS
USP Betamethasone Sodium Phosphate RS

Identification—
A: Thin-layer chromatographic identification test ⟨201⟩—
Test solution—Dilute 2 mL with 2 mL of methanol.
Standard solution—Prepare a solution of USP Betamethasone Sodium Phosphate RS in a mixture of methanol and water (1:1) having a concentration of 2 mg per mL.
Developing solvent system, Spray reagent, and *Procedure* —Proceed as directed for *Identification* test *B* under *Betamethasone Sodium Phosphate.*
B: Test solution—Use the *Test solution* prepared for *Identification* test *A.*
Standard solution—Prepare a solution of USP Betamethasone Acetate RS in a mixture of methanol and water (1:1) having a concentration of 1.5 mg per mL.
Developing solvent system and *Procedure*—Proceed as directed for *Identification* test *B* under *Betamethasone*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 29.2 USP Endotoxin Units per mg of betamethasone.

www.webofpharma.com

pH ⟨791⟩: between 6.8 and 7.2.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of methanol and 0.075 M monobasic potassium phosphate (7:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer about 50 mg of methyltestosterone to a 50-mL volumetric flask, add methanol to volume, and mix.

Standard preparation—Transfer about 63 mg of USP Betamethasone Sodium Phosphate RS, accurately weighed, to a 25-mL volumetric flask, add *Mobile phase* to volume, and mix (*Solution 1*). Transfer about 45 mg of USP Betamethasone Acetate RS, accurately weighed, to a 25-mL volumetric flask, add methanol to volume, and mix (*Solution 2*). Pipet 5 mL each of *Solution 1* and *Solution 2* into a 100-mL volumetric flask. Add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having known concentrations of about 126 µg of USP Betamethasone Sodium Phosphate RS per mL and 90 µg of USP Betamethasone Acetate RS per mL.

Assay preparation—Using a "To contain" pipet transfer an accurately measured volume of the well-mixed Injectable Suspension, equivalent to about 9 mg of betamethasone acetate, to a 100-mL volumetric flask. Rinse the pipet with about 10 mL of *Mobile phase*, collecting the rinse in the volumetric flask. Add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the betamethasone phosphate and betamethasone acetate peaks is not less than 5.0, and the resolution, *R*, between the betamethasone acetate and internal standard peaks is not less than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for betamethasone phosphate, 1.7 for methyltestosterone, and 1.0 for betamethasone acetate. Calculate the quantity, in mg, of betamethasone acetate ($C_{24}H_{31}FO_6$) in each mL of the Injectable Suspension taken by the formula:

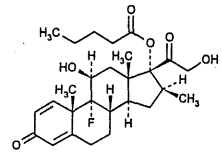
$$0.1C/V(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Betamethasone Acetate RS in the *Standard preparation*; V is the volume, in mL, of Injectable Suspension taken; and R_U and R_S are the peak response ratios obtained for betamethasone acetate and methyltestosterone from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of betamethasone ($C_{22}H_{29}FO_5$) equivalent to the quantity of betamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$), in each mL of the Injectable Suspension taken by the formula:

$$(392.46/516.41)(0.1C/V)(R_U/R_S)$$

in which 392.46 and 516.41 are the molecular weights of betamethasone and betamethasone sodium phosphate, respectively; C is the concentration, in µg per mL, of USP Betamethasone Sodium Phosphate RS in the *Standard preparation*; V is the volume, in mL, of Injectable Suspension taken; and R_U and R_S are the peak response ratios obtained for betamethasone phosphate and methyltestosterone from the *Assay preparation* and the *Standard preparation*, respectively.

Betamethasone Valerate



$C_{27}H_{37}FO_6$ 476.58

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-dihydroxy-16-methyl-17-[(1-oxopentyl)oxy]-, (11β,16β)-.
9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-valerate [2152-44-5].

» Betamethasone Valerate contains not less than 97.0 percent and not more than 103.0 percent of $C_{27}H_{37}FO_6$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Beclomethasone Dipropionate RS
USP Betamethasone Valerate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: Thin-Layer Chromatographic Identification Test ⟨201⟩—
Test solution: 1 mg per mL, in alcohol.
Developing solvent system: a mixture of toluene and ethyl acetate (1:1).
Procedure—Proceed as directed in the chapter. Spray the plate with a mixture of sulfuric acid, methanol, and nitric acid (10:10:1), and heat at 105° for 15 minutes.
Specific rotation ⟨781S⟩: between +75° and +82°.
Test solution: 10 mg per mL, in dioxane.
Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%, a platinum crucible being used.

Chromatographic purity—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and glacial acetic acid (550:450:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Test solution—Transfer about 4 mg of Betamethasone Valerate, accurately weighed, to a suitable flask. Add 10 mL of *Mobile phase*, and shake until dissolved.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between betamethasone valerate

USP Monographs

www.webofpharma.com

and any impurity is not less than 1.5; and the column efficiency is not less than 9000 theoretical plates.

Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Betamethasone Valerate taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of all the peak responses: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer about 40 mg of beclomethasone dipropionate to a 100-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix.

Standard preparation—Transfer about 30 mg of USP Betamethasone Valerate RS, accurately weighed, to a 50-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix. Transfer 5.0 mL of this solution to a suitable stoppered vial, add 10.0 mL of *Internal standard solution*, and mix to obtain a solution having a known concentration of about 0.2 mg of USP Betamethasone Valerate RS per mL.

Assay preparation—Transfer about 60 mg of Betameth asone Valerate, accurately weighed, to a 100-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix. Transfer 5.0 mL of this solution to a suitable stoppered vial, add 10.0 mL of *Internal standard solution*, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.7 for beclomethasone dipropionate and 1.0 for betamethasone valerate; the resolution, *R,* between betamethasone valerate and beclomethasone dipropionate is not less than 4.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{27}H_{37}FO_6$ in the portion of Betamethasone Valerate taken by the formula:

$$300C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Betamethasone Valerate Cream

DEFINITION

Betamethasone Valerate Cream contains an amount of betamethasone valerate ($C_{27}H_{37}FO_6$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$), in a suitable cream base.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	63	37
7.0	63	37
15.0	30	70
19.0	30	70
19.1	10	90
21.0	10	90
21.1	63	37
25.0	63	37

Diluent A: Tetrahydrofuran and water (50:50)
Diluent B: Acetonitrile and water (40:60)
System suitability solution: 25 µg/mL of USP Betamethasone Valerate RS and 10 µg/mL of USP Betamethasone Valerate Related Compound A RS in *Diluent B.* Sonicate to dissolve if necessary.
Standard solution: 25 µg/mL of USP Betamethasone Valerate RS in *Diluent B.* Sonicate to dissolve if necessary.
Sample solution: Nominally 20 µg/mL of betamethasone, prepared as follows. Transfer 1.0 mg of betamethasone from a portion of Cream to a suitable glass centrifuge tube. Add 15.0 mL of *Diluent A* and mix with a vortex mixer to disperse the sample thoroughly. Add 35.0 mL of *Diluent B* and sonicate for 10 min with intermittent shaking. Centrifuge to obtain a clear supernatant. Pass through a suitable filter of 0.2-µm pore size using a glass syringe. Discard the first 1 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 240 nm. For *Identification B,* use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Temperatures
Autosampler: 4°
Column: Ambient
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*

USP Monographs

www.webofpharma.com

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of betamethasone, 392.46
M_{r2} = molecular weight of betamethasone valerate, 476.58

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent A, Diluent B, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.25 µg/mL each of USP Betamethasone RS, USP Betamethasone Valerate RS, and USP Betamethasone Valerate Related Compound A RS in *Diluent B*. Sonicate to dissolve if necessary.

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*

Relative standard deviation: NMT 5%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each specified degradation product in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified degradation product from the *Sample solution*
r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (µg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (µg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of betamethasone valerate from the *Standard solution*
C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of betamethasone, 392.46
M_{r2} = molecular weight of betamethasone valerate, 476.58

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Betamethasone	0.30	1.0
Betamethasone valerate	1.00	—
Betamethasone valerate related compound A	1.04	1.0
Any individual unspecified degradation product	—	1.0
Total degradation products	—	2.0

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Betamethasone RS
USP Betamethasone Valerate RS
USP Betamethasone Valerate Related Compound A RS
9-Fluoro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-21-yl valerate.
$C_{27}H_{37}FO_6$ 476.58

Betamethasone Valerate Lotion

DEFINITION

Betamethasone Valerate Lotion contains an amount of betamethasone valerate ($C_{27}H_{37}FO_6$) equivalent to NLT 95.0% and NMT 115.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	63	37

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
7.0	63	37
15.0	30	70
19.0	30	70
19.1	10	90
21.0	10	90
21.1	63	37
25.0	63	37

Diluent: Acetonitrile and water (40:60)
System suitability solution: 0.05 mg/mL of USP Betamethasone Valerate RS and 0.01 mg/mL of USP Betamethasone Valerate Related Compound A RS in *Diluent*. Sonicate to dissolve if necessary.
Standard solution: 0.05 mg/mL of USP Betamethasone Valerate RS in *Diluent*. Sonicate to dissolve if necessary.
Sample solution: Nominally 0.04 mg/mL of betamethasone in *Diluent*, prepared as follows. Accurately weigh and transfer a portion of Lotion to a suitable volumetric flask. Add about 80% of the final flask volume of *Diluent*. Sonicate for about 5 min. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.2-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Temperatures
Column: Ambient
Autosampler: 4°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Lotion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of betamethasone, 392.46
M_{r2} = molecular weight of betamethasone valerate, 476.58

Acceptance criteria: 95.0%–115.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.001 mg/mL each of USP Betamethasone RS, USP Betamethasone Valerate RS, and USP Betamethasone Valerate Related Compound A RS in *Diluent*. Sonicate to dissolve if necessary.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each specified degradation product in the portion of Lotion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified degradation product from the *Sample solution*
r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Lotion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of betamethasone valerate from the *Standard solution*
C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of betamethasone, 392.46
M_{r2} = molecular weight of betamethasone valerate, 476.58

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Betamethasone	0.30	1.0
Betamethasone valerate	1.00	—
Betamethasone valerate related compound A	1.04	10.0

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified degradation product	—	1.0
Total degradation products	—	12.0

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*
- **MINIMUM FILL** ⟨755⟩: Meets the requirements
- **pH** ⟨791⟩: 4.0–6.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Betamethasone RS
  USP Betamethasone Valerate RS
  USP Betamethasone Valerate Related Compound A RS
  9-Fluoro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-21-yl valerate.
  $C_{27}H_{37}FO_6$ 476.58

Betamethasone Valerate Ointment

DEFINITION

Betamethasone Valerate Ointment contains an amount of betamethasone valerate ($C_{27}H_{37}FO_6$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$), in a suitable ointment base.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	63	37
7.0	63	37
15.0	30	70
19.0	30	70
19.1	10	90
21.0	10	90
21.1	63	37
25.0	63	37

Diluent A: Tetrahydrofuran and water (50:50)
Diluent B: Acetonitrile and water (40:60)
System suitability solution: 25 µg/mL of USP Betamethasone Valerate RS and 10 µg/mL of USP Betamethasone Valerate Related Compound A RS in *Diluent B*. Sonicate to dissolve if necessary.
Standard solution: 25 µg/mL of USP Betamethasone Valerate RS in *Diluent B*. Sonicate to dissolve if necessary.
Sample solution: Nominally 20 µg/mL of betamethasone, prepared as follows. Transfer 1.0 mg of betamethasone from a portion of Ointment to a suitable glass centrifuge tube. Add 15.0 mL of *Diluent A* and mix with a vortex mixer to disperse the sample thoroughly. Add 35.0 mL of *Diluent B* and sonicate for 10 min with intermittent shaking. Centrifuge to obtain a clear supernatant and use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
Autosampler temperature: 4°
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of betamethasone, 392.46
M_{r2} = molecular weight of betamethasone valerate, 476.58

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent A, Diluent B, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.25 µg/mL each of USP Betamethasone RS, USP Betamethasone Valerate RS, and USP Betamethasone Valerate Related Compound A RS in *Diluent B*. Sonicate to dissolve if necessary.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each specified degradation product in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified degradation product from the *Sample solution*
r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (µg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (µg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of betamethasone valerate from the *Standard solution*
C_S = concentration of USP Betamethasone Valerate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of betamethasone, 392.46
M_{r2} = molecular weight of betamethasone valerate, 476.58

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.1%.

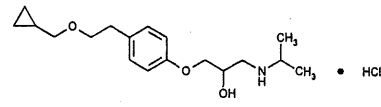
Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Betamethasone	0.30	1.0
Betamethasone valerate	1.00	—
Betamethasone valerate related compound A	1.04	1.0
Any individual unspecified degradation product	—	1.0
Total degradation products	—	2.0

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*
- **MINIMUM FILL** ⟨755⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Betamethasone RS
USP Betamethasone Valerate RS
USP Betamethasone Valerate Related Compound A RS
9-Fluoro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-21-yl valerate.
$C_{27}H_{37}FO_6$ 476.58

Betaxolol Hydrochloride



$C_{18}H_{29}NO_3 \cdot HCl$ 343.89
2-Propanol, 1-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-3-[(1-methylethyl)amino]-, hydrochloride, (±)-;
(±)-1-[*p*-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-3-(isopropylamino)-2-propanol hydrochloride [63659-19-8].

DEFINITION
Betaxolol Hydrochloride contains NLT 98.0% and NMT 102.0% of betaxolol hydrochloride ($C_{18}H_{29}NO_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197K▲ (CN 1-May-2020)

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

Change to read:

- **▲C.▲** (USP 1-May-2020) **IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*
Analysis: Proceed as directed for test *B*.
Acceptance criteria: Meets the requirements

ASSAY

Change to read:

- **PROCEDURE**
▲Buffer: 3.4 g/L of monobasic potassium phosphate in water, adjusted with phosphoric acid to a pH of 3.0
Mobile phase: Acetonitrile, methanol, and *Buffer* (175:175:650)
Standard solution: 1 mg/mL of USP Betaxolol Hydrochloride RS in *Mobile phase*. Sonicate if necessary.
Sample solution: 1 mg/mL of Betaxolol Hydrochloride in *Mobile phase*. Sonicate if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 222 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*

USP Monographs

www.webofpharma.com

Suitability requirements
Tailing factor: NMT 3.0
Relative standard deviation: NMT 0.73% ▲(USP 1-May-2020)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of betaxolol hydrochloride ($C_{18}H_{29}NO_3 \cdot HCl$) in the portion of Betaxolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Betaxolol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Betaxolol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

Change to read:

• **ORGANIC IMPURITIES**
▲**Buffer, Mobile phase, Sample solution,** and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 1 mg/mL of USP Betaxolol Hydrochloride RS and 30 µg/mL of USP Betaxolol Related Compound A RS in *Mobile phase*
Standard solution: 0.003 mg/mL of USP Betaxolol Hydrochloride RS in *Mobile phase*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for betaxolol related compound A and betaxolol are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the betaxolol related compound A and betaxolol peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Identify the impurities in the *Sample solution* on the basis of the relative retention times in *Table 1*.
Calculate the percentage of each impurity in the portion of Betaxolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of betaxolol from the *Standard solution*
C_S = concentration of USP Betaxolol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Betaxolol Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Betaxolol hydroxyethyl analog[a]	0.3	1.3	0.5
Betaxolol related compound A[b]	0.9	1.2	0.5
Betaxolol	1.0	1.0	—
Betaxolol phenol analog[c]	1.6	1.3	0.5
Betaxolol open chain analog[d]	1.7	0.95	0.5
Betaxolol oxirane analog[e]	3.7	1.3	0.5
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 1-[4-(2-Hydroxyethyl)phenoxy]-3-(isopropylamino)propan-2-ol.
[b] 1-(4-Ethylphenoxy)-3-(isopropylamino)propan-2-ol.
[c] 4-[2-(Cyclopropylmethoxy)ethyl]phenol.
[d] 1-[4-(2-Butoxyethyl)phenoxy]-3-(isopropylamino)propan-2-ol.
[e] 2-({[4-(2-Cyclopropylmethoxy)ethyl]phenoxy}methyl)oxirane. ▲(USP 1-May-2020)

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 20 mg/mL
Acceptance criteria: 4.5–6.5
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 65° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Betaxolol Hydrochloride RS
▲USP Betaxolol Related Compound A RS
1-(4-Ethylphenoxy)-3-(isopropylamino)propan-2-ol.
$C_{14}H_{23}NO_2$ 237.34 ▲(USP 1-May-2020)

Betaxolol Ophthalmic Solution

DEFINITION
Betaxolol Ophthalmic Solution is a sterile, aqueous, isotonic solution of Betaxolol Hydrochloride. It contains a suitable antimicrobial preservative. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of betaxolol ($C_{18}H_{29}NO_3$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: Dissolve 7.1 g of anhydrous dibasic sodium phosphate in 800 mL of water, adjust with phosphoric acid to a pH of 3.0, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (1:1)

Standard solution: 0.11 mg/mL of USP Betaxolol Hydrochloride RS in *Buffer*

Sample solution: Nominally 0.1 mg/mL of betaxolol in *Buffer* from Ophthalmic Solution

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV or diode array 280 nm. [NOTE—Use the diode array detector to perform *Identification* test *B*.]

Column: 4-mm × 25-cm; packing L1

Flow rate: 1.1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betaxolol ($C_{18}H_{29}NO_3$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Betaxolol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of betaxolol in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of betaxolol, 307.43

M_{r2} = molecular weight of betaxolol hydrochloride, 343.89

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase: Add 5 mL of phosphoric acid to 990 mL of water. Adjust with 2 M ammonium hydroxide to a pH of 3.0, and dilute with water to 1000 mL. Prepare a mixture of this solution and acetonitrile (45:55). Dissolve 3 g of sodium dodecyl sulfate in 450 mL of the mixture.

Standard solution: 2.2 µg/mL of USP Betaxolol Hydrochloride RS in *Mobile phase*

Sample solution: Nominally equivalent to 0.2 mg/mL of betaxolol in *Mobile phase* from Ophthalmic Solution

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: 220 nm

Column: 4.6-mm × 25-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5%

Tailing factor: NMT 2.5

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of betaxolol from the *Standard solution*

C_S = concentration of USP Betaxolol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of betaxolol in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of betaxolol, 307.43

M_{r2} = molecular weight of betaxolol hydrochloride, 343.89

Acceptance criteria

Single largest individual impurity: NMT 1%

Any other individual impurity: NMT 0.3%

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **pH** ⟨791⟩**:** 4.0–8.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Betaxolol Hydrochloride RS

Betaxolol Tablets

DEFINITION

Betaxolol Tablets contain an amount of Betaxolol Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betaxolol hydrochloride ($C_{18}H_{29}NO_3 \cdot HCl$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

ASSAY

Change to read:

• **PROCEDURE**

▲**Buffer:** 3.4 g/L of monobasic potassium phosphate in water, adjusted with phosphoric acid to a pH of 3.0

Mobile phase: Acetonitrile, methanol, and *Buffer* (175:175:650)

Standard solution: 1 mg/mL of USP Betaxolol Hydrochloride RS in *Mobile phase*

Sample solution: Nominally 1 mg/mL of betaxolol hydrochloride prepared as follows. Powder NLT 30 Tablets, transfer a portion of the powder containing nominally 20 mg of betaxolol hydrochloride to a 20-mL volumetric flask,

USP Monographs

www.webofpharma.com

and add 15 mL of *Mobile phase*. Sonicate for 10 min, and centrifuge for 30 min. Use the clear supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 222 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 3.0
Relative standard deviation: NMT 1.0% ▲(USP 1-May-2020)

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of betaxolol hydrochloride ($C_{18}H_{29}NO_3 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Betaxolol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of betaxolol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨**711**⟩
Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: USP Betaxolol Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: 274 nm
Cell path length: A 5-cm path length cell may be used for lower dosage levels.
▲**Analysis**
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of betaxolol hydrochloride ($C_{18}H_{29}NO_3 \cdot HCl$) dissolved:

$$(A_U/A_S) \times C_S \times D \times (V/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet) ▲(USP 1-May-2020)

Tolerances: NLT 80% (Q) of the labeled amount of betaxolol hydrochloride ($C_{18}H_{29}NO_3 \cdot HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨**905**⟩
Procedure for content uniformity
Standard solution: 0.1 mg/mL of USP Betaxolol Hydrochloride RS in 0.1 N hydrochloric acid
Sample solution: Place 1 Tablet in a suitable volumetric flask to obtain a concentration of betaxolol hydrochloride, based on the label claim, of 0.1 mg/mL. Add an amount of 0.1 N hydrochloric acid equal to 70% of the volume of the flask. Shake by mechanical means until dissolved, dilute with 0.1 N hydrochloric acid to volume, and mix. Filter, and discard the first 20 mL of the filtrate.
Instrumental conditions
Mode: UV
Analytical wavelength: 274 nm
Cell path length: 1 cm
Blank: 0.1 N hydrochloric acid
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of betaxolol hydrochloride ($C_{18}H_{29}NO_3 \cdot HCl$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Betaxolol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of betaxolol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

Add the following:

▲• **Organic Impurities**
Buffer, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability solution: 1 mg/mL of USP Betaxolol Hydrochloride RS and 30 µg/mL of USP Betaxolol Related Compound A RS in *Mobile phase*
Standard solution: 6 µg/mL of USP Betaxolol Hydrochloride RS in *Mobile phase*
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for betaxolol related compound A and betaxolol are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between betaxolol related compound A and betaxolol, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Betaxolol Hydrochloride RS in the *Standard solution* (mg/mL)

Supplement 2

www.webofpharma.com

C_U = nominal concentration of betaxolol hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Betaxolol hydroxyethyl analog[a]	0.3	1.3	0.5
Betaxolol related compound A[b, c]	0.9	1.2	—
Betaxolol	1.0	1.0	—
Betaxolol phenol analog[d]	1.6	1.3	0.5
Betaxolol open chain analog[e]	1.7	0.95	0.5
Betaxolol oxirane analog[c, f]	3.7	1.3	—
Any unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.0

[a] 1-[4-(2-Hydroxyethyl)phenoxy]-3-(isopropylamino)propan-2-ol.
[b] 1-(4-Ethylphenoxy)-3-(isopropylamino)propan-2-ol.
[c] It is a process impurity and is listed for identification only. It is controlled in the drug substance, is not reported for the drug product, and should not be included in the total degradation products.
[d] 4-[2-Cyclopropylmethoxy)ethyl]phenol.
[e] 1-[4-(2-Butoxyethyl)phenoxy]-3-(isopropylamino)propan-2-ol.
[f] 2-(([4-(2-Cyclopropylmethoxy)ethyl]phenoxy]methyl)oxirane. ▲(USP 1-May-2020)

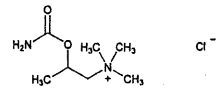
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label the Tablets to state both the content of the betaxolol active moiety and the content of betaxolol hydrochloride used in formulating them.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Betaxolol Hydrochloride RS
  ▲USP Betaxolol Related Compound A RS
  1-(4-Ethylphenoxy)-3-(isopropylamino)propan-2-ol.
  $C_{14}H_{23}NO_2$ 237.34 ▲(USP 1-May-2020)

Bethanechol Chloride



$C_7H_{17}ClN_2O_2$ 196.68
1-Propanaminium, 2-[(aminocarbonyl)oxy]-*N*,*N*,*N*-trimethyl-, chloride, (±)-;
(±)-(2-Hydroxypropyl)trimethylammonium chloride carbamate [590-63-6].

DEFINITION

Bethanechol Chloride contains NLT 98.0% and NMT 101.5% of bethanechol chloride ($C_7H_{17}ClN_2O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: 29 mg/L of edetic acid in a solution prepared as follows. Transfer a portion of edetic acid to a suitable volumetric flask. Dissolve in water, using 50% of the final flask volume. Add 0.3 mL of nitric acid per L, and dilute with water to volume.
Mobile phase: Acetonitrile and *Buffer* (5:95)
System suitability solution: 0.1 mg/mL of bethanechol chloride in a solution prepared as follows. Transfer a portion of bethanechol chloride to a suitable volumetric flask. Add 4% of the final flask volume of 0.1 N sodium hydroxide, and allow to stand for 15 min. Add 4% of the final flask volume of 0.1 N hydrochloric acid. Dissolve in and dilute with *Mobile phase* to volume.
Standard solution: 0.1 mg/mL of USP Bethanechol Chloride RS in *Mobile phase*
Sample solution: 0.1 mg/mL of Bethanechol Chloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity
Column: 3.9-mm × 15.0-cm; packing L55
Temperatures
Detector: 35°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 0.8 between desacetyl methacholine and bethanechol chloride, *System suitability solution*
Tailing factor: NMT 3.5, *Standard solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of bethanechol chloride ($C_7H_{17}ClN_2O_2$) in the portion of Bethanechol Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Bethanechol Chloride in the *Sample solution* (mg/mL)

www.webofpharma.com

Acceptance criteria: 98.0%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer: 0.48 g/L of methanesulfonic acid in water
Mobile phase: Acetonitrile and *Buffer* (5:95)
System suitability solution: 0.1 mg/mL of bethanechol chloride in a solution prepared as follows. Transfer a portion of bethanechol chloride to a suitable volumetric flask. Add 4% of the final flask volume of 0.1 N sodium hydroxide, and allow to stand for 15 min. Add 4% of the final flask volume of 0.1 N hydrochloric acid. Dissolve in and dilute with *Mobile phase* to volume.
Standard solution: 1 µg/mL of USP Bethanechol Chloride RS in *Mobile phase*
Sample solution: 0.1 mg/mL of Bethanechol Chloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity
Column: 3.9-mm × 15.0-cm; packing L55
Temperatures
Detector: 35°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 0.8 between desacetyl methacholine and bethanechol chloride, *System suitability solution*
Relative standard deviation: NMT 10.0% for bethanechol chloride, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Bethanechol Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of bethanechol chloride from the *Standard solution*
C_S = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Bethanechol Chloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desacetyl methacholine[a]	0.9	1.2	1.0
Bethanechol chloride	1.0	—	—
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	1.5

[a] 2-Hydroxypropyltrimethyl ammonium chloride.

SPECIFIC TESTS

- **PH** ⟨791⟩

Sample solution: 10 mg/mL of Bethanechol Chloride in water
Acceptance criteria: 5.5–6.5

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Bethanechol Chloride RS

Bethanechol Chloride Injection

DEFINITION

Bethanechol Chloride Injection is a sterile solution of Bethanechol Chloride in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of bethanechol chloride ($C_7H_{17}ClN_2O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: 20 mM methanesulfonic acid
Diluent: 0.1 mg/mL of calcium chloride and 0.1 mg/mL of magnesium chloride in water
System suitability solution: 1 mg/mL of USP Bethanechol Chloride RS in solution prepared as follows. Transfer a portion of USP Bethanechol Chloride RS to a suitable volumetric flask. Add 60% of the final volume of water, 8% of the final volume of *Diluent*, and 2% of the final volume of 0.1 N of sodium hydroxide. Dilute with water to volume.
Standard solution: 1.0 mg/mL of USP Bethanechol Chloride RS in water
Sample solution: Nominally 1.0 mg/mL of bethanechol chloride from a volume of Injection in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity
Column: 4-mm × 25-cm; packing L53
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between the calcium ion and desacetyl methacholine
Tailing factor: NMT 4.5 for bethanechol chloride
Relative standard deviation: NMT 2.0% for bethanechol chloride
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bethanechol chloride ($C_7H_{17}ClN_2O_2$) in each mL of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of desacetyl methacholine in each mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desacetyl methacholine from the *Sample solution*
r_S = peak response of bethanechol chloride from the *Standard solution*
C_S = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bethanechol chloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sodium[a]	1.0	—
Magnesium[a]	1.4	—
Calcium[a]	1.6	—
Desacetyl methacholine[b]	2.0	4.0
Bethanechol chloride	2.8	—

[a] Included for identification purposes only.
[b] 2-Hydroxypropyltrimethyl ammonium chloride.

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 25.0 USP Endotoxin Units/mg of bethanechol chloride
• **PH** ⟨791⟩: 5.5–7.5
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Bethanechol Chloride RS

Bethanechol Chloride Compounded Oral Solution

DEFINITION

Bethanechol Chloride Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of bethanechol chloride ($C_7H_{17}ClN_2O_2$).

Prepare Bethanechol Chloride Compounded Oral Solution 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Bethanechol Chloride	500 mg
Vehicle for Oral Solution (regular or sugar-free), *NF*, a sufficient quantity to make	100 mL

Add *Bethanechol Chloride* powder and about 20 mL of *Vehicle for Oral Solution* to a mortar, and mix. Add the *Vehicle for Oral Solution* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle for Oral Solution* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (33:67)

Standard solution: 500 µg/mL of USP Bethanechol Chloride RS in *Mobile phase*

Sample solution: Agitate the container of Oral Solution for 30 min on a rotating mixer, remove a 10-mL sample, and store in a clear glass vial at −70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Dilute a suitable volume of Oral Solution with *Mobile phase* to obtain a nominal concentration of 500 µg/mL of bethanechol chloride.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Flow rate: 0.7 mL/min
Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time of bethanechol chloride is about 3 min.]

Suitability requirements

Relative standard deviation: NMT 3.1% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bethanechol chloride ($C_7H_{17}ClN_2O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bethanechol Chloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of bethanechol chloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

USP Monographs

www.webofpharma.com

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.9–4.9

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store at room temperature or in a refrigerator.
- **Beyond-Use Date:** NMT 60 days after the day on which it was compounded when stored at room temperature or in a refrigerator
- **Labeling:** Label it to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
  USP Bethanechol Chloride RS

Bethanechol Chloride Compounded Oral Suspension

DEFINITION
Bethanechol Chloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of bethanechol chloride ($C_7H_{17}ClN_2O_2$).
Prepare Bethanechol Chloride Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Bethanechol Chloride	500 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

If using *Bethanechol Chloride* tablets, add to a suitable mortar and comminute to a fine powder, or add the *Bethanechol Chloride* powder to the mortar. Add about 20 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to final volume, and mix well.

ASSAY
- **Procedure**

Mobile phase: Acetonitrile and water (33:67)
Standard solution: 500 µg/mL of USP Bethanechol Chloride RS in *Mobile phase*
Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 10-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Dilute a suitable volume of Oral Suspension with *Mobile phase* to obtain a nominal concentration of 500 µg/mL of bethanechol chloride.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Flow rate: 0.7 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[Note—The retention time for bethanechol chloride is about 3 min.]
Suitability requirements
Relative standard deviation: NMT 3.1% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bethanechol chloride ($C_7H_{17}ClN_2O_2$) in the volume of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bethanechol Chloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of bethanechol chloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.9–4.9

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store at room temperature, or in a refrigerator.
- **Beyond-Use Date:** NMT 60 days after the day on which it was compounded when stored at room temperature, or in a refrigerator
- **Labeling:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
  USP Bethanechol Chloride RS

Bethanechol Chloride Tablets

DEFINITION
Bethanechol Chloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bethanechol chloride ($C_7H_{17}ClN_2O_2$).

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M▲** (CN 1-May-2020)

Sample: Nominally 100 mg of bethanechol chloride from a suitable portion of pulverized Tablets prepared as follows. Pulverize a portion of Tablets equivalent to 100 mg of bethanechol chloride. Add 15 mL of ether, and allow to digest for 15 min. Decant the ether, again extract the residue with 10 mL of ether, and discard the ether extracts. Add 30 mL of alcohol to the residue. Shake for 10 min, and allow to stand for 1 h with frequent agitation. Filter with suction, and evaporate the filtrate on a steam bath to dryness: the bethanechol chloride so obtained is recrystallized from alcohol and dried at 105° for 2 h.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY
- **Procedure**

Buffer: 29 mg/L of edetic acid in solution prepared as follows. Transfer a portion of edetic acid to a suitable volumetric flask. Dissolve with water, using 50% of the final volume. Add 0.3 mL of nitric acid per L, and dilute with water to volume.
Mobile phase: Acetonitrile and *Buffer* (5:95)

USP Monographs

www.webofpharma.com

System suitability solution: 0.1 mg/mL of bethanechol chloride in solution prepared as follows. Transfer a portion of bethanechol chloride to a suitable volumetric flask. Add 4% of the final volume of 0.1 N sodium hydroxide, and allow to stand for 15 min. Add 4% of the final volume of 0.1 N hydrochloric acid. Dissolve in and dilute with *Mobile phase* to volume.

Standard solution: 0.1 mg/mL of USP Bethanechol Chloride RS in *Mobile phase*

Sample solution: Nominally 0.1 mg/mL of bethanechol chloride from a suitable amount of powdered Tablets in solution prepared as follows. Add a portion of fine powder, equivalent to 1 Tablet, from NLT 20 Tablets to a suitable volumetric flask. Dissolve in *Mobile phase*, using 60%–70% of the final volume. Sonicate for 20 min. Shake by mechanical means for 15 min. Dilute with *Mobile phase* to volume, and mix. Allow to stand for 10 min, and pass through a 1-µm glass filter, discarding the first 3 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Conductivity

Column: 3.9-mm × 15.0-cm; packing L55

Temperatures

Detector: 35°

Column: 30°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 0.8 between desacetyl methacholine and bethanechol chloride, *System suitability solution*

Tailing factor: NMT 3.5, *Standard solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bethanechol chloride ($C_7H_{17}ClN_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Buffer, Mobile phase, and **System suitability solution:** Proceed as directed in the *Assay*.

Standard solution: (*L*/900) mg/mL of USP Bethanechol Chloride RS in *Medium*, where *L* is the label claim in mg/Tablet

Sample solution: A portion of solution under test

Chromatographic system and **System suitability:** Proceed as directed in the *Assay*, except for the following parameters:

Injection volumes

For the System suitability solution: 50 µL

For the Standard solution and **Sample solution:** 100 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount (*Q*) of bethanechol chloride ($C_7H_{17}ClN_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of bethanechol chloride ($C_7H_{17}ClN_2O_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer: 0.48 g/L of methanesulfonic acid in water

Mobile phase: Acetonitrile and *Buffer* (5:95)

System suitability solution: 0.1 mg/mL of bethanechol chloride in solution prepared as follows. Transfer the bethanechol chloride to a suitable volumetric flask. Add 4% of the final volume of 0.1 N sodium hydroxide, and allow to stand for 15 min. Add 4% of the final volume of 0.1 N hydrochloric acid. Dissolve in and dilute with *Mobile phase* to volume.

Standard solution: 1 µg/mL of USP Bethanechol Chloride RS in *Mobile phase*

Sample solution: Nominally 0.1 mg/mL of bethanechol chloride from a suitable amount of powdered Tablets in solution prepared as follows. Add a portion of fine powder, equivalent to 1 Tablet, from NLT 20 Tablets to a suitable volumetric flask. Dissolve in *Mobile phase*, using 60%–70% of the final volume. Sonicate for 20 min. Shake by mechanical means for 15 min. Dilute with *Mobile phase* to volume, and mix. Allow to stand for 10 min, and pass through a 1-µm glass filter, discarding the first 3 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Conductivity

Column: 3.9-mm × 15.0-cm; packing L55

Temperatures

Detector: 35°

Column: 30°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 0.8 between desacetyl methacholine and bethanechol chloride, *System suitability solution*

Relative standard deviation: NMT 10.0% for bethanechol chloride, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

www.webofpharma.com

r_U = peak response for any impurity in the *Sample solution*
r_S = peak response of bethanechol chloride from the *Standard solution*
C_S = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bethanechol chloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

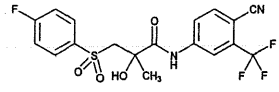
Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desacetyl methacholine[a]	0.9	1.2	1.0
Bethanechol chloride	1.0	—	—
Any unspecified degradation product	—	1.0	0.2
Total impurities	—	—	1.5

[a] 2-Hydroxypropyltrimethyl ammonium chloride.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bethanechol Chloride RS

Bicalutamide



$C_{18}H_{14}F_4N_2O_4S$ 430.37
Propanamide, *N*-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methyl-, (±)-;
(±)-4′-Cyano-α,α,α-trifluoro-3-[(*p*-fluorophenyl)sulfonyl]-2-methyl-*m*-lactotoluidide [90357-06-5].

DEFINITION
Bicalutamide contains NLT 98.0% and NMT 102.0% of $C_{18}H_{14}F_4N_2O_4S$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: 0.01% (v/v) of trifluoroacetic acid in water
Solution B: 0.01% (v/v) of trifluoroacetic acid in acetonitrile
Mobile phase: *Solution A* and *Solution B* (52:48)
Diluent: *Solution A* and *Solution B* (1:2)
System suitability solution: 5 µg/mL of USP Bicalutamide Related Compound A RS and 50 µg/mL of USP Bicalutamide RS in *Diluent*
Standard solution: 0.05 mg/mL of USP Bicalutamide RS in *Diluent*
Sample solution: 0.05 mg/mL of Bicalutamide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for bicalutamide related compound A isomer A and bicalutamide related compound A isomer B are 0.75 and 0.78, respectively.]
Suitability requirements
Resolution: NLT 2.0 between bicalutamide related compound A isomer B and bicalutamide, *System suitability solution*
Relative standard deviation: NMT 2%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{18}H_{14}F_4N_2O_4S$ in the portion of Bicalutamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES
INORGANIC IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES
- **Procedure**

Solution A, Solution B, Diluent, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	67	33
16.5	67	33
26.5	40	60
32.5	5	95
32.6	67	33
35	67	33

Standard solution: 1 µg/mL of USP Bicalutamide RS in *Diluent*
Sample solution: 1 mg/mL of Bicalutamide in *Diluent*
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution 1: NLT 0.8 between bicalutamide related compound A isomer A and bicalutamide related compound A isomer B

USP Monographs

www.webofpharma.com

Resolution 2: NLT 8.5 between bicalutamide related compound A isomer B and bicalutamide

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Bicalutamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of bicalutamide from the *Standard solution*

C_S = concentration of bicalutamide in the *Standard solution* (mg/mL)

C_U = concentration of Bicalutamide in the *Sample solution* (mg/mL)

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bicalutamide aminobenzonitrile[a]	0.30	1.4	0.1
Bicalutamide related compound A isomer A[b]	0.64	1.0	0.1
Bicalutamide related compound A isomer B[b]	0.67	1.0	0.1
Desfluoro bicalutamide[c]	0.83	1.1	0.2
2-Fluoro bicalutamide[d]	0.94	1.0	0.2
Bicalutamide	1.00	—	—
Deoxybicalutamide[e]	1.33	1.0	0.2
Bicalutamide sulfide[f]	1.56	1.0	0.1
Any unspecified impurity	—	1.0	0.1

[a] 4-Amino-2-(trifluoromethyl)benzonitrile.

[b] *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfinyl]-2-hydroxy-2-methylpropanamide.

[c] *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-2-hydroxy-2-methyl-3-(phenylsulfonyl)propanamide.

[d] *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(2-fluorophenylsulfonyl)-2-hydroxy-2-methylpropanamide.

[e] *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylsulfonyl)-2-methylpropanamide.

[f] *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylthio)-2-hydroxy-2-methylpropanamide.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.2%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Bicalutamide RS

USP Bicalutamide Related Compound A RS

[*N*-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfinyl]-2-hydroxy-2-methylpropanamide]

($C_{18}H_{14}F_4N_2O_3S$ 414.37)

Bicalutamide Tablets

DEFINITION

Bicalutamide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: Tetrahydrofuran, acetonitrile, and water (20:15:65)

System suitability stock solution: 0.8 mg/mL of USP Bicalutamide RS and 0.4 mg/mL of USP Bicalutamide Related Compound B RS in tetrahydrofuran

System suitability solution: 0.04 mg/mL of USP Bicalutamide RS and 0.02 mg/mL of USP Bicalutamide Related Compound B RS in *Mobile phase* from the *System suitability stock solution*

Standard stock solution: 0.8 mg/mL of USP Bicalutamide RS in tetrahydrofuran

Standard solution: 0.04 mg/mL of USP Bicalutamide RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: 0.5 mg/mL of bicalutamide in tetrahydrofuran prepared as follows. Transfer an equivalent to 50 mg of bicalutamide from finely powdered Tablets (NLT 20) into a 100-mL volumetric flask. Add 50 mL of tetrahydrofuran, and sonicate for NLT 10 min to complete dissolution. Allow to cool to room temperature, and dilute with tetrahydrofuran to volume. Pass through a suitable filter of 0.45-µm pore size.

Sample solution: 0.04 mg/mL of bicalutamide in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm. ▲For *Identification B*, use a diode array detector in the range of 190–400 nm.▲ 2S (USP41)

Column: 5-mm × 12.5-cm; 3-µm packing L1

Column temperature: 50°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for bicalutamide and bicalutamide related compound B are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: Greater than 1.9 between bicalutamide and bicalutamide related compound B

Tailing factor: Less than 1.3 for bicalutamide

Relative standard deviation: NMT ▲1.0%▲ 2S (USP41) for bicalutamide

Analysis

Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of the labeled amount of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bicalutamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩
Test 1
Medium: 1.0% w/v sodium lauryl sulfate in water; 1000 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: 0.05 mg/mL of USP Bicalutamide RS in *Medium* prepared as follows. Transfer USP Bicalutamide RS to a suitable volumetric flask, dissolve in tetrahydrofuran equivalent to 1% of the final volume, and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 270 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL)
V = volume of *Medium* (mL)
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium, Apparatus 2, Time, Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed for *Test 1*.
Tolerances: NLT 75% (Q) of the labeled amount of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$) is dissolved.
▲**Test 3:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
Medium: 1.0% (w/v) sodium lauryl sulfate in water; 1000 mL
Apparatus 2: 75 rpm
Time: 60 min
Standard solution: 0.01 mg/mL of USP Bicalutamide RS in *Medium*, sonicate to aid dissolution. Pass a portion of the solution through a suitable filter of 0.45-µm pore size. Discard the first few milliliters of the filtrate.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard the first few milliliters of the filtrate. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 270 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium* (mL)
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$) is dissolved.▲ (RB 1-Nov-2018)

• **Uniformity of Dosage Units** ⟨905⟩
Procedure for content uniformity
Diluent: 10 mg/mL of sodium lauryl sulfate in water
Standard solution: 0.05 mg/mL of USP Bicalutamide RS in *Diluent*. [Note—Dissolve USP Bicalutamide RS in a minimum volume of tetrahydrofuran before dilution with *Diluent*.]
Sample stock solution: Transfer 1 Tablet to a 100-mL volumetric flask. Add 10 mL of water, and sonicate for approximately 30 min. Add 80 mL of tetrahydrofuran, and sonicate for 30 min to complete dissolution of bicalutamide. Allow to cool to room temperature, and dilute with tetrahydrofuran to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Sample solution: Transfer 10.0 mL of the *Sample stock solution* into a 100-mL volumetric flask, and dilute with *Diluent* to volume.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 270 nm
Blank: *Diluent*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bicalutamide ($C_{18}H_{14}F_4N_2O_4S$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bicalutamide in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

USP Monographs

www.webofpharma.com

IMPURITIES

• **LIMIT OF 4-AMINO-2-(TRIFLUOROMETHYL)BENZONITRILE**

Mobile phase and **System suitability solution:** Prepare as directed in the *Assay*.

Standard stock solution: 0.2 mg/mL of USP Bicalutamide RS in tetrahydrofuran

Standard solution: 0.02 mg/mL of USP Bicalutamide RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Transfer the equivalent to 50 mg of bicalutamide from powdered Tablets (NLT 20) to a 25-mL volumetric flask. Add 2 mL of tetrahydrofuran, and allow to stand for 5 min. Add 20 mL of *Mobile phase*, sonicate for 10 min, and allow to cool to room temperature. Dilute with *Mobile phase* to volume, and pass through a suitable filter of 0.2-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 5-mm × 12.5-cm; 3-µm packing L1

Column temperature: 50°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times of 4-amino-2-(trifluoromethyl)benzonitrile, bicalutamide, and bicalutamide related compound B are about 0.4, 1.0, and about 1.1, respectively.]

Suitability requirements

Resolution: Greater than 1.9 between bicalutamide and bicalutamide related compound B

Tailing factor: Less than 1.3 for bicalutamide

Relative standard deviation: NMT 2.0% for bicalutamide

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 4-amino-2-(trifluoromethyl) benzonitrile in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of 4-amino-2-(trifluoromethyl) benzonitrile from the *Sample solution*

r_S = peak area of bicalutamide from the *Standard solution*

C_S = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of bicalutamide in the *Sample solution* (mg/mL)

F = relative response factor for 4-amino-2-(trifluoromethyl)benzonitrile, 1.4

Acceptance criteria: NMT 0.1%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

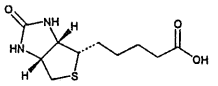
USP Bicalutamide RS

USP Bicalutamide Related Compound B RS

(*RS*)-*N*-(4-Cyano-3-(trifluoromethyl)phenyl)-3-(3-fluorophenylsulfonyl)-2-hydroxy-2-methylpropanamide.

$C_{18}H_{14}F_4N_2O_4S$ 430.37

Biotin



$C_{10}H_{16}N_2O_3S$ 244.31

1*H*-Thieno[3,4-*d*]imidazole-4-pentanoic acid, hexahydro-2-oxo-, [3a*S*-(3aα,4β,6aα)]-;

(3a*S*,4*S*,6a*R*)-Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-valeric acid [58-85-5].

DEFINITION

Biotin contains NLT 97.5% and NMT 102.0% of biotin ($C_{10}H_{16}N_2O_3S$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

• **B.** It meets the requirements in *Specific Tests* for *Optical Rotation* ⟨781S⟩, *Specific Rotation*.

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer solution: Dissolve 1 g of sodium perchlorate monohydrate in 500 mL of water, add 1 mL of phosphoric acid, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer solution* (8.5: 91.5)

Diluent: Acetonitrile and water (1:4)

Standard solution: 0.1 mg/mL of USP Biotin RS in *Diluent*. Sonicate if necessary to dissolve.

Sample solution: 0.1 mg/mL of Biotin in *Diluent*. Sonicate if necessary to dissolve.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 15-cm; 3-µm packing L7

Flow rate: 1.2 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of biotin ($C_{10}H_{16}N_2O_3S$) in the portion of Biotin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Biotin RS in the *Standard solution* (mg/mL)

C_U = concentration of Biotin in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.0%

USP Monographs

www.webofpharma.com

IMPURITIES

• **RELATED COMPOUNDS**

Buffer solution, Mobile phase, Diluent, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Measure the peak responses of the *Sample solution*.

Calculate the percentage of each impurity in the portion of Biotin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the peak responses of all the peaks from the *Sample solution*

Acceptance criteria

Individual impurity: NMT 1.0%

Total impurities: NMT 2.0%

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 20 mg/mL in 0.1 N sodium hydroxide

Acceptance criteria: +89° to +93°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Biotin RS

Biotin Capsules

DEFINITION

Biotin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of biotin ($C_{10}H_{16}N_2O_3S$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer solution: Dissolve 1 g of sodium perchlorate monohydrate in 500 mL of water, add 1 mL of phosphoric acid, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer solution* (8.5: 91.5)

Diluent: Acetonitrile and water (1:4)

Standard solution: 0.05 mg/mL of USP Biotin RS in *Diluent*. Sonicate, if necessary, to dissolve.

Sample solution: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to a nominal amount of 5 mg of biotin to a 100-mL volumetric flask, add 60 mL of water, and shake in a water bath at 65° for 20 min. Sonicate for 5 min, shake by mechanical means for 15 min, and cool to room temperature. Add 20 mL of acetonitrile, dilute with water to volume, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 15-cm; 3-µm packing L7

Flow rate: 1.2 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of biotin ($C_{10}H_{16}N_2O_3S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Biotin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of biotin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Buffer solution: 0.02 N anhydrous disodium hydrogen phosphate adjusted with phosphoric acid to a pH of 7.4

Medium: *Buffer solution*; 500 mL

Apparatus 1: 100 rpm

Time: 1 h

Standard solution: Dissolve a suitable amount of USP Biotin RS in *Buffer solution* to obtain a concentration similar to that expected in the *Sample solution*.

Sample solution: Withdraw a portion of the solution under test, pass through a suitable filter, and use the pooled sample as the test specimen.

Analysis: Proceed as directed in the *Assay*, making any necessary adjustments.

Calculate the percentage of the labeled amount of biotin ($C_{10}H_{16}N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times V/L) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Biotin RS in the *Standard solution* (µg/mL)

V = volume of *Medium*, 500 mL

L = labeled amount of biotin (µg/Capsule)

Tolerances: NLT 75% (Q) of the labeled amount of biotin ($C_{10}H_{16}N_2O_3S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Biotin RS

USP Monographs

www.webofpharma.com

Add the following:

▲Biotin Compounded Oral Suspension

DEFINITION

Biotin Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of biotin ($C_{10}H_{16}N_2O_3S$).

Prepare Biotin Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Biotin powder	1 g
Vehicle: 1:1 mixture of Ora-Plus[a] and Ora-Sweet,[a] a sufficient quantity to make	100 mL

[a] Perrigo Pharmaceuticals, Allegan, MI.

Place the *Biotin powder* in a suitable container and triturate to a fine powder. Add a small amount of *Vehicle* and mix well to form a smooth paste. Add a sufficient amount of *Vehicle* to make the mortar contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Mix well.

ASSAY

• **PROCEDURE**

Solution A: Add 1 g of sodium perchlorate monohydrate to 500 mL of water. Add 1 mL of phosphoric acid and then an additional 500 mL of water.

Mobile phase: Acetonitrile and *Solution A* (8.5: 91.5)

Diluent: Acetonitrile and water (20:80)

Standard solution: 0.1 mg/mL of USP Biotin RS in *Diluent*

Sample solution: Transfer 1.0 mL of Oral Suspension into a 100-mL volumetric flask, and add *Diluent* to volume. Pass through a filter of 0.22-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Flow rate: 1.2 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for biotin is about 11.8 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of biotin ($C_{10}H_{16}N_2O_3S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of biotin from the *Sample solution*

r_S = peak response of biotin from the *Standard solution*

C_S = concentration of USP Biotin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of biotin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.7–4.7

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Biotin RS▲ 2S (USP41)

Biotin Tablets

DEFINITION

Biotin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of biotin ($C_{10}H_{16}N_2O_3S$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer solution: Dissolve 1 g of sodium perchlorate monohydrate in 500 mL of water, add 1 mL of phosphoric acid, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer solution* (8.5: 91.5)

Diluent: Acetonitrile and water (1:4)

Standard solution: 0.05 mg/mL of USP Biotin RS in *Diluent*. Sonicate, if necessary, to dissolve.

Sample solution: Transfer a portion equivalent to 5 mg of biotin from NLT 30 finely powdered Tablets to a 100-mL volumetric flask, add 60 mL of water, and shake in a water bath at 65° for 20 min. Sonicate for 5 min, shake by mechanical means for 15 min, and cool to room temperature. Add 20 mL of acetonitrile, dilute with water to volume, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 15-cm; 3-µm packing L7

Flow rate: 1.2 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of biotin ($C_{10}H_{16}N_2O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Biotin RS in the *Standard solution* (mg/mL)

www.webofpharma.com

C_U = nominal concentration of biotin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Buffer solution: 0.02 N anhydrous disodium hydrogen phosphate adjusted with phosphoric acid to a pH of 7.4
Medium: *Buffer solution*; 500 mL
Apparatus 2: 75 rpm
Time: 1 h
Standard solution: Dissolve a suitable amount of USP Biotin RS in *Buffer solution* to obtain a concentration similar to that expected in the *Sample solution*.
Sample solution: Withdraw a portion of the solution under test, pass through a suitable filter, and use the pooled sample as the test specimen.
Analysis: Proceed as directed in the *Assay*, making any necessary adjustments.
Calculate the percentage of the labeled amount of biotin ($C_{10}H_{16}N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times V/L) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Biotin RS in the *Standard solution* (µg/mL)
V = volume of *Medium*, 500 mL
L = labeled amount of biotin (µg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of biotin ($C_{10}H_{16}N_2O_3S$) is dissolved.

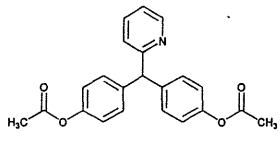
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Biotin RS

Bisacodyl



$C_{22}H_{19}NO_4$ 361.39
Phenol, 4,4′-(2-pyridinylmethylene)bis-, diacetate (ester); 4,4′-(2-Pyridylmethylene)diphenol diacetate (ester); 4,4′-(Pyridin-2-ylmethylene)diphenyl diacetate [603-50-9].

DEFINITION

Bisacodyl contains NLT 98.0% and NMT 102.0% of bisacodyl ($C_{22}H_{19}NO_4$), calculated on the dried basis.
[**CAUTION**—Avoid inhalation and contact with the eyes, skin, and mucous membranes.]

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*, 197A or 197K▲ (CN 1-May-2020): Meets the requirements.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 1.58 g/L of ammonium formate in water; adjusted with formic acid to a pH of 5.0
Mobile phase: Acetonitrile and *Buffer* (45:55)
Diluent: Acetonitrile, acetic acid, and water (30:4:66)
Standard solution: 0.5 mg/mL of USP Bisacodyl RS in *Diluent*
Sample solution: 0.5 mg/mL of Bisacodyl in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of bisacodyl
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of bisacodyl ($C_{22}H_{19}NO_4$) in the portion of Bisacodyl taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of bisacodyl from the *Sample solution*
r_S = peak response of bisacodyl from the *Standard solution*
C_S = concentration of USP Bisacodyl RS in the *Standard solution* (mg/mL)
C_U = concentration of Bisacodyl in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**

Buffer: 1.58 g/L of ammonium formate in water; adjusted with formic acid to a pH of 5.0
Mobile phase: Acetonitrile and *Buffer* (45:55)
Diluent: Acetonitrile and water (35:5)
System suitability solution: 0.8 mg/mL of USP Bisacodyl RS; 2 µg/mL each of USP Bisacodyl Related Compound A RS, USP Bisacodyl Related Compound C RS, and USP Bisacodyl Related Compound E RS; and 4 µg/mL of USP Bisacodyl Related Compound B RS in *Diluent*
Sensitivity solution: 0.0003 mg/mL of USP Bisacodyl RS in *Diluent*
Standard stock solution: 1.0 mg/mL of USP Bisacodyl RS in *Diluent*
Standard solution: 1.0 µg/mL of USP Bisacodyl RS in *Diluent*
Sample solution: 1.0 mg/mL of Bisacodyl in *Diluent*

USP Monographs

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 25-cm; 4- or 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: NLT 3.5 times the retention time of bisacodyl
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between the bisacodyl related compound E and bisacodyl peaks, *System suitability solution*
Tailing factor: NMT 2.0 for the bisacodyl peak, *System suitability solution*
Relative standard deviation: NMT 5.0% for the bisacodyl peak, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Bisacodyl taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of bisacodyl from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bisacodyl related compound A	0.20	1.7	0.15
Bisacodyl related compound B	0.40	1.5	0.15
Bisacodyl related compound C	0.45	1.3	0.50
Specified unidentified impurity 1	0.85	1.0	0.20
Bisacodyl related compound E	0.90	1.0	0.50
Bisacodyl	1.0	—	—
Specified unidentified impurity 2	2.6	1.0	0.30
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Bisacodyl RS
USP Bisacodyl Related Compound A RS
4,4′-(Pyridin-2-ylmethylene)diphenol.
$C_{18}H_{15}NO_2$ 277.32
USP Bisacodyl Related Compound B RS
2,4′-(Pyridin-2-ylmethylene)diphenol.
$C_{18}H_{15}NO_2$ 277.32
USP Bisacodyl Related Compound C RS
4-[(4-Hydroxyphenyl)(pyridin-2-yl)methyl]phenyl acetate.
$C_{20}H_{17}NO_3$ 319.35
USP Bisacodyl Related Compound E RS
2-[(4-Acetoxyphenyl)(pyridin-2-yl)methyl]phenyl acetate.
$C_{22}H_{19}NO_4$ 361.39

Bisacodyl Suppositories

» Bisacodyl Suppositories contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{22}H_{19}NO_4$.

Packaging and storage—Preserve in well-closed containers at a temperature not exceeding 30°.
USP Reference standards ⟨11⟩—
USP Bisacodyl RS

Identification—
A: Transfer a quantity of Suppositories, equivalent to about 150 mg of bisacodyl, to a 500-mL conical flask, add 75 mL of solvent hexane, and heat on a steam bath until they are melted. Filter the solution, with the aid of vacuum, through a medium-porosity, sintered-glass funnel, and wash the residue with about 100 mL of warm solvent hexane until it is free from fat. Continue the vacuum until the residue appears dry. Dissolve the residue by rinsing the filter with about 50 mL of warm acetone, collecting the filtrate in a 150-mL beaker, and evaporate the filtrate on a steam bath to a volume of about 5 mL. To the residual liquid add about 75 mL of water, heat on a steam bath for 15 minutes, and cool. Scratch the sides of the beaker to induce crystallization, filter the crystals, and dry at 100° for about 15 minutes: the bisacodyl so obtained melts between 129° and 135°, and responds to *Identification* test *A* under *Bisacodyl*
B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for bisacodyl, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of 0.074 M sodium acetate in water [adjusted with 2.5% (v/v) acetic acid to a pH of 7.4] and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Bisacodyl RS in acetonitrile to obtain a *Standard preparation* having a known concentration of about 0.5 mg per mL.
Assay preparation—Transfer a number of Suppositories, equivalent to about 100 mg of bisacodyl, to a 500-mL

www.webofpharma.com

separator, add 150 mL of *n*-hexane, and shake until all the suppositories are dissolved. Add 50 mL of acetonitrile, shake for 1 minute, and allow the layers to separate. Drain the lower layer into a 200-mL volumetric flask, and extract the *n*-hexane layer remaining in the separator with two 50-mL portions of acetonitrile, combining the lower layers in the volumetric flask. Dilute the combined extracts in the volumetric flask with acetonitrile to volume, mix, and filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 265-nm detector, a 3.9-mm × 30-cm column that contains packing L1, and a guard column that contains packing L2. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{22}H_{19}NO_4$ in the Suppositories taken by the formula:

$$200C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Bisacodyl RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Bisacodyl Rectal Suspension

» Bisacodyl Rectal Suspension is a suspension of Bisacodyl in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{22}H_{19}NO_4$.

Packaging and storage—Preserve in unit-dose containers at a temperature not exceeding 30°.

USP Reference standards ⟨11⟩—
USP Bisacodyl RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay.*

pH ⟨791⟩: between 5.0 and 6.8.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of methanol and 0.01 M monobasic potassium phosphate (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve a suitable quantity of ethylparaben in methanol, and dilute with an equal volume of water to obtain a solution containing about 5.0 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Bisacodyl RS in methanol, add an accurately measured volume of *Internal standard solution*, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having known concentrations of about 67 µg per mL and 250 µg per mL for bisacodyl and ethylparaben, respectively.

Assay preparation—Transfer an accurately measured volume of Rectal Suspension, equivalent to 6.7 mg of bisacodyl, to a 100-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 2.0 for bisacodyl and 1.0 for ethylparaben; the resolution, *R*, between bisacodyl and the internal standard is not less than 7.0; the column efficiency, determined for the analyte peak, is not less than 2000 theoretical plates; the tailing factor is not more than 1.2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{22}H_{19}NO_4$ in the portion of Rectal Suspension taken by the formula:

$$100C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Bisacodyl RS in the *Standard preparation;* and R_U and R_S are the peak response ratios of the bisacodyl peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Bisacodyl Delayed-Release Tablets

DEFINITION

Bisacodyl Delayed-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bisacodyl ($C_{22}H_{19}NO_4$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)

Cell: 1.0 mm

Sample solution: Macerate a portion of powdered Tablets, equivalent to 300 mg of bisacodyl, with 100 mL of acetone. Heat on a steam bath to boiling, filter, and evaporate to about 20 mL. Add 200 mL of water, and warm the mixture on the steam bath, passing a stream of nitrogen over the surface to evaporate the acetone. After 30 min, cool the mixture, and filter through a sintered-glass funnel. Discard the filtrate, and dissolve the crystals in 50 mL of acetone. Evaporate the solution to about 15 mL, add about 75 mL of water, heat on a steam bath for 15 min, and then cool. Scratch the sides of the beaker to induce crystallization, filter the crystals, and dry at 100° for about 15 min. Using the crystals, prepare a solution (1 in 200) in chloroform.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer: 0.074 M sodium acetate in water, adjusted with 2.5% (v/v) acetic acid to a pH of 7.4

Mobile phase: Acetonitrile and *Buffer* (45:55)

Standard solution: 0.5 mg/mL of USP Bisacodyl RS in acetonitrile

www.webofpharma.com

Sample solution: Transfer a portion of finely powdered Tablets equivalent to 100 mg of bisacodyl, to a 200-mL volumetric flask, add 25 mL of water, and shake by mechanical means for 15 min followed by sonication for 15 min. Add 100 mL of acetonitrile, and shake by mechanical means for 15 min followed by sonication for 15 min. Dilute with acetonitrile to volume, mix, and filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Columns
Guard: Packing L2
Analytical: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bisacodyl ($C_{22}H_{19}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bisacodyl RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bisacodyl in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISINTEGRATION** ⟨701⟩**:** Proceed as directed for *Delayed-Release (Enteric-Coated) Tablets*. The Tablets do not disintegrate after 1 h of agitation in simulated gastric fluid TS, but then disintegrate within 45 min in simulated intestinal fluid TS.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers at a temperature not exceeding 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Bisacodyl RS

Milk of Bismuth

» Milk of Bismuth contains bismuth hydroxide and Bismuth Subcarbonate in suspension in water, and yields not less than 5.2 percent and not more than 5.8 percent (w/w) of bismuth trioxide (Bi_2O_3).

Bismuth Subnitrate	80 g
Nitric Acid	120 mL
Ammonium Carbonate	10 g
Strong Ammonia Solution,	
Purified Water, each, a sufficient quantity, to make	1000 mL

Mix the Bismuth Subnitrate with 60 mL of Purified Water and 60 mL of the Nitric Acid in a suitable container, and agitate, warming gently until solution is effected. Pour this solution, with constant stirring, into 5000 mL of Purified Water containing 60 mL of the Nitric Acid. Dilute 160 mL of Strong Ammonia Solution with 4300 mL of Purified Water in a glazed or glass vessel of at least 12,000-mL capacity. Dissolve the Ammonium Carbonate in this solution, and then pour the bismuth solution quickly into it with constant stirring. Add sufficient 6 N ammonium hydroxide, if necessary, to render the mixture distinctly alkaline, allow to stand until the precipitate has settled, then pour or siphon off the supernatant, and wash the precipitate twice with Purified Water, by decantation. Transfer the magma to a strainer of close texture, so as to provide continuous washing with Purified Water, the outlet tube being elevated to prevent the surface of the magma from becoming dry. When the washings no longer yield a pink color with phenolphthalein TS, drain the moist preparation, transfer to a graduated vessel, add sufficient Purified Water to make 1000 mL, and mix.
[NOTE—This method of preparation may be varied, provided the product meets the following requirements.]

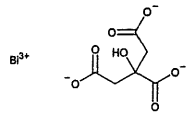
Packaging and storage—Preserve in tight containers, and protect from freezing.

Identification—
A: It responds to the tests for *Bismuth* ⟨191⟩ and for *Carbonate* ⟨191⟩.
B: Add 1 mL of 3 N hydrochloric acid to 1 mL of Milk of Bismuth: a clear solution is produced. Pour the clear solution into 10 volumes of water: a white precipitate is formed.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—The total bacterial count does not exceed 100 cfu per mL and the test for *Escherichia coli* is negative.
Water-soluble substances—Boil 10 mL with 90 mL of water for 10 minutes, cool, add water to make the total volume 100 mL, mix, and filter. Evaporate 50 mL of the filtrate to dryness, and ignite it gently: the weight of the residue does not exceed 5 mg (0.1%).
Arsenic, *Method I* ⟨211⟩—Evaporate 3.75 mL on a steam bath to dryness, add 2 mL of sulfuric acid, and heat until copious fumes of sulfur trioxide are evolved. The limit is 0.8 ppm.
Lead—To 5 mL add warm nitric acid, dropwise, until it is just dissolved, and pour the solution into 50 mL of water: a white precipitate may form. Filter, if necessary, evaporate the filtrate on a steam bath to 15 mL, again filter, and to 10 mL of the filtrate add an equal volume of 2 N sulfuric acid: no precipitate is formed.
Limit of alkalies and alkaline earths—Dissolve 2.0 mL in 5 mL of hydrochloric acid, dilute with water to 100 mL, add hydrogen sulfide to precipitate the bismuth completely, and

www.webofpharma.com

filter. To 50 mL of the clear filtrate add 5 drops of sulfuric acid, evaporate to dryness, and ignite: the weight of the residue does not exceed 3 mg (0.3%).
Assay—Evaporate an accurately weighed quantity of Milk of Bismuth to dryness, and ignite the residue to constant weight. From the weight of the Bi_2O_3 so obtained determine the percentage in the assay specimen.

Bismuth Citrate



$BiC_6H_5O_7$ 398.08
[813-93-4].

» Bismuth Citrate contains not less than 49 percent and not more than 54 percent of bismuth (Bi).

Packaging and storage—Preserve in tight, light-resistant containers, store at controlled room temperature, and prevent exposure to excessive heat.
USP Reference standards ⟨11⟩—
USP Bismuth Citrate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020): on the undried specimen.
B: When strongly heated, the salt chars, and on ignition leaves a more or less blackened residue having a yellow surface. The residue is soluble in warm nitric acid, and this solution, when dropped into a large excess of water, produces a white turbidity.
C: Dissolve 1 g in ammonia TS. When treated with hydrogen sulfide in excess, a black precipitate is obtained. Filter this mixture, drive off the excess hydrogen sulfide by heating, and allow to cool. To a portion of this cooled solution add an excess of calcium hydroxide TS, and boil: a white precipitate is formed. Reserve a second portion of the cooled solution for the test for *Limit of nitrate*.
Arsenic, *Method I* ⟨211⟩—Prepare the *Test Preparation* as follows. Triturate 300 mg with an equal weight of calcium hydroxide, and ignite. Dissolve the residue in 5 mL of 3 N hydrochloric acid: the limit is 10 µg per g.
Limit of nitrate—To the second portion of cooled solution reserved from *Identification* test *C*, add an equal volume of sulfuric acid, mix, and allow to cool. Into the liquid, drop a crystal of ferrous sulfate, and allow to stand for 30 minutes: no brown or brownish black color appears around the crystal.

Limit of copper, lead, and silver—
Standard solution—Prepare a solution containing 1000 µg of copper per mL, a solution containing 1000 µg of lead per mL, and a solution containing 1000 µg of silver per mL. Transfer 3.0 mL of each solution to a 2000-mL volumetric flask, dilute with 1 N nitric acid to volume, and mix. [NOTE—The concentrations of copper, lead, and silver in this solution may be modified by using a different quantity or by further dilution to bring the absorption responses within the working range of the atomic absorption spectrophotometer.]
Test solution—Ignite about 3 g of Bismuth Citrate, accurately weighed, in a porcelain crucible, cool, and cautiously add 6 N nitric acid to dissolve the residue. Add 100 mL of water, and mix. A white precipitate forms. Filter this mixture, evaporate on a steam bath to obtain about 15 mL of solution, and filter again. Dilute the filtrate with water to 20.0 mL.
Procedure—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the emission lines of 324.7 nm, 217 nm, and 328.1 nm for copper, lead, and silver, respectively, with an atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with copper, lead, and silver hollow-cathode lamps and an oxidizing flame. The absorbances of the *Test solution* do not exceed those of the *Standard solution* for each element (10 µg per g).

Limit of soluble bismuth—
Standard solution—Transfer 242.0 mg of bismuth nitrate pentahydrate to a 100-mL volumetric flask. Add 3 mL of 1.5 N nitric acid, swirl to dissolve, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a 500-mL volumetric flask, add 250 mL of 1.5 N nitric acid, dilute with water to volume, and mix. This solution contains 2.0 µg of bismuth (Bi) per mL. [NOTE—The concentration of bismuth in this solution may be modified by using a different quantity or by further dilution to bring the absorption responses within the working range of the atomic absorption spectrophotometer.]
Test solution—Prepare a mixture of 5.0 g of Bismuth Citrate and 100 mL of water, and stir by mechanical means the suspension thus obtained for 2 hours. Pass through filter paper. Pass the filtrate thus obtained through a filter having a 0.1-µm or finer porosity. To 10.0 mL of the filtrate add 0.1 mL of nitric acid.
Procedure—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the emission line of 223.06 nm for bismuth with an atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a bismuth hollow-cathode lamp and an oxidizing flame. The absorbances of the *Test solution* do not exceed those of the *Standard solution* (40 µg per g).
Assay—Transfer about 300 mg of Bismuth Citrate, accurately weighed, to a porcelain crucible, and ignite. Allow to cool, add 2 mL of nitric acid to the residue, dropwise, and warm until complete solution has been effected. Add about 60 mL of water and 0.3 mL of xylenol orange TS, and titrate with 0.05 N edetate disodium VS to a yellow endpoint. Each mL of 0.05 N edetate disodium is equivalent to 10.45 mg of bismuth (Bi).

Bismuth Subcarbonate

DEFINITION
Bismuth Subcarbonate contains NLT 97.6% and NMT 100.7% of bismuth subcarbonate $[(BiO)_2CO_3]$, calculated on the dried basis.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Bismuth* and *Carbonate* ⟨191⟩

ASSAY
• **PROCEDURE**
Sample solution: 500 mg of Bismuth Subcarbonate in 3 mL of nitric acid. Dilute with water to 250 mL, and add 0.3 mL of xylenol orange TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS

USP Monographs

www.webofpharma.com

Endpoint detection: Visual
Analysis: Titrate with *Titrant* to a yellow endpoint. Each mL of 0.05 M edetate disodium is equivalent to 12.75 mg of bismuth subcarbonate [$(BiO)_2CO_3$].
Acceptance criteria: 97.6%–100.7% on the dried basis

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample stock solution: 5.0 g in 10 mL of water. Add 20 mL of nitric acid, warm to achieve dissolution, and allow to cool. Dilute with water to obtain 100 mL of solution.
Sample solution: To 6.6 mL of the *Sample stock solution* add 4 mL of nitric acid, and dilute with water to 50 mL.
Acceptance criteria: A 15.0-mL portion of the *Sample solution* shows no more chloride than corresponds to 70 µL of 0.020 N hydrochloric acid (0.05%).

• **LIMIT OF ALKALIES AND ALKALINE EARTHS**
Sample solution: Boil 1.0 g with 20 mL of a mixture of acetic acid and water (1:1). After 2 min, cool, and filter.
Analysis: Collect the filtrate, wash the residue with 20 mL of water, and add the washing to the filtrate. To this solution add 2 mL of 2 N hydrochloric acid and 20 mL of water. Heat to boiling, and precipitate the bismuth by adding hydrogen sulfide. Cool the mixture, and filter. Collect the filtrate, wash the residue with water, and add the washing to the filtrate. Evaporate this solution to dryness on a water bath. To the residue add 0.5 mL of sulfuric acid, dry slowly, and cool.
Acceptance criteria: The weight of the residue does not exceed 10 mg (1.0%).

• **LIMIT OF NITRATE**
Indigo carmine titrant: To 4 g of indigo carmine in 900 mL of water add 2 mL of sulfuric acid, and dilute with water to 1000 mL.
Standard solution: 0.0815 mg/mL of potassium nitrate (equivalent to 0.05 mg/mL of nitrate) in water. Place 20.0 mL in a 125-mL conical flask.
Sample solution: To 250 mg of Bismuth Subcarbonate in a 125-mL conical flask add 20 mL of water, and swirl to suspend.
Analysis: To the *Standard solution* and the *Sample solution* add 0.05 mL of *Indigo carmine titrant*. Carefully add 30 mL of sulfuric acid, and immediately titrate with *Indigo carmine titrant* to a stable blue endpoint.
Acceptance criteria: The volume of *Indigo carmine titrant* consumed by the *Sample solution* does not exceed that consumed by the *Standard solution* (0.4%).

• **LIMIT OF SILVER**
Standard solution: 7.87 µg/mL of silver nitrate
Sample solution: To 2.0 g of Bismuth Subcarbonate add 1 mL of water and 4 mL of nitric acid.
Analysis: Heat the *Sample solution* gently to achieve dissolution, add water to obtain 11 mL of solution, and cool. Add 2 mL of 1 N hydrochloric acid, and allow to stand in a dark place for 5 min. Treat the *Standard solution* concomitantly with 1 mL of nitric acid and 2 mL of 1 N hydrochloric acid.
Acceptance criteria: The turbidity produced from the *Sample solution* is NMT that produced from the *Standard solution* (0.0025%).

• **LIMIT OF ARSENIC,** *Method I* ⟨211⟩
Test preparation: 600 mg in 35 mL of 3 N hydrochloric acid
Acceptance criteria: NMT 5 ppm

• **LIMIT OF COPPER**
Standard stock solution 1: 5 mg/mL of copper prepared as follows. To a 100-mL volumetric flask add 1.34 g of cupric chloride, 10 g of ammonium chloride, and 3 mL of sodium metabisulfite solution (275 mg/mL), and dilute with water to volume.
Standard stock solution 2: 10 µg/mL of copper in 2 N nitric acid from *Standard stock solution 1*
Standard solution: Mix 0.25 mL of *Standard stock solution 2* and 9.75 mL of water.
Sample solution: To 5 mL of the *Sample stock solution* retained from the test for *Chloride and Sulfate, Chloride* add 2 mL of 6 N ammonium hydroxide, dilute with water to 50 mL, mix, and filter.
Analysis: To 10 mL each of the *Standard solution* and the *Sample solution* add 1 mL of a solution of sodium diethyldithiocarbamate (1 in 1000).
Acceptance criteria: No more color is obtained from the *Sample solution* than is obtained from the *Standard solution* (0.005%).

• **LIMIT OF LEAD**
Diluent: 6 N nitric acid, lead-free
Standard stock solution: 0.1598 mg/mL of lead nitrate in *Diluent*. This solution contains 100 µg/mL of lead.
Standard solutions: 1.0, 2.0, and 3.0 µg/mL of lead from the *Standard stock solution* in *Diluent*
Sample solution: 12.5 g of Bismuth Subcarbonate in 75 mL of *Diluent*. Heat to boiling for 1 min, cool, and dilute with water to 100 mL.
Analysis: Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* at the lead emission line of 283.3 nm with an atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a lead hollow-cathode lamp and an air–acetylene flame, using a 1:5 dilution of the *Diluent* as the blank. Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of lead, and draw the straight line best fitting the three plotted points. From the graph, determine the concentration, *C*, in µg/mL, of lead in the *Sample solution*.
Calculate the percentage of lead (Pb) in the portion of Bismuth Subcarbonate taken:

$$\text{Result} = C/1250$$

C = concentration of lead in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.002%

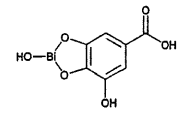
SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 1.0% of its weight

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.

Bismuth Subgallate



$C_7H_5BiO_6$ 394.09
Gallic acid bismuth basic salt [99-26-3].

DEFINITION

Bismuth Subgallate is a basic salt that, when dried at 105° for 3 h, contains the equivalent of NLT 52.0% and NMT 57.0% of bismuth trioxide (Bi_2O_3).

www.webofpharma.com

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Bismuth* ⟨191⟩
Sample: When heated to redness, it at first chars, leaving finally a yellow residue. Use the residue for analysis.
Acceptance criteria: Meets the requirements

• **B.**
Sample: 100 mg
Analysis: Agitate the *Sample* thoroughly with an excess of hydrogen sulfide TS, filter, and boil the filtrate to expel the dissolved gas. Cool, and add 1 drop of ferric chloride TS.
Acceptance criteria: A purplish blue mixture is produced.

ASSAY

• **PROCEDURE**
Sample solution: Dry 1 g of Bismuth Subgallate at 105° for 3 h, then weigh and ignite in a porcelain crucible. Allow it to cool, and add nitric acid to the residue, dropwise, warming until complete solution has been effected.
Analysis: Evaporate the *Sample solution* to dryness, and carefully ignite the residue to constant weight. From the weight of the residue, determine the percentage of Bi_2O_3 in the portion of Bismuth Subgallate taken.
Acceptance criteria: 52.0%–57.0% on the dried basis

IMPURITIES

• **ARSENIC** ⟨211⟩
Test preparation: 400 mg
Analysis: Triturate the *Test preparation* with 400 mg of calcium hydroxide, and ignite. Dissolve the residue in 5 mL of 3 N hydrochloric acid.
Acceptance criteria: NMT 7.5 ppm; the solution, without further treatment, meets the requirements.

• **LIMIT OF NITRATE**
Sample: 100 mg
Analysis: Mix the *Sample* with 5 mL of 2 N sulfuric acid and 5 mL of ferrous sulfate TS, filter the mixture, and carefully superimpose the filtrate, without mixing, on 5 mL of sulfuric acid, in a test tube.
Acceptance criteria: No reddish brown color appears at the zone of contact of the two liquids.

• **LIMITS OF COPPER, LEAD, AND SILVER**
Sample: 3 g
Analysis: Ignite the *Sample* in a porcelain crucible, cool, and cautiously add, dropwise, just sufficient nitric acid to dissolve the residue upon warming. Evaporate the solution to dryness, again ignite, and cool. Cautiously dissolve the residue in just sufficient nitric acid with the aid of gentle heat, concentrate the solution to about 4 mL, and pour it into 100 mL of water. Filter, evaporate the filtrate on a steam bath to 20 mL, again filter, and divide this filtrate into portions of 5 mL each.
Acceptance criteria
Copper: To 5 mL of the filtrate add a slight excess of 6 N ammonium hydroxide: the liquid does not exhibit a bluish color.
Lead: To 5 mL of the filtrate add 5 mL of 2 N sulfuric acid: the liquid does not become cloudy.
Silver: To 5 mL of the filtrate add hydrochloric acid, dropwise: no precipitate is formed that is insoluble in a slight excess of hydrochloric acid, but that is soluble in 6 N ammonium hydroxide.

• **LIMIT OF ALKALIES AND ALKALINE EARTHS**
Sample: 1.0 g
Analysis: Boil the *Sample* with 20 mL of a mixture of equal volumes of 6 N acetic acid and water, cool, and filter. Precipitate the bismuth from the filtrate by the addition of hydrogen sulfide, boil the mixture, and filter. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness, and ignite to constant weight. Weigh the residue.
Acceptance criteria: NMT 5 mg (0.5%)

• **LIMIT OF FREE GALLIC ACID**
Sample: 1.0 g
Analysis: Shake the *Sample* with 20 mL of alcohol for 1 min, filter, and evaporate the filtrate to dryness on a steam bath, then dry the residue at 105° for 1 h. Weigh the residue.
Acceptance criteria: NMT 5 mg (0.5%)

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 3 h.
Acceptance criteria: NMT 7.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Bismuth Subnitrate

$Bi_5O(OH)_9(NO_3)_4$ 1461.99
Bismuth hydroxide nitrate oxide $Bi_5O(OH)_9(NO_3)_4$.
Bismuth hydroxide nitrate oxide $Bi_5O(OH)_9(NO_3)_4$ [1304-85-4].

» Bismuth Subnitrate is a basic salt that contains the equivalent of not less than 79.0 percent of bismuth trioxide (Bi_2O_3), calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
Identification—It responds to the tests for *Bismuth* ⟨191⟩ and for *Nitrate* ⟨191⟩.
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 3.0% of its weight.
Carbonate—Add 3 g to 3 mL of warm nitric acid: no effervescence occurs. Pour the solution into 100 mL of water: a white precipitate forms. Filter, evaporate the filtrate on a steam bath to 30 mL, again filter the liquid, divide the latter filtrate into portions of 5 mL each, and use these several portions in the tests for *Chloride, Sulfate, Copper, Lead,* and *Silver.*
Chloride ⟨221⟩—A 10-mL portion of the test liquid retained in the test for *Carbonate* shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.035%).
Sulfate ⟨221⟩—To a 5-mL portion of the test liquid retained in the test for *Carbonate* add 5 drops of barium nitrate TS: no turbidity is produced immediately.
Limit of ammonium salts—Boil about 100 mg with 5 mL of 1 N sodium hydroxide: the vapor does not turn moistened red litmus paper blue.
Arsenic, *Method I* ⟨211⟩—Mix 375 mg with 5 mL of water, cautiously add 2 mL of sulfuric acid, and heat the mixture until fumes of sulfur trioxide are copiously evolved. Cool, cautiously add 10 mL of water, and again evaporate to strong fuming, repeating, if necessary, to remove any trace of nitric acid. The limit is 8 ppm.
Copper—To a 5-mL portion of the test liquid retained in the test for *Carbonate* add a slight excess of 6 N ammonium hydroxide: the liquid does not exhibit a bluish color.
Lead—Mix a 5-mL portion of the test liquid retained in the test for *Carbonate* with an equal volume of 2 N sulfuric acid: the liquid does not become cloudy.
Silver—To a 5-mL portion of the test liquid retained in the test for *Carbonate* add hydrochloric acid, dropwise: no precipitate is formed that is insoluble in a slight excess of hydrochloric acid, but that is soluble in 6 N ammonium hydroxide.

USP Monographs

www.webofpharma.com

Limit of alkalies and alkaline earths—Boil 1.0 g with 20 mL of a mixture of equal volumes of 6 N acetic acid and water, cool, and filter. Add 2 mL of 3 N hydrochloric acid, precipitate the bismuth by the addition of hydrogen sulfide, boil the mixture, and filter it. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness, and ignite to constant weight: the weight of the residue does not exceed 5 mg (0.5%).
Assay—Transfer about 400 mg of Bismuth Subnitrate, accurately weighed, to a 250-mL beaker. Add 5 mL of water, then add 2 mL of nitric acid, and warm, if necessary, to effect solution. Dilute with water to 100 mL, add 0.3 mL of xylenol orange TS, and titrate with 0.05 M edetate disodium VS to a yellow endpoint. Each mL of 0.05 M edetate disodium is equivalent to 11.65 mg of Bi_2O_3.

Bismuth Subsalicylate

$C_7H_5BiO_4$ 362.09
(2-Hydroxybenzoato-O^1)-oxobismuth;
2-Hydroxybenzoic acid bismuth (3+) salt, basic [14882-18-9].

DEFINITION
Bismuth Subsalicylate is a basic salt that contains NLT 56.0% and NMT 59.4% of bismuth (Bi) and NLT 36.5% and NMT 39.3% of total salicylates on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Bismuth* ⟨191⟩: Meets the requirements

ASSAY
- **BISMUTH**

Sample solution: Transfer an equivalent to 300 mg of Bismuth Subsalicylate, previously dried at 105° for 3 h, to a porcelain crucible, and ignite. Allow it to cool, and add about 2 mL of nitric acid to the residue, dropwise, warming until dissolved. Add about 60 mL of water and 0.3 mL of xylenol orange TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Titrate the *Sample solution* with *Titrant* to a yellow endpoint. Each mL of *Titrant* is equivalent to 10.45 mg of bismuth (Bi).
Acceptance criteria: 56.0%–59.4% of bismuth (Bi) on the dried basis

- **TOTAL SALICYLATES**

Solution A: Ferric ammonium sulfate TS, 1 N hydrochloric acid, and water (4:1:15)
Standard stock solution: 0.2 mg/mL of USP Salicylic Acid RS in water
Standard solution: 0.05 mg/mL of USP Salicylic Acid RS in water, prepared by adding 25.0 mL of *Standard stock solution* and 70 mL of water to a 100-mL volumetric flask. Adjust with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5, before dilution with water to volume.
Reacted standard solution: To 25.0 mL of *Standard solution* add 1.0 mL of *Solution A*.
Unreacted standard solution: To 25.0 mL of the *Standard solution* add 1.0 mL of 0.05 N hydrochloric acid.
Sample solution: Transfer 52 mg of Bismuth Subsalicylate, previously dried at 105° for 3 h, into a 200-mL volumetric flask. Add 10 mL of 0.5 N sodium hydroxide, heat on a steam bath for 15 min, allow to cool, and dilute with water to volume. Centrifuge 70 mL, and then transfer 50.0 mL of the clear supernatant to a beaker. Add about 40 mL of water, and adjust with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.
Reacted sample solution: To 25.0 mL of *Sample solution* add 1.0 mL of *Solution A*.
Unreacted sample solution: To 25.0 mL of the *Sample solution* add 1.0 mL of 0.05 N hydrochloric acid.
Blank: Water, adjusted with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5
Reacted blank solution: To 25.0 mL of *Blank* add 1.0 mL of *Solution A*.
Unreacted blank: To 25.0 mL of *Blank* add 1.0 mL of 0.05 N hydrochloric acid.
Instrumental conditions
Mode: UV
Analytical wavelength: 525 nm
Analysis
Samples: *Reacted standard solution*, *Unreacted standard solution*, *Reacted sample solution*, *Unreacted sample solution*, *Reacted blank solution*, and *Unreacted blank*
Concomitantly determine the absorbances of the *Samples*.
Calculate the percentage of total salicylates in the portion of dried Bismuth Subsalicylate taken:

$$\text{Result} = [(A_{UR} - A_{UU} - B)/(A_{SR} - A_{SU} - B)] \times (C_S/C_U) \times 100$$

A_{UR} = absorbance of the *Reacted sample solution*
A_{UU} = absorbance of the *Unreacted sample solution*
B = difference in the absorption of the *Reacted blank solution* and the absorption of the *Unreacted blank*
A_{SR} = absorbance of the *Reacted standard solution*
A_{SU} = absorbance of the *Unreacted standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of Bismuth Subsalicylate in the *Sample solution* (mg/mL)

Acceptance criteria: 36.5%–39.3% of total salicylates on the dried basis

IMPURITIES
- **ARSENIC,** *Method I* ⟨211⟩

Sample: 300 mg of Bismuth Subsalicylate with 300 mg of calcium hydroxide
Analysis: Triturate the *Sample*, and ignite. Dissolve the residue in 5 mL of 3 N hydrochloric acid.
Acceptance criteria: 10 ppm

- **LIMIT OF COPPER, LEAD, AND SILVER**

Standard stock solution: Add 3.0 mL each of 1000-µg/mL solutions of copper, lead, and silver, respectively, to a 2000-mL flask, and dilute with 1 M nitric acid to volume.
Standard solution: 1.5 µg/mL of copper, 1.5 µg/mL of lead, and 1.5 µg/mL of silver, in 1 M nitric acid from the *Standard stock solution*. The concentrations of copper, lead, and silver may be modified by using different volumes or concentrations to bring the absorption response within the working range of the atomic absorption spectrophotometer.
Sample solution: Ignite 3 g of sample in a porcelain crucible, cool, and cautiously add 6 M nitric acid to dissolve the residue, and evaporate on a steam bath. Ignite the residue, cool, transfer the residue to a tared conical flask,

USP Monographs

www.webofpharma.com

and wash the flask with about 5 mL of 6 M nitric acid, adding the wash to the conical flask. Dissolve the residue with the aid of heat, and add water to obtain a solution weighing 20.0 g. The concentrate of Bismuth Subsalicylate may be modified by using the same proportions used for modifying the *Standard solution*, by using a different quantity, or by further dilution.

Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 324.7 nm for copper; 217 nm for lead; 328.1 nm for silver
Lamps: Copper, lead, and silver hollow-cathode, and oxidizing flames

Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: 10 ppm; the absorbances of the *Sample solutions* do not exceed those of the *Standard solutions* for each element.

• **LIMIT OF SOLUBLE BISMUTH**
Standard solution: 2 µg/mL of bismuth (Bi), prepared as follows. Add 242.0 mg of bismuth nitrate pentahydrate to a 100-mL volumetric flask, add 3 mL of 1.5 M nitric acid, swirl to dissolve, and dilute with water to volume. Add 1.0 mL of this solution to a 500-mL volumetric flask, add 250 mL of 1.5 M nitric acid, and dilute with water to volume. The concentration of bismuth in this solution may be modified by using a lesser dilution or by further dilution to bring the absorption response within the working range of the atomic absorption spectrophotometer.
Sample solution: 5.0 g of Bismuth Subsalicylate in 100 mL of water, and stir the suspension thus obtained for 2 h at 20°–23°. Pass through filter paper. Pass the filtrate thus obtained through a filter of 0.1-µm or less pore size. Add 0.1 mL of nitric acid to 10.0 mL of the filtrate. The concentrate of Bismuth Subsalicylate may be modified by using the same proportions used for modifying the *Standard solution*, by using a different quantity, or by further dilution.

Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 223.06 nm for bismuth
Lamp: Bismuth hollow-cathode and an oxidizing flame

Analysis
Samples: *Standard solution* and *Sample solution*
Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*.
Acceptance criteria: 40 ppm; the absorbances of the *Sample solution* do not exceed those of the *Standard solution*.

• **LIMIT OF NITRATE**
Standard solution: To 0.1 g of salicylic acid add 6 mL of water, 4.0 mL of a solution containing 100 µg of nitrate per mL, and 20 mL of sulfuric acid. Prepare concomitantly with the *Sample solution*.
Sample solution: Add 10 mL of water to 0.1 g of Bismuth Subsalicylate. Carefully add 20 mL of sulfuric acid.
Acceptance criteria: 0.4%; the *Sample solution* should not be more yellow than the *Standard solution*.

• **LIMIT OF FREE SALICYLIC ACID**
Mobile phase: Methanol and 0.06 M acetic acid (55:45)
Diluent: Acetonitrile and water (1:1)
Standard solution: 0.02 mg/mL of USP Salicylic Acid RS in *Diluent*
Sample solution: Add 260 mg of Bismuth Subsalicylate to a glass centrifuge tube, add about 12 mL of acetonitrile, shake by mechanical means for 20 min, and centrifuge. Decant the supernatant into a suitable container. Repeat the acetonitrile addition, shaking, centrifuging, and decanting. Combine the decanted liquid with the first decantate. Pass the combined liquid through a filter of 0.5-µm or finer pore size, and collect the filtrate in a 50-mL volumetric flask. Wash the container with 5 mL of acetonitrile, and filter the wash, collecting the filtrate in the volumetric flask. Dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm
Columns
Guard: 3.2-mm × 1.5-cm; 5-µm packing L1
Analytical: 4.6-mm × 30-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of free salicylic acid in the portion of Bismuth Subsalicylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of salicylic acid from the *Sample solution*
r_S = peak area of salicylic acid from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of the Bismuth Subsalicylate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2%

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 10 g of Bismuth Subsalicylate in 90 mL of water
Analysis: Shake by mechanical means for 10 min, and filter.
Acceptance criteria: 2.7–5.0
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Bismuth Subsalicylate RS
USP Salicylic Acid RS

Bismuth Subsalicylate Magma

DEFINITION
Bismuth Subsalicylate Magma is a suspension of Bismuth Subsalicylate in water that contains NLT 90.0% and NMT 110.0% of the labeled amount of bismuth subsalicylate ($C_7H_5BiO_4$). Bismuth subsalicylate is a basic salt that when dried at 105° for 3 h contains NLT 56.0% and NMT 59.4% bismuth (Bi) and NLT 36.5% and NMT 39.3% of total salicylates.

www.webofpharma.com

Dry at 105° for 3 h to determine the solids content and, after determining the solids content, perform all tests on a portion of the dried Magma.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Bismuth* ⟨191⟩: Meets the requirements

ASSAY

- **BISMUTH**

Sample solution: Transfer an equivalent to 300 mg of bismuth subsalicylate, previously dried at 105° for 3 h, to a porcelain crucible, and ignite. Allow it to cool, and add about 2 mL of nitric acid to the residue, dropwise, warming until dissolved. Add about 60 mL of water and 0.3 mL of xylenol orange TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with *Titrant* to a yellow endpoint. Each mL of *Titrant* is equivalent to 10.45 mg of bismuth (Bi).

Acceptance criteria: 56.0%–59.4% of bismuth on the previously dried basis

- **TOTAL SALICYLATES**

Solution A: Ferric ammonium sulfate TS, 1 N hydrochloric acid, and water (4:1:15)

Standard stock solution: 0.2 mg/mL of USP Salicylic Acid RS in water

Standard solution: 0.05 mg/mL of USP Salicylic Acid RS in water, prepared by adding 25.0 mL of *Standard stock solution* and 70 mL of water to a 100-mL volumetric flask. Adjust with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5, before dilution with water to volume.

Reacted standard solution: To 25.0 mL of *Standard solution* add 1.0 mL of *Solution A*.

Unreacted standard solution: To 25.0 mL of the *Standard solution* add 1.0 mL of 0.05 N hydrochloric acid.

Sample solution: Transfer an equivalent to 52 mg of bismuth subsalicylate from previously dried Magma at 105° for 3 h to a 200-mL volumetric flask. Add 10 mL of 0.5 N sodium hydroxide, heat on a steam bath for 15 min, allow to cool, and dilute with water to volume. Centrifuge 70 mL, and then transfer 50.0 mL of the clear supernatant to a beaker. Add about 40 mL of water, and adjust with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.

Reacted sample solution: To 25.0 mL of *Sample solution* add 1.0 mL of *Solution A*.

Unreacted sample solution: To 25.0 mL of the *Sample solution* add 1.0 mL of 0.05 N hydrochloric acid.

Blank: Water, adjusted with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5

Reacted blank solution: To 25.0 mL of *Blank* add 1.0 mL of *Solution A*.

Unreacted blank: To 25.0 mL of *Blank* add 1.0 mL of 0.05 N hydrochloric acid.

Instrumental conditions

Mode: UV

Analytical wavelength: 525 nm

Analysis

Samples: *Reacted standard solution*, *Unreacted standard solution*, *Reacted sample solution*, *Unreacted sample solution*, *Reacted blank solution*, and *Unreacted blank*

Concomitantly determine the absorbances of the *Samples*.

Calculate the percentage of total salicylates in the portion of dried Magma taken:

$$\text{Result} = [(A_{UR} - A_{UU} - B)/(A_{SR} - A_{SU} - B)] \times (C_S/C_U) \times 100$$

A_{UR} = absorbance of the *Reacted sample solution*

A_{UU} = absorbance of the *Unreacted sample solution*

B = difference in the absorption of the *Reacted blank solution* and the absorption of the *Unreacted blank*

A_{SR} = absorbance of the *Reacted standard solution*

A_{SU} = absorbance of the *Unreacted standard solution*

C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of bismuth subsalicylate in the *Sample solution* (mg/mL)

Acceptance criteria: 36.5%–39.3% of total salicylates on the previously dried basis

IMPURITIES

- **LIMIT OF COPPER, LEAD, AND SILVER**

Standard stock solution: Add 3.0 mL each of 1000-µg/mL solutions of copper, lead, and silver, respectively, to a 2000-mL flask, and dilute with 1 M nitric acid to volume.

Standard solution: 1.5 µg/mL of copper, 1.5 µg/mL of lead, and 1.5 µg/mL of silver, in 1 M nitric acid from the *Standard stock solution*. The concentrations of copper, lead, and silver may be modified by using different volumes or concentrations to bring the absorption response within the working range of the atomic absorption spectrophotometer.

Sample solution: Ignite 3 g of sample in a porcelain crucible, cool, cautiously add 6 M nitric acid to dissolve the residue, and evaporate on a steam bath. Ignite the residue, cool, transfer the residue to a tared conical flask, and wash the flask with about 5 mL of 6 M nitric acid, adding the wash to the conical flask. Dissolve the residue with the aid of heat, and add water to obtain a solution weighing 20.0 g. The concentrate of bismuth subsalicyclate may be modified by using the same proportions used for modifying the *Standard solution*, by using a different quantity, or by further dilution.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 324.7 nm for copper; 217 nm for lead; 328.1 nm for silver

Lamps: Copper, lead and silver hollow-cathode, and oxidizing flames

Analysis

Samples: *Standard solution* and *Sample solution*

Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*

Acceptance criteria: 10 ppm; the absorbances of the *Sample solutions* do not exceed those of the *Standard solutions* for each element.

- **LIMIT OF SOLUBLE BISMUTH**

Standard solution: 2 µg/mL of bismuth (Bi), prepared as follows. Add 242.0 mg of bismuth nitrate pentahydrate to a 100-mL volumetric flask, add 3 mL of 1.5 M nitric acid, swirl to dissolve, and dilute with water to volume. Add 1.0 mL of this solution to a 500-mL volumetric flask, add 250 mL of 1.5 M nitric acid, and dilute with water to volume. The concentration of bismuth in this solution may be

www.webofpharma.com

modified by using a lesser dilution or by further dilution to bring the absorption response within the working range of the atomic absorption spectrophotometer.

Sample solution: 5.0 g of bismuth subsalicylate from dried Magma in 100 mL of water, and stir the suspension thus obtained for 2 h at 20°–23°. Pass through filter paper. Pass the filtrate thus obtained through a filter of 0.1-µm or less pore size. Add 0.1 mL of nitric acid to 10.0 mL of the filtrate. The concentrate of bismuth subsalicyclate may be modified by using the same proportions used for modifying the *Standard solution*, by using a different quantity, or by further dilution.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 223.06 nm for bismuth

Lamp: Bismuth hollow-cathode and an oxidizing flame

Analysis

Samples: *Standard solution* and *Sample solution*

Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*.

Acceptance criteria: 40 ppm; the absorbances of the *Sample solution* do not exceed those of the *Standard solution*.

• **LIMIT OF NITRATE**

Standard solution: To 0.1 g of salicylic acid add 6 mL of water, 4.0 mL of a solution containing 100 µg of nitrate per mL, and 20 mL of sulfuric acid. Prepare concomitantly with the *Sample solution*.

Sample solution: Add 10 mL of water to 0.1 g of Magma. Carefully add 20 mL of sulfuric acid, and mix.

Acceptance criteria: 0.4%; the *Sample solution* should not be more yellow than the *Standard solution*.

• **LIMIT OF FREE SALICYLIC ACID**

Mobile phase: Methanol and 0.06 M acetic acid (550:450)

Diluent: Acetonitrile and water (1:1)

Standard solution: 0.02 mg/mL of USP Salicylic Acid RS in *Diluent*

Sample solution: Add 260 mg of bismuth subsalicylate from dried Magma to a glass centrifuge tube, add about 12 mL of acetonitrile, shake by mechanical means for 20 min, and centrifuge. Decant the supernatant into a suitable container. Repeat the acetonitrile addition, shaking, centrifuging, and decanting, combining the decanted liquid with the first decantate. Pass the combined liquid through a filter of 0.5-µm pore size, collecting the filtrate in a 50-mL volumetric flask. Wash the container with 5 mL of acetonitrile, and filter the wash, collecting the filtrate in the volumetric flask. Dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 300 nm

Columns

Guard: 3.2-mm × 1.5-cm; 5-µm packing L1

Analytical: 4.6-mm × 30-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of free salicylic acid in the portion of Magma taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of salicylic acid from the *Sample solution*

r_S = peak area of salicylic acid from of the *Standard solution*

C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of the bismuth subsalicylate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **LABELING:** The label states that this article is not intended for direct administration to humans or animals.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Bismuth Subsalicylate RS

USP Salicylic Acid RS

Bismuth Subsalicylate Oral Suspension

DEFINITION

Bismuth Subsalicylate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of bismuth subsalicylate ($C_7H_5BiO_4$). It may contain one or more suitable buffers, coloring agents, flavors, preservatives, stabilizers, sweeteners, and suspending agents.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Bismuth*: Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Salicylate*: Meets the requirements of test *A* after acidifying with nitric acid

ASSAY

• **PROCEDURE**

Standard stock solution: 2.5 mg/mL of bismuth in nitric acid. Prepare by dissolving in 6% of the flask volume of nitric acid and diluting with 0.01 N nitric acid to volume.

Standard solution: 0.05 mg/mL of bismuth in 1 N nitric acid from the *Standard stock solution*

Sample solution: Transfer 10 g of Oral Suspension, previously well shaken in its original container to ensure homogeneity, to a 200-mL volumetric flask. Add about 100 mL of 1 N nitric acid, and dilute with 1 N nitric acid to volume. Mix well without shaking, transfer 10.0 mL of this mixture to a 100-mL volumetric flask, and dilute with 1 N nitric acid to volume. Centrifuge about 20 mL at 4500 rpm for at least 10 min.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 463 nm

Cell: 1 cm

Blank: 1 N nitric acid

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Transfer a measured volume of the *Sample solution* that contains 0.9 mg of bismuth subsalicylate and 10 mL of the *Standard solution* to separate 50-mL volumetric flasks. Add 10.0 mL of 10% ascorbic acid solution and 25.0 mL of 20% potassium iodide solution to each volumetric flask, and dilute with water to volume. Concomitantly determine the absorbances of both solutions, using the *Blank* to set the spectrophotometer.

USP Monographs

www.webofpharma.com

Calculate the percentage of the labeled amount of bismuth subsalicylate ($C_7H_5BiO_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of bismuth in the *Standard solution* (mg/mL)
C_U = nominal concentration of bismuth subsalicylate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of bismuth subsalicylate, 362.09
M_{r2} = molecular weight of bismuth, 208.98

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 10^2 cfu/g, and the total combined molds and yeasts count is NMT 5×10^1 cfu/g. It meets the requirements of the test for the absence of *Escherichia coli.*
- **pH** ⟨791⟩**:** 3.0–5.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from freezing. Avoid excessive heat (over 40°).

Bismuth Subsalicylate Tablets

DEFINITION

Bismuth Subsalicylate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bismuth subsalicylate ($C_7H_5BiO_4$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Bismuth* ⟨191⟩**:** Meet the requirements
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩**,** *Salicylate***:** After acidifying with nitric acid, it meets the requirements of test A.

ASSAY

- **PROCEDURE**

Standard stock solution: 2.5 mg/mL of bismuth in nitric acid. Prepare by dissolving in 6% of the flask volume of nitric acid, and diluting with 0.01 N nitric acid to volume.
Standard solution: 0.05 mg/mL of bismuth in 1 N nitric acid from the *Standard stock solution*
Sample stock solution: Equivalent to 90 mg of bismuth subsalicylate from finely powdered Tablets in a 200-mL volumetric flask. Add 150 mL of 1 N nitric acid, and sonicate for 2 min. Dilute with 1 N nitric acid to volume.
Sample solution: Transfer 20.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with 1 N nitric acid to volume. Centrifuge a portion at 4500 rpm for at least 10 min.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 463 nm
Cell: 1 cm
Blank: 10% ascorbic acid solution, 20% potassium iodide solution, and 1 N nitric acid (2:5:1)
Analysis
Samples: *Standard solution* and *Sample solution*

Transfer 10.0 mL of the *Standard solution* and the *Sample solution* to separate 50.0-mL volumetric flasks, and dilute with the *Blank* to volume. Concomitantly determine the absorbance of the solutions at the wavelength of maximum absorbance at 463 nm with a suitable spectrophotometer, using the combined reagent solutions as the blank.
Calculate the percentage of the labeled amount of bismuth subsalicylate ($C_7H_5BiO_4$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of bismuth in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of bismuth subsalicylate, 362.09
M_{r2} = molecular weight of bismuth, 208.98

Acceptance criteria: 90.0%–110.0%

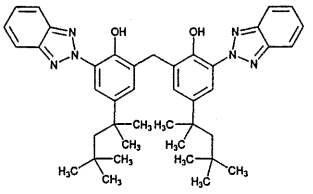
PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩
This test does not apply to Tablets labeled as chewable.
Time: 10 min
Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Avoid excessive heat (over 40°).
- **LABELING:** Label chewable Tablets to indicate that they are to be chewed before swallowing.

Bisoctrizole



$C_{41}H_{50}N_6O_2$ 658.87

Phenol, 2,2′-methylenebis[6-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)]-;
2,2′-Methylenebis[6-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol] [103597-45-1].

DEFINITION

Bisoctrizole contains NLT 96.0% and NMT 102.0% of bisoctrizole ($C_{41}H_{50}N_6O_2$), calculated on the as-is basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩**,** *Infrared Spectroscopy***: 197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Diluent: Tetrahydrofuran and 0.2% (w/v) aqueous solution of 1-pentane sulfonic acid sodium salt (60:40)

Solution A: 0.4 g of 1-pentane sulfonic acid sodium salt, 800 mL of methanol, 200 mL of water, and 0.5 mL of phosphoric acid

Solution B: 0.4 g of 1-pentane sulfonic acid sodium salt, 1000 mL of methanol, and 0.5 mL of phosphoric acid

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
1	70	30
11	3	97
40	3	97

System suitability solution: 0.8 mg/mL of bisoctrizole from USP Bisoctrizole Resolution Mixture RS prepared as follows. Transfer USP Bisoctrizole Resolution Mixture RS to a suitable volumetric flask, dissolve in tetrahydrofuran, and dilute with *Diluent* to volume.

Standard solution: 0.8 mg/mL of USP Bisoctrizole RS prepared as follows. Transfer USP Bisoctrizole RS to a suitable volumetric flask, dissolve in tetrahydrofuran equivalent to 60% of the final volume, and dilute with *Diluent* to volume.

Sample solution: Transfer 80 mg of Bisoctrizole to a 100-mL volumetric flask. Dissolve in 60 mL of tetrahydrofuran, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 346 nm

Column: 3.0-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 0.8 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times for bisoctrizole and the bisoctrizole isomer.]

Suitability requirements

Resolution: NLT 1.5 between bisoctrizole and the bisoctrizole isomer, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of bisoctrizole ($C_{41}H_{50}N_6O_2$) in the portion of Bisoctrizole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Bisoctrizole RS in the *Standard solution* (mg/mL)

C_U = concentration of Bisoctrizole in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–102.0% on the as-is basis

IMPURITIES

• **LIMIT OF BISOCTRIZOLE RELATED COMPOUND A AND BISOCTRIZOLE ISOMER**

Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution A: 0.65 mg/mL of USP Bisoctrizole RS in tetrahydrofuran

Standard stock solution B: 0.40 mg/mL of USP Bisoctrizole Related Compound A RS in tetrahydrofuran

Standard solution: Transfer 5 mL of *Standard stock solution A* and 1.0 mL of *Standard stock solution B* to a 100-mL volumetric flask. Add 60 mL of tetrahydrofuran, and dilute with *Diluent* to volume.

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 2* for the relative retention times for bisoctrizole related compound A and the bisoctrizole isomer.]

Suitability requirements

Resolution: NLT 1.5 between bisoctrizole and the bisoctrizole isomer

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of bisoctrizole related compound A in the portion of Bisoctrizole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of bisoctrizole related compound A from the *Sample solution*

r_S = peak response of bisoctrizole related compound A from the *Standard solution*

C_S = concentration of USP Bisoctrizole Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Bisoctrizole in the *Sample solution* (mg/mL)

Calculate the percentage of bisoctrizole isomer in the portion of Bisoctrizole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of bisoctrizole isomer from the *Sample solution*

r_S = peak response of bisoctrizole from the *Standard solution*

C_S = concentration of USP Bisoctrizole RS in the *Standard solution* (mg/mL)

C_U = concentration of Bisoctrizole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

• **ORGANIC IMPURITIES**

Diluent, Solution A, Solution B, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual unspecified impurity in the portion of Bisoctrizole taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity

r_T = sum of the responses of all the peaks

Acceptance criteria: See *Table 2*.

www.webofpharma.com

Table 2

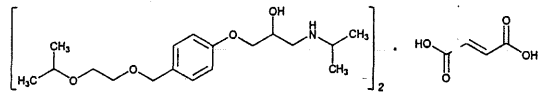
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bisoctrizole related compound A[a]	0.42	0.5
Bisoctrizole	1.0	—
Bisoctrizole isomer[b]	1.1	4.0
Any individual unspecified impurity	—	0.10
Total impurities	—	4.0

[a] 2-(2*H*-Benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol.
[b] Phenol, 2,2′-methylenebis[6-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)]

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bisoctrizole RS
  USP Bisoctrizole Related Compound A RS
  2-(2*H*-Benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol.
  $C_{20}H_{25}N_3$ 323.43
  USP Bisoctrizole Resolution Mixture RS
  A mixture of approximately 1.5% of bisoctrizole isomer [phenol, 2,2′-methylenebis[6-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)]] in a matrix of bisoctrizole.

Bisoprolol Fumarate



$(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ 766.96
2-Propanol, 1-[4-[[2-(1-methylethoxy)ethoxy]methyl]phenoxy]-3-[(1-methylethyl)amino]-, (±)-, (*E*)-2-butenedioate (2:1) (salt).
(±)-1-[[α-(2-Isoproproxyethoxy)-*p*-tolyl]oxy]-3-(isopropylamino)-2-propanol fumarate (2:1) (salt) [104344-23-2].

» Bisoprolol Fumarate contains not less than 97.5 percent and not more than 102.0 percent of $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Bisoprolol Fumarate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Specific rotation ⟨781⟩: between –2° and +2°.
Test solution: 10 mg per mL, in methanol.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%.
Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Diluent, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay.*
Standard solution—Prepare as directed for *Standard preparation* in the *Assay.*
Test solution—Prepare as directed for *Assay preparation* in the *Assay.*
Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak areas. Calculate the percentage of total impurities in the portion of Bisoprolol Fumarate taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the sum of areas for all the peaks, excluding the fumaric acid and bisoprolol peaks; and r_s is the sum of the areas of all the peaks in the chromatogram: not more than 0.5% of total impurities is found.
Content of fumaric acid—Transfer about 500 mg of Bisoprolol Fumarate, accurately weighed, to a beaker, and dissolve in 70 mL of dehydrated alcohol. Add 8.0 mL of 0.1 N tetrabutylammonium hydroxide VS, and stir for 2 minutes. Titrate with 0.1 N tetrabutylammonium hydroxide VS, determining the endpoint potentiometrically, using a glass-calomel electrode system. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 5.804 mg of fumaric acid: not less than 14.8% and not more than 15.4% of fumaric acid is found, calculated on the anhydrous basis.

Assay—
Diluent—Prepare a mixture of water and acetonitrile (65:35).
Mobile phase—To a 1-L portion of *Diluent* add 5 mL of heptafluorobutyric acid, 5 mL of diethylamine, and 2.5 mL of formic acid. Mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Prepare a solution in *Diluent* containing about 0.5 mg of propranolol hydrochloride and 1 mg of Bisoprolol Fumarate per mL.
Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Bisoprolol Fumarate RS in *Diluent* to obtain a solution having a known concentration of about 1 mg per mL.
Assay preparation—Transfer about 50 mg of Bisoprolol Fumarate, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 273-nm detector and a 4.6-mm × 12.5-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak areas as directed for *Procedure:* the resolution, *R,* between bisoprolol and propranolol is not less than 7.0. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of (C

www.webofpharma.com

$_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ in the portion of Bisoprolol Fumarate taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Bisoprolol Fumarate RS in the *Standard preparation;* and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Bisoprolol Fumarate Tablets

DEFINITION

Bisoprolol Fumarate Tablets contain NLT 90.0% and NMT 105.0% of the labeled amount of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$].

IDENTIFICATION

• **Thin-Layer Chromatographic Identification Test** ⟨201⟩

Sample solution: Equivalent to 40 mg of bisoprolol fumarate, from powdered Tablets (NLT 5), in a 50-mL flask. Add about 20 mL of a mixture of dichloromethane and methanol (7:3), shake for 30 min, centrifuge, and use the clear solution.

Application volume: 20 µL

Developing solvent system: Dichloromethane, methanol, and ammonia TS, stronger (70: 10: 0.8)

Analysis

Sample: *Sample solution*

Proceed as directed in the chapter, except to develop the chromatogram until the solvent front has moved about two-thirds of the length of the plate and to dry the plate in a current of cold air.

ASSAY

• **Procedure**

Diluent: Acetonitrile and water (7:13)

Mobile phase: A 1-L portion of *Diluent.* Add 5 mL of heptafluorobutyric acid, 5 mL of diethylamine, and 2.5 mL of formic acid.

System suitability solution: 0.5 mg/mL of propranolol hydrochloride and 1 mg/mL of bisoprolol fumarate in *Diluent*

Standard solution: 1 mg/mL of USP Bisoprolol Fumarate RS in *Diluent*

Sample solution: Transfer an equivalent of 25 mg of bisoprolol fumarate, from powdered Tablets (NLT 20), to a 25-mL volumetric flask. Add 10 mL of *Diluent,* and sonicate for 10 min. Cool, dilute with *Diluent* to volume, and mix. Centrifuge for 20 min, and use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 273 nm

Column: 4.6-mm × 12.5-cm; packing L7

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 7.0 between bisoprolol and propranolol, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Bisoprolol Fumarate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of bisoprolol fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–105.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Test 1

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 20 min

Determine the amount of $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ dissolved by using the following method.

Diluent: Methanol, triethylamine, phosphoric acid, and water (160: 5: 2.5: 35)

Mobile phase: Methanol, triethylamine, and water (34:1:50). Adjust with phosphoric acid to a pH of 4.0 ± 0.1.

Standard stock solution: USP Bisoprolol Fumarate RS in water to obtain a solution having a known concentration of about twice the concentration of bisoprolol fumarate in the *Sample solution*

Standard solution: *Standard stock solution* and *Diluent* (1:1)

Sample solution: Sample per *Dissolution* ⟨711⟩. Withdraw a portion of the solution under test, filter, and dilute with an equal volume of *Diluent.*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm × 33-mm; packing L7

Flow rate: 1 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 80% (*Q*) of the labeled amount of $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.*

Medium: 0.5 M sodium chloride; 900 mL

Apparatus 2: 75 rpm

Time: 20 min

Analysis: Proceed as directed for *Test 1* with the following modifications.

Diluent: Prepare a mixture of methanol, 0.1 N hydrochloric acid, triethylamine, and phosphoric acid (160: 35: 5: 2.5). The dimensions of the column are 4.6 mm × 25 cm.

Tolerances: NLT 80% (*Q*) of the labeled amount of $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

USP Monographs

www.webofpharma.com

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Bisoprolol Fumarate RS
2-Propanol, 1-[4-[[2-(1-methylethoxy)ethoxy]methyl] phenoxy]-3-[(1-methylethyl)amino]-, (±)-, (*E*)-2-butenedioate (2:1) (salt).
$(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ 766.96

Bisoprolol Fumarate and Hydrochlorothiazide Tablets

» Bisoprolol Fumarate and Hydrochlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of bisoprolol fumarate $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ and hydrochlorothiazide $(C_7H_8ClN_3O_4S_2)$.

Packaging and storage—Preserve in tight, light-resistant containers. Store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Bisoprolol Fumarate RS
USP Chlorothiazide RS
USP Hydrochlorothiazide RS

Identification—
A: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Test solution*—Finely powder 1 Tablet, and transfer the powder to a 5-mL volumetric flask. Dilute with methanol to volume, sonicate for 5 minutes, centrifuge, and use the supernatant.
Standard solution 1—Dissolve a suitable quantity of USP Bisoprolol Fumarate RS in methanol to obtain a solution containing 1 mg per mL.
Standard solution 2—Dissolve a suitable quantity of USP Hydrochlorothiazide RS in methanol to obtain a solution containing 1 mg per mL.
Application volume: 25 µL.
Developing solvent system: a mixture of methylene chloride, methanol, and 14.5 M ammonium hydroxide solution (43:20:8).
Procedure—Locate the spots on the plate under short-wavelength UV light and by exposure to iodine vapors: the R_F values of the principal spots in the chromatogram obtained from the *Test solution* correspond to those of the principal spots in the chromatograms obtained from *Standard solution 1* and *Standard solution 2*.
B: The retention times of the major peaks in the chromatograms of the *Bisoprolol fumarate assay preparation* and the *Hydrochlorothiazide assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 2: 75 rpm.
Times: 20 minutes for bisoprolol fumarate; 30 minutes for hydrochlorothiazide.
Triethylamine solution—Mix 2 mL of triethylamine with 1000 mL of water, and adjust with phosphoric acid to a pH of 3.0.
Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *Triethylamine solution* (1:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard stock solution 1—Quantitatively dissolve an accurately weighed quantity of USP Bisoprolol Fumarate RS in *Medium* to obtain a solution having a known concentration of about 0.5 mg per mL.
Standard stock solution 2—Transfer about 30 mg of USP Hydrochlorothiazide RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Medium* to volume, and mix.
Standard solution—Dilute accurately measured volumes of *Standard stock solution 1* and *Standard stock solution 2* with *Medium* to obtain a solution having known concentrations of bisoprolol fumarate and hydrochlorothiazide corresponding to those of the solution under test.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a UV detector capable of measuring peak responses at 227 nm and 272 nm, simultaneously, and a 3.9-mm × 15-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the filtered portions of the solution under test into the chromatograph, record the chromatograms, and measure the peak areas for bisoprolol at 227 nm and for hydrochlorothiazide at 272 nm. Calculate the quantities, in mg, of bisoprolol fumarate $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ and hydrochlorothiazide $(C_7H_8ClN_3O_4S_2)$ dissolved.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ is dissolved in 20 minutes and not less than 80% *(Q)* of the labeled amount of $C_7H_8ClN_3O_4S_2$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩**:** Meet the requirements with respect to bisoprolol fumarate and to hydrochlorothiazide.

Chromatographic purity—
Diluent, Solution A, Solution B, Mobile phase, and *System suitability solution*—Proceed as directed in the *Assay*.
Standard solution—Dissolve an accurately weighed quantity of USP Hydrochlorothiazide RS in *Diluent*, and quantitatively dilute with *Diluent*, if necessary, to obtain a solution having a known concentration of about 2 µg per mL.
Test stock solution—Proceed as directed for *Assay stock preparation* in the *Assay*.
Test solution—Quantitatively dilute an accurately measured volume of the *Test stock solution* with *Diluent* to obtain a solution having a concentration of about 100 µg of bisoprolol fumarate per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay*, but use a 260-nm detector. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between chlorothiazide and hydrochlorothiazide is not less than 1.5. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.3; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$(100/F)(W_B/W_H)(C_S/C_B)(r_i/r_S)$$

in which *F* is the response factor, equal to 1.2 for the peak with a relative retention time of 0.69 and 1.4 for the peak with a relative retention time of 1.2, both retention times relative to

www.webofpharma.com

that of the hydrochlorothiazide peak; W_B and W_H are the labeled quantities, in mg, of bisoprolol fumarate and hydrochlorothiazide, respectively, in each Tablet; C_S is the concentration, in mg per mL, of USP Hydrochlorothiazide RS in the *Standard solution;* C_B is the concentration, in mg per mL, of bisoprolol fumarate in the *Test solution;* r_i is the peak response of each of the two impurities obtained from the *Test solution;* and r_S is the response for the hydrochlorothiazide peak obtained from the *Standard solution:* not more than 1.0% for the impurity with a relative retention time of 0.69 is found; and not more than 2.0% for the impurity with a relative retention time of 1.2 is found.

Assay—

Diluent—Mix 10 mL of 1 M dibutylammonium phosphate with 1000 mL of a mixture of water and acetronitrile (1:1).

Solution A—Mix 10 mL of 1 M dibutylammonium phosphate with 1000 mL of water.

Solution B—Prepare a mixture of acetonitrile and water (3:2). Add 10 mL of 1 M dibutylammonium phosphate per liter, stir vigorously for 2 minutes, filter, and degas.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare a solution of USP Chlorothiazide RS and USP Hydrochlorothiazide RS in *Diluent* containing 40 µg of each per mL.

Standard preparation—Dissolve suitable quantities of USP Bisoprolol Fumarate RS and USP Hydrochlorothiazide RS in *Diluent* to obtain a solution having known concentrations of about 100 µg of each per mL. Stir by mechanical means for 1 hour.

Assay stock preparation—Weigh 10 Tablets, and transfer to a 100-mL volumetric flask. Add about 50 mL of *Diluent,* sonicate for 10 minutes, and cool. Dilute with *Diluent* to volume, stir by mechanical means for 1 hour, and centrifuge.

Bisoprolol fumarate assay preparation—Quantitatively transfer a portion of the *Assay stock preparation* to a 50-mL volumetric flask, and dilute with *Diluent* to volume to obtain a solution having a concentration of about 100 µg of bisoprolol fumarate per mL.

Hydrochlorothiazide assay preparation—Quantitatively transfer a portion of the *Assay stock preparation* to a 50-mL volumetric flask, and dilute with *Diluent* to volume to obtain a solution having a concentration of about 62.5 µg of hydrochlorothiazide per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with 225-nm detector and an 8-mm × 10-cm column that contains packing L11. The flow rate is about 3 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–9.0	100→40	0→60	linear gradient
9.0–9.1	40→100	60→0	linear gradient
9.1–12.0	100	0	re-equilibration

Chromatograph the *System suitability solution,* and record the peak areas as directed for *Procedure:* the resolution, *R,* between chlorothiazide and hydrochlorothiazide is not less than 1.5. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the tailing factor for the hydrochlorothiazide peak is not more than 1.3; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation, Bisoprolol fumarate assay preparation,* and *Hydrochlorothiazide assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of bisoprolol fumarate $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken by the formula:

$$5000(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Bisoprolol Fumarate RS or USP Hydrochlorothiazide RS in the *Standard preparation,* as appropriate; *V* is the volume of the *Assay stock preparation* used to prepare the *Bisoprolol fumarate assay preparation* or the *Hydrochlorothiazide assay preparation;* r_U is the peak area obtained from the *Bisoprolol fumarate assay preparation* or the *Hydrochlorothiazide assay preparation,* as appropriate; and r_S is the corresponding peak area obtained from the *Standard preparation.*

Bleomycin for Injection

» Bleomycin for Injection contains an amount of Bleomycin Sulfate equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of bleomycin.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*

USP Reference standards ⟨11⟩—
USP Bleomycin Sulfate RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: It responds to the tests for *Sulfate* ⟨191⟩.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 10.0 USP Endotoxin Units per Bleomycin Unit.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined,* the entire contents of each container being used.

Water Determination, *Method Ic* ⟨921⟩ **:** not more than 6.0%. Prepare the specimen for test as follows. Use a dry syringe to inject 4 mL of anhydrous methanol through the stoppers of two tared containers, respectively, and shake to dissolve. Using the same syringe, aspirate the contents of the two containers, transfer to the titration vessel, and titrate. Perform a blank determination on 8 mL of the anhydrous methanol. Determine the weights of the empty containers, and calculate the percentage of water.

Other requirements—It meets the requirements for *pH, Copper,* and *Content of bleomycins* under *Bleomycin Sulfate.* It meets also the requirements for *Uniformity of Dosage Units*

USP Monographs

www.webofpharma.com

⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—

Assay preparation—Constitute Bleomycin for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Buffer B.16* to obtain a solution having a convenient concentration.

Procedure—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with *Buffer B.16* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Bleomycin Sulfate

Bleomycin sulfate (salt).
Bleomycin sulfate (salt) [9041-93-4].

» Bleomycin Sulfate is the sulfate salt of bleomycin, a mixture of basic cytotoxic glycopeptides produced by the growth of *Streptomyces verticillus*, or produced by other means. It has a potency of not less than 1.5 Bleomycin Units and not more than 2.0 Bleomycin Units per mg.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Bleomycin Sulfate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: It responds to the tests for *Sulfate* ⟨191⟩.

pH ⟨791⟩: between 4.5 and 6.0, in a solution containing 10 Bleomycin Units per mL.

Loss on drying ⟨731⟩—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 6.0% of its weight.

Copper—

Dilute nitric acid—Dilute 20 mL of nitric acid to 2000 mL with water.

Copper stock solution—Transfer 1.000 g of copper to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, dilute with *Dilute nitric acid* to volume, and mix. Store in a polyethylene bottle. This solution contains 1000 µg of copper per mL.

Standard preparations—Transfer 5.0 mL of *Copper stock solution* to a 100-mL volumetric flask, dilute with *Dilute nitric acid* to volume, and mix. Transfer 3.0, 9.0, and 15.0 mL, respectively, of this solution to separate 100-mL volumetric flasks, dilute the contents of each flask with *Dilute nitric acid* to volume, and mix. These *Standard preparations* contain, respectively, 1.5, 4.5, and 7.5 µg of copper per mL.

Test preparation—Dissolve about 75 mg of Bleomycin Sulfate, accurately weighed, in 10.0 mL of *Dilute nitric acid*.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Test preparation* at the copper emission line at 324.8 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a copper hollow-cathode lamp and an air–acetylene flame, using *Dilute nitric acid* as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of copper, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, *C*, in µg per mL, of copper in the *Test preparation*. Calculate the percentage of copper in the portion of Bleomycin Sulfate taken by the formula:

$$C/W$$

in which *W* is the weight, in mg, of Bleomycin Sulfate taken to prepare the *Test preparation:* not more than 0.1% is found.

Content of bleomycins—

Mobile phase—Dissolve 960 mg of sodium 1-pentane sulfonate in 1000 mL of deaerated 0.08 N acetic acid, adjust with ammonium hydroxide to a pH of 4.3, filter, and degas. [NOTE—1.86 g of edetate disodium may be included if needed to obtain satisfactory chromatography.] Use a linear gradient of 10% to 40% methanol mixed with this solution, with a gradient mixing time of 60 minutes, and allow chromatography to proceed with the final gradient mixture for a further 20 minutes or until demethylbleomycin A_2 has been eluted.

Test preparation—Dissolve Bleomycin Sulfate in deaerated water to obtain a solution having a concentration of about 2.5 Bleomycin Units per mL. Store this solution in a refrigerator until just prior to use.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 250-mm stainless steel column containing packing L1. The flow rate is about 1.2 mL per minute.

Procedure—Inject about 10 µL of the *Test preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatogram, and measure the peak responses for all peaks. The elution order is bleomycinic acid, bleomycin A_2 (major peak), bleomycin A_5, bleomycin B_2 (major peak), bleomycin B_4, and demethylbleomycin A_2. Calculate the percentage contents of bleomycin A_2, bleomycin B_2, and bleomycin B_4 taken by the formula:

$$100 r_i / r_t$$

in which r_i is the peak response corresponding to the particular bleomycin and r_t is the total of the responses of all peaks: the content of bleomycin A_2 is between 55% and 70%; the content of bleomycin B_2 is between 25% and 32%; the content of bleomycin B_4 is not more than 1%; and the combined percentage of bleomycin A_2 and bleomycin B_2 is not less than 90%.

Other requirements—Where the label states that Bleomycin Sulfate is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Bleomycin for Injection*. Where the label states that Bleomycin Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Bleomycin for Injection*.

Assay—

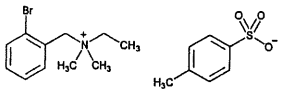
Assay preparation—Dissolve a suitable quantity of Bleomycin Sulfate, accurately weighed, in *Buffer B.16*, and

www.webofpharma.com

quantitatively dilute with *Buffer B.16* to obtain a solution having a convenient concentration.

Procedure—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with *Buffer B.16* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Bretylium Tosylate



$C_{18}H_{24}BrNO_3S$ 414.36

Benzenemethanaminium, 2-bromo-*N*-ethyl-*N*,*N*-dimethyl-, salt with 4-methylbenzenesulfonic acid (1:1).
(*o*-Bromobenzyl)ethyldimethylammonium *p*-toluenesulfonate [61-75-6].

» Bretylium Tosylate contains not less than 98.0 percent and not more than 101.0 percent of $C_{18}H_{24}BrNO_3S$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Bretylium Tosylate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K.*▲ (CN 1-May-2020)

B: The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution,* as obtained in the test for *Related compounds.*

Loss on drying ⟨731⟩—Dry it in vacuum at 75° for 2 hours: it loses not more than 3.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—

0.01 M Sodium 1-octanesulfonate solution—Dissolve 1.0814 g of 1-sodium octanesulfonate in 500 mL of water.

Mobile phase—Prepare a mixture of *0.01 M Sodium 1-octanesulfonate solution,* acetonitrile, glacial acetic acid, and triethylamine (81: 19: 2: 0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Bretylium Tosylate RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 20 µg per mL.

Test solution—Transfer about 200 mg of Bretylium Tosylate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 265-nm detector and a 4.6-mm × 25-cm column that contains packing L11. The flow rate is about 1.9 mL per minute. Chromatograph the *Standard solution,* record the chromatograms, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 30 µL) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the peak responses. The relative retention times are about 0.25, 0.74, 1.0, 1.27, 1.40 for tosylate ion, *o*-bromobenzyldimethylamine, bretylium, *m*-bromobenzyldimethylamine, and *p*-bromobenzyldimethylamine, respectively. The sum of the responses for all the peaks, excluding those of the bretylium and tosylate peaks, from the *Test solution* is not more than two times the bretylium response from the *Standard solution* (2%); and no individual peak response is greater than that of the bretylium peak from the *Standard solution* (1%).

Assay—Dissolve about 300 mg of Bretylium Tosylate, accurately weighed, in 50 mL of dioxane in a conical flask. Add 2 drops of crystal violet TS, and titrate with 0.025 N perchloric acid in dioxane to a blue-green endpoint. Perform a blank determination (see *Titrimetry* ⟨541⟩), and make any necessary correction. Each mL of 0.025 N perchloric acid is equivalent to 10.36 mg of $C_{18}H_{24}BrNO_3S$.

Bretylium Tosylate Injection

» Bretylium Tosylate Injection is a sterile solution of Bretylium Tosylate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{18}H_{24}BrNO_3S$.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Bretylium Tosylate RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation,* both relative to the internal standard, as obtained in the *Assay.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.20 USP Endotoxin Unit per mg of bretylium tosylate.
pH ⟨791⟩: between 3.5 and 7.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

pH 3.1 Tetramethylammonium phosphate buffer—Dissolve 1.38 g of monobasic sodium phosphate and 2.0 mL of 25% tetra-methylammonium hydroxide solution in methanol in 800 mL of water, adjust with phosphoric acid to a pH of 3.1 ± 0.1, dilute with water to 1000 mL, and mix.

Mobile phase—Transfer 15 mL of tetrahydrofuran and 75 mL of acetonitrile to a 1000-mL volumetric flask, and dilute with *pH 3.1 Tetramethylammonium phosphate buffer* to volume.

Standard preparation—Dissolve an accurately weighed quantity of USP Bretylium Tosylate RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of bretylium tosylate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 3.9-mm × 30-cm column that contains packing

USP Monographs

www.webofpharma.com

L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for tosylate and 1.0 for bretylium; the resolution, *R*, between the bretylium and tosylate peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 1.4%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{24}BrNO_3S$ in each mL of the Injection taken by the formula:

$$50(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Bretylium Tosylate RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; and r_U and r_S are the bretylium peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Bretylium Tosylate in Dextrose Injection

» Bretylium Tosylate in Dextrose Injection is a sterile solution of Bretylium Tosylate and Dextrose in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of bretylium tosylate ($C_{18}H_{24}BrNO_3S$) and dextrose ($C_6H_{12}O_6 \cdot H_2O$). It contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

USP Reference standards ⟨11⟩—
USP Bretylium Tosylate RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for bretylium tosylate.*

B: Add a few drops of a solution (1 in 20) to 5 mL of hot alkaline cupric tartrate TS. A copious red precipitate of cuprous oxide is formed.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.20 USP Endotoxin Unit per mg of bretylium tosylate.

pH ⟨791⟩**:** between 3.0 and 6.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for bretylium tosylate—

pH 3.1 Tetramethylammonium phosphate buffer, Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Bretylium Tosylate Injection.*

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of bretylium tosylate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of bretylium tosylate ($C_{18}H_{24}BrNO_3S$) in each mL of the Injection taken by the formula:

$$50(C/V)(r_U/r_S)$$

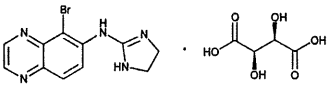
in which *C* is the concentration, in mg per mL, of USP Bretylium Tosylate RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; and r_U and r_S are the bretylium peak responses from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for dextrose—Transfer an accurately measured volume of Injection, containing 2 to 5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix. Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩). Calculate the percentage (g per 100 mL) of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively; *A* is 100 mm divided by the length of the polarimeter tube, in mm; and *R* is the observed rotation, in degrees.

Brimonidine Tartrate



$C_{11}H_{10}BrN_5 \cdot C_4H_6O_6$ 442.22

6-Quinoxalinamine, 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-, [*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1); 5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline L-tartrate (1:1). [70359-46-5].

DEFINITION

Brimonidine Tartrate contains NLT 98.0% and NMT 102.0% of brimonidine tartrate ($C_{11}H_{10}BrN_5 \cdot C_4H_6O_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M.▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Protect the solutions containing brimonidine tartrate from light.

Mobile phase: In a 1-L volumetric flask, dissolve 2.6 g of sodium 1-heptanesulfonate in 310 mL of methanol, add 2.5 mL of triethylamine and 7.5 mL of glacial acetic acid, and dilute with water to volume.

Standard solution: 1.3 mg/mL of USP Brimonidine Tartrate RS in water

Sample solution: 1.3 mg/mL of Brimonidine Tartrate in water

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 264 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of brimonidine tartrate ($C_{11}H_{10}BrN_5 \cdot C_4H_6O_6$) in the portion of Brimonidine Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Brimonidine Tartrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Brimonidine Tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%
- **ORGANIC IMPURITIES**

Protect the solutions containing brimonidine tartrate and related substances from light.
Mobile phase: Proceed as directed in the *Assay*.
System suitability solution: 1.3 mg/mL of USP Brimonidine Tartrate RS and 1.3 µg/mL of USP Brimonidine Related Compound E RS in water
Sensitivity solution: 0.65 µg/mL of USP Brimonidine Tartrate RS in water
Standard solution: 1.3 µg/mL of USP Brimonidine Tartrate RS in water
Sample solution: 1.3 mg/mL of Brimonidine Tartrate in water
Chromatographic system: Proceed as directed in the *Assay*, except for the *Run time*.
Run time: NLT 3 times the retention time of brimonidine
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
[NOTE—The relative retention times for brimonidine related compound E and brimonidine are about 0.93 and 1.00, respectively.]
Suitability requirements
Resolution: NLT 1.5 between brimonidine related compound E and brimonidine, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Brimonidine Tartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of brimonidine from the *Standard solution*
C_S = concentration of USP Brimonidine Tartrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Brimonidine Tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard the tartrate peak at a relative retention time of 0.09. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desbromobrimonidine[a]	0.86	0.1
Brimonidine	1.00	—
Any individual unspecified impurity	—	0.1
Total impurities	—	0.3

[a] *N*-(Quinoxalin-6-yl)imidazolidin-2-imine.

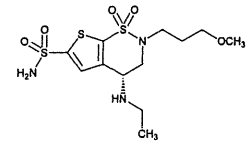
SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL in water
Acceptance criteria: +9.0° to +10.5°
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry 1 g at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Brimonidine Tartrate RS
USP Brimonidine Related Compound E RS
2-(5-Bromoquinoxalin-6-yl)guanidine.
$C_9H_8BrN_5$ 266.10

Brinzolamide



$C_{12}H_{21}N_3O_5S_3$ 383.51
2*H*-Thieno[3,2-*e*]-1,2-thiazine-6-sulfonamide, 4-(ethylamino)-3,4-dihydro-2-(3-methoxypropyl)-, 1,1-dioxide, (*R*)-;
(*R*)-4-(Ethylamino)-3,4-dihydro-2-(3-methoxypropyl)-2*H*-thieno[3,2-*e*]-1,2-thiazine-6-sulfonamide 1,1-dioxide [138890-62-7].

DEFINITION

Brinzolamide contains NLT 98.0% and NMT 102.0% of brinzolamide ($C_{12}H_{21}N_3O_5S_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability*

www.webofpharma.com

solution, as obtained in *Limit of Brinzolamide Related Compound A*.

ASSAY

• **PROCEDURE**

Buffer: Add 4.0 mL of triethylamine to 1000 mL of water, and adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (25:75)
Standard solution: 0.1 mg/mL of USP Brinzolamide RS in *Mobile phase*
Sample solution: 0.1 mg/mL of Brinzolamide in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1200 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of brinzolamide ($C_{12}H_{21}N_3O_5S_3$) in the portion of Brinzolamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Brinzolamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Brinzolamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **LIMIT OF BRINZOLAMIDE RELATED COMPOUND A**

Mobile phase: Dehydrated alcohol, chromatographic hexane, methanol, and diethylamine (55: 40: 5: 0.2)
System suitability solution: 0.4 mg/mL of USP Brinzolamide RS and 0.02 mg/mL of USP Brinzolamide Related Compound A RS in dehydrated alcohol
Sample solution: 0.5 mg/mL of Brinzolamide in dehydrated alcohol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L51
Flow rate: 0.75 mL/min
Injection volume: 5 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for brinzolamide and brinzolamide related compound A are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 1.8 between brinzolamide and brinzolamide related compound A peaks
Column efficiency: NLT 2000 theoretical plates for the brinzolamide peak
Tailing factor: NMT 1.8 for the brinzolamide peak
Analysis
Sample: *Sample solution*
Calculate the percentage of brinzolamide related compound A in the portion of Brinzolamide taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for brinzolamide related compound A
r_T = sum of the peak responses for brinzolamide and brinzolamide related compound A

Acceptance criteria: NMT 0.5%

• **ORGANIC IMPURITIES**

Buffer: Prepare as directed in the *Assay*.
Mobile phase A: Prepare as directed for *Mobile phase* in the *Assay*.
Mobile phase B: Acetonitrile and *Buffer* (35:65)
System suitability solution: 0.1 mg/mL each of USP Brinzolamide RS and USP Brinzolamide Related Compound B RS in *Mobile phase A*
Sample solution: 1 mg/mL of Brinzolamide in *Mobile phase A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Use *Mobile phase A*.
[NOTE—The relative retention times for brinzolamide related compound B and brinzolamide are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the brinzolamide and brinzolamide related compound B peaks
Column efficiency: NLT 1200 theoretical plates for the brinzolamide peak
Tailing factor: NMT 2.0 for the brinzolamide peak
Analysis 1
Use *Mobile phase A*.
Sample: *Sample solution*
Allow the elution to continue for 20 min, and measure the areas for all the peaks, excluding the peaks of *Mobile phase A*.
Calculate the percentage of each impurity in the portion of Brinzolamide taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria 1: NMT 0.3% for any individual impurity
Analysis 2
Use *Mobile phase B*.
Sample: *Sample solution*
Allow the elution to continue for 20 min, and measure the areas for brinzolamide and all the peaks having a relative retention greater than 6.
Calculate the percentage of each impurity in the portion of Brinzolamide taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity

www.webofpharma.com

r_T = sum of all the peak responses

Acceptance criteria 2: NMT 0.3% for any individual impurity; NMT 1.0% for total impurities from *Analysis 1* and *Analysis 2*

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 100°–105° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Brinzolamide RS

USP Brinzolamide Related Compound A RS
Brinzolamide (*S*)-isomer.
$C_{12}H_{21}N_3O_5S_3$ 383.52

USP Brinzolamide Related Compound B RS
(*R*)-4-Amino-2-(3-methoxypropyl)-3,4-dihydro-2*H*-thieno[3,2-e][1,2]thiazine-6-sulfonamide 1,1-dioxide oxalate.
$C_{10}H_{17}N_3O_5S_3 \cdot C_2H_2O_4$ 445.49

Brinzolamide Ophthalmic Suspension

DEFINITION

Brinzolamide Ophthalmic Suspension is a sterile, aqueous suspension of Brinzolamide containing a suitable antimicrobial preservative. It contains NLT 90.0% and NMT 110.0% of the labeled amount of brinzolamide ($C_{12}H_{21}N_3O_5S_3$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of *Standard solution A*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 11.75 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 5.2.

Mobile phase: Methanol and *Buffer* (35:65)

Standard solution A: 0.2 mg/mL of USP Brinzolamide RS in *Mobile phase*

System suitability solution: 0.06 mg/mL of USP Brinzolamide Related Compound B RS in *Standard solution A*

Sample solution: Nominally 0.2 mg/mL of brinzolamide in *Mobile phase* prepared as follows. Transfer a volume of Ophthalmic Suspension, equivalent to 10 mg of brinzolamide, into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL

System suitability
Samples: *Standard solution A* and *System suitability solution*
[NOTE—The relative retention times for brinzolamide related compound B are between 0.48 and 0.61, and the relative retention time for brinzolamide is 1.0.]

Suitability requirements
Resolution: NLT 4.5 between the brinzolamide and brinzolamide related compound B peaks, *System suitability solution*
Tailing factor: NMT 2.0, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution A*

Analysis
Samples: *Standard solution A* and *Sample solution*
Calculate the percentage of the labeled amount of brinzolamide ($C_{12}H_{21}N_3O_5S_3$) in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from *Standard solution A*
C_S = concentration of USP Brinzolamide RS in *Standard solution A* (mg/mL)
C_U = nominal concentration of brinzolamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **LIMIT OF BRINZOLAMIDE RELATED COMPOUND A**

Mobile phase: Dehydrated alcohol, chromatographic hexane, methanol, and diethylamine (55: 40: 5: 0.2)

System suitability solution: 0.4 mg/mL of USP Brinzolamide RS and 0.02 mg/mL of USP Brinzolamide Related Compound A RS in dehydrated alcohol

Sample solution: Transfer a volume of Ophthalmic Suspension, equivalent to 10 mg of brinzolamide, to a 25-mL volumetric flask. Dilute with alcohol to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L51
Flow rate: 0.75 mL/min
Injection volume: 5 µL

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for brinzolamide and brinzolamide related compound A are 1.0 and 1.2, respectively.]

Suitability requirements
Resolution: NLT 1.8 between the brinzolamide and brinzolamide related compound A peaks
Column efficiency: NLT 2000 theoretical plates for the brinzolamide peak
Tailing factor: NMT 1.8 for the brinzolamide peak

Analysis
Sample: *Sample solution*
Calculate the percentage of brinzolamide related compound A in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for brinzolamide related compound A
r_T = sum of the peak responses for brinzolamide and brinzolamide related compound A

Acceptance criteria: NMT 1.5%

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, Standard solution A, System suitability solution, Sample solution, Chromatographic

www.webofpharma.com

system, and **System suitability:** Proceed as directed in the *Assay.*
Standard solution B: 2.5 µg/mL of USP Brinzolamide Related Compound B RS in *Mobile phase*
Analysis
Samples: *Sample solution* and *Standard solution B*
Calculate the percentage of each impurity in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

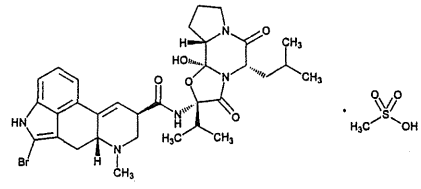
r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for brinzolamide related compound B from *Standard solution B*
C_S = concentration of USP Brinzolamide Related Compound B RS in *Standard solution B* (mg/mL)
C_U = nominal concentration of brinzolamide in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of des-ethyl brinzolamide, 356.46
M_{r2} = molecular weight of des-ethyl brinzolamide oxalate, 445.49

Acceptance criteria
Any individual impurity: NMT 0.5%
Total impurities: NMT 2.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **pH** ⟨791⟩**:** 6.5–8.5

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at a temperature between 4° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Brinzolamide RS
USP Brinzolamide Related Compound A RS
Brinzolamide (*S*)-isomer.
$C_{12}H_{21}N_3O_5S_3$ 383.52
USP Brinzolamide Related Compound B RS
(*R*)-4-Amino-2-(3-methoxypropyl)-3,4-dihydro-2*H*-thieno[3,2-e][1,2]thiazine-6-sulfonamide 1,1-dioxide oxalate.
$C_{10}H_{17}N_3O_5S_3 \cdot C_2H_2O_4$ 445.49

Bromocriptine Mesylate



$C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ 750.70
Ergotaman-3′,6′,18-trione, 2-bromo-12′-hydroxy-2′-(1-methylethyl)-5′-(2-methylpropyl)-, monomethanesulfonate (salt), (5′α)-;
2-Bromoergocryptine monomethanesulfonate (salt) [22260-51-1].

DEFINITION
Bromocriptine Mesylate contains NLT 98.0% and NMT 102.0% of $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)**:** Undried

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 50 µg/mL in 0.1 M methanolic methanesulfonic acid
Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**
Sample solution: 600 mg of Bromocriptine Mesylate
Analysis: Dissolve with 80 mL of a mixture of acetic anhydride and glacial acetic acid (7:1). Titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 75.07 mg of $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$.
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
INORGANIC IMPURITIES
- **Residue on Ignition** ⟨281⟩**:** NMT 0.1%

ORGANIC IMPURITIES
- **Procedure 1: Limit of Methanesulfonic Acid Content**

Sample solution: 400 mg of Bromocriptine Mesylate
Analysis: Dissolve with 70 mL of methanol. Titrate under nitrogen with 0.1 N methanolic potassium hydroxide VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N methanolic potassium hydroxide is equivalent to 9.61 mg of CH_3SO_3H.
Acceptance criteria: NLT 12.5% and NMT 13.4% of CH_3SO_3H on the dried basis
- **Procedure 2**

Solution A: 0.1 N citric acid solution. Adjust with hydrochloric acid to a pH of 2.0.
Diluent: Methanol and *Solution A* (1:1)
Solution B: Acetonitrile and 0.01 M phosphate buffer, pH 7.0 (2:3)
Solution C: Acetonitrile and 0.01 M phosphate buffer, pH 7.0 (3:2)
Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
18	100	0
30	0	100
40	0	100
41	100	0

System suitability solution: 2.0 mg/mL each of α-ergocryptine and Bromocriptine Mesylate in *Diluent*
Standard stock solution: 46 µg/mL of USP Bromocriptine Mesylate RS in methanol and *Solution A* (1:1).

www.webofpharma.com

[NOTE—Dissolve in 50% of the flask volume of methanol and dilute with *Solution A* to volume.]
Standard solution: 4.6 µg/mL of USP Bromocriptine Mesylate RS in *Diluent* from the *Standard stock solution*
Sample solution: 4.6 mg/mL of Bromocriptine Mesylate in methanol and *Solution A* (1:1). [NOTE—Dissolve in 50% of the flask volume of methanol and dilute with *Solution A* to volume.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for α-ergocryptine and bromocriptine mesylate are 0.46 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 15 between α-ergocryptine and bromocriptine mesylate, *System suitability solution*
Tailing factor: NMT 1.5, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for bromocriptine and bromocriptinine are 1.0 and 1.7, respectively.]
Calculate the percentage of each impurity in the portion of Bromocriptine Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of bromocriptine from the *Standard solution*
C_S = concentration of USP Bromocriptine Mesylate RS in the *Standard solution* (mg/mL)
C_U = concentration of Bromocriptine Mesylate in the *Sample solution* (mg/mL)
F = relative response factor equal to 1.4 for any peak eluting at a relative retention time of about 0.9 or less, and equal to 1.0 for all other peaks

Acceptance criteria
Individual impurities: NMT 0.4% of bromocriptinine is found; NMT 0.1% of any individual impurity is found.
Total impurities: NMT 1.0%

SPECIFIC TESTS
• **COLOR OF SOLUTION** ⟨631⟩
Matching solutions: Prepare three solutions, A, B, and C, containing, respectively, the following parts of cobaltous chloride CS, ferric chloride CS, cupric sulfate CS, and dilute hydrochloric acid (1 in 40).
A: 3.0:3.0:2.4:31.6
B: 1.0:2.4:0.4:36.2
C: 0.6:2.4:0:37.0
Sample solution: 10 mg/mL of Bromocriptine Mesylate in methanol
Analysis: Compare the *Sample solution* with 10-mL portions of the *Matching solutions* in suitable matched tubes.
Acceptance criteria: The solution is clear and not darker in color than *Matching solutions A, B,* and *C*.
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL, in a mixture of methylene chloride and methanol (1:1)
Acceptance criteria: +95° to +105°
• **LOSS ON DRYING**
(See *Thermal Analysis* ⟨891⟩.)
Analysis: Determine the percentage of volatile substances by thermogravimetric analysis using 10 mg of Bromocriptine Mesylate. Heat the specimen under test at the rate of 10°/min in an atmosphere of nitrogen at a flow rate of 45 mL/min. Record the thermogram from ambient temperature to 160°.
Acceptance criteria: It loses NMT 4.0% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, in a cold place.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Bromocriptine Mesylate RS

Bromocriptine Mesylate Capsules

DEFINITION
Bromocriptine Mesylate Capsules contain bromocriptine mesylate ($C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of bromocriptine ($C_{32}H_{40}BrN_5O_5$).

IDENTIFICATION
• **A.** The principal spot of the *Sample solution* corresponds, in R_f value and color, to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY
• **PROCEDURE**
Conduct this procedure without exposure to daylight and with minimum exposure to artificial light.
Buffer: 0.125 g/L of ammonium carbonate in water
Mobile phase: Acetonitrile and *Buffer* (3:2)
Standard solution: 1.0 mg/mL of bromocriptine from USP Bromocriptine Mesylate RS in dehydrated alcohol. Sonicate as needed.
Sample solution: 1.0 mg/mL of bromocriptine in methanol, prepared as follows. Remove, as completely as possible, the contents of NLT 10 Capsules. Weigh and determine the average weight per Capsule. Mix the combined contents, and transfer a weighed quantity of the powder, nominally equivalent to 50 mg of bromocriptine, to a 50-mL volumetric flask. Add 30 mL of dehydrated alcohol, and shake for 15 min. Dilute with dehydrated alcohol to volume, mix, and filter. Use this solution without delay.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm
Column: 4-mm × 25-cm; packing L7
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount bromocriptine ($C_{32}H_{40}BrN_5O_5$) in the portion of Capsules taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of bromocriptine, from USP Bromocriptine Mesylate RS, in the *Standard solution* (mg/mL)
C_U = nominal concentration of bromocriptine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 60 min
Standard solution: USP Bromocriptine Mesylate RS in *Medium*, at a concentration similar to the *Sample solution*. [Note—A volume of alcohol not to exceed 5% of the total volume of the *Standard solution* may be used to bring the Standard into solution before dilution with *Medium*.]
Sample solution: Sample per *Dissolution* ⟨711⟩, passed through a glass-fiber filter.
Instrumental conditions
(See *Fluorescence Spectroscopy* ⟨853⟩.)
Mode: Fluorometry
Excitation wavelength: 315 nm
Emission wavelength: 445 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bromocriptine mesylate ($C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of bromocriptine mesylate ($C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩
Procedure for content uniformity
Protect all solutions from light.
Diluent: Dissolve 1.0 g of tartaric acid in 500 mL of water, add 500 mL of methanol, and mix.
Standard solution: 0.04 mg/mL of USP Bromocriptine Mesylate RS in *Diluent*
Sample solution: Transfer the contents of 1 Capsule into a 25-mL volumetric flask. Add 15 mL of *Diluent*, and shake by mechanical means for 20 min. Dilute with *Diluent* to volume, and mix. Filter, and dilute 10.0 mL of the clear filtrate with *Diluent* to 50.0 mL.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Maximum absorbance (about 306 nm)
Cell: 1 cm
Blank: *Diluent*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of the labeled amount of bromocriptine ($C_{32}H_{40}BrN_5O_5$) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Bromocriptine Mesylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bromocriptine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of bromocriptine, 654.59
M_{r2} = molecular weight of bromocriptine mesylate, 750.70

Acceptance criteria: Meet the requirements

IMPURITIES

• **Organic Impurities**
Conduct this test without exposure to daylight and with minimum exposure to artificial light. Perform the test rapidly, preparing and spotting the *Sample solution* last.
Standard stock solution: 2.3 mg/mL of USP Bromocriptine Mesylate RS in methanol, equivalent to 2 mg/mL of bromocriptine
Standard solution 1: 0.06 mg/mL (3.0%) of bromocriptine in methanol, from *Standard stock solution*
Standard solution 2: 0.04 mg/mL (2.0%) of bromocriptine in methanol, from *Standard stock solution*
Standard solution 3: 0.02 mg/mL (1.0%) of bromocriptine in methanol, from *Standard stock solution*
Standard solution 4: 0.01 mg/mL (0.50%) of bromocriptine in methanol, from *Standard stock solution*
Sample solution: 2.0 mg/mL of bromocriptine in methanol, prepared as follows. Transfer a quantity of the Capsule contents, equivalent to 20 mg of bromocriptine, to a conical flask. Add 10 mL of methanol, and stir by mechanical means for 20 min. Centrifuge the suspension for 10 min at about 3500 rpm. Use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 50 µL as 1.5-cm bands
Developing solvent: Methylene chloride, dioxane, alcohol, and ammonium hydroxide (180:15:5:1)
Spray reagent: 0.2% *o*-phthalaldehyde in sulfuric acid
Analysis
Samples: *Standard stock solution*, *Standard solutions*, and *Sample solution*
Develop under the exclusion of light in a tank lined with filter paper, previously equilibrated for 30 min, using *Developing solvent* until the solvent front has moved a distance of 15 cm on the plate. Dry the plate briefly in a current of cold air. Spray evenly with the *Spray reagent*, and view the plate under long-wavelength UV light.
Acceptance criteria: Any major secondary spot, other than the principal spot, obtained from the *Sample solution* is not greater in size and intensity than the spot obtained from *Standard solution 1* (3.0%). Any remaining spots are not greater in size and intensity than the spot obtained from *Standard solution 3* (1.0%). The sum of the organic impurities is NMT 5.0%.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Bromocriptine Mesylate RS

Bromocriptine Mesylate Tablets

DEFINITION

Bromocriptine Mesylate Tablets contain bromocriptine mesylate ($C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$) equivalent to NLT 90.0%

USP Monographs

www.webofpharma.com

and NMT 110.0% of the labeled amount of bromocriptine ($C_{32}H_{40}BrN_5O_5$).

IDENTIFICATION

• **A.** The principal spot of the *Sample solution* corresponds, in R_F value and color, to that of the *Standard stock solution*, as obtained in the test for *Organic Impurities*.

ASSAY

• **PROCEDURE**

Buffer: 0.01 M ammonium carbonate in water
Mobile phase: Acetonitrile and *Buffer* (65:35)
Standard solution: 0.22 mg/mL of USP Bromocriptine Mesylate RS in methanol
Sample solution: Transfer a quantity of powdered Tablets (NLT 20), equivalent to 10 mg of bromocriptine, to an appropriate container. Add 40 mL of methanol, and stir for 20 min, protected from light. Quantitatively filter through a fine glass filtering funnel into a 50-mL volumetric flask. Rinse the filter with methanol, adding the rinsing to the filtrate, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm
Column: 4-mm × 25-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Coefficient of variation: NMT 3.0% for 3 replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bromocriptine ($C_{32}H_{40}BrN_5O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bromocriptine Mesylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bromocriptine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of bromocriptine, 654.59
M_{r2} = molecular weight of bromocriptine mesylate, 750.70

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 1: 120 rpm
Time: 60 min
Standard solution: USP Bromocriptine Mesylate RS at a known concentration in *Medium*
[NOTE—A volume of alcohol not to exceed 5% of the total volume of the *Standard solution* may be used to dissolve the Standard before dilution with *Medium*.]
Sample solution: Sample per *Dissolution* ⟨711⟩, passed through a glass-fiber filter.
Blank: *Medium*
Instrumental conditions
(See *Fluorescence Spectroscopy* ⟨853⟩.)
Mode: Fluorometry
Excitation wavelength: 315 nm
Emission wavelength: 445 nm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of the labeled amount of bromocriptine ($C_{32}H_{40}BrN_5O_5$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amount of bromocriptine ($C_{32}H_{40}BrN_5O_5$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer: 0.01 M ammonium carbonate in water
Mobile phase: Acetonitrile and *Buffer* (65:35)
Standard solution: Dissolve USP Bromocriptine Mesylate RS in methanol, and quantitatively dilute with *Medium* to obtain a solution having a known concentration similar to the expected concentration of the *Sample solution*.
Sample solution: Sample per *Dissolution* ⟨711⟩.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bromocriptine ($C_{32}H_{40}BrN_5O_5$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amount of bromocriptine ($C_{32}H_{40}BrN_5O_5$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity
[NOTE—Protect all solutions from light.]
Diluent: Dissolve 1.0 g of tartaric acid in 500 mL of water, add 500 mL of methanol, and mix.
Standard solution: 0.04 mg/mL of USP Bromocriptine Mesylate RS in *Diluent*
Sample solution: Transfer 1 Tablet into a 25-mL volumetric flask. Add 15 mL of *Diluent*, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume, and mix. Filter, and dilute 10.0 mL of the clear filtrate with *Diluent* to 50.0 mL.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 306 nm
Cell: 1 cm
Blank: *Diluent*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of the labeled amount of bromocriptine ($C_{32}H_{40}BrN_5O_5$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Bromocriptine Mesylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bromocriptine in the *Sample solution* (mg/mL)

USP Monographs

www.webofpharma.com

M_{r1} = molecular weight of bromocriptine, 654.59
M_{r2} = molecular weight of bromocriptine mesylate, 750.70

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

[NOTE—Conduct this test without exposure to daylight and with minimum exposure to artificial light. Perform the test rapidly, preparing and spotting the *Sample solution* last.]

Standard stock solution: 1.2 mg/mL of USP Bromocriptine Mesylate RS in methanol, equivalent to 1 mg/mL of bromocriptine

Standard solution 1: 0.50 mg/mL (5%) of bromocriptine in methanol, from *Standard stock solution*

Standard solution 2: 0.30 mg/mL (3%) of bromocriptine in methanol, from *Standard stock solution*

Standard solution 3: 0.10 mg/mL (1%) of bromocriptine in methanol, from *Standard stock solution*

Sample solution: Transfer an equivalent to 20 mg of bromocriptine, from powdered Tablets, to a conical flask. Add 10 mL of methanol, and mix for 20 min. Centrifuge the suspension for 10 min at 4000 rpm. Use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volumes

Standard solutions: 10-µL, as 1.5-cm bands

Sample solution: 50-µL, as 1.5-cm bands

Developing solvent system: Methylene chloride, dioxane, alcohol, and ammonium hydroxide (180: 15: 5: 0.1)

Spray reagent: 0.2% *o*-phthalaldehyde in sulfuric acid

Analysis

Samples: *Standard stock solution*, *Standard solutions*, and *Sample solution*

Proceed as directed in *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*. Dry the plate for 5 min in a current of cold air. Develop in a tank lined with filter paper, previously equilibrated for 20 min, using *Developing solvent system* until the solvent front has moved a distance of 10 cm on the plate. Dry the plate under vacuum at room temperature for 15 min. Spray evenly with the *Spray reagent*, and view the plate under long-wavelength UV light.

Acceptance criteria: Any spot, other than the principal spot, from the *Sample solution* is not greater in size and intensity than the spot from *Standard solution 2* (3.0%). Any remaining spots are not greater in size and intensity than the spot obtained from *Standard solution 3* (1.0%). The sum of the organic impurities is NMT 5.0%.

ADDITIONAL REQUIREMENTS

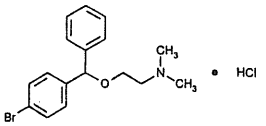
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **LABELING:** The labeling indicates the *Dissolution* test with which the product complies.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Bromocriptine Mesylate RS

Bromodiphenhydramine Hydrochloride



$C_{17}H_{20}BrNO \cdot HCl$ 370.71

Ethanamine, 2-(4-bromophenyl)phenylmethoxy-*N*,*N*-dimethyl-, hydrochloride.

2-(*p*-Bromo-α-phenylbenzyl)oxy-*N*,*N*-dimethylethylamine hydrochloride [1808-12-4].

» Bromodiphenhydramine Hydrochloride contains not less than 98.0 percent and not more than 101.0 percent of $C_{17}H_{20}BrNO \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Bromodiphenhydramine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—

Solution: 15 µg per mL.

Medium: 0.1 N sulfuric acid.

Absorptivities at 228 nm, calculated on the dried basis, do not differ by more than 3.0%.

Melting range ⟨741⟩: between 148° and 152°.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Assay—Dissolve about 700 mg of Bromodiphenhydramine Hydrochloride, accurately weighed, in 50 mL of glacial acetic acid, and add 10 mL of benzene and 15 mL of mercuric acetate TS. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 37.07 mg of $C_{17}H_{20}BrNO \cdot HCl$.

Bromodiphenhydramine Hydrochloride Oral Solution

» Bromodiphenhydramine Hydrochloride Oral Solution contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of bromodiphenhydramine hydrochloride ($C_{17}H_{20}BrNO \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.

www.webofpharma.com

USP Reference standards ⟨11⟩—
USP Bromodiphenhydramine Hydrochloride RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197K***▲ (CN 1-May-2020) —

Test specimen—Transfer the final solution obtained from the titration in the *Assay* to a separator, add about 1 mL of 0.1 N sulfuric acid, and shake with 25 mL of ether. (Methyl red enters the ether phase.) Drain the aqueous layer into another separator, add 5 mL of 1 N sodium hydroxide, and shake with 10 mL of chloroform. Drain the chloroform layer into a small flask containing 2 g of anhydrous sodium sulfate, and swirl. Pour the chloroform solution through a small cotton pledget, pre-rinsed with chloroform, into a beaker, and evaporate to about 5 mL. Apply a few drops of the solution directly to a potassium bromide plate, and completely remove the chloroform by warming for 2 to 3 minutes under an IR lamp.
Alcohol Determination, *Method I* ⟨611⟩: between 12.0% and 15.0% of C_2H_5OH.

Assay—Evaporate an accurately measured volume of Oral Solution, equivalent to about 250 mg of bromodiphenhydramine hydrochloride, to about half the original volume, using a suitable vacuum evaporator. Transfer the concentrated solution to a 250-mL separator, with the aid of sufficient warm water to bring the volume to the original volume. Add 20 g of sodium chloride, and shake until dissolved. Add 5 mL of 1 N sodium hydroxide, shake with 100 mL of ether, and drain the aqueous layer into a second separator containing 50 mL of ether. Shake, and discard the aqueous layer. Wash the ether solutions with two 20-mL portions of water, shaking each aqueous portion successively in the two separators, and then discard the aqueous solutions. Extract the ether solutions successively with 10.0 mL of 0.1 N sulfuric acid VS, followed by two 5-mL portions of water, and collect the aqueous extracts in a conical flask. Add methyl red TS to the solution in the flask, and titrate the excess acid with 0.02 N sodium hydroxide VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 0.1 N sulfuric acid is equivalent to 37.07 mg of bromodiphenhydramine hydrochloride ($C_{17}H_{20}BrNO \cdot HCl$).

Bromodiphenhydramine Hydrochloride and Codeine Phosphate Oral Solution

» Bromodiphenhydramine Hydrochloride and Codeine Phosphate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of bromodiphen hydramine hydrochloride ($C_{17}H_{20}$ BrNO · HCl) and codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$).

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Label it to indicate the alcohol content.
USP Reference standards ⟨11⟩—
USP Bromodiphenhydramine Hydrochloride RS
USP Codeine Phosphate RS

Identification—
A: Thin-Layer Chromatographic Identification Test ⟨201⟩—
Test solution—Transfer a volume of Oral Solution, equivalent to about 10 mg of codeine phosphate, to a separator, and add 5 mL of water, 5 mL of methylene chloride, and 1 mL of ammonium hydroxide. Shake for 1 minute, allow the layers to separate, and use the clear, lower layer.
Standard solution—Prepare a solution of USP Bromodiphenhydramine Hydrochloride RS and USP Codeine Phosphate RS in methanol containing 10 mg of each per mL.
Developing solvent system: a mixture of alcohol and ammonium hydroxide (49:1).
B: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.* The total aerobic microbial count does not exceed 100 cfu per mL, and the total combined molds and yeasts count does not exceed 50 cfu per mL.
pH ⟨791⟩: between 4.5 and 6.5.
Alcohol Determination, *Method II* ⟨611⟩: between 4.0% and 6.0% is found.

Assay—
Diluent—Prepare a mixture of methanol and water (80:20).
Mobile phase—Prepare a filtered and degassed mixture of methanol, water, 0.1 N ammonium hydroxide solution, and 0.1 N ammonium nitrate solution (27:3:2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve accurately weighed quantities of USP Bromodiphenhydramine Hydrochloride RS and USP Codeine Phosphate RS in *Diluent,* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having known concentrations of about 100 µg per mL and 80 µg per mL, respectively.
Assay preparation—Using a pipet calibrated "to contain", transfer an accurately measured volume of Oral Solution, equivalent to about 10 mg of bromodiphenhydramine hydrochloride and 8 mg of codeine phosphate, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30.0-cm column that contains packing L3. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for bromodiphenhydramine and 1.4 for codeine; the resolution, *R,* between bromodiphenhydramine and codeine is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for bromodiphenhydramine and codeine. Calculate the quantity, in mg, of bromodiphen hydramine hydrochloride ($C_{17}H_{20}BrNO \cdot HCl$) in each mL of the Oral Solution taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Bromodiphenhydramine Hydrochloride RS in the *Standard preparation; V* is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation;* and r_U and r_S are the bromodiphenhydramine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

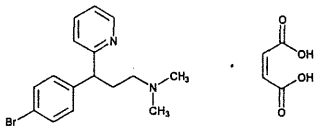
www.webofpharma.com

Calculate the quantity, in mg, of codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) in each mL of the Oral Solution taken by the formula:

$$(406.37/397.36)(100C/V)(r_U/r_S)$$

in which 406.37 and 397.36 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively; C is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation*; and r_U and r_S are the codeine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Brompheniramine Maleate



$C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ 435.31

2-Pyridinepropanamine, γ-(4-bromophenyl)-*N*,*N*-dimethyl-, (±)-, (*Z*)-2-butenedioate (1:1);
(±)-2-*p*-Bromo-α-2-(dimethylamino)ethylbenzylpyridine maleate (1:1) [980-71-2].

DEFINITION

Brompheniramine Maleate, dried at 105° for 3 h, contains NLT 98.0% and NMT 102.0% of brompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention times of the maleic acid and brompheniramine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 5.44 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0 ± 0.1.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
1	95	5
20	70	30
30	70	30
31	95	5
40	95	5

Diluent: Acetonitrile and *Solution A* (5:95)
System suitability stock solution: 0.02 mg/mL each of USP Pheniramine Maleate RS, USP Chlorpheniramine Maleate RS, and USP Chlorpheniramine Related Compound B RS in *Diluent*. Sonicate for 1 min.
System suitability solution: 0.5 mg/mL of USP Brompheniramine Maleate RS and 2 µg/mL each of USP Pheniramine Maleate RS, USP Chlorpheniramine Maleate RS, and USP Chlorpheniramine Related Compound B RS in *Diluent*, prepared as follows. Transfer 5.0 mg of USP Brompheniramine Maleate RS to a 10-mL volumetric flask, add 5.0 mL of *Diluent*, and 1.0 mL of the *System suitability stock solution*, and dilute with *Diluent* to volume.
Standard solution: 0.5 mg/mL of USP Brompheniramine Maleate RS in *Diluent*. Sonicate for 1 min.
Sample solution: 0.5 mg/mL of Brompheniramine Maleate in *Diluent*. Sonicate for 1 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
[NOTE—The relative retention times for maleic acid and brompheniramine are 0.18 and 1.0, respectively.]
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between chlorpheniramine and brompheniramine; and NLT 2.0 between chlorpheniramine related compound B and pheniramine, *System suitability solution*
Tailing factor: NMT 2.0 for brompheniramine, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of brompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$) in the portion of Brompheniramine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of brompheniramine from the *Sample solution*
r_S = peak response of brompheniramine from the *Standard solution*
C_S = concentration of USP Brompheniramine Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of Brompheniramine Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: NLT 98.0%–NMT 102.0% on the previously dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Diluent, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 2.7 µg/mL of USP Brompheniramine Maleate RS in *Diluent*, equivalent to 2.0 µg/mL of brompheniramine. Sonicate 1 min.
Sensitivity solution: 0.74 µg/mL of USP Pheniramine Maleate RS in *Diluent*
Sample solution: 0.5 mg/mL of Brompheniramine Maleate in *Diluent*. Sonicate for 1 min.

www.webofpharma.com

System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between chlorpheniramine and brompheniramine; and NLT 2.0 between chlorpheniramine related compound B and pheniramine, *System suitability solution*
Signal-to-noise ratio: NLT 10 for pheniramine, *Sensitivity solution*
Relative standard deviation: NMT 5.0% for brompheniramine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Brompheniramine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of brompheniramine from the *Standard solution*
C_S = concentration of brompheniramine in the *Standard solution* (mg/mL)
C_U = concentration of Brompheniramine Maleate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak having areas less than 0.05% of brompheniramine.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Maleic acid[a]	0.18	—	—
Chlorpheniramine related compound B[b]	0.46	—	—
Pheniramine	0.53	0.45	0.4
Chlorpheniramine	0.94	1.1	0.4
Brompheniramine	1.0	—	—
Any other unspecified impurity	—	1.0	0.10
Total impurities	—	—	1

[a] Salt counter ion is included in the table for identification purposes only.
[b] Di(pyridin-2-yl)amine. Used only to establish the system suitability.

SPECIFIC TESTS
- **Optical Rotation** ⟨781⟩
  Sample: 100 mg/mL in water at 20°
  Acceptance criteria: –0.2° to +0.2°, measured in a 20-cm tube
- **pH** ⟨791⟩
  Sample: 10 mg/mL
  Acceptance criteria: 4.0–5.0
- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩
  USP Brompheniramine Maleate RS
  USP Chlorpheniramine Maleate RS
  USP Chlorpheniramine Related Compound B RS
  Di(pyridin-2-yl)amine.
  $C_{10}H_9N_3$ 171.20
  USP Pheniramine Maleate RS

Brompheniramine Maleate Injection

» Brompheniramine Maleate Injection is a sterile solution of Brompheniramine Maleate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Brompheniramine Maleate RS
Identification—Dilute a volume of Injection, equivalent to about 50 mg of brompheniramine maleate, with dilute hydrochloric acid (1 in 1200) to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Transfer the liquid to a separator:" the Injection meets the requirements of the test.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 35.7 USP Endotoxin Units per mg of brompheniramine maleate.
pH ⟨791⟩: between 6.3 and 7.3.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Proceed with Injection as directed under *Salts of Organic Nitrogenous Bases* ⟨501⟩, to prepare the solution employed for the determination of the absorbance, A_U, at 262 nm. For the determination of A_S, dissolve about 25 mg of USP Brompheniramine Maleate RS, accurately weighed, in 20 mL of dilute sulfuric acid (1 in 350), and treat this solution the same as the portion of Injection being assayed. Calculate the quantity, in mg, of $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ in each mL of the Injection taken by the formula:

$$(W/V)(A_U/A_S)$$

in which W is the weight, in mg, of USP Brompheniramine Maleate RS in the *Standard Preparation*, and V is the volume, in mL, of Injection taken.

Brompheniramine Maleate Oral Solution

» Brompheniramine Maleate Oral Solution contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of brompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$).

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Brompheniramine Maleate RS
Identification—Transfer a volume of Oral Solution, equivalent to about 50 mg of brompheniramine maleate, to a

www.webofpharma.com

separator, render distinctly alkaline with 1 N sodium hydroxide, and extract with two 50-mL portions of chloroform, shaking gently to avoid emulsification. Wash the combined chloroform extracts with 10 mL of water, and discard the aqueous phase. Filter the combined chloroform extracts into a conical flask, and evaporate the solvent on a steam bath, with the aid of a current of air. To the residue add 25 mL of dilute hydrochloric acid (1 in 1200), and proceed as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Transfer the liquid to a separator." The Oral Solution meets the requirements of the test.

pH ⟨791⟩: between 2.5 and 3.5.

Alcohol Determination, *Method I* ⟨611⟩: between 2.7% and 3.3% of C_2H_5OH.

Assay—Transfer an accurately measured volume of Oral Solution, equivalent to about 20 mg of brompheniramine maleate, to a separator, render distinctly alkaline with 1 N sodium hydroxide, and extract with ten 10-mL portions of chloroform, shaking gently to avoid emulsification. Wash the combined chloroform extracts with 10 mL of water, wash the latter with 20 mL of chloroform, and discard the aqueous phase. Quantitatively filter the combined chloroform extracts and washings into a conical flask, and evaporate the solvent on a steam bath, with the aid of a current of air. To the residue add 25 mL of glacial acetic acid and 5 mL of acetic anhydride, agitate, and allow to stand for about 15 minutes. Add 1 drop of crystal violet TS, and titrate with 0.01 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.01 N perchloric acid is equivalent to 2.177 mg of brompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$).

Brompheniramine Maleate Tablets

» Brompheniramine Maleate Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Brompheniramine Maleate RS

Identification—Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 264 nm on filtered portions of the solution under test, suitably diluted with 3 N hydrochloric acid, using 5-cm cuvettes, in comparison with a Standard solution having a known concentration of USP Brompheniramine Maleate RS in the same *Medium*.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Standard preparation—Dissolve an accurately weighed quantity of USP Brompheniramine Maleate RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 160 µg per mL. Transfer 25.0 mL of this solution to a separator containing 25 mL of water, mix, and proceed as directed under *Assay preparation,* beginning with "adjust with sodium hydroxide solution (1 in 10) to a pH of 11." The concentration of USP Brompheniramine Maleate RS in the *Standard preparation* is about 20 µg per mL.
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 4 mg of brompheniramine maleate, mix with 50 mL of water for 10 minutes, adjust with sodium hydroxide solution (1 in 10) to a pH of 11, and cool to room temperature. Extract the mixture with two 75-mL portions of solvent hexane, and combine the extracts in a second separator. Extract the solvent hexane solution with three 50-mL portions of dilute hydrochloric acid (1 in 120), combining the acid extracts in a 200-mL volumetric flask. Add dilute hydrochloric acid (1 in 120) to volume, and mix.
Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation,* in 1-cm cells at the wavelength of maximum absorbance at about 264 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 120) as the blank. Calculate the quantity, in mg, of $C_{16}H_{19}Br\,N_2 \cdot C_4H_4O_4$ in the portion of Tablets taken by the formula:

$$0.2C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Brompheniramine Maleate RS in the *Standard preparation;* and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation,* respectively.

Brompheniramine Maleate and Pseudoephedrine Sulfate Oral Solution

» Brompheniramine Maleate and Pseudoephedrine Sulfate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of brompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$) and pseudoephedrine sulfate [$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$].

USP Reference standards ⟨11⟩—
USP Brompheniramine Maleate RS
USP Pseudoephedrine Sulfate RS

Identification—
A: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
B: A solution of it meets the requirements of the test for *Sulfate* ⟨191⟩.
C: Transfer a volume of Oral Solution, equivalent to about 6 mg of brompheniramine maleate, to a separator, add 0.5 mL of ammonium hydroxide and 5 mL of methylene chloride, shake for 1 minute, and allow the layers to separate. Use the clear, lower layer as the test solution. Prepare separate Standard solutions in methanol containing, respectively, 1.2 mg of USP Brompheniramine Maleate RS and 9 mg of USP Pseudoephedrine Sulfate RS per mL. Separately apply 5 µL of each solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and

www.webofpharma.com

develop the chromatogram in a solvent system consisting of a mixture of ethyl ether, methanol, and ammonium hydroxide (16:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the R_F values of the two principal spots obtained from the test solution correspond to those obtained from the Standard solutions.

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Assay—
Mobile phase—Prepare a mixture of water, acetonitrile, methanol, and tetrahydrofuran (550:320:80:50). Transfer 1.0 mL of phosphoric acid, followed by 4.33 g of dodecyl sulfate sodium to this mixture, and mix. Adjust with ammonium hydroxide to a pH of 3.50 ± 0.05, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). [NOTE—The pH of the *Mobile phase* is critical and may cause 1 to 4 minutes of differences in the retention times of internal standard and brompheniramine maleate.]

Internal standard solution—Transfer about 50 mg of naphazoline hydrochloride to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Brompheniramine Maleate RS in *Mobile phase,* and quantitatively dilute with *Mobile phase* to obtain a solution having a known concentration of about 6000*J* µg per mL, *J* being the ratio of the labeled amount, in mg, of brompheniramine maleate to the labeled amount, in mg, of pseudoephedrine sulfate per mL *(Solution P).* Transfer about 30 mg of USP Pseudoephedrine Sulfate RS, accurately weighed, to a 25-mL volumetric flask, add 5.0 mL each of *Solution P* and *Internal standard solution,* dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having known concentrations of about 1200*J* µg of USP Brompheniramine Maleate RS per mL and about 1.2 mg of USP Pseudoephedrine Sulfate RS per mL.

Assay preparation—Using a "To contain" pipet transfer an accurately measured volume of Oral Solution, equivalent to about 30 mg of pseudoephedrine sulfate, to a 25-mL volumetric flask. Rinse the pipet with about 5 mL of *Mobile phase,* collecting the rinse in the volumetric flask. Add 5.0 mL of *Internal standard solution,* dilute with *Mobile phase* to volume, and mix.

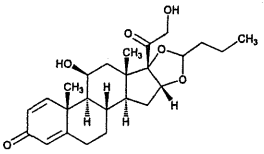
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for pseudoephedrine sulfate, 1.5 for naphazoline hydrochloride, and 2.5 for brompheniramine maleate; the resolution, *R,* between the pseudoephedrine sulfate and naphazoline hydrochloride peaks is not less than 3, and between the brompheniramine maleate and naphazoline hydrochloride peaks is not less than 3; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of brompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$) in each mL of the Oral Solution taken by the formula:

$$25CV(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Brompheniramine Maleate RS in the *Standard preparation; V* is the volume, in mL, of Oral Solution taken; and R_U and R_S are the peak response ratios obtained for brompheniramine maleate and naphazoline hydrochloride from the *Assay preparation* and the *Standard preparation,* respectively. Calculate the quantity, in mg, of pseudoephedrine sulfate $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ in each mL of the Oral Solution taken by the same formula, changing the terms to refer to pseudoephedrine sulfate.

Budesonide



$C_{25}H_{34}O_6$ 430.53
Pregna-1,4-diene-3,20-dione, 16,17-butylidenebis(oxy)-11,21-dihydroxy-,[11b,16a(*R*)], and 16a,17-[(*S*)-Butylidenebis(oxy)]-11b,21-dihydroxypregna-1,4-diene-3,20-dione;
(*RS*)-11b,16a,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with butyraldehyde [51333-22-3].
S epimer [51372-28-2].
R epimer [51372-29-3].

DEFINITION
Budesonide is a mixture of two epimeric forms, epimer A (C-22S) and epimer B (C-22R). It contains NLT 40.0% and NMT 51.0% of epimer A, and the sum of both epimers is NLT 98.0% and NMT 102.0% of budesonide ($C_{25}H_{34}O_6$), calculated on the dried basis.
[NOTE—Protect all solutions containing Budesonide from light.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy:* **197U**▲ (CN 1-May-2020)
Sample solution: 25 µg/mL
Medium: Methanol
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Buffer: 0.5 mL of glacial acetic acid in 1 L of water. Adjust with potassium hydroxide to a pH of 3.9.
Mobile phase: Acetonitrile and *Buffer* (45:55)
Standard solution: 0.06 mg/mL of USP Budesonide RS prepared as follows. Transfer USP Budesonide RS to a

www.webofpharma.com

suitable volumetric flask, dissolve in acetonitrile equivalent to 30% of the flask volume, and dilute with water to volume.

Sample solution: 0.06 mg/mL of Budesonide prepared as follows. Transfer Budesonide to a suitable volumetric flask, dissolve in acetonitrile equivalent to 30% of the flask volume, and dilute with water to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 3-µm packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention time for epimer B is 0.96 with respect to epimer A.]
Suitability requirements
Resolution: NLT 1.2 between the two budesonide epimer peaks
Relative standard deviation NMT 1.0%, for the sum of the peak areas of the two budesonide epimers

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of budesonide epimer A ($C_{25}H_{34}O_6$) in the portion of Budesonide taken:

$$\text{Result} = [r_{UA}/(r_{UA} + r_{UB})] \times 100$$

r_{UA} = peak area of epimer A from the *Sample solution*
r_{UB} = peak area of epimer B from the *Sample solution*

Calculate the percentage of budesonide ($C_{25}H_{34}O_6$) in the portion of Budesonide taken:

$$\text{Result} = [(r_{UA} + r_{UB})/(r_{SA} + r_{SB})] \times (C_S/C_U) \times 100$$

r_{UA} = peak area of epimer A from the *Sample solution*
r_{UB} = peak area of epimer B from the *Sample solution*
r_{SA} = peak area of epimer A from the *Standard solution*
r_{SB} = peak area of epimer B from the *Standard solution*
C_S = concentration of USP Budesonide RS in the *Standard solution* (mg/mL)
C_U = concentration of Budesonide in the *Sample solution* (mg/mL)

Acceptance criteria
Epimer A: 40.0%–51.0%
Both epimers: 98.0%–102.0% on the dried basis

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: 0.5 mL of glacial acetic acid in 1 L of water. Adjust with potassium hydroxide to a pH of 3.9.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
5	75	25
35	68	32
42	59	41
59	25	75
60	75	25
70	75	25

Diluent: Acetonitrile and water (3:7)
System suitability stock solution A: 0.3 mg/mL of USP Budesonide Related Compound E RS in acetonitrile
System suitability stock solution B: 0.3 mg/mL of USP Budesonide Related Compound G RS in acetonitrile
System suitability stock solution C: 0.3 mg/mL of USP Budesonide Related Compound L RS in acetonitrile
System suitability solution: 0.6 mg/mL of USP Budesonide RS and 3 µg/mL each of USP Budesonide Related Compound E RS, USP Budesonide Related Compound G RS, and USP Budesonide Related Compound L RS in *Diluent* prepared as follows. Transfer USP Budesonide RS to a suitable volumetric flask and add suitable quantities of *System suitability stock solution A*, *System suitability stock solution B*, and *System suitability stock solution C*. Dilute with acetonitrile equivalent to 30% of the final volume and dilute with water to volume.
Standard stock solution: 0.6 mg/mL of USP Budesonide RS prepared as follows. Transfer USP Budesonide RS to a suitable volumetric flask, dissolve in acetonitrile equivalent to 30% of the flask volume, and dilute with water to volume.
Sensitivity solution: 0.3 µg/mL of USP Budesonide RS in *Diluent* from the *Standard stock solution*
Standard solution: 6 µg/mL of USP Budesonide RS in *Diluent* from the *Standard stock solution*
Sample solution: 0.6 mg/mL of Budesonide prepared as follows. Transfer Budesonide to a suitable volumetric flask, dissolve in acetonitrile equivalent to 30% of the flask volume, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 3-µm packing L1
Temperatures
Autosampler: 4°
Column: 50°. [NOTE—The resolution between budesonide related compound E and budesonide related compound L may be improved by lowering the temperature, but to NLT 40°.]
Flow rate: 1.5 mL/min
Injection volume: 50 µL

System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[NOTE—The relative retention times of the two epimers of budesonide are 0.96 (epimer B) and 1.00 (epimer A), respectively.]
Suitability requirements
Resolution: NLT 1.2 between budesonide related compound E and budesonide related compound L and NLT 3.0 between budesonide epimer A and the first epimer of budesonide related compound G, *System suitability solution*
Tailing factor: NMT 1.5 for the budesonide epimer B peak, *Standard solution*
Relative standard deviation: NMT 5.0%, for the sum of the peak areas of the two budesonide epimers, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Budesonide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of the sum of budesonide epimers from the *Standard solution*
C_S = concentration of USP Budesonide RS in the *Standard solution* (mg/mL)
C_U = concentration of Budesonide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.05% of the total peak areas of the budesonide epimers. Disregard peaks eluting after 60 min, which, if present, are due to the change in the gradient.

Table 2

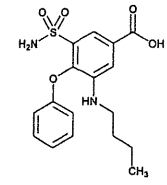
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
16α-Hydroxyprednisolone[a]	0.12	0.2
Budesonide acetaldehyde acetal (epimers)[b]	0.39, 0.40	0.10[c]
Budesonide D-homo analog[d, e]	0.47	0.10
Desonide[e, f]	0.51	0.10
Budesonide glyoxal (epimers)[g]	0.76, 0.78	0.07[c]
Budesonide related compound E[h]	0.86	0.10
Budesonide related compound L[i]	0.88	0.2
Budesonide epimer B	0.96	—
Budesonide epimer A	1.00	—
Budesonide related compound G (epimers)[j]	1.07, 1.08	0.10[c]
Budesonide 21-acetate (epimers)[k]	1.39, 1.40	0.10[c]
Budesonide 21-butyrate[l]	1.48	0.10
Any other individual impurity	—	0.10
Total specified impurities	—	0.4
Total unspecified impurities	—	0.4

[a] 11β,16α,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione.
[b] 16α,17-[Ethylidenebis(oxy)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione.
[c] Limit includes both epimers.
[d] 16α,17-[Butylidenebis(oxy)]-11β-hydroxy-17-(hydroxymethyl)-D-homoandrosta-1,4-diene-3,17a-dione; also known as D-homobudesonide.
[e] This impurity is to be reported under total unspecified impurities. Do not report it under total specified impurities.
[f] 16α,17-[1-Methylethylidenebis(oxy)]-11β, 21-dihydroxypregna-1,4-diene-3,20-dione.
[g] 16α,17-[Butylidenebis(oxy)]-11β-hydroxy-3,20-dioxopregna-1,4-dien-21-al; also known as 21-dehydrobudesonide.
[h] Also known as 14,15-dehydrobudesonide or budesonide 14-ene.
[i] Also known as 11-ketobudesonide.
[j] Also known as 1,2-dihydrobudesonide.
[k] 16α,17-[Butylidenebis(oxy)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione-21-acetate.
[l] 16α,17-[Butylidenebis(oxy)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione-21-butyrate.

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count is NMT 10^3 cfu/g, and the total combined molds and yeast count is NMT 10^2 cfu/g.
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° to constant weight.
  Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Budesonide RS
  USP Budesonide Related Compound E RS
  16α,17-[Butylidenebis(oxy)]-11β,21-dihydroxypregna-1,4,14-triene-3,20-dione.
  $C_{25}H_{32}O_6$ 428.52
  USP Budesonide Related Compound G RS
  16α,17-[Butylidenebis(oxy)]-11β,21-dihydroxypregna-4-ene-3,20-dione.
  $C_{25}H_{36}O_6$ 432.55
  USP Budesonide Related Compound L RS
  16α,17-[Butylidenebis(oxy)]-21-hydroxypregna-1,4-diene-3,11,20-trione.
  $C_{25}H_{32}O_6$ 428.52

Bumetanide



$C_{17}H_{20}N_2O_5S$ 364.42
Benzoic acid, 3-(aminosulfonyl)-5-(butylamino)-4-phenoxy-; 3-(Butylamino)-4-phenoxy-5-sulfamoylbenzoic acid [28395-03-1].

DEFINITION
Bumetanide contains NLT 98.0% and NMT 102.0% of bumetanide ($C_{17}H_{20}N_2O_5S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Sample solution: 50 µg/mL in isopropyl alcohol
  Acceptance criteria: Meets the requirements
- **C.** The principal spot of the *Sample solution* exhibits an R_F value corresponding to that of *Standard solution A*, as obtained in the test for *Organic Impurities*.

ASSAY
- **PROCEDURE**
  Sample solution: Dissolve 1 g of Bumetanide in 150 mL of alcohol, and add phenol red TS.
  Titrimetric system
  Mode: Direct titration
  Titrant: 0.1 N sodium hydroxide VS

www.webofpharma.com

Analysis: Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N sodium hydroxide is equivalent to 36.44 mg of bumetanide ($C_{17}H_{20}N_2O_5S$.)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%, on a 1-g specimen

• **ORGANIC IMPURITIES**

Standard solution A: 25 mg/mL of USP Bumetanide RS in methanol

Standard solution B: 50 µg/mL of USP Bumetanide RS from *Standard solution A* in methanol

Standard solution C: 50 µg/mL of USP Bumetanide Related Compound B RS in methanol

Standard solution D: 25 µg/mL of USP Bumetanide Related Compound A RS in methanol

Standard solution E: 25 µg/mL of USP Butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate RS in methanol

Sample solution: 25 mg/mL of Bumetanide in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Developing solvent system: Chloroform, cyclohexane, glacial acetic acid, and methanol (160:20:20:5)

Application volume: 20 µL

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, *Standard solution D*, *Standard solution E*, and *Sample solution*

Proceed as directed in *Chromatography* ⟨621⟩. After drying the application spots, place the plate in an unlined and unsaturated chromatographic chamber. Examine the plate under short-wavelength UV light.

Acceptance criteria: See *Table 1*. Any secondary spots from the *Sample solution* are not larger or more intense than the corresponding principal spots from the corresponding standard solution identified in *Table 1*.

Table 1

Name	Corresponding Standard Solution	Acceptance Criteria, NMT (%)
Bumetanide related compound A[a]	Standard solution D	0.1
Bumetanide related compound B[a]	Standard solution C	0.2
Butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate	Standard solution E	0.1
Other individual impurities	Standard solution B	0.2
Sum of other individual impurities[a]	—	0.4

[a] Excluding bumetanide related compound A, bumetanide related compound B, and butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Sample: Dry a sample at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Bumetanide RS

USP Bumetanide Related Compound A RS
3-Amino-4-phenoxy-5-sulfamoylbenzoic acid.
$C_{13}H_{12}N_2O_5S$ 308.31

USP Bumetanide Related Compound B RS
3-Nitro-4-phenoxy-5-sulfamoylbenzoic acid.
$C_{13}H_{10}N_2O_7S$ 338.29

USP Butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate RS
$C_{21}H_{28}N_2O_5S$ 420.53

Bumetanide Injection

» Bumetanide Injection is a sterile solution of Bumetanide in Water for Injection, prepared with the aid of Sodium Hydroxide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of bumetanide ($C_{17}H_{20}N_2O_5S$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—

USP Bumetanide RS

USP Bumetanide Related Compound A RS
3-Amino-4-phenoxy-5-sulfamoylbenzoic acid.
$C_{13}H_{12}N_2O_5S$ 308.31

Identification—

A: The relative retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

B: The principal spot obtained from the chromatogram of the *Test solution* exhibits an R_f value corresponding to that of the *Identification solution*, as obtained in the test for *Related compounds*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 350 USP Endotoxin Units per mg of bumetanide.

pH ⟨791⟩: between 6.8 and 7.8.

Related compounds—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Test solution—Pipet a volume of Injection, equivalent to 5 mg of bumetanide, into a 125-mL separator, and adjust with 0.1 N sodium hydroxide to a pH of 12. Extract with two 20-mL portions of ethyl ether, discard the ethyl ether extracts, and adjust the aqueous layer with 1 N acetic acid to a pH of 4. Extract with two 20-mL portions of ethyl ether, passing the extracts through anhydrous sodium sulfate. Wash the sodium sulfate with about 5 mL of ethyl ether. Evaporate the combined ethyl ether extracts with the aid of a stream of nitrogen to dryness, and dissolve the residue in 0.5 mL of methanol.

Identification solution—Dissolve USP Bumetanide RS in methanol to obtain a solution having a concentration of about 10 mg per mL.

Standard solutions—Dilute a volume of the *Identification solution* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.08 mg of USP Bumetanide RS per mL. Quantitatively dilute with methanol to obtain *Standard solutions* having the following compositions.

www.webofpharma.com

Standard solution	Dilution	Concentration (µg of RS per mL)	Percentage (%, for comparison with test specimen)
1	undiluted	80	0.8
2	3 in 4	60	0.6
3	1 in 2	40	0.4
4	1 in 4	20	0.2
5	1 in 8	10	0.1

Standard solution 6—Dissolve an accurately weighed quantity of USP Bumetanide Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.02 mg per mL.

Application volume: 50 µL.

Developing solvent system: a mixture of chloroform, cyclohexane, glacial acetic acid, and methanol (80: 10: 10: 2.5).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. Examine the plate under short-wavelength UV light. Any secondary spot obtained from the chromatogram of the *Test solution* having an R_F value corresponding to the R_F value of the principal spot obtained from the chromatogram of *Standard solution 6* is not larger or more intense than the principal spot obtained from the chromatogram of *Standard solution 6:* not more than 0.2% of bumetanide related compound A is found. For all other secondary spots obtained from the chromatogram of the *Test solution*, compare the intensity of each spot with the principal spots obtained from the chromatograms of *Standard solutions 1* through *5:* not more than 0.2% of any individual other impurity is found; and not more than 0.8% of the sum of all other impurities is found (excluding bumetanide related compound A).

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of methanol, water, tetrahydrofuran, and glacial acetic acid (50:45:5:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer about 50 mg of 4-ethylbenzaldehyde to a 100-mL volumetric flask. Dissolve in and dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 100-mL volumetric flask, add 10.0 mL of tetrahydrofuran and 4.0 mL of glacial acetic acid, dilute with methanol to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Bumetanide RS in *Internal standard solution*, and quantitatively dilute with *Internal standard solution* to obtain a solution having a known concentration of about 250 µg per mL. Transfer 5.0 mL of the resulting solution to a 10-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a known concentration of about 125 µg of USP Bumetanide RS per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 0.25 mg of bumetanide, to a flask. Add an equal volume of *Internal standard solution*, accurately measured, insert the stopper, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for 4-ethylbenzaldehyde and 1.0 for bumetanide; the resolution, *R*, between the analyte and internal standard peaks is not less than 1.5, the tailing factor for the analyte peak is not more than 1.4, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{17}H_{20}N_2O_5S$ in each mL of the Injection taken by the formula:

$$(2C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Bumetanide RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Bumetanide Tablets

DEFINITION

Bumetanide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bumetanide ($C_{17}H_{20}N_2O_5S$).

IDENTIFICATION

- **A.** The relative retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The principal spot of the *Sample solution* exhibits an R_F value corresponding to that of the *Identification solution*, as obtained in the test for *Organic Impurities*.

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: Methanol, tetrahydrofuran, glacial acetic acid, and water (50: 5:2: 45)

Internal standard stock solution: 0.5 mg/mL of 4-ethylbenzaldehyde in methanol

Internal standard solution: Add 10.0 mL of *Internal standard stock solution*, 10.0 mL of tetrahydrofuran, and 4.0 mL of glacial acetic acid to a 100-mL volumetric flask, and dilute with methanol to volume.

Standard stock solution: 250 µg/mL of USP Bumetanide RS in *Internal standard solution*

Standard solution: 125 µg/mL from *Standard stock solution* in water

Sample solution: Nominally ▲125 µg/mL▲ (ERR 1-Apr-2019) of bumetanide prepared as follows. Transfer a nominal equivalent to 0.5 mg of bumetanide, from finely powdered Tablets (NLT 20), to a 10-mL volumetric flask. Add 2.0 mL of *Internal standard solution* and sonicate for 5 min. Add 2.0 mL of water. Cool and filter, discarding the first 1 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

www.webofpharma.com

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for 4-ethylbenzaldehyde and bumetanide are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between 4-ethylbenzaldehyde and bumetanide
Tailing factor: NMT 1.4
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of bumetanide ($C_{17}H_{20}N_2O_5S$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of bumetanide to the internal standard from the *Sample solution*
R_S = peak response ratio of bumetanide to the internal standard from the *Standard solution*
C_S = concentration of USP Bumetanide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the bumetanide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Solution A: 7.505 g/L of glycine and 5.85 g/L of sodium chloride in water
Solution B: *Solution A*, 0.1 N hydrochloric acid, and water (4:1:45). Adjust, if necessary, with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 2.9.
Standard solution: USP Bumetanide RS at a known concentration in *Medium*
Sample solution: Dilute with *Solution B* as needed.
Instrumental conditions
Mode: Fluorescence
Detectors
Excitation wavelength: 350 nm
Emission wavelength: 450 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of bumetanide ($C_{17}H_{20}N_2O_5S$) dissolved.
Tolerances: NLT 85% (*Q*) of the labeled amount of bumetanide ($C_{17}H_{20}N_2O_5S$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium, Apparatus 2, and **Time:** Proceed as directed in *Test 1*.
Buffer: 2.72 g/L of potassium phosphate, monobasic in water. Adjust with 1.8 N potassium hydroxide to a pH of 7.0.
Mobile phase: Acetonitrile and *Buffer* (30:70)
Diluent: Acetonitrile and water (50:50)
Standard stock solution: 55.5 µg/mL of USP Bumetanide RS in *Diluent*
Standard solution: (*L*/1000) µg/mL of USP Bumetanide RS in *Medium*, from *Standard stock solution*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 222 nm
Column: 4.6-mm x 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 100 µL
Run time: NLT 1.7 times retention time of bumetanide
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bumetanide ($C_{17}H_{20}N_2O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of bumetanide from the *Sample solution*
r_S = peak response of bumetanide from the *Standard solution*
C_S = concentration of USP Bumetanide RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of bumetanide ($C_{17}H_{20}N_2O_5S$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

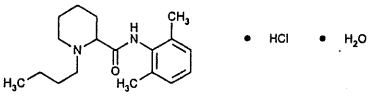
ORGANIC IMPURITIES
Identification solution: 20 mg/mL of USP Bumetanide RS in methanol
Standard solution 1: 160 µg/mL of USP Bumetanide RS from *Identification solution* in methanol
Standard solution 2: 120 µg/mL of USP Bumetanide RS from *Standard solution 1* in methanol
Standard solution 3: 80 µg/mL of USP Bumetanide RS from *Standard solution 1* in methanol
Standard solution 4: 40 µg/mL of USP Bumetanide RS from *Standard solution 1* in methanol
Standard solution 5: 20 µg/mL of USP Bumetanide RS from *Standard solution 1* in methanol
Standard solution 6: 40 µg/mL of USP Bumetanide Related Compound A RS in methanol
Sample solution: Nominally 20 mg/mL of bumetanide prepared as follows. Equivalent to 10 mg of bumetanide from powdered Tablets in a 50-mL centrifuge tube. Add 20 mL of acetone (spectrophotometric or HPLC quality), and shake by mechanical means for 10 min. Centrifuge for 10 min, decant the supernatant into a glass-stoppered, 25-mL conical flask, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 0.5 mL of methanol.
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 25 µL
Visualization: Short-wavelength UV light
Developing solvent system: Methanol, cyclohexane, glacial acetic acid, and chloroform (2.5: 10: 10: 80)

www.webofpharma.com

Analysis
Samples: *Standard solutions 1–6* and *Sample solution*
Acceptance criteria
Bumetanide related compound A: Any secondary spot from the *Sample solution* with an R_F value corresponding to the R_F value of the principal spot from *Standard solution 6* is not larger or more intense than the principal spot from *Standard solution 6*; NMT 0.2%.
Any individual other impurity: For all other secondary spots from the *Sample solution*, compare the intensity of each spot with the principal spots from *Standard solutions 1–5*; NMT 0.2% of any individual other impurity is found.
Sum of all other impurities: NMT 0.8% of the sum of all other impurities is found (excluding bumetanide related compound A).

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bumetanide RS
  USP Bumetanide Related Compound A RS
  3-Amino-4-phenoxy-5-sulfamoylbenzoic acid.
  $C_{13}H_{12}N_2O_5S$ 308.31

Bupivacaine Hydrochloride



$C_{18}H_{28}N_2O \cdot HCl \cdot H_2O$ 342.90
2-Piperidinecarboxamide, 1-butyl-*N*-(2,6-dimethylphenyl)-, monohydrochloride, monohydrate, (±)-.
(±)-1-Butyl-2′,6′-pipecoloxylidide monohydrochloride, monohydrate [73360-54-0].
Anhydrous 324.90
[18010-40-7].

» Bupivacaine Hydrochloride contains not less than 98.5 percent and not more than 101.5 percent of $C_{18}H_{28}N_2O \cdot HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Bupivacaine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197S*▲ (CN 1-May-2020)—
Solution—Dissolve about 230 mg in 15 mL of water in a separator, add 1 mL of 6 N ammonium hydroxide, and extract with three 30-mL portions of chloroform. Evaporate the chloroform at room temperature with the aid of a stream of nitrogen, and dry the residue in vacuum. Add 2 mL of chloroform to the residue, and dissolve.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 500 µg per mL.
Medium: 0.1 N hydrochloric acid.
Absorptivities at 271 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.
C: Dissolve about 50 mg in 10 mL of water in a small separator, render alkaline with 6 N ammonium hydroxide, and extract with 10 mL of ether: the aqueous layer meets the requirements of the tests for *Chloride* ⟨191⟩.
pH ⟨791⟩**:** between 4.5 and 6.0, in a solution (1 in 100).
Water Determination, *Method I* ⟨921⟩ **:** between 4.0% and 6.0%.
Residue on ignition ⟨281⟩**:** not more than 0.1%.

Limit of residual solvents—
Alcohol standard solution—Pipet 2 mL of dehydrated alcohol into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix. The resulting solution contains 0.08% of alcohol.
Isopropyl alcohol standard solution—Pipet 2 mL of isopropyl alcohol into a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. The resulting solution contains 0.004% of isopropyl alcohol.
Test solution—Transfer 1.0 g of Bupivacaine Hydrochloride, accurately weighed, to a 25-mL volumetric flask, dilute with water to volume, and mix.
Chromatographic system—Under typical conditions, the instrument is equipped with a flame-ionization detector and a 4-mm × 2-m column that contains packing S3. The carrier gas is nitrogen, flowing at a rate of about 40 mL per minute. The column temperature is maintained at about 175°, the injection port temperature is maintained at about 200°, and the detector temperature is maintained at about 280°.
Procedure—Inject equal volumes (about 5 µL) of the *Test solution*, the *Alcohol standard solution*, and the *Isopropyl alcohol standard solution* successively into the gas chromatograph. Measure the responses of the alcohol peak and the isopropyl alcohol peak in each chromatogram. Determine the percentage of alcohol taken by the formula:

$$2(r_U/r_S)$$

and determine the percentage of isopropyl alcohol taken by the formula:

$$0.1(r_U/r_S)$$

in which r_U and r_S are the responses of the respective analytes in the *Test solution* and of the corresponding analytes in the *Alcohol standard solution* and the *Isopropyl alcohol standard solution*, respectively. The sum of the content of alcohol and the content of isopropyl alcohol does not exceed 2%.

Chromatographic purity—
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.
Solvent: a mixture of chloroform and isopropylamine (99:1).
Test solution—Dissolve a suitable quantity of Bupivacaine Hydrochloride in *Solvent* to obtain a solution containing 20.0 mg per mL.

USP Monographs

www.webofpharma.com

Standard solution—Dissolve a suitable quantity of USP Bupivacaine Hydrochloride RS, accurately weighed, in *Solvent* to obtain a solution containing 20.0 mg per mL.

Diluted standard solution—Quantitatively dilute a portion of the *Standard solution* in *Solvent* to obtain a solution having a concentration of 100 µg per mL.

Developing solvent system: a mixture of hexanes and isopropylamine (97:3).

Procedure—Apply separate 10-µL portions of the *Test Solution*, the *Standard solution*, and the *Diluted standard solution* on the starting line of suitable thin-layer chromatographic plate as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. Develop the chromatogram in a suitable chamber until the solvent has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and dry it in warm air. Place the plate in a closed chamber with a dish containing 1 g of iodine in a shallow layer, and allow to remain for about 5 minutes. Remove the plate from the chamber, spray it with 7 N sulfuric acid, and examine the chromatogram: the R_F value of the principal spot from the *Test solution* corresponds to that of the *Standard solution*, and the estimated size and intensity of any other spot obtained from the *Test solution* does not exceed that of the principal spot obtained from the *Diluted standard solution* (0.5%); and the total of the estimated sizes and intensities of all of the other spots obtained from the *Test solution* does not exceed four times that of the principal spot obtained from the *Diluted standard solution* (2.0%).

Assay—Transfer about 600 mg of Bupivacaine Hydrochloride, accurately weighed, to a 250-mL conical flask, and dissolve in 20 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS and 3 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 32.49 mg of $C_{18}H_{28}N_2O \cdot HCl$.

Bupivacaine Hydrochloride Injection

DEFINITION

Bupivacaine Hydrochloride Injection is a sterile solution of Bupivacaine Hydrochloride in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$).

IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩
  Sample solution: 2 mg/mL of bupivacaine hydrochloride in 0.01 N hydrochloric acid, from Injection
  Analysis: Proceed as directed in the chapter beginning with "Transfer the liquid to a separator".
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the bupivacaine peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 1.94 g/L of monobasic potassium phosphate and 2.48 g/L of dibasic potassium phosphate in water. Adjust, if necessary, with 1 N potassium hydroxide or 1 M phosphoric acid to a pH of 6.8.

Mobile phase: Acetonitrile and *Buffer* (65:35). Adjust, if necessary, with 1 M phosphoric acid to a pH of 7.7 ± 0.2. Filter the solution through a membrane filter of 1-µm or finer pore size, and degas.

Internal standard solution: 1.3 mg/mL of dibutyl phthalate in methanol

Standard solution: 0.5 mg/mL of USP Bupivacaine Hydrochloride RS, prepared as follows. In a 100-mL volumetric flask, dissolve 50 mg of USP Bupivacaine Hydrochloride RS in 10.0 mL of water, using sonication if necessary. Add 10 mL of *Internal standard solution*, and dilute with methanol to volume.

Sample solution: Nominally 0.5 mg/mL of bupivacaine hydrochloride, prepared as follows. In a 100-mL volumetric flask, transfer an amount of Injection equivalent to 50 mg of bupivacaine hydrochloride, add 10.0 mL of *Internal standard solution*, and dilute with methanol to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 263 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for bupivacaine hydrochloride and dibutyl phthalate are about 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between bupivacaine hydrochloride and dibutyl phthalate
Relative standard deviation: NMT 1.0% for the ratio of bupivacaine to the internal standard from three replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of bupivacaine to the internal standard from the *Sample solution*
R_S = peak response ratio of bupivacaine to the internal standard from the *Standard solution*
C_S = concentration of USP Bupivacaine Hydrochloride RS, calculated on the anhydrous basis, in the *Standard solution* (mg/mL)
C_U = nominal concentration of bupivacaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 2.5 USP Endotoxin Units/mg of bupivacaine hydrochloride
- **pH** ⟨791⟩: 4.0–6.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass. Injection labeled to contain 0.5% or less of bupivacaine hydrochloride may be packaged in 50-mL, multiple-dose containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bupivacaine Hydrochloride RS

www.webofpharma.com

Bupivacaine Hydrochloride in Dextrose Injection

» Bupivacaine Hydrochloride in Dextrose Injection is a sterile solution of Bupivacaine Hydrochloride and Dextrose in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amounts of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$) and dextrose ($C_6H_{12}O_6$). It contains no preservative.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Bupivacaine Hydrochloride RS
USP Dextrose RS

Identification—
A: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Adsorbent:* chromatographic silica gel mixture; 0.25 mm. *Developing solvent:* mixture of butyl alcohol, water, dehydrated alcohol, and glacial acetic acid (6:2:1:1). *Test preparation:* Bupivacaine Hydrochloride in Dextrose Injection.
Standard preparations A, B, and *C*—Separately prepare (*A*) a solution of USP Bupivacaine Hydrochloride RS in water, (*B*) a solution of USP Dextrose RS in water, and (*C*) a solution of USP Bupivacaine Hydrochloride RS in (*B*) to obtain solutions having concentrations corresponding to the labeled concentrations of bupivacaine hydrochloride and dextrose in the Injection. *Naphthalenediol reagent*—Dissolve 20 mg of 1,3-naphthalenediol in 10 mL of dehydrated alcohol containing 0.2 mL of sulfuric acid. *Iodoplatinate reagent*—Mix equal volumes of platinic chloride solution (3 in 1000) and potassium iodide solution (6 in 100). *Procedure*—Separately apply 10 µL each of the *Test preparation* and *Standard preparations A* and *C* to a portion of the chromatographic plate, and separately apply 1 µL each of the *Test preparation* and *Standard preparation B* to the remaining portion of the plate. Dry the applications in a current of warm air, develop the chromatograms, remove the plate from the developing chamber, and mark the solvent front. Dry the plate in warm circulating air, and examine the plate under short-wavelength UV light: the R_F value of the principal spot obtained from the *Test preparation* corresponds to the spots obtained from the adjacent chromatograms of *Standard preparations A* and *C*. Spray the plate with *Naphthalenediol reagent*, heat at 90° for 5 minutes, and examine the plate: the R_F value of the principal blue-purple spot obtained from the *Test preparation* corresponds to that obtained in the adjacent chromatogram of *Standard preparation B*. Cool the plate, spray it with *Iodoplatinate reagent*, and examine the plate: bupivacaine appears as a blue-purple spot on a salmon-colored background, and the dextrose spots fade slightly: the R_F value of the bupivacaine spot obtained from the *Test preparation* corresponds to those obtained from the adjacent chromatograms of *Standard preparations A* and *C*.
B: It responds to *Identification* test *B* under *Bupivacaine Hydrochloride Injection*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.8 USP Endotoxin Units per mg of bupivacaine hydrochloride.
pH ⟨791⟩: between 4.0 and 6.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for bupivacaine hydrochloride—
pH 6.8 Phosphate buffer, Mobile phase, Internal standard solution, Standard preparation, Chromatographic system, and *Procedure*—Proceed as directed in the *Assay* under *Bupivacaine Hydrochloride Injection*.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of bupivacaine hydrochloride, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.
Assay for dextrose—Determine the angular rotation of Injection in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩). Calculate the percentage (g per 100 mL) of dextrose ($C_6H_{12}O_6$) in the portion of Injection taken by the formula:

$$(100/52.9)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; *A* is 100 mm divided by the length of the polarimeter tube, in mm; and *R* is the observed rotation, in degrees.

Bupivacaine Hydrochloride and Epinephrine Injection

DEFINITION
Bupivacaine Hydrochloride and Epinephrine Injection is a sterile solution of Bupivacaine Hydrochloride and Epinephrine or Epinephrine Bitartrate in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$). The content of epinephrine ($C_9H_{13}NO_3$) does not exceed 0.001% (1 in 100,000). It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of epinephrine ($C_9H_{13}NO_3$).

IDENTIFICATION
• **A.**
Procedure 1
Sample solution: Nominally 2 mg/mL of bupivacaine hydrochloride in 0.01 N hydrochloric acid from Injection
Analysis: Proceed as directed in *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Transfer the liquid to a separator".
Acceptance criteria: Meets the requirements
Procedure 2
Sample solution: Use the *Sample solution* from *Procedure 1: Bupivacaine Hydrochloride* in the *Assay*.
Acceptance criteria: The retention time of the bupivacaine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Procedure 1: Bupivacaine Hydrochloride* in the *Assay*.
• **B.**
Sample: Nominally equivalent to 50 µg of epinephrine from Injection
Analysis: Pipet the *Sample* into a suitable container, add 0.1 mL of *Ferro-citrate solution* and 2.0 mL of *Buffer solution* (prepared as directed in *Epinephrine Assay* ⟨391⟩), mix, and allow the solution to stand for 10 min. Filter the solution.
Acceptance criteria: The filtrate is violet in color and may turn brownish.

ASSAY
• **PROCEDURE 1: BUPIVACAINE HYDROCHLORIDE**
Buffer: 1.94 g/L of monobasic potassium phosphate and 2.48 g/L of dibasic potassium phosphate in water. Adjust,

www.webofpharma.com

if necessary, with 1 N potassium hydroxide or 1 M phosphoric acid to a pH of 6.8.

Mobile phase: Acetonitrile and *Buffer* (65:35). Adjust, if necessary, with 1 M phosphoric acid to a pH of 7.7 ± 0.2. Pass the solution through a membrane filter of 1-µm or finer pore size, and degas.

Internal standard solution: 1.3 mg/mL of dibutyl phthalate in methanol

Standard solution: 0.5 mg/mL of USP Bupivacaine Hydrochloride RS, prepared as follows. In a 100-mL volumetric flask, dissolve 50 mg of USP Bupivacaine Hydrochloride RS in 10.0 mL of water, using sonication if necessary. Add 10 mL of *Internal standard solution*, and dilute with methanol to volume.

Sample solution: Nominally 0.5 mg/mL of bupivacaine hydrochloride, prepared as follows. In a 100-mL volumetric flask, transfer an amount of Injection equivalent to 50 mg of bupivacaine hydrochloride, add 10.0 mL of *Internal standard solution*, and dilute with methanol to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 263 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for bupivacaine and dibutyl phthalate are about 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between bupivacaine and dibutyl phthalate
Relative standard deviation: NMT 1.0% for the ratio of bupivacaine to the internal standard from three replicate Injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of bupivacaine to the internal standard from the *Sample solution*
R_S = peak response ratio of bupivacaine to the internal standard from the *Standard solution*
C_S = concentration of USP Bupivacaine Hydrochloride RS, calculated on the anhydrous basis, in the *Standard solution* (mg/mL)
C_U = nominal concentration of bupivacaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

• **PROCEDURE 2: EPINEPHRINE**

Mobile phase: Prepare a mixture of water, methanol, and 2 M monobasic sodium phosphate (900:50:50), containing 40 mg/L of edetate disodium, 0.4 mL/L of phosphoric acid, and 0.4 g/L of sodium 1-octanesulfonate. Make adjustments, if necessary, to obtain a retention time of NLT 11 min for the epinephrine peak.

System suitability solution: 2 µg/mL each of epinephrine bitartrate and dopamine hydrochloride in *Mobile phase*

Standard solution: 2 µg/mL of USP Epinephrine Bitartrate RS in *Mobile phase*

Sample solution: Nominally 1 µg/mL of epinephrine, prepared as follows. In a 25-mL volumetric flask, transfer an amount of Injection equivalent to 25 µg of epinephrine, and dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Electrochemical held at a potential of +0.75 V
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for epinephrine and dopamine are about 1.0 and 2, respectively.]
Suitability requirements
Resolution: NLT 6.0 between the epinephrine and dopamine peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of epinephrine ($C_9H_{13}NO_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of epinephrine from the *Sample solution*
r_S = peak response of epinephrine from the *Standard solution*
C_S = concentration of USP Epinephrine Bitartrate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of epinephrine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of epinephrine, 183.21
M_{r2} = molecular weight of epinephrine bitartrate, 333.30

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

• **COLOR AND CLARITY**

Standard solution: Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask and dilute with water to volume.

Sample solution: Injection

Analysis 1: Visually examine a portion of the *Sample solution* in a suitable clear glass test tube against a white background.

Acceptance criteria 1: The *Sample solution* is not pinkish, and it contains no precipitate.

Analysis 2: Perform *Analysis 2* if any yellow color is observed in the *Sample solution*. Concomitantly determine the absorbances of the *Sample solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm.

Acceptance criteria 2: The absorbance of the *Sample solution* does not exceed that of the *Standard solution*.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 1.6 USP Endotoxin Units/mg of bupivacaine hydrochloride

• **pH** ⟨791⟩: 3.3–5.5

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

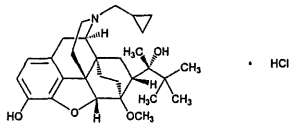
• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Injection labeled to contain 0.5% or less of bupivacaine hydrochloride may be packaged in 50-mL multiple-dose containers.

USP Monographs

www.webofpharma.com

- **LABELING:** The label indicates that Injection is not to be used if its color is pinkish or darker than slightly yellow, or if it contains a precipitate.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bupivacaine Hydrochloride RS
  USP Epinephrine Bitartrate RS

Buprenorphine Hydrochloride



$C_{29}H_{41}NO_4 \cdot HCl$ 504.11

6,14-Ethenomorphinan-7-methanol, 17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-, hydrochloride, [5α,7α(*S*)]-;
21-Cyclopropyl-7α-[(*S*)-1-hydroxy-1,2,2-trimethylpropyl]-6,14-*endo*-ethano-6,7,8,14-tetrahydrooripavine hydrochloride [53152-21-9].

DEFINITION

Buprenorphine Hydrochloride contains NLT 98.0% and NMT 102.0% of buprenorphine hydrochloride ($C_{29}H_{41}NO_4 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the buprenorphine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*
  Sample stock solution: 50 mg/mL of Buprenorphine Hydrochloride in methanol
  Sample solution: 10 mg/mL of Buprenorphine Hydrochloride in carbon dioxide-free water from the *Sample stock solution*
  Analysis: Use 3 mL of the *Sample solution*.
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
  Buffer: 5.44 g/L of monobasic potassium phosphate prepared as follows. Initially dissolve in 90% volume of water, and adjust with 5% (v/v) of phosphoric acid to a pH of 4.5. Dilute with water to volume.
  Solution A: Acetonitrile and *Buffer* (10:90)
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*. Return to the original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	89	11
2	89	11
12	64	36
15	41	59
20	39	61

Diluent: Methanol and water (80:20)
Standard solution: 2.0 mg/mL of USP Buprenorphine Hydrochloride RS in *Diluent*
Sample solution: 2.0 mg/mL of Buprenorphine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 1.3 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of buprenorphine hydrochloride ($C_{29}H_{41}NO_4 \cdot HCl$) in the portion of Buprenorphine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of buprenorphine from the *Sample solution*
r_S = peak response of buprenorphine from the *Standard solution*
C_S = concentration of USP Buprenorphine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Buprenorphine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Buffer, Solution A, Solution B, and **Mobile phase:** Prepare as directed in the *Assay*.
  System suitability solution: 5.0 mg/mL of USP Buprenorphine System Suitability Mixture RS in methanol
  Standard solution: 0.005 mg/mL of USP Buprenorphine Hydrochloride RS and 0.01 mg/mL of USP Buprenorphine Related Compound A RS in methanol
  Sample solution: 5.0 mg/mL of Buprenorphine Hydrochloride in methanol
  Chromatographic system: Proceed as directed in the *Assay* except for the *Column*.
  Column: 4.6-mm × 5.0-cm; 3.5-µm packing L1
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Suitability requirements
  Resolution: NLT 1.5 between buprenorphine and ethenobuprenorphine, *System suitability solution*
  Relative standard deviation: NMT 5% for buprenorphine and buprenorphine related compound A, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of buprenorphine related compound A in the portion of Buprenorphine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of buprenorphine related compound A from the *Sample solution*
r_S = peak response of buprenorphine related compound A from the *Standard solution*
C_S = concentration of USP Buprenorphine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Buprenorphine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each other individual impurity in the portion of Buprenorphine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each other individual impurity from the *Sample solution*
r_S = peak response of buprenorphine from the *Standard solution*
C_S = concentration of USP Buprenorphine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Buprenorphine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Buprenorphine	1.0	—	—
Ethenobuprenorphine[a]	1.1	1.0	0.10
Buprenorphine related compound A	1.4	—	0.20
Buprenorphine 2,2′-dimer[b]	1.8	3.3	0.10
Any other individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.65

[a] (*S*)-2-[17-(Cyclopropylmethyl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethenomorphinan-7α-yl]-3,3-dimethylbutan-2-ol.
[b] 2,2′-Bi{17-(cyclopropylmethyl)-4,5α-epoxy-3-hydroxy-7α-[(*S*)-2-hydroxy-3,3-dimethylbutan-2-yl]-6-methoxy-6α,14-ethanomorphinan}.

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 20 mg/mL in methanol
  Acceptance criteria: −92° to −98°
- **ACIDITY OR ALKALINITY**
  Sample stock solution: 50 mg/mL of Buprenorphine Hydrochloride in methanol
  Sample solution: 10 mg/mL of Buprenorphine Hydrochloride in carbon dioxide-free water from *Sample stock solution*
  Analysis: Add 0.05 mL of methyl red TS 2 to 10 mL of *Sample solution* and titrate with 0.02 N sodium hydroxide or 0.02 N hydrochloric acid.
  Acceptance criteria: NMT 0.2 mL of 0.02 N sodium hydroxide or 0.02 N hydrochloric acid is required to change the color of the indicator.
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Buprenorphine Hydrochloride RS
  USP Buprenorphine Related Compound A RS
  (*S*)-2-[17-(But-3-en-1-yl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethanomorphinan-7α-yl]-3,3-dimethylbutan-2-ol.
  $C_{29}H_{41}NO_4$ 467.65
  USP Buprenorphine System Suitability Mixture RS
  It contains buprenorphine and about 0.5% of ethenobuprenorphine. The chemical information for ethenobuprenorphine is as follows:
  (*S*)-2-[17-(Cyclopropylmethyl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethenomorphinan-7α-yl]-3,3-dimethylbutan-2-ol.
  $C_{29}H_{39}NO_4$ 465.63

Buprenorphine Compounded Buccal Solution, Veterinary

DEFINITION

Buprenorphine Compounded Buccal Solution, Veterinary, contains NLT 90.0% and NMT 110.0% of the labeled amount of buprenorphine ($C_{29}H_{41}NO_4$).
Prepare Buprenorphine Compounded Buccal Solution, Veterinary 3 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Buprenorphine (as hydrochloride)	30 mg (32.4 mg)
Dextrose	500 mg
Sodium Citrate (anhydrous)	20 mg
Citric Acid Monohydrate	25 mg
Purified Water, a sufficient quantity to make	10 mL

Dissolve the *Dextrose*, *Sodium Citrate Anhydrous*, and *Citric Acid Monohydrate* in 5 mL of *Purified Water* in a suitable calibrated container. Add the *Buprenorphine hydrochloride* powder into the mixture and add sufficient *Purified Water* to bring to final volume, and mix well.

ASSAY

- **PROCEDURE**
  Mobile phase: Acetonitrile and 10 mM ammonium acetate (80:20)
  Standard solution: 0.3 mg/mL of buprenorphine prepared from USP Buprenorphine Hydrochloride RS in methanol
  Sample solution: Transfer 1 mL of Buccal Solution, Veterinary into a 10-mL volumetric flask, dilute with methanol to volume, and mix well.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 2.1-mm × 5-cm; 5-µm packing L7
  Column temperature: 40°
  Flow rate: 0.25 mL/min

www.webofpharma.com

Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for buprenorphine is about 5.8 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of buprenorphine ($C_{29}H_{41}NO_4$) in the portion of Buccal Solution, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of buprenorphine from the *Sample solution*
r_S = peak response of buprenorphine from the *Standard solution*
C_S = concentration of buprenorphine in the *Standard solution* (mg/mL)
C_U = nominal concentration of buprenorphine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.5–4.5

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8°.
- **LABELING:** Label it to indicate that it is for veterinary use only. Label to indicate that it is for buccal administration, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8°
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Buprenorphine Hydrochloride RS

Buprenorphine and Naloxone Sublingual Tablets

DEFINITION
Buprenorphine and Naloxone Sublingual Tablets contain amounts of buprenorphine hydrochloride and naloxone hydrochloride equivalent to NLT 94.0% and NMT 106.0% of the labeled amount of buprenorphine ($C_{29}H_{41}NO_4$) and naloxone ($C_{19}H_{21}NO_4$).

IDENTIFICATION
- **A.** The retention times of the buprenorphine and naloxone peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the buprenorphine and naloxone peaks of the *Sample solution* and those of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
[NOTE—It is suggested to protect all solutions containing buprenorphine and naloxone from light.]

Buffer: 9 mM of dibasic ammonium phosphate in water. Adjust with a solution of phosphoric acid and water (1:1) to a pH of 6.2.
Solution A: Acetonitrile, methanol, and *Buffer* (7:3:90)
Solution B: Acetonitrile, methanol, and *Buffer* (56:24:20)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	99	1
30	1	99
45	1	99
45.1	99	1
55	99	1

Solution C: Phosphoric acid and water (1:1000)
Diluent: Acetonitrile, methanol, and *Solution C* (7:3:90)
Standard solution: 0.57 mg/mL of USP Buprenorphine Hydrochloride RS and 0.13 mg/mL of USP Naloxone RS in *Diluent*
Sample solution: Nominally 0.52 mg/mL of buprenorphine and 0.13 mg/mL of naloxone prepared as follows. Transfer NLT 13 Tablets to a suitable volumetric flask, and add about 70% of the final volume of *Diluent*. Sonicate for 15 min with occasional swirling and shake for 15 min. Dilute with *Diluent* to volume. Pass a portion through a suitable filter of 0.45-µm pore size. Discard the first 5 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L11
Column temperature: 60°
Flow rate: 0.8 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both buprenorphine and naloxone
Relative standard deviation: NMT 2.0% for both buprenorphine and naloxone
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of buprenorphine ($C_{29}H_{41}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of buprenorphine from the *Sample solution*
r_S = peak response of buprenorphine from the *Standard solution*
C_S = concentration of USP Buprenorphine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of buprenorphine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of buprenorphine, 467.65
M_{r2} = molecular weight of buprenorphine hydrochloride, 504.11

www.webofpharma.com

Calculate the percentage of the labeled amount of naloxone ($C_{19}H_{21}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naloxone from the *Sample solution*
r_S = peak response of naloxone from the *Standard solution*
C_S = concentration of USP Naloxone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naloxone in the *Sample solution* (mg/mL)

Acceptance criteria: 94.0%–106.0% of the labeled amount of buprenorphine ($C_{29}H_{41}NO_4$) and naloxone ($C_{19}H_{21}NO_4$)

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: Water (deaerated for 5 min); 500 mL
Apparatus 1: 100 rpm
Time: 10 min
Buffer: 0.018 M monobasic potassium phosphate in water prepared as follows. Dissolve 2.4 g of monobasic potassium phosphate and 0.5 g of sodium hydroxide in each liter of water. Adjust with phosphoric acid to a pH of 6.8.
Solution A: Acetonitrile, methanol, and *Buffer* (40:20:40)
Solution B: Acetonitrile and *Buffer* (78:22)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2.0	100	0
3.0	0	100
6.0	0	100
6.1	100	0
8.0	100	0

Diluent: Methanol and water (50:50)
Standard solution: 0.01 mg/mL of USP Buprenorphine Hydrochloride RS and 0.0025 mg/mL of USP Naloxone RS in *Diluent*. Sonicate if necessary. Pass a portion through a suitable filter of 0.45-µm pore size. Discard the first 4 mL of filtrate.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 5-cm; 5-µm packing L7
Column temperature: 25°
Flow rate: 1.0 mL/min
Injection volume: 40 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both buprenorphine and naloxone
Relative standard deviation: NMT 2.0% for both buprenorphine and naloxone
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of buprenorphine ($C_{29}H_{41}NO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response of buprenorphine from the *Sample solution*
r_S = peak response of buprenorphine from the *Standard solution*
C_S = concentration of USP Buprenorphine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
M_{r1} = molecular weight of buprenorphine, 467.65
M_{r2} = molecular weight of buprenorphine hydrochloride, 504.11
L = label claim of buprenorphine (mg/Tablet)

Calculate the percentage of the labeled amount of naloxone ($C_{19}H_{21}NO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of naloxone from the *Sample solution*
r_S = peak response of naloxone from the *Standard solution*
C_S = concentration of USP Naloxone RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim of naloxone (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of buprenorphine ($C_{29}H_{41}NO_4$) and naloxone ($C_{19}H_{21}NO_4$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
[Note—It is suggested to protect all solutions containing buprenorphine and naloxone from light.]
Buffer, Solution A, Solution B, Mobile phase, Solution C, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.0015 mg/mL of USP Buprenorphine Hydrochloride RS and 0.0004 mg/mL of USP Naloxone RS in *Diluent*
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5% for buprenorphine and naloxone
Analysis
Samples: *Sample solution* and *Standard solution*
Identify the buprenorphine degradation products using the relative retention times given in *Table 3*.
Calculate the percentage of each buprenorphine related degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual buprenorphine related degradation product from the *Sample solution*
r_S = peak response of buprenorphine from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Buprenorphine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of buprenorphine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of buprenorphine, 467.65
M_{r2} = molecular weight of buprenorphine hydrochloride, 504.11

Identify the naloxone degradation products using the relative retention times given in *Table 3*.
Calculate the percentage of each naloxone related degradation product and any other degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each naloxone related degradation product or any other degradation product from the *Sample solution*
r_S = peak response of naloxone from the *Standard solution*
C_S = concentration of USP Naloxone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of naloxone in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard any peaks below 0.05%.

Table 3

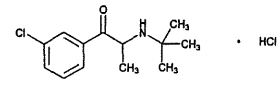
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Naloxone degradation product 1[a]	0.30	0.5
Naloxone degradation product 2[a]	0.54	0.5
Dealkyl buprenorphine[b, c]	0.55	—
Naloxone	0.61	—
Naloxone degradation product 3[a]	0.67	0.5
Buprenorphine nitrile[c, d]	0.90	—
6-*O*-Desmethylbuprenorphine[c, e]	0.91	—
Buprenorphine degradation product 1[f]	0.95	0.3
Buprenorphine 7-(*S*)-epimer[c, g]	0.99	—
Buprenorphine	1.00	—
Buprenorphine butenyl analog[c, h]	1.03	—
3-*O*-Methylbuprenorphine[c, i]	1.16	—
Any unspecified degradation product[a]	—	0.3
Total degradation products	—	3.0

[a] Quantified relative to naloxone.
[b] (*S*)-2-(4,5α-Epoxy-3-hydroxy-6-methoxy-6α,14-ethanomorphinan-7α-yl)-3,3-dimethylbutan-2-ol.
[c] These are process impurities and are excluded from the total degradation products.
[d] 4,5α-Epoxy-7α-[(*S*)-2-hydroxy-3,3-dimethylbutan-2-yl]-3,6-dimethoxy-6α,14-ethanomorphinan-17-carbonitrile.
[e] (*S*)-2-[17-(Cyclopropylmethyl)-4,5α-epoxy-3,6-dihydroxy-6α,14-ethanomorphinan-7α-yl]-3,3-dimethylbutan-2-ol.
[f] Quantified relative to buprenorphine.
[g] (*S*)-2-[17-(Cyclopropylmethyl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethanomorphinan-7β-yl]-3,3-dimethylbutan-2-ol.
[h] (*S*)-2-[17-(But-3-en-1-yl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethanomorphinan-7α-yl]-3,3-dimethylbutan-2-ol.
[i] (*S*)-2-[17-(Cyclopropylmethyl)-4,5α-epoxy-3,6-dimethoxy-6α,14-ethanomorphinan-7α-yl]-3,3-dimethylbutan-2-ol.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Buprenorphine Hydrochloride RS
  USP Naloxone RS

Bupropion Hydrochloride



$C_{13}H_{18}ClNO \cdot HCl$ 276.20

1-Propanone, 1-(3-chlorophenyl)-2-[(1,1-dimethylethyl) amino]-, hydrochloride, (±)-;
(±)-2-(*tert*-Butylamino)-3′-chloropropiophenone hydrochloride [31677-93-7].

DEFINITION

Bupropion Hydrochloride contains NLT 98.0% and NMT 102.0% of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*
  Sample solution: 1 mg/mL of Bupropion Hydrochloride
  Acceptance criteria: Meets the requirements ▲of test▲ (CN 1-May-2018) ▲*A*▲ (ERR 1-Aug-2018)

ASSAY

- **PROCEDURE**
  Diluent: Methanol and water (50:50)
  Buffer: 3.4 g/L of monobasic potassium phosphate in water. Adjust with 1 N sodium hydroxide to a pH of 7.0.

www.webofpharma.com

Mobile phase: Methanol, tetrahydrofuran, and *Buffer* (39:11:50)
Standard solution: 1 mg/mL of USP Bupropion Hydrochloride RS and 2 µg/mL each of USP Bupropion Hydrochloride Related Compound A RS and USP Bupropion Hydrochloride Related Compound B RS in *Diluent*
Sample solution: 1 mg/mL of Bupropion Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 3.9-mm × 15-cm; 5-µm packing L7
Flow rate: 1.1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.3 between bupropion hydrochloride related compound A and bupropion; NLT 1.3 between bupropion and bupropion hydrochloride related compound B
Relative standard deviation: NMT 2.0% for bupropion
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the portion of Bupropion Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Bupropion Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• LIMIT OF 3-CHLOROBENZOIC ACID

Protect all analytical solutions from light and use within one day.
Diluent: Methanol and 0.001 N hydrochloric acid (20:80)
Solution A: Acetonitrile and water (10:90). Add 0.4 mL of trifluoroacetic acid per L of the mixture.
Solution B: Acetonitrile and water (95:5). Add 0.3 mL of trifluoroacetic acid per L of the mixture.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3.4	87	13
10.0	15	85
10.1	0	100
13.0	0	100
13.2	90	10
19.0	90	10

System suitability stock solution: 0.02 mg/mL of USP Bupropion Hydrochloride Related Compound C RS, 0.02 mg/mL of USP Bupropion Hydrochloride Related Compound F RS, and 0.012 mg/mL of USP 3-Chlorobenzoic Acid RS in methanol
System suitability solution: 0.002 mg/mL of bupropion hydrochloride related compound C, 0.002 mg/mL of bupropion hydrochloride related compound F, and 0.0012 mg/mL of 3-chlorobenzoic acid from *System suitability stock solution* in *Diluent*
Standard stock solution: 0.06 mg/mL of USP 3-Chlorobenzoic Acid RS in methanol
Standard solution: 1.2 µg/mL of USP 3-Chlorobenzoic Acid RS from *Standard stock solution* in *Diluent*
Sample solution: 600 µg/mL of Bupropion Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.3 between bupropion hydrochloride related compound F and bupropion hydrochloride related compound C, *System suitability solution*; NLT 1.5 between bupropion hydrochloride related compound C and 3-chlorobenzoic acid, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 3-chlorobenzoic acid in the portion of Bupropion Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 3-chlorobenzoic acid from the *Sample solution*
r_S = peak response of 3-chlorobenzoic acid from the *Standard solution*
C_S = concentration of USP 3-Chlorobenzoic Acid RS in the *Standard solution* (µg/mL)
C_U = concentration of Bupropion Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bupropion	1.0	—
Bupropion hydrochloride related compound F[a]	1.71	—
Bupropion hydrochloride related compound C[a]	1.75	—
3-Chlorobenzoic acid	1.80	0.2

[a] Included for system suitability purposes only.

• ORGANIC IMPURITIES

Diluent, Buffer, Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

www.webofpharma.com

System suitability
Sample: *Standard solution*
[NOTE—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.3 between bupropion hydrochloride related compound A and bupropion; NLT 1.3 between bupropion and bupropion hydrochloride related compound B
Relative standard deviation: NMT 2.0% for bupropion; NMT 5.0% for bupropion hydrochloride related compound B
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Bupropion Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for bupropion from the *Standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Bupropion Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for each impurity relative to bupropion (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Deschloro bupropion[a]	0.38	1.5	0.5
Bupropion dione derivative[b]	0.58	1.0	0.2
o-Bupropion[c]	0.71	0.45	0.1
Chloropropiophenone[d]	0.78	1.2	0.1
Bupropion hydrochloride related compound A	0.92	1.4	0.2
Bupropion	1.0	—	—
Bupropion hydrochloride related compound B	1.14	0.81	0.2
Bromochloropropiophenone[e]	1.63	0.88	0.1
4-Chlorobupropion[f]	2.30	1.1	0.2
5-Chlorobupropion[g]	2.74	0.69	0.2
Any individual impurity	—	1.0	0.1
Total impurities[h]	—	—	1.0

[a] 2-(*tert*-Butylamino)-1-phenylpropan-1-one; also known as 2-(*tert*-butylamino) propiophenone.
[b] 1-(3-Chlorophenyl)propane-1,2-dione; also known as 1-(3-chlorophenyl)-1,2-propanedione.
[c] 2-(*tert*-Butylamino)-1-(2-chlorophenyl)propan-1-one; also known as 2-(*tert*-butylamino)-2′-chloropropiophenone.
[d] 1-(3-Chlorophenyl)propan-1-one; also known as 3′-chloropropiophenone.
[e] 2-Bromo-1-(3-chlorophenyl)propan-1-one; also known as 2-bromo-3′-chloropropiophenone.
[f] 2-(*tert*-Butylamino)-1-(3,4-dichlorophenyl)propan-1-one; also known as 2-(*tert*-butylamino)-3′,4′-dichloropropiophenone.
[g] 2-(*tert*-Butylamino)-1-(3,5-dichlorophenyl)propan-1-one; also known as 2-(*tert*-butylamino)-3′,5′-dichloropropiophenone.
[h] Sum of all impurities found in the tests for *Limit of 3-Chlorobenzoic Acid* and *Organic Impurities*.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Bupropion Hydrochloride RS
USP Bupropion Hydrochloride Related Compound A RS
2-(*tert*-Butylamino)-4′-chloropropiophenone hydrochloride.
$C_{13}H_{18}ClNO \cdot HCl$ 276.20
USP Bupropion Hydrochloride Related Compound B RS
2-(*tert*-Butylamino)-3′-bromopropiophenone hydrochloride.
$C_{13}H_{18}BrNO \cdot HCl$ 320.66
USP Bupropion Hydrochloride Related Compound C RS
1-(3-Chlorophenyl)-2-hydroxypropan-1-one.
$C_9H_9O_2Cl$ 184.62
USP Bupropion Hydrochloride Related Compound F RS
1-(3-Chlorophenyl)-1-hydroxypropan-2-one.
$C_9H_9O_2Cl$ 184.62
USP 3-Chlorobenzoic Acid RS
3-Chlorobenzoic acid.
$C_7H_5ClO_2$ 156.57

Bupropion Hydrochloride Tablets

DEFINITION
Bupropion Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Crush 1 Tablet using a mortar and pestle. Prepare an approximate 1% (w/w) dispersion of the sample in potassium bromide.
Acceptance criteria: The *Sample* shows strong bands at about 1690, 1560, and 1240 cm^{-1} and a weaker band at about 740 cm^{-1}, similar to the reference preparation.

www.webofpharma.com

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate and 1.164 g/L of sodium hydroxide in water

Mobile phase: Methanol and *Buffer* (65:35)

Diluent: Methanol and water (65:35)

Standard solution: 0.6 mg/mL of USP Bupropion Hydrochloride RS in *Diluent*

Sample stock solution: Nominally 3.0 mg/mL of bupropion hydrochloride in *Diluent* prepared as follows. Transfer an appropriate number of Tablets to a suitable volumetric flask. Add 50% of the flask volume of *Diluent*, and shake by mechanical means until the Tablets have disintegrated (30–60 min). Sonicate for 5 min, dilute with *Diluent* to volume, and mix. Allow to stand for at least 30 min. Use the supernatant.

Sample solution: Nominally 0.6 mg/mL of bupropion hydrochloride from the *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 224 nm

Column: 4.6-mm × 15-cm; 5-µm base-deactivated packing L1

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of bupropion hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: USP Bupropion Hydrochloride RS at a known concentration in 0.1 N hydrochloric acid

Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with 0.1 N hydrochloric acid, if necessary.

Instrumental conditions

Mode: UV

Analytical wavelength: 252 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 80% (*Q*) of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Bupropion Hydrochloride RS

Bupropion Hydrochloride Extended-Release Tablets

DEFINITION

Bupropion Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Crush 1 Tablet using a mortar and pestle. Prepare an approximate 1% (w/w) dispersion of the sample in potassium bromide.

Acceptance criteria: The *Sample* shows strong bands at about 1690, 1560, and 1240 cm^{-1} and a weaker band at about 740 cm^{-1}, similar to the reference preparation.

• **B.** The retention time of the major peak of *Sample solution A* or *Sample solution B* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**

Diluent 1: Methanol and 0.001 N hydrochloric acid (20:80)

Solution A: Acetonitrile, trifluoroacetic acid, and water (10: 0.04: 90)

Solution B: Acetonitrile, trifluoroacetic acid, and water (95: 0.03: 5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3.4	87	13
10.0	15	85
10.1	0	100
13.0	0	100
13.2	90	10
19.0	90	10

System suitability stock solution: 0.02 mg/mL of USP Bupropion Hydrochloride Related Compound C RS and 0.2 mg/mL of USP Bupropion Hydrochloride Related Compound F RS in methanol

System suitability solution: 0.002 mg/mL of bupropion hydrochloride related compound C and 0.02 mg/mL of bupropion hydrochloride related compound F from the *System suitability stock solution* in *Diluent 1*

Standard solution: 0.6 mg/mL of USP Bupropion Hydrochloride RS in *Diluent 1*

USP Monographs

www.webofpharma.com

Sample stock solution A: Transfer a number of Tablets, intact or crushed, to a suitable homogenizer vessel containing sufficient methanol to obtain a concentration of 3.0 mg/mL of bupropion hydrochloride. Immediately homogenize the sample for 30 s at 20,000 rpm. Allow extraction for 3 min, and follow by two additional 10-s pulses, each at 20,000 rpm, pausing 3 min between these pulses to ensure complete extraction. Pass a portion of the solution through a nylon filter of 0.45-µm pore size, discarding the first 2–4 mL of the filtrate.

Sample solution A: Nominally 0.6 mg/mL of bupropion hydrochloride from *Sample stock solution A* in 0.001 N hydrochloric acid

Alternatively, the *Sample solution* can be prepared as follows.

Buffer: Dissolve 100 g of anhydrous dibasic sodium phosphate in 1 L of water. Add 50 mL of phosphoric acid, stir or sonicate until dissolved, and mix. Adjust with phosphoric acid to a pH of 3.0.

Diluent 2: Methanol and *Buffer* (20:80)

Sample stock solution B: Weigh and grind NLT 20 Tablets to prepare a solution having a nominal concentration of 3 mg/mL. Initially add *Diluent 2* (75% of the volume of the flask), stir for 30 min, and sonicate for 15 min. Dilute with *Diluent 2* to volume. Centrifuge a portion of the resulting solution, and use the supernatant.

Sample solution B: Nominally 0.6 mg/mL of bupropion hydrochloride from *Sample stock solution B* in *Diluent 2*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 5 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See ▲*Table 25*▲ (RB 1-Feb-2019) for the relative retention times.]
Suitability requirements
Resolution: NLT 1.3 between bupropion hydrochloride related compound F and bupropion hydrochloride related compound C, *System suitability solution*
Tailing factor: NMT 1.9, *Standard solution*
Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution A* or *Sample solution B*
Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of bupropion hydrochloride from *Sample solution A* or *Sample solution B*
r_S = peak response of bupropion hydrochloride from the *Standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bupropion hydrochloride in *Sample solution A* or *Sample solution B* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
For products labeled for dosing every 12 h
Test 1
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Times: 1, 4, and 8 h
Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet. Dilute with *Medium*, if necessary.
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 298 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved.
Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
1	25–45
4	60–85
8	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid, pH 1.5 (prepared by transferring 50 mL of hydrochloric acid to 6000 mL of water, adding 18 g of sodium hydroxide, mixing, and adjusting with either diluted sodium hydroxide or hydrochloric acid to a pH of 1.5); 900 mL, deaerated
Apparatus 1: 50 rpm
Times: 1, 2, 4, and 6 h
Buffer: 3.45 g of monobasic sodium phosphate in 996 mL of water. Add 4.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.80.
Mobile phase: Methanol and *Buffer* (35:65)
Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet
Sample solution: Use portions of the solution under test, and pass through a nylon filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 298 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*

www.webofpharma.com

Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved.
Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved (%)
1	25–50
2	40–65
4	65–90
6	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm. Use wire coil sinkers, if necessary.
Times: 1, 2, 4, and 6 h
Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet. Dilute with *Medium*, if necessary.
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 250 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved.
Tolerances: See *Table 4*.

Table 4

Time (h)	Amount Dissolved (for Tablets that contain 200 mg of bupropion hydrochloride) (%)	Amount Dissolved (for Tablets that contain all other strengths of bupropion hydrochloride) (%)
1	30–50	30–55
2	45–65	50–75
4	65–85	70–90
6	NLT 78	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Times: 1, 3, and 6 h
Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet. Dilute with *Medium*, if necessary.
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 298 nm
Cell: 0.5 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved.
Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved (%)
1	35–55
3	65–85
6	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.
Medium: 0.1 N hydrochloric acid, pH 1.5 (prepared by transferring 50 mL of hydrochloric acid to 6000 mL of water, adding 18 g of sodium hydroxide, mixing, and adjusting with either diluted sodium hydroxide or hydrochloric acid to a pH of 1.5); 900 mL, deaerated
Apparatus 1: 50 rpm
Times: 1, 2, 4, and 6 h
Buffer: 3.45 g of monobasic sodium phosphate in 996 mL of water. Add 4.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.80.
Mobile phase: Methanol and *Buffer* (45:55)
Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet
Sample solution: Use portions of the solution under test, and pass through a nylon filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 298 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Determine the percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved.

Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Dissolved (%)
1	25–50
2	45–70
4	NLT 70
6	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 9: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.

Medium: 0.1 N hydrochloric acid, pH 1.5 (prepared by transferring 50 mL of hydrochloric acid to 6000 mL of water, adding 18 g of sodium hydroxide, mixing, and adjusting with either diluted sodium hydroxide or hydrochloric acid to a pH of 1.5); 900 mL

Apparatus 1: 50 rpm

Times: 1, 2, 4, and 8 h

Standard solution: (*L*/1000) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 298 nm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved.

Tolerances: See *Table 7*.

Table 7

Time (h)	Amount Dissolved (%)
1	20–45
2	35–55
4	55–85
8	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 10: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 10*.

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 1, 2, 4, and 8 h

Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 298 nm

Cell: 0.5 cm

Blank: *Medium*

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_i = (A_i/A_S) \times C_S \times V \times (1/L) \times 100$$

A_i = absorbance of bupropion hydrochloride from the *Sample solution* at time point *i*

A_S = absorbance of bupropion hydrochloride from the *Standard solution*

C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: See *Table 8*.

Table 8

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	20–40
2	2	35–60
3	4	55–85
4	8	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 17: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 17*.

Medium: 0.1 N hydrochloric acid, pH 1.5 (prepared by transferring 50 mL of hydrochloric acid to 6 L of water containing 18 g of sodium hydroxide, mixing, and adjusting with either diluted sodium hydroxide or diluted hydrochloric acid to a pH of 1.5); 900 mL, deaerated

Apparatus 1: 50 rpm

Times: 1, 2, 4, and 8 h

Buffer: To each liter of water add 6.8 g of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Methanol and *Buffer* (60:40)

Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet. Sonication may be used to promote dissolution.

Sample solution: Pass a portion of the solution under test through a suitable filter. [NOTE—A 0.45-µm nylon membrane filter may be suitable.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

www.webofpharma.com

Detector: UV 298 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 25 µL
Run time: NLT 1.5 times the retention time of bupropion
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S$$

r_i = peak response of bupropion from the *Sample solution* at time point *i*
r_S = peak response of bupropion from the *Standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 9*.

Table 9

Time Point (*i*)	Time (h)	Amount Dissolved (for Tablets that contain 100 mg of bupropion hydrochloride) (%)	Amount Dissolved (for Tablets that contain 150 mg or 200 mg of bupropion hydrochloride) (%)
1	1	20–40	15–35
2	2	40–60	35–55
3	4	60–85	55–80
4	8	NLT 85	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 19: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 19*.
Medium: Water, degassed; 900 mL
Apparatus 1: 50 rpm
Times: 1, 2, 4, and 8 h
Standard stock solution: 0.56 mg/mL of USP Bupropion Hydrochloride RS in *Medium*
Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 10-µm pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 298 nm
Cell: 1 cm
Blank: *Medium*
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_i = (A_i/A_S) \times C_S \times V \times (1/L) \times 100$$

A_i = absorbance of bupropion from the *Sample solution* at time point *i*
A_S = absorbance of bupropion from the *Standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: See *Table 10*.

Table 10

Time Point (*i*)	Time (h)	Amount Dissolved (for Tablets that contain 100 mg of bupropion hydrochloride) (%)	Amount Dissolved (for Tablets that contain 150 or 200 mg of bupropion hydrochloride) (%)
1	1	32–52	25–45
2	2	50–70	45–65
3	4	NLT 75	65–85
4	8	NLT 85	NLT 85

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
For products labeled for dosing every 24 h
Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.1 N hydrochloric acid; 900 mL, deaerated
Apparatus 1: 75 rpm
Times: 2, 4, 8, and 16 h
Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet. Dilute with *Medium*, if necessary.
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.

www.webofpharma.com

Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 252 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved.
Tolerances: See *Table 11*.

Table 11

Time (h)	Amount Dissolved (%)
2	NMT 20
4	20–45
8	65–90
16	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium: 0.1 N hydrochloric acid; 900 mL, deaerated
Apparatus 1: 75 rpm
Times: 1, 2, 4, 8, and 12 h
Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet. Dilute with *Medium*, if necessary.
Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 298 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved.
Tolerances: See *Table 12*.

Table 12

Time (h)	Amount Dissolved (%)
1	15–35
2	25–50
4	40–65
8	65–90
12	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 8: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.
Acid stage medium: 0.1 N hydrochloric acid; 900 mL
Buffer stage medium: pH 6.8 phosphate buffer; 900 mL
Apparatus 1: 75 rpm
Times: 2 h in *Acid stage medium*; 3, 8, and 16 h in *Buffer stage medium*. The time in the *Buffer stage medium* includes the time in the *Acid stage medium*.
Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Acid stage medium*, where *L* is the label claim, in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 298 nm
Cell: 0.5 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved.
Tolerances: See *Table 13*.

Table 13

Time (h)	Amount Dissolved (%)
2	NMT 10
3	10–30
8	60–90
16	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 11: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 11*.
Acid stage medium: 0.1 N hydrochloric acid; 750 mL
Buffer stage medium: pH 6.8 phosphate buffer (add 250 mL of 76 g/L tribasic sodium phosphate to the *Acid stage medium*, adjust with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8, if necessary); 1000 mL
Apparatus 2: 50 rpm
Times: 2 h in *Acid stage medium*; 3, 8, and 16 h in *Buffer stage medium*. The time in the *Buffer stage medium* includes the time in the *Acid stage medium*.
Acid stage standard solution: 0.06 mg/mL of USP Bupropion Hydrochloride RS in *Acid stage medium*. Sonication may be used to aid in dissolution.
Buffer stage standard solution: 0.15 mg/mL of USP Bupropion Hydrochloride RS in *Buffer stage medium*. Sonication may be used to aid in dissolution.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 298 nm
Cell: 0.5 cm
Blank: *Acid stage medium* or *Buffer stage medium*
Analysis
Samples: *Acid stage standard solution*, *Buffer stage standard solution*, and *Sample solution*
Calculate the concentration (C_i) of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (A_i/A_S) \times C_S$$

USP Monographs

www.webofpharma.com

A_i = absorbance of bupropion hydrochloride from the *Sample solution* at time point *i*
A_S = absorbance of bupropion hydrochloride from the *Acid stage standard solution* or *Buffer stage standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Acid stage standard solution* or *Buffer stage standard solution* (mg/mL)

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V_A \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V_B - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V_B - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V_B - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *i* (mg/mL)
V_A = volume of *Acid stage medium*, 750 mL
L = label claim (mg/Tablet)
V_B = volume of *Buffer stage medium*, 1000 mL
V_S = volume of *Sample solution* withdrawn from the *Acid stage medium* or *Buffer stage medium* (mL)

Tolerances: See *Table 14*.

Table 14

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	NMT 10
2	3	10–30
3	8	55–85
4	16	NLT 75

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 12: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 12*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 75 rpm
Times: 2, 4, 8, and 12 h
Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet
Sample solution: Withdraw at least 10 mL of the solution under test and pass through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 252 nm
Cell
For Tablets labeled to contain 150 mg: 0.1 cm
For Tablets labeled to contain 300 mg: 0.05 cm
Blank: *Medium*
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (A_i/A_S) \times C_S$$

A_i = absorbance of bupropion hydrochloride from the *Sample solution* at time point *i*
A_S = absorbance of bupropion hydrochloride from the *Standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 15*.

Table 15

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	NMT 25
2	4	25–50
3	8	60–85
4	12	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 13: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 13*.
Medium: 0.1 N hydrochloric acid; 900 mL, deaerated
Apparatus 1: 75 rpm
Times: 2, 4, 8, and 12 h
Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet
Sample solution: Withdraw at least 10 mL of the solution under test and centrifuge. Use the supernatant.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 252 nm
Cell: 0.1 cm
Blank: *Medium*
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%

www.webofpharma.com

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (A_i/A_S) \times C_S$$

A_i = absorbance of bupropion hydrochloride from the *Sample solution* at time point *i*

A_S = absorbance of bupropion hydrochloride from the *Standard solution*

C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *i* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_S = volume of *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 16*.

Table 16

Time Point (*i*)	Time (h)	Amount Dissolved (150 mg/Tablet) (%)	Amount Dissolved (300 mg/Tablet) (%)
1	2	NMT 25	NMT 25
2	4	30–55	25–45
3	8	65–90	60–80
4	12	NLT 80	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 14: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 14*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 75 rpm

Times: 2, 4, 8, and 16 h

Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet. If necessary, dilute the solution with *Medium*.

Sample solution: Pass a portion of the solution under test through a suitable filter. Replace the portion removed with the same volume of *Medium*. If necessary, dilute the filtrate with *Medium*.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 252 nm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (A_i/A_S) \times C_S \times D$$

A_i = absorbance from the *Sample solution* at time point *i*

A_S = absorbance from the *Standard solution*

C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*, if needed

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *i* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_S = volume of *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 17*.

Table 17

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	NMT 20
2	4	20–45
3	8	55–85
4	16	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 15: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 15*.

Acid stage

Acid stage medium: 0.1 N hydrochloric acid, degassed; 900 mL

Apparatus 1: 100 rpm

Time: 2 h in *Acid stage medium*

Buffer: 3.5 g/L of monobasic sodium phosphate prepared as follows. Dissolve 3.45 g of monobasic sodium phosphate in 996 mL of water, add 4.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.8.

Mobile phase: Methanol and *Buffer* (45:55)

Acid stage standard solution: 0.033 mg/mL of USP Bupropion Hydrochloride RS in *Acid stage medium*. Sonication may be used to promote dissolution.

Acid stage sample solution: Pass a portion of the solution under test through a suitable filter, discard the first 5 mL, and use the filtrate. Then discard the Tablets

USP Monographs

www.webofpharma.com

and remaining solution. [NOTE—A 0.45-µm nylon membrane filter may be suitable.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 298 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 1.5 times the retention time of bupropion

System suitability
Sample: *Acid stage standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Acid stage standard solution* and *Acid stage sample solution*
Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of bupropion from the *Acid stage sample solution*
r_S = peak response of bupropion from the *Acid stage standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Acid stage standard solution* (mg/mL)
V = volume of *Acid stage medium*, 900 mL
L = label claim (mg/Tablet)

Buffer stage: Use fresh Tablets.
Buffer stage medium: pH 6.8 tribasic sodium phosphate buffer and 0.5% sodium lauryl sulfate (Dissolve 19 g of tribasic sodium phosphate in 1 L of water, add 7 mL of hydrochloric acid, and adjust with 0.2 N sodium hydroxide or dilute hydrochloric acid to a pH of 6.8. Add 5 g of sodium dodecyl sulfate. To promote dissolution, the resulting solution can be continuously stirred and heated to 41°. Allow the solution to cool to 37° before use. Do not allow the temperature to fall below 36.5° before beginning the test.); 900 mL
Apparatus 1: 100 rpm
Times: 1, 2, 4, and 8 h
Buffer: 1.4 g/L of dibasic ammonium phosphate and 0.5 g/L of sodium 1-hexanesulfonate prepared as follows. Dissolve 1.4 g of dibasic ammonium phosphate and 0.5 g of sodium 1-hexanesulfonate in 1 L of water. To each 1 L of this solution, add 2.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 7.0.
Mobile phase: Acetonitrile and *Buffer* (60:40)
Buffer stage standard solution: 0.33 mg/mL of USP Bupropion Hydrochloride RS in *Buffer stage medium*
Buffer stage sample solution: Pass a portion of the solution under test through a suitable filter, discard the first 5 mL, and use the filtrate.
Chromatographic system: Proceed as directed under the *Acid stage*.
System suitability
Sample: *Buffer stage standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Buffer stage standard solution* and *Buffer stage sample solution*
Calculate the concentration (C_i) of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S$$

r_i = peak response of bupropion from the *Buffer stage sample solution* at time point *i*
r_S = peak response of bupropion from the *Buffer stage standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Buffer stage standard solution* (mg/mL)

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *i* (mg/mL)
V = volume of *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of *Buffer stage sample solution* withdrawn at each time point (mL)

Tolerances
Acid stage: NMT 10%; the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the time specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 3*.
Buffer stage: See *Table 18*.

Table 18

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	5–25
2	2	25–45
3	4	60–85
4	8	NLT 85

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 16: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 16*.
Medium: 0.1 N hydrochloric acid; 900 mL, deaerated
Apparatus 1: 75 rpm
Times: 2, 5, 8, and 16 h
Buffer: 3.5 g/L of monobasic sodium phosphate prepared as follows. Dissolve 3.45 g of monobasic sodium phosphate in 996 mL of water, add 4.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.8.
Mobile phase: Methanol and *Buffer* (35:65)

www.webofpharma.com

Standard solution: 0.17 mg/mL of USP Bupropion Hydrochloride RS in *Medium*. Sonication may be used to promote dissolution
Sample solution: Pass a portion of the solution under test through a suitable filter, and discard NLT 1 mL. Dilute the filtrate with *Medium* if necessary. Replace the portion removed with the same volume of *Medium*. [NOTE—A 0.45-µm nylon membrane filter may be suitable.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 298 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of bupropion
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S \times D$$

r_i = peak response of bupropion from the *Sample solution* at time point *i*
r_S = peak response of bupropion from the *Standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 19*.

Table 19

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	NMT 10
2	5	30–60
3	8	65–88
4	16	NLT 85

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 18: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 18*.
Medium: 0.1 N hydrochloric acid; 900 mL, deaerated
Apparatus 1: 75 rpm
Times: 2, 4, 8, and 16 h
Buffer: 6.8 g/L of monobasic potassium phosphate in water adjusted with phosphoric acid to a pH of 3.0
Mobile phase: Methanol and *Buffer* (60:40)
Standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where *L* is the label claim, in mg/Tablet. Sonication may be used to promote dissolution.
Sample solution: Centrifuge a portion of the solution under test for 15 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 298 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 25 µL
Run time: NLT 1.5 times the retention time of bupropion
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S$$

r_i = peak response of bupropion from the *Sample solution* at time point *i*
r_S = peak response of bupropion from the *Standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 20*.

www.webofpharma.com

Table 20

Time Point (*i*)	Time (h)	Amount Dissolved (for Tablets that contain 150 mg of bupropion hydrochloride) (%)	Amount Dissolved (for Tablets that contain 300 mg of bupropion hydrochloride) (%)
1	2	NMT 20	NMT 20
2	4	25–50	25–50
3	8	65–95	60–85
4	16	NLT 80	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 20:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 20*.

Medium: 0.1 N hydrochloric acid VS; 900 mL, deareated

Apparatus 1: 75 rpm

Times: 2, 4, 8, and 16 h

Standard solution: 0.1 mg/mL of USP Bupropion Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary. Replace the portion removed with the same volume of *Medium*.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 298 nm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (A_i/A_S) \times C_S \times D$$

A_i = absorbance from the *Sample solution* at time point *i*

A_S = absorbance from the *Standard solution*

C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*, if needed

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *i* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_S = volume of *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 21*.

Table 21

Time Point (*i*)	Time (h)	Amount Dissolved (for Tablets that contain 150 mg of bupropion hydrochloride) (%)	Amount Dissolved (for Tablets that contain 300 mg of bupropion hydrochloride) (%)
1	2	NMT 15	NMT 15
2	4	10–35	10–35
3	8	55–80	50–75
4	16	NLT 80	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 21: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 21*.

Medium: 0.1 N hydrochloric acid VS; 900 mL, deareated

Apparatus 1: 75 rpm

Times: 4, 8, and 16 h

Standard stock solution 1: 0.84 mg/mL of USP Bupropion Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Bupropion Hydrochloride RS to an appropriate volumetric flask. Add 50% of the flask volume of acetonitrile. Dilute with water to volume.

Standard stock solution 2: 0.17 mg/mL of USP Bupropion Hydrochloride RS from *Standard stock solution 1* in *Medium*

Standard solution: 0.017 mg/mL of USP Bupropion Hydrochloride RS from *Standard stock solution 2* in *Medium* passed through a suitable filter of 0.45-µm pore size

Sample solution: Dilute a portion of the solution under test with *Medium*. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size. Replace the portion removed with the same volume of *Medium*.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 252 nm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (A_i/A_S) \times C_S \times D$$

A_i = absorbance from the *Sample solution* at time point *i*

A_S = absorbance from the *Standard solution*

C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*, if needed

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

USP Monographs

www.webofpharma.com

$$Result_3 = ((C_3 \times V) + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 22*.

Table 22

Time Point (*i*)	Time (h)	Amount Dissolved (for Tablets that contain 150 mg of bupropion hydrochloride) (%)	Amount Dissolved (for Tablets that contain 300 mg of bupropion hydrochloride) (%)
1	4	NMT 20	NMT 30
2	8	35–60	50–70
3	16	NLT 80	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 22: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 22*.
Acid stage medium: 0.1 N hydrochloric acid VS; 750 mL
Buffer stage medium: Sodium phosphate buffer, pH 6.8 (after 2 h, add 250 mL of 76 g/L of tribasic sodium phosphate, previously heated to 37 ± 0.5°, to the *Acid stage medium* and adjust with 2 N hydrochloric acid TS or 2 N sodium hydroxide TS, if necessary, to a pH of 6.8); 1000 mL
Apparatus 2: 50 rpm
Times: 2 h in *Acid stage medium*; 4 and 12 h in *Buffer stage medium*. The time in the *Buffer stage medium* includes the time in the *Acid stage medium*.
Acid stage standard solution: 0.08 mg/mL of USP Bupropion Hydrochloride RS in *Acid stage medium*
Buffer stage standard solution: 0.3 mg/mL of USP Bupropion Hydrochloride RS in *Buffer stage medium*
Acid stage sample solution and **Buffer stage sample solution:** Use a portion of the solution under test.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 298 nm
Blank: *Acid stage medium* or *Buffer stage medium*
System suitability
Samples: *Acid stage standard solution* and *Buffer stage standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%, *Acid stage standard solution* and *Buffer stage standard solution*
Analysis
Samples: *Acid stage standard solution*, *Buffer stage standard solution*, *Acid stage sample solution*, and *Buffer stage sample solution*
Calculate the concentration (C_i) of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the sample withdrawn from the vessel at time point *i*:

$$Result_i = (A_i/A_S) \times C_S \times D$$

A_i = absorbance from the *Acid stage sample solution* or *Buffer stage sample solution* at time point *i*
A_S = absorbance from the *Acid stage standard solution* or *Buffer stage standard solution* at time point *i*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Acid stage standard solution* or *Buffer stage standard solution* (mg/mL)
D = dilution factor, if needed

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved in *Acid stage medium*:

$$Result_1 = C_1 \times V_A \times (1/L) \times 100$$

C_1 = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *1*
V_A = volume of *Acid stage medium*, 750 mL
L = label claim (mg/Tablet)

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$Result_2 = \{[C_2 \times (V_B - V_{SA})] + (C_1 \times V_{SA})\} \times (1/L) \times 100$$
$$Result_3 = \{[C_3 \times (V_B - V_{SB} - V_{SA})] + (C_2 \times V_{SB}) + (C_1 \times V_{SA})\} \times (1/L) \times 100$$

C_i = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *i* (mg/mL)
V_B = volume of *Buffer stage medium*, 1000 mL
V_{SA} = volume of *Acid stage sample solution* withdrawn at time point 1 (mL)
L = label claim (mg/Tablet)
V_{SB} = volume of *Buffer stage sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 23*.

Table 23

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	NMT 15
2	4	40–60
3	12	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 23: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 23*.
Acid stage medium: 0.1 N hydrochloric acid VS; 900 mL, deaerated
Buffer stage medium: pH 6.8 phosphate buffer; 900 mL, deaerated
Apparatus 1: 75 rpm
Times: 2 h in *Acid stage medium*; 6 and 16 h in *Buffer stage medium*. The time in the *Buffer stage medium* includes the time in the *Acid stage medium*.
Acid stage standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Acid stage medium*, where *L* is the label claim, in mg/Tablet

USP Monographs

www.webofpharma.com

Buffer stage standard solution: (*L*/900) mg/mL of USP Bupropion Hydrochloride RS in *Buffer stage medium*, where *L* is the label claim, in mg/Tablet

Acid stage sample solution and **Buffer stage sample solution:** Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 298 nm

Cell: 0.5 cm, flow cell

Blank: *Acid stage medium* or *Buffer stage medium*

System suitability

Samples: *Acid stage standard solution* and *Buffer stage standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%, *Acid stage standard solution* and *Buffer stage standard solution*

Analysis

Samples: *Acid stage standard solution*, *Buffer stage standard solution*, *Acid stage sample solution*, and *Buffer stage sample solution*

Calculate the concentration (C_i) of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (A_i/A_S) \times C_S$$

A_i = absorbance from the *Acid stage sample solution* or *Buffer stage sample solution* at time point *i*

A_S = absorbance from the *Acid stage standard solution* or *Buffer stage standard solution* at time point *i*

C_S = concentration of USP Bupropion Hydrochloride RS in the *Acid stage standard solution* or *Buffer stage standard solution* (mg/mL)

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved in *Acid stage medium* (Q_A):

$$\text{Result}_1 = C_1 \times V_A \times (1/L) \times 100$$

C_1 = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *1*

V_A = volume of *Acid stage medium*, 900 mL

L = label claim (mg/Tablet)

Calculate the percentage of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_2 = [C_2 \times V_B \times (1/L) \times 100] + Q_A$$
$$\text{Result}_3 = [C_3 \times V_B \times (1/L) \times 100] + Q_A$$

C_i = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point *i* (mg/mL)

V_B = volume of *Buffer stage medium*, 900 mL

L = label claim (mg/Tablet)

Q_A = percentage of the labeled amount of bupropion hydrochloride dissolved in the *Acid stage medium*

Tolerances: See *Table 24*.

Table 24

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	NMT 15
2	6	50–75
3	16	NLT 80

The percentages of the labeled amount of bupropion hydrochloride ($C_{13}H_{18}ClNO \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 1-Feb-2019)

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**

Diluent 1, Solution A, Solution B, Mobile phase, and **Sample solution A** or **Sample solution B:** Proceed as directed in the *Assay*.

System suitability stock solution A: 0.02 mg/mL of USP Bupropion Hydrochloride Related Compound C RS, 0.02 mg/mL of USP Bupropion Hydrochloride Related Compound F RS, and 0.012 mg/mL of USP 3-Chlorobenzoic Acid RS in methanol

System suitability solution A: 0.002 mg/mL of bupropion hydrochloride related compound C, 0.002 mg/mL of bupropion hydrochloride related compound F, and 0.0012 mg/mL of 3-chlorobenzoic acid from *System suitability stock solution A* in *Diluent 1*

System suitability stock solution B: 0.012 mg/mL of USP 3-Chlorobenzoic Acid RS in methanol

System suitability solution B: 0.0012 mg/mL of 3-chlorobenzoic acid from *System suitability stock solution B* in *Diluent 1*

Standard solution: 0.0012 mg/mL of USP Bupropion Hydrochloride RS in *Diluent 1*

Chromatographic system: Proceed as directed in the *Assay* except use a *Detector* as follows.

Detector: UV 226 nm, adjusted ±2 nm so that the relative response factor requirement is met. [Note—The peak responses of the compounds of interest are very sensitive to changes in the detection wavelength.]

System suitability

Samples: *System suitability solution A*, *System suitability solution B*, and *Standard solution*

[Note—See ▲*Table 25*▲ (RB 1-Feb-2019) for the relative retention times.]

Suitability requirements

Resolution: NLT 1.3 between bupropion hydrochloride related compound F and bupropion hydrochloride related compound C, *System suitability solution A*; NLT 1.3 between bupropion hydrochloride C and 3-chlorobenzoic acid, *System suitability solution A*

Relative standard deviation: NMT 10%, *Standard solution*

Relative response factor: 3.8–4.5 for the peak response of 3-chlorobenzoic acid in *System suitability solution B* divided by the peak response from bupropion in the *Standard solution*

Analysis

Samples: *System suitability solution B*, *Standard solution*, and *Sample solution A* or *Sample solution B*

Calculate the percentage of 3-chlorobenzoic acid in the portion of Tablets taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 3-chlorobenzoic acid from *Sample solution A* or *Sample solution B*
r_S = peak response of 3-chlorobenzoic acid from *System suitability solution B*
C_S = concentration of USP 3-Chlorobenzoic Acid RS in *System suitability solution B* (mg/mL)
C_U = nominal concentration of bupropion hydrochloride in *Sample solution A* or *Sample solution B* (mg/mL)

Calculate the percentage of each other degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each other degradation product from *Sample solution A* or *Sample solution B*
r_S = peak response of bupropion hydrochloride from the *Standard solution*
C_S = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of bupropion hydrochloride in *Sample solution A* or *Sample solution B* (mg/mL)
F = relative response factor for each other degradation product (see ▲*Table 25*▲ (RB 1-Feb-2019))

Acceptance criteria: See ▲*Table 25*.

Table 25▲ (RB 1-Feb-2019)

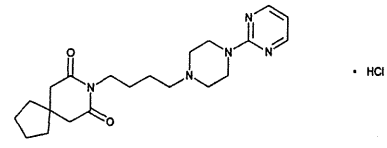
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)	
			100 mg or less	150 mg or greater
Bupropion amine[a]	0.38	1.2	0.3	0.3
S,S,S-Thiomorpholine derivative[b]	0.56	1.1	1.0	1.5
S,R,R-Thiomorpholine derivative[c]	0.78	1.1	0.5	0.4
Bupropion	1.0	—	—	—
Bupropion related compound F	1.71	1.8	1.2	2.3
Bupropion related compound C	1.75	1.7	0.3	0.3
3-Chlorobenzoic acid	1.80	—	0.3	0.3
Bupropion dione derivative[d]	2.25	1.00	0.4	0.4
Any unspecified degradation product	—	1.00	0.2	0.2
Total impurities	—	—	3.2	3.3

[a] 2-Amino-1-(3-chlorophenyl)-1-propanone.
[b] (3*S*,5*S*,6*S*)-6-(3-Chlorophenyl)-6-hydroxy-5-methyl-3-thiomorpholine carboxylic acid.
[c] (3*S*,5*R*,6*R*)-6-(3-Chlorophenyl)-6-hydroxy-5-methyl-3-thiomorpholine carboxylic acid.
[d] 1-(3-Chlorophenyl)propane-1,2-dione.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature. Protect from light.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bupropion Hydrochloride RS
  USP Bupropion Hydrochloride Related Compound C RS
  1-(3-Chlorophenyl)-2-hydroxypropan-1-one.
  $C_9H_9O_2Cl$ 184.62
  USP Bupropion Hydrochloride Related Compound F RS
  1-(3-Chlorophenyl)-1-hydroxypropan-2-one.
  $C_9H_9O_2Cl$ 184.62
  USP 3-Chlorobenzoic Acid RS
  3-Chlorobenzoic acid.
  $C_7H_5ClO_2$ 156.57

Buspirone Hydrochloride



$C_{21}H_{31}N_5O_2 \cdot HCl$ 421.96
8-Azaspiro[4,5]decane-7,9-dione, 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-, monohydrochloride;
N-[4-[4-(2-Pyrimidinyl)-1-piperazinyl]butyl]-1,1-cyclopentanediacetamide monohydrochloride [33386-08-2].

DEFINITION

Buspirone Hydrochloride contains NLT 97.5% and NMT 102.5% of buspirone hydrochloride ($C_{21}H_{31}N_5O_2 \cdot HCl$), calculated on the as-is basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The relative retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
  Sample solution: 10 mg/mL in water

www.webofpharma.com

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Buffer A: 6.8 g/L of monobasic potassium phosphate and 0.93 g/L of sodium 1-hexanesulfonate monohydrate. Adjust with phosphoric acid to a pH of 3.4.

Buffer B: 3.4 g/L of monobasic potassium phosphate and 3.52 g/L of sodium 1-hexanesulfonate monohydrate. Adjust with phosphoric acid to a pH of 2.2.

Solution A: Acetonitrile and *Buffer A* (5:95)

Solution B: Acetonitrile and *Buffer B* (75:25)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
6	90	10
34	42	58
45	42	58
55	0	100
56	100	0
60	100	0
61	90	10

Diluent: *Solution A*

Impurities stock solution: 0.25 mg/mL each of USP Buspirone Related Compound A RS, USP Buspirone Related Compound G RS, USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, and USP Buspirone Related Compound N RS in acetonitrile

System suitability solution: 1.0 mg/mL of USP Buspirone Hydrochloride RS and 0.001 mg/mL each of USP Buspirone Related Compound A RS, USP Buspirone Related Compound G RS, USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, and USP Buspirone Related Compound N RS in *Diluent*, from *Impurities stock solution*

Standard solution: 0.1 mg/mL of USP Buspirone Hydrochloride RS in *Diluent*

Sample solution: 0.1 mg/mL of Buspirone Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between buspirone and buspirone related compound G peaks, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 0.92%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of buspirone hydrochloride ($C_{21}H_{31}N_5O_2 \cdot HCl$) in the portion of Buspirone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Buspirone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.5% on the as-is basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%

• **ORGANIC IMPURITIES**

Buffer A, Buffer B, Solution A, Solution B, Mobile phase, Diluent, Impurities stock solution, and **System suitability solution:** Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL each of USP Buspirone Hydrochloride RS, USP Buspirone Related Compound A RS, USP Buspirone Related Compound G RS, USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, and USP Buspirone Related Compound N RS in *Diluent*

Sample solution: 1.0 mg/mL of Buspirone Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 and 240 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* and *Table 3* for relative retention times.]

Suitability requirements

Resolution at 240 nm: NLT 2.0 between buspirone and buspirone related compound G peaks, *System suitability solution*

Resolution at 210 nm: NLT 4.0 between buspirone related compound L and buspirone related compound N peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% for each peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

For impurities detected at UV 240 nm

Calculate the percentage of buspirone related compound A or buspirone related compound G in the portion of Buspirone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of buspirone related compound A or buspirone related compound G from the *Sample solution*

r_S = peak response of buspirone related compound A or buspirone related compound G from the *Standard solution*

C_S = concentration of USP Buspirone Related Compound A RS or USP Buspirone Related Compound G RS in the *Standard solution* (mg/mL)

C_U = concentration of Buspirone Hydrochloride in the *Sample solution* (mg/mL)

www.webofpharma.com

Calculate the percentage of specified impurities and any other individual impurity in the portion of Buspirone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of specified impurities and any other individual impurity from the *Sample solution*
r_S = peak response of buspirone from the *Standard solution*
C_S = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Buspirone Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria

For impurities detected at UV 240 nm: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Buspirone related compound A[a]	0.2	—	0.10
Spiroammonium salt[b]	0.3	1.0	0.10
Bispyrimidinylpiperazinyl butane[c]	0.6	1.0	0.10
Bispyrimidinylpiperazinylbutyl ether[d]	0.7	1.0	0.10
Buspirone open ring[e]	0.8	1.0	0.3
Buspirone open ring dimer[f]	0.9	1.0	0.10
Buspirone	1.0	—	—
Buspirone related compound G[g]	1.05	—	0.10
Buspirone diester dimer[h]	1.1	1.0	0.10
Chlorobuspirone[i]	1.2	1.0	0.10
Buspirone open ring spirodimer[j]	1.5	0.5	0.2
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.4

[a] 2-(Piperazin-1-yl)pyrimidine.
[b] 8-(Pyrimidin-2-yl)-8-aza-5-azoniaspiro[4.5]decane.
[c] 1,4-Bis[4-(pyrimidin-2-yl)piperazin-1-yl]butane.
[d] Bis{4-[1-(pyrimidine-2-yl)piperazine-4-yl]butane-1-yl} ether.
[e] 2-{1-[2-Oxo-2-({4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl}amino)ethyl] cyclopentyl}acetic acid.
[f] 4-[4-(Pyrimidin-2-yl)piperazin-1-yl]butyl 2-{1-[2-oxo-2-({4-[4-(pyrimidin-2-yl) piperazin-1-yl]butyl}amino)ethyl]cyclopentyl}acetate.
[g] 1,4-Di(pyrimidin-2-yl)piperazine.
[h] Bis{4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl} 2,2′-(cyclopentane-1,1-diyl) diacetate.
[i] 8-{4-[4-(5-Chloropyrimidin-2-yl)piperazin-1-yl]butyl}-8-azaspiro[4.5]decane-7,9-dione.
[j] 4-(7,9-Dioxo-8-azaspiro[4.5]decan-8-yl)butyl 2-{1-[2-oxo-2-({4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl}amino)ethyl]cyclopentyl}acetate.

For impurities detected at UV 210 nm

Calculate the percentage of buspirone related compound K, buspirone related compound L, or buspirone related compound N in the portion of Buspirone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of buspirone related compound K, buspirone related compound L, or buspirone related compound N from the *Sample solution*
r_S = peak response of buspirone related compound K, buspirone related compound L, or buspirone related compound N from the *Standard solution*
C_S = concentration of USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, or USP Buspirone Related Compound N RS in the *Standard solution* (mg/mL)
C_U = concentration of Buspirone Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of buspirone bromobutyl analog and any other individual impurity in the portion of Buspirone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of buspirone bromobutyl analog and any other individual impurity from the *Sample solution*
r_S = peak response of buspirone from the *Standard solution*
C_S = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Buspirone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria

For impurities detected at UV 210 nm: See *Table 3*. Disregard any peak below 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Buspirone related compound K[a]	0.6	0.1
Buspirone	1.0	—
Buspirone related compound L[b]	1.7	0.10
Buspirone bromobutyl analog[c]	1.8	0.10
Buspirone related compound N[d]	1.9	0.10
Any other individual impurity	—	0.10
Total impurities	—	0.2

[a] 8-Azaspiro[4,5]decane-7,9-dione.
[b] 8-(4-Chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione.
[c] 8-(4-Bromobutyl)-8-azaspiro[4.5]decane-7,9-dione.
[d] 8,8′-(Butane-1,4-diyl)bis(8-azaspiro[4.5]decane-7,9-dione).

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Buspirone Hydrochloride RS
  USP Buspirone Related Compound A RS
  2-(Piperazin-1-yl)pyrimidine.
  $C_8H_{12}N_4$ 164.21
  USP Buspirone Related Compound G RS
  1,4-Di(pyrimidin-2-yl)piperazine.
  $C_{12}H_{14}N_6$ 242.28

USP Monographs

www.webofpharma.com

USP Buspirone Related Compound K RS
8-Azaspiro[4.5]decane-7,9-dione.
$C_9H_{13}NO_2$ 167.21
USP Buspirone Related Compound L RS
8-(4-Chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione.
$C_{13}H_{20}ClNO_2$ 257.76
USP Buspirone Related Compound N RS
8,8′-(Butane-1,4-diyl)bis(8-azaspiro[4.5]decane-7,9-dione).
$C_{22}H_{32}N_2O_4$ 388.50

Buspirone Hydrochloride Tablets

DEFINITION

Buspirone Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of buspirone hydrochloride ($C_{21}H_{31}N_5O_2 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Grind 20 Tablets to a fine powder, add 50 mL of chloroform, stir for 3–5 min, and filter into a 250-mL evaporating flask. Evaporate the solution with the aid of a rotary evaporator to dryness at low heat. Use the residue.
Acceptance criteria: Meet the requirements

- **B.** The relative retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer A: 6.8 g/L of monobasic potassium phosphate and 0.93 g/L of sodium 1-hexanesulfonate monohydrate, adjusted with phosphoric acid to a pH of 3.4
Buffer B: 3.4 g/L of monobasic potassium phosphate and 3.52 g/L of sodium 1-hexanesulfonate monohydrate, adjusted with phosphoric acid to a pH of 2.2
Solution A: Acetonitrile and *Buffer A* (5:95)
Solution B: Acetonitrile and *Buffer B* (75:25)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
6	90	10
34	42	58
45	42	58
55	0	100
56	100	0
60	100	0
61	90	10

Diluent: *Solution A*
Impurities stock solution: 0.25 mg/mL each of USP Buspirone Related Compound A RS, USP Buspirone Related Compound G RS, USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, and USP Buspirone Related Compound N RS in acetonitrile
System suitability solution: 1.0 mg/mL of USP Buspirone Hydrochloride RS and 0.001 mg/mL each of USP Buspirone Related Compound A RS, USP Buspirone Related Compound G RS, USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, and USP Buspirone Related Compound N RS in *Diluent* from the *Impurities stock solution*
Standard solution: 0.1 mg/mL of USP Buspirone Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of buspirone hydrochloride from NLT 20 finely powdered Tablets in *Diluent*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 60% of the flask volume of *Diluent*, and sonicate for 30 min. Allow the solution to cool to room temperature, and then dilute with *Diluent* to volume. Centrifuge the solution and filter the supernatant. Further dilute the filtrate with *Diluent* as needed.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between the buspirone and buspirone related compound G peaks, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of buspirone hydrochloride ($C_{21}H_{31}N_5O_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of buspirone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
Sample solution: Filter a portion of the solution under test, and dilute with *Medium* as needed.
Standard solution: USP Buspirone Hydrochloride RS in *Medium* having a concentration similar to that expected in the *Sample solution*
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum at about 235 nm
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of the labeled amount of buspirone hydrochloride ($C_{21}H_{31}N_5O_2 \cdot HCl$) dissolved:

www.webofpharma.com

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of buspirone hydrochloride from the *Standard solution*
C_S = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of buspirone hydrochloride ($C_{21}H_{31}N_5O_2 \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer A, Buffer B, Solution A, Solution B, Mobile phase, Diluent, Impurities stock solution, and **System suitability solution:** Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL each of USP Buspirone Hydrochloride RS, USP Buspirone Related Compound A RS, USP Buspirone Related Compound G RS, USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, and USP Buspirone Related Compound N RS in *Diluent*

Sample solution: Nominally 1.0 mg/mL of buspirone hydrochloride from NLT 20 finely powdered Tablets in *Diluent*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 60% of the flask volume of *Diluent*, and sonicate for 30 min. Allow the solution to cool to room temperature, and then dilute with *Diluent* to volume. Centrifuge the solution and filter the supernatant. Use the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 and 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution at 240 nm: NLT 2.0 between the buspirone and buspirone related compound G peaks, *System suitability solution*
Resolution at 210 nm: NLT 4.0 between the buspirone related compound L and buspirone related compound N peaks, *System suitability solution*
Relative standard deviation: NMT 2.0% for each peak, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
For impurities detected at UV 240 nm
Calculate the percentage of buspirone related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of buspirone related compound A from the *Sample solution*
r_S = peak response of buspirone related compound A from the *Standard solution*
C_S = concentration of USP Buspirone Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of buspirone hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified degradation product from the *Sample solution*
r_S = peak response of buspirone from the *Standard solution*
C_S = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of buspirone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria
For impurities detected at UV 240 nm: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Buspirone related compound A[a]	0.2	0.20
Spiroammonium salt[b, c]	0.3	—
Bispyrimidinylpiperazinyl butane[c, d]	0.6	—
Bispyrimidinylpiperazinylbutyl ether[c, e]	0.7	—
Buspirone open ring[c, f]	0.8	—
Buspirone open ring dimer[c, g]	0.9	—
Buspirone	1.0	—
Buspirone related compound G[c, h]	1.05	—
Buspirone diester dimer[c, i]	1.1	—
Chlorobuspirone[c, j]	1.2	—
Buspirone open ring spirodimer[c, k]	1.5	—
Any individual unspecified degradation product	—	0.2
Total impurities	—	See *Table 3*

[a] 2-(Piperazin-1-yl)pyrimidine.
[b] 8-(Pyrimidin-2-yl)-8-aza-5-azoniaspiro[4.5]decane.
[c] Process impurity included for identification only and not included in the calculation of total degradation products.
[d] 1,4-Bis[4-(pyrimidin-2-yl)piperazin-1-yl]butane.
[e] Bis{4-[1-(pyrimidine-2-yl)piperazine-4-yl]butane-1-yl} ether.
[f] 2-{1-[2-Oxo-2-({4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl}amino)ethyl] cyclopentyl}acetic acid.
[g] 4-[4-(Pyrimidin-2-yl)piperazin-1-yl]butyl 2-{1-[2-oxo-2-({4-[4-(pyrimidin-2-yl) piperazin-1-yl]butyl}amino)ethyl]cyclopentyl}acetate.
[h] 1,4-Di(pyrimidin-2-yl)piperazine.
[i] Bis{4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl} 2,2′-(cyclopentane-1,1-diyl) diacetate.
[j] 8-{4-[4-(5-Chloropyrimidin-2-yl)piperazin-1-yl]butyl}-8-azaspiro[4.5]decane-7,9-dione.
[k] 4-(7,9-Dioxo-8-azaspiro[4.5]decan-8-yl)butyl 2-{1-[2-oxo-2-({4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl}amino)ethyl]cyclopentyl}acetate.

For impurities detected at UV 210 nm
Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified degradation product from the *Sample solution*
r_S = peak response of buspirone from the *Standard solution*

USP Monographs

www.webofpharma.com

C_S = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of buspirone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria
For impurities detected at UV 210 nm: See *Table 3*. Disregard any peak below 0.05%.

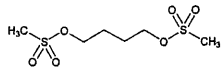
Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Buspirone related compound K[a, b]	0.6	—
Buspirone	1.0	—
Buspirone related compound L[b, c]	1.7	—
Buspirone bromobutyl analog[b, d]	1.8	—
Buspirone related compound N[b, e]	1.9	—
Any individual unspecified degradation product	—	0.2
Total impurities	—	2.0[f]

[a] 8-Azaspiro[4.5]decane-7,9-dione.
[b] Process impurity included for identification only and not included in the calculation of total degradation products.
[c] 8-(4-Chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione.
[d] 8-(4-Bromobutyl)-8-azaspiro[4.5]decane-7,9-dione.
[e] 8,8′-(Butane-1,4-diyl)bis(8-azaspiro[4.5]decane-7,9-dione).
[f] Total impurities include impurities detected at UV 240 nm.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Buspirone Hydrochloride RS
  USP Buspirone Related Compound A RS
  2-(Piperazin-1-yl)pyrimidine.
  $C_8H_{12}N_4$ 164.21
  USP Buspirone Related Compound G RS
  1,4-Di(pyrimidin-2-yl)piperazine.
  $C_{12}H_{14}N_6$ 242.28
  USP Buspirone Related Compound K RS
  8-Azaspiro[4.5]decane-7,9-dione.
  $C_9H_{13}NO_2$ 167.21
  USP Buspirone Related Compound L RS
  8-(4-Chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione.
  $C_{13}H_{20}ClNO_2$ 257.76
  USP Buspirone Related Compound N RS
  8,8′-(Butane-1,4-diyl)bis(8-azaspiro[4.5]decane-7,9-dione).
  $C_{22}H_{32}N_2O_4$ 388.50

Busulfan



$C_6H_{14}O_6S_2$ 246.30
1,4-Butanediol, dimethanesulfonate;
1,4-Butanediol dimethanesulfonate [55-98-1].

DEFINITION
Busulfan contains NLT 98.0% and NMT 100.5% of busulfan ($C_6H_{14}O_6S_2$), calculated on the dried basis.

IDENTIFICATION
- **A.**
  Sample: 100 mg
  Analysis: Fuse the *Sample* with 100 mg of potassium nitrate and a pellet of potassium hydroxide weighing 250 mg. Cool, dissolve the residue in water, acidify with 3 N hydrochloric acid, and add a few drops of barium chloride TS.
  Acceptance criteria: A white precipitate is formed.
- **B.**
  Sample: 100 mg
  Analysis: Add 10 mL of water and 5 mL of 1 N sodium hydroxide to the *Sample*. Heat until a clear solution is obtained.
  Acceptance criteria: An odor characteristic of methanesulfonic acid is perceptible.
- **C.**
  Sample solution: Use the solution from the *Analysis* in *Identification* test *B*.
  Analysis: Cool the *Sample solution*, and divide it into two equal portions. To the first portion add 1 drop of potassium permanganate TS. Acidify the second portion of the solution with 2 N sulfuric acid, and add 1 drop of potassium permanganate TS.
  Acceptance criteria
  For first portion: The purple color changes to violet, then to blue, and finally to emerald-green.
  For second portion: The color of the permanganate is not discharged.

ASSAY
- **PROCEDURE**
  Sample solution: Transfer 80 mg of Busulfan into a 250-mL conical flask. Add 30 mL of water, swirl, add phenolphthalein TS, and neutralize with 0.05 N sodium hydroxide. Connect the flask to a reflux air condenser, and boil the mixture gently for NLT 30 min, adding water occasionally to maintain the volume. Cool to room temperature.
  Titrimetric system
  Mode: Direct titration
  Titrant: 0.05 N sodium hydroxide VS
  Endpoint detection: Visual
  Analysis: Add phenolphthalein TS to the *Sample solution*, and titrate with *Titrant*. Each mL of *Titrant* is equivalent to 6.158 mg of busulfan ($C_6H_{14}O_6S_2$).
  Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 115°–118°
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry a sample under vacuum at 60° to constant weight.
  Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The label bears a warning that great care should be taken to prevent inhaling particles of Busulfan and exposing the skin to it.

www.webofpharma.com

Busulfan Tablets

DEFINITION

Busulfan Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of busulfan ($C_6H_{14}O_6S_2$).

IDENTIFICATION

- **A.**

  Sample: A suitable number of Tablets

  Analysis: Pulverize the *Sample* and extract the powder with several portions of acetone. Evaporate the combined acetone extracts, with the aid of a current of air, on a steam bath.

  Acceptance criteria: The dry residue melts at about 115°.

- **B.**

  Sample: 100 mg of the powder obtained in *Identification* test *A*

  Analysis: Fuse the *Sample* with 100 mg of potassium nitrate and a pellet of potassium hydroxide weighing 250 mg. Cool, dissolve the residue in water, acidify with 3 N hydrochloric acid, and add a few drops of barium chloride TS.

  Acceptance criteria: A white precipitate is formed.

- **C.**

  Sample: 100 mg of the powder obtained in *Identification* test *A*

  Analysis: Add 10 mL of water and 5 mL of 1 N sodium hydroxide to the *Sample*. Heat until a clear solution is obtained.

  Acceptance criteria: An odor characteristic of methanesulfonic acid is perceptible.

- **D.**

  Sample solution: Use the solution from *Identification* test *C*.

  Analysis: Cool the *Sample solution*, and divide it into two equal portions. To the first portion add 1 drop of potassium permanganate TS. Acidify the second portion of the solution with 2 N sulfuric acid, and add 1 drop of potassium permanganate TS.

  Acceptance criteria

  For first portion: The purple color changes to violet, then to blue, and finally to emerald-green.

  For second portion: The color of the permanganate is not discharged.

ASSAY

- **PROCEDURE**

Guard against accidental inhalation of the fine powder.

Sample solution: Transfer an equivalent to 80 mg of busulfan, from finely powdered Tablets (NLT 40), to a 100-mL beaker. Extract with four 20-mL portions of acetone, each time stirring the mixture well. Allow the insoluble matter to settle, and decant the supernatant through a sintered-glass filter into a 250-mL conical flask. Evaporate the combined acetone extracts to about 10 mL, add phenolphthalein TS, and neutralize with 0.05 N sodium hydroxide. Evaporate to dryness, and add about 30 mL of water. Connect the flask to a reflux air condenser, and boil the mixture gently for NLT 30 min, adding water occasionally to maintain the volume. Cool to room temperature.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 N sodium hydroxide VS

Endpoint detection: Visual

Analysis: Add phenolphthalein TS to the *Sample solution*, and titrate with *Titrant*. Each mL of *Titrant* is equivalent to 6.158 mg of the labeled amount of busulfan ($C_6H_{14}O_6S_2$).

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩

Time: 30 min, the use of disks being omitted

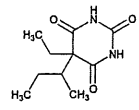
Acceptance criteria: Meet the requirements

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Butabarbital



$C_{10}H_{16}N_2O_3$ 212.25

2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-5-(1-methylpropyl)-;

5-*sec*-Butyl-5-ethylbarbituric acid [125-40-6].

DEFINITION

Butabarbital contains NLT 98.5% and NMT 101.0% of butabarbital ($C_{10}H_{16}N_2O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**

Internal standard solution: 2 mg/mL of tetracosane in chloroform

Standard stock solution: 2 mg/mL of USP Butabarbital RS in chloroform

Standard solution: 1 mg/mL of USP Butabarbital RS from *Standard stock solution* in *Internal standard solution* prepared as follows. Combine 10.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution*.

Sample stock solution: 2 mg/mL of Butabarbital in chloroform

Sample solution: 1 mg/mL of Butabarbital from *Sample stock solution* in *Internal standard solution* prepared as follows. Combine 10.0 mL of *Sample stock solution* and 10.0 mL of *Internal standard solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 4-mm × 1.8-m; 10% phase G37 on support S1AB

Temperatures

Injection port: 260°

Detector: 300°

Column: 260°

Carrier gas: Dry nitrogen

Flow rate: 50 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for butabarbital and tetracosane are 0.6 and 1.0, respectively.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 3.0 between butabarbital and tetracosane
Tailing factor: NMT 1.3 for butabarbital and NMT 1.2 for tetracosane
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of butabarbital ($C_{10}H_{16}N_2O_3$) in the portion of Butabarbital taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of butabarbital to the internal standard from the *Sample solution*
R_S = peak response ratio of butabarbital to the internal standard from the *Standard solution*
C_S = concentration of USP Butabarbital RS in the *Standard solution* (mg/mL)
C_U = concentration of Butabarbital in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the dried basis

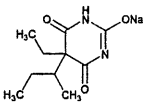
IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **ORGANIC IMPURITIES**
Diluent: Chloroform and methanol (50:50)
Standard solution A: 4.0 mg/mL of USP Butabarbital RS in *Diluent*
Standard solution B: 0.4 mg/mL of USP Butabarbital RS from *Standard solution A* in *Diluent*
Sample solution: 40 mg/mL of Butabarbital in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Acetone, methylene chloride, methanol, and ammonium hydroxide (5:3:1:1)
Spray reagent: A solution of mercurous nitrate dihydrate in 0.15 N nitric acid (1 in 100)
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Proceed as directed in the chapter. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and dry under a current of air. Spray the plate with *Spray reagent*, and immediately estimate the intensities of any spots of the *Sample solution*, other than the principal spot, in comparison with *Standard solution B*.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*; and the sum of the intensities of any secondary spots of the *Sample solution* is NMT the intensity of the principal spot produced by *Standard solution B*, corresponding to NMT a total of 1% of impurities.

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE,** *Class Ia* ⟨741⟩**:** 164°–167°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Butabarbital RS

Butabarbital Sodium



$C_{10}H_{15}N_2NaO_3$ 234.23
2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-5-(1-methylpropyl)-, monosodium salt;
Sodium 5-*sec*-butyl-5-ethylbarbiturate [143-81-7].

DEFINITION
Butabarbital Sodium contains NLT 98.2% and NMT 100.5% of butabarbital sodium ($C_{10}H_{15}N_2NaO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: 150 mg of Butabarbital Sodium
Analysis: Transfer the *Sample* to a suitable separator, dissolve in 10 mL of water, and add 15 mL of 3 N hydrochloric acid. Extract with three 20-mL portions of chloroform, filter the extracts through anhydrous sodium sulfate, and collect the extracts in a suitable beaker. Evaporate the combined extracts on a steam bath with the aid of a current of air to dryness, and dry the residue at 105° for 2 h.
Acceptance criteria: Meets the requirements

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Analytical wavelength: 240 nm
Buffer: pH 9.6 alkaline borate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)
Standard solution: 10 µg/mL of USP Butabarbital RS in *Buffer*
Sample solution: 10 µg/mL of Butabarbital Sodium in *Buffer*
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.
• **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩
Sample: 100 mg of Butabarbital Sodium
Analysis: Ignite the *Sample*, and proceed as directed in the chapter using the residue.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Buffer: pH 9.6 alkaline borate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)
Standard stock solution: 0.125 mg/mL of USP Butabarbital RS in *Buffer*
Standard solution: 0.0125 mg/mL of USP Butabarbital RS from *Standard stock solution* in *Buffer*
Sample stock solution: 0.140 mg/mL of Butabarbital Sodium in *Buffer*
Sample solution: 0.0140 mg/mL from *Sample stock solution* in *Buffer*

www.webofpharma.com

Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 240 nm
Blank: *Buffer*
Analysis
Samples: *Standard solution, Sample solution,* and *Blank*
Concomitantly determine the absorbances of the solutions. Calculate the percentage of butabarbital sodium ($C_{10}H_{15}N_2NaO_3$) in the portion of Butabarbital Sodium taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Butabarbital RS in the *Standard solution* (µg/mL)
C_U = concentration of Butabarbital Sodium in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of butabarbital sodium, 234.23
M_{r2} = molecular weight of butabarbital, 212.25

Acceptance criteria: 98.2%–100.5% on the dried basis

IMPURITIES
• **ORGANIC IMPURITIES**
Diluent: Chloroform and methanol (50:50)
Standard solution A: 4.0 mg/mL of USP Butabarbital RS in *Diluent*
Standard solution B: 0.4 mg/mL of USP Butabarbital RS from *Standard solution A* in *Diluent*
Sample solution: 44 mg/mL of Butabarbital Sodium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Acetone, methylene chloride, methanol, and ammonium hydroxide (5:3:1:1)
Spray reagent: A solution of mercurous nitrate dihydrate in 0.15 N nitric acid (1 in 100)
Analysis
Samples: *Standard solution A, Standard solution B,* and *Sample solution*
Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and dry under a current of air. Spray the plate with *Spray reagent,* and immediately estimate the intensities of any spots of the *Sample solution,* other than the principal spot, in comparison with *Standard solution B.*
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A;* and the sum of the intensities of any secondary spots of the *Sample solution* is NMT the intensity of the principal spot produced by *Standard solution B,* corresponding to NMT a total of 1% of impurities.

SPECIFIC TESTS
• **COMPLETENESS OF SOLUTION**
Sample solution: Dissolve 1.0 g of Butabarbital Sodium in 10 mL of carbon dioxide-free water.
Acceptance criteria: After 1 min, the solution is clear and free from undissolved solid.
• **pH** ⟨791⟩
Sample solution: Use the *Sample solution* from the test for *Completeness of Solution.*
Acceptance criteria: 10.0–11.2
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 150° to constant weight.
Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Butabarbital RS

Butabarbital Sodium Oral Solution

DEFINITION
Butabarbital Sodium Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of butabarbital sodium ($C_{10}H_{15}N_2NaO_3$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
Sample: Transfer an equivalent to 150 mg of butabarbital sodium from a volume of Oral Solution, to a separator. Render it distinctly alkaline by the addition of 1 N sodium hydroxide, and saturate it with sodium chloride. Extract the mixture with two 15-mL portions of ether, and discard the ether. Acidify the solution with hydrochloric acid, and render it just alkaline to litmus by adding small portions of sodium bicarbonate (carbonate-free). Extract the liberated acid barbiturate using five 20-mL portions of chloroform. Wash the combined chloroform extracts with 10 mL of water acidified with 1 drop of hydrochloric acid, then extract the water with 10 mL of chloroform, adding the latter to the main chloroform solution. Pass the chloroform solution through a pledget of cotton or other suitable filter, previously washed with chloroform, into a tared beaker, and finally wash the separator and the filter with three 5-mL portions of chloroform. Evaporate the combined chloroform solution and washings on a steam bath with the aid of a current of air to dryness, and dry the residue at 105° for 2 h.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the butabarbital peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Solution A: Dissolve 2.0 mL of bromine and 10 g of potassium bromide in 60 mL of water.
Solution B: Sodium metabisulfite in water (1 in 10)
Internal standard solution: 0.7 mg/mL of secobarbital in chloroform
Standard solution: 1 mg/mL of USP Butabarbital RS and 1.4 mg/mL of secobarbital in chloroform
Sample stock solution: Nominally 0.3 mg/mL of butabarbital sodium from Oral Solution prepared as follows. Transfer a volume of Oral Solution, equivalent to 30 mg of butabarbital sodium, to a separator. Add 1 mL of *Solution A,* and swirl. Allow to stand for 5 min, add 1 mL of *Solution B,* and swirl. Add 300 mg of sodium bicarbonate in small portions, with mixing, and extract with four 10-mL portions of chloroform. Pass the extracts through about 15

USP Monographs

www.webofpharma.com

g of anhydrous sodium sulfate that is supported on a funnel by a small pledget of glass wool. Collect the combined filtrates in a 50-mL volumetric flask, wash the sodium sulfate with 5 mL of chloroform, collecting the washing with the filtrate, dilute with chloroform to volume, and mix. [NOTE—This solution includes a bromination step for elimination of parabens and a carbonate–chloroform extraction for elimination of benzoic acid.]

Sample solution: Combine 2.0 mL of *Sample stock solution* with 2.0 mL of *Internal standard solution* in a suitable container, and reduce the volume to about 1 mL by evaporation, with the aid of a stream of dry nitrogen, at room temperature.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 4-mm × 0.9-m glass; packed with 3% liquid phase G10 support on 80- to 10-mesh S1A
Temperatures
Injection port: 225°
Detector: 225°
Column: 200 ± 10°
Carrier gas: A suitable gas such as dry nitrogen
Flow rate: 60–80 mL/min
Injection volume: 5 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for butabarbital and secobarbital are 0.6 and 1.0, respectively.]
Resolution: NLT 2.4 between butabarbital and secobarbital
Tailing factor: NMT 2.0 each for butabarbital and secobarbital
Relative standard deviation: NMT 1.5% for the peak response ratio of butabarbital to the internal standard

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of butabarbital sodium ($C_{10}H_{15}N_2NaO_3$) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of butabarbital to the internal standard from the *Sample solution*
R_S = peak response ratio of butabarbital to the internal standard from the *Standard solution*
C_S = concentration of USP Butabarbital RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of butabarbital sodium in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of butabarbital sodium, 234.23
M_{r2} = molecular weight of butabarbital, 212.25

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS
• **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩**:** Between 95.0% and 115.0% of the labeled amount of alcohol (C_2H_5OH)

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Butabarbital RS

Butabarbital Sodium Tablets

DEFINITION
Butabarbital Sodium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of butabarbital sodium ($C_{10}H_{15}N_2NaO_3$).

IDENTIFICATION
• **A.** The retention time of the butabarbital peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: Ammonium hydroxide in water (1 in 25)
Internal standard solution: 1.2 mg/mL of secobarbital in chloroform
Standard solution: 0.8 mg/mL of USP Butabarbital RS and 1 mg/mL of secobarbital in chloroform
Sample stock solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 50 mg of butabarbital sodium, to a 50-mL volumetric flask. Add 35 mL of *Solution A*, and dilute with water to volume. Filter, if necessary, discarding the first 15 mL of the filtrate, and transfer 25.0 mL of the clear solution to a separator. Add 2 mL of hydrochloric acid, and extract with three 25-mL portions of chloroform. Filter the extracts through about 15 g of anhydrous sodium sulfate that is supported on a funnel by a small pledget of glass wool. Collect the combined filtrate in a 100-mL volumetric flask, and wash the sodium sulfate with 15 mL of chloroform, collecting the washing with the filtrate. Dilute with chloroform to volume.
Sample solution: Combine 4.0 mL of *Sample stock solution* with 1.0 mL of *Internal standard solution* in a suitable container. Reduce the volume to about 1 mL by evaporation, with the aid of a stream of nitrogen, at room temperature.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 4-mm × 0.9-m glass; packed with 3% liquid phase G10 support on 80- to 10-mesh S1A
Temperatures
Injection port: 225°
Detector: 225°
Column: 200 ± 10°
Carrier gas: A suitable gas such as dry nitrogen
Flow rate: 60–80 mL/min
Injection volume: 5 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for butabarbital and secobarbital are 0.6 and 1.0, respectively.]
Resolution: NLT 2.4 between butabarbital and secobarbital
Tailing factor: NMT 2.0 each for butabarbital and secobarbital
Relative standard deviation: NMT 1.5% for the peak response ratio of butabarbital to the internal standard

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of butabarbital sodium ($C_{10}H_{15}N_2NaO_3$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of butabarbital to the internal standard from the *Sample solution*

www.webofpharma.com

R_S = peak response ratio of butabarbital to the internal standard from the *Standard solution*
C_S = concentration of USP Butabarbital RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of butabarbital sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of butabarbital sodium, 234.23
M_{r2} = molecular weight of butabarbital, 212.25

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: USP Butabarbital RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter, and mix with sufficient ammonium hydroxide to provide a concentration of 0.5 N ammonium hydroxide. Dilute with *Medium*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: 239 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of butabarbital sodium ($C_{10}H_{15}N_2NaO_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times D \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Butabarbital RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of butabarbital sodium in the *Sample solution* (mg/mL)
D = dilution factor for the *Sample solution*
M_{r1} = molecular weight of butabarbital sodium, 234.23
M_{r2} = molecular weight of butabarbital, 212.25

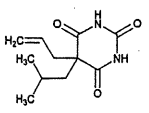
Tolerances: NLT 75% (Q) of the labeled amount of butabarbital sodium ($C_{10}H_{15}N_2NaO_3$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Butabarbital RS

Butalbital



$C_{11}H_{16}N_2O_3$ 224.26
2,4,6(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-(2-methylpropyl)-5-(2-propenyl)-;
5-Allyl-5-isobutylbarbituric acid [77-26-9].

DEFINITION

Butalbital contains NLT 98.0% and NMT 102.0% of butalbital ($C_{11}H_{16}N_2O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**
Buffer: 1.0 mL of phosphoric acid diluted with water to 1 L
Mobile phase: Acetonitrile and *Buffer* (25:75)
System suitability solution: 0.1 mg/mL each of USP Butalbital RS and USP Salicylic Acid RS in *Mobile phase*. Sonication may be used to aid in dissolution.
Standard solution: 0.1 mg/mL of USP Butalbital RS in *Mobile phase*. Sonication may be used to aid in dissolution.
Sample solution: 0.1 mg/mL of Butalbital in *Mobile phase*. Sonication may be used to aid in dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times of salicylic acid and butalbital are 0.86 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between salicylic acid and butalbital, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of butalbital ($C_{11}H_{16}N_2O_3$) in the portion of Butalbital taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Butalbital RS in the *Standard solution* (mg/mL)
C_U = concentration of Butalbital in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%
• **Organic Impurities**
Buffer: 4.1 g/L of monobasic potassium phosphate adjusted with 1 N sodium hydroxide to a pH of 6.0
Mobile phase: Acetonitrile and *Buffer* (22:78)
System suitability solution: 10 µg/mL each of USP Butalbital RS and USP Butabarbital RS in *Mobile phase*. Sonication may be used to aid in dissolution.
Sample solution: 1 mg/mL of Butalbital in *Mobile phase*. Sonication may be used to aid in dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm

USP Monographs

www.webofpharma.com

Column: 4.6-mm × 15-cm; 5-µm packing L78
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times of butabarbital and butalbital are 0.83 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between butabarbital and butalbital
Tailing factor: NMT 1.5 for butalbital
Relative standard deviation: NMT 5.0% for butalbital
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Butalbital taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the peak responses from the *Sample solution*

Acceptance criteria
Any individual unspecified impurity: NMT 0.10%
Total impurities: NMT 1%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at room temperature to constant weight.
Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Butabarbital RS
USP Butalbital RS
USP Salicylic Acid RS

Butalbital, Acetaminophen, and Caffeine Capsules

» Butalbital, Acetaminophen, and Caffeine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$), acetaminophen ($C_8H_9NO_2$), and caffeine ($C_8H_{10}N_4O_2$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Acetaminophen RS
USP Butalbital RS
USP Caffeine RS
Identification—The retention times of the butalbital, acetaminophen, and caffeine peaks in the chromatogram of the *Assay preparation* correspond to those of the butalbital, acetaminophen, and caffeine peaks in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Time: 60 minutes.
Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay* under *Butalbital, Acetaminophen, and Caffeine Tablets.*
Standard preparation—Prepare a solution in methanol having known concentrations of about 0.02*A* mg of USP Acetaminophen RS per mL, 0.02*B* mg of USP Butalbital RS per mL, and 0.02*C* mg of USP Caffeine RS per mL, in which *A*, *B*, and *C* are the labeled amounts, in mg of acetaminophen, butalbital, and caffeine, respectively, per Capsule. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Pass a portion of the solution under test through a filter of 10-µm or finer porosity. Separately inject equal volumes (about 20 µL) of the filtrate and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of butalbital ($C_{11}H_{16}N_2O_3$), acetaminophen ($C_8H_9NO_2$), and caffeine ($C_8H_{10}N_4O_2$) dissolved by the same formula:

$$900C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation;* and r_U and r_S are the peak responses of the corresponding analyte obtained from the solution under test and the *Standard preparation*, respectively.
Tolerances—Not less than 80% *(Q)* of the labeled amounts of $C_{11}H_{16}N_2O$, $C_8H_9NO_2$, and $C_8H_{10}N_4O_2$ is dissolved in 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase, Internal standard solution, Butalbital standard stock solution, Caffeine standard stock solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Butalbital, Acetaminophen, and Caffeine Tablets*
Assay preparation—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about the weight of the contents of 1 Capsule, to a 200-mL volumetric flask, add *Internal standard solution* to volume, and mix. Sonicate for 15 minutes, mix, and allow to cool and settle. Transfer 20.0 mL of the clear supernatant to a 50-mL volumetric flask, dilute with water to volume, and mix. Pass a portion of this solution through a filter of 0.5 µm or finer porosity, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation.*
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the major peaks. Calculate the quantities, in mg, of butalbital ($C_{11}H_{16}N_2O_3$), acetaminophen ($C_8H_9NO_2$), and caffeine ($C_8H_{10}N_4O_2$) in the portion of Capsules taken by the formula:

$$500D(R_U/R_S)$$

in which D is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation;* and R_U and R_S are the peak response ratios of the corresponding analyte to phenacetin obtained from the *Assay preparation* and the *Standard preparation*, respectively.

www.webofpharma.com

Butalbital, Acetaminophen, and Caffeine Tablets

» Butalbital, Acetaminophen, and Caffeine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$), acetaminophen ($C_8H_9NO_2$), and caffeine ($C_8H_{10}N_4O_2$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Acetaminophen RS
USP Butalbital RS
USP Caffeine RS
Identification—The retention times of the butalbital peak, the acetaminophen peak, and the caffeine peak in the chromatogram of the *Assay preparation* correspond to those of the butalbital peak, the acetaminophen peak, and the caffeine peak in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay.*
Standard preparation—Prepare a solution in methanol having known concentrations of about 0.02*A* mg of USP Acetaminophen RS per mL, 0.02*B* mg of USP Butalbital RS per mL, and 0.02*C* mg of USP Caffeine RS per mL, in which *A, B,* and *C* are the labeled amounts, in mg, of acetaminophen, butalbital, and caffeine, respectively, per Tablet. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Pass a portion of the solution under test through a suitable filter having a 10-µm or finer porosity. Separately inject equal volumes (about 20 µL) of the filtrate and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of butalbital ($C_{11}H_{16}N_2O_3$), acetaminophen ($C_8H_9NO_2$), and caffeine ($C_8H_{10}N_4O_2$) dissolved by the same formula:

$$900C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation;* and r_U and r_S are the peak responses of the corresponding analyte obtained from the solution under test and the *Standard preparation,* respectively.
Tolerances—Not less than 80% *(Q)* of the labeled amounts of $C_{11}H_{16}N_2O_3$, $C_8H_9NO_2$, and $C_8H_{10}N_4O_2$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Assay—
Mobile phase—Transfer 800 mg of monobasic potassium phosphate to a 2000-mL volumetric flask. Dissolve in 1100 mL of water, dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Prepare a solution of phenacetin in methanol containing 0.65 mg per mL.
Butalbital standard stock solution—Dissolve an accurately weighed quantity of USP Butalbital RS in *Internal standard solution* to obtain a solution having a known concentration of about 0.01*B* mg per mL, *B* being the labeled amount, in mg, of butalbital per Tablet, sonicating and shaking the solution, if necessary, to achieve complete dissolution.
Caffeine standard stock solution—Dissolve an accurately weighed quantity of USP Caffeine RS in *Internal standard solution* to obtain a solution having a known concentration of about 0.01*C* mg per mL, *C* being the labeled amount, in mg, of caffeine per Tablet, sonicating and shaking the solution, if necessary, to achieve complete dissolution.
Standard preparation—Transfer to a 50-mL volumetric flask about 0.1*A* mg of USP Acetaminophen RS, *A* being the labeled amount, in mg, of acetaminophen per Tablet, 10.0 mL of *Butalbital standard stock solution,* and 10.0 mL of *Caffeine standard stock solution,* sonicate for 5 minutes, dilute with water to volume, and mix. This solution contains about 0.002*B* mg of butalbital, 0.002*A* mg of acetaminophen, and 0.002*C* mg of caffeine per mL. Pass a portion of this solution through a suitable filter having a 0.5-µm or finer porosity, and use the filtrate as the *Standard preparation.*
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 average Tablet weight, to a 200-mL volumetric flask, add *Internal standard solution* to volume, and mix. Sonicate for 15 minutes, mix, and allow to cool and settle. Transfer 20.0 mL of the clear supernatant to a 50-mL volumetric flask, dilute with water to volume, and mix. Pass a portion of this solution through a suitable filter having a 0.5-µm or finer porosity, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 216-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.16 for acetaminophen, 0.33 for caffeine, 0.77 for phenacetin, and 1.0 for butalbital; the resolution, *R,* between any two peaks is not less than 1.2; the column efficiency, calculated from the butalbital peak, is not less than 1000 theoretical plates; and the relative standard deviations of the acetaminophen, caffeine, and butalbital responses for replicate injections are not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the major peaks. Calculate the quantities, in mg, of butalbital ($C_{11}H_{16}N_2O_3$), acetaminophen ($C_8H_9NO_2$), and caffeine ($C_8H_{10}N_4O_2$) in the portion of Tablets taken by the same formula:

$$500D(R_U/R_S)$$

in which D is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation;* and R_U and R_S are the peak response ratios of the corresponding analyte to phenacetin obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Butalbital and Aspirin Tablets

DEFINITION
Butalbital and Aspirin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$) and aspirin ($C_9H_8O_4$).

USP Monographs

www.webofpharma.com

IDENTIFICATION

• **A.** The retention times of the butalbital and aspirin peaks of the *Sample solution* correspond to that of the butalbital peak of *Standard solution A* and the aspirin peak of *Standard solution B*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, water, and phosphoric acid (725:3100:4)

Diluent: Add 10 mL of formic acid to each L of acetonitrile.

System suitability solution: 260*J* µg/mL of USP Butalbital RS and 12 µg/mL of USP Salicylic Acid RS in *Diluent*, where *J* is the ratio of the labeled amount of butalbital, in mg/Tablet, relative to the labeled amount of aspirin, in mg/Tablet

Standard solution A: 325*J* µg/mL of USP Butalbital RS and 2.4 µg/mL of USP Salicylic Acid RS in *Diluent*, where *J* is the ratio of the labeled amount of butalbital, in mg/Tablet, relative to the labeled amount of aspirin, in mg/Tablet

Standard solution B: 325 µg/mL of USP Aspirin RS in *Diluent*

Sample solution: Nominally 320 µg/mL of aspirin from a suitable amount of powdered Tables in solution prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer a portion of this fine powder to a suitable volumetric flask. Dilute with *Diluent* to volume, and sonicate for 15 min. Pass a portion of the solution through a filter of 0.5-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution*, *Standard solution A*, and *Standard solution B*

[NOTE—The relative retention times for aspirin, salicylic acid, and butalbital are about 0.6, 0.85, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between salicylic acid and butalbital, *System suitability solution*

Relative standard deviation

Standard solution A: NMT 3.0% for butalbital; NMT 6.0% for salicylic acid

Standard solution B: NMT 3.0% for aspirin

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of butalbital ($C_{11}H_{16}N_2O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of butalbital from the *Sample solution*

r_S = peak response of butalbital from *Standard solution A*

C_S = concentration of USP Butalbital RS in *Standard solution A* (µg/mL)

C_U = nominal concentration of butalbital in the *Sample solution* (µg/mL)

Calculate the percentage of aspirin ($C_9H_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aspirin from the *Sample solution*

r_S = peak response of aspirin from *Standard solution B*

C_S = concentration of USP Aspirin RS in *Standard solution B* (µg/mL)

C_U = nominal concentration of aspirin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$) and aspirin ($C_9H_8O_4$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 60 min

Butalbital

Mobile phase: Acetonitrile, water, and phosphoric acid (725:3100:4).

Standard solution: *L* µg/mL of USP Butalbital RS and 30 µg/mL of salicylic acid in *Mobile phase*, where *L* is the labeled amount of butalbital, in mg/Tablet

Sample solution: Use portions of the solution under test passed through a suitable filter of 0.5-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10–25 µL; equal volumes of the *Standard solution* and *Sample solution*

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for aspirin, salicylic acid, and butalbital are about 0.6, 0.85, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between salicylic acid and butalbital

Relative standard deviation: NMT 3.0% for butalbital

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of butalbital ($C_{11}H_{16}N_2O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of butalbital from the *Sample solution*

r_S = peak response of butalbital from the *Standard solution*

C_S = concentration of USP Butalbital RS in the *Standard solution* (µg/mL)

V = volume of *Medium*, 900 mL

L = label claim of butalbital (mg/Tablet)

Aspirin

Buffer: Dissolve 5.98 g of sodium acetate trihydrate in 500 mL of water, add 2.5 mL of glacial acetic acid, dilute with water to 1000 mL, and adjust with glacial acetic acid to a pH of 4.5.

Sample solution: Use a filtered portion of the solution under test diluted with 4 volumes of *Buffer*.

Standard solution: A known concentration of USP Aspirin RS in water diluted with 4 volumes of *Buffer*. Prepare the *Standard solution* at the time of use.

[NOTE—An amount of alcohol not to exceed 1% of the total volume of the *Standard solution* may be used to bring the Reference Standard into solution before

www.webofpharma.com

dilution first with water and then with 4 volumes of *Buffer*.]

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: The isosbestic point of aspirin and salicylic acid at 265 ± 2 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of aspirin ($C_9H_8O_4$) dissolved.

Tolerances: NLT 75% (Q) of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$) and aspirin ($C_9H_8O_4$) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Analysis: Proceed as directed in the chapter.

Acceptance criteria

Butalbital: Meet the requirements for *Content Uniformity*

Aspirin: Meet the requirements for *Weight Variation*

IMPURITIES

• **LIMIT OF FREE SALICYLIC ACID**

Mobile phase, System suitability solution, Standard solution A, Standard solution B, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution A* and *Sample solution*

Calculate the percentage of free salicylic acid in the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*

r_S = peak response of salicylic acid from *Standard solution A*

C_S = concentration of USP Salicylic Acid RS in *Standard solution A* (µg/mL)

C_U = nominal concentration of aspirin in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 3.0% of free salicylic acid

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Aspirin RS

USP Butalbital RS

USP Salicylic Acid RS

Butalbital, Aspirin, and Caffeine Capsules

DEFINITION

Butalbital, Aspirin, and Caffeine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$).

IDENTIFICATION

• **A.** The retention times of the butalbital, aspirin, and caffeine peaks of the *Sample solution* correspond to those of the butalbital, aspirin, and caffeine peaks of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 1.36 g/L of monobasic potassium phosphate in water

Mobile phase: Methanol and *Buffer* (45:55) initially adjusted with phosphoric acid to a pH of 3.9. If the retention time of the salicylic acid peak differs from that of the aspirin peak, adjust the pH of the *Mobile phase* with 0.2 N potassium hydroxide or 1 M phosphoric acid so that the salicylic acid peak has the same retention time as that of the aspirin peak. [NOTE—The retention time of the salicylic acid peak decreases about 0.3 min for each 0.1 pH increase. The retention time of the aspirin peak is essentially unaffected by such pH adjustments.]

Diluent: Methanol and *Buffer* (45:55) adjusted with phosphoric acid to a pH of 2.5 ± 0.05.

Salicylic acid solution: 0.1 mg/mL of salicylic acid in *Diluent*. Pass this solution through a suitable filter of 0.5-µm or finer pore size.

Standard stock solution: 1.6 mg/mL of USP Aspirin RS in *Diluent*. Sonication and shaking may be used to promote dissolution. Use this solution within 24 h.

Standard solution: USP Reference Standards in *Standard stock solution* as listed below. Sonication and shaking the solution may be used to promote dissolution. Use this solution within 24 h.

Butalbital: 1.6*J* mg/mL of USP Butalbital RS, where *J* is the ratio of the labeled amount, in mg, of butalbital relative to the labeled amount of aspirin in mg/Capsule

Caffeine: 1.6*J′* mg/mL of USP Caffeine RS, where *J′* is the ratio of the labeled amount, in mg, of caffeine relative to the labeled amount of aspirin in mg/Capsule

Sample solution: Nominally 1.6 mg/mL of aspirin from the contents of Capsules in solution prepared as follows. Transfer a suitable portion of the contents of NLT 20 Capsules to an appropriate volumetric flask. Dilute with *Diluent* to volume, and sonicate for 30 min. Pass a portion of this solution through a suitable filter of 0.5-µm or finer pore size. Use the filtrate within 24 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detectors

Butalbital: UV 210 nm

Aspirin and caffeine: UV at the wavelength of the isosbestic point of aspirin and salicylic acid at about 277 nm

Column: 3.9-mm × 30-cm; packing L1

Column temperature: 35 ± 1°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Salicylic acid solution* and *Standard solution*

[NOTE—The relative retention times for caffeine, aspirin, salicylic acid, and butalbital are about 0.45, 0.6, 0.6, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between caffeine and aspirin, *Standard solution*

Column efficiency: NLT 2000 theoretical plates from butalbital, *Standard solution*

Relative standard deviation: NMT 2.0% each for caffeine, aspirin, and butalbital responses, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$) and caffeine ($C_8H_{10}N_4O_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

www.webofpharma.com

r_U = peak response of butalbital or caffeine from the *Sample solution*
r_S = peak response of butalbital or caffeine from the *Standard solution*
C_S = concentration of USP Butalbital RS or USP Caffeine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of butalbital or caffeine in the *Sample solution* (mg/mL)

Determine the amount, in mg, of aspirin and salicylic acid in the portion of Capsules taken (*W*):

$$\text{Result} = (r_U/r_S) \times C_S \times V$$

r_U = peak response of aspirin and salicylic acid from the *Sample solution*
r_S = peak response of aspirin and salicylic acid from the *Standard solution*
C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)
V = volume of the *Sample solution* (mL)

Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Capsules taken:

$$\text{Result} = \{W - [(F/100) \times W]\}/(C_U \times V) \times 100$$

W = amount of aspirin and salicylic acid in the portion of Capsules taken to prepare the *Sample solution* (mg)
F = percentage of salicylic acid obtained in the *Limit of Free Salicylic Acid* procedure (%)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)
V = volume of the *Sample solution* (mL)

Acceptance criteria: 90.0%–110.0% each of butalbital, aspirin, and caffeine

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: Water; 1000 mL
Apparatus 2: 50 rpm
Time: 60 min
Buffer, Mobile phase, Diluent, Salicylic acid solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: Use a portion of solution under test.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Limit of Free Salicylic Acid**
Use glassware throughout this procedure. Perform this procedure on the same day the powder is removed from the Capsules.
Diluent: Add 1 mL of phosphoric acid to each L of methanol.
Standard solution: 0.0012 mg/mL of USP Salicylic Acid RS in *Diluent*. Use this solution promptly.
Sample solution: Nominally 0.65 mg/mL of aspirin from the contents of Capsules in solution prepared as follows. Transfer a suitable portion of the contents of NLT 20 Capsules, equivalent to about 65 mg of aspirin, to an appropriate container. Add 100.0 mL of *Diluent*, and shake for 1 min. Promptly filter a portion of this solution, discarding the first 15 mL of the filtrate, and use the clear filtrate within 20 min after the addition of the *Diluent*. If the intensity of the *Sample solution* greatly exceeds that of the *Standard solution*, the solution may be suitably diluted with *Diluent*.
Instrumental conditions
Mode: Fluorescence
Excitation wavelength: 305 nm
Emission wavelength: 444 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the *Samples* to equilibrate for 2 min in the fluorimeter.
Calculate the percentage of salicylic acid in the portion of Capsules taken (*F*):

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence intensity readings from the *Sample solution*
I_S = fluorescence intensity readings from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.5% of salicylic acid

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Aspirin RS
USP Butalbital RS
USP Caffeine RS
USP Salicylic Acid RS

Butalbital, Aspirin, and Caffeine Tablets

DEFINITION

Butalbital, Aspirin, and Caffeine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$).

IDENTIFICATION

• **A.** The retention times of the butalbital, aspirin, and caffeine peaks of the *Sample solution* correspond to those of the butalbital, aspirin, and caffeine peaks of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**
Buffer: 1.36 g/L of monobasic potassium phosphate in water
Mobile phase: Methanol and *Buffer* (45:55) initially adjusted with phosphoric acid to a pH of 3.9. If the retention time of the salicylic acid peak differs from that of the aspirin peak, adjust the pH of the *Mobile phase* with 0.2 N potassium hydroxide or 1 M phosphoric acid so that the salicylic acid peak has the same retention time as that of the aspirin peak. [Note—The retention time of the salicylic acid peak decreases about 0.3 min for each 0.1 pH increase. The retention time of the aspirin peak is essentially unaffected by such pH adjustments.]

www.webofpharma.com

Diluent: Methanol and *Buffer* (45:55) adjusted with phosphoric acid to a pH of 2.5 ± 0.05.
Salicylic acid solution: 0.1 mg/mL of salicylic acid in *Diluent*. Pass this solution through a suitable filter of 0.5-µm or finer pore size.
Standard stock solution: 1.6 mg/mL of USP Aspirin RS in *Diluent*. Sonication and shaking may be used to aid in dissolution. Use this solution within 24 h.
Standard solution: USP Reference Standards in *Standard stock solution* as listed below. Sonication and shaking the solution may be used to promote dissolution. Use this solution within 24 h.
Butalbital: 1.6*J* mg/mL of USP Butalbital RS, where *J* is the ratio of the labeled amount, in mg, of butalbital relative to the labeled amount of aspirin, in mg/Tablet
Caffeine: 1.6*J′* mg/mL of USP Caffeine RS, where *J′* is the ratio of the respective labeled amount, in mg, of caffeine relative to the labeled amount of aspirin in mg/Tablet
Sample solution: Nominally 1.6 mg/mL of aspirin from a suitable amount of powdered Tablets in solution prepared as follows. Finely powder NLT 20 Tablets, and transfer a portion of this fine powder to an appropriate volumetric flask. Dilute with *Diluent* to volume, and sonicate for 30 min. Pass a portion of this solution through a suitable filter of 0.5-µm or finer pore size, and use the filtrate within 24 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Butalbital: UV 210 nm
Aspirin and caffeine: UV at the wavelength of the isosbestic point of aspirin and salicylic acid at about 277 nm
Column: 3.9-mm × 30-cm; packing L1
Column temperature: 35 ± 1°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Salicylic acid solution* and *Standard solution*
[NOTE—The relative retention times for caffeine, aspirin, salicylic acid, and butalbital are about 0.45, 0.6, 0.6, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between caffeine and aspirin, *Standard solution*
Column efficiency: NLT 2000 theoretical plates from butalbital, *Standard solution*
Relative standard deviation: NMT 2.0% each for caffeine, aspirin, and butalbital responses, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$) and caffeine ($C_8H_{10}N_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of butalbital or caffeine from the *Sample solution*
r_S = peak response of butalbital or caffeine from the *Standard solution*
C_S = concentration of USP Butalbital RS or USP Caffeine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of butalbital or caffeine in the *Sample solution* (mg/mL)

Determine the amount, in mg, of aspirin and salicylic acid in the portion of Tablets taken (*W*):

$$\text{Result} = (r_U/r_S) \times C_S \times V$$

r_U = peak response of aspirin and salicylic acid from the *Sample solution*
r_S = peak response of aspirin and salicylic acid from the *Standard solution*
C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)
V = volume of the *Sample solution* (mL)

Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Tablets taken:

$$\text{Result} = \{W - [(F/100) \times W]\}/(C_U \times V) \times 100$$

W = amount of aspirin and salicylic acid in the portion of Tablets taken to prepare the *Sample solution* (mg)
F = percentage of salicylic acid obtained in the *Limit of Free Salicylic Acid* procedure (%)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)
V = volume of the *Sample solution* (mL)

Acceptance criteria: 90.0%–110.0% each of butalbital, aspirin, and caffeine

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 60 min
Buffer, Mobile phase, Diluent, Salicylic acid solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Sample solution: Use a portion of solution under test.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **LIMIT OF FREE SALICYLIC ACID**
Use glassware throughout this procedure. Perform this procedure on the same day the Tablets are powdered.
Diluent: Add 1 mL of phosphoric acid to each L of methanol.
Standard solution: 0.0012 mg/mL of USP Salicylic Acid RS in *Diluent*. Use this solution promptly.
Sample solution: Nominally 0.65 mg/mL of aspirin from a suitable amount of powdered Tablets in solution prepared as follows. Finely powder NLT 20 Tablets, and transfer a suitable portion of fine powder, equivalent to 65 mg of aspirin, to an appropriate container. Add 100.0 mL of *Diluent*, and shake by mechanical means for 15 min. Filter a portion of this solution, discarding the first 15 mL of the filtrate, and use the clear filtrate within 20 min after the addition of the *Diluent*. If the intensity of the *Sample solution* greatly exceeds that of the *Standard solution*, the solution may be suitably diluted with *Diluent*.
Instrumental conditions
Mode: Fluorescence

USP Monographs

www.webofpharma.com

Excitation wavelength: 305 nm
Emission wavelength: 444 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the *Samples* to equilibrate for 2 min in the fluorimeter.

Calculate the percentage of salicylic acid in the portion of Tablets taken (*F*):

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence intensity readings from the *Sample solution*

I_S = fluorescence intensity readings from the *Standard solution*

C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0% of salicylic acid

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Aspirin RS
USP Butalbital RS
USP Caffeine RS
USP Salicylic Acid RS

Butalbital, Aspirin, Caffeine, and Codeine Phosphate Capsules

DEFINITION

Butalbital, Aspirin, Caffeine, and Codeine Phosphate Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$), aspirin ($C_9H_8O_4$), caffeine ($C_8H_{10}N_4O_2$), and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$).

IDENTIFICATION

- **A.** The retention times of the butalbital, aspirin, caffeine, and codeine peaks of the *Sample solution* correspond to those of the butalbital, aspirin, caffeine, and codeine peaks of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 1.36 g/L of monobasic potassium phosphate in water

Mobile phase: Methanol and *Buffer* (45:55) initially adjusted with phosphoric acid to a pH of 3.9. If the retention time of the salicylic acid peak differs from that of the aspirin peak, adjust the pH of the *Mobile phase* with 0.2 N potassium hydroxide or 1 M phosphoric acid so that the salicylic acid peak has the same retention time as that of the aspirin peak. [NOTE—The retention time of the salicylic acid peak decreases about 0.3 min for each 0.1 pH increase. The retention time of the aspirin peak is essentially unaffected by such pH adjustments.]

Diluent: Methanol and *Buffer* (45:55) adjusted with phosphoric acid to a pH of 2.5 ± 0.05.

Salicylic acid solution: 0.1 mg/mL of salicylic acid in *Diluent*. Pass this solution through a suitable filter of 0.5-µm or finer pore size.

Standard stock solution: 1.6 mg/mL of USP Aspirin RS in *Diluent*. Sonication and shaking may be used to promote dissolution. Use this solution within 24 h.

Standard solution: USP Reference Standards in *Standard stock solution* as listed below. Sonication and shaking the solution may be used to promote dissolution. Use this solution within 24 h.

Butalbital: 1.6*J* mg/mL of USP Butalbital RS, where *J* is the ratio of the labeled amount of butalbital relative to the labeled amount of aspirin in mg/Capsule

Caffeine: 1.6*J′* mg/mL of USP Caffeine RS, where *J′* is the ratio of the labeled amount of caffeine relative to the labeled amount of aspirin in mg/Capsule

Codeine phosphate: 1.6*J″* mg/mL of USP Codeine Phosphate RS, where *J″* is the ratio of the labeled amount of codeine phosphate relative to the labeled amount of aspirin in mg/Capsule

Sample solution: Nominally 1.6 mg/mL of aspirin from the contents of Capsules in solution prepared as follows. Transfer a suitable portion of the contents of NLT 20 Capsules to an appropriate volumetric flask. Dilute with *Diluent* to volume, and sonicate for 30 min. Pass a portion of this solution through a suitable filter of 0.5-µm pore size, and use the filtrate within 24 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector

Butalbital and codeine: UV 210 nm

Caffeine and aspirin: UV at the wavelength of the isosbestic point of aspirin and salicylic acid at about 277 nm

Column: 3.9-mm × 30-cm; packing L1

Column temperature: 35 ± 1°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Salicylic acid solution* and *Standard solution*

[NOTE—The relative retention times for codeine, caffeine, aspirin, salicylic acid, and butalbital are about 0.3, 0.45, 0.6, 0.6, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between caffeine and aspirin, *Standard solution*

Column efficiency: NLT 2000 theoretical plates from butalbital, *Standard solution*

Relative standard deviation: NMT 2.0% each for codeine, caffeine, aspirin, and butalbital responses, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$) and caffeine ($C_8H_{10}N_4O_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of butalbital or caffeine from the *Sample solution*

r_S = peak response of butalbital or caffeine from the *Standard solution*

C_S = concentration of USP Butalbital RS or USP Caffeine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of butalbital or caffeine in the *Sample solution* (mg/mL)

Determine the amount of aspirin and salicylic acid in the portion of Capsules taken (*W*):

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times C_S \times V$$

r_U = peak response of aspirin and salicylic acid from the *Sample solution*
r_S = peak response of aspirin and salicylic acid from the *Standard solution*
C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)
V = volume of the *Sample solution* (mL)

Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Capsules taken:

$$\text{Result} = \{W - [(F/100) \times W]\}/(C_U \times V) \times 100$$

W = amount of aspirin and salicylic acid in the portion of Capsules taken to prepare the *Sample solution* (mg)
F = percentage of salicylic acid obtained in the *Limit of Free Salicylic Acid* procedure (%)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)
V = volume of the *Sample solution* (mL)

Calculate the percentage of the labeled amount of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of codeine from the *Sample solution*
r_S = peak response of codeine from the *Standard solution*
C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of codeine phosphate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of codeine phosphate hemihydrate, 406.37
M_{r2} = molecular weight of codeine phosphate anhydrous, 397.37

Acceptance criteria: 90.0%–110.0% each of butalbital, aspirin, caffeine, and codine phosphate

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 1000 mL
Apparatus 2: 50 rpm
Time: 60 min
Buffer, Mobile phase, and **Diluent:** Prepare as directed in the *Assay*.
Salicylic acid solution: 0.01 mg/mL of salicylic acid in *Diluent*. Pass this solution through a suitable filter of 0.5-µm or finer pore size.
Standard stock solution: 0.16 mg/mL of USP Aspirin RS in a mixture of *Diluent* and *Medium* (50:50). Use this solution within 24 h.
Standard solution: USP Reference Standards in *Standard stock solution* as listed below. Sonication and shaking the solution may be used to promote dissolution. Pass a portion of the resulting solution through a suitable filter of 0.5-µm or finer pore size. Use this solution within 24 h.
Butalbital: 0.16J mg/mL of USP Butalbital RS, where J is the ratio of the labeled amount of butalbital relative to the labeled amount of aspirin in mg/Capsule
Caffeine: 0.16J' mg/mL of USP Caffeine RS, where J' is the ratio of the labeled amount of caffeine relative to the labeled amount of aspirin in mg/Capsule
Codeine phosphate: 0.16J'' mg/mL of USP Codeine Phosphate RS, where J'' is the ratio of the labeled amount of codeine phosphate relative to the labeled amount of aspirin in mg/Capsule
Sample stock solution: Pass 20 mL of the solution under test through a suitable filter of 0.5-µm or finer pore size, discarding the first 2 mL of the filtrate.
Sample solution: A portion of the *Sample stock solution* diluted with an equal volume *Diluent*
Chromatographic system and **System suitability:** Proceed as directed in the *Assay*, except use an *Injection volume* of 100 µL.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times 100$$

r_U = peak response of the butalbital, aspirin, or caffeine from the *Sample solution*
r_S = peak response of the butalbital, aspirin, or caffeine from the *Standard solution*
C_S = concentration of USP Butalbital RS, USP Aspirin RS, or USP Caffeine RS in the *Standard solution* (mg/mL)
V = volume of the *Medium*, 1000 mL

Calculate the percentage of the labeled amount of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of codeine from the *Sample solution*
r_S = peak response of codeine from the *Standard solution*
C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)
V = volume of the *Medium*, 1000 mL
M_{r1} = molecular weight of codeine phosphate hemihydrate, 406.37
M_{r2} = molecular weight of codeine phosphate anhydrous, 397.37

Tolerances: NLT 75% (Q) of the labeled amounts of butalbital ($C_{11}H_{16}N_2O_3$), aspirin ($C_9H_8O_4$), caffeine ($C_8H_{10}N_4O_2$), and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **LIMIT OF FREE SALICYLIC ACID**

Use glassware throughout this procedure. Perform this procedure on the same day the powder is removed from the Capsules.
Diluent: To each L of methanol add 1 mL of phosphoric acid.
Standard solution: 0.0012 mg/mL of USP Salicylic Acid RS in *Diluent*. Use this solution promptly.
Sample solution: Nominally 0.65 mg/mL of aspirin from the contents of Capsules in solution prepared as follows. Transfer a suitable portion of the contents of NLT 20 Capsules, equivalent to about 65 mg of aspirin, to an appropriate container. Add 100.0 mL of *Diluent*, and shake for 1 min. Promptly filter a portion of this solution, discarding the first 15 mL of the filtrate, and use the clear filtrate within 20 min after the addition of the *Diluent*. If the

USP Monographs

www.webofpharma.com

intensity of the *Sample solution* greatly exceeds that of the *Standard solution*, the solution may be suitably diluted with *Diluent*.

Instrumental conditions
Mode: Fluorescence
Excitation wavelength: 305 nm
Emission wavelength: 444 nm

Analysis
Samples: *Standard solution* and *Sample solution*
Allow the *Samples* to equilibrate for 2 min in the fluorimeter.
Calculate the percentage of salicylic acid in the portion of Capsules taken (*F*):

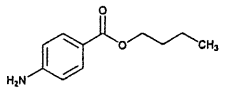
$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence intensity readings from the *Sample solution*
I_S = fluorescence intensity readings from the *Standard solution*
C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0% of salicylic acid

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Aspirin RS
  USP Butalbital RS
  USP Caffeine RS
  USP Codeine Phosphate RS
  USP Salicylic Acid RS

Butamben



$C_{11}H_{15}NO_2$ 193.24
Benzoic acid, 4-amino-, butyl ester;
Butyl *p*-aminobenzoate [94-25-7].

DEFINITION
Butamben contains NLT 98.0% and NMT 102.0% of butamben ($C_{11}H_{15}NO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: Add 1.0 mL of 98 percent formic acid to 1 L of water, and mix well.
Solution B: Add 1.0 mL of 98 percent formic acid to 1 L of acetonitrile, and mix well.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
1.0	90	10
6.0	50	50
8.5	50	50
8.6	90	10
10.0	90	10

Standard solution: 0.2 mg/mL of USP Butamben RS prepared as follows. Transfer a suitable quantity of USP Butamben RS to an appropriate volumetric flask. Add 10% of the total flask volume of acetonitrile to dissolve, then dilute with water to volume.
Sample solution: 0.2 mg/mL of Butamben prepared as follows. Transfer a suitable quantity of Butamben to an appropriate volumetric flask. Add 10% of the total flask volume of acetonitrile to dissolve, then dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 2.1-mm × 10-cm; 5-µm packing L1
Flow rate: 0.4 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of butamben ($C_{11}H_{15}NO_2$) in the portion of Butamben taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Butamben RS in the *Standard solution* (mg/mL)
C_U = concentration of Butamben in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Diluent: Acetonitrile and water (10:90)
Standard stock solution: 0.1 mg/mL each of USP Butamben RS and USP Aminobenzoic Acid RS prepared as follows. Transfer a suitable quantity of each Reference Standard to an appropriate volumetric flask. Add 10% of the total flask volume of acetonitrile to dissolve, then dilute with water to volume.
Standard solution: 0.2 µg/mL each of USP Butamben RS and USP Aminobenzoic Acid RS from the *Standard stock solution* in *Diluent*

www.webofpharma.com

[NOTE—The use of blank injections of *Diluent* may be suitable to reduce carryover between injections of the *Sample solution* and the *Standard solution*, if observed.]

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 10 between the aminobenzoic acid and butamben peaks

Relative standard deviation: NMT 5% for each peak corresponding to aminobenzoic acid and butamben

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of aminobenzoic acid in the portion of Butamben taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aminobenzoic acid from the *Sample solution*

r_S = peak response of aminobenzoic acid from the *Standard solution*

C_S = concentration of USP Aminobenzoic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Butamben in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Butamben taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*

r_S = peak response of butamben from the *Standard solution*

C_S = concentration of USP Butamben RS in the *Standard solution* (mg/mL)

C_U = concentration of Butamben in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.2	0.10
Butamben	1.0	—
Any unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry over phosphorus pentoxide for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

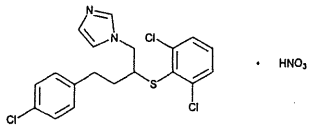
USP Aminobenzoic Acid RS

Benzoic acid, 4-amino.

$C_7H_7NO_2$ 137.14

USP Butamben RS

Butoconazole Nitrate



$C_{19}H_{17}Cl_3N_2S \cdot HNO_3$ 474.79

1*H*-Imidazole, 1-[4-(4-chlorophenyl)-2-[(2,6-dichlorophenyl) thio]butyl-, mononitrate, (±)-;

(±)-1-[4-(*p*-Chlorophenyl)-2-[(2,6-dichlorophenyl) thio]butyl] imidazole mononitrate [64872-77-1].

DEFINITION

Butoconazole Nitrate contains NLT 98.0% and NMT 102.0% of butoconazole nitrate ($C_{19}H_{17}Cl_3N_2S \cdot HNO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Buffer: 2.18 g/L of monobasic potassium phosphate and 4.18 g/L of dibasic potassium phosphate in water

Mobile phase: Methanol and *Buffer* (3:1)

Standard solution: 0.2 mg/mL of USP Butoconazole Nitrate RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Butoconazole Nitrate in *Mobile phase*. Filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 229 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 40°

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2800 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of butoconazole nitrate ($C_{19}H_{17}Cl_3N_2S \cdot HNO_3$) in the portion of Butoconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Butoconazole Nitrate RS in the *Standard solution* (mg/mL)

C_U = concentration of Butoconazole Nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

USP Monographs

www.webofpharma.com

• **ORDINARY IMPURITIES** ⟨466⟩
Standard solutions: USP Butoconazole Nitrate RS in a mixture of methylene chloride and methanol (2:1)
Test solution: Butoconazole Nitrate in a mixture of methylene chloride and methanol (2:1)
Eluant: Chloroform, tetrahydrofuran, cyclohexane, and ammonium hydroxide (18:18:13:1)
Visualization: 22
Acceptance criteria: Meets the requirements

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 60° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Butoconazole Nitrate RS

Butoconazole Nitrate Vaginal Cream

DEFINITION
Butoconazole Nitrate Vaginal Cream contains Butoconazole Nitrate in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of butoconazole nitrate ($C_{19}H_{17}Cl_3N_2S \cdot HNO_3$).

IDENTIFICATION
• **A.**
Sample solution: Prepare a mixture of the *Standard solution* and the *Sample solution* (1:1), as directed in the *Assay*.
Analysis: Chromatograph the *Sample solution*, as directed in the *Assay*.
Acceptance criteria: The chromatogram exhibits two main peaks that correspond to butoconazole nitrate and the internal standard.

ASSAY
• **PROCEDURE**
Buffer: 1.4 g of potassium acetate in 980 mL of water. Adjust with about 2 mL of glacial acetic acid to a pH of 4.3 ± 0.1, dilute with water to 1000 mL. Adjust the buffer molarity (0.018–0.072 M) as necessary to obtain suitable chromatographic performance. Increased retention time may be achieved by a decrease in the buffer molarity.
Diluent: Methanol and *Buffer* (60:40)
Mobile phase: Methanol and *Buffer* (65:35)
Internal standard solution: 1.6 mg/mL of 1-benzylimidazole in methanol
Standard stock solution: 0.4 mg/mL of USP Butoconazole Nitrate RS in methanol
Standard solution: Transfer 2.0 mL of the *Standard stock solution* and 3.0 mL of the *Internal standard solution* to a 50-mL flask, and add 35.0 mL of *Diluent*.
Sample stock solution: Nominally 0.4 mg/mL of butoconazole nitrate in methanol from Vaginal Cream prepared as follows. Add 200 mL of methanol to a 250-mL volumetric flask. Transfer to the flask a weighed quantity of Vaginal Cream equivalent to about 100 mg of butoconazole nitrate. Sonicate to dissolve, and cool to room temperature. Dilute with methanol to volume.
Sample solution: Transfer 2.0 mL of the *Sample stock solution* and 3.0 mL of the *Internal standard solution* to a 50-mL flask, and add 35.0 mL of *Diluent*. Allow the precipitated excipients that form to rise to the top of the solution, remove them by aspiration, and discard. Centrifuge or filter the remaining solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; packing L9 that has been converted to the potassium form by the use of 0.555 M potassium acetate solution
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for butoconazole nitrate and 1-benzylimidazole are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between the analyte and internal standard peaks
Column efficiency: NLT 1100 theoretical plates for the analyte peak
Tailing factor: NMT 2.1 for the analyte peak
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of butoconazole nitrate ($C_{19}H_{17}Cl_3N_2S \cdot HNO_3$) in the portion of Vaginal Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

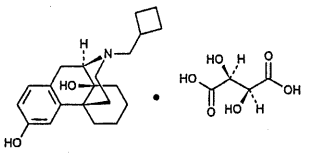
R_U = peak response ratio of butoconazole nitrate to the internal standard from the *Sample solution*
R_S = peak response ratio of butoconazole nitrate to the internal standard from the *Standard solution*
C_S = concentration of USP Butoconazole Nitrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of butoconazole nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers. Avoid excessive heat, and avoid freezing.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Butoconazole Nitrate RS

Butorphanol Tartrate



$C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ 477.55
Morphinan-3,14-diol, 17-(cyclobutylmethyl)-, (–)-, [*S*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1) (salt).
(–)-17-(Cyclobutylmethyl)morphinan-3,14-diol D-(–)-tartrate (1:1) (salt) [58786-99-5].

www.webofpharma.com

» Butorphanol Tartrate contains not less than 98.0 percent and not more than 102.0 percent of $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Butorphanol Tartrate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The R_F value of the principal spot in the chromatogram of the *Test preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained for test *A* in the *Chromatographic purity* test.

Specific rotation ⟨781S⟩: between –60° and –66°.
Test solution: 4 mg per mL, in methanol.

Water Determination, *Method I* ⟨921⟩ : not more than 2.0%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
METHOD A (Thin-Layer Chromatography)—
Standard solution—Prepare a solution in methanol containing 1 mg of USP Butorphanol Tartrate RS per mL.
Test solution—Transfer 100 mg of Butorphanol Tartrate to a 10-mL volumetric flask. Dissolve in methanol, dilute with methanol to volume, and mix.
Iodoplatinate spray reagent—Prepare a 1 in 10 solution of chloroplatinic acid in water. To 0.5 mL of this solution add 33 mL of water and 1 g of potassium iodide to obtain the spray reagent. Prepare fresh daily.
Procedure—Apply 50 µL of the *Test solution,* containing 500 µg of butorphanol tartrate, and 5 µL and 10 µL of the *Standard solution,* containing 5 µg and 10 µg of USP Butorphanol Tartrate RS, respectively, about 2 cm apart to a line parallel to and about 2 cm from the bottom of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a developing chamber containing, and equilibrated with, a mixture of chloroform, methanol, benzene, and ammonium hydroxide (85:25:20:5). Develop the chromatogram until the solvent front has moved about 10 cm above the line of application. Remove the plate, mark the solvent front, and allow the solvent to evaporate. Spray the plate with *Iodoplatinate spray reagent.* Estimate the percentage of the impurities present in the *Test solution* by comparing the intensities of secondary spots, if present, with the intensities of the principal spots obtained from the chromatograms of the *Standard solution.* The sum of the impurities observed is not greater than 2.0%.

METHOD B (Gas Chromatography)—Dissolve a suitable quantity of Butorphanol Tartrate in methanol to obtain a solution containing about 10 mg per mL. Inject 1 µL of this solution into a suitable gas chromatograph equipped with a flame-ionization detector and a 1.8-m × 4-mm glass column containing 3% liquid phase G3 on support S1AB. The temperatures of the injection port, column, and detector are maintained at about 280°, 250°, and 290°, respectively. The carrier gas is nitrogen. Record a 30-minute chromatogram. Preferably using an electronic integrator, determine the areas of all peaks in the chromatogram excluding the area of the solvent. In a suitable chromatogram, the retention time for the alpha isomer of butorphanol tartrate is 1.2 relative to 1.0 for butorphanol tartrate; and the retention time of butorphanol tartrate is not less than 15 minutes. Calculate the percentage of synthesis precursors in the test specimen by the formula:

$$100A_V/A_S$$

in which A_V is the sum of the areas of all minor peaks; and A_S is the sum of the areas of the major and minor peaks. The limit is 2.0%.

Assay—Dissolve about 500 mg of Butorphanol Tartrate, accurately weighed, in 75 mL of glacial acetic acid. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 47.76 mg of $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$.

Butorphanol Tartrate Injection

» Butorphanol Tartrate Injection is a sterile solution of Butorphanol Tartrate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$. It may contain a suitable preservative and a buffer.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Butorphanol Tartrate RS
Identification—Apply 10-µL portions of the Injection and a Standard solution of USP Butorphanol Tartrate RS having the same concentration about 2 cm apart to a line parallel to and about 2 cm from the bottom of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a developing chamber containing a mixture of chloroform, ethyl acetate, and methanol (40:10:9), and develop the chromatogram until the solvent front has moved about 10 cm above the line of application. Remove the plate, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light: the R_F value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution. Benzethonium chloride, if present, is observed as a streaked zone near the point of application. Visualize the butorphanol spots by lightly spraying the plate with a 1 in 250 solution of bromocresol purple in dehydrated alcohol: butorphanol appears as a blue spot against a light yellow background.
pH ⟨791⟩: between 3.0 and 5.5.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 88.0 USP Endotoxin Units per mg of butorphanol tartrate.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase—Prepare a mixture of 0.05 M ammonium acetate and acetonitrile (3:1) adjusted by the addition of glacial acetic acid to a pH of 4.1. The mixture is appropriately filtered and degassed.
Internal standard solution—Dissolve about 50 mg of propylparaben in 5.0 mL of methanol contained in a 250-mL volumetric flask. Add water to volume, and mix.
Standard preparation—Transfer about 50 mg of USP Butorphanol Tartrate RS, accurately weighed, to a 25-mL volumetric flask containing 1.0 mL of 1 N sulfuric acid. Swirl

USP Monographs

www.webofpharma.com

the flask to dissolve the powder completely, add water to volume, and mix. Pipet 5 mL of the resulting solution into a 50-mL volumetric flask containing 10.0 mL of *Internal standard solution*. Add water to volume, mix, and filter through a microporous filter, discarding the first 5 mL of the filtrate and collecting the remainder in a suitable container.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of butorphanol tartrate, to a 50-mL volumetric flask. Add 10.0 mL of *Internal standard solution*, mix, add water to volume, and mix. Filter through a microporous filter, discarding the first 5 mL of the filtrate and collecting the remainder in a suitable container.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 1.5%, and the capacity factor for butorphanol tartrate is not less than 2.0.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, adjusting the flow rate and other operating parameters, if necessary, until satisfactory chromatography and peak responses are obtained. Record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 1.7 for propylparaben and 1.0 for butorphanol tartrate. Calculate the quantity, in mg, of $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ in each mL of the Injection taken by the formula:

$$50(C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Butorphanol Tartrate RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and R_U and R_S are the peak response ratios of the butorphanol tartrate peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Butorphanol Tartrate Nasal Spray

DEFINITION

Butorphanol Tartrate Nasal Spray is an aqueous solution of butorphanol tartrate for administration as a metered spray to the nasal mucosa. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of butorphanol tartrate ($C_{21}H_{29}NO_2 \cdot C_4H_6O_6$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 1.0 mg/mL of USP Butorphanol Tartrate RS in methanol

Sample solution: Prepare a composite solution by pooling the contents of three containers of Nasal Spray into a suitable vessel. Transfer 1.0 mL of pooled sample to a 10-mL volumetric flask, and dilute with methanol to volume.

Developing solvent system: Chloroform, methanol, benzene, and ammonium hydroxide (17:5:4:1)

[**CAUTION**—Prepare in a hood while wearing appropriate safety gloves, lab coat, and protective eyewear.]

Spray reagent: Prepare a 1-in-10 solution of chloroplatinic acid in water. To 0.5 mL of this solution, add 33 mL of water and 1 g of potassium iodide. Prepare fresh daily.

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter, except spray the plate with *Spray reagent*.

Acceptance criteria: The typical R_F value is 0.7 for butorphanol tartrate.

ASSAY

• **PROCEDURE**

Buffer: 3.4 g/L of 0.025 M monobasic potassium phosphate. Filter.

Mobile phase: Acetonitrile, triethylamine, and *Buffer* (15:2:85). Mix thoroughly, and adjust with 85.0% phosphoric acid to a pH of 3.0 ± 0.1.

Standard solution: 0.2 mg/mL of USP Butorphanol Tartrate RS in *Mobile phase*. Mix, and filter, discarding the first 2 mL of the filtrate. The *Standard solution* is stable for at least 108 h.

Sample solution: Nominally 0.2 mg/mL of butorphanol tartrate in *Mobile phase* prepared as follows. Prepare a composite solution by pooling a minimum of four containers of Nasal Spray into a suitable glass vessel. Transfer the equivalent of 20 mg of butorphanol tartrate to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 2 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Columns

Analytical: 4.6-mm × 15-cm; 5-µm packing L11

Guard: 4.6-mm × 1-cm; 5-µm packing L11

Column temperature: 30°

Flow rate: 2.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of butorphanol tartrate ($C_{21}H_{29}NO_2 \cdot C_4H_6O_6$) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Butorphanol Tartrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of butorphanol tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer: Prepare as directed in the *Assay*.

Mobile phase: Acetonitrile, triethylamine, and *Buffer* (15:5.1:85). Mix thoroughly, and adjust with 85.0% phosphoric acid to a pH of 3.0 ± 0.1.

Standard solution: 0.005 mg/mL of USP Butorphanol Tartrate RS

USP Monographs

www.webofpharma.com

Sensitivity solution: Transfer 2.5 mL of the *Standard solution* to a 50-mL volumetric flask, and dilute with water to volume. Do not filter.
Sample solution: Nominally 1 mg/mL of butorphanol tartrate in water prepared as follows. Prepare a composite solution by pooling a minimum of four containers of Nasal Spray into a suitable glass vessel. Transfer the equivalent of 50 mg of butorphanol tartrate to a 50-mL volumetric flask. Dilute with water to volume. Do not filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Columns
Analytical: 4.6-mm × 25-cm; 5-µm packing L11
Guard: 4.6-mm × 1-cm; 5-µm packing L11
Column temperature: 40°
Flow rate: 2.0 mL/min
Injection volume: 60 µL
Run time: 40 min
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Relative standard deviation: NMT 10.0%, *Standard solution*
Sensitivity: The peak height for butorphanol tartrate is greater than or equal to three times the baseline noise, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms, and measure the responses for the butorphanol tartrate peak in the *Standard solution*, and for all known and unknown related compounds in the *Sample solution*.
Calculate the percentage of each related compound (see *Table 1*) and each unknown impurity in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each known or unknown related compound from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Butorphanol Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of butorphanol tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
3,14-Dihydroxymorphinan	0.3	0.3
Δ6-Butorphanol	0.7	0.5
Butorphanol tartrate	1.0	—
Unknown impurity	—	0.3
Total impurities	—	1.0

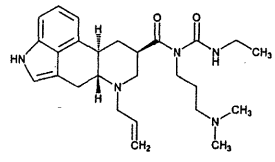
SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^3 cfu/g or mL, and the total combined molds and yeasts count does not exceed 10^2 cfu/g or mL. It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **pH** ⟨791⟩**:** 4.0–6.0
- **OSMOLALITY AND OSMOLARITY** ⟨785⟩**:** 252–292 mOsmol/kg

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature. Store at 25°; excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Butorphanol Tartrate RS

Cabergoline



$C_{26}H_{37}N_5O_2$ 451.60
Ergoline-8β-carboxamide, *N*-[3-(dimethylamino)propyl]-*N*-[(ethylamino)carbonyl]-6-(2-propenyl)-;
1-[(6-Allylergolin-8β-yl)carbonyl]-1-[3-(dimethylamino) propyl]-3-ethylurea [81409-90-7].

DEFINITION

Cabergoline contains NLT 98.0% and NMT 102.0% of cabergoline ($C_{26}H_{37}N_5O_2$), calculated on the anhydrous basis for the crystalline form and on the anhydrous and solvent-free basis for the amorphous form.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.**
Proceed as directed for *Procedure 1* or *Procedure 2*. The criteria for *Procedure 1* or *Procedure 2* must be met.
Procedure 1: *Crystallinity* ⟨695⟩
Acceptance criteria
For the crystalline form: Meets the requirements
For the amorphous form: Does not meet the requirements
Procedure 2: *X-Ray Diffraction* ⟨941⟩
Acceptance criteria
For the crystalline form: A diffraction pattern is present.
For the amorphous form: No diffraction pattern is present.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
Prepare solutions immediately before use, and protect from light.
Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 2.0, and dilute to 1 L. Add 0.2 mL of triethylamine to the resulting solution and mix.
Mobile phase: Acetonitrile and *Buffer* (4:21)
Standard solution: 0.25 mg/mL of USP Cabergoline RS in *Mobile phase*. Sonicate if needed.

www.webofpharma.com

Sample solution: 0.25 mg/mL of Cabergoline in *Mobile phase*. Sonicate if needed.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 25-cm; 10-µm packing L1
Flow rate: 1.3 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Relative standard deviation: NMT 2.0% for five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cabergoline ($C_{26}H_{37}N_5O_2$) in the portion of Cabergoline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cabergoline RS in the *Standard solution* (mg/mL)
C_U = concentration of Cabergoline in the *Sample solution* (mg/mL)

Acceptance criteria
For the crystalline form: 98.0%–102.0% on the anhydrous basis
For the amorphous form: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩**:** NMT 0.1%
- **Organic Impurities**

Prepare solutions immediately before use, and protect from light.
Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
System suitability solution: To 10 mL of 0.1 M sodium hydroxide add 50 mg of Cabergoline and stir for about 15 min. To 1 mL of the suspension add 1 mL of 0.1 M hydrochloric acid, and dilute with *Mobile phase* to 10.0 mL. Sonicate until dissolution is complete. [Note—The main degradation product obtained is cabergoline related compound A.]
Sample solution: 0.25 mg/mL of Cabergoline in *Mobile phase*. Sonicate if needed.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 25-cm; 10-µm packing L1
Flow rate: 1.3 mL/min
Injection volume: 100 µL
System suitability
Sample: *System suitability solution*
[Note—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between cabergoline and cabergoline related compound A
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Cabergoline taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cabergoline related compound D[a]	0.3	0.1
Cabergoline related compound B[b]	0.6	0.1
Cabergoline related compound A[c]	0.8	0.3
Cabergoline	1.0	—
Cabergoline related compound C[d]	2.9	0.3
Any other individual unidentified impurity	—	0.10
Total impurities	—	0.8

[a] (6a*R*,9*R*,10a*R*)-*N*-[3-(Dimethylamino)propyl]-7-(prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide.
[b] (6a*R*,9*R*,10a*R*)-*N* ^{9}-[3-(Dimethylamino)propyl]-*N* ^{4}-ethyl-7-(prop-2-enyl)6a,7,8,9,10,10a-hexahydroindolo[4,3-*fg*]quinoline-4,9(6*H*)-dicarboxamide.
[c] (6a*R*,9*R*,10a*R*)-7-(Prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid.
[d] (6a*R*,9*R*,10a*R*)-*N* ^{9}-[3-(Dimethylamino)propyl]-*N* ^{4}-ethyl-*N* ^{9}-(ethylcarbamoyl)-7-(prop-2-enyl)-6a,7,8,9,10,10a-hexahydroindolo[4,3-*fg*]quinoline-4,9(6*H*)-dicarboxamide.

SPECIFIC TESTS

- **Optical Rotation,** *Specific Rotation* ⟨781S⟩
Sample solution: 1 mg/mL in alcohol
Acceptance criteria
For the crystalline form: –77° to –83° on the anhydrous basis
For the amorphous form: –77° to –83° on the anhydrous and solvent-free basis
- **Water Determination,** *Method I* ⟨921⟩
Acceptance criteria
For the crystalline form: NMT 0.5%
For the amorphous form: NMT 1.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, protected from light. If it is labeled as amorphous, preserve under nitrogen in tight containers, store cold, and protect from light.
- **Labeling:** Where it is the amorphous form, the label so indicates.
- **USP Reference Standards** ⟨11⟩
USP Cabergoline RS

Cabergoline Tablets

DEFINITION

Cabergoline Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cabergoline ($C_{26}H_{37}N_5O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Identification sample solution* corresponds to that of the *Identification standard solution*, as obtained in the *Assay*.
- **B.** The UV-Vis spectrum of the major peak of the *Identification sample solution* corresponds to that of the *Identification standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Prepare solutions immediately before use, and protect from light.

Buffer: Transfer 6.8 g of monobasic potassium phosphate to a 1-L volumetric flask. Dissolve the contents in 900 mL of water. Adjust with phosphoric acid to a pH of 2.0. Dilute with water to volume, and add 0.2 mL of triethylamine.

Mobile phase: Acetonitrile and *Buffer* (16:84)

Standard solution: 0.25 mg/mL of USP Cabergoline RS in *Mobile phase*. Sonication may be used to aid in the dissolution of cabergoline.

Identification standard solution: 0.1 mg/mL of USP Cabergoline RS from the *Standard solution* in *Mobile phase*. [NOTE—This solution is used for *Identification A* and *Identification B*.]

Sample solution: Nominally 0.25 mg/mL of cabergoline from finely powdered Tablets in solution prepared as follows. Finely powder NLT 20 Tablets, and transfer a suitable portion of this fine powder to an appropriate volumetric flask. Dilute with *Mobile phase* to volume, and sonicate until completely dissolved. The resulting solution may be passed through a PVDF-type filter of 0.45-µm pore size before analysis.

Identification sample solution: Nominally 0.1 mg/mL of cabergoline from the *Sample solution* in *Mobile phase*. [NOTE—This solution is used for *Identification A* and *Identification B*.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.

Column: 4.0-mm × 25-cm; 10-µm packing L1

Flow rate: 1.3 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution*, *Identification standard solution*, *Sample solution*, and *Identification sample solution*

Calculate the percentage of the labeled amount of cabergoline ($C_{26}H_{37}N_5O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Cabergoline RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cabergoline in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 500 mL, degassed with helium

Apparatus 2: 50 rpm

Time: 15 min

Buffer, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL of USP Cabergoline RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter, discarding the first few mL.

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cabergoline ($C_{26}H_{37}N_5O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Cabergoline RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of cabergoline ($C_{26}H_{37}N_5O_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Prepare solutions immediately before use, and protect from light.

Buffer, Mobile phase, and Sample solution: Prepare as directed in the *Assay*.

System suitability solution: To 10 mL of 0.1 N sodium hydroxide, add 50 mg of cabergoline. Stir for 15 min. To 1 mL of the suspension, add 1 mL of 0.1 N hydrochloric acid, and dilute with *Mobile phase* to 10 mL. Sonicate until dissolution is complete. The main degradation product obtained is cabergoline acid.

Chromatographic system: Proceed as directed in the *Assay*, except for the *Injection volume*.

Injection volume

System suitability solution: 20 µL

Sample solution: 100 µL

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between cabergoline and cabergoline acid

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of peak responses of all impurities and cabergoline from the *Sample solution*

Calculate the percentage of total impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = sum of peak responses of all impurities from the *Sample solution*

r_T = sum of peak responses of all impurities and cabergoline from the *Sample solution*

Acceptance criteria: See *Table 1*.

www.webofpharma.com

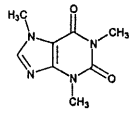
Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cabergoline acid[a]	0.8	2.0
Cabergoline	1.0	—
Cabergoline *N*-oxide[b]	1.4	1.0
Any unspecified degradation product	—	0.5
Total impurities	—	2.5

[a] (6a*R*,9*R*,10a*R*)-7-Allyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid.
[b] (6a*R*,9*R*,10a*R*)-7-Allyl-*N*-(3-(dimethylazinoyl)propyl)-*N*-(ethylcarbamoyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in light-resistant, tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cabergoline RS

Caffeine



$C_8H_{10}N_4O_2 \cdot H_2O$ 212.21
$C_8H_{10}N_4O_2$ 194.19
1*H*-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-; 1,3,7-Trimethylxanthine [58-08-2].
Monohydrate [5743-12-4].

DEFINITION
Caffeine is anhydrous or contains one molecule of water of hydration. It contains NLT 98.5% and NMT 101.0% of $C_8H_{10}N_4O_2$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M▲** (CN 1-May-2020)
- **B.** The retention time of the caffeine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: 0.82 g/L of anhydrous sodium acetate
Mobile phase: Acetonitrile, tetrahydrofuran, and *Buffer* (25:20:955). Adjust with glacial acetic acid to a pH of 4.5.
System suitability solution: 0.02 mg/mL of theophylline in *Mobile phase*. Shake, and sonicate if necessary, to dissolve.
Standard solution: Transfer 5.0 mg of USP Caffeine RS to a 25-mL volumetric flask. Add 5.0 mL of the *System suitability solution* and 10 mL of *Mobile phase*. Shake, and sonicate if necessary. Dilute with *Mobile phase* to volume, and filter.
Sample solution: 0.2 mg/mL of Caffeine in *Mobile phase*. [NOTE—Shake, and sonicate if necessary, to dissolve.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for theophylline and caffeine are 0.69 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6.0 between theophylline and caffeine
Tailing factor: NMT 2.0 for theophylline and caffeine peaks
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of caffeine ($C_8H_{10}N_4O_2$) in the portion of Caffeine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of caffeine from the *Sample solution*
r_S = peak response of caffeine from the *Standard solution*
C_S = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)
C_U = concentration of Caffeine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Caffeine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Acceptance criteria
Individual impurities: NMT 0.1%
Total impurities: NMT 0.1%

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method III* ⟨921⟩: Dry a sample at 80° for 4 h: the anhydrous form loses NMT 0.5% of its weight, and the hydrous form loses NMT 8.5% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve hydrous Caffeine in tight containers. Preserve anhydrous Caffeine in well-closed containers.
- **LABELING:** Label it to indicate whether it is anhydrous or hydrous.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Caffeine RS

www.webofpharma.com

Caffeine Citrate Injection

DEFINITION

Caffeine Citrate Injection is a sterile solution containing Caffeine and citric acid in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of caffeine citrate ($C_{14}H_{18}N_4O_9$). It contains no bacteriostat or other preservative.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL,** *Citrate* ⟨191⟩**:** Meets the requirements
- **C.**

Solution A: Transfer 4 g of potassium iodide to a 100-mL volumetric flask, and add 10 mL of water. Shake until the potassium iodide is dissolved. Add 2 g of iodine to the volumetric flask, and shake until dissolved. Dilute with water to volume.

Sample solution: 5.0 mL of Injection

Analysis: Transfer the *Sample solution* to a 25-mL centrifuge tube, and add 5 drops of *Solution A*. Add 0.5 mL of 2.0 M hydrochloric acid.

Acceptance criteria: A brown precipitate that dissolves on neutralization with 0.5 mL of sodium hydroxide TS is produced.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, tetrahydrofuran, and 0.01 M sodium acetate (5:4:191) adjusted with glacial acetic acid to a pH of 4.5

Theophylline stock solution: 0.02 mg/mL of theophylline in water

Standard solution: 0.2 mg/mL of USP Caffeine RS and 0.004 mg/mL of theophylline from *Theophylline stock solution* in water

Sample solution: Nominally 0.4 mg/mL of caffeine citrate (equivalent to 0.2 mg/mL of caffeine) from Injection in water prepared as follows. Transfer a volume of Injection, equivalent to 50 mg of caffeine, to a 250-mL volumetric flask. Dilute with water to volume, pass through a polyvinylidene difluoride or equivalent membrane of 0.45-µm pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 6.0 between theophylline and caffeine

Tailing factor: NMT 2.0 each for theophylline and caffeine

Relative standard deviation: NMT 2.0% for caffeine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_A), in mg/mL, of caffeine citrate in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of caffeine citrate, 386.31

M_{r2} = molecular weight of caffeine, 194.19

Calculate the percentage of the labeled amount of caffeine citrate ($C_{14}H_{18}N_4O_9$) in the portion of Injection taken:

$$\text{Result} = (C_A/C_U) \times 100$$

C_A = concentration of caffeine citrate in the *Sample solution*

C_U = nominal concentration of caffeine citrate in the *Sample solution*

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, Theophylline stock solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity solution: 5 µg/mL of caffeine and 0.1 µg/mL of theophylline from the *Standard solution* in water

System suitability

Sample: *Sensitivity solution*

Suitability requirements

Sensitivity: The theophylline peak is discernible.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_A) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of caffeine from the *Standard solution*

C_S = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)

C_A = concentration of caffeine citrate in the *Sample solution* as determined in the *Assay*

M_{r1} = molecular weight of caffeine citrate, 386.31

M_{r2} = molecular weight of caffeine, 194.19

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Theobromine	0.4	1.13	0.10
Paraxanthine	0.6	0.909	0.10
Theophylline	0.7	1.10	0.10
Caffeine	1.0	—	—
Any individual impurity	—	1.0	0.10
Total impurities	—	—	0.1

SPECIFIC TESTS

- **COLOR AND CLARITY**

Analysis: Transfer a suitable portion of the Injection to a clear glass test tube, and visually examine the solution in a well-lighted area.

USP Monographs

www.webofpharma.com

Acceptance criteria: The solution is colorless and free of haze, obvious turbidity, and precipitate.

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.25 USP Endotoxin Units/mg of caffeine
- **STERILITY TESTS** ⟨71⟩: It meets the requirements of the *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **pH** ⟨791⟩: 4.2–5.2
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: NMT 150 particles are equal to or greater than 10 µm, and NMT 25 particles are equal to or greater than 25 µm.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose, tight containers of Type I glass, and store between 15°–30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Caffeine RS

Caffeine Citrate Oral Solution

DEFINITION

Caffeine Citrate Oral Solution is a sterile aqueous solution containing Caffeine and citric acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of caffeine citrate ($C_{14}H_{18}N_4O_9$). It contains no bacteriostat or other preservative.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL,** *Citrate* ⟨191⟩: Meets the requirements
- **C.**

Solution A: Transfer 4 g of potassium iodide to a 100-mL volumetric flask, and add 10 mL of water. Shake until the potassium iodide is dissolved. Add 2 g of iodine to the volumetric flask, and shake until dissolved. Dilute with water to volume.

Sample solution: 5.0 mL of Oral Solution

Analysis: Transfer the *Sample solution* to a 25-mL centrifuge tube, and add 5 drops of *Solution A*. Add 0.5 mL of 2.0 M hydrochloric acid.

Acceptance criteria: A brown precipitate that dissolves on neutralization with 0.5 mL of sodium hydroxide TS is produced.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, tetrahydrofuran, and 0.01 M sodium acetate (5:4:191) adjusted with glacial acetic acid to a pH of 4.5

Theophylline stock solution: 0.02 mg/mL of theophylline in water

Standard solution: 0.2 mg/mL of USP Caffeine RS and 0.004 mg/mL of theophylline from *Theophylline stock solution* in water

Sample solution: Nominally 0.4 mg/mL of caffeine citrate (equivalent to 0.2 mg/mL of caffeine) from Oral Solution in water prepared as follows. Transfer a volume of Oral Solution, equivalent to 50 mg of caffeine, to a 250-mL volumetric flask. Dilute with water to volume, pass through a polyvinylidene difluoride or equivalent membrane of 0.45-µm pore size, and use the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 6.0 between theophylline and caffeine
Tailing factor: NMT 2.0 each for the theophylline and caffeine
Relative standard deviation: NMT 2.0% for caffeine

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_A), in mg/mL, of caffeine citrate in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of caffeine citrate, 386.31
M_{r2} = molecular weight of caffeine, 194.19

Calculate the percentage of the labeled amount of caffeine citrate ($C_{14}H_{18}N_4O_9$) in the portion of Oral Solution taken:

$$\text{Result} = (C_A/C_U) \times 100$$

C_A = concentration of caffeine citrate in the *Sample solution*
C_U = nominal concentration of caffeine citrate in the *Sample solution*

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, Theophylline stock solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 5 µg/mL of caffeine and 0.1 µg/mL of theophylline from the *Standard solution* in water

System suitability
Sample: *Sensitivity solution*
Suitability requirements
Sensitivity: The theophylline peak is discernible.

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_A) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of caffeine from the *Standard solution*
C_S = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)
C_A = concentration of caffeine citrate in the *Sample solution* as determined in the *Assay*
M_{r1} = molecular weight of caffeine citrate, 386.31
M_{r2} = molecular weight of caffeine, 194.19
F = relative response factor, (see *Table 1*)

Acceptance criteria: See *Table 1*.

www.webofpharma.com

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Theobromine	0.4	1.13	0.10
Paraxanthine	0.6	0.909	0.10
Theophylline	0.7	1.10	0.10
Caffeine	1.0	—	—
Any individual impurity	—	1.0	0.10
Total impurities	—	—	0.1

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements of the *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **pH** ⟨791⟩**:** 4.2–5.2

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose, tight containers, and store at a temperature between 15°–30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Caffeine RS

Caffeine and Sodium Benzoate Injection

DEFINITION
Caffeine and Sodium Benzoate Injection is a sterile solution containing equal amounts of Caffeine and Sodium Benzoate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of anhydrous caffeine ($C_8H_{10}N_4O_2$) and sodium benzoate ($C_7H_5NaO_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
  Sample: Use the residue from the *Assay* for *Caffeine.*
  Acceptance criteria: Meets the requirements
- **B.**
  Analysis: Dip the end of a platinum wire into a portion of Injection, and introduce it into a nonluminous flame.
  Acceptance criteria: The flame is colored intensely yellow.
- **C.**
  Analysis
  Part 1: Add a few drops of ferric chloride TS to a 0.5-mL portion of Injection.
  Part 2: Add 3 N hydrochloric acid to another portion of Injection.
  Acceptance criteria: The criteria in *Part 1* and *Part 2* must both be met.
  Part 1: A salmon-colored precipitate is formed.
  Part 2: A white precipitate is formed.

ASSAY
- **CAFFEINE**
  Sample solution: A volume of Injection equivalent to 250 mg each of caffeine and sodium benzoate
  Analysis: Transfer the *Sample solution* with the aid of 5 mL of water to a small separator, add 1 drop of phenolphthalein TS, and add 0.1 N sodium hydroxide, dropwise, until a permanent pink color is just produced. Shake the mixture with three or more 20-mL portions of chloroform to effect complete extraction of the caffeine, passing each chloroform extract through a small filter previously moistened with chloroform into a tared dish. Retain the water layer for the *Assay* for *Sodium Benzoate.* Wash the stem of the separator, the filter, and the funnel with 10 mL of hot chloroform, adding the washings to the dish. Evaporate the combined chloroform solutions on a steam bath, adding 2 mL of alcohol just before the last trace of chloroform is expelled. Complete the evaporation of the solvent, dry the residue, consisting of caffeine ($C_8H_{10}N_4O_2$), at 80° for 4 h. Cool, and weigh.
  Acceptance criteria: 90.0%–110.0%
- **SODIUM BENZOATE**
  Sample solution: Use the water layer obtained in the *Assay* for *Caffeine.*
  Titrimetric system
  Mode: Direct titration
  Titrant: 0.1 N hydrochloric acid VS
  Endpoint detection: Visual
  Analysis: Add 75 mL of ether and 5 drops of methyl orange TS to the *Sample solution.* Titrate with *Titrant,* and shake vigorously until a permanent pink color is produced in the water layer. Each mL of 0.1 N hydrochloric acid is equivalent to 14.41 mg of sodium benzoate ($C_7H_5NaO_2$).
  Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.7 USP Endotoxin Unit/mg of caffeine and sodium benzoate, based on the total, in mg, of the labeled amounts
- **pH** ⟨791⟩**:** 6.5–8.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Caffeine RS

Calamine

Iron oxide (Fe_2O_3), mixture with zinc oxide;
Calamine (pharmaceutical preparation) [8011-96-9].

DEFINITION
Calamine is Zinc Oxide with a small proportion of ferric oxide, and contains, after ignition, NLT 98.0% and NMT 100.5% of zinc oxide (ZnO).

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Zinc* ⟨191⟩
  Sample: 1 g
  Analysis: Treat the *Sample* with 10 mL of 3 N hydrochloric acid, and filter.
  Acceptance criteria: The filtrate meets the requirements.
- **B.**
  Sample: 1 g
  Analysis: Add 10 mL of 3 N hydrochloric acid to the *Sample,* heat to boil, and filter.
  Acceptance criteria: The filtrate assumes a reddish color upon the addition of ammonium thiocyanate TS.

ASSAY
- **PROCEDURE**
  Sample solution: Digest 1.5 g of freshly ignited Calamine in 50.0 mL of 1 N sulfuric acid VS, applying gentle heat until no further solution occurs. Filter the mixture, and wash the

www.webofpharma.com

residue on the filter with hot water until the last washing is neutral to litmus paper. To the combined filtrate and washings add 2.5 g of ammonium chloride. Cool, and add methyl orange TS.
Titrimetric system
Mode: Back titration
Titrant: 1 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Titrate the excess sulfuric acid in the *Sample solution* with *Titrant*. Each mL of 1 N sulfuric acid comsumed is equivalent to 40.69 mg of zinc oxide (ZnO).
Acceptance criteria: 98.0%–100.5% on the ignited basis

IMPURITIES
• **ARSENIC,** *Method I* ⟨211⟩: NMT 8 ppm
• **CALCIUM**
Sample: 1 g
Analysis: Digest the *Sample* in 25 mL of 3 N hydrochloric acid for 30 min, filter to remove the insoluble ferric oxide, and add 6 N ammonium hydroxide to the filtrate until the precipitate first formed is redissolved, then add 5 mL more of 6 N ammonium hydroxide. To 10 mL of this solution add 2 mL of ammonium oxalate TS.
Acceptance criteria: NMT a slight turbidity is produced.
• **CALCIUM OR MAGNESIUM**
Analysis: To another 10-mL portion of the solution prepared in the test for *Calcium*, add 2 mL of dibasic sodium phosphate TS.
Acceptance criteria: NMT a slight turbidity is produced.
• **LEAD**
Sample: 1 g
Analysis: Add 15 mL of water to the *Sample*, stir, then add 3 mL of glacial acetic acid, and warm on a steam bath until dissolved. Filter, and add 5 drops of potassium chromate TS.
Acceptance criteria: No turbidity is produced.

SPECIFIC TESTS
• **ACID-INSOLUBLE SUBSTANCES**
Sample: 2.0 g
Analysis: Dissolve the *Sample* in 50 mL of 3 N hydrochloric acid. If an insoluble residue remains, collect it on a tared filter, wash with water, and dry at 105° for 1 h. Cool, and weigh.
Acceptance criteria: NMT 40 mg (2.0%)
• **ALKALINE SUBSTANCES**
Sample: 1.0 g
Analysis: Digest the *Sample* with 20 mL of water on a steam bath for 15 min, filter, and add 2 drops of phenolphthalein TS.
Acceptance criteria: If a red color is produced, NMT 0.20 mL of 0.10 N sulfuric acid is required to discharge it.
• **LOSS ON IGNITION** ⟨733⟩
Sample: 2 g
Analysis: Ignite the *Sample* at 500° to constant weight.
Acceptance criteria: NMT 2.0%
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Calamine Topical Suspension

» Prepare Calamine Topical Suspension as follows.

Calamine	80 g
Zinc Oxide	80 g
Glycerin	20 mL
Bentonite Magma	250 mL
Calcium Hydroxide Topical Solution, a sufficient quantity to make	1000 mL

Dilute the Bentonite Magma with an equal volume of Calcium Hydroxide Topical Solution. Mix the powders intimately with the Glycerin and about 100 mL of the diluted magma, triturating until a smooth, uniform paste is formed. Gradually incorporate the remainder of the diluted magma. Finally add enough Calcium Hydroxide Topical Solution to make 1000 mL, and shake.
If a more viscous consistency in the Calamine Topical Suspension is desired, the quantity of Bentonite Magma may be increased to not more than 400 mL.
[NOTE—Shake the Calamine Topical Suspension before dispensing.]

Packaging and storage—Preserve in tight containers.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Phenolated Calamine Topical Suspension

» Prepare Phenolated Calamine Topical Suspension as follows:

Liquefied Phenol	10 mL
Calamine Topical Suspension	990 mL
To make	1000 mL

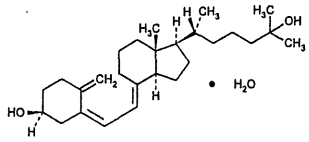
Mix the ingredients.
[NOTE—Shake Phenolated Calamine Topical Suspension before dispensing.]

Packaging and storage—Preserve in tight containers.

USP Monographs

www.webofpharma.com

Calcifediol



$C_{27}H_{44}O_2 \cdot H_2O$ 418.65

9,10-Secocholesta-5,7,10(19)-triene-3,25-diol monohydrate, (3β,5*Z*,7*E*)-.

25-Hydroxycholecalciferol monohydrate [63283-36-3].

» Calcifediol contains not less than 97.0 percent and not more than 103.0 percent of $C_{27}H_{44}O_2 \cdot H_2O$.

Packaging and storage—Preserve in tight, light-resistant containers at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Calcifediol RS

Change to read:

▲ ***Identification, Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: 197M▲ (CN 1-May-2020)

Water Determination, *Method Ia* ⟨921⟩: between 3.8% and 5.0%, determined on a 0.2-g specimen.

Assay—

Internal standard solution—Dissolve testosterone in ethyl acetate to obtain a solution having a concentration of about 0.10 mg per mL.

Mobile phase—Prepare a suitable degassed solution of about 6 volumes of heptane, 6 volumes of water-saturated heptane, 3 volumes of methylene chloride, and 5 volumes of ethyl acetate.

Standard preparation—Dissolve an accurately weighed quantity of USP Calcifediol RS in *Internal standard solution*, and dilute quantitatively and stepwise with *Internal standard solution* to obtain a solution having a known concentration of about 20 µg per mL.

Assay preparation—Transfer about 10 mg of Calcifediol, accurately weighed, to a 50-mL volumetric flask, dissolve in *Internal standard solution*, dilute with *Internal standard solution* to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 4-mm × 30-cm column that contains packing L3, a detector that monitors absorption at 254 nm, and a pump capable of providing constant flow up to a minimum of 2000 psi.

System suitability—The relative standard deviation for peak response ratios for four replicate injections of *Standard preparation* is not more than 3.0%, and the resolution factor is not less than 3.0.

Procedure—Introduce equal volumes of the *Standard preparation* and the *Assay preparation* into the chromatograph (see *Chromatography* ⟨621⟩), and measure the peak responses obtained. Calculate the quantity, in mg, of $C_{27}H_{44}O_2 \cdot H_2O$ in the portion of Calcifediol taken by the formula:

$$0.5C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Calcifediol RS in the *Standard preparation;* and R_U and R_S are the ratios of the peak responses of calcifediol to testosterone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Calcifediol Capsules

» Calcifediol Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{27}H_{44}O_2 \cdot H_2O$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Calcifediol RS

Identification—Transfer the contents of a number of Capsules, equivalent to about 150 µg of calcifediol, to a suitable container, add 1 mL of methanol, and shake vigorously for 1 minute. Separate the layers by centrifugation, and transfer as much of the top, methanol layer as possible to a second container. Evaporate this extract to dryness, and dissolve the residue in about 1 mL of chloroform. Proceed as directed under *Thin-layer Chromatographic Identification Test* ⟨201⟩, applying 20 µL of this solution and 20 µL of a solution containing about the same concentration of USP Calcifediol RS in chloroform, and using a solvent system consisting of 60 parts of cyclohexane and 40 parts of ethyl acetate.

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Time: 15 minutes.

Procedure—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each capsule shell to rupture.

Tolerances—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Internal standard solution—Dissolve testosterone in ethyl acetate to obtain a solution having a concentration of about 35 µg per mL.

Mobile phase—Prepare as directed in the *Assay*— under *Calcifediol.*

Standard preparation—Dissolve an accurately weighed quantity of USP Calcifediol RS in *Internal standard solution*, and dilute quantitatively and stepwise with *Internal standard solution* to obtain a solution having a known concentration of about 7 µg of USP Calcifediol RS per mL.

Assay preparation—Transfer a number of Calcifediol Capsules to a suitable container. Using a suitable implement, shear open a number of Capsules inside the container. Wash the implement with an accurately measured volume of *Internal standard solution* that will yield a solution having a concentration of about 7 µg of calcifediol per mL. Collect the rinsings in the container, and mix to obtain a homogeneous solution of the Capsule contents.

Chromatographic system and *System suitability*—Proceed as directed in the *Assay*— under *Calcifediol.*

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Calcifediol.* Calculate the quantity, in µg, of

USP Monographs

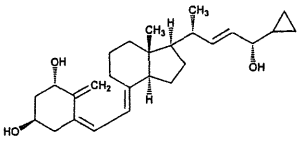
www.webofpharma.com

$C_{27}H_{44}O_2 \cdot H_2O$ in the portion of Capsule contents taken by the formula:

$$CV_U(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Calcifediol RS in the *Standard preparation*; V_U is the volume, in mL, of *Internal standard solution* taken for the *Assay preparation*; and R_U and R_S are the peak response ratios of calcifediol to testosterone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Calcipotriene



$C_{27}H_{40}O_3$ 412.60

9,10-Secochola-5,7,10(19),22-tetraene-1,3,24-triol, 24-cyclopropyl-, (1α,3β,5*Z*,7*E*,22*E*,24*S*)-; (5*Z*,7*E*,22*E*,24*S*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol [112965-21-6].

DEFINITION

Calcipotriene contains NLT 97.0% and NMT 102.0% of calcipotriene ($C_{27}H_{40}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Assay*.

ASSAY

- **PROCEDURE**

Protect solutions containing calcipotriene from light and air. Prepare the *Standard solution* and the *Sample solution* NMT 1 h before use. Prepare the *System suitability solution* daily.

Buffer: 1.0 g/L of tris(hydroxymethyl)aminomethane adjusted with phosphoric acid to pH 7.25 ± 0.25

Mobile phase: Acetonitrile and *Buffer* (45:55)

System suitability solution: 0.1 mg/mL of USP Calcipotriene RS and 0.01 mg/mL of USP Calcipotriene Related Compound C RS in *Mobile phase*

Standard solution: 0.1 mg/mL of USP Calcipotriene RS dissolved in 10% of acetonitrile, then diluted in *Mobile phase*

Sample solution: 0.1 mg/mL of Calcipotriene dissolved in 10% of acetonitrile, then diluted in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 264 nm

Column: 4.0-mm × 25-cm; 5-µm packing L7

Autosampler temperature: 4°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for calcipotriene related compound C and calcipotriene are 0.94 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between calcipotriene related compound C and calcipotriene

Relative standard deviation: NMT 1.0% from the calcipotriene peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of calcipotriene ($C_{27}H_{40}O_3$) in the portion of Calcipotriene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Calcipotriene RS in the *Standard solution* (mg/mL)

C_U = concentration of Calcipotriene in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES BY HPLC**

Protect solutions containing calcipotriene from light and air. Prepare the *Standard solution* and the *Sample solution* NMT 1 h before use. Prepare the *System suitability solution* daily.

Buffer, Mobile phase, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard stock solution: Use the *Standard solution* in the *Assay*.

Standard solution: 0.004 mg/mL of USP Calcipotriene RS in *Mobile phase* from the *Standard stock solution*

Sample solution: 0.4 mg/mL of Calcipotriene dissolved in 10% of acetonitrile, then diluted in *Mobile phase*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any impurity in the portion of Calcipotriene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any impurity from the *Sample solution*

r_S = peak response of calcipotriene from the *Standard solution*

C_S = concentration of USP Calcipotriene RS in the *Standard solution* (mg/mL)

C_U = concentration of Calcipotriene in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Calcipotriene related compound C[a]	0.92–0.96	1.00
Calcipotriene	1.00	—
Calcipotriene impurity D[b]	1.13–1.17	1.00
Any individual unspecified impurity	—	0.10

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	2.50

[a] (5*E*,7*E*,22*E*,24*S*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol.
[b] (5*Z*,7*E*,22*E*,24*R*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol.

• **ORGANIC IMPURITIES BY TLC**
Prepare solutions containing calcipotriene in low actinic glassware, and keep from air. Carry out the test as rapidly as possible.
Diluent: Chloroform and triethylamine (9:1)
System suitability solution: 10 mg/mL of USP Calcipotriene RS in *Diluent*. Heat in a water bath at 60° for 2 h to form precalcipotriene.
Standard solution 1: 0.025 mg/mL of USP Calcipotriene RS in *Diluent* (0.25%)
Standard solution 2: 0.01 mg/mL of USP Calcipotriene RS in *Diluent* (0.10%)
Sample solution: 10 mg/mL of Calcipotriene in *Diluent*
Developing solvent system: Methylene chloride and isobutyl alcohol (80:20)
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic plate coated with silica gel mixture
Application volume: 10 µL
Spray reagent: Transfer 20 mL of sulfuric acid into a 100-mL volumetric flask, and dilute with alcohol to volume.
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: The secondary spot precalcipotriene and principle spot calcipotriene are clearly separated.
Analysis
Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*
Develop with *Developing solvent system* until the solvent system has moved 2/3 of the plate from the point of spotting. Remove the plate, and let the plate air-dry. Dry it again at 140° for 10 min followed by spraying the hot plate with the *Spray reagent*. Dry the plate for NMT 1 min at 140°. Examine the plate under UV light at 366 nm.
Acceptance criteria: The spot of any impurity in the *Sample solution* is not more intense than the spot of calcipotriene in the appropriate *Standard solution* specified in *Table 2*.

Table 2

Name	Relative Retardation (R_{ret})	Comparison Solution	Acceptance Criteria, NMT (%)
Calcipotriene impurity G[a] and calcipotriene impurity H[b]	0.4	*Standard solution 1*	0.25
Precalcipotriene[c]	0.9	—	—
Calcipotriene	1.0	—	—
Calcipotriene impurity A[d]	1.2	*Standard solution 1*	0.25

Table 2 *(continued)*

Name	Relative Retardation (R_{ret})	Comparison Solution	Acceptance Criteria, NMT (%)
Any other individual impurity	—	*Standard solution 2*	0.10

[a] 24,24′-Oxybis[(5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β-diol].
[b] (5*Z*,7*E*,22*E*,24*R*)-24-Cyclopropyl-24-{[(5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-1α,3β-dihydroxy-9,10-secochola-5,7,10(19),22-tetraen-24-yl]oxy}-9,10-secochola-5,7,10(19),22-tetraene-1α,3β-diol.
[c] (5*E*,6*E*,22*E*,24*S*)-24-Cyclopropyl-9,10-secochola-5(10),6,22-triene-1α,3β,24-triol.
[d] (5*Z*,7*Z*,22*E*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-24-one-1α,3β-diol.

SPECIFIC TESTS
• **LOSS ON DRYING**
(See *Thermal Analysis* ⟨891⟩.)
Sample: 5 mg
Analysis: Heat the *Sample* to 105° at a rate of 10°/min, and hold at 105° for 60 min.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers and store at 2°–8° or at –20° or below. Protect from light.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Calcipotriene RS
USP Calcipotriene Related Compound C RS
(5*E*,7*E*,22*E*,24*S*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol.
$C_{27}H_{40}O_3$ 412.60

Calcipotriene Ointment

DEFINITION
Calcipotriene Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of calcipotriene ($C_{27}H_{40}O_3$), in a suitable ointment base.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Buffer, Diluent, Standard stock solution, Standard solution, Sample stock solution, and **Sample solution:** Proceed as directed in the *Assay*.
Blank: Dilute 5 mL of tetrahydrofuran with *Diluent* to 25 mL.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
The solutions containing calcipotriene are stable up to 24 h at room temperature.
Mobile phase: Methanol and water (70:30)
Buffer: 132 g/L of monobasic ammonium phosphate in water
Diluent: Methanol, *Buffer*, and water (700:3:297)
Standard stock solution: 0.1 mg/mL of USP Calcipotriene RS in *Diluent*. Sonicate if necessary.

USP Monographs

www.webofpharma.com

Standard solution: 2 µg/mL of USP Calcipotriene RS prepared as follows. Transfer 5 mL of *Standard stock solution* into a 250-mL volumetric flask, add 50 mL of tetrahydrofuran, and dilute with *Diluent* to volume.
Sample stock solution: 0.01 mg/mL of calcipotriene in tetrahydrofuran prepared as follows. Transfer Ointment equivalent to 0.25 mg of calcipotriene into a 25-mL volumetric flask. Add 15 mL of tetrahydrofuran, and sonicate, with intermittent shaking, for 20 min in a cold water bath. Dilute with tetrahydrofuran to volume.
Sample solution: 2 µg/mL of calcipotriene prepared as follows. Transfer 5 mL of the *Sample stock solution* into a suitable container. Add 20 mL of *Diluent*, mix, and sonicate for 10 min. Pass through a suitable filter of 0.45-µm pore size. Inject immediately after preparation.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 264 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of calcipotriene ($C_{27}H_{40}O_3$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Calcipotriene RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of calcipotriene in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Minimum Fill** ⟨755⟩**:** Meets the requirements

IMPURITIES

• **Organic Impurities**
The solutions containing calcipotriene are stable for up to 24 h at room temperature.
Mobile phase, Buffer, and **Diluent:** Proceed as directed in the *Assay*.
System suitability solution: 10.0 µg/mL of USP Calcipotriene RS and 0.1 µg/mL of USP Calcipotriene Related Compound C RS in *Diluent*
Standard stock solution: 1.0 µg/mL of USP Calcipotriene RS in *Diluent*
Standard solution: 0.1 µg/mL of USP Calcipotriene RS prepared as follows. Transfer 1.0 mL of the *Standard stock solution* into a 10-mL volumetric flask, add 1.0 mL tetrahydrofuran, and dilute with *Diluent* to volume.
Sample solution: Nominally equivalent to 0.01 mg/mL of calcipotriene prepared as follows. Transfer Ointment equivalent to 0.1 mg of calcipotriene into a glass-stoppered test tube, and add 1 mL of tetrahydrofuran. Sonicate for 20 min with intermittent shaking. Add 9 mL of *Diluent*, and sonicate for 5 min. Shake the test tube vigorously, and then place it in a beaker containing ice cold water for 2–3 min. Pass the liquid portion through a nylon filter of 0.45-µm pore size, and discard the first few mL of the solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 264 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 100 µL
Run time: NLT 1.25 times of retention time of the calcipotriene peak
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between calcipotriene related compound C and calcipotriene peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any impurity in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of calcipotriene from the *Standard solution*
C_S = concentration of USP Calcipotriene RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of calcipotriene in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Calcipotriene impurity B[a]	0.86	0.50
Calcipotriene related compound C[b]	0.92	1.00
Calcipotriene	1.0	—
Calcipotriene impurity D[c]	1.31	1.00
Specified unknown impurity	1.8	0.50
Any individual unspecified impurity	—	0.50
Total impurities	—	3.50

[a] (5*Z*,7*Z*,22*E*,24*S*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol.
[b] (5*E*,7*E*,22*E*,24*S*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol.
[c] (5*Z*,7*E*,22*E*,24*R*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol.

SPECIFIC TESTS

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count is NMT 10^2 cfu/g. The total yeasts and molds count is NMT 5×10^1 cfu/g. It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* species.
• **pH** ⟨791⟩
Sample solution: Transfer 1 g of Ointment in a centrifuge test tube. Add 10 mL of water, and heat in a water bath at

www.webofpharma.com

60° for 30 min with stirring. Cool to room temperature, and centrifuge at 2500 rpm for 10 min.
Acceptance criteria: 5.5–8.5

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature. Do not freeze.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Calcipotriene RS
  USP Calcipotriene Related Compound C RS
  (5*E*,7*E*,22*E*,24*S*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol.
  $C_{27}H_{40}O_3$ 412.60

Calcitonin Salmon

CSNLSTCVLG KLSQELHKLQ TYPRTNTGSG TP —NH_2

$C_{145}H_{240}N_{44}O_{48}S_2$ 3432 daltons
[47931-85-1].

DEFINITION
Calcitonin Salmon is a polypeptide that has the same sequence as that of the hormone that regulates calcium metabolism and is secreted by the ultimobranchial gland of salmon. It is produced from either synthetic processes or microbial processes using recombinant DNA (rDNA) technology. The host cell-derived protein content and the host cell- or vector-derived DNA content of Calcitonin Salmon produced from an rDNA process are determined by validated methods. It contains NLT 90.0% and NMT 105.0% of calcitonin salmon, calculated on an acetic acid-free and anhydrous basis. [NOTE—1 mg of acetic acid-free, anhydrous Calcitonin Salmon is equivalent to 6000 USP Calcitonin Salmon Units. 1 USP Calcitonin Salmon Unit = 1 Calcitonin IU.]

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: Dissolve 3.26 g of tetramethylammonium hydroxide pentahydrate in 900 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 100 mL of acetonitrile, and mix.
  Solution B: Dissolve 1.45 g of tetramethylammonium hydroxide pentahydrate in 400 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 600 mL of acetonitrile, and mix.
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	72	28
30	48	52
32	72	28
55	72	28

Standard solution: 1.0 mg/mL of USP Calcitonin Salmon RS in *Solution A*
System suitability solution: Prepare a solution in *Solution A* containing about 0.2 mg/mL of USP Calcitonin Salmon Related Compound A RS and 0.2 mg/mL of USP Calcitonin Salmon RS.
Sample solution: 1.0 mg/mL of Calcitonin Salmon in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 65°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for calcitonin salmon and calcitonin salmon related compound A are 1.0 and 1.15, respectively.]
Suitability requirements
Resolution: NLT 3 between calcitonin salmon and calcitonin salmon related compound A
Tailing factor: NMT 2.5 for calcitonin salmon
Relative standard deviation: NMT 3%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of calcitonin salmon ($C_{145}H_{240}N_{44}O_{48}S_2$) in the portion of Calcitonin Salmon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of calcitonin salmon from the *Sample solution*
r_S = peak response of calcitonin salmon from the *Standard solution*
C_S = concentration of USP Calcitonin Salmon RS in the *Standard solution* (corrected for water and acetic acid content) (mg/mL)
C_U = concentration of the *Sample solution* (corrected for water and acetic acid content) (mg/mL)

Acceptance criteria: 90.0%–105.0% on an acetic acid-free and anhydrous basis

OTHER COMPONENTS
- **ACETIC ACID CONTENT** ⟨503⟩
  Sample solution: 1 mg/mL of Calcitonin Salmon in *Diluent*, prepared as directed in the chapter
  Acceptance criteria: 4%–15%

IMPURITIES
- **PROCEDURE: RELATED PEPTIDES AND OTHER RELATED SUBSTANCES**
  Test 1
  [NOTE—This test is performed on material produced by both chemical synthesis processes and rDNA processes.]
  Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
  Analysis
  Sample: *Sample solution*
  Calculate the percentage of each impurity in the portion of Calcitonin Salmon taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area response of each impurity from the *Sample solution*
r_T = sum of the area responses of all the peaks from the *Sample solution*

USP Monographs

www.webofpharma.com

Acceptance criteria
Individual impurities: NMT 3.0% of the total area of all peaks
Total impurities: NMT 5.0% of the sum of the areas of all the peaks including the principal peak
[NOTE—Disregard any peaks due to the solvent and any peaks whose area is less than 0.1% of the principal peak.]

Test 2
[NOTE—This test needs to be performed only on material produced using rDNA technology.]
Buffer A: Dissolve 2.72 g of monobasic potassium phosphate in 1000 mL of water.
Buffer B: Dissolve 2.72 g of monobasic potassium phosphate and 29.2 g of sodium chloride in 1000 mL of water.
Buffer C (pH 3.0 citrate buffer): Dissolve 4.8 g of citric acid in 80 mL of water. Adjust with 1 M sodium hydroxide to a pH of 3.0, and dilute with water to 100.0 mL.
Solution A: Acetonitrile and *Buffer A* (15:85). Adjust with 45% (w/w) potassium hydroxide to a pH of 5.0.
Solution B: Acetonitrile and *Buffer B* (15:85). Adjust with 45% (w/w) potassium hydroxide to a pH of 4.6.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	0	100
15	0	100
15.1	100	0
22.1	100	0

Resolution solution: Prepare a solution in water containing about 0.5 mg/mL each of USP Calcitonin Salmon RS and USP Calcitonin Salmon Related Compound B RS. To 1 mL of this solution add 100 µL of pH 3.0 citrate buffer.
Sample solution: To 1 mL of a 0.5-mg/mL solution of Calcitonin Salmon add 100 mL of *Buffer C*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 20-cm; packing L9
Flow rate: 1.2 mL/min
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for [1,7-bis(3-sulfo-L-alanine)] calcitonin salmon-glycine, [1,7-bis(3-sulfo-L-alanine)] calcitonin salmon, and calcitonin salmon related compound B (calcitonin salmon-glycine) are 0.4, 0.6, and 0.9, respectively; and the retention time for calcitonin salmon is about 9 min.]
Suitability requirements
Resolution: NLT 3.0 between calcitonin salmon and calcitonin salmon related compound B
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Calcitonin Salmon taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of the responses of all the peaks

Acceptance criteria
Individual impurities: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria NMT (%)
[1,7-Bis(3-sulfo-L-alanine)] calcitonin salmon-glycine	0.4	0.2
[1,7-Bis(3-sulfo-L-alanine)] calcitonin salmon	0.6	0.2
Calcitonin salmon related compound B	0.9	0.6

SPECIFIC TESTS

• **AMINO ACID PROFILE**
(See *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩.)
[NOTE—This test needs to be performed only on material of synthetic origin. The concentration of amino acids in the *Internal standard solution*, *Standard stock solution*, and *Standard solution* and the amount of material used to prepare the *Sample solution* can be adjusted depending on the method used for amino acid analysis. The concentrations given are based on analysis using *Method 1*.]
Internal standard solution: 1 mM solution of γ-aminobutyric acid
Standard stock solution: Prepare a mixture containing equimolar amounts of ammonia and the L form of lysine, histidine, arginine, aspartic acid, threonine, serine, proline, valine, glutamic acid, glycine, leucine, and tyrosine, together with half the equimolar amount of L-cystine, in 0.1 M hydrochloric acid. The final concentration is about 2.5 mM for each amino acid.
Standard solution: Transfer 5 mL of the *Internal standard solution* and 2 mL of the *Standard stock solution* into a 50-mL volumetric flask, and dilute with 0.1 M hydrochloric acid to volume.
Sample solution: Place 1.5 mg of Calcitonin Salmon into a heavy-wall ignition tube. Add 1.0 mL of 6 N hydrochloric acid, and allow to cool. Immerse the lower half of the tube in a freezing mixture until the contents are frozen, then evacuate to approximately 10 µm of Hg, purge with nitrogen (repeat the evacuation and nitrogen purge three times), and seal the tube while it is under a 10 µm of Hg vacuum. Heat for 16 h at 110°–115° in an air oven. Cool, open the tube, dry in a vacuum desiccator, remove the contents, and allow to cool to room temperature. Dissolve in 0.1 M hydrochloric acid. Transfer to a 10-mL volumetric flask, add 1 mL of *Internal standard solution*, and dilute with 0.1 M hydrochloric acid to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Standardize the amino acid analyzer using the *Standard solution*. Inject the *Sample solution* into the amino acid analyzer, and determine the relative proportion of amino acids.
Express the content of each amino acid in moles using an internal standard calibration technique. Calculate the relative proportions of the amino acids by taking as equivalent to one the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, valine, leucine, histidine, arginine, and lysine divided by 20.
Acceptance criteria: The requirements are met if the values fall within the limits in *Table 4*.

www.webofpharma.com

Table 4

Name	Acceptance Criteria
Aspartic acid	1.8–2.2
Glutamic acid	2.7–3.3
Proline	1.7–2.3
Glycine	2.7–3.3
Valine	0.9–1.1
Leucine	4.5–5.3
Histidine	0.9–1.1
Arginine	0.9–1.1
Lysine	1.8–2.2
Serine	3.2–4.2
Threonine	4.2–5.2
Tyrosine	0.7–1.1
Half cystine	1.4–2.1

• **PEPTIDE MAPPING**

(See *Biotechnology-Derived Articles—Peptide Mapping* ⟨1055⟩.)

[NOTE—This test needs to be performed only on material produced using rDNA technology.]

Solution A: Water and trifluoroacetic acid (1000:1)

Solution B: Acetonitrile, water, and trifluoroacetic acid (800: 200: 0.85)

Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	100	0
50	65	35
60	40	60
60.1	0	100
65.1	0	100
65.2	100	0
80.2	100	0

Trypsin solution: Freshly prepare a solution containing 0.1 mg/mL of trypsin (previously treated with L-1-tosylamido-2-phenylethyl chloromethyl ketone [TPCK] to remove chymotrypsin activity) in water.

Tris buffer: 1 M tris(hydroxymethyl) aminomethane and 10 mM calcium chloride. Adjust with hydrochloric acid to a pH of 8.0.

Stopping solution: Water and trifluoroacetic acid (1:1)

Standard solution: Prepare a 1.0-mg/mL solution of USP Calcitonin Salmon RS. Transfer 1 mL of this solution to a clean vial. Add 100 µL of *Tris buffer* and 50 µL of *Trypsin solution*. Mix, and incubate at 2°–8° for 16–20 h. Quench the digestion by adding 10 µL of *Stopping solution*.

Sample solution: 1.0 mg/mL of Calcitonin Salmon. Transfer 1 mL of this solution to a clean vial. Add 100 µL of *Tris buffer* and 50 µL of *Trypsin solution*. Mix, and incubate at 2°–8° for 16–20 h. Quench the digestion by adding 10 µL of *Stopping solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Condition the chromatographic system by running a blank gradient program before injecting the digests.]

Acceptance criteria: The chromatographic profile of the *Sample solution* is similar to that of the *Standard solution*.

• **BIOIDENTITY**

RPMI 1640 with L-glutamine: Prepare a mixture of the ingredients in the quantities shown in *Table 6* in sufficient water to obtain 1 L of *RPMI 1640 with L-glutamine* solution, and sterilize by filtration.

Table 6

Calcium nitrate	100.00 mg
Potassium chloride	400.00 mg
Magnesium sulfate, anhydrous	48.84 mg
Potassium chloride	400 mg
Sodium chloride	6000 mg
Sodium phosphate, dibasic, anhydrous	800 mg
Sodium bicarbonate	2000 mg
Glucose	2000 mg
Glycine	10 mg
L-Arginine	200 mg
L-Asparagine	50 mg
L-Aspartic acid	20 mg
L-Cystine dihydrochloride	65 mg
L-Glutamic acid	20 mg
L-Glutamine	300 mg
L-Histidine	15 mg
L-Hydroxyproline	20 mg
L-Isoleucine	50 mg
L-Leucine	50 mg
L-Lysine hydrochloride	40 mg
L-Methionine	15 mg
L-Phenylalanine	15 mg
L-Proline	20 mg
L-Serine	30 mg
L-Threonine	20 mg
L-Tryptophan	5 mg
L-Tyrosine disodium salt dihydrate	29 mg
L-Valine	20 mg
Biotin	0.2 mg
Choline chloride	3 mg
D-Calcium pantothenate	0.25 mg
Folic acid	1 mg
i-Inositol	35 mg
Niacinamide	1 mg
para-Aminobenzoic acid	1 mg

www.webofpharma.com

Table 6 *(continued)*

Pyridoxine hydrochloride	1 mg
Riboflavin	0.2 mg
Thiamine hydrochloride	1 mg
Vitamin B_{12}	0.005 mg

Medium A (growth medium): Using aseptic technique prepare the following tissue culture medium.

Table 7

RPMI 1640 with L-glutamine	500 mL
Fetal bovine serum	50 mL
1 M HEPES	5 mL
Penicillin/streptomycin solution (10,000 IU/mL per 10 mg/mL)	5 mL
Human insulin	10 IU
Hydrocortisone	0.5 mg

Medium B (stimulation medium): Dissolve 5 g of bovine serum albumin (BSA) in 500 mL of 2 mM *RPMI 1640 with L-glutamine*.

Solution A (0.2% BSA solution): Dissolve 50 mg of BSA in 25 mL of water. [NOTE—Use within one day.]

Solution B (formic acid/BSA solution): Add 25 mL of 0.1 M formic acid and 5 mL of *Solution A* to a 50-mL volumetric flask, dilute with water to volume, and mix. [NOTE—Use within two days.]

Solution C (trypsin/EDTA solution): Prepare a sterile filtered solution containing 0.25% trypsin and 0.53 mM EDTA (tetrasodium ethylenediaminetetraacetate).

Solution D (Dulbecco's phosphate buffered saline): Dissolve 8 g of sodium chloride, 1.15 g of dibasic sodium phosphate, 0.2 g of monobasic potassium phosphate, 0.2 g of potassium chloride, 0.1 g of calcium chloride, and 0.1 g of magnesium chloride in 1 L of water.

Standard stock solution: 20 µg/mL of USP Calcitonin Salmon RS in *Solution B*

Positive control solution: Quantitatively dilute the *Standard stock solution* in *Medium B* to obtain a 1-ng/mL solution of USP Calcitonin Salmon RS.

Negative control solution: *Medium B*

[NOTE—Prior analysis should be performed to identify the linear portion of the dose-response curve. For example, the *Standard solutions* and *Sample solutions* given below.]

Standard solution A: 0.8 ng/mL of USP Calcitonin Salmon RS from the *Standard stock solution* in *Medium B*

Standard solution B: 0.4 ng/mL of USP Calcitonin Salmon RS from *Standard solution A* in *Medium B*

Standard solution C: 0.2 ng/mL of USP Calcitonin Salmon RS from *Standard solution B* in *Medium B*

Standard solution D: 0.1 ng/mL of USP Calcitonin Salmon RS from *Standard solution C* in *Medium B*

Sample stock solution: 20 µg/mL of Calcitonin Salmon in *Solution B*

Sample solution A: 0.8 ng/mL of Calcitonin Salmon from the *Sample stock solution* in *Medium B*

Sample solution B: 0.4 ng/mL of Calcitonin Salmon from *Sample solution A* in *Medium B*

Sample solution C: 0.2 ng/mL of Calcitonin Salmon from *Sample solution B* in *Medium B*

Sample solution D: 0.1 ng/mL of Calcitonin Salmon from *Sample solution C* in *Medium B*

Cell culture preparation: Prepare a cell culture of the human mammary tumor cell line T-47D. Cells are propagated using *Medium A* at 37° and 5% carbon dioxide. The medium is changed every two days, and cells are passaged every 5–9 days using *Solution C* with a 1:4 subculture.

Cell suspension: For the test use a cell culture that is 5–9 days old. Remove the cell culture medium from the flask by aspiration, add 10 mL of *Solution D,* and rock the culture flask to rinse the entire monolayer. Remove the liquid by aspiration, add 2 mL of *Solution C,* spread over the entire monolayer, allow to stand for 3–5 min, and add 10 mL of *Medium A.* Homogenize the cell suspension using a pipet, transfer to a 15-mL polypropylene tube, centrifuge at about 220 × *g* for 5 min, pour off the supernatant, and resuspend the cell pellet in 10 mL of *Medium A.* Count the cells, and adjust the cell density through dilution using *Medium A* to 2.5×10^4 cells/mL.

Procedure: Place 200 µL of the *Cell suspension* into each well of a 96-well culture plate (the tissue culture plate), and incubate for 18–24 h at 37° and 5% carbon dioxide. Fill each well of an empty, round-bottomed, 96-well culture plate (the prepared plate) with 150 µL of one of the following solutions: *Positive control solution, Negative control solution, Standard solutions A–D,* and *Sample solutions A–D,* so that each solution fills at least five wells on the prepared plate. After incubation remove the culture medium from the tissue culture plate. Using an 8-channel or 12-channel pipet, rapidly transfer 100 µL of solution from each well of the prepared plate to each well of the tissue culture plate. Incubate for 15 min at ambient temperature, remove the solution from each well, stop stimulation by immediately adding an appropriate cell-lysis buffer, and quantitate cAMP produced within the cells, using a validated kit. Perform the test three times, using three different 96-well culture plates. [NOTE—Some kits include a cell-lysis reagent and a sequestering agent for the cell-lysis reagent. The range of the test kit is between 0.05 and 10 ng/mL of cAMP. The number of cells used in the assay may vary, depending on the validated kit used to quantitate cAMP.]

Analysis: Potency is determined by a 3-dose, 6-point parallel-line assay, using standard statistical methods. The calculation is carried out using both the lower three concentrations and the upper three concentrations. For the assay to be valid, the requirements for regression, parallelism, and difference of quadratics must be met. If the requirements for validity are met to the same extent in both assessments (the lower and the higher assessments) the final result is determined from the concentration range that shows the higher value when the common slope is divided by the root mean square error.

Acceptance criteria: Combine the three potency values by using an unweighted mean on the log scale. Determine a 95% confidence interval in the log scale using standard statistical methods. Convert the average and confidence interval to the potency scale using antilogs to obtain a geometric mean and its confidence interval. The potency levels determined from all three performances of the test are valid if the data analysis indicates the three determinations to be homogeneous, and the confidence interval is fully contained within 64% and 156% of the geometric mean. If the confidence interval requirement is not met, additional assays may be performed to increase the number of assays and make the confidence interval narrower. The determination of whether it meets the identity requirement should be done only after the confidence interval requirement is met. The acceptance criterion for identity is that the geometric mean is within 80% and 125% of the *Assay* value.

www.webofpharma.com

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩
  Sample: 25 mg
  Acceptance criteria: The total aerobic microbial count is NMT 10^2 cfu/g, and the total combined molds and yeasts count is NMT 10^2 cfu/g.
- **STERILITY TESTS** ⟨71⟩**:** Where the label states that Calcitonin Salmon is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 10%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store in a refrigerator or maintain in a frozen state, protected from light.
- **LABELING:** The labeling states that the material is synthetic or of recombinant DNA origin.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Calcitonin Salmon RS
  $C_{145}H_{240}N_{44}O_{48}S_2$ 3432 daltons
  USP Calcitonin Salmon Related Compound A RS
  N-Acetyl-cys[1]-calcitonin salmon.
  USP Calcitonin Salmon Related Compound B RS (calcitonin salmon-glycine)
  Calcitonin salmon-glycine.

Calcitonin Salmon Injection

DEFINITION
Calcitonin Salmon Injection is a sterile solution of Calcitonin Salmon in a suitable diluent. Each mL of Calcitonin Salmon Injection possesses an activity of NLT 80% and NMT 120% of that stated on the label.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **PROCEDURE**
  Solution A: Dissolve 3.26 g of tetramethylammonium hydroxide pentahydrate in 900 mL of water, add 100 mL of acetonitrile, and mix. Adjust with phosphoric acid to a pH of 2.5, pass through a filter of 0.5-µm or finer pore size, and degas.
  Solution B: Dissolve 1.45 g of tetramethylammonium hydroxide pentahydrate in 400 mL of water, add 600 mL of acetonitrile, and mix. Adjust with phosphoric acid to a pH of 2.5, pass through a filter of 0.5-µm or finer pore size, and degas.
  Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	72	28
30	48	52
32	72	28
55	72	28

System suitability solution: Prepare a solution in *Solution A* containing about 0.2 mg/mL of USP Calcitonin Salmon Related Compound A RS and 0.2 mg/mL of USP Calcitonin Salmon RS. Take 0.1 mL of this solution, add 0.9 mL of *Solution A,* and mix.
Standard stock solution: 1.0 mg/mL of USP Calcitonin Salmon RS in *Solution A*
Standard solution: 0.1 mg/mL of USP Calcitonin Salmon RS from *Standard stock solution* diluted with *Solution A*
Sample solution: Use the solution from an undiluted Injection vial.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability.*)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 65°
Flow rate: 1 mL/min
Injection volume: 200 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for calcitonin salmon and calcitonin salmon related compound A are 1.0 and 1.15, respectively.]
Suitability requirements
Resolution: NLT 3 between calcitonin salmon and calcitonin salmon related compound A
Tailing factor: NMT 2.5
Relative standard deviation: NMT 3%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the potency, in USP Calcitonin Salmon Units/mL, in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Calcitonin Salmon RS in the *Standard solution* (USP Calcitonin Salmon Units/mL)

Acceptance criteria: 80%–120%

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.625 USP Endotoxin Units/USP Calcitonin Salmon Unit
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **PH** ⟨791⟩**:** 3.9–4.5
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Avoid freezing. Store in a refrigerator.
- **LABELING:** Label it to indicate the activity in USP Calcitonin Salmon Units/mL. The labeling states that the material is synthetic. Label it to state that it is to be stored in a refrigerator, and that freezing is to be avoided.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Calcitonin Salmon RS
  USP Calcitonin Salmon Related Compound A RS
  N-Acetyl-cys[1]-calcitonin.
  $C_{146}H_{243}N_{44}O_{49}S_2$ 3463

USP Monographs

www.webofpharma.com

Calcitonin Salmon Nasal Solution

DEFINITION

Calcitonin Salmon Nasal Solution is a solution of Calcitonin Salmon in a suitable diluent. It contains suitable preservatives, and is packaged in a form suitable for nasal administration so that the required dosage can be controlled as required. Each mL of Calcitonin Salmon Nasal Solution possesses an activity of NLT 80% and NMT 115% of that stated on the label.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 3.26 mg/mL of tetramethylammonium hydroxide pentahydrate in water and acetonitrile (9:1). Adjust with phosphoric acid to a pH of 2.5.

Solution B: 1.45 mg/mL of tetramethylammonium hydroxide pentahydrate in acetonitrile and water (3:2). Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	72	28
30	48	52
32	72	28
55	72	28

Diluent: 7.5 mg/mL of sodium chloride, 2 mg/mL of sodium acetate, and 2 mg/mL of glacial acetic acid in water

System suitability stock solution: Prepare a solution in *Solution A* containing about 0.2 mg/mL of USP Calcitonin Salmon Related Compound A RS and 0.2 mg/mL of USP Calcitonin Salmon RS.

System suitability solution: *System suitability stock solution* and *Solution A* (1:9)

Standard stock solution: 1.0 mg/mL of USP Calcitonin Salmon RS in *Solution A*

Standard solution: 0.1 mg/mL of USP Calcitonin Salmon RS from the *Standard stock solution* in *Solution A*

Sample solution: Nasal Solution in *Diluent* (1:9)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 65°

Flow rate: 1 mL/min

Injection volume: 200 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for calcitonin salmon and calcitonin salmon related compound A are 1.0 and 1.15, respectively.]

Suitability requirements

Resolution: NLT 3 between calcitonin salmon and calcitonin salmon related compound A, *System suitability solution*

Tailing factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcitonin salmon ($C_{145}H_{240}N_{44}O_{48}S_2$) in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Calcitonin Salmon RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of calcitonin salmon in the *Sample solution* (mg/mL)

Acceptance criteria: 80%–115%

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 10^2 cfu/g, and the total combined molds and yeasts count is NMT 5×10^1 cfu/g. It meets the requirements for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

• **pH** ⟨791⟩**:** 3.5–4.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in containers suitable for spraying the contents into the nasal cavities in a controlled individualized dosage. Store unopened containers in a refrigerator and opened containers at room temperature.

• **LABELING:** Label it to indicate that it is for intranasal administration only. The labeling also states that it has been prepared either with Calcitonin Salmon of synthetic origin or Calcitonin Salmon of rDNA origin. Label it to state that it is to be stored in a refrigerator, and that freezing is to be avoided. Label it to indicate the activity in USP Calcitonin Salmon Units/mL.

• **USP REFERENCE STANDARDS** ⟨11⟩

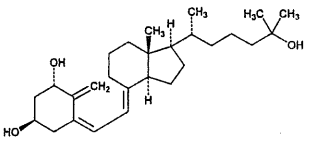
USP Calcitonin Salmon RS

USP Calcitonin Salmon Related Compound A RS

N-Acetyl-cys[1]-calcitonin.

$C_{146}H_{243}N_{44}O_{49}S_2$ 3463

Calcitriol



$C_{27}H_{44}O_3$ 416.64

$C_{27}H_{44}O_3 \cdot H_2O$ 434.65

9,10-Secocholesta-5,7,10(19)-triene-1,3,25-triol, (1α,3β,5*Z*,7*E*)-;

(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-triene-1α,3β,25-triol [32222-06-3].

Monohydrate [77326-95-5].

DEFINITION

Calcitriol is anhydrous or contains 1 molecule of hydration. The anhydrous form contains NLT 97.0% and NMT 103.0% of calcitriol ($C_{27}H_{44}O_3$), calculated on the solvent-free basis. The monohydrate form contains NLT

USP Monographs

www.webofpharma.com

97.0% and NMT 103.0% of calcitriol ($C_{27}H_{44}O_3$), calculated on the anhydrous basis.

[**CAUTION**—Care should be taken to prevent inhaling particles of calcitriol, and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Carry out the procedure as rapidly as possible, and protect all solutions containing calcitriol from light.

Buffer: 1.0 mg/mL of tris(hydroxymethyl)aminomethane in water, adjusted with phosphoric acid to a pH of 7.0–7.5 before final dilution

Mobile phase: Acetonitrile and *Buffer* (55:45)

Standard solution: 0.1 mg/mL of USP Calcitriol RS prepared as follows. Transfer an appropriate amount of USP Calcitriol RS to a suitable volumetric flask, dissolve in acetonitrile, using 55% of the final volume, then dilute with *Buffer* to volume.

System suitability solution: Heat 2.0 mL of the *Standard solution* at 80° for 30 min.

Sample solution: 0.1 mg/mL of Calcitriol prepared as follows. Transfer an appropriate amount of Calcitriol to a suitable volumetric flask, dissolve in acetonitrile, using 55% of the final volume, then dilute with *Buffer* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time: NLT 2 times the retention time of calcitriol

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for pre-calcitriol and calcitriol are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.5 between the pre-calcitriol and calcitriol peaks, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of calcitriol ($C_{27}H_{44}O_3$) in the portion of Calcitriol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses of calcitriol and pre-calcitriol from the *Sample solution*

r_S = sum of the peak responses of calcitriol and pre-calcitriol from the *Standard solution*

C_S = concentration of USP Calcitriol RS in the *Standard solution* (mg/mL)

C_U = concentration of Calcitriol in the *Sample solution* (mg/mL)

Acceptance criteria

Anhydrous form: 97.0%–103.0% on the solvent-free basis

Monohydrate form: 97.0%–103.0% on the anhydrous basis

IMPURITIES

- **ORGANIC IMPURITIES**

Carry out the procedure as rapidly as possible, and protect all solutions containing calcitriol from light.

Buffer, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of any individual impurity in the portion of Calcitriol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any individual peak other than the main calcitriol peak and the pre-calcitriol peak from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Triazoline adduct of pre-calcitriol[a]	0.43	0.1
trans-Calcitriol[b]	0.96	0.25
Calcitriol	1.0	—
1β-Calcitriol[c]	1.15	0.1
Methylene calcitriol[d]	1.5	0.25
Any unspecified impurity	—	0.1
Total impurities	—	1.0

[a] (6a*R*,7*R*,9a*R*)-11-[(3*S*,5*R*)-3,5-Dihydroxy-2-methylcyclohex-1-enyl]-7-[(*R*)-6-hydroxy-6-methylheptan-2-yl]-6a-methyl-2-phenyl-4a,5,6,6a,7,8,9,9a-octahydrocyclopenta[*f*][1,2,4]triazolo[1,2-*a*]cinnoline-1,3(2*H*,11*H*)-dione.

[b] (5*E*,7*E*)-9,10-Secocholesta-5,7,10(19)-triene-1α,3β,25-triol.

[c] (5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-triene-1β,3β,25-triol.

[d] (5*Z*,7*E*)-1α,3β-Dihydroxy-17-[(*R*)-7-hydroxy-7-methyloctan-2-yl]-9,10-secoandrosta-5,7,10(19)-triene.

SPECIFIC TESTS

- **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ic*: 3.5%–5.5%, where it is labeled as a monohydrate

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store as per labeling instructions.
- **LABELING:** Where it is a monohydrate form, the label so indicates.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Calcitriol RS

USP Monographs

www.webofpharma.com

Calcitriol Injection

» Calcitriol Injection is a sterile solution of Calcitriol. It contains an amount of Calcitriol equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of calcitriol ($C_{27}H_{44}O_3$). It contains no antimicrobial agents.

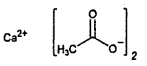
Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Calcitriol Solution RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 100 USP Endotoxin Units per µg of calcitriol.
pH ⟨791⟩**:** between 5.9 and 8.0, determined on a portion to which, if necessary, 0.30 mL of saturated potassium chloride solution has been added for each 100 mL of Injection.
Particulate Matter in Injections ⟨788⟩ **:** meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
[NOTE—Avoid unnecessary exposure of solutions to light or air.]
Mobile phase—Prepare a filtered and degassed mixture of methanol and water (74:26). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩) so that the retention time for calcitriol is not less than 20 minutes.
Standard preparation—Transfer 3.0 mL of USP Calcitriol Solution RS, equilibrated to room temperature, to a container; add 3.0 mL of water; and mix.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 3 µg of calcitriol, to a container; add a sufficient amount of water to dilute to a total volume of 3.0 mL; add 3.0 mL of methanol; and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 264-nm detector, a 4.6-mm × 4.5-cm guard column that contains 5-µm packing L1, and a 4.6-mm × 7.5-cm analytical column that contains 3-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of calcitriol ($C_{27}H_{44}O_3$) in each mL of the Injection taken by the formula:

$$C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of calcitriol in the USP Calcitriol Solution RS; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Calcium Acetate



$C_4H_6CaO_4$ 158.17
Acetic acid, calcium salt;
Calcium acetate [62-54-4].

DEFINITION
Calcium Acetate contains NLT 99.0% and NMT 100.5% of calcium acetate ($C_4H_6CaO_4$), calculated on the anhydrous basis.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩ and *Acetate* ⟨191⟩
Sample solution: 50 mg/mL
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Sample: 300 mg
Analysis: Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 7.909 mg of calcium acetate ($C_4H_6CaO_4$).
Acceptance criteria: 99.0%–100.5% on the anhydrous basis

IMPURITIES
• **ARSENIC,** *Method I* ⟨211⟩**:** NMT 3 ppm
• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Standard: 0.70 mL of 0.020 N hydrochloric acid
Sample: 1.0 g
Acceptance criteria: 0.05%
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Standard: 0.15 mL of 0.020 N sulfuric acid
Sample: 0.25 g
Acceptance criteria: 0.06%
• **LEAD** ⟨251⟩**:** NMT 10 ppm
• **LIMIT OF ALUMINUM**
[NOTE—Use where it is labeled as intended for parenteral use or for use in hemodialysis or peritoneal dialysis.]
Buffer: Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, and dilute with water to 250 mL.
Aluminum standard solution: 1.0 µg/mL of aluminum. Prepare as directed for *Standard Preparations* in *Aluminum* ⟨206⟩.
Standard solution: Prepare a solution containing 2.0 mL of *Aluminum standard solution,* 5 mL of *Buffer,* and 48 mL of water, and extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume.
Sample solution: Dissolve 1.0 g of Calcium Acetate in 50 mL of water, and add 5 mL of *Buffer.* Extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume.
Blank solution: Prepare a solution containing 50 mL of water and 5 mL of *Buffer.* Extract this solution with

www.webofpharma.com

successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume.

Instrumental conditions
(See *Fluorescence Spectroscopy* ⟨853⟩.)
Mode: Fluorescence
Excitation wavelength: 392 nm
Emission wavelength: 518 nm

Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank solution*
Use the *Blank solution* to zero the instrument.

Acceptance criteria: 2 ppm; the fluorescence of the *Sample solution* is NMT that of the *Standard solution*.

• **LIMIT OF BARIUM**
[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis.]

Barium chloride solution: 500 µg/mL of barium in water from anhydrous barium chloride

Buffer: Ammonium sulfate solution (1 in 10)

Standard solution: To a tube add 1 g of ammonium acetate, 2 mL of 1 N hydrochloric acid, 3.0 mL of *Barium chloride solution*, and sufficient water to bring the volume to 40 mL.

Sample stock solution: 250 mg/mL of Calcium Acetate and 25 mg/mL of ammonium acetate in 1 N hydrochloric acid. The pH of this solution is 4.5–5.5. Filter, and cover the solution.

Sample solutions: To four separate tubes add 1.0, 1.5, 2.0, and 2.5 mL of *Barium chloride solution*. To each tube add a sufficient volume of the *Sample stock solution* to bring the volume to 40 mL.

Analysis: To the *Sample solutions* and the *Standard solution* add, with brisk stirring, 3.0 mL of *Buffer*, and allow to stand for 20 min.

Acceptance criteria: The *Sample solutions* containing 1.0 and 1.5 mL of *Barium chloride solution* remain clear or are only faintly turbid. The *Sample solution* containing 2.0 mL of *Barium chloride solution* is not more turbid than the *Standard solution*.

• **LIMIT OF FLUORIDE**
[NOTE—Prepare and store all solutions in plastic containers.]

Buffer: 294 mg/mL of sodium citrate dihydrate in water

Standard stock solution: 1.11 mg/mL of USP Sodium Fluoride RS in water

Standard solution: Combine 20.0 mL of *Standard stock solution* with 50.0 mL of *Buffer*, and dilute with water to 100.0 mL. Equivalent to 100 µg/mL of fluoride

Sample solution: Transfer 2.0 g of Calcium Acetate to a beaker containing a plastic-coated stirring bar. Add 20.0 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer* and sufficient water to make 100 mL.

Electrode system: Use a fluoride-specific, ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see *pH* ⟨791⟩).

Analysis
Samples: *Standard solution* and *Sample solution*
Transfer 50.0 mL of *Buffer* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of the *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.
Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in mV. From the measured potential and the standard response line determine the concentration, *C*, in µg/mL, of fluoride ion in the *Sample solution*.
Calculate the amount of fluoride (ppm) in the sample taken by multiplying *C* by 50.

Acceptance criteria: 50 ppm

• **LIMIT OF MAGNESIUM**
[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis. The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

Standard stock solution: 1000 µg/mL of magnesium in 1 N nitric acid from magnesium oxide

Standard solution: 5.0 µg/mL of magnesium from the *Standard stock solution*

Sample solution: 2 mg/mL of Calcium Acetate

Linearity solution A: Dilute 20.0 mL of the *Sample solution* with water to 25.0 mL (0 µg/mL of magnesium).

Linearity solution B: Dilute 2.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (0.4 µg/mL of magnesium).

Linearity solution C: Dilute 4.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (0.8 µg/mL of magnesium).

Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 285.2 nm
Flame: Air–acetylene
Lamp: Magnesium hollow-cathode
Blank: Water

Analysis
Samples: *Linearity solutions A*, *B*, and *C*
Plot the absorbances of the *Linearity solutions* versus their content of magnesium (0, 0.4, and 0.8 µg/mL), draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg/mL, of magnesium in the *Sample solution*.
Calculate the percentage of magnesium in the sample by multiplying this value by 0.0625.

Acceptance criteria: NMT 0.05%

• **LIMIT OF NITRATE**

Sample solution: 100 mg/mL of Calcium Acetate in water

Analysis: To 10 mL of the *Sample solution* add 5 mg of sodium chloride, 0.05 mL of indigo carmine TS, and, with stirring, 10 mL of nitrogen-free sulfuric acid.

Acceptance criteria: The blue color persists for NLT 10 min.

• **LIMIT OF POTASSIUM**
[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis. The *Standard solution* and *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

Standard stock solution: 23.84 mg/mL of potassium chloride, using potassium chloride previously dried at 105° for 2 h, equivalent to 12.5 mg/mL of potassium

Standard solution: 31.25 µg/mL of potassium from the *Standard stock solution*

Sample solution: 12.5 mg/mL of Calcium Acetate

Linearity solution A: Dilute 20.0 mL of the *Sample solution* with water to 25.0 mL (0 µg/mL of potassium).

www.webofpharma.com

Linearity solution B: Dilute 2.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (2.5 µg/mL of potassium).
Linearity solution C: Dilute 4.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (5.0 µg/mL of potassium).
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 766.7 nm
Lamp: Potassium hollow-cathode
Flame: Air–acetylene
Blank: Water
Analysis
Samples: *Linearity solutions A, B,* and *C*
Plot the absorbances of the *Linearity solutions* versus their content of potassium (0, 2.5, and 5.0 µg/mL), draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg/mL, of potassium in the *Sample solution.*
Calculate the percentage of potassium in the sample by multiplying this value by 0.01.
Acceptance criteria: NMT 0.05%

• **LIMIT OF SODIUM**
[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis. The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]
Standard stock solution: 25.42 mg/mL of sodium chloride, using sodium chloride previously dried at 105° for 2 h, equivalent to 10.0 mg/mL of sodium
Standard solution: 250 µg/mL of sodium from the *Standard stock solution*
Sample solution: 10 mg/mL of Calcium Acetate
Linearity solution A: Dilute 20.0 mL of the *Sample solution* with water to 25.0 mL (0 µg/mL of sodium).
Linearity solution B: Dilute 2.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (20 µg/mL of sodium).
Linearity solution C: Dilute 4.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (40 µg/mL of sodium).
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 589.0 nm
Lamp: Sodium hollow-cathode
Flame: Air–acetylene
Blank: Water
Analysis
Samples: *Linearity solutions A, B,* and *C*
Plot the absorbances of the *Linearity solutions* versus their content of sodium (0, 20, and 40 µg/mL), draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg/mL, of sodium in the *Sample solution.*
Calculate the percentage of sodium in the sample by multiplying this value by 0.0125.
Acceptance criteria: NMT 0.5%

• **LIMIT OF STRONTIUM**
[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis. The *Standard solution* and *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]
Standard stock solution: 2.45 mg/mL of strontium acetate in water, equivalent to 1000 µg/mL of strontium
Standard solution: 50.0 µg/mL of strontium from the *Standard stock solution*
Sample solution: 20 mg/mL of Calcium Acetate
Linearity solution A: Dilute 20.0 mL of the *Sample solution* with water to 25.0 mL (0 µg/mL of strontium).
Linearity solution B: Dilute 2.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (4 µg/mL of strontium).
Linearity solution C: Dilute 4.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (8 µg/mL of strontium).
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 460.7 nm
Lamp: Strontium hollow-cathode
Flame: Nitrous oxide–acetylene
Blank: Water
Analysis
Samples: *Linearity solutions A, B,* and *C*
Plot the absorbances of the *Linearity solutions* versus their content of strontium (0, 4, and 8 µg/mL), draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg/mL, of strontium in the *Sample solution.*
Calculate the percentage of strontium in the sample by multiplying this value by 0.00625.
Acceptance criteria: NMT 0.05%

• **READILY OXIDIZABLE SUBSTANCES**
Sample solution: 20 mg/mL of Calcium Acetate in boiling water
Analysis: Add a few glass beads to 100 mL of the *Sample solution,* 6 mL of 10 N sulfuric acid, and 0.3 mL of 1 N potassium permanganate. Mix, boil gently for 5 min, and allow the precipitate to settle.
Acceptance criteria: The pink color in the supernatant is not completely discharged.

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: 50 mg/mL
Acceptance criteria: 6.3–9.6

• **WATER DETERMINATION,** *Method I* ⟨921⟩
Sample: 0.100 g
Analysis: Proceed as directed in the chapter, adding 2 mL of glacial acetic acid to the titration vessel in addition to the methanol.
Acceptance criteria: NMT 7.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** Where Calcium Acetate is intended for use in hemodialysis or peritoneal dialysis, it is so labeled.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sodium Fluoride RS

USP Monographs

www.webofpharma.com

Add the following:

▲Calcium Acetate Capsules

DEFINITION

Calcium Acetate Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium acetate ($C_4H_6CaO_4$).

IDENTIFICATION

- **A.** The retention time of the calcium peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Acetate*

Sample solution: 67 mg/mL of calcium acetate from Capsule contents

Acceptance criteria: Meet the requirements for test *B*

ASSAY

- **PROCEDURE**

Solution A: 0.75 mM dipicolinic acid and 1.7 mM nitric acid in water. [NOTE—Warm water may be required to dissolve dipicolinic acid.]

Mobile phase: Acetone and *Solution A* (100:900). Pass through a suitable filter of 0.2-µm pore size.

Standard solution: 0.08 mg/mL of USP Calcium Acetate RS in water

Sample stock solution: Nominally 6.7 mg/mL of calcium acetate prepared as follows. Transfer an appropriate portion of the contents of NLT 20 Capsules to a suitable volumetric flask. Add water to about 40% of the final volume of the flask and sonicate for 20 min with intermittent shaking. Dilute with water to volume. Pass through a suitable filter of 0.45-µm pore size.

Sample solution: Nominally 0.08 mg/mL of calcium acetate in water from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: Ion chromatography

Detector: Conductivity

Column: 4.0-mm × 15-cm; 5-µm packing L76

Column temperature: 35°

Flow rate: 0.9 mL/min

Injection volume: 10 µL

Run time: NLT 1.5 times the retention time of the calcium peak

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcium acetate ($C_4H_6CaO_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of calcium from the *Sample solution*

r_S = peak response of calcium from the *Standard solution*

C_S = concentration of USP Calcium Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of calcium acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

▲Test 1▲ (RB 1-May-2019)

Medium: Water; 900 mL

Apparatus 2: 50 rpm, with sinkers

Time: 10 min

Mobile phase, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute with *Medium* to a concentration similar to the *Standard solution*, if necessary.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcium acetate ($C_4H_6CaO_4$) dissolved at time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S \times V \times D \times (1/L) \times 100$$

r_U = peak response of calcium from the *Sample solution*

r_S = peak response of calcium from the *Standard solution*

C_S = concentration of USP Calcium Acetate RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

D = dilution factor for the *Sample solution*, if needed

L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of calcium acetate ($C_4H_6CaO_4$) is dissolved.

▲Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 15 min

Blank: 0.2% (v/v) nitric acid

Standard solutions: 4.0, 5.0, 6.0, 7.0, and 8.0 µg/mL of calcium [from commercially available, National Institute of Standards and Technology (NIST) traceable standard solution for calcium] in *Blank*

Sample solution: Pass a portion of the solution under test through a suitable filter of 1.0-µm pore size. Dilute with *Blank* to a concentration similar to 6.0-µg/mL *Standard solution*, if necessary.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrometry

Analytical wavelength: 422.8 nm

Lamp: Calcium hollow-cathode

Flame: Air–acetylene oxidizing flame

System suitability

Samples: *Blank* and *Standard solutions*

Suitability requirements

Linearity: Use the *Blank* to set the instrument to zero. Concomitantly determine the responses for each of the *Standard solutions*. Construct a linear calibration curve by plotting the absorbance values of the *Standard solutions* versus their corresponding concentrations, in micrograms per milliliter.

Correlation coefficient: NLT 0.995

Drift: Within ±2%, 7.0-µg/mL *Standard solution* . See *Atomic Absorption Spectroscopy* ⟨852⟩, *Procedure, Analysis*.

USP Monographs

www.webofpharma.com

Analysis
Sample: *Sample solution*
From the linear calibration curve, determine the concentration (C), in µg/mL, for calcium in the *Sample solution*.
Calculate the percentage of the labeled amount of calcium acetate ($C_4H_6CaO_4$) dissolved:

$$\text{Result} = C \times V \times F \times D \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

C = concentration of calcium in the *Sample solution* determined (µg/mL)
V = volume of *Medium*, 900 mL
F = equivalency factor, 0.001 mg/µg
D = dilution factor for the *Sample solution*, if needed
M_{r1} = molecular weight of calcium acetate, 158.17
M_{r2} = molecular weight of calcium, 40.08
L = label claim (mg/Capsule)

Tolerances: NLT 85% (Q) of the labeled amount of calcium acetate ($C_4H_6CaO_4$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Tier 1
Medium 1: Water; 900 mL
Apparatus 2: 100 rpm, with sinkers
Time: 15 min
Tier 2
Medium 2: Simulated gastric fluid TS; 900 mL
Apparatus 2: 100 rpm, with sinkers
Time: 15 min
Determine the amount of calcium acetate dissolved using *Analytical procedure 1* or *Analytical procedure 2* for *Tier 1* and *Analytical procedure 3* for *Tier 2*.
Sample stock solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Dissolution procedure: Perform the test using the conditions under *Tier 1*. In the presence of cross-linking, repeat the test with a new set of Capsules using the conditions under *Tier 2*.
Analytical procedure 1
Blank: 0.02 N nitric acid
Standard solutions: 2.4, 3.2, 4.0, 4.8, and 5.6 µg/mL of USP Calcium Acetate RS in *Blank*
Sample solution: Nominally 3.7 µg/mL of calcium acetate from *Sample stock solution*, dilute with *Blank* if necessary
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrometry
Analytical wavelength: 422.8 nm
Lamp: Calcium hollow-cathode
Flame: Nitrous oxide–acetylene
Replicates: 4
System suitability
Samples: *Blank*, *Standard solutions*, and *Sample solution*
Suitability requirements
Relative standard deviation: NMT 3.0% in 4 replicate measurements, *Standard solutions* and *Sample solution*
Correlation coefficient: NLT 0.995, use the *Blank* to set the instrument to zero. Concomitantly determine the responses for each of the *Standard solutions*. Construct a quadratic calibration curve by plotting the absorbance values of the *Standard solutions* versus their corresponding concentrations, in micrograms per milliter.
Drift: Within ±5%, the absorbance value of 5.6 µg/mL of USP Calcium Acetate RS from the *Standard solutions*. See *Atomic Absorption Spectroscopy* ⟨852⟩, *Procedure, Analysis*.
Analysis
Sample: *Sample solution*
From the quadratic calibration curve obtained from the *Correlation coefficient*, determine the concentration (C), in µg/mL, for calcium acetate in the *Sample solution*.
Calculate the percentage of the labeled amount of calcium acetate ($C_4H_6CaO_4$) dissolved:

$$\text{Result} = C \times V \times F \times D \times (1/L) \times 100$$

C = concentration of calcium acetate in the *Sample solution* determined (µg/mL)
V = volume of *Medium*, 900 mL
F = equivalency factor, 0.001 mg/µg
D = dilution factor for the *Sample solution*, if needed
L = label claim (mg/Capsule)

Analytical procedure 2
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Complexometric titration
Titrant: 0.005 M edetic acid (EDTA)
Endpoint detection: Photometric at 610 nm
Analysis: To an aliquot of the *Sample stock solution* equivalent to about 7.4 mg of calcium acetate, add 60 mL of 0.1 N sodium hydroxide and 0.2 g of hydroxynaphthol blue indicator. Titrate with *Titrant*, determining the endpoint photometrically using a suitable autotitrator.
Calculate the percentage of the labeled amount of calcium acetate ($C_4H_6CaO_4$) dissolved:

$$\text{Result} = V_S \times M \times F \times (V_M/V_A) \times (1/L) \times 100$$

V_S = volume of *Titrant* consumed by the aliquot of *Sample stock solution* (mL)
M = actual *Titrant* concentration, in molarity (mmol/mL)
F = equivalency factor of calcium acetate, 158.17 mg/mmol
V_M = volume of *Medium*, 900 mL
V_A = volume of the aliquot taken for *Analysis* (mL)
L = label claim (mg/Capsule)

Analytical procedure 3
Blank: *Medium*
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Complexometric titration
Titrant: 0.005 M edetic acid (EDTA)
Endpoint detection: Visual
Analysis: To an aliquot of the *Sample stock solution* equivalent to about 7.4 mg of calcium acetate, add 50 mL of water, 10 mL of 0.1 N sodium hydroxide, and 0.2 g of hydroxynaphthol blue indicator. Titrate with *Titrant* to a blue endpoint while stirring using a magnetic stirring bar. Perform a *Blank* determination in the same manner.
Calculate the percentage of the labeled amount of calcium acetate ($C_4H_6CaO_4$) dissolved:

$$\text{Result} = (V_S - V_B) \times M \times F \times (V_M/V_A) \times (1/L) \times 100$$

www.webofpharma.com

V_S = volume of *Titrant* consumed by the aliquot of *Sample stock solution* (mL)
V_B = volume of *Titrant* consumed by the *Blank* (mL)
M = actual *Titrant* concentration, in molarity (mmol/mL)
F = equivalency factor of calcium acetate, 158.17 mg/mmol
V_M = volume of *Medium*, 900 mL
V_A = volume of the aliquot taken for *Analysis* (mL)
L = label claim (mg/Capsule)

Tolerances: NLT 85% (*Q*) of the labeled amount of calcium acetate ($C_4H_6CaO_4$) is dissolved. ▲ (RB 1-May-2019)

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeast count does not exceed 10^2 cfu/g. It meets the requirements of the test for the absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers and store at controlled room temperature.

Add the following:

▲• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. ▲ (RB,1-May-2019)

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Calcium Acetate RS ▲ (USP 1-May-2019)

Calcium Acetate Tablets

DEFINITION

Calcium Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium acetate ($C_4H_6CaO_4$).

IDENTIFICATION

• **IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩and*Acetate* ⟨191⟩
Sample solution: 100 mg/mL of calcium acetate from powdered Tablets
Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**
Sample: Amount equivalent to 300 mg of calcium acetate from NLT 20 powdered Tablets
Analysis: Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, add 30 mL of 0.05 M edetate disodium VS from a 50-mL buret, and add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue. Continue the titration with the 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 7.909 mg of calcium acetate ($C_4H_6CaO_4$).
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrometry
Analytical wavelength: 422.8 nm
Standard solution: Calcium at a known concentration in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.
Acceptance criteria: NLT 80% (Q) of the labeled amount of calcium acetate ($C_4H_6CaO_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **LIMIT OF ALUMINUM**
Buffer: Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, and dilute with water to 250 mL.
Aluminum standard solution 1.0 µg/mL of aluminum. Prepare as directed for *Standard Preparation* under *Aluminum* ⟨206⟩.
Standard solution: Prepare a solution containing 2.0 mL of *Aluminum standard solution*, 5 mL of *Buffer*, and 48 mL of water, and extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask, and dilute the combined extracts with chloroform to volume.
Sample solution: Dissolve a portion of powdered Tablets (NLT 10) equivalent to 1.0 g of calcium acetate in 50 mL of water, and add 5 mL of *Buffer*. Extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask, and dilute the combined extracts with chloroform to volume.
Blank solution: Prepare a solution containing 50 mL of water and 5 mL of *Buffer*. Extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask, and dilute the combined extracts with chloroform to volume.
Instrumental conditions
(See *Fluorescence Spectroscopy* ⟨853⟩.)
Mode: Fluorescence
Excitation wavelength: 392 nm
Emission wavelength: 518 nm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank solution*. Use the *Blank solution* to zero the instrument.
Acceptance criteria: The fluorescence of the *Sample solution* does not exceed that of the *Standard solution* (NMT 2 ppm).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Calcium Ascorbate

$C_{12}H_{14}CaO_{12} \cdot 2H_2O$ 426.34

USP Monographs

www.webofpharma.com

DEFINITION
Calcium Ascorbate contains NLT 98.0% and NMT 101.0% of calcium ascorbate dihydrate ($C_{12}H_{14}CaO_{12} \cdot 2H_2O$), calculated on the as-is basis.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩: A 100 mg/mL solution meets the requirements.
- **B.** A 100 mg/mL solution decolorizes a 100 mg/mL solution of dichlorophenol–indophenol.

Change to read:

- **C. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

ASSAY
- **PROCEDURE**

Sample: 300 mg of Calcium Ascorbate
Blank: 50 mL of water
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N iodine VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* into a 250-mL conical flask, add 50 mL of water, and mix to dissolve. Immediately titrate with the *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a *Blank* determination. Calculate the percentage of calcium ascorbate dihydrate ($C_{12}H_{14}CaO_{12} \cdot 2H_2O$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = *Titrant* normality (mEq/mL)
F = equivalency factor, 106.6 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.0%–101.0% on the as-is basis

IMPURITIES
- **ARSENIC,** *Method I* ⟨211⟩: NMT 3 µg/g
- **LIMIT OF FLUORIDE**

[NOTE—Prepare and store all solutions in plastic containers.]
Buffer solution: 294 mg/mL of sodium citrate dihydrate in water
Standard stock solution: 1.1052 mg/mL of USP Sodium Fluoride RS in water
Standard solution: Transfer 20.0 mL of *Standard stock solution* to a 100-mL volumetric flask containing 50.0 mL of *Buffer solution*, dilute with water to volume, and mix. Each mL of *Standard solution* contains 100 µg of fluoride ion.
Sample solution: Transfer 2.0 g of Calcium Ascorbate to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer solution* and sufficient water to make 100 mL.
Electrode system: Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ± 0.2 mV (see *pH* ⟨791⟩).
Analysis
Samples: *Standard solution* and *Sample solution*
Standard response line: Transfer 50.0 mL of *Buffer solution* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.
Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard response line* determine the concentration, C (in µg/mL), of fluoride ion in the *Sample solution*.
Calculate the content of fluoride in the portion of Calcium Ascorbate taken:

$$\text{Result} = (C \times V)/W$$

C = concentration of fluoride ion in the *Sample solution* (µg/mL), obtained from the *Standard response line*
V = volume of the *Sample solution* (mL)
W = weight of Calcium Ascorbate taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 10 ppm

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 50 mg/mL in carbon dioxide-free water [NOTE—Perform measurements immediately after preparation.]
Acceptance criteria: +95° to +97°
- **pH** ⟨791⟩

Sample solution: 100 mg/mL
Acceptance criteria: 6.8–7.4
- **LOSS ON DRYING** ⟨731⟩: Dry 3 g at 105° for 2 h: it loses NMT 0.1% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Calcium Ascorbate RS
USP Sodium Fluoride RS

Calcium Carbonate

$CaCO_3$ 100.09
Carbonic acid, calcium salt (1:1);
Calcium carbonate (1:1) [471-34-1].

DEFINITION
Calcium Carbonate, dried at 200° for 4 h, contains calcium equivalent to NLT 98.0% and NMT 100.5% of calcium carbonate ($CaCO_3$).

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩: The addition of acetic acid to it produces effervescence (presence of carbonate), and the resulting solution, after boiling, meets the requirements of the tests.

ASSAY
- **TITRIMETRY** ⟨541⟩

Sample: 200 mg of Calcium Carbonate, previously dried at 200° for 4 h
Blank: 100 mL of water and 15 mL of 1 N sodium hydroxide
Titrimetric system
(See *Titrimetry* ⟨541⟩.)

USP Monographs

www.webofpharma.com

Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Indicator: 300 mg of hydroxy naphthol blue
Endpoint detection: Visual, change to distinct blue
Analysis: Transfer the *Sample* to a 250-mL beaker. Moisten thoroughly with a few mL of water, and add, dropwise, sufficient 3 N hydrochloric acid to dissolve. Add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue. Titrate with the *Titrant*. Calculate the percentage of calcium carbonate ($CaCO_3$) in the *Sample* taken:

$$\text{Result} = [(V - B) \times M \times F \times 100]/W$$

V = *Sample* titrant volume (mL)
B = *Blank* titrant volume (mL)
M = titrant molarity (mmol/mL)
F = equivalency factor, 100.09 mg/mmol
W = weight of the *Sample* (mg)

Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES

• **ACID-INSOLUBLE SUBSTANCES**
Sample: 5.0 g
Analysis: Mix the *Sample* with 10 mL of water, and add hydrochloric acid, dropwise, with agitation, until it ceases to cause effervescence, then add water to make the mixture measure 200 mL, and filter. Wash the insoluble residue with water until the last washing shows no chloride, and ignite and weigh the residue.
Acceptance criteria: NMT 0.2%; the weight of the residue does not exceed 10 mg.

• **ARSENIC,** *Method I* ⟨211⟩
Sample solution: Slowly dissolve 1.0 g in 15 mL of hydrochloric acid, and dilute with water to 55 mL.
Analysis: Omit the addition of 20 mL of 7 N sulfuric acid specified in *Arsenic* ⟨211⟩, *Method I, Procedure.*
Acceptance criteria: NMT 3 ppm

• **BARIUM:** A platinum wire, dipped in the filtrate obtained in the test for *Acid-Insoluble Substances* and held in a nonluminous flame, does not impart a green color.

• **IRON** ⟨241⟩
Sample solution: 40 mg in 5 mL of 2 N hydrochloric acid. Transfer to a beaker with the aid of water, and dilute with water to 10 mL.
Standard solution: Transfer 4.0 mL of the *Standard Iron Solution*, prepared as directed in *Iron* ⟨241⟩, to a beaker, and dilute with water to 10 mL.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Analytical wavelength: 530 nm
Blank: Water
Analysis: Separately to the *Sample solution* and *Standard solution* add 2 mL of citric acid solution (1 in 5) and 2 drops of thioglycolic acid, adjust with ammonia TS to a pH of 9.5 ± 0.1, dilute with water to 20 mL, and allow to stand for 5 min. Dilute with water to 50 mL. Concomitantly determine the absorbances of the solutions from the *Sample solution* and the *Standard solution.*
Acceptance criteria: NMT 0.1%; the absorbance of the solution from the *Sample solution* does not exceed that of the *Standard solution.*

• **LEAD** ⟨251⟩
Sample solution: 1.0 g in 5 mL of water
Analysis: To the *Sample solution* slowly add 8 mL of 3 N hydrochloric acid, evaporate on a steam bath to dryness, and dissolve the residue in 5 mL of water.
Acceptance criteria: NMT 3 ppm

• **LIMIT OF FLUORIDE**
[NOTE—Prepare and store all solutions in plastic containers.]
Solution A: 294 mg/mL of sodium citrate dihydrate in water
Sample: 2.0 g
Standard stock solution: 1.11 mg/mL of USP Sodium Fluoride RS in water
Standard solution: Combine 20.0 mL of the *Standard stock solution* with 50.0 mL of *Solution A*, and dilute with water to 100.0 mL. [NOTE—Each mL of this solution contains 100 µg of fluoride ion]
Electrode system: Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see *pH* ⟨791⟩).
Standard response line: Transfer 50.0 mL of *Solution A* and 4.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of the *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.
Analysis: Transfer the *Sample* to a beaker containing a plastic-coated stirring bar, add 20 mL of water and 4.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Solution A* and sufficient water to make 100 mL of test solution. Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard response line*, determine the concentration, C, in µg/mL, of fluoride ion in the *Sample solution.* Calculate the content of fluoride in the specimen taken:

$$\text{Result} = (V \times C)/W$$

V = volume of the *Sample solution* (mL)
C = concentration of fluoride in the *Sample solution* (µg/mL)
W = weight of *Sample* (g)

Acceptance criteria: NMT 50 ppm

• **LIMIT OF MAGNESIUM AND ALKALI SALTS**
Sample solution: 1.0 g
Analysis: Mix the *Sample* with 35 mL of water. Carefully add 3 mL of hydrochloric acid, heat the solution, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well-established. Add immediately to the warm mixture 2 drops of methyl red TS and then 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline. Cool to room temperature, transfer to a 100-mL graduated cylinder, dilute with water to 100 mL, mix, and allow to stand for 4 h or overnight. Filter, and to 50 mL of the clear filtrate in a platinum dish add 0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of ammonium salts. Finally, ignite the residue to constant weight.
Acceptance criteria: NMT 1.0%; the weight of the residue is NMT 5 mg.

• **MERCURY,** *Method IIa* ⟨261⟩
Mercury stock solution and **Standard mercury solution:** Proceed as directed in *Mercury* ⟨261⟩.
Standard solution: Proceed as directed in *Mercury* ⟨261⟩, except use 3 mL of hydrochloric acid instead of 3 mL of sulfuric acid.
Sample stock solution: 4.0 g in a 100-mL beaker, and cautiously dissolve in 14 mL of 6 N hydrochloric acid

USP Monographs

www.webofpharma.com

Sample solution: Proceed as directed in *Mercury* ⟨261⟩ using the *Sample stock solution*, except use 3 mL of hydrochloric acid instead of 3 mL of sulfuric acid.
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in *Mercury* ⟨261⟩.
Acceptance criteria: NMT 0.5 ppm

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩**:** Dry a sample at 200° for 4 h: it loses NMT 2.0% of its weight.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Sodium Fluoride RS

Calcium Carbonate Lozenges

DEFINITION
Calcium Carbonate Lozenges contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ($CaCO_3$).

IDENTIFICATION
• **A. Identification Tests—General,** *Calcium* ⟨191⟩**:** The addition of 6 N hydrochloric acid to a Lozenge produces effervescence, and the resulting solution, after being boiled to expel carbon dioxide and then neutralized with 6 N ammonium hydroxide, meets the requirements of the tests.

ASSAY
• **Procedure**
[Note—The *Standard solutions* and the *Sample solution* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]
Lanthanum chloride solution: Transfer 10 g of potassium chloride and 20 g of lanthanum chloride to a 2000-mL volumetric flask. Add 1000 mL of water and 40 mL of hydrochloric acid, mix, and allow to cool. Dilute with water to volume.
Standard stock solution: Transfer 250 mg of chelometric standard calcium carbonate, previously dried at 110° for 2 h and then cooled in a desiccator, to a 500-mL volumetric flask. Add 100 mL of water and 12 mL of 1 N hydrochloric acid, swirl to dissolve the calcium carbonate, and allow to cool. Dilute with water to volume. This stock solution contains about 500 µg/mL of calcium carbonate.
Standard solutions: To three separate 100-mL volumetric flasks add 2.0, 3.0, and 4.0 mL of the *Standard stock solution*, and dilute each with *Lanthanum chloride solution* to volume. These *Standard solutions* contain 10, 15, and 20 µg/mL of calcium carbonate, respectively.
Sample stock solution: Transfer the equivalent to 3000 mg of calcium carbonate, from powdered Lozenges, to a 1000-mL volumetric flask. Add 100 mL of 1 N hydrochloric acid and 300 mL of water, and sonicate to dissolve the powder. Dilute with water to volume.
Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 1000-mL volumetric flask, and dilute with *Lanthanum chloride solution* to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Lamp: Calcium hollow-cathode
Flame: Nitrous oxide–acetylene
Analytical wavelength: Calcium emission line at 422.7 nm
Blank: *Lanthanum chloride solution*
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbances of the *Standard solutions* versus their concentrations of calcium carbonate, in µg/mL, by drawing a straight line best fitting the three plotted points. From the graph determine the concentration, *C*, in µg/mL, of calcium carbonate in the *Sample solution*.
Calculate the percentage of label claim of calcium carbonate ($CaCO_3$) in the portion of Lozenges taken:

$$\text{Result} = (C/C_U) \times 100$$

C = measured concentration of calcium carbonate in the *Sample solution* (µg/mL), as calculated above
C_U = nominal concentration of calcium carbonate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS
• **Sodium Content** (if so labeled)
[Note—The *Standard solutions* and the *Sample solution* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]
Standard stock solution: Transfer 2.542 g of sodium chloride, previously dried at 105° for 2 h, to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.
Standard solutions: To three separate 100-mL volumetric flasks, add 1.0, 3.0, and 5.0 mL of the *Standard stock solution*, and dilute each with water to volume. These *Standard solutions* contain 1.0, 3.0, and 5.0 µg/mL of sodium, respectively.
Sample stock solution: Prepare as directed in the *Assay*. Pass a portion of it, if necessary, through a filter of 0.5-µm or finer pore size, and use the clear solution.
Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Lamp: Sodium hollow-cathode
Flame: Air–acetylene
Analytical wavelength: Sodium emission line at 589.6 nm
Blank: Water
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbances of the *Standard solutions* versus their contents of sodium, in µg/mL, by drawing a straight line best fitting the three plotted points. From the graph determine the quantity, *C*, in µg, of sodium in each mL of the *Sample solution*.
Calculate the percentage of label claim of sodium in the portion of Lozenges taken:

$$\text{Result} = (C/C_U) \times 100$$

C = measured concentration of sodium in the *Sample solution* (µg/mL), as calculated above
C_U = nominal concentration of sodium in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 115.0% of the labeled amount

www.webofpharma.com

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

SPECIFIC TESTS

• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩

Analysis: The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq of acid and NLT the number of mEq calculated by:

$$\text{Result} = (F_C \times C) \times 0.9$$

F_C = theoretical acid-neutralizing capacity of $CaCO_3$, 0.02 mEq

C = quantity of $CaCO_3$ in the sample tested (mg), based on the labeled quantity

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Calcium Carbonate Oral Suspension

DEFINITION

Calcium Carbonate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ($CaCO_3$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩: The addition of acetic acid to it produces effervescence (presence of carbonate). The resulting solution, after boiling, meets the requirements.

ASSAY

• **PROCEDURE**

Sample solution: Transfer a portion of Oral Suspension, equivalent to 1 g of calcium carbonate, previously well shaken in its original container, to a beaker with the aid of 25 mL of water. Add 20 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min. Allow to cool, and transfer with the aid of water to a 100-mL volumetric flask. Dilute with water to volume. Mix, and filter.

Blank: 100 mL of water, 15 mL of 1 N sodium hydroxide, and 5 mL of triethanolamine

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Indicator: 100 mg of hydroxy naphthol blue

Endpoint detection: Visual, change to distinct blue

Analysis: Transfer 20.0 mL of the *Sample solution* to a suitable container. Dilute with water to 100 mL. Add 15 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with the *Titrant*. Calculate the percentage of the labeled amount of calcium carbonate ($CaCO_3$) in the sample taken:

$$\text{Result} = [(V_S - V_B) \times M \times F \times 100]/W$$

V_S = volume of the *Titrant* consumed by the *Sample solution* (mL)

V_B = volume of the *Titrant* consumed by the *Blank* (mL)

M = *Titrant* molarity (mmol/mL)

F = equivalency factor, 100.09 mg/mmol

W = nominal amount of calcium carbonate taken for the *Analysis* (mg)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **LIMIT OF FLUORIDE**

[NOTE—Prepare and store all solutions in plastic containers.]

Solution A: 294 mg/mL of sodium citrate dihydrate in water

Standard stock solution: 1.1 mg/mL of USP Sodium Fluoride RS in water

Standard solution: Combine 20.0 mL of the *Standard stock solution* with 50.0 mL of *Solution A*, and dilute with water to 100.0 mL. [NOTE—Each mL of this solution contains 100 µg of fluoride ion.]

Sample solution: Transfer a portion of Oral Suspension, equivalent to 2.0 g of calcium carbonate, to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 4.0 mL of hydrochloric acid. Stir until dissolved. Add 50.0 mL of *Solution A* and sufficient water to make 100.0 mL.

Electrode system: Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see *pH* ⟨791⟩).

Standard response line: Transfer 50.0 mL of *Solution A* and 4.0 mL of hydrochloric acid to a beaker. Add water to make 100.0 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, and stir for 15 min. Read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of the *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.

Analysis: Rinse and dry the electrodes, and insert them into the *Sample solution*. Stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard response line*, determine the concentration, C, in µg/mL, of fluoride ion in the *Sample solution*. Calculate the content of fluoride in the sample taken:

$$\text{Result} = (V \times C)/W$$

V = volume of the *Sample solution* (mL)

C = determined concentration of fluoride in the *Sample solution* (µg/mL)

W = nominal weight of calcium carbonate taken (g)

Acceptance criteria: 50 µg/g, with respect to the labeled amount of calcium carbonate

• **ARSENIC,** *Method I* ⟨211⟩

Test preparation: Slowly dissolve a portion of Oral Suspension equivalent to 1.0 g of calcium carbonate in 15 mL of hydrochloric acid. Dilute with water to 55 mL.

Analysis: Proceed as directed in the chapter, except omit the addition of 20 mL of 7 N sulfuric acid specified under *Procedure*.

Acceptance criteria: NMT 3 µg/g, with respect to the labeled amount of calcium carbonate

• **LEAD** ⟨251⟩

Test preparation: Mix a portion of Oral Suspension equivalent to 1.0 g of calcium carbonate in 5 mL of water.

Analysis: To the *Test preparation* slowly add 8 mL of 3 N hydrochloric acid. Evaporate on a steam bath to dryness, and dissolve the residue in 5 mL of water.

Acceptance criteria: NMT 3 µg/g, with respect to the labeled amount of calcium carbonate

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count is NMT 10^2 cfu/mL. It meets the

www.webofpharma.com

requirements of the tests for absence of *Escherichia coli* and *Pseudomonas aeruginosa*.
- **pH** ⟨791⟩: 7.5–8.7

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers, and avoid freezing.
- **USP Reference Standards** ⟨11⟩
  USP Sodium Fluoride RS

Calcium Carbonate Tablets

DEFINITION
Calcium Carbonate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ($CaCO_3$). For Tablets labeled for any indication other than, or in addition to, antacid use, the Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of calcium carbonate.

IDENTIFICATION
- **A. Identification Tests—General,** *Calcium* ⟨191⟩: The addition of 6 N acetic acid to the Tablets produces effervescence, and the resulting solution, after being boiled to expel carbon dioxide and neutralized with 6 N ammonium hydroxide, meets the requirements.

ASSAY
- **Procedure**

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 200 mg of calcium carbonate, to a suitable crucible. Ignite to constant weight. Cool the crucible, add 10 mL of water, and dissolve the residue by adding sufficient 3 N hydrochloric acid, dropwise, to achieve complete solution.
Blank: 150 mL of water and 15 mL of 1 N sodium hydroxide
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Indicator: 300 mg of hydroxy naphthol blue
Endpoint detection: Visual, change to distinct blue
Analysis: Transfer the *Sample solution* completely to a suitable container, and dilute with water to 150 mL. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue. Titrate with the *Titrant*.
Calculate the percentage of calcium carbonate ($CaCO_3$) in the sample taken:

$$\text{Result} = [(V_S - V_B) \times M \times F \times 100]/W$$

V_S = volume of the *Titrant* consumed by the *Sample solution* (mL)
V_B = volume of the *Titrant* consumed by the *Blank* (mL)
M = *Titrant* molarity (mmol/mL)
F = equivalency factor, 100.09 mg/mmol
W = weight of calcium carbonate taken (mg)

Acceptance criteria: 90.0%–110.0% of the labeled amount of $CaCO_3$. For Tablets labeled for any indication other than, or in addition to, antacid use, 90.0%–115.0% of the labeled amount of $CaCO_3$

PERFORMANCE TESTS
- **Dissolution** ⟨711⟩
  [Note—For Tablets labeled for any indication other than, or in addition to, antacid use.]

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Lanthanum chloride solution: 50 mg/mL of lanthanum chloride in 0.1 N hydrochloric acid
Standard stock solution: 100 µg/mL of calcium in 0.1 N hydrochloric acid
Standard solutions: Into separate 100-mL volumetric flasks containing 10.0 mL of *Lanthanum chloride solution* pipet 3-, 4-, 5-, and 6-mL portions of *Standard stock solution* and dilute each with 0.1 N hydrochloric acid to volume to obtain solutions with calcium concentrations of 3, 4, 5, and 6 µg/mL, respectively.
Sample solution: Filter a portion of the solution under test. Pipet a volume of the filtrate, estimated to contain 1 mg of calcium, into a 250-mL volumetric flask. Add 25.0 mL of *Lanthanum chloride solution*, and dilute with 0.1 N hydrochloric acid to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 422.8 nm
Lamp: Calcium hollow-cathode
Flame: Air–acetylene
Blank: *Lanthanum chloride solution* and 0.1 N hydrochloric acid (1:9)
Analysis
Samples: *Standard solutions* and *Sample solution*
Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* against the *Blank*. Construct a standard curve by plotting absorbances versus calcium concentrations of the *Standard solutions*, then from it obtain the concentration, C, in µg/mL of calcium, of the *Sample solution*.
Calculate the percentage of the labeled amount of calcium carbonate ($CaCO_3$) dissolved:

$$\text{Result} = (M_r/A_r) \times (C \times D \times V/L) \times 100$$

M_r = molecular weight of calcium carbonate, 100.09
A_r = atomic weight of calcium, 40.08
C = measured concentration of calcium in the *Sample solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of calcium carbonate ($CaCO_3$) is dissolved.
- **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

SPECIFIC TESTS
- **Acid-Neutralizing Capacity** ⟨301⟩: For Tablets labeled for antacid use

Analysis: Proceed as directed in the chapter.
Acceptance criteria: NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated as follows:

$$\text{Result} = (C \times A_{NC}) \times F$$

C = quantity of $CaCO_3$ in the sample tested (mg), based on the labeled amount
A_{NC} = theoretical acid-neutralizing capacity of $CaCO_3$, 0.02 mEq/mg
F = acceptance factor for the lower limit of the required acid-neutralizing capacity, 0.9

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate whether it is for use as an antacid, or as a dietary supplement, or both.

Calcium Carbonate and Magnesia Tablets

DEFINITION
Calcium Carbonate and Magnesia Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ($CaCO_3$) and NLT 90.0% and NMT 115.0% of the labeled amount of magnesium hydroxide [$Mg(OH)_2$].

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩**:** The addition of 3 N hydrochloric acid to the Tablets produces effervescence. The resulting solution, after being boiled to expel carbon dioxide and neutralized with 6 N ammonium hydroxide, meets the requirements of the tests.
- **B. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
  Sample solution: Heat 2 Tablets in 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 min. Filter this solution, and add 2 mL of 1 N hydrochloric acid to the filtrate.
  Acceptance criteria: The solution meets the requirements.

ASSAY
- **CALCIUM CARBONATE**
  Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 400 mg of calcium carbonate, to a beaker with 25 mL of water. Add 40 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min, allow to cool, and transfer with the aid of water to a 100-mL volumetric flask. Dilute with water to volume, mix, filter, and use the filtrate. [NOTE—Reserve a portion of it for the test for *Magnesium Hydroxide*.]
  Analysis: Transfer 20.0 mL of the *Sample solution* to a suitable container, dilute with water to 100 mL, and add 30 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue in color. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of $CaCO_3$.
  Acceptance criteria: 90.0%–110.0%
- **MAGNESIUM HYDROXIDE**
  Sample solution: Use the *Sample solution* from the test for *Calcium Carbonate*.
  Analysis: Transfer a portion of the *Sample solution*, equivalent to 120 mg of calcium carbonate and magnesium hydroxide combined, to a suitable container. Dilute with water to 100 mL, and add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. The volume, in mL, of 0.05 M edetate disodium consumed, less the volume of 0.05 M edetate disodium corresponding to the content of calcium carbonate in the volume, in mL, of the *Sample solution* taken, represents the volume, in mL, of 0.05 M edetate disodium equivalent to the quantity of magnesium hydroxide present. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of $Mg(OH)_2$.
  Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Weight Variation* with respect to calcium carbonate and to magnesia

SPECIFIC TESTS
- **ACID-NEUTRALIZING CAPACITY** ⟨301⟩
  Analysis: NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated by the formula:

$$\text{Result} = [0.8 \times (F_M \times M)] + [0.9 \times (F_C \times C)]$$

F_M = theoretical acid-neutralizing capacity of $Mg(OH)_2$, 0.0343 mEq
M = quantity of $Mg(OH)_2$ in the sample tested (mg), based on the labeled quantity
F_C = theoretical acid-neutralizing capacity of $CaCO_3$, 0.02 mEq
C = quantity of $CaCO_3$ in the sample tested (mg), based on the labeled quantity

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Calcium Carbonate and Magnesia Chewable Tablets

DEFINITION
Calcium Carbonate and Magnesia Chewable Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ($CaCO_3$) and NLT 90.0% and NMT 115.0% of the labeled amount of magnesium hydroxide [$Mg(OH)_2$].

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩**:** The addition of 3 N hydrochloric acid to the Chewable Tablets produces effervescence. The resulting solution, after being boiled to expel carbon dioxide and neutralized with 6 N ammonium hydroxide, meets the requirements of the tests.
- **B. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩
  Sample solution: Heat 2 Chewable Tablets in 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 min. Filter this solution, and add 2 mL of 1 N hydrochloric acid to the filtrate.
  Acceptance criteria: The solution meets the requirements.

ASSAY
- **CALCIUM CARBONATE**
  Sample solution: Finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent to 400 mg of calcium carbonate, to a beaker with 25 mL of water. Add 40 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min, allow to cool, and transfer with the aid of water to a 100-mL volumetric flask. Dilute with water to volume, mix, filter, and use the filtrate. [NOTE—Reserve a portion of it for the test for *Magnesium Hydroxide*.]
  Analysis: Transfer 20.0 mL of the *Sample solution* to a suitable container, dilute with water to 100 mL, and add 30 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue in color. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of $CaCO_3$.
  Acceptance criteria: 90.0%–110.0%

USP Monographs

www.webofpharma.com

• **MAGNESIUM HYDROXIDE**
Sample solution: Use the *Sample solution* from the test for *Calcium Carbonate.*
Analysis: Transfer a portion of the *Sample solution,* equivalent to 120 mg of calcium carbonate and magnesium hydroxide combined, to a suitable container. Dilute with water to 100 mL, and add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. The volume, in mL, of 0.05 M edetate disodium consumed, less the volume of 0.05 M edetate disodium corresponding to the content of calcium carbonate in the volume, in mL, of the *Sample solution* taken, represents the volume, in mL, of 0.05 M edetate disodium equivalent to the quantity of magnesium hydroxide present. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of $Mg(OH)_2$.
Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Weight Variation* with respect to calcium carbonate and to magnesia

SPECIFIC TESTS
• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩
Analysis: NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated by the formula:

$$\text{Result} = [0.8 \times (F_M \times M)] + [0.9 \times (F_C \times C)]$$

F_M = theoretical acid-neutralizing capacity of $Mg(OH)_2$, 0.0343 mEq
M = quantity of $Mg(OH)_2$ in the sample tested (mg), based on the labeled quantity
F_C = theoretical acid-neutralizing capacities of $CaCO_3$, 0.02 mEq
C = quantity of $CaCO_3$ in the sample tested (mg), based on the labeled quantity

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** Label the Chewable Tablets to indicate that they must be chewed before being swallowed.

Calcium Carbonate, Magnesia, and Simethicone Chewable Tablets

Former title: Calcium Carbonate, Magnesia, and Simethicone Tablets

» Calcium Carbonate, Magnesia, and Simethicone Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of calcium carbonate ($CaCO_3$) and magnesium hydroxide [$Mg(OH)_2$], and an amount of polydimethylsiloxane [$-(CH_3)_2SiO-]_n$ that is not less than 85.0 percent and not more than 115.0 percent of the labeled amount of simethicone.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label it to indicate that the Chewable Tablets are to be chewed before swallowing. Label the Chewable Tablets to state the sodium content, in mg per Chewable Tablet, if it is greater than 5 mg per Chewable Tablet.
USP Reference standards ⟨11⟩—
USP Polydimethylsiloxane RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy:197S*▲ (CN 1-May-2020)—*Solution*—Using Chewable Tablets, proceed to obtain IR absorption spectra as directed in the *Assay for polydimethylsiloxane* under *Alumina, Magnesia, and Simethicone Chewable Tablets*

B: The addition of 1 N hydrochloric acid to a Chewable Tablet produces effervescence, and the resulting solution, after having been filtered, meets the requirements of the tests for *Calcium* ⟨191⟩.

C: Heat 2 Chewable Tablets in 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 minutes. Filter this solution, and to the filtrate add 2 mL of 1 N hydrochloric acid: this solution meets the requirements of the tests for *Magnesium* ⟨191⟩.
Uniformity of dosage units ⟨905⟩**:** meet the requirements for *Weight Variation* with respect to calcium carbonate and to magnesium hydroxide.
Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

Content of sodium (if so labeled)—
Lanthanum chloride solution—Prepare as directed in the *Assay for calcium carbonate and magnesium hydroxide.*
Dilute hydrochloric acid—Prepare as directed in the *Assay for polydimethylsiloxane.*
Standard solution—Transfer 2.542 g of sodium chloride, previously dried at 105° for 2 hours, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 4.0 mL of this solution to a second 100-mL volumetric flask containing 6.0 mL of *Dilute hydrochloric acid* and 2.0 mL of *Lanthanum chloride solution,* dilute with water to volume, and mix. This solution contains 2.0 µg of sodium (Na) per mL.
Test solution—Transfer 3.0 mL of the aqueous layer retained from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane* to a 50-mL volumetric flask containing 1.0 mL of *Lanthanum chloride solution,* dilute with water to volume, and mix.
Blank solution—Transfer 15.0 mL of *Dilute hydrochloric acid* and 5.0 mL of *Lanthanum chloride solution* to a 250-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the sodium emission line at 589.0 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a sodium hollow-cathode lamp and an air–acetylene flame, using the *Blank solution* as the blank. Calculate the mg of sodium (Na) in each Chewable Tablet taken by the formula:

$$(5C/6)(A/W)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of sodium in the *Standard solution; A* is the average weight, in mg, of each

www.webofpharma.com

Chewable Tablet; *W* is the weight, in mg, of the portion of Chewable Tablets from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane* used to prepare the *Test solution;* and A_U and A_S are the absorbances of the *Test solution* and the *Standard solution,* respectively. Each Chewable Tablet contains not more than the number of mg of sodium stated on the label.

Assay for polydimethylsiloxane—

Saccharin solution—Prepare a solution of saccharin in 4-methyl-2-pentanone containing 12.5 mg per mL.

Dilute hydrochloric acid—Mix 200 mL of hydrochloric acid with sufficient water to make 1000 mL.

Standard preparation—Dissolve a suitable quantity of USP Polydimethylsiloxane RS in 4-methyl-2-pentanone to obtain a stock solution having a known concentration of about 1 mg per mL. On the day of use, transfer 20.0 mL of this solution and 5.0 mL of *Saccharin solution* to a 250-mL volumetric flask, dilute with 4-methyl-2-pentanone to volume, and mix. This solution contains about 0.08 mg of USP Polydimethylsiloxane RS per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of polydimethylsiloxane, to a 125-mL separator. Cautiously add 50.0 mL of *Dilute hydrochloric acid,* and swirl until the reaction subsides. Insert the stopper, and mix. Carefully release the pressure, add 50.0 mL of 4-methyl-2-pentanone, and mix for 10 minutes. Allow the layers to separate, and drain the aqueous layer into a suitable stoppered container. [NOTE—Retain this aqueous layer for use in preparing the *Assay preparation* in the *Assay for calcium carbonate and magnesium hydroxide* and for the preparation of the *Test solution* in the test for *Content of sodium.*] Filter the organic layer through a filter containing 50 g of anhydrous sodium sulfate. Transfer 10.0 mL of the filtrate to a 50-mL volumetric flask, add 1.0 mL of *Saccharin solution,* dilute with methyl isobutyl ketone to volume, and mix.

Blank solution—Transfer 1.0 mL of *Saccharin solution* to a 50-mL volumetric flask, dilute with 4-methyl-2-pentanone to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the silicon emission line at 251.6 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a silicon hollow-cathode lamp and a nitrous oxide–acetylene flame, using the *Blank solution* as the blank. Calculate the quantity, in mg, of polydimethylsiloxane in each Chewable Tablet taken by the formula:

$$250C(A/W)(A_U/A_S)$$

in which *C* is the concentration, in mg per mL, of USP Polydimethylsiloxane RS in the *Standard preparation; A* is the average weight, in mg, of each Chewable Tablet; *W* is the weight, in mg, of the portion of Chewable Tablets taken to prepare the *Assay preparation;* and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation,* respectively.

Assay for calcium carbonate and magnesium hydroxide—

Lanthanum chloride solution—Transfer 26.8 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, and carefully add 50 mL of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume, and mix.

Dilute hydrochloric acid—Prepare as directed in the *Assay for polydimethylsiloxane.*

Calcium stock standard solution—Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of *Dilute hydrochloric acid,* and swirl to dissolve the calcium carbonate. Dilute with water to volume, and mix. This solution contains 1000 µg of calcium (Ca) per mL.

Magnesium stock standard solution—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water, slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with water to volume, and mix. This solution contains 1000 µg of magnesium (Mg) per mL.

Calcium and magnesium standard preparation—To a 250-mL volumetric flask add 10.0 mL of *Calcium stock standard solution* and 5.0 mL of *Magnesium stock standard solution,* dilute with water to volume, and mix. This solution contains 40 µg of calcium (Ca) and 20 µg of magnesium (Mg) per mL. On the day of use, transfer 4.0 mL of this solution to a 100-mL volumetric flask containing 2.0 mL of *Lanthanum chloride solution,* dilute with water to volume, and mix. This solution contains 1.6 µg of calcium (Ca) and 0.8 µg of magnesium (Mg) per mL.

Assay preparation—Transfer an accurately measured volume of the aqueous layer retained from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane,* equivalent to about 28 mg of calcium carbonate, to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 3.0 mL of this solution to a 100-mL volumetric flask containing 2.0 mL of *Lanthanum chloride solution,* dilute with water to volume, and mix.

Blank solution—Transfer 5.0 mL of *Lanthanum chloride solution* to a 250-mL volumetric flask, dilute with water to volume, and mix.

Procedure for calcium carbonate—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the calcium emission line at 422.7 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a calcium hollow-cathode lamp and a nitrous oxide–acetylene flame, using the *Blank solution* as the blank. Calculate the quantity, in mg, of calcium carbonate ($CaCO_3$) in each Chewable Tablet taken by the formula:

$$(100.09/40.08)(1000C/3V)(A/W)(A_U/A_S)$$

in which 100.09 is the molecular weight of calcium carbonate; 40.08 is the atomic weight of calcium; *C* is the concentration, in µg per mL, of calcium in the *Standard preparation; V* is the volume, in mL, of the aqueous layer retained from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane* used to prepare the *Assay preparation; A* is the average weight, in mg, of each Chewable Tablet; *W* is the weight, in mg, of the portion of Chewable Tablets taken to prepare the *Assay preparation* in the *Assay for polydimethylsiloxane;* and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation,* respectively.

Procedure for magnesium hydroxide—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a magnesium hollow-cathode lamp and a nitrous oxide–acetylene flame, using the *Blank solution* as the blank. Calculate the quantity, in mg, of magnesium hydroxide [$Mg(OH)_2$] in each Chewable Tablet taken by the formula:

$$(58.34/24.305)(1000C/3V)(A/W)(A_U/A_S)$$

www.webofpharma.com

in which 58.34 is the molecular weight of magnesium hydroxide; 24.305 is the atomic weight of magnesium; *C* is the concentration, in µg per mL, of magnesium in the *Standard preparation*; *V* is the volume, in mL, of the aqueous layer retained from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane* used to prepare the *Assay preparation*; *A* is the average weight, in mg, of each Chewable Tablet taken; *W* is the weight, in mg, of the portion of Chewable Tablets taken to prepare the *Assay preparation* in the *Assay for polydimethylsiloxane*; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Calcium and Magnesium Carbonates Oral Suspension

DEFINITION

Calcium and Magnesium Carbonates Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ($CaCO_3$) and NLT 85.0% and NMT 115.0% of the labeled amount of magnesium carbonate ($MgCO_3$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩**:** The addition of 3 N hydrochloric acid to a quantity of Oral Suspension, equivalent to 500 mg of calcium carbonate, produces effervescence, and the resulting solution, after having been filtered, meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩

Sample solution: Heat a quantity of Oral Suspension, equivalent to 800 mg of magnesium carbonate, with 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 min. Filter this solution, and add 2 mL of 1 N hydrochloric acid to the filtrate.

Acceptance criteria: Meets the requirements

ASSAY

- **CALCIUM CARBONATE**

Sample solution: Transfer a portion of Oral Suspension equivalent to 400 mg of calcium carbonate, previously well shaken in its original container and free of air bubbles, to a beaker, with the aid of 20 mL of water, and add 10 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min, allow to cool, transfer with the aid of water to a 100-mL volumetric flask, dilute with water to volume, filter and use the filtrate. [NOTE—Reserve a portion of the filtrate for the *Sample solution* in the *Magnesium Carbonate* test.]

Analysis: Transfer 20.0 mL of *Sample solution* to a suitable container. Dilute with water to 100 mL, and add 15 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of $CaCO_3$.

Acceptance criteria: 90.0%–110.0%

- **MAGNESIUM CARBONATE**

Sample solution: Use a portion of the filtrate from the *Sample solution* in the *Calcium Carbonate* test.

Analysis: Transfer the *Sample solution*, equivalent to 120 mg of calcium carbonate and magnesium carbonate combined, to a suitable container. Dilute with water to 100 mL, and add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. From the volume of 0.05 M edetate disodium consumed, subtract the volume of 0.05 M edetate disodium corresponding to the content of calcium carbonate in the portion of the *Sample solution* taken. The difference is the volume of 0.05 M edetate disodium equivalent to the quantity of magnesium carbonate present. Each mL of 0.05 M edetate disodium is equivalent to 4.216 mg of $MgCO_3$.

Acceptance criteria: 85.0%–115.0%

PERFORMANCE TESTS

- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 100 cfu/mL, and it meets the requirements of the tests for absence of *Escherichia coli* and *Pseudomonas aeruginosa*.
- **pH** ⟨791⟩**:** 7.0–8.6
- **ACID-NEUTRALIZING CAPACITY** ⟨301⟩**:** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated:

$$\text{Result} = [(F_M \times M) \times 0.8] + [(F_C \times C) \times 0.9]$$

F_M	= theoretical acid-neutralizing capacity of $MgCO_3$, 0.024 mEq
M	= quantity of $MgCO_3$ in the specimen tested, based on the labeled quantity (mg)
F_C	= theoretical acid-neutralizing capacity of $CaCO_3$, 0.02 mEq
C	= quantity of $CaCO_3$ in the specimen tested, based on the labeled quantity (mg)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.

Calcium and Magnesium Carbonates Tablets

DEFINITION

Calcium and Magnesium Carbonates Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ($CaCO_3$) and NLT 85.0% and NMT 115.0% of the labeled amount of magnesium carbonate ($MgCO_3$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩**:** The addition of 1 N hydrochloric acid to 1 Tablet produces effervescence, and the resulting solution, after having been filtered, meets the requirements of the tests.
- **B. IDENTIFICATION TESTS—GENERAL,** *Magnesium* ⟨191⟩

Sample solution: Heat 2 Tablets in 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 min. Filter this solution, and add 2 mL of 1 N hydrochloric acid to the filtrate.

Acceptance criteria: Meet the requirements

ASSAY

- **CALCIUM CARBONATE**

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 400 mg of calcium carbonate, to a beaker with the aid of 25 mL of water, and add 10 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min, allow to cool, and transfer to a 100-mL volumetric flask with the aid of water. Dilute with water to volume, mix, filter, and use the filtrate. [NOTE—Reserve a

www.webofpharma.com

portion of the filtrate for the *Sample solution* in the *Magnesium Carbonate* test.]

Analysis: Transfer 20.0 mL of *Sample solution* to a suitable container. Dilute with water to 100 mL, and add 15 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of $CaCO_3$.

Acceptance criteria: 90.0%–110.0%

• **MAGNESIUM CARBONATE**

Sample solution: Use a portion of the filtrate from the *Sample solution* in the *Calcium Carbonate* test.

Analysis: Transfer the *Sample solution* equivalent to 120 mg of calcium carbonate and magnesium carbonate combined to a suitable container. Dilute with water to 100 mL, and add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. From the volume of 0.05 M edetate disodium consumed, subtract the volume of 0.05 M edetate disodium corresponding to the content of calcium carbonate in the portion of the *Sample solution* taken. The difference is the volume of 0.05 M edetate disodium equivalent to the quantity of magnesium carbonate present. Each mL of 0.05 M edetate disodium is equivalent to 4.216 mg of $MgCO_3$.

Acceptance criteria: 85.0%–115.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩

Time: NMT 10 min, except that where Tablets are labeled as gelatin-coated, the time is NMT 30 min, simulated gastric fluid TS being substituted for water in the test

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Weight Variation* with respect to calcium carbonate and to magnesium carbonate

SPECIFIC TESTS

• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩

Analysis: NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated:

$$\text{Result} = [(F_M \times M) \times 0.8] + [(F_C \times C) \times 0.9]$$

F_M = theoretical acid-neutralizing capacity of $MgCO_3$, 0.024 mEq

M = quantity of $MgCO_3$ in the specimen tested, based on the labeled quantity (mg)

F_C = theoretical acid-neutralizing capacity of $CaCO_3$, 0.02 mEq

C = quantity of $CaCO_3$ in the specimen tested, based on the labeled quantity (mg)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** Tablets that are gelatin-coated are so labeled.

Calcium Chloride

$CaCl_2 \cdot 2H_2O$ 147.01

Calcium chloride dihydrate [10035-04-8].

$CaCl_2$ 110.98

Anhydrous [10043-52-4].

DEFINITION

Calcium Chloride contains an amount of $CaCl_2$ equivalent to NLT 99.0% and NMT 107.0% of $CaCl_2 \cdot 2H_2O$.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩

Sample solution: 100 mg/mL

Acceptance criteria: Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

Sample solution: 100 mg/mL

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Sample solution: Dissolve 1 g of Calcium Chloride in a mixture of water and 3 N hydrochloric acid (100:5). Transfer the solution to a 250-mL volumetric flask, and dilute with water to volume.

Analysis: Pipet 50 mL of the *Sample solution* into a suitable container, and add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 7.351 mg of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$).

Acceptance criteria: 99.0%–107.0% of $CaCl_2 \cdot 2H_2O$

IMPURITIES

• **ALUMINUM** ⟨206⟩

[NOTE—Perform if labeled as intended for use in hemodialysis.]

Test preparation: Use a 2.0-g sample.

Acceptance criteria: NMT 1 ppm

• **IRON, ALUMINUM, AND PHOSPHATE**

Sample solution: 50 mg/mL

Analysis: Add 2 drops of 3 N hydrochloric acid and 1 drop of phenolphthalein TS to the *Sample solution*. Then add ammonium chloride–ammonium hydroxide TS, dropwise, until the solution is faintly pink. Add 2 drops in excess, and heat the liquid to boiling.

Acceptance criteria: No turbidity or precipitate is produced.

• **LIMIT OF MAGNESIUM AND ALKALI SALTS**

Sample solution: 20 mg/mL

Analysis: To 50 mL of *Sample solution* add 500 mg of ammonium chloride. Heat the solution, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Add immediately to the warm mixture 2 drops of methyl red TS and then 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline. Cool to room temperature. Transfer to a 100-mL graduated cylinder, dilute with water to 100 mL, mix, and allow to stand for 4 h or overnight. Filter, and to 50 mL of the clear filtrate in a platinum dish, add 0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of ammonium salts. Finally, ignite the residue to constant weight.

Acceptance criteria: The weight of the residue is NMT 5 mg (1.0%).

SPECIFIC TESTS

• **PH** ⟨791⟩

Sample solution: 50 mg/mL

Acceptance criteria: 4.5–9.2

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Where Calcium Chloride is intended for use in hemodialysis, it is so labeled.

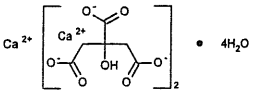
www.webofpharma.com

Calcium Chloride Injection

» Calcium Chloride Injection is a sterile solution of Calcium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $CaCl_2 \cdot 2H_2O$.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.
Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.
Identification—It responds to the tests for *Calcium* and *Chloride* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.2 USP Endotoxin Unit per mg of calcium chloride.
pH ⟨791⟩: between 5.5 and 7.5 in the undiluted Injection, except where the concentration is greater than 1 in 20, in which case this range applies to the Injection diluted with water to yield a concentration of 1 in 20.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer an accurately measured volume of Injection, equivalent to about 1 g of calcium chloride, to a 250-mL volumetric flask, add 5 mL of 3 N hydrochloric acid, dilute with water to volume, and mix. Pipet 50 mL of the resulting solution into a suitable container, add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue, and titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 7.351 mg of $CaCl_2 \cdot 2H_2O$.

Calcium Citrate



$C_{12}H_{10}Ca_3O_{14} \cdot 4H_2O$ 570.49
1,2,3-Propanetricarboxylic acid, 2-hydroxy-, calcium salt (2:3), tetrahydrate;
Calcium citrate (3:2), tetrahydrate [5785-44-4].

DEFINITION
Calcium Citrate contains four molecules of water of hydration. When dried at 150° to constant weight, it contains NLT 97.5% and NMT 100.5% of $Ca_3(C_6H_5O_7)_2$.

IDENTIFICATION
• **A.**
Analysis: Dissolve 0.5 g in a mixture of 10 mL of water and 2.5 mL of 2 N nitric acid. Add 1 mL of mercuric sulfate TS, heat to boiling, and add 1 mL of potassium permanganate TS.
Acceptance criteria: A white precipitate is formed.
• **B.**
Sample: 0.5 g of Calcium Citrate
Analysis: Ignite completely the *Sample* at as low a temperature as possible, cool, and dissolve the residue in dilute glacial acetic acid (1:10). Filter, and add 10 mL of ammonium oxalate TS to the filtrate.
Acceptance criteria: A voluminous white precipitate that is soluble in hydrochloric acid is formed.

ASSAY
• **PROCEDURE**
Sample solution: Dissolve 350 mg of Calcium Citrate, previously dried at 150° to constant weight, in 12 mL of 0.5 M hydrochloric acid, and dilute with water to about 100 mL.
Analysis: While stirring the *Sample solution*, add 30 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 8.307 mg of calcium citrate $(Ca_3(C_6H_5O_7)_2)$.
Acceptance criteria: 97.5%–100.5% on the dried basis

IMPURITIES
• **ARSENIC,** *Method I* ⟨211⟩
Test preparation: Dissolve 1 g of Calcium Citrate in 5 mL of 3 N hydrochloric acid, and dilute with water to 35 mL.
Acceptance criteria: NMT 3 ppm
• **LEAD** ⟨251⟩
Test preparation: Dissolve 0.5 g of Calcium Citrate in 20 mL of 3 N hydrochloric acid. Evaporate this solution on a steam bath to 10 mL, dilute with water to 20 mL, and cool. Use 5 mL of *Diluted Standard Lead Solution* (5 µg of Pb) for the test.
Acceptance criteria: NMT 10 ppm
• **LIMIT OF FLUORIDE**
[NOTE—Prepare and store all solutions in plastic containers.]
Standard stock solution: 1000 µg/mL of fluoride ion from USP Sodium Fluoride RS in water
Standard solution: 5 µg/mL of fluoride ion from *Standard stock solution*. [NOTE—Prepare on the day of use.]
Linearity solution A: Transfer 1.0 mL of the *Standard solution* to a 250-mL plastic beaker. Add 50 mL of water, 5 mL of 1 N hydrochloric acid, 10 mL of 1.0 M sodium citrate, and 10 mL of 0.2 M edetate disodium. If necessary, adjust with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of 5.5. Transfer to a 100-mL volumetric flask, and dilute with water to volume. This solution contains 0.05 µg/mL of fluoride.
Linearity solution B: Transfer 5.0 mL of the *Standard solution* to a 250-mL plastic beaker, and proceed as directed for *Linearity solution A* beginning with "Add 50 mL of water". This solution contains 0.25 µg/mL of fluoride.
Linearity solution C: Transfer 10.0 mL of the *Standard solution* to a 250-mL plastic beaker, and proceed as directed for *Linearity solution A* beginning with "Add 50 mL of water". This solution contains 0.50 µg/mL of fluoride.
Sample solution: Transfer 1.0 g of Calcium Citrate to a 100-mL beaker. Add 10 mL of water and, while stirring, 10 mL of 1 N hydrochloric acid. When dissolved, boil rapidly for 1 min, transfer the solution to a 250-mL plastic beaker, and cool in ice water. Add 15 mL of 1.0 M sodium citrate and 10 mL of 0.2 M edetate disodium, and adjust with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of 5.5. Transfer this solution to a 100-mL volumetric flask, and dilute with water to volume.
Electrode system: Use a fluoride-specific, ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see *pH* ⟨791⟩).
Analysis
Samples: *Linearity solution A, Linearity solution B, Linearity solution C,* and *Sample solution*

www.webofpharma.com

Transfer 50 mL of each *Linearity solution A*, *Linearity solution B*, and *Linearity solution C* to separate 250-mL plastic beakers, and measure the potential of each solution with the *Electrode system*. Between each reading wash the electrodes with water, and absorb any residual water by blotting the electrodes dry. Plot the logarithms of the fluoride concentrations (0.05, 0.25, and 0.50 µg/mL, respectively) versus potential to obtain a Standard response line.

Transfer 50 mL of the *Sample solution* to a 250-mL plastic beaker, and measure the potential with the *Electrode system*. From the measured potential and the Standard response line determine the concentration, *C*, in µg/mL, of fluoride ion in the *Sample solution*. Calculate the percentage of fluoride in the specimen taken by multiplying *C* by 0.01.

Acceptance criteria: NMT 0.003%

• **LIMIT OF ACID-INSOLUBLE SUBSTANCES**

Sample solution: Dissolve 5 g of Calcium Citrate by heating with a mixture of hydrochloric acid and water (10:50) for 30 min.

Analysis: Filter, wash, and dry at 105° for 2 h the residue so obtained.

Acceptance criteria: The weight of the residue is NMT 10 mg (0.2%).

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 150° for 4 h: it loses from 10.0% to 13.3% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Sodium Fluoride RS

Add the following:

▲Calcium Citrate Malate

$Ca_3(C_6H_5O_7)_2 \cdot 3CaC_4H_4O_5 \cdot 6H_2O$ 1122.97

1,2,3-Propanetricarboxylic acid, 2-hydroxy-, calcium salt mixture with 2-hydroxybutanedioic acid calcium salt (2:3:6), hexahydrate;

Calcium citrate malate (6:2:3), hexahydrate.

DEFINITION

Calcium Citrate Malate is a complex of calcium, citrate, and malate. It contains NLT 23.0% and NMT 24.2% of calcium, calculated on the dried basis. The sum of citrate and malate is NLT 73.0%, calculated on the dried basis.

IDENTIFICATION

• **A.**

Sample: 0.5 g of Calcium Citrate Malate

Analysis: Ignite completely the *Sample* at as low a temperature as possible, cool, and dissolve the residue in dilute glacial acetic acid (1:10). Filter, and add 10 mL of ammonium oxalate TS to the filtrate.

Acceptance criteria: A voluminous white precipitate that is soluble in hydrochloric acid is formed.

• **B.** The retention times of the citric acid peak and the malic acid peak of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Content of Citrate, Malate, and Fumarate*.

ASSAY

• **PROCEDURE**

Sample: 350 mg of Calcium Citrate Malate

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a 250-mL beaker, add 10 mL of water, 3 mL of 3 N hydrochloric acid, and swirl to dissolve. Add 90 mL of water and stir using a magnetic stirring bar. While stirring, add 30 mL of *Titrant* from the titration buret, and 25 mL of 1 N sodium hydroxide. Add 150 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform a blank determination. Calculate the percentage of calcium in the portion of Calcium Citrate Malate taken:

$$\text{Result} = \{[(V_S - V_B) \times M_A \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the blank (mL)
M_A = actual molarity of the *Titrant* (mmol/mL)
F = equivalency factor, 40.08 mg/mmol
W = *Sample* weight (mg)

Acceptance criteria: 23.0%–24.2% on the dried basis

IMPURITIES

Change to read:

• **LIMIT OF FLUORIDE**

[NOTE—Prepare and store all solutions in plastic containers.]

Buffer solution: 294 mg/mL of sodium citrate dihydrate in water

Standard stock solution: 1.1052 mg/mL of USP Sodium Fluoride RS in water

Standard solution: Transfer ▲2.0 mL▲ (ERR 1-Aug-2018) of *Standard stock solution* to a 1000-mL volumetric flask, dilute with water to volume, and mix. Each milliliter of this solution contains 1 µg of fluoride ion.

Sample solution: Transfer 1.0 g of Calcium Citrate Malate to a 100-mL beaker containing a plastic-coated stirring bar. Add 10 mL of water and place on a hot plate (medium heat). While stirring, add 10 mL of 1 N hydrochloric acid to dissolve. When dissolved, boil rapidly for 1 min, and transfer the solution to a 250-mL beaker. Cool rapidly to room temperature in an ice bath. Add 15 mL of *Buffer solution* and 10 mL of 0.2 M edetic acid, and mix. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 5.5 ± 0.1. Transfer the solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Pour the solution back to the 250-mL beaker for performing the titration.

Electrode system: Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see *pH* ⟨791⟩).

Analysis

Samples: *Standard solution* and *Sample solution*

Standard response line: Transfer 5.0, 25.0, and 50.0 mL of the *Standard solution* to three individual 250-mL beakers containing a plastic-coated stirring bar. Add 10 mL of 1 N hydrochloric acid, 15 mL of *Buffer solution*, and 0.2 M edetic acid to each beaker and mix. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 5.5 ± 0.1. Transfer the solutions to individual 100-mL

www.webofpharma.com

volumetric flasks, dilute with water to volume, and mix. Pour the solutions back to the individual 250-mL beakers for performing the titration. Insert the electrodes into the solutions starting with the lowest concentration, stir for 15 min, and read the potential in millivolts. Between measurements, wash both reference and ion-selective electrodes with water and dry with cloth or paper towel. Plot the logarithms of the fluoride ion concentrations (0.05, 0.25, and 0.5 µg/mL) versus potential, in millivolts. Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in millivolts. From the measured potential and the *Standard response line* determine the concentration (*C*), in µg/mL, of fluoride ion in the *Sample solution*.

Calculate the content of fluoride in the portion of Calcium Citrate Malate taken:

$$\text{Result} = (V \times C)/W$$

V = volume of the *Sample solution* (mL)
C = concentration of fluoride ion in the *Sample solution* determined from the *Standard response line* (µg/mL)
W = weight of Calcium Citrate Malate taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 30 µg/g

• **ELEMENTAL IMPURITIES—PROCEDURES** ⟨233⟩

Acceptance criteria

Arsenic: NMT 3.0 µg/g

Lead: NMT 1.0 µg/g

SPECIFIC TESTS

• **CONTENT OF CITRATE, MALATE, AND FUMARATE**

Diluent: 0.2 N hydrochloric acid

Mobile phase: 50 mM of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

System suitability solution: 5 mg/mL of USP Calcium Citrate Malate RS in *Diluent*

Citric acid standard solution: 1.8 mg/mL of USP Citric Acid RS in *Diluent*

Malic acid standard solution: 1.8 mg/mL of USP Malic Acid RS in *Diluent*

Fumaric acid standard solution: 20 µg/mL of USP Fumaric Acid RS in *Diluent*

Sample solution: 5 mg/mL of Calcium Citrate Malate in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 226 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution*, *Citric acid standard solution*, *Malic acid standard solution*, and *Fumaric acid standard solution*

[NOTE—The relative retention times for malic acid, citric acid, and fumaric acid are 0.6, 1.0, and 1.4, respectively.]

Suitability requirements

Resolution: NLT 8.0 between malic acid and citric acid, *System suitability solution*

Relative standard deviation: NMT 2.0% for each *Standard solution*

Chromatogram similarity: The chromatogram of the *System suitability solution* is similar to the reference chromatogram provided with the lot of USP Calcium Citrate Malate RS being used.

Analysis

Samples: *Citric acid standard solution*, *Malic acid standard solution*, *Fumaric acid standard solution*, and *Sample solution*

Separately calculate the percentage of citrate, malate, and fumarate in the portion of Calcium Citrate Malate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak response of citric acid, malic acid, or fumaric acid from the *Sample solution*
r_S = peak response of citric acid, malic acid, or fumaric acid from the respective *Standard solutions*
C_S = concentration of USP Citric Acid RS, USP Malic Acid RS, or USP Fumaric Acid RS in the respective *Standard solutions* (mg/mL)
C_U = concentration of Calcium Citrate Malate in the *Sample solution* (mg/mL)
F = factor for converting molecular weight of the acid form to that of the salt form, 0.98

Acceptance criteria

Sum of citrate and malate: NLT 73.0% on the dried basis

Fumarate: NMT 0.1% on the dried basis

• **pH** ⟨791⟩

Sample solution: 30 mg/mL in water. [NOTE—The solution is a slurry.]

Acceptance criteria: 4.0–8.0

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 150° for 4 h.

Acceptance criteria: 8.0%–11.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Calcium Citrate Malate RS
USP Citric Acid RS
USP Fumaric Acid RS
USP Malic Acid RS
USP Sodium Fluoride RS▲ 1S (USP41)

Calcium Glubionate Syrup

DEFINITION

Calcium Glubionate Syrup is a solution containing equimolar amounts of Calcium Gluconate and Calcium Lactobionate or with Calcium Lactobionate predominating. It contains NLT 95.0% and NMT 105.0% of the labeled amount of calcium (Ca).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩

Sample solution: Syrup in water (1 in 10)

Acceptance criteria: Meets the requirements

• **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 2 mg/mL of calcium gluconate and 4 mg/mL of calcium lactobionate in water

Sample solution: Equivalent to 0.4 mg/mL of calcium, from Syrup in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

USP Monographs

www.webofpharma.com

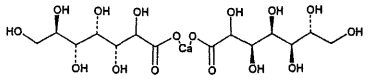
Application volume: 5 µL
Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)
Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber and dry at 100° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for 10 min.
Acceptance criteria: The two principal spots of the *Sample solution* correspond in color, size, and R_F value to those obtained from the *Standard solution*. [NOTE—Sucrose, if present in the *Sample solution*, appears in the chromatogram as a spot between the two principal spots.]

ASSAY
• **PROCEDURE**
Sample solution: Transfer a portion of Syrup equivalent to 70 mg of Ca into a suitable beaker. Add 2 mL of 3 N hydrochloric acid, and dilute with water to 150 mL.
Analysis: While stirring the *Sample solution* with a magnetic stirrer, add 20 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of calcium (Ca).
Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 3.4–4.5

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, at a temperature not exceeding 30°, and avoid freezing.

Calcium Gluceptate



$C_{14}H_{26}CaO_{16}$ (anhydrous) 490.42
Glucoheptonic acid, calcium salt (2:1);
Calcium glucoheptonate (1:2) [29039-00-7].

DEFINITION
Calcium Gluceptate is anhydrous or contains varying amounts of water of hydration. It consists of the calcium salt of the alpha epimer of glucoheptonic acid or of a mixture of the alpha and beta epimers of glucoheptonic acid. It contains NLT 95.0% and NMT 102.0% of calcium gluceptate ($C_{14}H_{26}CaO_{16}$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩: A 20-mg/mL solution meets the requirements.

ASSAY
• **PROCEDURE**
Sample: 800 mg of Calcium Gluceptate
Blank: 150 mL of water containing 2 mL of 3 N hydrochloric acid
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* with 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, preferably with a magnetic stirrer, add 25 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.
Calculate the percentage of calcium gluceptate ($C_{14}H_{26}CaO_{16}$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mM/mL)
F = equivalency factor, 490.4 mg/mM
W = *Sample* weight (mg)

Acceptance criteria: 95.0%–102.0% on the dried basis

IMPURITIES
• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Standard: 1.0 mL of 0.020 N hydrochloric acid
Sample: 1.0 g
Acceptance criteria: NMT 0.07%
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Standard: 1.0 mL of 0.020 N sulfuric acid
Sample: 2.0 g
Acceptance criteria: NMT 0.05%
• **REDUCING SUGARS**
Sample: 0.50 g
Analysis: Dissolve the *Sample* in 10 mL of hot water, add 2 mL of 3 N hydrochloric acid, boil for about 2 min, and cool. Add 5 mL of sodium carbonate TS, allow to stand for 5 min, dilute with water to 20 mL, and filter. Add 5 mL of the clear filtrate to 2 mL of alkaline cupric tartrate TS, and boil for 1 min.
Acceptance criteria: No red precipitate is formed immediately.

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 100 mg/mL
Acceptance criteria: 6.0–8.0
• **LOSS ON DRYING** ⟨731⟩
(See *Thermal Analysis* ⟨891⟩.)
[NOTE—The quantity taken for the determination may be adjusted, if necessary, for instrument sensitivity. Weight loss occurring at temperatures above about 160°, indicative of decomposition, is not to be interpreted as *Loss on Drying*.]
Sample: 10–25 mg
Analysis: Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument. Heat the specimen under test at a rate of 5°/min in an atmosphere of nitrogen, at a flow rate of 40 mL/min. Record the thermogram to 150°.
Acceptance criteria: See *Table 1*.

www.webofpharma.com

Table 1

Form	Loss on Drying (%)
Anhydrous	NMT 1.0
$2H_2O$ (dihydrate)	NMT 6.9
$3\frac{1}{2}H_2O$	NMT 11.4

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label to indicate whether hydrous or anhydrous; if hydrous, label to indicate also the degree of hydration.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Calcium Gluceptate RS

Calcium Gluceptate Injection

» Calcium Gluceptate Injection is a sterile solution of Calcium Gluceptate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of calcium (Ca).

Packaging and storage—Preserve in tight, single-dose containers, preferably of Type I or Type II glass.
Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.
USP Reference standards ⟨11⟩—
USP Calcium Gluceptate RS

Identification—

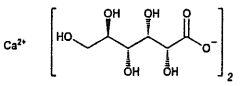
Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—Prepare the test specimen as follows. Transfer 5 mL of Injection to a separator, add 10 mL of chloroform, shake, and allow the layers to separate. Draw off and discard the chloroform layer, and repeat the extraction with a second 10-mL portion of chloroform. Drain the water layer into a small beaker, evaporate to dryness, and dry in vacuum at 60° for 16 hours.

B: A dilution of the Injection with water (1 in 5) responds to the tests for *Calcium* ⟨191⟩.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.32 USP Endotoxin Unit per mg of calcium gluceptate.
pH ⟨791⟩: between 5.6 and 7.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—To an accurately measured volume of Injection, equivalent to about 45 mg of calcium, add 2 mL of 3 N hydrochloric acid and 148 mL of water. While stirring, preferably with a magnetic stirrer, add about 15 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of calcium (Ca).

Calcium Gluconate



$C_{12}H_{22}CaO_{14}$ 430.37
$C_{12}H_{22}CaO_{14} \cdot H_2O$ 448.39
D-Gluconic acid, calcium salt (2:1);
Calcium D-gluconate (1:2) [299-28-5].
Monohydrate [66905-23-5].

DEFINITION
Calcium Gluconate is anhydrous or contains one molecule of water of hydration. The anhydrous form contains NLT 98.0% and NMT 102.0% of calcium gluconate ($C_{12}H_{22}CaO_{14}$), calculated on the dried basis. The monohydrate form contains NLT 99.0% and NMT 101.0% of calcium gluconate monohydrate ($C_{12}H_{22}CaO_{14} \cdot H_2O$) where labeled as intended for use in preparing injectable dosage forms, and NLT 98.5% and NMT 102.0% of calcium gluconate monohydrate ($C_{12}H_{22}CaO_{14} \cdot H_2O$) where labeled as not intended for use in preparing injectable dosage forms.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Calcium*
  Sample solution: 20 mg/mL
  Acceptance criteria: Meets the requirements
- **B. INFRARED ABSORPTION**
  Standard preparation: Prepare USP Calcium Gluconate Anhydrous RS or USP Calcium Gluconate Monohydrate RS in the form of a potassium bromide pellet.
  Sample preparation: Prepare calcium gluconate anhydrous or calcium gluconate monohydrate in the form of a potassium bromide pellet.
  Analysis
  Samples: *Standard preparation* and *Sample preparation*
  Record the spectra over the range from about 2.6 to 15 µm (3800–650 cm^{-1}).
  Acceptance criteria: The spectrum of Calcium Gluconate labeled as anhydrous exhibits the differential maxima at the wavenumbers in *Table 1* that are consistent with those of the spectrum of USP Calcium Gluconate Anhydrous RS. The spectrum of Calcium Gluconate labeled as monohydrate exhibits the differential maxima at the wavenumbers in *Table 1* that are consistent with those of the spectrum of USP Calcium Gluconate Monohydrate RS.

Table 1

Spectral Region	Wavenumber (cm^{-1}) of Characteristic Absorptions for Anhydrous	Wavenumber (cm^{-1}) of Characteristic Absorptions for Monohydrate
O-H stretching	No sharp band at about 3485, only broad absorption	At about 3485 (medium sharp over broad absorption)
C=O stretching	1618 (strong)	1595 (strong)

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Spectral Region	Wavenumber (cm^{-1}) of Characteristic Absorptions for Anhydrous	Wavenumber (cm^{-1}) of Characteristic Absorptions for Monohydrate
	1329 (medium)	Not observed
	Not observed	1305 (medium)
	1263–1250 (strong, two fused bands)	Not observed
	Not observed	1236 (medium)
	1007 (medium)	Not observed
	Not observed	1045 (medium)
	948 (duplet, medium)	Not observed
	Not observed	972 (weak)
	865 (weak)	Not observed
	Not observed	878 (weak)
Fingerprint	766 (medium)	Not observed

ASSAY

• **PROCEDURE**

Sample: 800 mg of calcium gluconate anhydrous or calcium gluconate monohydrate

Blank: 150 mL of water containing 2 mL of 3 N hydrochloric acid

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, add 30 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.

Calculate the percentage of calcium gluconate ($C_{12}H_{22}CaO_{14}$) or calcium gluconate monohydrate ($C_{12}H_{22}CaO_{14} \cdot H_2O$) in the *Sample* taken:

$$\text{Result (anhydrous form)} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

$$\text{Result (monohydrate form)} = \{[(V_S - V_B) \times M \times F]/W\} \times (M_{r2}/M_{r1}) \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = *Titrant* molarity (mmol/mL)
F = equivalency factor, 430.4 mg/mmol
W = *Sample* weight (mg)
M_{r2} = molecular weight of calcium gluconate monohydrate, 448.4
M_{r1} = molecular weight of calcium gluconate anhydrous, 430.4

Acceptance criteria

Anhydrous: 98.0%–102.0% on the dried basis

Monohydrate: 99.0%–101.0% where labeled as intended for use in preparing injectable dosage forms; 98.5%–102.0% where labeled as not intended for use in preparing injectable dosage forms

IMPURITIES

• **ARSENIC** ⟨211⟩, *Method I*

Test preparation: Dissolve 1.0 g in a mixture of 10 mL of hydrochloric acid and 20 mL of water, and dilute with water to 55 mL.

Analysis: Proceed as directed in the chapter, except to omit the addition of 20 mL of 7 N sulfuric acid.

Acceptance criteria: NMT 3 ppm

• **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*: A 1.0-g portion shows no more chloride than corresponds to 0.07 mL of 0.020 N hydrochloric acid (0.005%). Where it is labeled as not intended for use in the preparation of injectable dosage forms, a 1.0-g portion shows no more chloride than corresponds to 1 mL of 0.020 N hydrochloric acid (0.07%).

• **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*: A 2.0-g portion dissolved in boiling water shows no more sulfate than corresponds to 0.1 mL of 0.020 N sulfuric acid (0.005%). Where it is labeled as not intended for use in the preparation of injectable dosage forms, a 2.0-g portion dissolved in boiling water shows no more sulfate than corresponds to 1 mL of 0.020 N sulfuric acid (0.05%).

• **LIMIT OF IRON**

[NOTE—Calcium Gluconate labeled as not intended for use in the preparation of injectable dosage forms is exempt from this requirement.]

Internal standard solution: 2 µg/mL of yttrium in 10% nitric acid, prepared from commercially prepared yttrium standard suitable for ICP–OES. [NOTE—The yttrium concentration can be varied to optimize the analysis.]

Standard solutions: 0.05, 0.1, 0.2, and 0.4 µg/mL of iron in 10% nitric acid, prepared from commercially prepared iron standard suitable for ICP–OES

Sample solution: 40 mg/mL of Calcium Gluconate in 10% nitric acid

Blank solution: 10% Nitric acid solution

Instrumental conditions

(See *Plasma Spectrochemistry* ⟨730⟩.)

Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)

Emission wavelengths: 239.562 nm or optimized wavelength for iron; 371.029 nm or optimized wavelength for yttrium

System suitability

Samples: *Internal standard solution*, *Standard solutions*, and *Blank solution*

Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check. Generate the calibration curve using the *Blank solution* and *Standard solutions* as follows. Scan the *Internal standard solution* while running the *Blank solution* to measure the intensity of the yttrium emission. Hold this value constant throughout the remainder of the test. Separately scan the *Blank solution*; *Standard solutions* of 0.05, 0.1, 0.2, and 0.4 µg/mL of iron; and *Internal standard solution*. [NOTE—Add the *Internal standard solution* via an in-line mixing chamber.] Normalize the yttrium intensity to the value of the *Internal standard solution*. Apply this normalization factor to the iron intensity, which is then referred to as the corrected iron intensity. Construct a calibration curve by plotting the corrected iron intensity versus the known concentrations, in µg/mL, of the iron: the linear regression coefficient is NLT 0.999.

Analysis

Sample: *Sample solution*

Similarly, analyze the *Sample solution* on the ICP. Plot the intensity of the emission of the *Sample solution* on the calibration curve. Obtain the concentration of iron, C, in

www.webofpharma.com

µg/mL, in the *Sample solution* through the calibration curve.

Calculate the content, in µg/g (ppm), of iron in the portion of Calcium Gluconate taken:

$$\text{Result} = (C \times V)/W$$

C = concentration of iron in the *Sample solution* obtained from the calibration curve (µg/mL)
V = volume of the *Sample solution* (mL)
W = weight of Calcium Gluconate taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 5 ppm

• **LIMIT OF MAGNESIUM AND ALKALI METALS**

[NOTE—Calcium Gluconate labeled as not intended for use in preparing injectable dosage forms is exempt from this requirement.]

Sample: 1.0 g

Analysis: Dissolve the *Sample* completely in 100 mL of boiling water. Add 10 mL of ammonium chloride TS, 1 mL of ammonium hydroxide, and 50 mL of hot (maintained at 70°–80°) ammonium oxalate TS. Allow to stand for 4 h, dilute with water to 200 mL, and filter. Evaporate 100 mL of the filtrate to dryness, and ignite to constant weight.

Acceptance criteria: NMT 0.4%; the weight of the residue does not exceed 2 mg.

• **LIMIT OF PHOSPHATE**

[NOTE—Calcium Gluconate labeled as not intended for use in the preparation of injectable dosage forms is exempt from this requirement.]

Standard stock solution 1: 0.716 mg/mL of monobasic potassium phosphate

Standard stock solution 2: Dilute 1.0 mL of *Standard stock solution 1* with water to 100 mL.

Standard solution: Dilute 2.0 mL of *Standard stock solution 2* with water to 100 mL.

Sample stock solution: To 10.0 g of Calcium Gluconate add 90 mL of hot water (70°–80°), and heat to boiling, with swirling, for 10 s to obtain a clear solution.

Sample solution: Dilute 1 mL of the hot *Sample stock solution* with water to 100 mL.

Analysis

Samples: *Standard solution* and *Sample solution*

To the *Standard solution* and *Sample solution* add 4 mL of sulfomolybdic acid TS, and mix. To both solutions add 0.1 mL of a freshly prepared mixture of 3 N hydrochloric acid and stronger acid stannous chloride TS (10:1), and mix.

Acceptance criteria: NMT 0.01%; after 10 min any color in the *Sample solution* is not more intense than that in the *Standard solution*.

• **LIMIT OF OXALATE**

[NOTE—Calcium Gluconate labeled as not intended for use in the preparation of injectable dosage forms is exempt from this requirement.]

[NOTE—Use deionized water where water is indicated.]

Solution A: 0.0125 M sulfuric acid in water

Solution B: Dilute 1 mL of hydrochloric acid with water to 1200 mL.

Mobile phase: 0.0017 M sodium bicarbonate and 0.0018 M sodium carbonate in water

Standard solution: 1.5 µg/mL of sodium oxalate in *Solution B*

Sample solution: 20 mg/mL of Calcium Gluconate in *Solution B*. Sonicate if necessary.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: Ion chromatography

Detector: Conductance

Columns

Guard: 4-mm × 5-cm; 15-µm packing L12

Analytical: 4-mm × 25-cm; 15-µm packing L12

Anion suppressor: The micromembrane anion suppressor column is connected in series with the guard and analytical columns. The anion suppressor column is equipped with a micromembrane that separates *Mobile phase* from *Solution A* flowing countercurrent to *Mobile phase* at a rate of about 7 mL/min. [NOTE—Condition the system for about 15 min with *Mobile phase* at a flow rate of 2 mL/min.]

Flow rate: 2 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2500 theoretical plates

Tailing factor: NMT 1.2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxalate in the portion of Calcium Gluconate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times F \times 100$$

r_U = peak response of oxalate from the *Sample solution*
r_S = peak response of oxalate from the *Standard solution*
C_S = concentration of sodium oxalate in the *Standard solution* (µg/mL)
C_U = concentration of Calcium Gluconate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of oxalate, 88.03
M_{r2} = molecular weight of sodium oxalate, 134.00
F = conversion factor, 0.001 mg/µg

Acceptance criteria: NMT 0.01%

• **REDUCING SUBSTANCES**

Sample: 1.0 g of Calcium Gluconate

Blank: 20 mL of water

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Residual titration

Titrant: 0.1 N iodine VS

Back titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 250-mL conical flask, dissolve in 20 mL of hot water, cool, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid. Titrate with the *Back titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the *Blank* determination.

Calculate the percentage of reducing substances (as dextrose) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F]/W\} \times 100$$

V_B = *Back titrant* volume consumed by the *Blank* (mL)
V_S = *Back titrant* volume consumed by the *Sample* (mL)
N = *Back titrant* normality (mEq/mL)
F = equivalency factor, 27 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 1.0%

www.webofpharma.com

SPECIFIC TESTS
- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° for 16 h.
  Acceptance criteria
  Anhydrous: NMT 3.0%
  Monohydrate: NMT 1.0%, where labeled as intended for use in preparing injectable dosage forms; NMT 2.0%, where labeled as not intended for use in preparing injectable dosage forms

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed containers.
- **Labeling:** Label it to indicate whether it is anhydrous or monohydrate. Where the quantity of calcium gluconate is indicated in the labeling of any solution containing Calcium Gluconate, this shall be understood to be in terms of anhydrous calcium gluconate ($C_{12}H_{22}CaO_{14}$). Calcium Gluconate intended for use in preparing injectable dosage forms is so labeled. Calcium Gluconate not intended for use in preparing injectable dosage forms is so labeled; in addition, it may be labeled also as intended for use in preparing oral dosage forms.
- **USP Reference Standards** ⟨11⟩
  USP Calcium Gluconate Anhydrous RS
  USP Calcium Gluconate Monohydrate RS

Calcium Gluconate Injection

DEFINITION
Calcium Gluconate Injection is a sterile solution of Calcium Gluconate in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of total calcium. The calcium is in the form of calcium gluconate, except that a small amount may be replaced with an equal amount of calcium in the form of Calcium Saccharate, or other suitable calcium salts, for the purpose of stabilization. It may contain sodium hydroxide or hydrochloric acid added for adjustment of the pH.
Injection intended for veterinary use only may be prepared from Calcium Gluconate solubilized with Boric Acid, or from Gluconolactone, Boric Acid, and Calcium Carbonate.

IDENTIFICATION
- **A. Thin-Layer Chromatography**
  Standard solution: 10 mg/mL of USP Potassium Gluconate RS
  Sample solution: Dilute a volume of injection, if necessary, with water to obtain a concentration of 10 mg/mL of calcium gluconate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel
  Application volume: 5 µL
  Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)
  Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 N sulfuric acid to volume, and mix.
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the *Spray reagent*. Heat the plate at 110° for about 10 min.
  Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.
- **B. Identification Tests—General,** *Calcium* ⟨191⟩
  Sample solution: Dilute a volume of Injection with water to obtain a concentration of 20 mg/mL of calcium gluconate.
  Acceptance criteria: Meets the requirements

ASSAY
- **Procedure**
  Sample: Accurately measured volume of Injection, equivalent to 46.5 mg of calcium
  Blank: 150 mL of water containing 2 mL of 3 N hydrochloric acid
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.05 M edetate disodium VS
  Endpoint detection: Visual
  Analysis: Add 2 mL of 3 N hydrochloric acid to the *Sample*, and dilute with water to 150 mL. While stirring, add 20 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform a blank determination.
  Calculate the percentage of the labeled amount of total calcium in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = *Titrant* molarity (mmol/mL)
F = equivalency factor, 40.08 mg/mmol
W = weight of calcium in the *Sample* (mg)

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 0.17 USP Endotoxin Units/mg of calcium gluconate
- **Particulate Matter in Injections** ⟨788⟩**:** Meets the requirements for small-volume injections
- **pH** ⟨791⟩**:** 6.0–8.2; 2.5–4.5 where labeled as intended for veterinary use only and as containing boric acid
- **Injections and Implanted Drug Products** ⟨1⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass.
- **Labeling:** Label the Injection to indicate its content, if any, of added calcium salts, calculated as percentage of calcium in the Injection. The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol/mL. The labeling indicates that the Injection must be clear at the time of use, and that if crystallization has occurred, warming may redissolve the precipitate. Injection intended for veterinary use only is so labeled. If Injection contains boric acid, it is so labeled.
- **USP Reference Standards** ⟨11⟩
  USP Potassium Gluconate RS

USP Monographs

www.webofpharma.com

Calcium Gluconate Tablets

DEFINITION

Calcium Gluconate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of calcium gluconate ($C_{12}H_{22}CaO_{14}$).

IDENTIFICATION

• **A. Identification Tests—General,** *Calcium* ⟨191⟩

Sample stock solution: A warm, filtered solution in water, equivalent to 100 mg/mL of calcium gluconate from powdered Tablets

Sample solution: Equivalent to 20 mg/mL of calcium gluconate from a dilution of the *Sample stock solution*

Acceptance criteria: Meet the requirements

• **B. Thin-Layer Chromatographic Identification Test**

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: Equivalent to 10 mg/mL of calcium gluconate from a dilution of the *Sample stock solution* obtained from *Identification* test *A*

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.

ASSAY

• **Procedure**

Sample: A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 500 mg of calcium gluconate

Blank: Proceed as directed in the *Analysis*, without the *Sample*.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Indicator: Hydroxy naphthol blue, 300 mg

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a suitable crucible. Ignite, gently at first, until free from carbon. Cool the crucible. Add 10 mL of water, and dissolve the residue by adding sufficient 3 N hydrochloric acid, dropwise, to achieve complete solution. Transfer the solution to a suitable container, and add about 150 mL of water. While stirring, preferably with a magnetic stirrer, add 20 mL of *Titrant* from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide, then add the *Indicator*. Continue the titration to a blue endpoint. Perform a *Blank* determination.

Calculate the percentage of the labeled amount of calcium gluconate ($C_{12}H_{22}CaO_{14}$) in the portion of Tablets taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mM/mL)
F = equivalency factor, 430.4 (mg/mM)
W = nominal weight of calcium gluconate in the *Sample* (mg)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: Solution having a known concentration of calcium in *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium* if necessary

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 422.8 nm

Lamp: Calcium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcium gluconate ($C_{12}H_{22}CaO_{14}$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S \times D \times V/L) \times (M_r/A_r) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of calcium in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
M_r = molecular weight of calcium gluconate, 430.4
A_r = atomic weight of calcium, 40.078

Tolerances: NLT 75% (Q) of the labeled amount of calcium gluconate ($C_{12}H_{22}CaO_{14}$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards** ⟨11⟩

USP Potassium Gluconate RS

Calcium Hydroxide

$Ca(OH)_2$ 74.09

Calcium hydroxide [1305-62-0].

DEFINITION

Calcium Hydroxide contains NLT 95.0% and NMT 100.5% of calcium hydroxide [$Ca(OH)_2$].

IDENTIFICATION

• **A.**

Sample solution: Mix with three to four times its weight of water.

Acceptance criteria: It forms a smooth magma. The clear supernatant from the magma is alkaline to litmus.

www.webofpharma.com

• **B. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩
Sample solution: Mix 1 g with 20 mL of water, and add sufficient 6 N acetic acid to effect the solution.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Sample solution: To 1.5 g of Calcium Hydroxide in a beaker, gradually add 30 mL of 3 N hydrochloric acid. When dissolved, transfer the solution to a 500-mL volumetric flask, and rinse the beaker thoroughly, adding the rinsings to the flask. Dilute with water to volume, and mix. Transfer 50 mL of the solution into a suitable container, and add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue.
Titrimetric system
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Titrate the *Sample solution* with *Titrant* to a blue endpoint. Each mL of *Titrant* is equivalent to 3.705 mg of calcium hydroxide [$Ca(OH)_2$].
Acceptance criteria: 95.0%–100.5%

IMPURITIES
• **LIMIT OF MAGNESIUM AND ALKALI SALTS**
Sample solution: Dissolve 0.50 g in a mixture of 30 mL of water and 10 mL of 3 N hydrochloric acid.
Analysis: Heat the solution, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Add immediately to the warm mixture 2 drops of methyl red TS and then 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline. Cool to room temperature, transfer to a 100-mL graduated cylinder, dilute with water to 100 mL, mix, and allow to stand for 4 h or overnight. Filter, and to 50 mL of the clear filtrate in a platinum dish add 0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of ammonium salts. Finally, ignite the residue to constant weight.
Acceptance criteria: NMT 12 mg (4.8%)
• **LIMIT OF ACID-INSOLUBLE SUBSTANCES**
Sample solution: Dissolve 2.0 g in 30 mL of 4 N hydrochloric acid, and heat to boiling.
Analysis: Filter the mixture, wash the residue with hot water, and ignite.
Acceptance criteria: 0.5%; the weight of the residue is NMT 10 mg.
• **CARBONATE**
Sample solution: Mix 2 g with 50 mL of water.
Analysis: Add an excess of 3 N hydrochloric acid to the *Sample solution*.
Acceptance criteria: The *Analysis* does not cause more than a slight effervescence.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.

Calcium Hydroxide Topical Solution

» Calcium Hydroxide Topical Solution is a solution containing, in each 100 mL, not less than 140 mg of calcium hydroxide [$Ca(OH)_2$].

Prepare Calcium Hydroxide Topical Solution as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

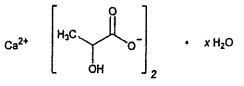
Calcium Hydroxide	3 g
Purified Water	1000 mL

Add the Calcium Hydroxide to 1000 mL of cool Purified Water, and agitate the mixture vigorously and repeatedly during 1 hour. Allow the excess calcium hydroxide to settle. Dispense only the clear supernatant.
[NOTE—The solubility of calcium hydroxide, which varies with the temperature at which the solution is stored, is about 170 mg per 100 mL at 15° and less at a higher temperature. The official concentration is based upon a temperature of 25°.]
The undissolved portion of the mixture is not suitable for preparing additional quantities of Calcium Hydroxide Topical Solution.

Packaging and storage—Preserve in well-filled, tight containers, at a temperature not exceeding 25°.

Identification—
A: It absorbs carbon dioxide from the air, a film of calcium carbonate forming on the surface of the liquid.
B: When heated, it becomes turbid, owing to the separation of calcium hydroxide.
C: It responds to the tests for *Calcium* ⟨191⟩.
Alkalies and their carbonates—A portion of it, saturated with carbon dioxide and subsequently boiled, is neutral in reaction.
Assay—Pipet 100 mL of Topical Solution into a suitable container, add 50 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 3.705 mg of calcium hydroxide [$Ca(OH)_2$].

Calcium Lactate



$C_6H_{10}CaO_6 \cdot 5H_2O$ 308.30
$C_6H_{10}CaO_6$ 218.22
Propanoic acid, 2-hydroxy-, calcium salt (2:1), hydrate;
Calcium lactate (1:2) hydrate [41372-22-9].
Calcium lactate (1:2) pentahydrate [5743-47-5].
Anhydrous [814-80-2].

DEFINITION
Calcium Lactate contains NLT 98.0% and NMT 101.0% of calcium lactate ($C_6H_{10}CaO_6$), calculated on the dried basis.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩**:** Meets the requirements

www.webofpharma.com

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Sample: Weighed portion of Calcium Lactate equivalent to 350 mg of $C_6H_{10}CaO_6$

Blank: 150 mL of water and 2 mL of 3 N hydrochloric acid

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in a mixture of water and 3 N hydrochloric acid (150:2). While stirring with a magnetic stirrer, add 30 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.

Calculate the percentage of calcium lactate ($C_6H_{10}CaO_6$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = *Titrant* molarity (mM/mL)
F = equivalency factor, 218.2 mg/mM
W = *Sample* weight (mg)

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

• **LIMIT OF MAGNESIUM AND ALKALI SALTS**

Sample: 1.0 g Calcium Lactate

Analysis: Mix the *Sample* with 40 mL of water, carefully add 1 mL of hydrochloric acid, and heat the solution to boiling. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Add immediately to the warm mixture 2 drops of methyl red TS and then 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline. Cool to room temperature, transfer to a 100-mL graduated cylinder, dilute with water to 100 mL, mix, and allow to stand for 4 h or overnight. Filter, and to 50 mL of the clear filtrate in a platinum dish add 0.5 mL of sulfuric acid. Evaporate the mixture on a steam bath to a small volume. Carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of ammonium salts. Finally, ignite the residue to constant weight.

Acceptance criteria: NMT 1.0%: The weight of the residue does not exceed 5.0 mg.

• **VOLATILE FATTY ACID**

Sample solution: Stir 500 mg of Calcium Lactate with 1 mL of sulfuric acid, and warm.

Acceptance criteria: The mixture does not emit an odor of volatile fatty acid.

SPECIFIC TESTS

• **ACIDITY**

Sample solution: 50 mg/mL

Analysis: Titrate 20 mL of *Sample solution* with 0.10 N sodium hydroxide, using phenolphthalein TS as the indicator.

Acceptance criteria: NMT 0.50 mL is required for neutralization, equivalent to NMT 0.45% as lactic acid.

• **LOSS ON DRYING** ⟨731⟩

Sample: 1–2 g

Analysis: Distribute the *Sample* evenly in a suitable weighing dish to a depth of NMT 3 mm, and dry at 120° for 4 h.

Acceptance criteria: See *Table 1*.

Table 1

Form	Loss on Drying (%)
Pentahydrate	22.0–27.0
Trihydrate	15.0–20.0
Monohydrate	5.0–8.0
Anhydrous	NMT 3.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** The label indicates whether it is the dried form or is hydrous; if the latter, the label indicates the degree of hydration. Where the quantity of Calcium Lactate is indicated in the labeling of any solution containing Calcium Lactate, this shall be understood to be in terms of calcium lactate pentahydrate ($C_6H_{10}CaO_6 \cdot 5H_2O$).

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Calcium Lactate RS

Calcium Lactate Tablets

DEFINITION

Calcium Lactate Tablets contain NLT 94.0% and NMT 106.0% of the labeled amount of calcium lactate pentahydrate ($C_6H_{10}CaO_6 \cdot 5H_2O$).

[NOTE—An equivalent amount of Calcium Lactate with less water of hydration may be used in place of calcium lactate pentahydrate ($C_6H_{10}CaO_6 \cdot 5H_2O$) in preparing Calcium Lactate Tablets.]

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩

Sample solution: A filtered solution, equivalent to 50 mg/mL of calcium lactate pentahydrate from powdered Tablets

Acceptance criteria: Meet the requirements

• **B. IDENTIFICATION TESTS—GENERAL,** *Lactate* ⟨191⟩

Sample solution: A filtered solution, equivalent to 50 mg/mL of calcium lactate pentahydrate from powdered Tablets

Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**

Sample: A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 350 mg of calcium lactate pentahydrate ($C_6H_{10}CaO_6 \cdot 5H_2O$)

Blank: Proceed as directed in the *Analysis* without the *Sample*.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Indicator: Hydroxy naphthol blue, 300 mg

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a suitable container, and add 150 mL of water and 2 mL of 3 N hydrochloric acid. Stir, using a magnetic stirrer, for 3–5 min. While stirring, add 15 mL of *Titrant* from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue,

www.webofpharma.com

and continue the titration to a blue endpoint. Perform a *Blank* determination.

Calculate the percentage of the labeled amount of calcium lactate pentahydrate ($C_6H_{10}CaO_6 \cdot 5H_2O$) in the portion of Tablets taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mM/mL)
F = equivalency factor, 308.4 (mg/mM)
W = nominal weight of calcium lactate pentahydrate in the *Sample* taken (mg)

Acceptance criteria: 94.0%–106.0%

PERFORMANCE TESTS

• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: Water; 500 mL
Apparatus 1: 100 rpm
Time: 45 min
Analysis: Determine the amount of calcium lactate pentahydrate ($C_6H_{10}CaO_6 \cdot 5H_2O$) dissolved, as directed in the *Assay*, making any necessary modifications.
Tolerances: NLT 75% (*Q*) of the labeled amount of calcium lactate pentahydrate ($C_6H_{10}CaO_6 \cdot 5H_2O$) is dissolved.

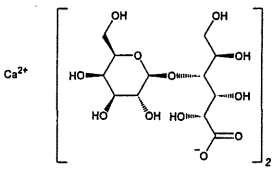
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** The quantity of Calcium Lactate stated in the labeling is in terms of calcium lactate pentahydrate.

Calcium Lactobionate



$C_{24}H_{42}CaO_{24} \cdot 2H_2O$ 790.68

D-Gluconic acid, 4-*O*-β-D-galactopyranosyl-, calcium salt (2:1), dihydrate;
Lactobionic acid, calcium salt (2:1), dihydrate;
Calcium lactobionate (1:2), dihydrate [110638-68-1].

DEFINITION

Calcium Lactobionate contains NLT 96.0% and NMT 102.0% of calcium lactobionate ($C_{24}H_{42}CaO_{24} \cdot 2H_2O$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩**:** A 20 mg/mL solution meets the requirements.

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **C. THIN LAYER CHROMATOGRAPHY**
Standard solution: 10 mg/mL of USP Calcium Lactobionate RS in water
Sample solution: 10 mg/mL of Calcium Lactobionate in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 5 µL
Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)
Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 N sulfuric acid to volume, and mix.
Analysis:
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 100° for 20 min. Allow to cool, and spray with the *Spray reagent*. Heat the plate at 110° for about 10 min.
Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.

ASSAY

• **PROCEDURE**
Sample: 800 mg of Calcium Lactobionate
Blank: 150 mL of water and 2 mL of 3 N hydrochloric acid
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in a mixture of water and 3 N hydrochloric acid (150:2). While stirring with a magnetic stirrer, add 15 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.
Calculate the percentage of calcium lactobionate ($C_{24}H_{42}CaO_{24} \cdot 2H_2O$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = *Titrant* molarity (mM/mL)
F = equivalency factor, 790.7 mg/mmol
W = *Sample* weight (mg)

Acceptance criteria: 96.0%–102.0%

IMPURITIES

• **HALIDES**
Standard solution: 0.7 mL of 0.020 N hydrochloric acid
Sample: 1.2 g
Analysis: Proceed as directed for *Chloride and Sulfate* ⟨221⟩, *Chloride*.
Acceptance criteria: NMT 0.04%

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Standard solution: 1 mL of 0.020 N sulfuric acid
Sample: 2.0 g
Analysis: Dissolve the *Sample* in boiling water.
Acceptance criteria: NMT 0.05%

• **REDUCING SUBSTANCES**
Sample: 1.0 g of Calcium Lactobionate
Blank: 20 mL of water
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 0.1 N iodine VS
Back-titrant: 0.1 N sodium thiosulfate VS

www.webofpharma.com

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 250-mL conical flask, dissolve in the *Blank*, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid, and titrate with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the *Blank* determination.

Calculate the percentage of reducing substances (as dextrose) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F]/W\} \times 100$$

V_B = *Back-titrant* volume consumed by the *Blank* (mL)
V_S = *Back-titrant* volume consumed by the *Sample* (mL)
N = *Back-titrant* normality (mEq/mL)
F = equivalency factor, 27 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 1.0%

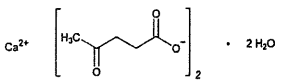
SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 100 mg/mL
  Acceptance criteria: +22.0° to +26.5°
- **PH** ⟨791⟩
  Sample solution: 50 mg/mL
  Acceptance criteria: 5.4–7.4

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Calcium Lactobionate RS

Calcium Levulinate



$C_{10}H_{14}CaO_6 \cdot 2H_2O$ 306.32

$C_{10}H_{14}CaO_6$ 270.30

Pentanoic acid, 4-oxo-, calcium salt (2:1), dihydrate; Calcium levulinate (1:2) dihydrate [5743-49-7].
Anhydrous [591-64-0].

DEFINITION

Calcium Levulinate contains NLT 97.5% and NMT 100.5% of calcium levulinate ($C_{10}H_{14}CaO_6$), calculated on the dried basis.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩**:** A 100 mg/mL solution meets the requirements.
- **B.**
  Sample solution: 0.5 g in 5 mL of water
  Analysis: To the *Sample solution* add 5 mL of 1 N sodium hydroxide, and filter. To the filtrate add 5 mL of iodine TS.
  Acceptance criteria: A precipitate of iodoform is produced.

ASSAY

- **PROCEDURE**
  Sample: 600 mg of Calcium Levulinate
  Blank: 150 mL of water containing 2 mL of 3 N hydrochloric acid
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.05 M edetate disodium VS
  Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring with a magnetic stirrer, add 30 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.

Calculate the percentage of calcium levulinate ($C_{10}H_{14}CaO_6$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = *Titrant* molarity (mM/mL)
F = equivalency factor, 270.3 mg/mM
W = *Sample* weight (mg)

Acceptance criteria: 97.5%–100.5% on the dried basis

IMPURITIES

- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Standard: 1.0 mL of 0.020 N hydrochloric acid
  Sample: 1.0 g
  Acceptance criteria: NMT 0.07%
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Standard: 1.0 mL of 0.020 N sulfuric acid
  Sample: 2.0 g
  Acceptance criteria: NMT 0.05%
- **REDUCING SUGARS**
  Sample: 0.50 g
  Analysis: Dissolve the *Sample* in 10 mL of water, add 2 mL of 3 N hydrochloric acid, boil for about 2 min, and cool. Add 5 mL of sodium carbonate TS, allow to stand for 5 min, dilute with water to 20 mL, and filter. Add 5 mL of the clear filtrate to 2 mL of alkaline cupric tartrate TS, and boil for 1 min.
  Acceptance criteria: No red precipitate is formed immediately.

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩**:** 119°–125°
- **PH** ⟨791⟩
  Sample solution: 100 mg/mL
  Acceptance criteria: 7.0–8.5
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at a pressure not exceeding 5 mm of mercury at 60° for 5 h: it loses 10.5%–12.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

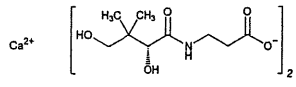
Calcium Levulinate Injection

» Calcium Levulinate Injection is a sterile solution of Calcium Levulinate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{10}H_{14}CaO_6 \cdot 2H_2O$.

www.webofpharma.com

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.
Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.
Identification—It responds to the *Identification* tests under *Calcium Levulinate*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 35.70 USP Endotoxin Units per mg of calcium levulinate.
pH ⟨791⟩: between 6.0 and 8.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer an accurately measured volume of Injection, equivalent to about 600 mg of calcium levulinate, to a 400-mL beaker, add 2 mL of hydrochloric acid, and proceed as directed in the *Assay* under *Calcium Levulinate*, beginning with "While stirring with a magnetic stirrer." Each mL of 0.05 M edetate disodium is equivalent to 15.32 mg of $C_{10}H_{14}CaO_6 \cdot 2H_2O$.

Calcium Pantothenate



$C_{18}H_{32}CaN_2O_{10}$ 476.53
β-Alanine, *N*-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-, calcium salt (2:1), (*R*)-;
Calcium D-pantothenate (1:2) [137-08-6].

DEFINITION
Calcium Pantothenate is the calcium salt of the dextrorotatory isomer of pantothenic acid. It contains NLT 98.0% and NMT 102.0% of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩: A 50-mg/mL solution meets the requirements.
- **C. OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 50 mg/mL in water
  Acceptance criteria: +25.0° to +27.5°

ASSAY
- **PROCEDURE**
  Buffer solution: Dissolve 3.2 g of monobasic sodium phosphate in 1 L of water; adjust with 1 N sodium hydroxide to a pH of 5.5.
  Mobile phase: Acetonitrile and *Buffer solution* (2:98)
  System suitability solution: 0.1 mg/mL of USP Calcium Pantothenate RS and 0.5 mg/mL each of USP β-Alanine RS, USP Sodium D-Pantoate RS, and USP Pantolactone RS in water
  Standard solution: 0.5 mg/mL of USP Calcium Pantothenate RS in water
  Sample solution: 0.5 mg/mL of Calcium Pantothenate in water
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 200 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 35°
  Flow rate: 2 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for β-alanine, pantoic acid, pantothenic acid, and pantolactone are 0.3, 0.6, 1.0, and 1.9 min, respectively.]
  Suitability requirements
  Resolution: NLT 5.0 between pantothenic acid and pantoic acid peaks, *System suitability solution*
  Tailing factor: NMT 2.0, *Standard solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of Calcium Pantothenate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Calcium Pantothenate RS in the *Standard solution* (mg/mL)
C_U = concentration of Calcium Pantothenate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

OTHER COMPONENTS
- **CONTENT OF CALCIUM**
  Sample: 800 mg of Calcium Pantothenate
  Blank: 150 mL of water containing 2 mL of 3 N hydrochloric acid
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.05 M edetate disodium VS
  Endpoint detection: Visual
  Analysis: Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and titrate with *Titrant* to a distinct blue endpoint. Perform the *Blank* determination.
  Calculate the percentage of calcium (Ca) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mmol/mL)
F = equivalency factor, 40.08 mg/mmol
W = *Sample* weight (mg)

Acceptance criteria: 8.2%–8.6% on the dried basis

SPECIFIC TESTS
- **ALKALINITY**
  Sample: 1.0 g
  Analysis: Dissolve the *Sample* in 15 mL of carbon dioxide-free water in a small flask. As soon as the solution

www.webofpharma.com

is complete, add 1.0 mL of 0.10 N hydrochloric acid, then add 0.05 mL of phenolphthalein TS.
Acceptance criteria: No pink color is produced within 5 s.
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP β-Alanine RS
USP Calcium Pantothenate RS
USP Pantolactone RS
USP Sodium D-Pantoate RS

Calcium Pantothenate Tablets

DEFINITION
Calcium Pantothenate Tablets contain NLT 95.0% and NMT 115.0% of the labeled amount of the dextrorotatory isomer of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$).

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩
Sample solution: Digest a quantity of powdered Tablets, equivalent to 150 mg of calcium pantothenate, with 15 mL of 1 N sodium hydroxide, and filter.
Acceptance criteria: Meet the requirements
• **B.**
Sample solution: 5 mL of the filtrate obtained in *Identification* test *A*
Analysis: Add 5 mL of 1 N hydrochloric acid and 2 drops of ferric chloride TS to the *Sample solution*.
Acceptance criteria: A strong yellow color is produced.

ASSAY
• **CALCIUM PANTOTHENATE**
Buffer solution: Dissolve 10.0 g of monobasic potassium phosphate in 2000 mL of water, and adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Methanol and *Buffer solution* (1:9)
System suitability solution: 0.5 mg/mL of USP Calcium Pantothenate RS and 0.1 mg/mL of USP Racemic Panthenol RS in water
Standard solution: 0.5 mg/mL of USP Calcium Pantothenate RS in water
Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 50 mg of calcium pantothenate, to a 100-mL volumetric flask. Add 5 mL of methanol, and swirl the flask to disperse. Dilute with water to volume, mix, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 2 mL/min
Injection volume: 25 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for pantothenate and panthenol are 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between pantothenate and panthenol
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the peak areas for calcium pantothenate.
Calculate the percentage of the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Calcium Pantothenate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of calcium pantothenate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–115.0%

OTHER COMPONENTS
• **CONTENT OF CALCIUM**
Sample: A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 500 mg of calcium pantothenate
Blank: Proceed as directed in the *Analysis*, without the *Sample*.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Indicator: Hydroxy naphthol blue, 300 mg
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a suitable crucible. Ignite, gently at first, until free from carbon. Cool the crucible. Add 10 mL of water, and dissolve the residue by adding sufficient 3 N hydrochloric acid, dropwise, to completely dissolve. Transfer the solution to a suitable container, and dilute with water to 150 mL. Add 15 mL of 1 N sodium hydroxide, then add the *Indicator*. Titrate with *Titrant* to a distinct blue endpoint. Perform a blank determination.
Calculate the percentage of calcium in the content of calcium pantothenate, as determined by the *Assay*, in the portion of Tablets taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mM/mL)
F = equivalency factor, 40.08 mg/mM
W = weight of calcium pantothenate in the *Sample* taken, as determined by the *Assay* (mg)

Acceptance criteria: 7.9%–9.7% of the weight of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the Tablets, as determined by the *Assay*

PERFORMANCE TESTS
• **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase: Phosphoric acid and water (1:1000)
Standard solution: A known concentration of USP Calcium Pantothenate RS in *Medium*
Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium* if necessary, having a concentration of $C_{18}H_{32}CaN_2O_{10}$ similar to that of the *Standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

www.webofpharma.com

Mode: LC
Detector: UV 210 nm
Column: 3.9-mm × 15-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) dissolved:

$$\text{Result} = (r_U/r_S) \times [C_S \times D \times (V/L)] \times 100$$

r_U = peak area of calcium pantothenate from the *Sample solution*
r_S = peak area of calcium pantothenate from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **Labeling:** Label the Tablets to indicate the content of dextrorotatory calcium pantothenate.
• **USP Reference Standards** ⟨11⟩
USP Calcium Pantothenate RS
USP Racemic Panthenol RS

Racemic Calcium Pantothenate

$C_{18}H_{32}CaN_2O_{10}$ 476.53
β-Alanine, *N*-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-, calcium salt (2:1), (±)-;
Calcium DL-pantothenate (1:2) [6381-63-1].

DEFINITION
Racemic Calcium Pantothenate is a mixture of the calcium salts of the dextrorotatory and levorotatory isomers of pantothenic acid. It contains NLT 5.7% and NMT 6.0% of nitrogen (N), and NLT 8.2% and NMT 8.6% of calcium (Ca), both calculated on the dried basis.
[NOTE—The physiological activity of Racemic Calcium Pantothenate is approximately one-half that of Calcium Pantothenate.]

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B. Identification Tests—General,** *Calcium* ⟨191⟩: A 50-mg/mL solution meets the requirements.

COMPOSITION
• **Nitrogen Determination,** *Method I* ⟨461⟩
Sample: 500 mg
Analysis: Proceed as directed in the chapter.
Acceptance criteria: 5.7%–6.0% on the dried basis
• **Content of Calcium**
Sample: 800 mg
Blank: 150 mL of water containing 2 mL of 3 N hydrochloric acid
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and titrate with the *Titrant* to a distinct blue endpoint. Perform the *Blank* determination.
Calculate the percentage of calcium (Ca) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mM/mL)
F = equivalency factor, 40.08 mg/mM
W = *Sample* weight (mg)

Acceptance criteria: 8.2%–8.6% on the dried basis

SPECIFIC TESTS
• **Optical Rotation,** *Specific Rotation* ⟨781S⟩
Test solution: 50 mg/mL in water
Acceptance criteria: –0.05° to +0.05°
• **Alkalinity**
Sample: 1.0 g
Analysis: Dissolve the *Sample* in 15 mL of carbon dioxide-free water in a small flask. As soon as the solution is complete, add 1.6 mL of 0.10 N hydrochloric acid, and then add 0.05 mL of phenolphthalein TS.
Acceptance criteria: No pink color is produced within 5 s.
• **Loss on Drying** ⟨731⟩: Dry a sample at 105° for 3 h: it loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **Labeling:** Label preparations containing it in terms of the equivalent amount of dextrorotatory calcium pantothenate.
• **USP Reference Standards** ⟨11⟩
USP Calcium Pantothenate RS

Dibasic Calcium Phosphate Dihydrate

Pharmacopeial Discussion Group Sign-Off Document

Attribute	JP	EP	USP
Definition	+	+	+
Identification A	+	+	+
Identification B	+	+	+
Acid-Insoluble Substances	+	+	+
Chloride	+	+	+
Sulfate	+	+	+
Carbonate	+	+	+
Barium	+	+	+

USP Monographs

www.webofpharma.com

Pharmacopeial Discussion Group Sign-Off Document
(continued)

Attribute	JP	EP	USP
Loss on Ignition	+	+	+
Assay	+	+	+

Legend:+, will adopt and implement; –, will not stipulate.
Nonharmonized attributes: *Packaging and Storage, Limit of Fluoride, Iron*
Specific local attributes: Identification C (EP), Lead (USP), Description (JP)

$CaHPO_4 \cdot 2H_2O$ 172.09
Phosphoric acid, calcium salt (1:1);
Calcium phosphate, dihydrate (1:1) [7789-77-7].

DEFINITION
Dibasic Calcium Phosphate Dihydrate contains two molecules of water of hydration. It contains NLT 98.0% and NMT 105.0% of dibasic calcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$).

IDENTIFICATION
• **A.**
Sample: 0.1 g of Dibasic Calcium Phosphate Dihydrate
Analysis: Dissolve the *Sample* by warming in 10 mL of 2 N hydrochloric acid. Add 2.5 mL of ammonia TS dropwise, with shaking, and then add 5 mL of ammonium oxalate TS.
Acceptance criteria: A white precipitate is formed.
• **B.**
Sample: 0.1 g of Dibasic Calcium Phosphate Dihydrate
Analysis: Dissolve the *Sample* in 5 mL of diluted nitric acid. Warm the solution to 70°, and add 2 mL of 10% ammonium molybdate solution (freshly prepared).
Acceptance criteria: A yellow precipitate of ammonium phosphomolybdate is formed.

ASSAY
• **PROCEDURE**
Buffer: Dissolve 53.5 g of ammonium chloride with sufficient water in a 1000-mL volumetric flask. Add 570 mL of ammonia water, stronger, and dilute with water to volume. The pH of this solution is 10.7.
Sample solution: Transfer 400 mg of Dibasic Calcium Phosphate Dihydrate to a 200-mL volumetric flask. Dissolve in 12 mL of diluted hydrochloric acid with the aid of gentle heat, if necessary, and dilute with water to volume.
Blank: 20 mL of water containing 1.2 mL of diluted hydrochloric acid
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 0.02 M edetate disodium VS
Back-titrant: 0.02 M zinc sulfate VS
Endpoint detection: Visual
Analysis: To 20.0 mL of the *Sample solution* add 25.0 mL of *Titrant*, 50 mL of water, and 5 mL of *Buffer*. Add 25 mg of eriochrome black T–sodium chloride indicator. Titrate the excess *Titrant* with the *Back-titrant*. Perform a *Blank* determination in the same manner.
Calculate the percentage of dibasic calcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$) in the sample taken:

$$\text{Result} = \{[(V_B - V_S) \times M \times F]/W\} \times 100$$

V_B = *Back-titrant* volume consumed by the *Blank* (mL)
V_S = *Back-titrant* volume consumed by the *Sample* (mL)
M = actual molarity of the *Back-titrant* (mM/mL)
F = equivalency factor, 172.1 mg/mM
W = *Sample weight* (mg) in 20.0 mL of the *Sample solution*

Acceptance criteria: 98.0%–105.0%

IMPURITIES
• **CARBONATE**
Sample: 1.0 g of Dibasic Calcium Phosphate Dihydrate
Analysis: Mix the *Sample* with 5 mL of carbon dioxide-free water, and immediately add 2 mL of hydrochloric acid.
Acceptance criteria: No effervescence occurs.
• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Standard: 0.70 mL of 0.010 N hydrochloric acid
Sample: 0.2 g of of Dibasic Calcium Phosphate Dihydrate
Analysis: To the *Sample* add 20 mL of water and 13 mL of diluted nitric acid, and warm gently, if necessary, to completely dissolve. Dilute with water to 100 mL, and filter if necessary. To 50 mL of the filtrate add 1 mL of silver nitrate TS.
Accceptance criteria: The turbidity of the *Sample* does not exceed that of the *Standard* (NMT 0.25%).
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Standard: 1.0 mL of 0.010 N sulfuric acid
Sample: 0.5 g of Dibasic Calcium Phosphate Dihydrate
Analysis: To the *Sample* add 5 mL of water and 5 mL of diluted hydrochloric acid, and warm gently, if necessary, to completely dissolve. Dilute with water to 100 mL, and filter if necessary. To 20 mL of the filtrate add 1 mL of diluted hydrochloric acid, and dilute with water to 50 mL. Add 1 mL of barium chloride TS.
Accceptance criteria: The turbidity of the *Sample* does not exceed that of the *Standard* (NMT 0.5%).
• **ARSENIC,** *Method I* ⟨211⟩
Test preparation: 1.0 g in 25 mL of 3 N hydrochloric acid, diluted with water to 55 mL. Omit the addition of 20 mL of 7 N sulfuric acid specified in *Procedure*.
Acceptance criteria: NMT 3 µg/g
• **BARIUM**
Sample: 0.5 g Dibasic Calcium Phosphate Dihydrate
Analysis: Heat the *Sample* to boiling with 10 mL of water, and add 1 mL of hydrochloric acid dropwise, stirring after each addition. Allow to cool, and filter, if necessary. To the filtrate add 2 mL of potassium sulfate TS.
Acceptance criteria: No turbidity is produced within 10 min.
• **LIMIT OF ACID-INSOLUBLE SUBSTANCES**
Sample solution: Dissolve 5.0 g in a mixture of 40 mL of water and 10 mL of hydrochloric acid by boiling gently for 5 min.
Analysis: After cooling, collect the insoluble substance on ashless filter paper, and wash with water until the last washing does not give a reaction for chloride (no turbidity results from the addition of silver nitrate TS). Ignite to completely incinerate the residue and the ashless filter paper at 600 ± 50°.
Acceptance criteria: The weight of the residue does not exceed 10 mg (NMT 0.2%).
• **LIMIT OF FLUORIDE**
[NOTE—Prepare and store all solutions in plastic containers.]
Buffer solution: 294 mg/mL of sodium citrate dihydrate in water
Standard stock solution: 1.1052 mg/mL of USP Sodium Fluoride RS in water
Standard solution: Transfer 20.0 mL of *Standard stock solution* to a 100-mL volumetric flask containing 50.0 mL of *Buffer solution*, dilute with water to volume, and mix. Each mL of this solution contains 100 µg of fluoride ion.

USP Monographs

www.webofpharma.com

Sample solution: Transfer 2.0 g of Dibasic Calcium Phosphate dihydtrate to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer solution* and sufficient water to make 100 mL.

Electrode system: Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see *pH* ⟨791⟩).

Analysis

Samples: *Standard solution* and *Sample solution*

Standard response line: Transfer 50.0 mL of *Buffer solution* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential in mV.

Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential in mV. From the measured potential and the *Standard response line* determine the concentration, *C* (in µg/mL), of fluoride ion in the *Sample solution*.

Calculate the content of fluoride (ppm) in the portion of Dibasic Calcium Phosphate Dihydrate taken:

$$\text{Result} = (V \times C)/W$$

V = *Sample solution* volume (mL)
C = concentration of fluoride ion, determined from the *Standard response line*, in the *Sample solution* (µg/mL)
W = weight of Dibasic Calcium Phosphate Dihydrate taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 50 ppm

SPECIFIC TESTS

- **LOSS ON IGNITION** ⟨733⟩

Sample: 1 g of Dibasic Calcium Phosphate Dihydrate
Analysis: Ignite the *Sample* at 800°–825° to constant weight.
Acceptance criteria: 24.5%–26.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements specified.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sodium Fluoride RS

Anhydrous Dibasic Calcium Phosphate

Change to read:

▲Portions of this monograph that are national *USP* text, and are not part of the harmonized text, are marked with symbols (•▲) to specify this fact.▲ (USP 1-Dec-2019)

$CaHPO_4$ 136.06
Phosphoric acid, calcium salt (1:1);
Calcium phosphate (1:1) [7757-93-9].

Change to read:

DEFINITION

Anhydrous Dibasic Calcium Phosphate contains ▲NLT 97.5% and NMT 102.5%▲ (USP 1-Dec-2019) of anhydrous dibasic calcium phosphate ($CaHPO_4$).

IDENTIFICATION

- **A.**

Sample: 0.1 g of Anhydrous Dibasic Calcium Phosphate
Analysis: Dissolve the *Sample* by warming in 10 mL of 2 N hydrochloric acid. Add 2.5 mL of ammonia TS dropwise, with shaking, and then add 5 mL of ammonium oxalate TS.
Acceptance criteria: A white precipitate is formed.

- **B.**

Sample: 0.1 g of Anhydrous Dibasic Calcium Phosphate
Analysis: Dissolve the *Sample* in 5 mL of diluted nitric acid. Warm the solution to 70°, and add 2 mL of 10% ammonium molybdate solution (freshly prepared).
Acceptance criteria: A yellow precipitate of ammonium phosphomolybdate is formed.

ASSAY

Change to read:

- **PROCEDURE**

Buffer: Dissolve 53.5 g of ammonium chloride with sufficient water in a 1000-mL volumetric flask. Add 570 mL of ammonia water, stronger, and dilute with water to volume. The pH of this solution is 10.7.
Sample solution: Transfer 400 mg of Anhydrous Dibasic Calcium Phosphate to a 200-mL volumetric flask. Dissolve in 12 mL of diluted hydrochloric acid with the aid of gentle heat, if necessary, and dilute with water to volume.
Blank: 20 mL of water containing 1.2 mL of diluted hydrochloric acid
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 0.02 M edetate disodium VS
Back-titrant: 0.02 M zinc sulfate VS
Endpoint detection: Visual
Analysis: To 20.0 mL of the *Sample solution* add 25.0 mL of *Titrant*, 50 mL of water, and 5 mL of *Buffer*. Add 25 mg of eriochrome black T–sodium chloride indicator. Titrate the excess *Titrant* with the *Back-titrant*. Perform a *Blank* determination in the same manner.

Calculate the percentage of anhydrous dibasic calcium phosphate ($CaHPO_4$) in the sample taken:

$$\text{Result} = \{[(V_B - V_S) \times M \times F]/W\} \times 100$$

V_B = *Back-titrant* volume consumed by the *Blank* (mL)
V_S = *Back-titrant* volume consumed by the *Sample* (mL)
M = actual molarity of the *Back-titrant* (mM/mL)
F = equivalency factor, 136.06 mg/mM
W = sample weight (mg) in 20.0 mL of the *Sample solution*

Acceptance criteria: ▲97.5%–102.5%▲ (USP 1-Dec-2019)

IMPURITIES

- **CARBONATE**

Sample: 1.0 g of Anhydrous Dibasic Calcium Phosphate
Analysis: Mix the *Sample* with 5 mL of carbon dioxide-free water, and immediately add 2 mL of hydrochloric acid.
Acceptance criteria: No effervescence occurs.

USP Monographs

www.webofpharma.com

Change to read:

• **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*

Standard ▲solution:▲ (USP 1-Dec-2019) To 0.70 mL of ▲0.01▲ (USP 1-Dec-2019) N hydrochloric acid, ▲add 6 mL of diluted nitric acid, and dilute with water to 50 mL.▲ (USP 1-Dec-2019)

Sample ▲solution:▲ (USP 1-Dec-2019) To ▲0.20▲ (USP 1-Dec-2019) g of Anhydrous Dibasic Calcium Phosphate, add 20 mL of water and 13 mL of diluted nitric acid, and warm gently, if necessary, to completely dissolve. Dilute with water to 100 mL, and filter if necessary. ▲Use 50 mL of the solution as *Sample solution*.▲ (USP 1-Dec-2019)

Analysis

Samples: *Standard solution* and *Sample solution*

▲Add 1 mL of silver nitrate TS to the *Sample solution* and the *Standard solution*, respectively. Mix well, and allow to stand for 5 min protecting from direct sunlight. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely.▲ (USP 1-Dec-2019)

Acceptance criteria: The ▲opalescence▲ (USP 1-Dec-2019) of the *Sample* ▲*solution*▲ (USP 1-Dec-2019) does not exceed that of the *Standard* ▲*solution*▲ (USP 1-Dec-2019) (NMT 0.25%).

Change to read:

• **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*

Standard ▲solution:▲ (USP 1-Dec-2019) To 1.0 mL of 0.010 N sulfuric acid, ▲add 1 mL of diluted hydrochloric acid, and dilute with water to 50 mL.▲ (USP 1-Dec-2019)

Sample ▲solution:▲ (USP 1-Dec-2019) To 0.5 g of Anhydrous Dibasic Calcium Phosphate, add 5 mL of water and 5 mL of diluted hydrochloric acid, and warm gently, if necessary, to completely dissolve. Dilute with water to 100 mL, and filter if necessary. To 20 mL of the ▲solution▲ (USP 1-Dec-2019) add 1 mL of diluted hydrochloric acid, and dilute with water to 50 mL.

Analysis

Samples: *Standard solution* and *Sample solution*

▲Add 2 mL of barium chloride TS to the *Sample solution* and the *Standard solution*, respectively. Mix well, and allow to stand for 10 min. Compare the white turbidity produced in both solutions against a black background by viewing downward or transversely.▲ (USP 1-Dec-2019)

Acceptance criteria: The turbidity of the *Sample* ▲*solution*▲ (USP 1-Dec-2019) does not exceed that of the *Standard* ▲*solution*▲ (USP 1-Dec-2019) (NMT 0.5%).

Change to read:

• ▲♦▲ (USP 1-Dec-2019)**ARSENIC** ⟨211⟩, *Method I*

Test preparation: 1.0 g in 25 mL of 3 N hydrochloric acid, diluted with water to 55 mL. Omit the addition of 20 mL of 7 N sulfuric acid specified in *Procedure*.

Acceptance criteria: NMT 3 µg/g▲♦▲ (USP 1-Dec-2019)

• **BARIUM**

Sample: 0.5 g Anhydrous Dibasic Calcium Phosphate

Analysis: Heat the *Sample* to boiling with 10 mL of water, and add 1 mL of hydrochloric acid dropwise, stirring after each addition. Allow to cool, and filter if necessary. To the filtrate add 2 mL of potassium sulfate TS.

Acceptance criteria: No turbidity is produced within 10 min.

• **LIMIT OF ACID-INSOLUBLE SUBSTANCES**

Sample solution: Dissolve 5.0 g in a mixture of 40 mL of water and 10 mL of hydrochloric acid by boiling gently for 5 min.

Analysis: After cooling, collect the insoluble substance on ashless filter paper, and wash with water until the last washing does not give a reaction for chloride (no turbidity results from the addition of silver nitrate TS). Ignite to completely incinerate the residue and the ashless filter paper at 600 ± 50°.

Acceptance criteria: The weight of the residue does not exceed 10 mg (NMT 0.2%).

Change to read:

• ▲♦▲ (USP 1-Dec-2019)**LIMIT OF FLUORIDE**

[NOTE—Prepare and store all solutions in plastic containers.]

Buffer solution: 294 mg/mL of sodium citrate dihydrate in water

Standard stock solution: 1.1052 mg/mL of USP Sodium Fluoride RS in water

Standard solution: Transfer 20.0 mL of *Standard stock solution* to a 100-mL volumetric flask containing 50.0 mL of *Buffer solution*, dilute with water to volume, and mix. Each milliliter of this solution contains 100 µg of fluoride ion.

Sample solution: Transfer 2.0 g of Anhydrous Dibasic Calcium Phosphate to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer solution* and sufficient water to make 100 mL.

Electrode system: Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see *pH* ⟨791⟩).

Analysis

Samples: *Standard solution* and *Sample solution*

Standard response line: Transfer 50.0 mL of *Buffer solution* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential in millivolts. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential in millivolts.

Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential in millivolts. From the measured potential and the *Standard response line* determine the concentration (*C*), in µg/mL, of fluoride ion in the *Sample solution*.

Calculate the content of fluoride in ppm in the portion of Anhydrous Dibasic Calcium Phosphate taken:

$$\text{Result} = (V \times C)/W$$

V = *Sample solution* volume (mL)

C = concentration of fluoride ion, determined from the *Standard response line*, in the *Sample solution* (µg/mL)

W = weight of Anhydrous Dibasic Calcium Phosphate taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 50 ppm▲♦▲ (USP 1-Dec-2019)

www.webofpharma.com

SPECIFIC TESTS

Change to read:

- **LOSS ON IGNITION** ⟨733⟩
  Sample: 1 g of Anhydrous Dibasic Calcium Phosphate
  Analysis: Ignite the *Sample* at 800°–825° to constant weight.
  Acceptance criteria: ▲6.6%–8.7%▲ (USP 1-Dec-2019)

ADDITIONAL REQUIREMENTS

Change to read:

- ▲•▲ (USP 1-Dec-2019)**PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements specified.▲•▲ (USP 1-Dec-2019)
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Sodium Fluoride RS

Dibasic Calcium Phosphate Tablets

DEFINITION

Dibasic Calcium Phosphate Tablets contain NLT 92.5% and NMT 107.5% of the labeled amount of dibasic calcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$).

[NOTE—An equivalent amount of Dibasic Calcium Phosphate with less water of hydration may be used in place of $CaHPO_4 \cdot 2H_2O$ in preparing Dibasic Calcium Phosphate Tablets.]

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩
  Sample solution: A filtered portion of the *Sample solution* from the *Assay*
  Acceptance criteria: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL,** *Phosphate* ⟨191⟩
  Sample solution: A filtered portion of the *Sample solution* from the *Assay*, neutralized with ammonium hydroxide
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Sample solution: Transfer a portion of powder, equivalent to 1 g of dibasic calcium phosphate dihydrate, from NLT 20 powdered Tablets, to a 100-mL volumetric flask containing 15 mL of hydrochloric acid and 10 mL of water. Heat on a steam bath, with occasional mixing, to dissolve the dibasic calcium phosphate, but not longer than 30 min. Cool, add water to volume, and mix. If the solution is not clear, filter, discarding the first 10 mL of the filtrate.

Blank: Water

Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Indicator: Hydroxy naphthol blue
Endpoint detection: Visual

Analysis: Transfer 25.0 mL of the *Sample solution* to a 250-mL beaker equipped with a magnetic stirrer. With constant stirring, add, in the order named, 0.5 mL of triethanolamine, 300 mg of *Indicator*, and from a 50-mL buret, about 23 mL of *Titrant*. Add sodium hydroxide solution (45 in 100) until the initial red color changes to clear blue. Continue to add it dropwise until the color changes to violet, and add an additional 0.5 mL. The pH is 12.3–12.5. Continue the titration dropwise with the *Titrant* to the appearance of a clear blue endpoint that persists for NLT 60 s. Perform a blank determination.

Calculate the percentage of the labeled amount of dibasic calcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$) in the portion of Tablets taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample solution* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
M = actual molarity of the *Titrant* (mM/mL)
F = equivalency factor, 172.08 mg/mM
W = nominal weight of dibasic calcium phosphate dihydrate in the *Sample solution* taken for *Analysis* (mg)

Acceptance criteria: 92.5%–107.5%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 45 min
Standard solution: Solution having a known concentration of calcium in *Medium*
Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium* if necessary
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 422.7 nm
Lamp: Calcium hollow-cathode
Flame: Air-acetylene
Analysis
Samples: *Standard solution* and *Sample solution*

Determine the concentration of calcium (Ca) in the *Sample solution* in comparison with a *Standard solution*.

Calculate the percentage of the labeled amount of dibasic calcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S \times D \times V/L) \times (M_r/A_r) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of calcium in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
M_r = molecular weight of dibasic calcium phosphate, 172.08
A_r = atomic weight of calcium, 40.08

Tolerances: NLT 75% (Q) of the labeled amount of dibasic calcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** The quantity of dibasic calcium phosphate stated in the labeling is in terms of dibasic calcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$).

USP Monographs

www.webofpharma.com

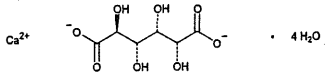
Calcium Polycarbophil

Calcium polycarbophil [9003-97-8].

» Calcium Polycarbophil is the calcium salt of polyacrylic acid cross-linked with divinyl glycol.

Packaging and storage—Preserve in tight containers.
Identification—When tested as directed in the test for *Absorbing power*, it absorbs about 35 times its original weight.
Loss on drying ⟨731⟩—Dry it in vacuum at 130° for 4 hours: it loses not more than 10.0% of its weight.
Absorbing power—Transfer about 250 mg, accurately weighed, to a tared 50-mL centrifuge tube fitted with a tight closure. Add 35 mL of 0.1 N hydrochloric acid to the tube, seal the tube, and shake by mechanical means for 30 minutes. Centrifuge at 2000 rpm for 20 minutes, and decant and discard the supernatant. [NOTE—Exercise care to avoid any loss of particles.] Add 35 mL of 0.1 N hydrochloric acid, and shake for 30 minutes. Centrifuge, decanting and discarding the supernatant. Repeat the foregoing steps, using water instead of acid. Add 35 mL of a sodium bicarbonate solution (15 in 1000), and shake, venting as necessary to release any carbon dioxide liberated. Shake for 1 hour, centrifuge, and decant the supernatant. Add 35 mL of sodium bicarbonate solution (15 in 1000), and shake for 1 hour. Allow the tube and contents to stand overnight or until the contents have settled, and centrifuge. Withdraw the supernatant, and weigh the tube and contents. Calculate the weight of sodium bicarbonate solution absorbed: not less than 35.0 g of the sodium bicarbonate solution is absorbed by 1.0 g of Calcium Polycarbophil, calculated on the dried basis.
Content of calcium—Transfer about 2 g of Calcium Polycarbophil, accurately weighed, to a tared crucible. Cover, leaving the lid slightly ajar, and place in a muffle furnace. Heat to 600° over 2 hours, increase the temperature to 1000° over 1 hour, and maintain at 1000° for 1 hour. Allow to cool slowly. Dissolve the residue in dilute hydrochloric acid (1 in 5), quantitatively transfer with the aid of dilute hydrochloric acid (1 in 5) to a 100-mL volumetric flask, and dilute with dilute hydrochloric acid (1 in 5) to volume. Pipet 15 mL of this solution into a 250-mL beaker, and add, while stirring with a magnetic stirrer, 100 mL of water, 20.0 mL of 0.05 M edetate disodium VS, and 300 mg of hydroxy naphthol blue. Adjust with 1 N sodium hydroxide solution to a pH of 9.0 to 9.5. Adjust with about 10 mL of 2 N sodium hydroxide to a pH of 12.4. Titrate with 0.05 M edetate disodium VS to a persistent blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of calcium (Ca). The content of Ca found is not less than 18.0% and not more than 22.0%, calculated on the dried basis.

Calcium Saccharate



$C_6H_8CaO_8 \cdot 4H_2O$ 320.26
D-Glucaric acid, calcium salt (1:1) tetrahydrate;
Calcium D-glucarate (1:1), tetrahydrate [5793-89-5].

DEFINITION
Calcium Saccharate is the calcium salt of D-saccharic acid. It contains NLT 98.5% and NMT 102.0% of $C_6H_8CaO_8 \cdot 4H_2O$.

IDENTIFICATION
• **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩
Sample: 0.2 g
Analysis: Dissolve the *Sample* in 10 mL of water by the addition of 2 mL of hydrochloric acid.
Acceptance criteria: Meets the requirements

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020) Meets the requirements

ASSAY
• **PROCEDURE**
Sample: 600 mg
Blank: 150.0 mL of water
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 150 mL of water with the aid of a sufficient volume of hydrochloric acid. While stirring, preferably with a magnetic stirrer, add 30 mL of *Titrant* from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue. Continue the titration to a blue endpoint. Perform a *Blank* determination and make any necessary corrections. Calculate the percentage of calcium saccharate ($C_6H_8CaO_8 \cdot 4H_2O$) in the portion of Calcium Saccharate taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 320.2 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–102.0%

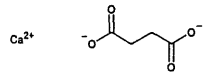
IMPURITIES
• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Standard: 0.50 mL of 0.020 N hydrochloric acid
Sample: 0.50 g dissolved in 10 mL of water by the addition of 2 mL of nitric acid
Acceptance criteria: NMT 0.07%
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Standard: 0.6 mL of 0.020 N sulfuric acid
Sample: 0.50 g dissolved in 10 mL of water by the addition of 2 mL of hydrochloric acid
Acceptance criteria: NMT 0.12%
• **SUCROSE AND REDUCING SUGARS**
Sample: 0.5 g
Analysis: Dissolve the *Sample* in 10 mL of water with the addition of 2 mL of hydrochloric acid, and boil the solution for about 2 min. Cool, add 15 mL of sodium carbonate TS, allow to stand for 5 min, and filter. Add 5 mL of the clear filtrate to about 2 mL of alkaline cupric tartrate TS, and boil for 1 min.
Acceptance criteria: No red precipitate is formed immediately.

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 60 mg/mL in 4.8 N hydrochloric acid that has been allowed to stand for 1 h
Acceptance criteria: +18.5° to +22.5°

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Calcium Saccharate RS

Calcium Succinate



$C_4H_4CaO_4 \cdot H_2O$ 174.17
Butanedioic acid, calcium salt (1:1), monohydrate;
Calcium succinate monohydrate [159389-75-0].
Anhydrous [140-99-8].

DEFINITION
Calcium Succinate contains NLT 98.0% and NMT 102.0% of calcium succinate ($C_4H_4CaO_4$), calculated on the dried basis.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩
  Sample solution: 20 mg/mL
  Acceptance criteria: Meets the requirements

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩**,** *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
- **PROCEDURE**
  Sample: 650 mg of Calcium Succinate
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 M edetate disodium VS
  Endpoint detection: Visual
  Analysis: Transfer the *Sample* into a 250-mL beaker, add 100 mL of water and 2 mL of concentrated hydrochloric acid, and stir using a magnetic stirring bar. While stirring, add 30 mL of *Titrant* from the titration buret, and 25 mL of 2 N sodium hydroxide. Check the pH of the solution and make sure it is between 12.0 and 13.0. Add 300–500 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform a blank determination.
  Calculate the percentage of calcium succinate ($C_4H_4CaO_4$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the blank (mL)
M = actual molarity of the *Titrant* (mmol/mL)
F = equivalency factor, 156.15 mg/mmol
W = *Sample* weight (mg)

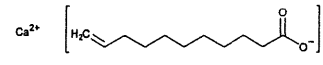
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Standard solution: 1.0 mL of 0.010 N hydrochloric acid
  Sample: 1.8 g
  Acceptance criteria: NMT 0.02%
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Standard solution: 1.0 mL of 0.020 N sulfuric acid
  Sample: 2.0 g
  Acceptance criteria: NMT 0.05%
- **ELEMENTAL IMPURITIES—PROCEDURES** ⟨233⟩
  Acceptance criteria
  Arsenic: NMT 3.0 µg/g
  Cadmium: NMT 2.0 µg/g
  Lead: NMT 2.0 µg/g
  Mercury: NMT 0.1 µg/g

SPECIFIC TESTS
- **pH** ⟨791⟩
  Sample solution: 50 mg/mL in water. [NOTE—The solution is slurry.]
  Acceptance criteria: 7.0–12.0
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 160° for 5 h.
  Acceptance criteria: 9.0%–13.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Calcium Succinate RS

Calcium Undecylenate



$C_{22}H_{38}O_4Ca$ 406.61
10-Undecenoic acid, calcium (2+) salt;
Calcium 10-undecenoate [1322-14-1].

DEFINITION
Calcium Undecylenate contains NLT 98.0% and NMT 102.0% of calcium undecylenate ($C_{22}H_{38}O_4Ca$), calculated on the dried basis.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩
  Sample solution: A filtered solution (1 in 20) of Calcium Undecylenate in 3 N hydrochloric acid
  Acceptance criteria: Meets the requirements
- **B. MELTING RANGE OR TEMPERATURE,** *Class Ia* ⟨741⟩
  Sample solution: Suspend 10 g of Calcium Undecylenate in 40 mL of water in a 250-mL separator. Cautiously and slowly add 10 mL of hydrochloric acid while swirling. Insert the stopper, and shake. The separator will become quite warm, and pressure must be carefully and frequently relieved through the stopcock. If a curdy, white material remains after 5 min of shaking, add additional hydrochloric acid, 1 mL at a time, and shake until a clear oily phase is formed.
  Analysis: Allow the phases to separate, drain, and discard the bottom aqueous layer. Drain and discard the middle oily layer, if present. Filter the top layer of undecylenic acid through a pledget of cotton, noting the volume obtained. To the filtrate add an equal volume of aniline. Reflux for 1 h, swirling the flask occasionally. Allow to cool, and pour 60 mL of alcohol through the condenser into the flask. Remove the flask from the condenser, add 1 g of charcoal, and stir. Filter the slurry. Add water dropwise until a few crystals form or the solution becomes slightly cloudy. If too much water is added, an oil will form. Add alcohol dropwise until the oil dissolves. Allow the mixture to stand or refrigerate until crystals are formed. Collect the crystals on a filter paper inserted in a 45-mm porous glass filter funnel. Wash the crystals with 75 mL of 25% alcohol: the crystals have a clean, white, glossy appearance. If not, recrystallize

www.webofpharma.com

by dissolving the crystals in about 50 mL of alcohol. Add about 1 g of charcoal, stir, filter, and continue as directed above, beginning with "Add water dropwise". Dry the crystals under vacuum at 50° for 2 h. If the melting point is low, additional drying or recrystallization may be necessary.
Acceptance criteria: The crystals so obtained melt at 66°–67.5°.

ASSAY

• **PROCEDURE**
Sample solution: Boil 40.0 mL of 0.1 N hydrochloric acid VS with 600 mg of Calcium Undecylenate for 10 min, or until the undecylenic acid layer is clear, adding water as necessary, to maintain the original volume. Transfer the mixture, with the aid of water, to a 500-mL separator. Dilute with water to about 75 mL, and extract with two 100-mL portions of solvent hexane. Wash the combined extracts with water until the last washing is neutral to litmus, and add the washings to the original water layer. Cool.
Titrimetric system
(See *Titrimetry* ⟨541⟩, *Residual Titrations*.)
Mode: Residual titration
Titrant: 0.1 N hydrochloric acid VS
Back-titrant: 0.1 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Add 3 drops of methyl orange TS to the *Sample solution*. Then titrate the excess *Titrant* with *Back-titrant*. Perform a blank determination. Each mL of hydrochloric acid is equivalent to 20.33 mg of calcium undecylenate ($C_{22}H_{38}O_4Ca$).
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **LIMIT OF FREE UNDECYLENIC ACID**
Sample solution: Transfer 10 g of Calcium Undecylenate to a 400-mL beaker, add 250 mL of solvent hexane, and mix for 2 h using a magnetic stirrer. Filter into a 500-mL flask, and evaporate with the aid of a current of air to about 20 mL.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: To the *Sample solution* add 100 mL of neutralized alcohol. Add 3 drops of phenolphthalein TS, and titrate with *Titrant*. Each mL of 0.1 N sodium hydroxide is equivalent to 18.43 mg of free undecylenic acid ($C_{11}H_{20}O_2$).
Acceptance criteria: NMT 0.1%

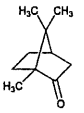
SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: 2.0%–5.7%
• **PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING** ⟨786⟩
Analysis: Test in accordance with this procedure, except use NMT 25 g and use a single No. 100 sieve that is to be shaken for NLT 30 min or until sifting is practically complete.
Acceptance criteria: NLT 99.0% of it passes through a No. 100 sieve.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Camphor



$C_{10}H_{16}O$ 152.23
Bicyclo[2.2.1]heptane-2-one, 1,7,7-trimethyl-;
Camphor;
2-Bornanone [76-22-2].

DEFINITION

Camphor is a ketone of *Cinnamomum camphora* (L.) Nees et Ebermaier (Fam. Lauraceae) (natural Camphor), or is produced synthetically (synthetic Camphor).

IMPURITIES

• **LIMIT OF NONVOLATILE RESIDUE**
Sample: 2.0 g of Camphor
Analysis: Heat the *Sample* in a tared dish on a steam bath until sublimation is complete. Dry the residue at 120° for 3 h, cool, and weigh.
Acceptance criteria: 0.05%; the weight of the residue does not exceed 1.0 mg.
• **HALOGENS**
Sample: Mix 100 mg of finely divided Camphor with 200 mg of sodium peroxide in a clean, dry, hard glass test tube about 25 mm in internal diameter and 20 cm in length. Suspend the tube at an angle of about 45°, using a clamp placed at the upper end. Gently heat the tube, starting near the upper end, but not heating the clamp. Gradually bring the heat toward the lower part of the tube until incineration is complete.
Analysis: Dissolve the residue in 25 mL of warm water, acidify with nitric acid, and filter the solution into a comparison tube. Wash the test tube and the filter with two 10-mL portions of hot water, adding the washings to the filtered solution. To the filtrate add 0.50 mL of 0.10 N silver nitrate, dilute with water to 50 mL, and mix.
Acceptance criteria: 0.035%; the turbidity does not exceed that produced in a blank test with the same quantities of the same reagents and 0.050 mL of 0.020 N hydrochloric acid.

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 174°–179°
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 100 mg/mL in alcohol
Synthetic Camphor is optically inactive.
Acceptance criteria: +41° to +43° for natural Camphor
• **APPEARANCE OF SOLUTION:** A 100-mg/mL solution in solvent hexane is clear.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid exposure to excessive heat.
• **LABELING:** Label it to indicate whether it is of natural sources or is prepared synthetically.

Camphor Spirit

DEFINITION

Camphor Spirit is an alcohol solution containing NLT 9.0 g and NMT 11.0 g of camphor ($C_{10}H_{16}O$) in each 100 mL.
Prepare Camphor Spirit as follows.

www.webofpharma.com

Camphor	100 g
Alcohol, a sufficient quantity to make	1000 mL

Dissolve the *Camphor* in 800 mL of the *Alcohol*, and add sufficient *Alcohol* to bring to final volume. Filter, if necessary.

ASSAY

• **PROCEDURE**

Sample: 2.0 mL

Analysis: Transfer the *Sample* to a suitable pressure bottle containing 50 mL of freshly prepared dinitrophenylhydrazine TS. Close the pressure bottle, immerse it in a water bath, and maintain at about 75° for 16 h. Cool to room temperature, and transfer the contents to a beaker with the aid of 100 mL of 3 N sulfuric acid. Allow to stand at room temperature for NLT 12 h, transfer the precipitate to a tared filter crucible, and wash with 100 mL of 3 N sulfuric acid followed by 75 mL of cold water in divided portions. Continue the suction until the excess water is removed, dry the crucible and precipitate at 80° for 2 h, cool, and weigh. The weight of the precipitate so obtained, multiplied by 0.4581, represents the weight of camphor ($C_{10}H_{16}O$) in the portion taken.

Acceptance criteria: 9.0–11.0 g of camphor in 100 mL

OTHER COMPONENTS

• **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩

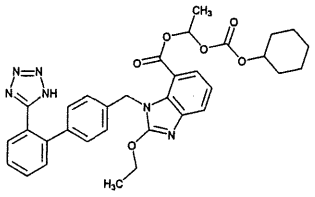
Test stock preparation: Dilute the *Spirit* with methanol to obtain a solution containing approximately 2% (v/v) of alcohol.

Acceptance criteria: 80.0%–87.0% of alcohol (C_2H_5OH)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight containers.

Candesartan Cilexetil



$C_{33}H_{34}N_6O_6$

1*H*-Benzimidazole-7-carboxylic acid, 2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-, 1-[[(cyclohexyloxy)carbonyl]oxy]ethyl ester, (±);
(±)-1-Hydroxyethyl 2-ethoxy-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl) benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester). 610.66

[145040-37-5].

DEFINITION

Candesartan Cilexetil contains NLT 98.7% and NMT 101.0% of $C_{33}H_{34}N_6O_6$, calculated on anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)**:** If the spectra obtained show differences, proceed with the samples prepared as follows. Separately dissolve a quantity of USP Candesartan Cilexetil RS and Candesartan Cilexetil in alcohol. [NOTE—Heating the solution may be necessary for complete dissolution.] Cool the solution in an ice bath, filter the crystals, and dry at 105°.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the procedure for *Organic Impurities*.

ASSAY

• **PROCEDURE**

Sample solution: 8.33 mg/mL of Candesartan Cilexetil in glacial acetic acid

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Potentiometric

Titrant: 0.1 N perchloric acid

Analysis: Titrate with 0.1 N perchloric acid VS using a blank determination under the same conditions. Each mL of the *Titrant* is equivalent to 61.07 mg of $C_{33}H_{34}N_6O_6$.

Acceptance criteria: 98.7%–101.0% on the anhydrous basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%, determined from a 1-g sample

ORGANIC IMPURITIES

• **Procedure**

Diluent: Acetonitrile and water (3:2)

Solution A: Acetonitrile, glacial acetic acid, and water (57:1:43)

Solution B: Acetonitrile, glacial acetic acid, and water (90:1:10)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	0	100

[NOTE—Equilibration for about 10 min may be necessary between injections.]

System suitability solution: 0.04 mg/mL of USP Candesartan Cilexetil RS and 0.125 mg/mL of acenaphthene in *Diluent*

Standard solution: 4 µg/mL of USP Candesartan Cilexetil RS in *Diluent*

Sample solution: 0.4 mg/mL of Candesartan Cilexetil in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 15-cm; 4-µm packing L1

Flow rate: 0.8 mL/min

Injection size: 10 µL

System suitability

[NOTE—The *Mobile phase* used for testing system suitability is 100% *Solution A* in an isocratic mode.]

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 5.0 between candesartan cilexetil and acenaphtene

Tailing factor: NMT 1.5 for candesartan cilexetil

Relative standard deviation: NMT 3.0% for the candesartan cilexetil peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Candesartan Cilexetil taken:

USP Monographs

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of candesartan cilexetil from the *Standard solution*
C_S = concentration of USP Candesartan Cilexetil RS in the *Standard solution* (mg/mL)
C_U = concentration of Candesartan Cilexetil in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Table 1*.
Total impurities: NMT 0.6%. [NOTE—Calculate the total impurities from the sum of all impurity peaks greater than or equal to 0.05%.]

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ethyl candesartan[a]	0.4	0.2
Desethyl candesartan cilexetil[b]	0.5	0.3
Candesartan cilexetil	1.0	—
N^2-Ethyl candesartan cilexetil[c]	2.0	0.2
Any other unknown impurity	—	0.10

[a] Ethyl 1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylate.
[b] ±1-(Cyclohexyloxycarbonyloxy)ethyl 1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-oxobenzimidazole-7-carboxylate.
[c] ±1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-{[2′-(*N*-ethyl-tetrazol-5-yl)biphenyl-4-yl]methyl}benzimidazole-7-carboxylate.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method 1* ⟨921⟩**:** NMT 0.3%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Candesartan Cilexetil RS
1*H*-Benzimidazole-7-carboxylic acid, 2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-, 1-[[(cyclohexyloxy)carbonyl]oxy]ethyl ester, (±); (±)-1-Hydroxyethyl 2-ethoxy-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester). 610.66

Candesartan Cilexetil Tablets

DEFINITION
Candesartan Cilexetil Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak from the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, trifluoroacetic acid, and water (550:1:450)
Diluent: Acetonitrile and water (70:30)
Standard solution: 0.8 mg/mL of USP Candesartan Cilexetil RS in *Diluent*. Sonication may be necessary for complete dissolution. Pass through a suitable filter of 0.45-µm pore size.
Sample solution: Nominally 0.8 mg/mL of candesartan cilexetil in *Diluent* prepared as follows. Transfer a number of Tablets (see *Table 1*) to a suitable volumetric flask.

Table 1

Tablet Strength (mg)	Number of Tablets (NLT)
4	10
8	10
16	5
32	5

Add *Diluent* to fill about 70% of the total volume, and sonicate for about 25 min with intermittent shaking. Allow to cool and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Assay: UV 282 nm
Identification test B: Diode array
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 2.7 times the retention time of candesartan cilexetil
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Candesartan Cilexetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of candesartan cilexetil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium for Tablets labeled to contain 4 mg, 8 mg, and 16 mg: 0.35% polysorbate 20 in 0.05 M phosphate buffer, pH 6.5; 900 mL
Medium for Tablets labeled to contain 32 mg: 0.70% polysorbate 20 in 0.05 M phosphate buffer, pH 6.5; 900 mL

www.webofpharma.com

Apparatus 2: 50 rpm
Time: 45 min
Mobile phase: Acetonitrile, trifluoroacetic acid, and water (550:1:450)
Standard stock solution: 0.45 mg/mL of USP Candesartan Cilexetil RS in acetonitrile. Sonication may be necessary for complete dissolution.
Standard solution: Prepare solutions in *Medium* from *Standard stock solution* (see *Table 2* for concentrations).

Table 2

Tablet Strength (mg)	Concentration (mg/mL)
4	0.0045
8	0.009
16	0.018
32	0.036

Sample solution: Pass a portion of solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
Run time: NLT 1.8 times the retention time of candesartan cilexetil
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of medium, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A: Acetonitrile, trifluoroacetic acid, and water (10:0.1:90)
Solution B: Acetonitrile, trifluoroacetic acid, and water (90:0.1:10)
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	65	35
30	5	95
45	5	95
50	65	35
55	65	35

System suitability stock solution A: 0.05 mg/mL each of USP Candesartan Cilexetil Related Compound A RS, USP Candesartan Cilexetil Related Compound B RS, USP Candesartan Cilexetil Related Compound D RS, and USP Candesartan Cilexetil Related Compound F RS in acetonitrile
System suitability stock solution B: 0.1 mg/mL of USP Candesartan Cilexetil RS in acetonitrile
System suitability stock solution C: 0.5 mg/mL of USP Candesartan Cilexetil Related Compound G RS in methanol
System suitability solution: 0.0015 mg/mL each of USP Candesartan Cilexetil Related Compound A RS, USP Candesartan Cilexetil Related Compound B RS, USP Candesartan Cilexetil Related Compound D RS, and USP Candesartan Cilexetil Related Compound F RS, 0.001 mg/mL of USP Candesartan Cilexetil RS, 0.005 mg/mL of USP Candesartan Cilexetil Related Compound G RS from *System suitability stock solution A*, *System suitability stock solution B*, and *System suitability stock solution C* in acetonitrile
Standard solution: 0.001 mg/mL of USP Candesartan Cilexetil RS in acetonitrile from *System suitability stock solution B*
Sample solution: Nominally 1 mg/mL of candesartan cilexetil in acetonitrile prepared as follows. Transfer a suitable quantity of candesartan cilexetil from NLT 20 powdered Tablets into a suitable volumetric flask. Add acetonitrile to fill 60% of the total volume and sonicate for 15 min with intermittent shaking in cold water. Dilute with acetonitrile to volume and pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L1
Sample cooler temperature: 10°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between candesartan cilexetil related compound B and candesartan cilexetil, *System suitability solution*
Tailing factor: NMT 2.0 for candesartan cilexetil peak, *Standard solution*
Relative standard deviation: NMT 10.0% for candesartan cilexetil peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of candesartan cilexetil from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Candesartan Cilexetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of candesartan cilexetil in the *Sample solution* (mg/mL)
F = relative response factor of each impurity (see *Table 4*)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Candesartan cilexetil related compound G[a]	0.17	1.30	1.0
Candesartan cilexetil related compound A[b,c]	0.46	1.16	—
Candesartan cilexetil related compound B[d]	0.77	1.00	1.5
Candesartan cilexetil	1.0	—	—
Candesartan cilexetil related compound D[e]	1.15	1.00	0.5
Candesartan cilexetil related compound F[f]	1.47	0.88	1.5
Any unspecified impurity	—	1.00	0.2
Total impurities	—	—	4.0

[a] 1-{[2′-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylic acid.
[b] Ethyl 1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylate.
[c] Process-related impurity not included in total impurities.
[d] 1-(Cyclohexyloxycarbonyloxy)ethyl 1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-hydroxybenzimidazole-7-carboxylate.
[e] 1-{[(Cyclohexyloxy)carbonyl]oxy}ethyl 3-({2′-(2-ethyl-1*H*-tetrazol-5-yl)-[1,1′-biphenyl]-4-yl}methyl)-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.
[f] 1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-{[2′-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl}benzimidazole-7-carboxylate.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Candesartan Cilexetil RS
  USP Candesartan Cilexetil Related Compound A RS
  Ethyl 1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylate.
  $C_{26}H_{24}N_6O_3$ 468.51
  USP Candesartan Cilexetil Related Compound B RS
  1-(Cyclohexyloxycarbonyloxy)ethyl 1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-hydroxybenzimidazole-7-carboxylate.
  $C_{31}H_{30}N_6O_6$ 582.61
  USP Candesartan Cilexetil Related Compound D RS
  1-{[(Cyclohexyloxy)carbonyl]oxy}ethyl 3-({2′-(2-ethyl-2*H*-tetrazol-5-yl)-[1,1′-biphenyl]-4-yl}methyl)-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.
  $C_{33}H_{34}N_6O_6$ 610.67
  USP Candesartan Cilexetil Related Compound F RS
  1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-{[2′-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl}benzimidazole-7-carboxylate.
  $C_{35}H_{38}N_6O_6$ 638.71
  USP Candesartan Cilexetil Related Compound G RS
  1-{[2′-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylic acid.
  $C_{24}H_{20}N_6O_3$ 440.45

Candesartan Cilexetil and Hydrochlorothiazide Tablets

DEFINITION

Candesartan Cilexetil and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% each of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the major peaks of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peaks of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Solution A: Acetonitrile, trifluoroacetic acid, and water (10: 0.1: 90)
Solution B: Acetonitrile, trifluoroacetic acid, and water (90: 0.1: 10)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4.0	90	10
6.0	30	70
15.0	30	70
17.0	90	10
20.0	90	10

Diluent: Acetonitrile and water (70:30)
Standard solution: Prepare solutions of USP Candesartan Cilexetil RS and USP Hydrochlorothiazide RS in *Diluent* at concentrations given in *Table 2* as follows. Transfer suitable amounts of USP Candesartan Cilexetil RS and USP Hydrochlorothiazide RS to a suitable volumetric flask. Add *Diluent*, about 50% of the total volume, and sonicate to dissolve. Dilute with *Diluent* to volume and pass through a suitable filter of 0.45-µm pore size.

Table 2

Tablet Strength Candesartan Cilexetil/ Hydrochlorothiazide (mg/mg)	Concentration of Candesartan Cilexetil (mg/mL)	Concentration of Hydrochlorothiazide (mg/mL)
16/12.5	0.32	0.25
32/12.5	0.64	0.25
32/25	0.32	0.25

USP Monographs

www.webofpharma.com

Sample solution: Nominally equivalent to the concentration mentioned in *Table 2* prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask. Add about 60% of the total volume of *Diluent*, and sonicate for about 25 min with intermittent shaking. Cool to room temperature and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 282 nm. For *Identification B*, use a diode array detector of UV 282 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L7▲ ▲(ERR 1-May-2019)
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both the candesartan cilexetil and hydrochlorothiazide peaks
Relative standard deviation: NMT 2.0% for both the candesartan cilexetil and hydrochlorothiazide peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount each of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of candesartan cilexetil or hydrochlorothiazide from the *Sample solution*
r_S = peak response of candesartan cilexetil or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Candesartan Cilexetil RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of candesartan cilexetil or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of candesartan cilexetil and hydrochlorothiazide

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Medium
For Tablets labeled to contain 16 mg/12.5 mg of candesartan cilexetil/hydrochlorothiazide: 0.35% polysorbate 20 in 0.05 M phosphate buffer pH 6.5; 900 mL
For Tablets labeled to contain 32 mg/12.5 mg and 32 mg/25 mg of candesartan cilexetil/hydrochlorothiazide: 0.70% polysorbate 20 in 0.05 M phosphate buffer pH 6.5; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Solution A and **Solution B:** Prepare as directed in the *Assay*.
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	80	20
3.0	80	20
5.0	30	70
10.0	30	70
13.0	80	20
16.0	80	20

Standard stock solution A: 0.72 mg/mL of USP Candesartan Cilexetil RS in acetonitrile prepared as follows. Transfer a quantity of USP Candesartan Cilexetil RS to a suitable volumetric flask. Add acetonitrile, about 50% of volume of the flask, and sonicate. Dilute with acetonitrile to volume.
Standard stock solution B: 0.28 mg/mL of USP Hydrochlorothiazide RS in acetonitrile prepared as follows. Transfer a quantity of USP Hydrochlorothiazide RS to a suitable volumetric flask. Add acetonitrile, about 50% of volume of the flask, and sonicate. Dilute with acetonitrile to volume.
Standard solution: Prepare solutions of concentrations per *Table 4*, from *Standard stock solution A* and *Standard stock solution B* in the appropriate *Medium*. Pass through a suitable filter of 0.45-µm pore size.

Table 4

Tablet Strength Candesartan Cilexetil/ Hydrochlorothiazide (mg/mg)	Concentration of Candesartan Cilexetil (mg/mL)	Concentration of Hydrochlorothiazide (mg/mL)
16/12.5	0.018	0.014▲ ▲(ERR 1-May-2019)
32/12.5 and 32/25	0.036	0.014▲/0.028▲ (ERR 1-May-2019)

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 264 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7▲ ▲(ERR 1-May-2019)
Autosampler temperature: 10°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both the candesartan cilexetil and hydrochlorothiazide peaks
Relative standard deviation: NMT 2.0% for both the candesartan cilexetil and hydrochlorothiazide peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) or hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of candesartan cilexetil or hydrochlorothiazide from the *Sample solution*

www.webofpharma.com

r_S = peak response of candesartan cilexetil or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Candesartan Cilexetil RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) each of the labeled amounts of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**

Solution A and **Solution B:** Prepare as directed in the *Assay*.

Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	95	5
8	95	5
15	60	40
20	60	40
30	40	60
35	30	70
45	20	80
50	0	100
60	0	100
62	95	5
70	95	5

Diluent A: Acetonitrile and water (70:30)

Diluent B: Acetonitrile and water (50:50)

Peak identification stock solution A: 0.05 mg/mL each of USP Candesartan Cilexetil Related Compound A RS, USP Candesartan Cilexetil Related Compound B RS, USP Candesartan Cilexetil Related Compound D RS, and USP Candesartan Cilexetil Related Compound F RS in acetonitrile

Peak identification stock solution B: 0.1 mg/mL of USP Candesartan Cilexetil RS in acetonitrile

Peak identification stock solution C: 0.5 mg/mL of USP Candesartan Cilexetil Related Compound G RS in methanol

Peak identification solution: 0.0015 mg/mL each of USP Candesartan Cilexetil Related Compound A RS, USP Candesartan Cilexetil Related Compound B RS, USP Candesartan Cilexetil Related Compound D RS, and USP Candesartan Cilexetil Related Compound F RS, 0.001 mg/mL of USP Candesartan Cilexetil RS, and 0.005 mg/mL of USP Candesartan Cilexetil Related Compound G RS from *Peak identification stock solution A*, *Peak identification stock solution B*, and *Peak identification stock solution C* in acetonitrile

System suitability stock solution: 0.05 mg/mL each of USP Benzothiadiazine Related Compound A RS and USP Hydrochlorothiazide RS, and 0.1 mg/mL of USP Chlorothiazide RS in *Diluent B*

System suitability solution: 2.5 µg/mL each of USP Benzothiadiazine Related Compound A RS and USP Hydrochlorothiazide RS, and 5 µg/mL of USP Chlorothiazide RS in *Diluent A* from *System suitability stock solution*

Standard stock solution: 1.6 mg/mL of USP Candesartan Cilexetil RS and 0.6 mg/mL of USP Hydrochlorothiazide RS in *Diluent A* prepared as follows. Transfer a quantity of USP Candesartan Cilexetil RS and USP Hydrochlorothiazide RS to a suitable volumetric flask. Add *Diluent A*, about 60% of the total volume, and sonicate to dissolve. Dilute with *Diluent A* to volume.

Standard solution: 0.008 mg/mL of USP Candesartan Cilexetil RS and 0.003 mg/mL of USP Hydrochlorothiazide RS in *Diluent A* from *Standard stock solution*. Pass through a suitable filter of 0.45-µm pore size.

Sample solution: Nominally 1.5 mg/mL of candesartan cilexetil in acetonitrile prepared as follows. Transfer about 75 mg of candesartan cilexetil, from NLT 20 finely powdered Tablets, to a 50-mL volumetric flask. Add 30 mL of *Diluent A* and sonicate for 20 min with intermittent shaking in cold water. Allow it to reach room temperature, dilute with *Diluent A* to volume, and pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples:

System suitability solution and *Standard solution*

[Note—The relative retention times for benzothiadiazine related compound A and chlorothiazide in *Table 6* are relative to hydrochlorothiazide.]

Suitability requirements

Resolution: NLT 1.5 between benzothiadiazine related compound A and chlorothiazide; NLT 1.5 between chlorothiazide and hydrochlorothiazide, *System suitability solution*

Tailing factor: NMT 2.0 for both the candesartan cilexetil and hydrochlorothiazide peaks, *Standard solution*

Relative standard deviation: NMT 10.0% for both the candesartan cilexetil and hydrochlorothiazide peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity of candesartan cilexetil and any unspecified impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of candesartan cilexetil from the *Standard solution*
C_S = concentration of USP Candesartan Cilexetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of candesartan cilexetil in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 6*)

Calculate the percentage of benzothiadiazine related compound A and chlorothiazide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

www.webofpharma.com

r_U = peak response of benzothiadiazine related compound A and chlorothiazide from the *Sample solution*
r_S = peak response of hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 6*)

Acceptance criteria: See *Table 6*.

Table 6

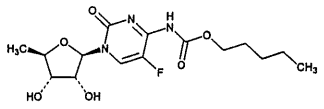
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Candesartan cilexetil related compound G[a]	0.51	1.11	1.0
Candesartan cilexetil related compound A[b, c]	0.73	—	—
Benzothiadiazine related compound A[d]	0.75	1.15	1.0
Chlorothiazide[e]	0.85	0.48	0.5
Candesartan cilexetil related compound B[f]	0.89	0.90	1.75
Candesartan cilexetil	1.00	—	—
Candesartan cilexetil related compound D[g]	1.06	0.91	0.5
Candesartan cilexetil related compound F[h]	1.24	0.83	1.5
Any unspecified degradation product	—	1.00	0.2
Total impurities	—	—	4.0

[a] 1-{[2′-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylic acid.
[b] Ethyl 1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylate.
[c] Process-related impurity not included in total impurities.
[d] 4-Amino-6-chloro-1,3-benzenedisulfonamide.
[e] 6-Chloro-2-*H*-1,2,4-benzothiadiazine-7-sulfonamide-1-1-dioxide.
[f] 1-{[(Cyclohexyloxy)carbonyl]oxy}ethyl 3-({2′-(1*H*-tetrazol-5-yl)-[1,1′-biphenyl-4-yl}methyl]-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-4-carboxylate.
[g] 1-{[(Cyclohexyloxy)carbonyl]oxy}ethyl 3-{[2′-(2-ethyl-2*H*-tetrazol-5-yl)-[1,1′-biphenyl]-4-yl}methyl)-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.
[h] 1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-{[2′-(2-ethyl-2*H*-tetrazol-5-yl) biphenyl-4-yl]methyl}-1*H*-benzimidazole-7-carboxylate

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzothiadiazine Related Compound A RS
  4-Amino-6-chloro-1,3-benzenedisulfonamide.
  $C_6H_8ClN_3O_4S_2$ 285.73
  USP Candesartan Cilexetil RS
  USP Candesartan Cilexetil Related Compound A RS
  Ethyl 1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylate.
  $C_{26}H_{24}N_6O_3$ 468.51
  USP Candesartan Cilexetil Related Compound B RS
  1-{[(Cyclohexyloxy)carbonyl]oxy}ethyl 3-({2′-(1*H*-tetrazol-5-yl)-[1,1′-biphenyl]-4-yl}methyl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-4-carboxylate.
  $C_{31}H_{30}N_6O_6$ 582.61
  USP Candesartan Cilexetil Related Compound D RS
  1-{[(Cyclohexyloxy)carbonyl]oxy}ethyl 3-{[2′(2-ethyl-2*H*-tetrazol-5-yl)-[1,1′-biphenyl]-4-yl}methyl)-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.
  $C_{33}H_{34}N_6O_6$ 610.67
  USP Candesartan Cilexetil Related Compound F RS
  1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-{[2′-(2-ethyl-2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-1*H*-benzimidazole-7-carboxylate.
  $C_{35}H_{38}N_6O_6$ 638.71
  USP Candesartan Cilexetil Related Compound G RS
  1-{[2′-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylic acid.
  $C_{24}H_{20}N_6O_3$ 440.45
  USP Chlorothiazide RS
  USP Hydrochlorothiazide RS

Capecitabine



$C_{15}H_{22}FN_3O_6$ 359.35
Carbamic acid, [1-(5-deoxy-β-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinyl]-, pentyl ester;
Pentyl 1-(5-deoxy-β-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinecarbamate [154361-50-9].

DEFINITION

Capecitabine contains NLT 98.0% and NMT 102.0% of $C_{15}H_{22}FN_3O_6$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: 2 mg of sample in 300 mg of potassium bromide
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Diluent: Methanol, acetonitrile, and water (7:1:12)
  Solution A: 0.1% mixture of glacial acetic acid in water
  Solution B: Methanol, acetonitrile, and *Solution A* (7:1:12)
  Solution C: Methanol, acetonitrile, and *Solution A* (16:1:3)
  Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
5	100	0
20	49	51
30	49	51

www.webofpharma.com

Time (min)	Solution B (%)	Solution C (%)
31	100	0
40	100	0

[NOTE—The following solutions may be sonicated if necessary.]
System suitability solution: 0.6 µg/mL each of USP Capecitabine RS, USP Capecitabine Related Compound A RS, USP Capecitabine Related Compound B RS, and USP Capecitabine Related Compound C RS in *Diluent*
Standard solution: 0.6 mg/mL of USP Capecitabine RS in *Diluent*
Sample solution: 0.6 mg/mL of Capecitabine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Autosampler temperature: 5°
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—For the purpose of peak identification, the approximate relative retention times are given in *Impurity Table 1* The relative retention times are measured with respect to capecitabine.]
Suitability requirements
Resolution: NLT 1.0 between capecitabine related compound A and capecitabine related compound B, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{15}H_{22}FN_3O_6$ in the portion of Capecitabine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)
C_U = concentration of Capecitabine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES
INORGANIC IMPURITIES
• **Residue on Ignition** ⟨281⟩: NMT 0.1%
ORGANIC IMPURITIES
• **Procedure**
Diluent, Solution B, Solution C, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capecitabine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100/F$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for capecitabine from the *Standard solution*
C_S = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)
C_U = concentration of Capecitabine in the *Sample solution* (mg/mL)
F = relative response factor for an impurity, from *Impurity Table 1*

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.5%
SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩**:** +96.0° to +100.0°
Sample solution: 10 mg/mL, on the anhydrous and solvent-free basis, in methanol, at 20°
• **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 0.3%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Capecitabine RS
USP Capecitabine Related Compound A RS
5′-Deoxy-5-fluorocytidine.
$C_9H_{12}FN_3O_4$ 245.21
USP Capecitabine Related Compound B RS
5′-Deoxy-5-fluorouridine.
$C_9H_{11}FN_2O_5$ 246.19
USP Capecitabine Related Compound C RS
2′,3′-*O*-Carbonyl-5′-deoxy-5-fluoro-*N*⁴-(pentyloxycarbonyl)cytidine.
$C_{16}H_{20}FN_3O_7$ 385.34

Capecitabine Tablets

DEFINITION
Capecitabine Tablets contain NLT 93.0% and NMT 105.0% of the labeled amount of capecitabine ($C_{15}H_{22}FN_3O_6$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Analytical wave number: 1500–1760 cm^{-1}
Sample: Grind 1 Tablet to a fine powder with a mortar and pestle. Mix 1 mg of this sample with 300 mg of potassium bromide.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Diluent: Methanol, acetonitrile, and water (7:1:12)
Solution A: 0.1% mixture of glacial acetic acid in water
Solution B: Methanol, acetonitrile, and *Solution A* (7:1:12)
Solution C: Methanol, acetonitrile, and *Solution A* (16:1:3)
Mobile phase: See the gradient table below.

www.webofpharma.com

Impurity Table 1 (Capecitabine)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Capecitabine related compound A	0.18	1.05	0.3
Capecitabine related compound B	0.19	0.81	0.3
2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluorocytidine	0.36	0.89	0.1
5′-Deoxy-5-fluoro-N4-(2-methyl-1-butyloxycarbonyl) cytidine + 5′-Deoxy-5-fluoro-N4-(3-methyl-1-butyloxycarbonyl)cytidine	0.95	1.01	0.5
Capecitabine	1.00	1.00	—
[1-[5-Deoxy-3-*O*-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester	1.06	1.00	0.3
[1-[5-Deoxy-2-*O*-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester	1.09	1.00	0.2
Capecitabine related compound C	1.11	0.91	0.3
[1-[5-Deoxy-3-*O*-(5-deoxy-α-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester	1.20	1.00	0.3
2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluoro-N4-(pentyloxycarbonyl)cytidine	1.37	0.85	0.1
Individual unspecified impurity	—	1.00	0.1

Time (min)	Solution B (%)	Solution C (%)
5	100	0
20	49	51
30	49	51
31	100	0
40	100	0
0	100	0

[NOTE—The following solutions may be sonicated as necessary.]

System suitability solution: Includes 0.6 µg/mL of USP Capecitabine RS, 0.6 µg/mL of USP Capecitabine Related Compound A RS, 0.6 µg/mL of USP Capecitabine Related Compound B RS, and 0.6 µg/mL of USP Capecitabine Related Compound C RS in *Diluent*

Standard solution: 0.6 mg/mL of USP Capecitabine RS in *Diluent*

Sample solution: Equivalent to 0.6 mg/mL of capecitabine, from powdered Tablets (NLT 20), in *Diluent*. [NOTE—Pass through a PVDF membrane filter of 0.45-µm pore size, and use the filtrate.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Autosampler temperature: 5°

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—For the purpose of peak identification, the approximate relative retention times are given in *Impurity Table 1*. The relative retention times are measured with respect to capecitabine.]

Suitability requirements

Resolution: NLT 1.0 between capecitabine related compound A and capecitabine related compound B, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{15}H_{22}FN_3O_6$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of capecitabine in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL, degassed

Apparatus 2: 50 rpm

Time: 30 min

Standard solutions

For Tablets labeled to contain 150 mg: 17 mg of USP Capecitabine RS in 100 mL of *Medium*

For Tablets labeled to contain 500 mg: 28 mg of USP Capecitabine RS in 50 mL of *Medium*

Sample solution: Pass a portion of the solution under test through a fiberglass filter of 0.45-µm pore size.

Analysis: Determine the amount of $C_{15}H_{22}FN_3O_6$ dissolved by selecting a wavelength with appropriate sensitivity between 300 and 330 nm on portions of the *Sample*

USP Monographs

www.webofpharma.com

solution, suitably diluted with *Medium*, if necessary, in comparison with the appropriate *Standard solution*, using a 1-mm quartz cell. Calculate the percentage of $C_{15}H_{22}FN_3O_6$ dissolved in each Tablet:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of capecitabine in the *Standard solution* (mg/mL)
V = volume of medium, 900 mL
L = Tablet label claim (mg)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{15}H_{22}FN_3O_6$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Diluent, Solution A, Solution B, Solution C, Mobile phase, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100/F$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for capecitabine from the *Standard solution*
C_S = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of capecitabine in the *Sample solution* (mg/mL)
F = relative response factor for each impurity, from *Impurity Table 1*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total degradation products: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

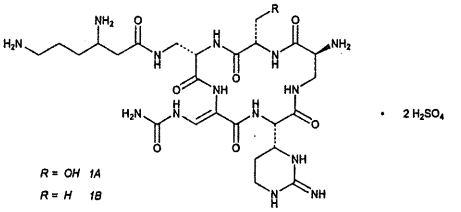
USP Capecitabine RS

USP Capecitabine Related Compound A RS
5′-Deoxy-5-fluorocytidine.
$C_9H_{12}FN_3O_4$ 245.21

USP Capecitabine Related Compound B RS
5′-Deoxy-5-fluorouridine.
$C_9H_{11}FN_2O_5$ 246.19

USP Capecitabine Related Compound C RS
2′,3′-*O*-Carbonyl-5′-deoxy-5-fluoro-*N*⁴-(pentyloxycarbonyl)cytidine.
$C_{16}H_{20}FN_3O_7$ 385.34

Capreomycin Sulfate



Capreomycin sulfate [1405-37-4].

$C_{25}H_{44}N_{14}O_8$ 668.71

Capreomycin 1A (free base);
3,6-Diamino-*N*-({(2*S*,5*S*,11*S*,15*S*,*Z*)-15-amino-2-(hydroxymethyl)-11-[(*R*)-iminohexahydropyrimidin-4-yl]-3,6,9,12,16-pentaoxo-8-(ureidomethylene)-1,4,7,10,13-pentaazacyclohexadecan-5-yl}methyl)hexanamide [37290-35-6].

$C_{25}H_{44}N_{14}O_7$ 652.71

Capreomycin 1B (free base);
3,6-Diamino-*N*-({(2*S*,5*S*,11*S*,15*S*,*Z*)-15-amino-11-[(*R*)-iminohexahydropyrimidin-4-yl]-2-methyl-3,6,9,12,16-pentaoxo-8-(ureidomethylene)-1,4,7,10,13-pentaazacyclohexadecan-5-yl}methyl)hexanamide [33490-33-4].

DEFINITION

Capreomycin Sulfate is the disulfate salt of capreomycin, a polypeptide mixture produced by the growth of *Streptomyces capreolus*, suitable for parenteral use. It has a potency equivalent to NLT 700 µg/mg and NMT 1050 µg/mg of capreomycin.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩

• **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Capreomycin 1 Content*.

ASSAY

• **PROCEDURE:** Proceed as directed in *Antibiotics—Microbial Assays* ⟨81⟩.

Acceptance criteria: 700–1050 µg/mg

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 3.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

SPECIFIC TESTS

• **CAPREOMYCIN 1 CONTENT**

Solution A: 0.4 mg/mL of ammonium bisulfate in water. Pass through a filter of 0.5-µm or less pore size.

Mobile phase: Methanol and *Solution A* (2:3)

System suitability solution: 0.25 mg/mL of USP Capreomycin Sulfate RS in water

Sample solution: 0.25 mg/mL of Capreomycin Sulfate in water

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 268 nm
Column: 4.6-mm × 25-cm; 5-µm packing L10
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection size: 20 µL

System suitability
Sample: *System suitability solution*

USP Monographs

www.webofpharma.com

Impurity Table 1 (Capecitabine Tablets)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Capecitabine related compound A	0.18	1.05	1.0
Capecitabine related compound B	0.19	0.81	1.0
2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluorocytidine*	0.36	0.89	—
5′-Deoxy-5-fluoro-N4-(2-methyl-1-butyloxycarbonyl) cytidine + 5′-Deoxy-5-fluoro-N4-(3-methyl-1-butyloxycarbonyl)cytidine*	0.95	1.01	—
Capecitabine	1.00	1.00	—
[1-[5-Deoxy-3-*O*-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.06	1.00	—
[1-[5-Deoxy-2-*O*-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.09	1.00	—
Capecitabine related compound C	1.11	0.91	0.5
[1-[5-Deoxy-3-*O*-(5-deoxy-α-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.20	1.00	—
2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluoro-N4-(pentyloxycarbonyl)cytidine*	1.37	0.85	—
Individual unspecified degradation product	—	1.00	0.1
The impurities marked with an "*" are process impurities and are not included in the total degradation products.			

[NOTE—The relative retention times of capreomycin 1A and capreomycin 1B are 0.85 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between capreomycin 1A and capreomycin 1B

Tailing factor: NMT 3.5 for the major peaks (capreomycin 1A and capreomycin 1B)

Analysis

[NOTE—The chromatographic run time is at least five times the retention time of the capreomycin 1A peak.]

Sample: *Sample solution*

Calculate the percentage of capreomycin 1 in the portion of Capreomycin Sulfate taken:

$$\text{Result} = [(r_{1A} + r_{1B})/r_T] \times 100$$

r_{1A} = peak area response of capreomycin 1A
r_{1B} = peak area response of capreomycin 1B
r_T = total response for all peaks

Acceptance criteria: NLT 90.0%

- **pH** ⟨791⟩: 4.5–7.5

Sample solution: 30 mg/mL

- **Loss on Drying** ⟨731⟩: Dry 100 mg in a vacuum at a pressure not exceeding 5 mm of mercury at 100° for 4 h: it loses NMT 10.0% of its weight.
- **Bacterial Endotoxins Test** ⟨85⟩: Where it is intended for use in preparing injectable dosage forms: NMT 0.35 USP Endotoxin Unit/mg of capreomycin
- **Other Requirements:** Where the label states that Capreomycin Sulfate is sterile, it meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP Reference Standards** ⟨11⟩
  USP Capreomycin Sulfate RS

Capreomycin for Injection

DEFINITION

Capreomycin for Injection contains an amount of Capreomycin Sulfate equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of capreomycin.

IDENTIFICATION

- **A. Identification Tests—General,** *Sulfate* ⟨191⟩
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *System suitability solution,* as obtained in the test for *Capreomycin 1 Content.*

ASSAY

- **Procedure:** Proceed as directed in *Antibiotics—Microbial Assays* ⟨81⟩.

Acceptance criteria: 90.0%–115.0%

IMPURITIES

Inorganic Impurities

- **Residue on Ignition** ⟨281⟩: NMT 3.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

SPECIFIC TESTS

- **Capreomycin 1 Content**

Solution A: 0.4 mg/mL of ammonium bisulfate in water. Pass through a filter of 0.5-µm or less pore size.

Mobile phase: Methanol and *Solution A* (2:3)

System suitability solution: 0.25 mg/mL of USP Capreomycin Sulfate RS in water

USP Monographs

www.webofpharma.com

Sample solution: 0.25 mg/mL of Capreomycin for Injection in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 268 nm
Column: 4.6-mm × 25-cm; 5-µm packing L10
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times of capreomycin 1A and capreomycin 1B are 0.85 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between capreomycin 1A and capreomycin 1B
Tailing factor: NMT 3.5 for the major peaks (capreomycin 1A and capreomycin 1B)
Analysis
[NOTE—The chromatographic run time is at least five times the retention time of the capreomycin 1A peak.]
Sample: *Sample solution*
Calculate the percentage of capreomycin 1 in the portion of Capreomycin for Injection taken:

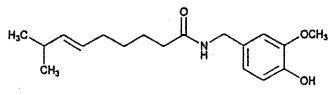
$$\text{Result} = (r_{1A} + r_{1B})/r_T \times 100$$

r_{1A} = peak area response for capreomycin 1A
r_{1B} = peak area response for capreomycin 1B
r_T = total response for all peaks

Acceptance criteria: The capreomycin 1 content is NLT 90.0%.
- **pH** ⟨791⟩: 4.5–7.5
Sample solution: 30 mg/mL
- **LOSS ON DRYING** ⟨731⟩: Dry 100 mg in a vacuum at a pressure not exceeding 5 mm of mercury at 100° for 4 h: it loses NMT 10.0% of its weight.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.35 USP Endotoxin Unit/mg of capreomycin
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Capreomycin Sulfate RS

Capsaicin



$C_{18}H_{27}NO_3$ 305.41
6-Nonenamide, (*E*)-*N*-[(4-Hydroxy-3-methoxy-phenyl) methyl]-8-methyl.
(*E*)-8-Methyl-*N*-vanillyl-6-nonenamide [404-86-4].

» Capsaicin contains not less than 90.0 percent and not more than 110.0 percent of the labeled percentage of total capsaicinoids. The content of capsaicin ($C_{18}H_{27}NO_3$) is not less than 55 percent, and the sum of the contents of capsaicin and dihydrocapsaicin ($C_{18}H_{29}NO_3$) is not less than 75 percent, and the content of other capsaicinoids is not more than 15 percent, all calculated on the dried basis.
[**CAUTION**—Handle Capsaicin with care. Prevent inhalation of particles of it and prevent its contact with any part of the body.]

Packaging and storage—Preserve in tight containers, protected from light, and store in a cool place.
Labeling—Label it to state the percentage content of total capsaicinoids.
USP Reference standards ⟨11⟩—
USP Capsaicin RS
USP Dihydrocapsaicin RS
Identification—Prepare a test solution of Capsaicin in methanol containing 1 mg per mL. Prepare a Standard solution of USP Capsaicin RS in methanol containing 1 mg per mL. Separately apply 10-µL portions of the test solution and the Standard solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatograms in a solvent system consisting of a mixture of ether and methanol (19:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow it to air-dry. Spray the plate with a 0.5% solution of 2,6-dibromoquinone-chlorimide in methanol, allow to stand in a chamber containing ammonia fumes, and examine the chromatograms: the blue color and the R_F value of the principal spot obtained from the test solution correspond to those properties of the principal spot obtained from the Standard solution.
Melting range ⟨741⟩: between 57° and 66°, but the range between beginning and end of melting does not exceed 5°.
Loss on drying ⟨731⟩: Dry it in vacuum over phosphorus pentoxide at 40° for 5 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 1.0%.

Content of capsaicin, dihydrocapsaicin, and other capsaicinoids—
Mobile phase—Prepare a mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (600:400). Pass through a filter having a porosity of 0.5 µm or finer, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard capsaicin solution—Quantitatively dissolve an accurately weighed quantity of USP Capsaicin RS in methanol to obtain a solution having a known concentration of about 0.1 mg per mL.
Standard dihydrocapsaicin solution—Quantitatively dissolve an accurately weighed quantity of USP Dihydrocapsaicin RS in methanol to obtain a solution having a known concentration of about 0.025 mg per mL.
Test solution—Transfer about 25 mg of Capsaicin, accurately weighed, to a 250-mL volumetric flask, dilute with methanol to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 281-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L11 and is maintained at a constant temperature of about 30°. Adjust the flow rate to obtain a retention time of about 20 minutes for the main capsaicin peak.

www.webofpharma.com

Chromatograph the *Standard capsaicin solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard capsaicin solution*, the *Standard dihydrocapsaicin solution*, and the *Test solution* into the chromatograph, record the chromatogram for a period of time that is twice that of the retention time of capsaicin, and measure the areas of the responses for all of the peaks. Calculate the percentage of capsaicin ($C_{18}H_{27}NO_3$) in the portion of Capsaicin taken by the formula:

$$25{,}000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Capsaicin RS in the *Standard capsaicin solution*; W is the weight, in mg, of Capsaicin taken to prepare the *Test solution*; and r_U and r_S are the capsaicin peak responses obtained from the *Test solution* and the *Standard capsaicin solution*, respectively. Not less than 55% is found. Calculate the percentage of dihydrocapsaicin ($C_{18}H_{29}NO_3$) in the portion of Capsaicin taken by the formula:

$$25{,}000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dihydrocapsaicin RS in the *Standard dihydrocapsaicin solution*; W is the weight, in mg, of Capsaicin taken to prepare the *Test solution*; and r_U and r_S are the dihydrocapsaicin peak responses obtained from the *Test solution* and the *Standard dihydrocapsaicin solution*, respectively. The sum of the percentage of capsaicin found and of the percentage of dihydrocapsaicin found is not less than 75%. Using the chromatograms obtained from the *Standard capsaicin solution* and the *Test solution*, calculate the percentage of other capsaicinoids in the portion of Capsaicin taken by the formula:

$$25{,}000(C/W)(r_T/r_S)$$

in which C is the concentration, in mg per mL, of USP Capsaicin RS in the *Standard capsaicin solution*; W is the weight, in mg, of Capsaicin taken to prepare the *Test solution*; r_T is the sum of the peak responses of the capsaicinoids other than capsaicin and dihydrocapsaicin in the chromatogram obtained from the *Test solution*; and r_S is the capsaicin peak response obtained from the *Standard capsaicin solution.* Not more than 15% of other capsaicinoids is found.

Capsicum

DEFINITION

Capsicum is the dried ripe fruit of various *Capsicum* species (Fam. Solanaceae). It contains NLT 0.3% of total capsaicinoids, calculated as the sum of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin; and the nonivamide content is NMT 5% of the total capsaicinoids; all calculated on the dried basis.

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution A: 0.4 mg/mL of USP Capsaicin RS in methanol

Standard solution B: 0.4 mg/mL of USP Dihydrocapsaicin RS in methanol

Sample solution: Shake for 5 min about 0.5 g of Capsicum, finely powdered, in 5 mL of hexanes, and centrifuge. Use the supernatant.

Chromatographic system

Adsorbent: Chromatographic reversed-phase octadecyl silyl silica gel with an average particle size of 5 µm (HPTLC plates)

Application volume: 2 µL, as 8-mm bands

Developing solvent system: A mixture of methanol and water (8:2)

Derivatization reagent A: 0.25 mg/mL of dichloroquinonechlorimide in ethyl acetate

Derivatization reagent B: Ammonium hydroxide solution

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable high-performance thin-layer chromatographic plate. Use a saturated chamber, and condition the plate to a relative humidity of about 33% using a suitable device. Develop the chromatograms over a distance of 6 cm. Remove the plate from the chamber, dry, derivatize with *Derivatization reagent A*, and expose to vapors of *Derivatization reagent B* until blue bands develop. Examine under white light.

System suitability: *Standard solution A* shows a blue band at about one-third of the chromatogram, and *Standard solution B* shows a blue band at an R_F right below that from *Standard solution A*.

Acceptance criteria: The *Sample solution* exhibits a blue band at about one-third of the chromatogram, similar in position and color to the capsaicin band in the chromatogram of *Standard solution A*, and exhibits a blue band at an R_F right below that of capsaicin, similar in position and color to the dihydrocapsaicin band in the chromatogram of *Standard solution B*. Other bands may be observed in the *Sample solution* chromatogram.

• **B. HPLC**

Analysis: Proceed as directed in the test for *Content of Total Capsaicinoids*.

Acceptance criteria: The *Sample solution* chromatogram exhibits the main capsaicinoid peak at the retention time corresponding to capsaicin in the chromatogram of *Standard solution A* and a peak of lower intensity corresponding to dihydrocapsaicin in the chromatogram of *Standard solution B*. The *Sample solution* chromatogram shows additional minor peaks corresponding to nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin.

COMPOSITION

• **Content of Total Capsaicinoids**

Mobile phase: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (2:3)

Standard solution A: 0.02 mg/mL of USP Capsaicin RS in methanol

Standard solution B: 0.01 mg/mL of USP Dihydrocapsaicin RS in methanol

Sample solution: To a glass centrifuge tube transfer about 0.5 g of Capsicum, powdered and accurately weighed, add 30 mL of methanol, shake for 15 min, and centrifuge. Transfer the supernatant to a 50-mL volumetric flask. To the residue add 10 mL of methanol, shake for 5 min, and centrifuge. Transfer the supernatant to the volumetric flask. Repeat the extraction one more time with 10 mL of methanol. Complete with methanol to volume, and mix. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

USP Monographs

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 281 nm
Column: 4.6-mm × 25-cm; end-capped, 5-µm, 150 Å, packing L11
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution A* and *Sample solution*
Suitability requirements
Resolution: NLT 1.5 between the capsaicin peak and the nonivamide peak occurring at a retention time of 0.95 relative to 1.0 for capsaicin, *Sample solution*
Relative standard deviation: NMT 2.0% for the capsaicin peak in repeated injections, *Standard solution A*
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Identify the capsaicin and dihydrocapsaicin peaks in the *Sample solution* chromatogram by comparison with the chromatograms of *Standard solution A* and *Standard solution B*, respectively. Identify the peaks corresponding to nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin using the approximate relative retention times provided in *Table 1*.

Table 1

Analyte	Approximate Relative Retention Time
Nordihydrocapsaicin	0.89
Nonivamide	0.95
Capsaicin	1.00
Decanylvanillinamide	1.34
Homocapsaicin	1.40

Calculate the percentage of capsaicin in the portion of Capsicum taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of capsaicin from the *Sample solution*
r_S = peak area of capsaicin from *Standard solution A*
C_S = concentration of capsaicin in *Standard solution A* (mg/mL)
V = volume of the *Sample solution* (mL)
W = weight of Capsicum taken to prepare the *Sample solution* (mg)

Calculate the percentage of dihydrocapsaicin in the portion of Capsicum taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of dihydrocapsaicin from the *Sample solution*
r_S = peak area of dihydrocapsaicin from *Standard solution B*
C_S = concentration of dihydrocapsaicin in *Standard solution B* (mg/mL)
V = volume of the *Sample solution* (mL)
W = weight of Capsicum taken to prepare the *Sample solution* (mg)

Calculate the percentage of nonivamide, expressed as capsaicin, in the portion of Capsicum taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of nonivamide from the *Sample solution*
r_S = peak area of capsaicin from *Standard solution A*
C_S = concentration of capsaicin in *Standard solution A* (mg/mL)
V = volume of the *Sample solution* (mL)
W = weight of Capsicum taken to prepare the *Sample solution* (mg)

Calculate the sum of the percentages of nordihydrocapsaicin, decanylvanillinamide, and homocapsaicin, expressed as capsaicin, in the portion of Capsicum taken:

$$\text{Result} = (\Sigma r_U/r_S) \times C_S \times (V/W) \times 100$$

Σr_U = sum of peak areas of nordihydrocapsaicin, decanylvanillinamide, and homocapsaicin from the *Sample solution*
r_S = peak area of capsaicin from *Standard solution A*
C_S = concentration of capsaicin in *Standard solution A* (mg/mL)
V = volume of the *Sample solution* (mL)
W = weight of Capsicum taken to prepare the *Sample solution* (mg)

Calculate the content of total capsaicinoids as the sum of the percentages of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin.
Acceptance criteria: NLT 0.3% on the dried basis

CONTAMINANTS

• **ELEMENTAL IMPURITIES—PROCEDURES** ⟨233⟩
Acceptance criteria
Arsenic: NMT 0.5 µg/g
Cadmium: NMT 1.0 µg/g
Lead: NMT 5.0 µg/g
Mercury: NMT 0.1 µg/g

Change to read:

• **▲ARTICLES OF BOTANICAL ORIGIN,** *Pesticide Residue Analysis* ⟨561⟩▲ (CN 1-May-2019)**:** Meets the requirements
• **ARTICLES OF BOTANICAL ORIGIN,** *Test for Aflatoxins* ⟨561⟩**:** Meets the requirements

SPECIFIC TESTS

• **LIMIT OF NONIVAMIDE**
Analysis: Use the chromatograms and calculations obtained in the test for *Content of Total Capsaicinoids*.
Calculate the content of nonivamide as a percentage of total capsaicinoids:

$$\text{Result} = (PN/PTC) \times 100$$

PN = percentage of nonivamide as calculated in the *Content of Total Capsaicinoids*
PTC = percentage of total capsaicinoids as calculated in the *Content of Total Capsaicinoids*

Acceptance criteria: NMT 5% on the dried basis
• **BOTANIC CHARACTERISTICS**
Macroscopic: Mature Capsicum fruit vary in length from a few cm to more than 10 cm; fruit diameter ranges from a few mm to a few cm; shape varies from elongate, ellipse, almost round, triangular, campanulate, tomato-like, or

USP Monographs

www.webofpharma.com

square (blocky); number of locules two, three, or four; the dissepiments being united at the base to a conical, central placenta; calyx margin is either entire, dentate, or intermediate dentate, and sometimes attached to a long, straight pedicel; the presence of calyx annular constriction, at junction of calyx and pedicel indicates *Capsicum chinense*; the seeds are light brown to weak yellowish-orange, suborbicular or irregular, flattened, from 2–4 mm in diameter, with a thickened edge and a prominent, pointed micropyle.

Microscopic: Powdered Capsicum shows fragments of epicarp cells, polygonal, triangular, rectangular, or irregular cells, with or without beaded walls; numerous fragments of thin-walled parenchyma cells of the pericarp containing oil globules and chromoplasts of the various colors similar to the color of the fruits; fragments of sclerenchymatous cells of the endocarp with slightly wavy, lignified walls and broad lumina, striations and pit canal distinct, cells appear bright white under a polarizing microscope; fragments of epidermal cells of testa, extremely thick wall, deeply sinuate, striations and pit canal distinct, cells appear bright yellowish-white under a polarizing microscope; fragments of parenchyma cells of the endosperm containing fixed oil and aleurone grains; and occasional fibrovascular elements and calyx tissues.

- **ARTICLES OF BOTANICAL ORIGIN,** *Foreign Organic Matter* ⟨561⟩: NMT 1%, other than stems and calyces, the proportion of which does not exceed 3%
- **LOSS ON DRYING** ⟨731⟩
  Sample: 1.0 g of finely powdered Capsicum
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 11%
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨561⟩: NMT 10.0%
- **ARTICLES OF BOTANICAL ORIGIN,** *Acid-Insoluble Ash* ⟨561⟩: NMT 1.25%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. A few drops of chloroform may be added from time to time to prevent attack by insects.
- **LABELING:** Label each container to indicate which variety of Capsicum is contained therein.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Capsaicin RS
  USP Dihydrocapsaicin RS

Capsicum Oleoresin

DEFINITION

Capsicum Oleoresin is an alcoholic extract of the dried ripe fruits of Capsicum. It contains NLT 6.5% of total capsaicinoids, calculated as the sum of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin, all calculated on the anhydrous basis. The nonivamide content is NMT 5% of the total capsaicinoids, calculated on the anhydrous basis. [**CAUTION**—Capsicum Oleoresin is a powerful irritant, and even in minute quantities produces an intense burning sensation when it comes in contact with the eyes and tender parts of the skin. Care should be taken to protect the eyes and to prevent contact of the skin with Capsicum Oleoresin.]

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**
  Standard solution A: 0.4 mg/mL of USP Capsaicin RS in methanol
  Standard solution B: 0.4 mg/mL of USP Dihydrocapsaicin RS in methanol
  Sample solution: 10 mg/mL of Capsicum Oleoresin in hexanes
  Chromatographic system
  Adsorbent: Chromatographic reverse phase octadecyl silyl silica gel with an average particle size of 5 µm (HPTLC plates)
  Application volume: 2 µL, as 8-mm bands
  Developing solvent system: A mixture of methanol and water (8:2)
  Derivatization reagent A: 0.25 mg/mL of dichloroquinonechlorimide in ethyl acetate
  Derivatization reagent B: Ammonium hydroxide solution
  Analysis
  Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
  Apply the *Samples* as bands to a suitable high-performance thin-layer chromatographic plate. Use a saturated chamber, and condition the plate to a relative humidity of about 33% using a suitable device. Develop the chromatograms over a distance of 6 cm. Remove the plate from the chamber, dry, derivatize with *Derivatization reagent A*, dry, expose to vapors of *Derivatization reagent B* until blue bands develop, and examine under white light.
  System suitability: *Standard solution A* shows a blue band at about one-third of the chromatogram, and *Standard solution B* shows a blue band at an R_F right below that from *Standard solution A*.
  Acceptance criteria: The *Sample solution* exhibits a blue band at about one-third of the chromatogram, similar in position and color to the capsaicin band in the chromatogram of *Standard solution A*, and exhibits a blue band at an R_F right below that of capsaicin, similar in position and color to the dihydrocapsaicin band in the chromatogram of *Standard solution B*. Other bands may be observed in the *Sample solution* chromatogram.
- **B. HPLC**
  Analysis: Proceed as directed in the test for *Content of Total Capsaicinoids*.
  Acceptance criteria: The *Sample solution* chromatogram exhibits the main capsaicinoid peak at the retention time corresponding to capsaicin in the chromatogram of *Standard solution A* and a peak of lower intensity corresponding to dihydrocapsaicin in the chromatogram of *Standard solution B*. The *Sample solution* chromatogram shows additional minor peaks corresponding to nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin.

ASSAY

- **CONTENT OF TOTAL CAPSAICINOIDS**
  Mobile phase: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (2:3)
  Standard solution A: 0.2 mg/mL of USP Capsaicin RS in methanol
  Standard solution B: 0.1 mg/mL of USP Dihydrocapsaicin RS in methanol
  Sample solution: 5 mg/mL of Capsicum Oleoresin in methanol. Pass a portion of this solution through a filter of 0.2-µm pore size, and use the filtrate as the *Sample solution*.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC

www.webofpharma.com

Detector: UV 281 nm
Column: 4.6-mm × 25-cm; end-capped, 5-µm, 150 Å, packing L11
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution A* and *Sample solution*
Suitability requirements
Resolution: NLT 1.5 between the capsaicin peak and the nonivamide peak that occurs at a retention time of 0.95 relative to 1.0 for capsaicin, *Sample solution*
Relative standard deviation: NMT 2.0% for the capsaicin peak, *Standard solution A*
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Identify the capsaicin and dihydrocapsaicin peaks in the *Sample solution* chromatogram by comparison with the chromatograms of *Standard solution A* and *Standard solution B*, respectively. Identify the peaks corresponding to nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin using the approximate relative retention times provided in *Table 1*.

Table 1

Analyte	Approximate Relative Retention Time
Nordihydrocapsaicin	0.89
Nonivamide	0.95
Capsaicin	1.00
Decanylvanillinamide	1.34
Homocapsaicin	1.40

Calculate the percentage of capsaicin in the portion of Capsicum Oleoresin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of capsaicin from the *Sample solution*
r_S = peak area of capsaicin from *Standard solution A*
C_S = concentration of capsaicin in *Standard solution A* (mg/mL)
C_U = concentration of Capsicum Oleoresin in the *Sample solution* (mg/mL)

Calculate the percentage of dihydrocapsaicin in the portion of Capsicum Oleoresin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of dihydrocapsaicin from the *Sample solution*
r_S = peak area of dihydrocapsaicin from *Standard solution B*
C_S = concentration of dihydrocapsaicin in *Standard solution B* (mg/mL)
C_U = concentration of Capsicum Oleoresin in the *Sample solution* (mg/mL)

Calculate the percentage of nonivamide, expressed as capsaicin, in the portion of Capsicum Oleoresin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of nonivamide from the *Sample solution*
r_S = peak area of capsaicin from *Standard solution A*
C_S = concentration of capsaicin in *Standard solution A* (mg/mL)
C_U = concentration of Capsaicin Oleoresin in the *Sample solution* (mg/mL)

Calculate the sum of the percentages of nordihydrocapsaicin, decanylvanillinamide, and homocapsaicin, expressed as capsaicin, in the portion of Capsicum Oleoresin taken:

$$\text{Result} = (\Sigma r_U/r_S) \times (C_S/C_U) \times 100$$

Σr_U = sum of the peak areas of nordihydrocapsaicin, decanylvanillinamide, and homocapsaicin from the *Sample solution*
r_S = peak area of capsaicin from *Standard solution A*
C_S = concentration of capsaicin in *Standard solution A* (mg/mL)
C_U = concentration of Capsicum Oleoresin in the *Sample solution* (mg/mL)

Calculate the content of total capsaicinoids as the sum of the percentages of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin.
Acceptance criteria: NLT 6.5% on the anhydrous basis

CONTAMINANTS

• **ELEMENTAL IMPURITIES—PROCEDURES** ⟨233⟩
Acceptance criteria
Arsenic: NMT 0.5 µg/g
Cadmium: NMT 1.0 µg/g
Lead: NMT 5.0 µg/g
Mercury: NMT 0.1 µg/g

Change to read:

• **▲ARTICLES OF BOTANICAL ORIGIN,** *Pesticide Residue Analysis* ⟨561⟩▲ (CN 1-May-2019)**:** Meets the requirements
• **ARTICLES OF BOTANICAL ORIGIN,** *Test for Aflatoxins* ⟨561⟩**:** Meets the requirements

SPECIFIC TESTS

• **LIMIT OF NONIVAMIDE**
Analysis: Use the chromatograms and calculations obtained in the test for *Content of Total Capsaicinoids*.
Calculate the content of nonivamide as percentage of total capsaicinoids:

$$\text{Result} = (PN/PTC) \times 100$$

PN = percentage of nonivamide as calculated in the test for *Content of Total Capsaicinoids*
PTC = percentage of total capsaicinoids as calculated in the test for *Content of Total Capsaicinoids*

Acceptance criteria: NMT 5% on the anhydrous basis
• **WATER DETERMINATION,** *Method Ia* ⟨921⟩
Sample: 5.0 g of Capsicum Oleoresin
Acceptance criteria: NMT 8%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** Label it to indicate that if separation occurs, it should be warmed and mixed before use.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Capsaicin RS
USP Dihydrocapsaicin RS

USP Monographs

www.webofpharma.com

Capsicum Tincture

DEFINITION

Capsicum Tincture is prepared as follows.

Capsicum	100 g
A mixture of Alcohol and Water (70:30) to (85:15), a sufficient quantity to make	1000 mL

Prepare the Tincture as directed for *Botanical Extracts* ⟨565⟩, *Tinctures, Maceration Process*. It contains NLT 0.02% (w/v) of total capsaicinoids, calculated as the sum of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin; and the nonivamide content is NMT 5% of the total capsaicinoids.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution A: 0.2 mg/mL of USP Capsaicin RS in methanol

Standard solution B: 0.2 mg/mL of USP Dihydrocapsaicin RS in methanol

Sample solution: Shake 10 mL of Tincture with 10 mL of hexanes, allow to separate, and use the lower layer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: Chromatographic reverse phase octadecyl silyl silica gel with an average particle size of 5 µm (HPTLC plates)

Application volume: 2 µL, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Developing solvent system: A mixture of methanol and water (8:2)

Derivatization reagent A: 5 mg/mL of dichloroquinonechlorimide in methanol

Derivatization reagent B: Ammonium hydroxide solution

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable high-performance thin-layer chromatographic plate. Use a saturated chamber, and develop the chromatograms over a distance of 6 cm. Remove the plate from the chamber, dry, derivatize with *Derivatization reagent A*, dry, expose to the vapors of *Derivatization reagent B* until blue bands develop, and then examine under white light.

System suitability: *Standard solution A* shows a blue band at about one-third of the chromatogram, and *Standard solution B* shows a blue band at an R_F just below that from *Standard solution A*. Separation between capsaicin and dihydrocapsaicin should be achieved with the reference standards; the bands appear at the intended location of the plate and they are visible as blue bands.

Acceptance criteria: The *Sample solution* chromatogram exhibits a blue band at about one-third of the chromatogram, corresponding in color and R_F to the capsaicin band in the chromatogram of *Standard solution A*, and exhibits a blue band at an R_F just below that of capsaicin, similar in position and color to the dihydrocapsaicin band in the chromatogram of *Standard solution B*. Other bands may be observed in the *Sample solution* chromatogram.

• **B. HPLC**

Analysis: Proceed as directed in the test for *Content of Total Capsaicinoids*.

Acceptance criteria: The *Sample solution* chromatogram exhibits the main capsaicinoid peak at the retention time corresponding to capsaicin in the chromatogram of *Standard solution A* and a peak of lower intensity corresponding to dihydrocapsaicin in the chromatogram of *Standard solution B*. The *Sample solution* chromatogram shows additional minor peaks corresponding to nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin.

STRENGTH

• **CONTENT OF TOTAL CAPSAICINOIDS**

Mobile phase: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (2:3)

Standard solution A: 0.1 mg/mL of USP Capsaicin RS in methanol

Standard solution B: 0.05 mg/mL of USP Dihydrocapsaicin RS in methanol

Sample solution: Dilute an accurately measured volume of Tincture in methanol (1:1), and mix. Before injection, pass through a membrane filter with a 0.45-µm or finer pore size, discarding the first few mL of the filtrate. [NOTE—The sample can be weighed and converted to volume using the density of the Tincture.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 281 nm

Column: 4.6-mm × 25-cm; end-capped, 5-µm, 150 Å, packing L11

Flow rate: 1.0 mL/min

Column temperature: 30°

Injection volume: 20 µL

System suitability

Samples: *Standard solution A* and *Sample solution*

Suitability requirements

Resolution: NLT 1.5 between the capsaicin peak and the nonivamide peak at a retention time of 0.95, relative to 1.0 for capsaicin, *Sample solution*

Relative standard deviation: NMT 2.0% for the capsaicin peak in repeated injections, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Identify the capsaicin and dihydrocapsaicin peaks in the *Sample solution* chromatogram by comparison with the chromatograms of *Standard solution A* and *Standard solution B*, respectively. Identify the peaks corresponding to nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin using the approximate relative retention times provided in *Table 1*.

Table 1

Analyte	Approximate Relative Retention Time
Nordihydrocapsaicin	0.89
Nonivamide	0.95
Capsaicin	1.00
Decanylvanillinamide	1.34
Homocapsaicin	1.40

Calculate the percentage of capsaicin in the portion of Tincture taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of capsaicin from the *Sample solution*

r_S = peak area of capsaicin from *Standard solution A*

www.webofpharma.com

C_S = concentration of capsaicin in *Standard solution A* (g/mL)
C_U = concentration of Tincture in the *Sample solution* (mL/mL)

Calculate the percentage of dihydrocapsaicin in the portion of Tincture taken:

$$\text{Result} = (r_U/r_S) \times C_S/C_U) \times 100$$

r_U = peak area of dihydrocapsaicin from the *Sample solution*
r_S = peak area of dihydrocapsaicin from *Standard solution B*
C_S = concentration of dihydrocapsaicin in *Standard solution B* (g/mL)
C_U = concentration of Tincture in the *Sample solution* (mL/mL)

Calculate the percentage of nonivamide, expressed as capsaicin, in the portion of Tincture taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of nonivamide from the *Sample solution*
r_S = peak area of capsaicin from *Standard solution A*
C_S = concentration of capsaicin in *Standard solution A* (g/mL)
C_U = concentration of Tincture in the *Sample solution* (mL/mL)

Calculate the sum of the percentages of nordihydrocapsaicin, decanylvanillinamide and homocapsaicin, expressed as capsaicin, in the portion of Tincture taken:

$$\text{Result} = (\Sigma r_U/r_S) \times (C_S/C_U) \times 100$$

Σr_U = sum of the peak areas of nordihydrocapsaicin, decanylvanillinamide, and homocapsaicin from the *Sample solution*
r_S = peak area of capsaicin from *Standard solution A*
C_S = concentration of capsaicin in *Standard solution A* (g/mL)
C_U = concentration of Tincture in the *Sample solution* (mL/mL)

Calculate the content of total capsaicinoids as the sum of the percentages of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanylvanillinamide, and homocapsaicin.

Acceptance criteria: NLT 0.02%

OTHER COMPONENTS

- **ALCOHOL DETERMINATION,** *Method I* ⟨611⟩: NLT 90.0% and NMT 110.0% of the labeled amount of C_2H_5OH

CONTAMINANTS

Change to read:

- **▲ARTICLES OF BOTANICAL ORIGIN,** *Pesticide Residue Analysis* ⟨561⟩▲ (CN 1-May-2019)**:** Meets the requirements

SPECIFIC TESTS

- **LIMIT OF NONIVAMIDE**

Analysis: Use the chromatograms and calculations obtained in the test for *Content of Total Capsaicinoids*.
Calculate the content of nonivamide as a percentage of total capsaicinoids:

$$\text{Result} = (PN/PTC) \times 100$$

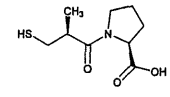
PN = percentage of nonivamide as calculated in the *Content of Total Capsaicinoids*
PTC = percentage of total capsaicinoids as calculated in the *Content of Total Capsaicinoids*

Acceptance criteria: NMT 5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.
- **LABELING:** The label states the official name of the article, the Latin binomial, and the part of the plant from which the article was prepared. Label it to indicate the content of capsaicinoids, the solvent mixture used for extraction, and the ratio of the starting crude plant material to Tincture.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Capsaicin RS
  USP Dihydrocapsaicin RS

Captopril



$C_9H_{15}NO_3S$ 217.29

L-Proline, 1-[(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-; 1-[(2*S*)-3-Mercapto-2-methylpropionyl]-L-proline [62571-86-2].

DEFINITION

Captopril contains NLT 97.5% and NMT 102.0% of captopril ($C_9H_{15}NO_3S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**

Sample solution: Dissolve about 300 mg of Captopril in 100 mL of water in a suitable glass-stoppered flask. Add 10 mL of 3.6 N sulfuric acid, 1 g of potassium iodide, and 2 mL of starch TS.

Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: Dissolve 3.567 g of potassium iodate, previously dried at 110° to constant weight, in water to make 1.0 L.
Endpoint detection: Visual

Analysis: Titrate with *Titrant* to a faint blue endpoint that persists for NLT 30 s. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 21.73 mg of captopril ($C_9H_{15}NO_3S$).

Acceptance criteria: 97.5%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Use low-actinic glassware to prepare the *Standard solution* and *Sample solution*.

www.webofpharma.com

Mobile phase: 9-in-100 solution of tetrahydrofuran in methanol and 1-in-2000 solution of phosphoric acid (33:67)
System suitability stock solution: 0.1 mg/mL each of USP Captopril RS, USP Captopril Disulfide RS, and 3-acetylthio-2-methylpropanoic acid in methanol
System suitability solution: 10 µg/mL each of USP Captopril RS, USP Captopril Disulfide RS, and 3-acetylthio-2-methylpropanoic acid in methanol from *System suitability stock solution*
Standard solution: 10 µg/mL of USP Captopril Disulfide RS in methanol
Sample solution: 2 mg/mL of Captopril in methanol. Use the solution promptly.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for captopril, 3-acetylthio-2-methylpropanoic acid, and captopril disulfide are 0.32, 0.42, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between captopril and 3-acetylthio-2-methylpropanoic acid
Analysis
Samples: *Standard solution* and *Sample solution*
Compare the peak responses from the *Sample solution*, excluding those of the solvent, captopril, and captopril disulfide, with the main peak response from the *Standard solution*.
Calculate the percentage of captopril disulfide in the portion of Captopril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of captopril disulfide from the *Sample solution*
r_S = peak response of captopril disulfide from the *Standard solution*
C_S = concentration of USP Captopril Disulfide RS in the *Standard solution* (µg/mL)
C_U = concentration of Captopril in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 1.0% of captopril disulfide. The peak response of each impurity does not exceed 40% of the main peak response from the *Standard solution* (0.2%), and the sum of the impurity peak responses does not exceed the main peak response from the *Standard solution* (0.5%).

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in dehydrated alcohol
Acceptance criteria: −125° to −134°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample under vacuum at 60° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Captopril RS
USP Captopril Disulfide RS
L-Proline, 1,1′-[dithiobis(2-methyl-1-oxo-3,1-propanediyl)]bis-[*S*-(*R**,*R**)]-.
$C_{18}H_{28}N_2O_6S_2$ 432.55

Captopril Compounded Oral Solution

DEFINITION
Captopril Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of captopril ($C_9H_{15}NO_3S$).
Prepare Captopril Compounded Oral Solution 0.75 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Captopril powder	75 mg
Vehicle for Oral Solution (regular or sugar-free), *NF*, a sufficient quantity to make	100 mL

Add *Captopril powder* and 10 mL of *Vehicle* to a mortar, and mix. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol and water (55:45) containing 0.5 mL/L of phosphoric acid. Filter, and degas.
Standard solution: 7.5 µg/mL of USP Captopril RS
Sample solution: Agitate the container of Oral Solution for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at −70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for captopril is about 5.0 min.]
Suitability requirements
Relative standard deviation: NMT 0.9% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of captopril ($C_9H_{15}NO_3S$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Captopril RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of captopril in the *Sample solution* (µg/mL)

USP Monographs

www.webofpharma.com

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.8–4.3

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store in a refrigerator.
- **Beyond-Use Date:** NMT 7 days after the date on which it was compounded when stored in a refrigerator
- **Labeling:** Label it to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
  USP Captopril RS

Captopril Compounded Oral Suspension

DEFINITION
Captopril Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of captopril ($C_9H_{15}NO_3S$).
Prepare Captopril Compounded Oral Suspension 0.75 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Captopril	75 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Place the required number of tablets in a suitable mortar and comminute to a fine powder, or use *Captopril* powder. Add 10 mL of *Vehicle*, and mix to form a uniform paste. Add the *Vehicle* in small portions, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY
- **Procedure**

Mobile phase: Methanol and water (55:45) containing 0.5 mL/L of phosphoric acid. Filter, and degas.
Standard solution: 7.5 µg/mL of USP Captopril RS
Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[Note—The retention time for captopril is about 5.0 min.]
Suitability requirements
Relative standard deviation: NMT 0.9% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of captopril ($C_9H_{15}NO_3S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Captopril RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of captopril in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.8–4.3

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store in a refrigerator.
- **Beyond-Use Date:** NMT 7 days after the date on which it was compounded when stored in a refrigerator
- **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
  USP Captopril RS

Captopril Tablets

DEFINITION
Captopril Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of captopril ($C_9H_{15}NO_3S$).

IDENTIFICATION

Delete the following:

▲• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Standard solution: 4 mg/mL in methanol
Sample solution: 4 mg/mL of captopril in methanol prepared as follows. Dissolve the equivalent to 100 mg of captopril from a portion of powdered Tablets, taken in a conical flask, in 25 mL of methanol. Stir for 30 min using a magnetic stirrer, and centrifuge. Use the clear supernatant.
Chromatographic system
Application volume: 50 µL as streaks
Developing solvent system: Toluene, methanol, and glacial acetic acid (75:1:25)
Spray reagent: Freshly prepared mixture of 1 volume of ammonium hydroxide and 6 volumes of a solution of 0.04% 5,5′-dithiobis(2-nitrobenzoic acid) in methanol
Analysis: Proceed as directed in the chapter. Locate the spots on the plate by lightly spraying with *Spray reagent.*
Acceptance criteria: Meet the requirements▲ 2S (USP41)

Add the following:

▲• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*▲ 2S (USP41)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*▲ 2S (USP41)

www.webofpharma.com

ASSAY

Change to read:

• **PROCEDURE**

Protect solutions from exposure to air and use within 8 h of preparation.

Mobile phase: 550 mL of methanol and 450 mL of water containing 0.50 mL of phosphoric acid

Standard solution: 1 mg/mL of USP Captopril RS and 0.05 mg/mL of USP Captopril Disulfide RS in *Mobile phase*

Sample solution: Nominally equivalent to 1 mg/mL of captopril prepared as follows. Transfer NLT 20 Tablets into a suitable volumetric flask and add *Mobile phase* to fill about 50% of the volume of the flask. Sonicate for 15 min. Dilute with *Mobile phase* to volume, shake by mechanical means for 15 min, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm. ▲For *Identification B*, use a diode array detector in the range of 190–400 nm.▲ 2S (USP41)

Column: 4.6-mm × 25-cm; ▲5-µm▲ 2S (USP41) packing L1▲ 2S (USP41)

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for captopril and captopril disulfide are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between captopril and captopril disulfide

Relative standard deviation: NMT 2.0% ▲for both the captopril and captopril disulfide peaks▲ 2S (USP41)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of captopril ($C_9H_{15}NO_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of captopril from the *Sample solution*

r_S = peak response of captopril from the *Standard solution*

C_S = concentration of USP Captopril RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of captopril in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

[NOTE—Completely deaerate the *Medium* to minimize exposure of captopril to air, and analyze the samples immediately.]

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 50 rpm

Time: 20 min

Standard solution: USP Captopril RS at a known concentration in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 205 nm

▲**Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of captopril ($C_9H_{15}NO_3S$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Captopril RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

D = dilution factor for the *Sample solution*▲ 2S (USP41)

Tolerances: NLT 80% (Q) of the labeled amount of captopril ($C_9H_{15}NO_3S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **LIMIT OF CAPTOPRIL DISULFIDE**

Protect solutions from exposure to air and use within 8 h of preparation.

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: Use the *Standard solution* prepared as directed in the *Assay*.

Standard solution: 0.05 mg/mL of USP Captopril Disulfide RS in *Mobile phase*

Sample solution: Nominally 1 mg/mL of captopril in *Mobile phase* prepared as follows. Transfer 25 mg of captopril from NLT 20 finely powdered Tablets into a suitable centrifuge tube. Add 25 mL of *Mobile phase*, sonicate for 15 min, and centrifuge. Use the clear supernatant.

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for captopril and captopril disulfide are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between captopril and captopril disulfide, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of captopril disulfide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of captopril disulfide from the *Sample solution*

r_S = peak response of captopril disulfide from the *Standard solution*

C_S = concentration of USP Captopril Disulfide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of captopril in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0%

www.webofpharma.com

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers. ▲Store at controlled room temperature.▲ 2S (USP41)

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Captopril RS
  USP Captopril Disulfide RS
  ▲(2′*S*)-[(2*S*,2′*S*)-3,3′-Disulfanediylbis(2-methylpropanoyl)]di-L-proline.▲ 2S (USP41)
  $C_{18}H_{28}N_2O_6S_2$ 432.55

Captopril and Hydrochlorothiazide Tablets

DEFINITION

Captopril and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount each of captopril ($C_9H_{15}NO_3S$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectra of the hydrochlorothiazide and captopril peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: Methanol, phosphoric acid, and water (250: 0.5: 750)

System suitability solution: 0.3 mg/mL each of USP Captopril RS, USP Hydrochlorothiazide RS, and USP Benzothiadiazine Related Compound A RS in *Mobile phase*

Standard solution: 0.3 mg/mL of USP Hydrochlorothiazide RS and about 0.3*J* mg/mL of USP Captopril RS in *Mobile phase*, *J* being the ratio of the labeled amount of captopril (mg) to the labeled amount of hydrochlorothiazide (mg) per Tablet

Sample solution: Nominally equivalent to 0.3 mg/mL of hydrochlorothiazide prepared as follows. Transfer 15 mg of hydrochlorothiazide from NLT 20 finely powdered Tablets into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. Sonicate for 15 min with occasional shaking, and centrifuge.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm. ▲For *Identification B*, use a diode array detector in the range of 190–400 nm.▲ (USP 1-Dec-2019)
Column: 4.6-mm × 30-cm; ▲10-µm▲ (USP 1-Dec-2019) packing L11
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for benzothiadiazine related compound A, hydrochlorothiazide, and captopril are 0.4, 0.5, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.7 between the void volume and benzothiadiazine related compound A; NLT 1.8 between benzothiadiazine related compound A and hydrochlorothiazide; and NLT 2.0 between captopril and hydrochlorothiazide, *System suitability solution*
Relative standard deviation: NMT 3.0% ▲for both the hydrochlorothiazide and captopril peaks,▲ (USP 1-Dec-2019) *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of captopril ($C_9H_{15}NO_3S$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of captopril or hydrochlorothiazide from the *Sample solution*
r_S = peak response of captopril or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Captopril RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of captopril or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 50 rpm
Times
Captopril: 20 min
Hydrochlorothiazide: 30 min
Standard solution: Known concentrations of USP Captopril RS and USP Hydrochlorothiazide RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.
Chromatographic system and **System suitability**▲▲ (USP 1-Dec-2019)**:** Proceed as directed in the *Assay*.

▲**Analysis**
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of captopril ($C_9H_{15}NO_3S$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times 100$$

r_U = peak response of captopril or hydrochlorothiazide from the *Sample solution*
r_S = peak response of captopril or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Captopril RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

USP Monographs

www.webofpharma.com

L = label claim of captopril or hydrochlorothiazide (mg/Tablet)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*▲ (USP 1-Dec-2019)

Tolerances: NLT 80% (Q) of the labeled amount of captopril ($C_9H_{15}NO_3S$) and NLT 60% (Q) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **LIMIT OF CAPTOPRIL DISULFIDE**
Mobile phase: Methanol, phosphoric acid, and water (450: 0.5: 550)
System suitability solution: 0.0075 mg/mL each of USP Captopril RS and USP Hydrochlorothiazide RS, and 0.015 mg/mL of USP Captopril Disulfide RS in *Mobile phase*
Standard solution: 0.015 mg/mL of USP Captopril Disulfide RS in *Mobile phase*
Sample solution: Nominally equivalent to 0.5 mg/mL of captopril prepared as follows. Transfer 25 mg of captopril from NLT 20 finely powdered Tablets into a 50-mL volumetric flask, add about 20 mL of *Mobile phase*, and sonicate for 15 min with occasional shaking. Dilute with *Mobile phase* to volume, and centrifuge. Use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: ▲4.6-mm × 30-cm; 10-µm▲ (USP 1-Dec-2019) packing L11
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for ▲hydrochlorothiazide,▲ (USP 1-Dec-2019) captopril, and captopril disulfide are ▲0.2,▲ (USP 1-Dec-2019) 0.3, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between captopril and captopril disulfide. Both peaks are resolved from hydrochlorothiazide, *System suitability solution*.
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of captopril disulfide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of captopril disulfide from the *Sample solution*
r_S = peak response of captopril disulfide from the *Standard solution*
C_S = concentration of USP Captopril Disulfide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of captopril in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0%

• **LIMIT OF BENZOTHIADIAZINE RELATED COMPOUND A**
Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 0.01 mg/mL of USP Benzothiadiazine Related Compound A RS in *Mobile phase*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzothiadiazine related compound A from the *Sample solution*
r_S = peak response of benzothiadiazine related compound A from the *Standard solution*
C_S = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

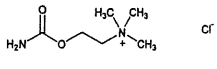
Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight containers. ▲Store at controlled room temperature.▲ (USP 1-Dec-2019)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73
USP Captopril RS
USP Captopril Disulfide RS
▲(2′*S*)-[(2*S*,2′*S*)-3,3′-Disulfanediylbis(2-methylpropanoyl)]di-L-proline.▲ (USP 1-Dec-2019)
$C_{18}H_{28}N_2O_6S_2$ 432.55
USP Hydrochlorothiazide RS

Carbachol



$C_6H_{15}ClN_2O_2$ 182.65
Ethanaminium, 2-[(aminocarbonyl)oxy]-*N*,*N*,*N*-trimethyl-, chloride;
Choline chloride, carbamate [51-83-2].

DEFINITION
Carbachol contains NLT 99.0% and NMT 101.0% of carbachol ($C_6H_{15}ClN_2O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*
Sample solution: Carbachol in water (1 in 20)
Acceptance criteria: Meets the requirements of test *A*

USP Monographs

www.webofpharma.com

ASSAY
• **PROCEDURE**
Sample: 150 mg of Carbachol
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric
Blank: 10 mL of glacial acetic acid TS and 40 mL of acetic anhydride
Analysis: Dissolve the *Sample* in a mixture of 10 mL of glacial acetic acid TS and 40 mL of acetic anhydride. Titrate with *Titrant*. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 18.27 mg of carbachol ($C_6H_{15}ClN_2O_2$).
Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **ORDINARY IMPURITIES** ⟨466⟩
Solvent: Methanol and water (80:20)
Eluant: Alcohol
Visualization: 17
Analysis
Samples: *Test Solution* and *Standard Solutions*
Proceed as directed in the chapter except use a thin-layer chromatographic plate coated with aluminum oxide.
Acceptance criteria: Meets the requirements

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 200°–204°, with some decomposition
• **LOSS ON DRYING** ⟨731⟩
Sample: 1 g of Carbachol
Analysis: Dry the *Sample* at 105° for 2 h.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Carbachol RS

Carbachol Intraocular Solution

DEFINITION
Carbachol Intraocular Solution is a sterile solution of Carbachol in an aqueous medium. It contains NLT 90.0% and NMT 115.0% of the labeled amount of carbachol ($C_6H_{15}ClN_2O_2$). It contains no preservatives or antimicrobial agents.

IDENTIFICATION
• **A.**
Solution A: A saturated (filtered) ammonium reineckate solution
Analysis: To 5 mL of Intraocular Solution add 4–5 drops of *Solution A*.
Acceptance criteria: A pink precipitate is formed that is soluble in acetone; the acetone solution is red.

ASSAY
• **PROCEDURE**
Solution A: Dilute 1 volume of sodium hypochlorite TS with water to 15 volumes, and allow to stand for 30 min.
Solution B: *Solution A* and 1 N sodium hydroxide (50:50). Prepare fresh daily.
Solution C: Phenol in water (1 in 200)
Solution D: Potassium iodide in water (3 in 1000)
Standard solution: 100 µg/mL of USP Carbachol RS in water
Sample solution: Nominally 100 µg/mL of carbachol from a volume of Intraocular Solution in water
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: Maximum absorbance at about 590 nm
Cell: 1 cm
Blank: Water
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Transfer 2.0-mL portions each of the *Standard solution*, *Sample solution*, and *Blank* to separate 50-mL conical flasks. To each flask add 1.0 mL of 0.1 N hydrochloric acid. Treat each as follows. Add 4.0 mL of *Solution B*, rinsing the inner walls of the flask with small portions of water, mix, and allow to stand for 15 min, accurately timed. Add 2.0 mL of *Solution C*, rinsing the walls of the flask with *Solution C* and with additional small portions of water. Mix, and allow to stand for 5 min. Add 2.0 mL of 3.5 N hydrochloric acid, washing the sides of the flask upon addition. Rinse the flask sparingly with 0.1 N hydrochloric acid to ensure complete acidification of all content, then mix. Add 1.0 mL of *Solution D*, and allow to stand for 5 min. Add 3.0 mL of starch TS, mix, and transfer the solutions to 50-mL volumetric flasks with the aid of several small portions of water, and dilute each solution with water to volume.
Concomitantly determine the absorbances of the solutions from the *Sample solution* and the *Standard solution* against the *Blank*.
Calculate the percentage of the labeled amount of carbachol ($C_6H_{15}ClN_2O_2$) in the portion of Intraocular Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Carbachol RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of carbachol in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
• **PH** ⟨791⟩**:** 5.0–7.5

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, at controlled room temperature, and protect from freezing.
• **LABELING:** Label it to indicate that it is for single-dose intraocular use only, and that the unused portion is to be discarded.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Carbachol RS

Carbachol Ophthalmic Solution

DEFINITION
Carbachol Ophthalmic Solution is a sterile solution of Carbachol in an isotonic, aqueous medium. It contains NLT 95.0% and NMT 105.0% of the labeled amount of carbachol ($C_6H_{15}ClN_2O_2$). It may contain suitable preservatives and antimicrobial agents.

www.webofpharma.com

IDENTIFICATION

• **A.**

Solution A: Ammonium reineckate in water (1 in 30)

Sample solution: 1 mg/mL of carbachol from Ophthalmic Solution in water

Analysis: Add 5 mL of *Solution A* to the *Sample solution*, and shake vigorously for 1 min.

Acceptance criteria: A red precipitate is formed; it is soluble in acetone.

ASSAY

• **PROCEDURE**

Solution A: Dilute 1 volume of sodium hypochlorite TS with water to 15 volumes, and allow to stand for 30 min.

Solution B: *Solution A* and 1 N sodium hydroxide (50:50). Prepare fresh daily.

Solution C: Phenol in water (1 in 200)

Solution D: Potassium iodide in water (3 in 1000)

Standard solution: 100 µg/mL of USP Carbachol RS in water

Sample solution: Nominally 100 µg/mL of carbachol from a volume of Ophthalmic Solution in water

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: Maximum absorbance at about 590 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Transfer 2.0-mL portions each of the *Standard solution*, *Sample solution*, and *Blank* to separate 50-mL conical flasks. To each flask add 1.0 mL of 0.1 N hydrochloric acid. Treat each as follows. Add 4.0 mL of *Solution B*, rinsing the inner walls of the flask with small portions of water, mix, and allow to stand for 15 min, accurately timed. Add 2.0 mL of *Solution C*, rinsing the walls of the flask with *Solution C* and with additional small portions of water. Mix, and allow to stand for 5 min. Add 2.0 mL of 3.5 N hydrochloric acid, washing the sides of the flask upon addition. Rinse the flask sparingly with 0.1 N hydrochloric acid to ensure complete acidification of all content, then mix. Add 1.0 mL of *Solution D*, and allow to stand for 5 min. Add 3.0 mL of starch TS, mix, and transfer the solutions to 50-mL volumetric flasks with the aid of several small portions of water, and dilute each solution with water to volume.

Concomitantly determine the absorbances of the solutions from the *Sample solution* and the *Standard solution* against the *Blank*.

Calculate the percentage of the labeled amount of carbachol ($C_6H_{15}ClN_2O_2$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Carbachol RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of carbachol in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩: Meets the requirements

• **PH** ⟨791⟩: 5.0–7.0

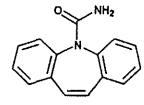
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Carbachol RS

Carbamazepine



$C_{15}H_{12}N_2O$ 236.27

5H-Dibenz[*b*,*f*]azepine, 5-carboxamide;
5H-Dibenz[*b*,*f*]azepine-5-carboxamide [298-46-4].

DEFINITION

Carbamazepine contains NLT 98.0% and NMT 102.0% of carbamazepine ($C_{15}H_{12}N_2O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Add 0.5 mL of triethylamine and 0.5 mL of formic acid to 1000 mL of water.

Solution B: Add 0.25 mL of formic acid to 1000 mL of methanol.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	80	20
3.0	80	20
12.0	60	40
18.0	45	55
20.0	45	55
20.1	80	20
23.0	80	20

Diluent: Methanol and water (50:50)

System suitability stock solution: 0.02 mg/mL each of USP Carbamazepine RS and USP Carbamazepine Related Compound A RS prepared as follows. First dissolve the Reference Standards in 50% of the final flask volume of methanol, then dilute with water to volume.

System suitability solution: 0.002 mg/mL each of USP Carbamazepine RS and USP Carbamazepine Related Compound A RS from *System suitability stock solution* in *Diluent*

Standard solution: 0.1 mg/mL of USP Carbamazepine RS prepared as follows. First dissolve the Reference Standard in 50% of the final flask volume of methanol, then dilute with water to volume.

Sample solution: 0.1 mg/mL of Carbamazepine prepared as follows. First dissolve the sample in 50% of the final flask volume of methanol, then dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 2.1-mm × 10-cm; 1.8-µm packing L10
Column temperature: 40°
Flow rate: 0.3 mL/min
Injection volume: 2 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.7 between carbamazepine related compound A and carbamazepine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carbamazepine ($C_{15}H_{12}N_2O$) in the portion of Carbamazepine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)
C_U = concentration of Carbamazepine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample solution: Boil 1.0 g of Carbamazepine in 20.0 mL of water for 10 min, cool, adjust the volume to 20 mL, and filter. Use a 10.0-mL portion of the filtrate.
Acceptance criteria: 0.014%; the *Sample solution* contains no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid.

• **RESIDUE ON IGNITION** ⟨281⟩
Sample: 2.0 g of Carbamazepine
Acceptance criteria: NMT 0.1%

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 0.02 mg/mL each of USP Carbamazepine RS, USP Carbamazepine Related Compound A RS, and USP Carbamazepine Related Compound B RS, prepared as follows. First dissolve the Reference Standards in 50% of the final flask volume of methanol, then dilute with water to volume.
Standard solution: 0.002 mg/mL each of USP Carbamazepine RS, USP Carbamazepine Related Compound A RS, and USP Carbamazepine Related Compound B RS from *Standard stock solution* in *Diluent*
Sample solution: 1.0 mg/mL of Carbamazepine prepared as follows. First dissolve the sample in 50% of the final flask volume of methanol, then dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.7 between carbamazepine related compound A and carbamazepine
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Carbamazepine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified impurity from the *Sample solution*
r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = concentration of Carbamazepine in the *Sample solution* (mg/mL)

Calculate the percentage of each individual unspecified impurity in the portion of Carbamazepine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of carbamazepine from the *Standard solution*
C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)
C_U = concentration of Carbamazepine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carbamazepine related compound A[a]	0.96	0.2
Carbamazepine	1.00	—
Carbamazepine related compound B[b]	1.45	0.2
Individual unspecified impurity	—	0.2
Total impurities	—	0.5

[a] 10,11-Dihydrocarbamazepine.
[b] 5*H*-Dibenz[*b*,*f*]azepine.

SPECIFIC TESTS

• **ACIDITY**
Sample solution: 50 mg/mL of Carbamazepine in water prepared as follows. Mix 2.0 g of Carbamazepine in 40.0 mL of water for 15 min, and filter through paper.
Analysis: To a 10.0-mL aliquot of *Sample solution* add 1 drop of phenolphthalein TS, and titrate with 0.01 N sodium hydroxide VS. Perform a blank determination, and make any necessary correction.
Acceptance criteria: NMT 1.0 mL of 0.01 N sodium hydroxide VS is required for each 1.0 g of Carbamazepine.

• **ALKALINITY**
Sample solution: 50 mg/mL of Carbamazepine in water prepared as follows. Mix 2.0 g of Carbamazepine in 40.0 mL of water for 15 min, and filter through paper.
Analysis: To a 10.0-mL aliquot of *Sample solution* add 1 drop of methyl red TS, and titrate with 0.01 N hydrochloric acid VS. Perform a blank determination, and make any necessary correction.
Acceptance criteria: NMT 1.0 mL of 0.01 N hydrochloric acid VS is required for each 1.0 g of Carbamazepine.

www.webofpharma.com

- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 0.5%
- **X-RAY DIFFRACTION** ⟨941⟩**:** The X-ray diffraction pattern conforms to that of USP Carbamazepine RS, similarly determined.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Carbamazepine RS
  USP Carbamazepine Related Compound A RS
  10,11-Dihydrocarbamazepine.
  $C_{15}H_{14}N_2O$ 238.28
  USP Carbamazepine Related Compound B RS
  5*H*-Dibenz[*b*,*f*]azepine.
  $C_{14}H_{11}N$ 193.24

Carbamazepine Oral Suspension

DEFINITION
Carbamazepine Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197S▲ (CN 1-May-2020)
  Sample solution: Place 5 mL of Oral Suspension in a separator containing 20 mL of 0.1 N sodium hydroxide, and extract with 25 mL of chloroform. Pass the extract through anhydrous sodium sulfate supported on filter paper into a beaker. Wash the anhydrous sodium sulfate with 10 mL of chloroform, and add the washing to the extract. Evaporate the chloroform extract to dryness with the aid of a stream of nitrogen. Dissolve the residue in 10 mL of methylene chloride.
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: Add 0.5 mL of triethylamine and 0.5 mL of formic acid to 1000 mL of water.
  Solution B: Add 0.25 mL of formic acid to 1000 mL of methanol.
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	80	20
3.0	80	20
12.0	60	40
18.0	45	55
20.0	45	55
20.1	80	20
23.0	80	20

Diluent: Methanol
System suitability solution: 0.002 mg/mL each of USP Carbamazepine RS and USP Carbamazepine Related Compound A RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Carbamazepine RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of carbamazepine from a volume of Oral Suspension prepared as follows. Weigh and transfer freshly mixed Oral Suspension equivalent to 20 mg of carbamazepine to a 200-mL volumetric flask. Add about 140 mL of *Diluent*, shake by mechanical means for about 30 min, sonicate for about 2 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.2-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 2.1-mm × 10-cm; 1.8-µm packing L10
Column temperature: 40°
Flow rate: 0.3 mL/min
Injection volume: 2 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.7 between carbamazepine related compound A and carbamazepine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements for oral suspension packaged in single-unit containers
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements for oral suspension packaged in multiple-unit containers

IMPURITIES
- **ORGANIC IMPURITIES**
  Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
  Standard solution: 0.002 mg/mL each of USP Carbamazepine RS, USP Carbamazepine Related Compound A RS, and USP Carbamazepine Related Compound B RS in *Diluent*
  Sample solution: Nominally 1.0 mg/mL of carbamazepine from a volume of Oral Suspension prepared as follows. Weigh and transfer freshly mixed Oral Suspension equivalent to 50 mg of carbamazepine to a 50-mL volumetric flask. Add about 35 mL of *Diluent*, shake by mechanical means for about 30 min, sonicate for about 2

www.webofpharma.com

min, dilute with *Diluent* to volume, and shake for about 5 min. Pass through a suitable filter of 0.2-µm pore size.

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.7 between carbamazepine related compound A and carbamazepine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of carbamazepine related compound B in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carbamazepine related compound B from the *Sample solution*

r_S = peak response of carbamazepine related compound B from the *Standard solution*

C_S = concentration of USP Carbamazepine Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

Calculate the percentage of each individual unspecified impurity in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response of carbamazepine from the *Standard solution*

C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carbamazepine related compound A[a,b]	0.96	—
Carbamazepine	1.00	—
Carbamazepine related compound B[c]	1.45	0.2
Individual unspecified impurity	—	0.2
Total impurities	—	0.5

[a] 10,11-Dihydrocarbamazepine.

[b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

[c] 5*H*-Dibenz[*b*,*f*]azepine.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total bacterial count does not exceed 1×10^2 cfu/g, and the tests for *Salmonella* species and *Escherichia coli* are negative.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, protected from freezing and from excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Carbamazepine RS

USP Carbamazepine Related Compound A RS
10,11-Dihydrocarbamazepine.
$C_{15}H_{14}N_2O$ 238.28

USP Carbamazepine Related Compound B RS
5*H*-Dibenz[*b*,*f*]azepine.
$C_{14}H_{11}N$ 193.24

Carbamazepine Tablets

DEFINITION

Carbamazepine Tablets contain NLT 92.0% and NMT 108.0% of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Sample solution: Nominally 250 mg of carbamazepine from powdered Tablets in 15 mL of acetone

Analysis: Boil the *Sample solution* for 5 min in a suitable beaker. Filter while hot, using two 5-mL portions of hot acetone to effect transfer. Evaporate the filtrate with the aid of nitrogen to 5 mL, and cool in an ice bath until crystals are formed. Filter the crystals, wash with 3 mL of cold acetone, and dry under vacuum at 70° for 30 min. Use the crystals.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, tetrahydrofuran, and water (12:3:85). Add 0.22 mL of formic acid and then 0.5 mL of triethylamine to each L.

Diluent: Methanol and water (50:50)

System suitability stock solution: 0.1 mg/mL of USP Carbamazepine RS and 0.5 mg/mL of USP Carbamazepine Related Compound A RS in methanol. Sonication may be used to aid in dissolution.

System suitability solution: 0.01 mg/mL of USP Carbamazepine RS and 0.05 mg/mL of USP Carbamazepine Related Compound A RS from *System suitability stock solution* in *Diluent*

Standard stock solution: 2 mg/mL of USP Carbamazepine RS in methanol. Sonication may be used to aid in dissolution.

Standard solution: 0.2 mg/mL of USP Carbamazepine RS from *Standard stock solution* in *Diluent*

Sample stock solution: Nominally 2 mg/mL of carbamazepine from NLT 20 Tablets prepared as follows. Finely powder the Tablets, and transfer a portion of the powder to a suitable volumetric flask. Add 80% of the final flask volume of methanol, sonicate for 15 min, and allow to cool to room temperature. Dilute with methanol to volume. Pass through a suitable filter and discard the first few mL of filtrate.

www.webofpharma.com

Sample solution: Nominally 0.2 mg/mL of carbamazepine from *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.0- or 4.6-mm × 25-cm; 7-µm packing L10
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: NLT 1.6 times the retention time of carbamazepine
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.7 between carbamazepine related compound A and carbamazepine, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

Acceptance criteria: 92.0%–108.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
For products labeled as 100-mg chewable Tablets
Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.
Medium: Water containing 1% sodium lauryl sulfate; 900 mL
Apparatus 2: 75 rpm
Time: 60 min
Standard solution: USP Carbamazepine RS in *Medium*. [NOTE—A volume of methanol NMT 1% of the final total volume of the *Standard solution* may be used to dissolve the carbamazepine.]
Sample solution: Filtered portion of the solution under test, diluted with *Medium* if necessary
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 288 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$) is dissolved. Use *Dissolution* ⟨711⟩, *Acceptance Table 1* with the following exceptions: at S_2, no unit is less than Q – 5%; at S_3, no unit is less than Q – 10%; and NMT 2 of the 24 units are less than Q – 5%.
For products labeled as 200-mg Tablets
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium, Apparatus 2, Standard solution, Sample solution, and **Instrumental conditions:** Proceed as directed in *Test 1*.
Times: 15 and 60 min
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of carbamazepine ($C_{15}H_{12}N_2O$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result} = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V_2) + (C_1 \times V_S)] \times (1/L) \times 100$$

C_i = concentration of carbamazepine in the portion of sample withdrawn at the specified time point (i) (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)
V_2 = volume of the *Medium* at time point 2 (mL)
V_S = volume of the *Sample solution* withdrawn at each time point (i) (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (*i*)	Time (min)	Amount Dissolved (%)
1	15	45–75
2	60	NLT 75

Use *Dissolution* ⟨711⟩, *Acceptance Table 2* with the following exceptions. At 15 min: at L_2, no unit is more than 5% outside the stated range; at L_3, no unit is more than 10% outside the stated range; and NMT 2 of the 24 units are more than 5% outside the stated range. At 60 min: at L_2, no unit is less than Q – 5%; at L_3, no unit is less than Q – 10%; and NMT 2 of the 24 units are less than Q – 5%.
Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium, Apparatus 2, Standard solution, Sample solution, and **Instrumental conditions:** Proceed as indicated in *Test 1*.
Times: 15 and 60 min
Analysis
Samples: *Standard solution* and *Sample solution*

Stimuli Pharmacopeial

www.webofpharma.com

Calculate the concentration (C_i) of carbamazepine ($C_{15}H_{12}N_2O$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V_2) + (C_i \times V_S)] \times (1/L) \times 100$$

C_i = concentration of carbamazepine in the portion of sample withdrawn at the specified time point (*i*) (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)
V_2 = volume of the *Medium* at time point 2 (mL)
V_S = volume of the *Sample solution* withdrawn at each time point (*i*) (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (min)	Amount Dissolved (%)
1	15	60–85
2	60	NLT 75

Use *Dissolution* ⟨711⟩, *Acceptance Table 2* with the following exceptions. At 15 min: at L_2, no unit is more than 5% outside the stated range; at L_3, no unit is more than 10% outside the stated range; and NMT 2 of the 24 units are more than 5% outside the stated range. At 60 min: at L_2, no unit is less than Q – 5%; at L_3, no unit is less than Q – 10%; and NMT 2 of the 24 units are less than Q – 5%.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, and **System suitability solution:** Proceed as directed in the *Assay*.

Standard stock solution: 0.02 mg/mL each of USP Carbamazepine RS, USP Carbamazepine Related Compound B RS, and USP 9-Methylacridine RS in methanol. Sonication may be used to aid in dissolution.

Standard solution: 0.001 mg/mL each of USP Carbamazepine RS, USP Carbamazepine Related Compound B RS, and USP 9-Methylacridine RS from *Standard stock solution* in *Diluent*

Sample solution: Nominally 1 mg/mL of carbamazepine from NLT 20 Tablets prepared as follows. Finely powder the Tablets, and transfer a portion of the powder to a suitable volumetric flask. Add about 50% of the final flask volume of *Diluent*, sonicate for 15 min, and allow to cool to room temperature. Dilute with *Diluent* to volume. Pass through a suitable filter and discard the first few mL of filtrate.

Chromatographic system: Proceed as directed in the *Assay* except use a *Run time* of NLT 3.5 times the retention time of carbamazepine.

System suitability

Samples: *System suitability solution* and *Standard solution* [NOTE—See *Table 3* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.7 between carbamazepine related compound A and carbamazepine, *System suitability solution*

Relative standard deviation: NMT 10.0% each for carbamazepine, carbamazepine related compound B, and 9-methylacridine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of carbamazepine related compound B and 9-methylacridine in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carbamazepine related compound B or 9-methylacridine from the *Sample solution*
r_S = peak response of carbamazepine related compound B or 9-methylacridine from the *Standard solution*
C_S = concentration of USP Carbamazepine Related Compound B RS or USP 9-Methylacridine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

Calculate the percentage of other degradation products in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified degradation product from the *Sample solution*
r_S = peak response of carbamazepine from the *Standard solution*
C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard peaks below 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
9-Methylacridine	0.54	0.2
Carbamazepine related compound A[a]	0.87	—
Carbamazepine	1.0	—
Carbamazepine related compound B	3.1	0.2
Any individual unspecified degradation product	—	0.2
Total degradation products	—	0.30

[a] This is a process impurity that is controlled in the drug substance. It is not to be reported or included in the total degradation products.

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, preferably of glass. Protect from light and moisture. Store at controlled room temperature.
- **LABELING:** The labeling indicates the *Dissolution* test with which the product complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Carbamazepine RS
  USP Carbamazepine Related Compound A RS
  10,11-Dihydrocarbamazepine.
  $C_{15}H_{14}N_2O$ 238.28
  USP Carbamazepine Related Compound B RS
  5*H*-Dibenz[*b*,*f*]azepine.
  $C_{14}H_{11}N$ 193.24
  USP 9-Methylacridine RS
  9-Methylacridine.
  $C_{14}H_{11}N$ 193.24

Carbamazepine Extended-Release Tablets

DEFINITION

Carbamazepine Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Standard solution: 10 µg/mL of USP Carbamazepine RS in methanol
  Sample solution: Finely powder 1 Tablet, and quantitatively transfer the powder, with the aid of methanol, to a 100-mL volumetric flask. Add about 70 mL of methanol, and shake by mechanical means for 60 min. Sonicate for 15 min, and dilute with methanol to volume. Allow to stand for 10–15 min. Dilute a portion of the clear solution with methanol to obtain a solution containing about 10 µg/mL of carbamazepine.
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: Methanol, methylene chloride, and water (450:45:600)
  Internal standard solution: 600 µg/mL of phenytoin in methanol
  Standard stock solution: 200 µg/mL of USP Carbamazepine RS in methanol
  Standard solution: 100 µg/mL of carbamazepine from *Standard stock solution* in *Internal standard solution*
  System suitability solution: 50 µg/mL of carbamazepine from *Standard solution* in *Internal standard solution*
  Sample stock solution A: Nominally 4 mg/mL of carbamazepine from finely powdered Tablets prepared as follows. Finely powder 10 Tablets. Transfer the powder to an appropriate volumetric flask with the aid of methanol. Add 70% of the flask volume of methanol. Shake by mechanical means for 60 min. Sonicate for 15 min, and dilute with methanol to volume. Allow to stand for 10–15 min, and then filter a portion of the supernatant. Use the clear filtrate.
  Sample stock solution B: Nominally 0.2 mg/mL of carbamazepine from *Sample stock solution A* in methanol
  Sample solution: Nominally 100 µg/mL of carbamazepine from *Sample stock solution B* in *Internal standard solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 230 nm
  Columns
  Guard: 4.6-mm × 30-mm; 7-µm packing L7
  Analytical: 3.9-mm × 30-cm; packing L1
  Flow rate: 2 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for phenytoin and carbamazepine are about 0.8 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2.8 between phenytoin and carbamazepine
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of carbamazepine to the internal standard from the *Sample solution*
R_S = peak response ratio of carbamazepine to the internal standard from the *Standard solution*
C_S = concentration of USP Carbamazepine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of carbamazepine in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Medium
  For Tablets labeled to contain 100 mg or 200 mg: Water; 900 mL
  For Tablets labeled to contain 400 mg: Water; 1800 mL
  Apparatus 1: 100 rpm
  Times: 3, 6, 12, and 24 h
  Standard solution: USP Carbamazepine RS in *Medium*
  Sample solution: Filtered portions of the solution under test, diluted with *Medium* if necessary
  Instrumental conditions
  Mode: UV
  Analytical wavelength: The wavelength of maximum absorbance at about 284 nm
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Determine the percentage of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$) dissolved at each time using the UV absorption.
  Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved
3	10%–35%

www.webofpharma.com

Table 1 *(continued)*

Time (h)	Amount Dissolved
6	35%–65%
12	65%–90%
24	NLT 75%

The percentages (*Q*) of the labeled amount of carbamazepine ($C_{15}H_{12}N_2O$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES: PROCEDURE 1**

Mobile phase: Methanol, methylene chloride, and water (450:45:600)

System suitability solution: 60 µg/mL of phenytoin and 20 µg/mL of USP Carbamazepine RS in methanol

Standard solution: 4 µg/mL of USP Carbamazepine RS in methanol

Sample solution: Use *Sample stock solution A* from the *Assay*.

Chromatographic system and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of carbamazepine from the *Standard solution*

C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

Acceptance criteria

Any individual unspecified degradation product: NMT 0.2%

- **ORGANIC IMPURITIES: PROCEDURE 2**

Mobile phase: Methanol, acetonitrile, and water (35:15:50)

System suitability solution: 12.5 µg/mL of iminostilbene and 5.0 µg/mL of USP Carbamazepine RS in methanol

Standard solution: 4 µg/mL of USP Carbamazepine RS in methanol

Sample solution: Use *Sample stock solution A* from the *Assay*.

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for carbamazepine and iminostilbene are about 0.3 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 10.0 between carbamazepine and iminostilbene

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of carbamazepine from the *Standard solution*

C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

Acceptance criteria

Any individual unspecified degradation product: NMT 0.2%

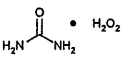
Total Impurities: NMT 0.5% for all impurities from *Procedure 1* and *Procedure 2*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Carbamazepine RS

Carbamide Peroxide



$CH_6N_2O_3$ 94.07

Urea, compd. with hydrogen peroxide (1:1).

Urea compound with hydrogen peroxide (1:1) [124-43-6].

» Carbamide Peroxide contains not less than 96.0 percent and not more than 102.0 percent of $CH_6N_2O_3$.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

Identification—

A: Mix 1 mL of a solution (1 in 10) of it with 1 mL of nitric acid: a white, crystalline precipitate is formed.

B: A solution of it (1 in 10) responds to the tests for *Peroxide* ⟨191⟩.

Assay—Transfer about 100 mg of Carbamide Peroxide, accurately weighed, to a 500-mL iodine flask with the aid of 25 mL of water, add 5 mL of glacial acetic acid, and mix. Add 2 g of potassium iodide and 1 drop of ammonium molybdate TS, insert the stopper, and allow to stand in the dark for 10 minutes. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 4.704 mg of $CH_6N_2O_3$.

Carbamide Peroxide Topical Solution

» Carbamide Peroxide Topical Solution is a solution in anhydrous glycerin of Carbamide Peroxide or of carbamide peroxide prepared from hydrogen peroxide and Urea. It contains not less than 78.0

www.webofpharma.com

percent and not more than 110.0 percent, by weight, of the labeled amount of $CH_6N_2O_3$.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

Identification—
A: Mix 1 mL with 1 mL of nitric acid: a white, crystalline precipitate is formed.
B: It responds to the tests for *Peroxide* ⟨191⟩.
Specific gravity ⟨841⟩: between 1.245 and 1.272.
pH ⟨791⟩: between 4.0 and 7.5.
Assay—Transfer an accurately weighed quantity of Topical Solution, equivalent to about 100 mg of carbamide peroxide, to a 500-mL iodine flask with the aid of 25 mL of water, add 5 mL of glacial acetic acid, and mix. Add 2 g of potassium iodide and 1 drop of ammonium molybdate TS, and allow to stand in the dark for 10 minutes. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 4.704 mg of $CH_6N_2O_3$.

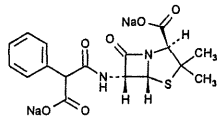
Carbenicillin for Injection

» Carbenicillin for Injection contains an amount of Carbenicillin Disodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of carbenicillin ($C_{17}H_{18}N_2O_6S$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Carbenicillin Monosodium Monohydrate RS
Identification—It responds to the tests for *Sodium* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.05 USP Endotoxin Unit per mg of carbenicillin.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 6 g being aseptically dissolved in 200 mL of *Fluid A.*
pH ⟨791⟩: between 6.5 and 8.0, in the solution constituted as directed in the labeling.
Water Determination, *Method I* ⟨921⟩: not more than 6.0%.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions* and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
Assay preparation 1 (where it is packaged for dispensing and where the package is represented as being a single-dose container)—Constitute Carbenicillin for Injection as directed in the labeling. Withdraw all of the withdrawable contents, and dilute quantitatively with *Buffer B.1* to obtain a solution having a convenient concentration of carbenicillin.
Assay preparation 2 (where the label states the quantity of carbenicillin in a given volume of constituted solution)—Constitute Carbenicillin for Injection as directed in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Buffer B.1* to obtain a solution having a convenient concentration of carbenicillin.
Procedure—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer B.1* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Carbenicillin Disodium



$C_{17}H_{16}N_2Na_2O_6S$ (anhydrous) 422.36
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[(carboxyphenylacetyl)amino]-3,3-dimethyl-7-oxo-, disodium salt, [2*S*-(2α,5α,6β)]-.
N-(2-Carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo. [3.2.0]- hept-6-yl)- 2-phenylmalonamic acid disodium salt [4800-94-6].

» Carbenicillin Disodium has a potency equivalent to not less than 770 µg of carbenicillin ($C_{17}H_{18}N_2O_6S$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Carbenicillin Monosodium Monohydrate RS
Identification—It responds to the tests for *Sodium* ⟨191⟩.
pH ⟨791⟩: between 6.5 and 8.0, in a solution containing 10 mg of carbenicillin per mL.
Water Determination, *Method I* ⟨921⟩: not more than 6.0%.
Other requirements—Where the label states that Carbenicillin Disodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Carbenicillin for Injection* Where the label states that Carbenicillin Disodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Carbenicillin for Injection*

Assay—
Assay preparation—Dissolve a suitable quantity of Carbenicillin Disodium, accurately weighed, in *Buffer B.1*, and dilute quantitatively with *Buffer B.1* to obtain a solution having a convenient concentration of carbenicillin.
Procedure—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer B.1* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Carbenicillin Indanyl Sodium

$C_{26}H_{25}N_2NaO_6S$ 516.54

www.webofpharma.com

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[3-[(2,3-dihydro-1*H*-inden-5-yl)oxy]-1,3-dioxo-2-phenylpropyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, [2*S*-(2α,5α,6β)]-.
1-(5-Indanyl)(2*S*,5*R*,6*R*)-*N*-(2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-6-yl)-2-phenylmalonamate monosodium salt [26605-69-6].

» Carbenicillin Indanyl Sodium has a potency equivalent to not less than 630 µg and not more than 769 µg of carbenicillin ($C_{17}H_{18}N_2O_6S$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers. For periods up to 18 months, store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Carbenicillin Indanyl Sodium RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: It responds to the tests for *Sodium* ⟨191⟩.
pH ⟨791⟩: between 5.0 and 8.0, in a solution containing 100 mg per mL.
Water Determination, *Method I* ⟨921⟩: not more than 2.0%.

Assay—
Mobile phase—Prepare a buffer of 0.0009 M tetrabutylammonium hydrogen phosphate and 0.05 M dibasic sodium phosphate as follows. Dissolve 604 mg of tetrabutylammonium phosphate and 26.8 g of dibasic sodium phosphate in 1800 mL of water, adjust with phosphoric acid to a pH of 3.8, and dilute with water to 2000 mL. Prepare a filtered and degassed mixture of this buffer and acetonitrile (116:84), allow to stand for 1 hour, and if necessary readjust with phosphoric acid to a pH of 3.8. Make any necessary adjustments (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluting solvent—Prepare a mixture of acetonitrile and 0.005 M monobasic potassium phosphate (85:15).
Standard preparation—Dissolve an accurately weighed quantity of USP Carbenicillin Indanyl Sodium RS quantitatively in *Diluting solvent* to obtain a solution having a known concentration of about 250 µg per mL. This *Standard preparation* contains the equivalent of about 222 µg of carbenicillin ($C_{17}H_{18}N_2O_6S$) per mL.
Assay preparation—Transfer about 125 mg of Carbenicillin Indanyl Sodium, accurately weighed, to a 50-mL volumetric flask, dissolve in *Diluting solvent* with the aid of sonication, dilute with *Diluting solvent* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluting* solvent to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the responses for the major peaks. Calculate the quantity, in µg, of carbenicillin ($C_{17}H_{18}N_2O_6S$) in each mg of the Carbenicillin Indanyl Sodium taken by the formula:

$$0.5(CP/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Carbenicillin Indanyl Sodium RS, calculated on the anhydrous basis, in the *Standard preparation*; P is the assigned potency, in µg per mg, of USP Carbenicillin Indanyl Sodium RS; W is the quantity, in mg, of Carbenicillin Indanyl Sodium taken to prepare the *Assay preparation*; and r_U and r_S are the carbenicillin indanyl peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Carbenicillin Indanyl Sodium Tablets

» Carbenicillin Indanyl Sodium Tablets contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of carbenicillin ($C_{17}H_{18}N_2O_6S$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Carbenicillin Indanyl Sodium RS
Identification—Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of carbenicillin, with 10 mL of a solvent mixture consisting of acetone, ethyl acetate, water, pyridine, and glacial acetic acid (200: 100: 75: 25: 1.5). Shake the mixture for 5 minutes, and dilute 1 volume of it with 9 volumes of the solvent mixture. Apply 10 µL each of this solution and of a solution of USP Carbenicillin Indanyl Sodium RS in the same solvent mixture containing 1 mg of carbenicillin per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of acetone, ethyl acetate, water, pyridine, and glacial acetic acid (400:300:75:25:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and heat the plate at 80° for 30 minutes. Allow the plate to cool, and expose it to iodine vapors in a closed chamber for about 30 seconds. Spray the plate with a reagent consisting of a 1 in 100 solution of ferric chloride in 0.1 N hydrochloric acid, potassium ferricyanide solution (1 in 100), and methanol (4:4:3): the principal spots from the test solution and the Standard solution are blue on a yellow-green background, and the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution (R_F about 0.5).

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Procedure—Determine the amount of carbenicillin ($C_{17}H_{18}N_2O_6S$) equivalent dissolved from the difference between UV absorbances at the wavelengths of maximum and minimum absorbance at about 267 nm and 254 nm, respectively, of filtered portions of the solution under test, suitably diluted with water, in comparison with a Standard solution having a known concentration of USP Carbenicillin Indanyl Sodium RS in the same medium.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{17}H_{18}N_2O_6S$ equivalent is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 2.0%.

www.webofpharma.com

Assay—

Mobile phase , *Diluting solvent*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Carbenicillin Indanyl Sodium*.

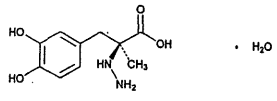
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 111 mg of carbenicillin ($C_{17}H_{18}N_2O_6S$), to a 50-mL volumetric flask, dissolve in *Diluting solvent* with the aid of sonication, dilute with *Diluting solvent* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Carbenicillin Indanyl Sodium*. Calculate the quantity, in mg, of carbenicillin ($C_{17}H_{18}N_2O_6S$) in the portion of Tablets taken by the formula:

$$0.5CP(r_U/r_S)$$

in which the terms are as defined therein.

Carbidopa



$C_{10}H_{14}N_2O_4 \cdot H_2O$ 244.24

$C_{10}H_{14}N_2O_4$ 226.23

Benzenepropanoic acid, α-hydrazino-3,4-dihydroxy-α-methyl-, monohydrate, (*S*)-;
(–)-L-α-Hydrazino-3,4-dihydroxy-α-methylhydrocinnamic acid monohydrate [38821-49-7].
Anhydrous [28860-95-9].

DEFINITION

Carbidopa contains NLT 98.0% and NMT 102.0% of carbidopa ($C_{10}H_{14}N_2O_4 \cdot H_2O$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.05 M monobasic sodium phosphate, adjusted with phosphoric acid to a pH of 2.7
Mobile phase: Alcohol and *Buffer* (5:95)
System suitability solution: 0.1 mg/mL of USP Carbidopa RS and 0.1 mg/mL of USP Methyldopa RS in *Mobile phase*
Standard solution: 0.5 mg/mL of USP Carbidopa RS in *Mobile phase*. [NOTE—Use gentle heat and ultrasonification, if necessary, to dissolve.]
Sample solution: 0.5 mg/mL of Carbidopa in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for methyldopa and carbidopa are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 0.9 between methyldopa and carbidopa, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, in mg/mL, of carbidopa ($C_{10}H_{14}N_2O_4 \cdot H_2O$) in the *Standard solution* (C_S):

$$\text{Result} = C_{S2} \times (M_{r1}/M_{r2})$$

C_{S2} = concentration of USP Carbidopa RS, as determined using the value on the USP Reference Standard label, in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of carbidopa monohydrate, 244.24
M_{r2} = molecular weight of anhydrous carbidopa, 226.23

Calculate the percentage of carbidopa ($C_{10}H_{14}N_2O_4 \cdot H_2O$) in the portion of Carbidopa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of carbidopa ($C_{10}H_{14}N_2O_4 \cdot H_2O$) in the *Standard solution* (mg/mL)
C_U = concentration of Carbidopa in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF METHYLDOPA AND CARBIDOPA RELATED COMPOUND A**

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Impurity standard solution: 2.5 µg/mL of USP Methyldopa RS and 2.5 µg/mL of USP Carbidopa RS in *Mobile phase*
Analysis
Samples: *Sample solution* and *Impurity standard solution*
[NOTE—The relative retention times for methyldopa, carbidopa, and carbidopa related compound A are about 0.8, 1.0, and 1.8, respectively.]
Calculate the percentage of methyldopa in the portion of Carbidopa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methyldopa from the *Sample solution*
r_S = peak response of methyldopa from the *Impurity standard solution*
C_S = concentration of USP Methyldopa RS in the *Impurity standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

Calculate the percentage of carbidopa related compound A in the portion of Carbidopa taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carbidopa related compound A from the *Sample solution*
r_S = peak response of carbidopa from the *Impurity standard solution*
C_S = concentration of USP Carbidopa RS in the *Impurity standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.5% of methyldopa and NMT 0.5% of carbidopa related compound A

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in 0.7 g/mL of aluminum chloride solution (prepared using the hexahydrate form of the aluminum salt) that has been filtered and adjusted with 0.25 N sodium hydroxide to a pH of 1.5
Acceptance criteria: −21.0° to −23.5° calculated as the monohydrate

• **LOSS ON DRYING** ⟨731⟩
Analysis: Heat 1 g in a suitable vacuum drying apparatus at 100° and a pressure of NMT 5 mm of mercury to constant weight. Cool, and weigh.
Acceptance criteria: 6.9%–7.9%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Carbidopa RS
USP Methyldopa RS

Carbidopa and Levodopa Tablets

DEFINITION

Carbidopa and Levodopa Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$).

IDENTIFICATION

• **A.** The UV spectra of the carbidopa and levodopa peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

• **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**
Protect solutions containing carbidopa or levodopa from light and maintain them at 2°–8° until they are injected. Use within 12 h.
Buffer: 6 g/L of anhydrous monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.2
Mobile phase: Alcohol and *Buffer* (5:95)
Standard solution: 125 µg/mL of USP Carbidopa RS and 125 µg/mL of USP Levodopa RS in *Mobile phase*
System suitability solution: 2.5 µg/mL of USP Levodopa Related Compound B RS in *Standard solution*
Sample solution A: Nominally 125 µg/mL of carbidopa from Tablets prepared as follows. Transfer a suitable portion of powder from NLT 10 Tablets to an appropriate volumetric flask. Add 80% of the total flask volume of *Mobile phase*. Sonicate for 15 min with intermittent shaking. Dilute with *Mobile phase*. Centrifuge the resulting solution and use the supernatant. [NOTE—The use of a centrifuge speed of 3000 rpm for 5 min may be suitable.]
Sample solution B: Nominally 125 µg/mL of levodopa from *Sample solution A* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. For ▲*Identification A*,▲ (ERR 1-Jun-2018) use a diode array detector in the range of 210–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Autosampler temperature: 5°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 5 times the retention time of carbidopa
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between levodopa related compound B and carbidopa, *System suitability solution*
Tailing factor: NMT 2.0 each for levodopa and carbidopa, *Standard solution*
Relative standard deviation: NMT 1.0% each for levodopa and carbidopa, *Standard solution*
Analysis
Samples: *Standard solution*, *Sample solution A*, and *Sample solution B*
Calculate the percentage of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carbidopa from *Sample solution A*
r_S = peak response of carbidopa from the *Standard solution*
C_S = concentration of USP Carbidopa RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of carbidopa in *Sample solution A* (µg/mL)

Calculate the percentage of the labeled amount of levodopa ($C_9H_{11}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levodopa from *Sample solution B*
r_S = peak response of levodopa from the *Standard solution*
C_S = concentration of USP Levodopa RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of levodopa in *Sample solution B* (µg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of carbidopa; 90.0%–110.0% of the labeled amount of levodopa

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 750 mL
Apparatus 1: 50 rpm
Time: 30 min
Diluent: 0.24 g/L of sodium 1-decanesulfonate in water
Mobile phase: 11.0 g/L of monobasic sodium phosphate in solution, prepared as follows. Transfer a sufficient

www.webofpharma.com

quantity of monobasic sodium phosphate into a container, and dissolve in water, using 95% of the total volume. Add 0.13% of the total volume of *Diluent*, and adjust with phosphoric acid to a pH of 2.8. Transfer to a suitable volumetric flask, and dilute with water to volume. Pass through a membrane filter.

Standard solution: (L_1/750) mg/mL of USP Levodopa RS and (L_2/750) mg/mL of USP Carbidopa RS in *Medium*, where L_1 and L_2 are the label claims of levodopa and carbidopa, respectively, in mg/Tablet

Sample solution: A filtered portion of solution under test

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min, adjusted as needed to obtain retention times for levodopa and carbidopa of 4 and 11 min, respectively

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 6 between levodopa and carbidopa

Relative standard deviation: NMT 2.0% for levodopa; NMT 2.0% for carbidopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*

r_S = peak response of carbidopa or levodopa from the *Standard solution*

C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 750 mL

L = label claim of carbidopa or levodopa (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) are dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 750 mL

Apparatus 1: 100 rpm

Time: 15 min

Solution A: 0.24 g/L of sodium 1-decanesulfonate in water

Mobile phase: 11.0 g/L of monobasic sodium phosphate in solution, prepared as follows. Transfer a sufficient quantity of monobasic sodium phosphate into a container, and dissolve in water, using 95% of the final volume. Add 0.13% of the final volume of *Solution A*, and adjust with phosphoric acid to a pH of 2.8. Transfer to a suitable volumetric flask, and dilute with water to volume. Pass through a membrane filter.

Standard solution: (L_1/750) mg/mL of USP Levodopa RS and (L_2/750) mg/mL of USP Carbidopa RS in *Medium*, where L_1 and L_2 are the label claims of levodopa and carbidopa, respectively, in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 40 µL

Run time: NLT 2 times the retention time for carbidopa

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for levodopa and carbidopa are 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6 between levodopa and carbidopa

Relative standard deviation: NMT 2.0% for levodopa; NMT 2.0% for carbidopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*

r_S = peak response of carbidopa or levodopa from the *Standard solution*

C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 750 mL

L = label claim of carbidopa or levodopa (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Protect solutions containing carbidopa or levodopa from light and maintain them at 2°–8° until they are injected. Use within 12 h.

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 125 µg/mL of USP Carbidopa RS, 0.25 µg/mL of dihydroxybenzaldehyde, 1.25 µg/mL of dihydroxyphenylacetone, and 2.5 µg/mL of USP Levodopa Related Compound B RS in *Mobile phase*

Standard solution: 1.25 µg/mL of USP Carbidopa RS and 5 µg/mL of USP Levodopa RS in *Mobile phase*

Sample solution: Nominally 125 µg/mL of carbidopa from Tablets prepared as follows. Transfer a suitable amount of powder from NLT 10 Tablets to an appropriate volumetric flask. Add 80% of the total flask volume of *Mobile phase*. Sonicate for 15 min with intermittent shaking. Dilute with *Mobile phase*. Centrifuge the resulting solution and use the supernatant. [NOTE—The use of a centrifuge speed of 3000 rpm for 5 min may be suitable.]

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between levodopa related compound B and carbidopa; NLT 1.5 between dihydroxybenzaldehyde and dihydroxyphenylacetone, *System suitability solution*

Relative standard deviation: NMT 3.0% each for levodopa and carbidopa, *Standard solution*

www.webofpharma.com

Signal-to-noise ratio: NLT 10 for dihydroxybenzaldehyde and dihydroxyphenylacetone, *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methyldopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of methyldopa from the *Sample solution*
r_S = peak response of carbidopa from the *Standard solution*
C_S = concentration of USP Carbidopa RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of carbidopa in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 1*)

Calculate the percentage of any other unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any other unspecified degradation product from the *Sample solution*
r_S = peak response of levodopa from the *Standard solution*
C_S = concentration of USP Levodopa RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of levodopa in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting thresholds are 0.1% and 0.05% for peaks associated with carbidopa and levodopa, respectively.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levodopa related compound A[a, b]	0.8	1.0	—
Levodopa	1.0	—	—
Methyldopa	1.8	1.0	0.6
Levodopa related compound B[a]	2.0	—	—
Carbidopa	2.3	—	—
Dihydroxybenzaldehyde[a, c]	5.6	6.2	—
Dihydroxyphenylacetone[a, d]	6.2	1.4	—
3-*O*-Methylcarbidopa[a, e]	6.6	—	—
Any unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	4.0

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.
[b] 3-(2,4,5-Trihydroxyphenyl)-L-alanine; also known as 3-(3,4,6-Trihydroxyphenyl)alanine.
[c] 3,4-Dihydroxybenzaldehyde.
[d] 3,4-Dihydroxyphenylacetone.
[e] (*S*)-2-Hydrazinyl-3-(4-hydroxy-3-methoxyphenyl)-2-methylpropanoic acid.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Dispense in a tight, light-resistant container. Store at controlled room temperature.
- **LABELING:** The labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Carbidopa RS
  USP Levodopa RS
  USP Levodopa Related Compound B RS
  3-Methoxy-L-tyrosine;
  Also known as 3-Methoxytyrosine.
  $C_{10}H_{13}NO_4$ 211.21

Carbidopa and Levodopa Extended-Release Tablets

DEFINITION

Carbidopa and Levodopa Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectra of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Protect the volumetric preparations from light.

Solution A: 0.24 g/L of sodium 1-decanesulfonate in water

Solution B: 11.6 g/L of monobasic sodium phosphate in water

Mobile phase: *Solution A*, *Solution B*, and water (0.13: 95: 4.87), prepared as follows. Add 0.13% of the final volume of *Solution A* to 95% of the final volume of *Solution B*. Adjust with phosphoric acid to a pH of 2.8. Dilute with water to final volume.

Standard solution: 0.1 mg/mL of USP Carbidopa RS and 0.4 mg/mL of USP Levodopa RS in solution, prepared as follows. Transfer accurately weighed portions of the Reference Standards into a suitable volumetric flask, and dissolve in 0.1 N phosphoric acid using 8% of the final volume. Sonication may be used to promote dissolution. Dilute with water to final volume.

Sample solution: Nominally 0.1 mg/mL of carbidopa and 0.4 mg/mL of levodopa from NLT 20 finely powdered Tablets, prepared as follows. Transfer an accurately weighed portion of the powder, equivalent to 1 Tablet weight, into a suitable volumetric flask, and dissolve in 0.1 N phosphoric acid, using 10% of the final volume. Sonicate for 10 min and then stir for 30 min. Dilute with water to volume and stir for another 20 min. Pass the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm. For *Identification B*, use a diode array detector in the range of 200–350 nm.

Column: 4.6-mm × 10-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

Run time: NLT 4 times the retention time of levodopa

www.webofpharma.com

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for levodopa and carbidopa are 1.0 and 2.8, respectively.]
Suitability requirements
Tailing factor: NMT 1.5 for carbidopa; NMT 1.5 for levodopa
Resolution: NLT 6 between levodopa and carbidopa
Relative standard deviation: NMT 1.0% for carbidopa; NMT 1.0% for levodopa
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) or levodopa ($C_9H_{11}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*
r_S = peak response of carbidopa or levodopa from the *Standard solution*
C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of carbidopa or levodopa in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of the labeled amounts of carbidopa and levodopa

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid; 900 mL degassed with helium
Apparatus 2: 50 rpm
Times
For Tablets that contain 25 mg of carbidopa and 100 mg of levodopa: 0.5, 1, and 4 h
For Tablets that contain 50 mg of carbidopa and 200 mg of levodopa: 0.5, 1, 2.5, and 4 h
Solution A: 0.24 g/L of sodium 1-decanesulfonate in water
Solution B: 12.7 g/L of monobasic sodium phosphate in water
Mobile phase: *Solution A*, *Solution B*, and water (0.13: 95: 4.87), prepared as follows. Add 0.13% of the final volume of *Solution A* to 95% of the final volume of *Solution B*. Adjust with phosphoric acid to a pH of 2.8. Dilute with water to final volume.
Standard solution: 0.03 mg/mL of USP Carbidopa RS and 0.1 mg/mL of USP Levodopa RS in *Medium*. Sonication may be used to aid in dissolution.
Sample solution
For Tablets that contain 25 mg of carbidopa and 100 mg of levodopa: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first 1–3 mL.
For Tablets that contain 50 mg of carbidopa and 200 mg of levodopa: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discard the first 1–3 mL, and dilute with *Medium* (50:50).
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
Run time: NLT 3 times the retention time of levodopa
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for levodopa and carbidopa are 0.4 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between levodopa and carbidopa
Relative standard deviation: NMT 2.0% for carbidopa and NMT 2.0% for levodopa for six replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of carbidopa ($C_{10}H_{14}N_2O_4$) or levodopa ($C_9H_{11}NO_4$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result} = (r_U/r_S) \times C_S \times D$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*
r_S = peak response of carbidopa or levodopa from the *Standard solution*
C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed

Calculate the percentage of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) or levodopa ($C_9H_{11}NO_4$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point i (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim of carbidopa or levodopa (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances
For Tablets that contain 25 mg of carbidopa and 100 mg of levodopa: See *Table 1*.

Table 1

Time Point (*i*)	Time (h)	Amount of Carbidopa Dissolved (%)	Amount of Levodopa Dissolved (%)
1	0.5	15–40	14–39
2	1	37–62	36–61
3	4	NLT 80	NLT 80

For Tablets that contain 50 mg of carbidopa and 200 mg of levodopa: See *Table 2*.

www.webofpharma.com

Table 2

Time Point (*i*)	Time (h)	Amount of Carbidopa Dissolved (%)	Amount of Levodopa Dissolved (%)
1	0.5	8–33	8–33
2	1	26–51	26–51
3	2.5	62–87	64–89
4	4	NLT 80	NLT 80

The percentages of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Simulated gastric fluid TS (prepared without enzymes); 900 mL

Apparatus 2: 50 rpm

Times: 0.5, 1, 2, and 3 h

Buffer: 6.8 g/L of monobasic potassium phosphate and 1.0 g/L of 1-hexanesulfonic acid in water. Adjust with phosphoric acid to a pH of 3.3.

Mobile phase: Filtered and degassed mixture of methanol and *Buffer* (20:80)

Standard solution: (*L*/900) mg/mL each of USP Carbidopa RS and USP Levodopa RS in *Medium*, where *L* is the label claim, in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 2.5 times the retention time of levodopa

System suitability

Sample: *Standard solution*
[NOTE—The relative retention times for levodopa and carbidopa are 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 2.0 between levodopa and carbidopa

Column efficiency: NLT 4000 theoretical plates for both carbidopa and levodopa

Tailing factor: NMT 2.0 for both carbidopa and levodopa

Relative standard deviation: NMT 1.0% for both carbidopa and levodopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of carbidopa ($C_{10}H_{14}N_2O_4$) or levodopa ($C_9H_{11}NO_4$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*

r_S = peak response of carbidopa or levodopa from the *Standard solution*

C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) or levodopa ($C_9H_{11}NO_4$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point *i* (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim of carbidopa or levodopa (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	0.5	20–35
2	1	35–60
3	2	65–95
4	3	NLT 80

The percentages of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium, Apparatus 2, Solution A, Solution B, Mobile phase, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in *Test 1*.

Times: 0.5, 1, 2.5, and 4 h

Sample solution: Pass a portion of the solution under test through a suitable filter.

Analysis: Proceed as directed in *Test 1*.

Tolerances: See *Table 4*.

Table 4

Time Point (*i*)	Time (h)	Amount Dissolved for Tablets That Contain 25 mg of Carbidopa and 100 mg of Levodopa (%)	Amount Dissolved for Tablets That Contain 50 mg of Carbidopa and 200 mg of Levodopa (%)
1	0.5	15–40	15–35
2	1	25–65	25–65
3	2.5	NLT 60	NLT 60
4	4	NLT 80	NLT 80

The percentages of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) dissolved at the

USP Monographs

www.webofpharma.com

times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Times: 1, 3, and 6 h
Solution A: 0.24 g/L of sodium 1-decanesulfonate in water
Solution B: 11.6 g/L of monobasic sodium phosphate in water
Mobile phase: *Solution A*, *Solution B*, and water (0.13:95:4.87), prepared as follows. Add 0.13% of the final volume of *Solution A* to 95% of the final volume of *Solution B*. Adjust with phosphoric acid to a pH of 2.8. Dilute with water to final volume.
Standard solution: (*L*/900) mg/mL each of USP Carbidopa RS and USP Levodopa RS in *Medium*, where *L* is the label claim, in mg/Tablet
Sample solution: Withdraw a 10.0-mL aliquot at each time point and pass a portion of the solution under test through a suitable filter. Replace the 10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Medium*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
Run time: NLT 3 times the retention time of levodopa
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for levodopa and carbidopa are 1.0 and 2.5, respectively.]
Suitability requirements
Resolution: NLT 2.0 between levodopa and carbidopa
Tailing factor: NMT 2.0 for both carbidopa and levodopa
Relative standard deviation: NMT 2.0% for both carbidopa and levodopa
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of carbidopa ($C_{10}H_{14}N_2O_4$) or levodopa ($C_9H_{11}NO_4$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*
r_S = peak response of carbidopa or levodopa from the *Standard solution*
C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) or levodopa ($C_9H_{11}NO_4$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = [(C_3 \times V) + (C_2 + C_1) \times V_S] \times (1/L) \times 100$$

C_i = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim of carbidopa or levodopa (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the vessel and replaced with *Medium*, 10 mL

Tolerances: See *Table 5*.

Table 5

Time Point (*i*)	Time (h)	Amount Dissolved for Tablets That Contain 25 mg of Carbidopa and 100 mg of Levodopa (%)	Amount Dissolved for Tablets That Contain 50 mg of Carbidopa and 200 mg of Levodopa (%)
1	1	35–70	25–60
2	3	NLT 65	NLT 65
3	6	NLT 80	NLT 80

The percentages of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Times: 0.5, 1, 2.5, and 4 h
Mobile phase: 13.6 g/L of monobasic potassium phosphate adjusted with phosphoric acid to a pH of 3.0
Standard solution: (*L*/900) mg/mL each of USP Carbidopa RS and USP Levodopa RS in *Medium*, where *L* is the label claim, in mg/Tablet. [NOTE—This solution is stable for 1 day if stored at 23°–27°.]
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and discard the first 4–5 mL.
[NOTE—This solution is stable for 1 day if stored at 23°–27°.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 282 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: NLT 3 times the retention time of levodopa
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for levodopa and carbidopa are 1.0 and 1.6, respectively.]
Suitability requirements
Resolution: NLT 2.0 between levodopa and carbidopa
Tailing factor: NMT 2.0 for both carbidopa and levodopa
Relative standard deviation: NMT 2.0% for both carbidopa and levodopa
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of carbidopa ($C_{10}H_{14}N_2O_4$) or levodopa ($C_9H_{11}NO_4$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*

www.webofpharma.com

r_S = peak response of carbidopa or levodopa from the *Standard solution*
C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) or levodopa ($C_9H_{11}NO_4$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim of carbidopa or levodopa (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 6*.

Table 6

Time Point (*i*)	Time (h)	Amount Dissolved for Tablets That Contain 25 mg of Carbidopa and 100 mg of Levodopa (%)	Amount Dissolved for Tablets That Contain 50 mg of Carbidopa and 200 mg of Levodopa (%)
1	0.5	25–45	20–40
2	1	40–65	30–60
3	2.5	NLT 65	NLT 55
4	4	NLT 80	NLT 75

The percentages of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium: 0.1 N hydrochloric acid; 900 mL degassed under vacuum
Apparatus 1: 75 rpm
Times: 0.5, 1, 2.5, and 3.5 h
Solution A: 0.24 g/L of sodium 1-decanesulfonate in water
Mobile phase: To each liter of 12.5 g/L of monobasic sodium phosphate dihydrate, add 1.3 mL of *Solution A* and adjust with phosphoric acid to a pH of 2.8.
Standard solution: 0.03 mg/mL of USP Carbidopa RS and 0.11 mg/mL of USP Levodopa RS in *Medium*
Sample solution
For Tablets that contain 25 mg of carbidopa and 100 mg of levodopa: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discard the first 2 mL, and use the remaining filtrate. Use within 24 h.
For Tablets that contain 50 mg of carbidopa and 200 mg of levodopa: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discard the first 2 mL, and dilute with *Medium* (50:50). Use within 24 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
Run time: NLT 3 times the retention time of levodopa
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for levodopa and carbidopa are 1.0 and 2.8, respectively.]
Suitability requirements
Resolution: NLT 2.0 between levodopa and carbidopa
Tailing factor: NMT 2.0 for both levodopa and carbidopa
Relative standard deviation: NMT 2.0% for both levodopa and carbidopa
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of carbidopa ($C_{10}H_{14}N_2O_4$) or levodopa ($C_9H_{11}NO_4$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S \times D$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*
r_S = peak response of carbidopa or levodopa from the *Standard solution*
C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed

Calculate the percentage of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) or levodopa ($C_9H_{11}NO_4$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim of carbidopa or levodopa (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 7*.

www.webofpharma.com

Table 7

Time Point (*i*)	Time (h)	Amount Dissolved for Tablets That Contain 25 mg of Carbidopa and 100 mg of Levodopa (%)	Amount Dissolved for Tablets That Contain 50 mg of Carbidopa and 200 mg of Levodopa (%)
1	0.5	15–40	10–30
2	1	35–60	25–50
3	2.5	NLT 70	NLT 65
4	3.5	NLT 85	NLT 80

The percentages of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**

Protect all analytical solutions from light and maintain them at 2°–8° until they are injected.

Buffer: 6 g/L of anhydrous monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 2.2.

Mobile phase: Alcohol and *Buffer* (5:95)

System suitability solution: 1 µg/mL of USP Levodopa Related Compound B RS and 125 µg/mL of USP Carbidopa RS in *Mobile phase*

Standard solution: 1.25 µg/mL of USP Carbidopa RS and 5 µg/mL of USP Levodopa RS in *Mobile phase*

Sensitivity solution: 0.125 µg/mL of USP Carbidopa RS and 0.5 µg/mL of USP Levodopa RS in *Mobile phase* from the *Standard solution*

Sample solution: Nominally 0.125 mg/mL of carbidopa and nominally 0.5 mg/mL of levodopa in *Mobile phase* from NLT 10 finely powdered Tablets, prepared as follows. Transfer an accurately weighed portion of the powder into a suitable volumetric flask, dissolve in *Mobile phase*, and pass through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Autosampler temperature: 6°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 6 times the retention time of carbidopa

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[Note—For the relative retention times, see *Table 8*.]

Suitability requirements

Resolution: NLT 1.5 between carbidopa and levodopa related compound B, *System suitability solution*

Relative standard deviation: NMT 3.0% for both carbidopa and levodopa for five replicate injections, *Standard solution*

Signal-to-noise ratio: NLT 10 for carbidopa, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dihydroxybenzaldehyde, dihydroxyphenylacetone, and any unspecified carbidopa degradant based on the label claim of carbidopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of dihydroxybenzaldehyde, dihydroxyphenylacetone, or any unspecified carbidopa degradant from the *Sample solution*

r_S = peak response of carbidopa from the *Standard solution*

C_S = concentration of USP Carbidopa RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of carbidopa in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 8*)

Calculate the percentage of levodopa related compound A and any unspecified levodopa degradant based on the label claim of levodopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of levodopa related compound A or any unspecified levodopa degradant from the *Sample solution*

r_S = peak response of levodopa from the *Standard solution*

C_S = concentration of USP Levodopa RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levodopa in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 8*)

Acceptance criteria: See *Table 8*. The reporting threshold is 0.05%, relative to the drug substance.

Table 8

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levodopa related compound A[a, b]	0.9	0.8	0.1
Levodopa	1.0	—	—
Methyldopa[c, d]	1.9	—	—
Levodopa related compound B[a, d]	2.1	—	—
Carbidopa	2.3	—	—
Dihydroxybenzaldehyde[c, e]	5.7	5.9	0.2
Dihydroxyphenylacetone[c, f]	6.3	1.0	1
3-*O*-Methylcarbidopa[d, g]	6.9	—	—
Any unspecified carbidopa degradant	—	1.0	0.2
Any unspecified levodopa degradant	—	1.0	0.1
Total degradants	—	—	4.0

[a] Individual impurity based on label claim of levodopa.
[b] 3-(3,4,6-Trihydroxyphenyl)alanine.
[c] Individual impurity based on label claim of carbidopa.
[d] This impurity is listed for information only. It is monitored in the drug substance. This impurity is not to be reported and is not to be included in the total degradants.
[e] 3,4-Dihydroxybenzaldehyde.
[f] 3,4-Dihydroxyphenylacetone.
[g] (*S*)-2-Hydrazinyl-3-(4-hydroxy-3-methoxyphenyl)-2-methylpropanoic acid.

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Carbidopa RS
  USP Levodopa RS
  USP Levodopa Related Compound B RS
  3-Methoxytyrosine.
  $C_{10}H_{13}NO_4$ 211.21

Carbidopa and Levodopa Orally Disintegrating Tablets

DEFINITION

Carbidopa and Levodopa Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Protect the volumetric solutions from light.
Buffer: 6.6 g/L of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.2
Mobile phase: Alcohol and *Buffer* (5:95)
Standard solution: 0.025 mg/mL of USP Carbidopa RS and 0.25 mg/mL of USP Levodopa RS in *Mobile phase*
Sample stock solution: Transfer NLT 10 Tablets to a 1-L volumetric flask. Add 750 mL of *Mobile phase*, sonicate for 20 min, and then stir for 20 min. Dilute with *Mobile phase* to volume.
Sample solution: Dilute the *Sample stock solution* with *Mobile phase* to obtain a nominal concentration of carbidopa of between 0.025 and 0.07 mg/mL and a nominal concentration of levodopa of 0.25 mg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Autosampler temperature: 6°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for levodopa and carbidopa are 0.42 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.4 for both the levodopa and carbidopa peaks
Relative standard deviation: NMT 2.0% for both carbidopa and levodopa
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*
r_S = peak response of carbidopa or levodopa from the *Standard solution*
C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of carbidopa or levodopa in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of the labeled amounts of carbidopa and levodopa

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩**:** NMT 60 s

Change to read:

- **DISSOLUTION** ⟨711⟩

▲**Test 1** ▲ (RB 1-Jun-2018)
Medium: 0.1 N hydrochloric acid; 750 mL
Apparatus 2: 50 rpm
Time: 10 min
Solution A: 0.24 g/L of sodium 1-decanesulfonate in water
Mobile phase: Dissolve 11.0 g of monobasic sodium phosphate monohydrate in 1 L of water. Add 1.3 mL of *Solution A*, and adjust with phosphoric acid to a pH of 2.8.
Standard solution: (*L*/800) mg/mL each of USP Carbidopa RS and USP Levodopa RS in *Medium*, where *L* is the label claim in mg/Tablet of carbidopa or levodopa
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and discard the first 3 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15.0-cm; 5-µm packing L1
Autosampler temperature: 4°
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for levodopa and carbidopa are 0.4 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0 for both levodopa and carbidopa
Relative standard deviation: NMT 2.0% for both levodopa and carbidopa
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*
r_S = peak response of carbidopa or levodopa from the *Standard solution*
C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)
V = volume of the *Medium*, 750 mL
L = label claim of carbidopa or levodopa (mg/Tablet)

www.webofpharma.com

Tolerances: NLT 75% (*Q*) of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) is dissolved, and NLT 75% (*Q*) of the labeled amount of levodopa ($C_9H_{11}NO_4$) is dissolved.

▲**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 750 mL, degassed

Apparatus 2: 75 rpm

Time: 15 min

Solution A: 0.24 g/L of sodium 1-decanesulfonate in water

Mobile phase: 12.5 g/L of monobasic sodium phosphate dihydrate prepared as follows. Transfer an appropriate amount of monobasic sodium phosphate dihydrate to a suitable volumetric flask. Dissolve in 95% of the flask volume of water. Add 0.13% of the flask volume of *Solution A*, and adjust with phosphoric acid to a pH of 2.8 ± 0.05. Dilute with water to volume.

Standard stock solution 1: 0.19 mg/mL of USP Carbidopa RS in *Medium*. Transfer an appropriate amount of USP Carbidopa RS to a suitable volumetric flask. Add about 60% of the flask volume of *Medium* and sonicate to promote dissolution. Allow the solution to cool to room temperature and dilute with *Medium* to volume.

Standard stock solution 2: 1.1 mg/mL of USP Levodopa RS in *Medium*. Transfer an appropriate amount of USP Levodopa RS to a suitable volumetric flask. Add about 60% of the flask volume of *Medium* and sonicate to promote dissolution. Allow the solution to cool to room temperature and dilute with *Medium* to volume.

Standard solution

For Tablets labeled to contain 10 mg of carbidopa and 100 mg of levodopa: 0.015 mg/mL of USP Carbidopa RS from *Standard stock solution 1* and 0.13 mg/mL of USP Levodopa RS from *Standard stock solution 2* in *Medium*

For Tablets labeled to contain 25 mg of carbidopa and 100 or 250 mg of levodopa: 0.038 mg/mL of USP Carbidopa RS from *Standard stock solution 1* and 0.22 mg/mL of USP Levodopa RS from *Standard stock solution 2* in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and discard the first 2 mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30.0-cm; 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

Run time: NLT 1.3 times the retention time of carbidopa

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for levodopa and carbidopa are 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6 between levodopa and carbidopa

Tailing factor: NMT 2.0 for both levodopa and carbidopa

Relative standard deviation: NMT 2.0% for both levodopa and carbidopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of carbidopa ($C_{10}H_{14}N_2O_4$) and levodopa ($C_9H_{11}NO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of carbidopa or levodopa from the *Sample solution*

r_S = peak response of carbidopa or levodopa from the *Standard solution*

C_S = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 750 mL

L = label claim of carbidopa or levodopa (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of carbidopa ($C_{10}H_{14}N_2O_4$) is dissolved, and NLT 75% (*Q*) of the labeled amount of levodopa ($C_9H_{11}NO_4$) is dissolved. ▲ (RB 1-Jun-2018)

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Protect all analytical solutions from light, and maintain them at 2°–8° until they are injected.

Diluent: Methanol and 0.1 N hydrochloric acid (30:70)

Mobile phase: 13.8 g/L of monobasic sodium phosphate monohydrate in water, adjusted with phosphoric acid to a pH of 2.7

System suitability solution: 0.025 mg/mL each of USP Carbidopa RS, USP Levodopa RS, USP Levodopa Related Compound A RS, USP Levodopa Related Compound B RS, and USP Methyldopa RS in *Diluent*

Standard solution: 0.025 mg/mL of USP Levodopa RS in *Diluent*

Sample solution: Transfer a weighed quantity of powder equivalent to 250 mg of levodopa from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add 80 mL of *Diluent*, sonicate for 10 min, and then stir for 30 min. Dilute with *Diluent* to volume. Centrifuge, and inject the supernatant within 2 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Autosampler temperature: 4°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Run time: 6 times the retention time of carbidopa

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—For the relative retention times, see *Table 1*. If peak fronting for levodopa related compound A is observed, lowering the column temperature to 15° is recommended to eliminate this problem.]

Suitability requirements

Resolution: NLT 1.5 between levodopa related compound A and levodopa, NLT 2.0 between carbidopa and levodopa related compound B, and NLT 1.5 between methyldopa and carbidopa; *System suitability solution*

Relative standard deviation: NMT 5.0% for levodopa, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of all impurities and any unspecified degradation product other than methyldopa and 3,4-dihydroxyphenylacetone, based on the label claim of levodopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

www.webofpharma.com

r_U = peak response of levodopa related compound A or any unspecified degradation product from the *Sample solution*
r_S = peak response of levodopa from the *Standard solution*
C_S = concentration of USP Levodopa RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levodopa in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Calculate the percentage of methyldopa and 3,4-dihydroxyphenylacetone based on the label claim of carbidopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of methyldopa or 3,4-dihydroxyphenylacetone from the *Sample solution*
r_S = peak response of levodopa from the *Standard solution*
C_S = concentration of USP Levodopa RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of carbidopa in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levodopa related compound A[a]	0.45	0.80	0.2
Levodopa	0.52	—	—
Methyldopa[b]	0.84	1.0	0.5
Carbidopa	1.0	—	—
Levodopa related compound B[c]	1.2	—	—
3-*O*-Methyl carbidopa[c, d]	3.1	—	—
3,4-Dihydroxyphenylacetone[b, d]	3.9	1.0	1.0
Any individual unspecified degradation product[a]	—	1.0	0.2
Total impurities[e]	—	—	1.0

[a] Individual impurity based on the label claim of levodopa.
[b] Individual impurity based on the label claim of carbidopa.
[c] Process-related impurities, included for identification only; not to be included in total impurities.
[d] (*S*)-2-Hydrazinyl-3-(4-hydroxy-3-methoxyphenyl)-2-methylpropanoic acid.
[e] Excluding all process impurities and 3,4-dihydroxyphenylacetone.

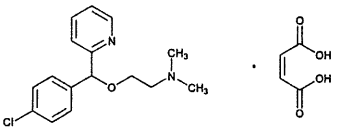
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.

Add the following:

▲• **LABELING:** The labeling states the *Dissolution* test used only if *Test 1* is not used.▲ (RB 1-Jun-2018)
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Carbidopa RS
USP Levodopa RS
USP Levodopa Related Compound A RS
3-(3,4,6-Trihydroxyphenyl)alanine.
$C_9H_{11}NO_5$ 213.19
USP Levodopa Related Compound B RS
3-Methoxytyrosine.
$C_{10}H_{13}NO_4$ 211.21
USP Methyldopa RS

Carbinoxamine Maleate



$C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$ 406.86
Ethanamine, 2-[(4-chlorophenyl)-2-pyridinylmethoxy]-*N*,*N*-dimethyl-, (*Z*)-2-butenedioate (1:1);
2-[*p*-Chloro-α-[2-(dimethylamino)ethoxy]benzyl]pyridine maleate (1:1) [3505-38-2].

DEFINITION

Carbinoxamine Maleate contains NLT 98.0% and NMT 102.0% of carbinoxamine maleate ($C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 2.72 g/L of monobasic potassium phosphate. Adjust with *phosphoric acid* to a pH of 4.0.
Solution B: Methanol and acetonitrile (80:20)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
2	75	25
10	25	75
15	25	75
16	75	25
20	75	25

Diluent: Methanol, acetonitrile, and water (200:50:750)
System suitability solution: 0.1 mg/mL of USP Carbinoxamine Maleate RS and 0.01 mg/mL each of USP Carbinoxamine Related Compound A RS and USP Carbinoxamine Related Compound B RS in *Diluent*

www.webofpharma.com

Standard solution: 0.1 mg/mL of USP Carbinoxamine Maleate RS in *Diluent*
Sample solution: 0.1 mg/mL of Carbinoxamine Maleate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between carbinoxamine related compound A and carbinoxamine related compound B, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carbinoxamine maleate ($C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$) in the portion of Carbinoxamine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carbinoxamine from the *Sample solution*
r_S = peak response of carbinoxamine from the *Standard solution*
C_S = concentration of USP Carbinoxamine Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of Carbinoxamine Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 0.07 mg/mL of USP Carbinoxamine Maleate RS (equivalent to 0.05 mg/mL of carbinoxamine) and 0.05 mg/mL each of USP Carbinoxamine Related Compound A RS, USP Carbinoxamine Related Compound B RS, and USP Carbinoxamine Related Compound C RS (as the free base) in *Diluent*
Standard solution: 0.0014 mg/mL of USP Carbinoxamine Maleate RS (equivalent to 0.001 mg/mL of carbinoxamine) and 0.001 mg/mL each of USP Carbinoxamine Related Compound A RS, USP Carbinoxamine Related Compound B RS, and USP Carbinoxamine Related Compound C RS (as the free base) in *Diluent*, from *Standard stock solution*
Sample solution: 1.0 mg/mL of Carbinoxamine Maleate in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between carbinoxamine related compound A and carbinoxamine related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0% for each corresponding compound, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carbinoxamine related compound A, carbinoxamine related compound B, and carbinoxamine related compound C in the portion of Carbinoxamine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carbinoxamine related compound A, carbinoxamine related compound B, or carbinoxamine related compound C from the *Sample solution*
r_S = peak response of the corresponding Reference Standard from the *Standard solution*
C_S = concentration of the corresponding Reference Standard in the *Standard solution* (mg/mL)
C_U = concentration of Carbinoxamine Maleate in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity in the portion of Carbinoxamine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified impurity from the *Sample solution*
r_S = peak response of carbinoxamine from the *Standard solution*
C_S = concentration of USP Carbinoxamine Maleate RS (as the free base) in the *Standard solution* (mg/mL)
C_U = concentration of Carbinoxamine Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carbinoxamine related compound C	0.68	0.1
Carbinoxamine	1.0	—
Carbinoxamine related compound B	1.25	0.1
Carbinoxamine related compound A	1.36	0.1
Any other individual unspecified impurity	—	0.10
Total impurities	—	1.5

SPECIFIC TESTS

- **PH** ⟨791⟩
Sample: In a solution (1 in 100)
Acceptance criteria: 4.6–5.1
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Carbinoxamine Maleate RS

USP Monographs

www.webofpharma.com

USP Carbinoxamine Related Compound A RS
(4-Chlorophenyl)(pyridin-2-yl)methanone.
$C_{12}H_8ClNO$ 217.65
USP Carbinoxamine Related Compound B RS
(4-Chlorophenyl)(pyridin-2-yl)methanol.
$C_{12}H_{10}ClNO$ 219.67
USP Carbinoxamine Related Compound C RS
N,N-Dimethyl-2-[phenyl(pyridin-2-yl)methoxy]ethan-1-amine oxalate.
$C_{16}H_{20}N_2O \cdot C_2H_2O_4$ 346.38

Carbinoxamine Maleate Tablets

DEFINITION

Carbinoxamine Maleate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of carbinoxamine maleate ($C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$).

IDENTIFICATION

Delete the following:

▲• **A.**
Standard solution: 0.02 mg/mL of USP Carbinoxamine Maleate RS in dilute sulfuric acid (1 in 70)
Sample solution: Nominally 0.02 mg/mL of carbinoxamine maleate in dilute sulfuric acid (1 in 70), from the Tablets, as directed under *Salts of Organic Nitrogenous Bases* ⟨501⟩.
Analytical wavelength: 263 ± 2 nm
Acceptance criteria: The absorptivity of the *Sample solution* at 263 nm is within 7.0% of that of the *Standard solution*. ▲2S (USP41)

Add the following:

▲• **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲2S (USP41)

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲2S (USP41)

ASSAY

Change to read:

• **PROCEDURE**
▲**Solution A:** 2.72 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 4.0.
Solution B: Methanol and acetonitrile (80:20)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
2	75	25
10	25	75
15	25	75
16	75	25
20	75	25

Diluent 1: 0.1 N hydrochloric acid
Diluent 2: Methanol, acetonitrile, and water (200:50:750)
System suitability solution: 0.1 mg/mL of USP Carbinoxamine Maleate RS and 0.01 mg/mL each of USP Carbinoxamine Related Compound A RS and USP Carbinoxamine Related Compound B RS in *Diluent 2*
Standard solution: 0.1 mg/mL of USP Carbinoxamine Maleate RS in *Diluent 2*
Sample solution: Nominally 0.1 mg/mL of carbinoxamine maleate prepared as follows. Transfer a suitable amount of powder from finely powdered Tablets (NLT 20) to a suitable volumetric flask. Add 70% of the flask volume of *Diluent 1* and shake for 15 min, then dilute with *Diluent 2* to volume. Centrifuge the solution and filter the supernatant by passing through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of filtrate. Inject the freshly prepared solution immediately.
Chromatographic system
(See Chromatography ⟨621⟩, System Suitability.)
Mode: LC
Detector: UV 225 nm. For *Identification A* , use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between carbinoxamine related compound A and carbinoxamine related compound B, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of carbinoxamine maleate ($C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carbinoxamine from the *Sample solution*
r_S = peak response of carbinoxamine from the *Standard solution*
C_S = concentration of USP Carbinoxamine Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of carbinoxamine maleate in the *Sample solution* (mg/mL)▲2S (USP41)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm

USP Monographs

www.webofpharma.com

Time: 45 min
Standard solution: USP Carbinoxamine Maleate RS in *Medium* with a concentration similar to that expected in the *Sample solution*
Sample solution: Filter a portion of the solution under test and dilute with *Medium* as needed.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 260 nm
Analysis
Samples: *Standard solution* and *Sample solution*
▲Calculate the percentage of the labeled amount of carbinoxamine maleate ($C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance of carbinoxamine maleate from the *Standard solution*
C_S = concentration of USP Carbinoxamine Maleate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*
L = label claim (mg/Tablet)▲ 2S (USP41)

Tolerances: NLT 75% (Q) of the labeled amount of carbinoxamine maleate ($C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$) is dissolved.

Change to read:

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: ▲Meet the requirements▲ 2S (USP41)

IMPURITIES

Change to read:

▲• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent 1, Diluent 2, and **System suitability solution:** Prepare as directed in the *Assay*.
Standard stock solution: 0.028 mg/mL of USP Carbinoxamine Maleate RS (equivalent to 0.02 mg/mL of ▲carbinoxamine)▲ (ERR 1-Mar-2019) and 0.02 mg/mL each of USP Carbinoxamine Related Compound A RS and USP Carbinoxamine Related Compound B RS in *Diluent 2*
Standard solution: 0.0014 mg/mL of USP Carbinoxamine Maleate RS (equivalent to 0.001 mg/mL of ▲carbinoxamine)▲ (ERR 1-Mar-2019) and 0.001 mg/mL each of USP Carbinoxamine Related Compound A RS and USP Carbinoxamine Related Compound B RS in *Diluent 2*, from *Standard stock solution*
Sample solution: Nominally 1.0 mg/mL of carbinoxamine maleate prepared as follows. Transfer a suitable quantity of powder from finely powdered Tablets (NLT 20) to a suitable volumetric flask. Add 75% of the flask volume of *Diluent 1*, shake for 15 min, and dilute with *Diluent 2* to volume. Centrifuge the solution and filter the supernatant by passing through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of filtrate. Inject the freshly prepared solution immediately.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between carbinoxamine related compound A and carbinoxamine related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0% for each corresponding compound present in the *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carbinoxamine related compound A and carbinoxamine related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carbinoxamine related compound A or carbinoxamine related compound B from the *Sample solution*
r_S = peak response of the corresponding Reference Standard from the *Standard solution*
C_S = concentration of the corresponding Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of carbinoxamine maleate in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of carbinoxamine from the *Standard solution*
C_S = concentration of USP Carbinoxamine Maleate RS▲(as the free base)▲ (ERR 1-Mar-2019) in the *Standard solution* (mg/mL)
C_U = nominal concentration of carbinoxamine maleate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carbinoxamine related compound C[a, b]	0.68	—
Carbinoxamine	1.0	—
Carbinoxamine related compound B	1.25	0.2
Carbinoxamine related compound A	1.36	0.2
Each unspecified degradation product	—	0.2

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total degradation products	—	2.0

[a] Process impurity included for identification only and not included in the calculation of total degradation products.
[b] *N,N*-Dimethyl-2-[phenyl(pyridin-2-yl)methoxy]ethan-1-amine. ▲2S (USP41)

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, ▲and store at controlled room temperature.▲ 2S (USP41)

Change to read:

- *USP Reference Standards* ⟨11⟩
  USP Carbinoxamine Maleate RS
  ▲USP Carbinoxamine Related Compound A RS
  (4-Chlorophenyl)(pyridin-2-yl)methanone.
  $C_{12}H_8ClNO$ 217.65
  USP Carbinoxamine Related Compound B RS
  (4-Chlorophenyl)(pyridin-2-yl)methanol.
  $C_{12}H_{10}ClNO$ 219.67▲ 2S (USP41)

Carbol-Fuchsin Topical Solution

» Prepare Carbol-Fuchsin Topical Solution as follows:

Basic Fuchsin	3 g
Phenol	45 g
Resorcinol	100 g
Acetone	50 mL
Alcohol	100 mL
Purified Water, a sufficient quantity, to make	1000 mL

Dissolve the Basic Fuchsin in a mixture of the Acetone and Alcohol, and add to this solution the Phenol and Resorcinol previously dissolved in 725 mL of Purified Water. Then add sufficient Purified Water to make the product measure 1000 mL, and mix.

Packaging and storage—Preserve in tight, light-resistant containers.
Specific gravity ⟨841⟩: not less than 0.990 and not more than 1.050.
Alcohol Determination ⟨611⟩: between 7.0% and 10.0% of C_2H_5OH.

Carbon Dioxide

CO_2 44.01
Carbon dioxide [124-38-9].

DEFINITION
Carbon Dioxide contains NLT 99.0%, by volume, of carbon dioxide (CO_2).
[NOTE—The following tests are designed to reflect the quality of Carbon Dioxide in both its vapor and liquid phases, which are present in previously unopened cylinders. Reduce the container pressure by means of a regulator. Withdraw the specimens for the tests with the least possible release of Carbon Dioxide consistent with proper purging of the sampling apparatus. Measure the gases with a gas volume meter downstream from the detector tubes to minimize contamination or change of the specimens.]

IDENTIFICATION
- **A.**
  Sample: 100 ± 5 mL, released from the vapor phase of the contents of the container
  Analysis: Pass the *Sample* through a carbon dioxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.
  Acceptance criteria: The indicator change extends throughout the entire indicating range of the tube.

ASSAY
- **PROCEDURE**
  [NOTE—Sampling for this *Assay* may be done from the vapor phase for convenience, but this method results in more residual volume. If the specification of 1 mL is exceeded from the vapor phase, a liquid specimen may be taken.]
  Sample: 100.0 mL of specimen taken from the liquid phase, as directed in the test for *Nitrogen Dioxide*
  Analysis: Assemble a 100-mL gas buret, provided with a leveling bulb and two-way stopcock, and a gas absorption pipet of suitable capacity by connecting the pipet to one of the buret outlets. Fill the buret with slightly acidified water (turned pink with methyl orange), and fill the pipet with potassium hydroxide solution (1 in 2). By manipulation of the leveling bulb and leveling water, draw the potassium hydroxide solution to fill the pipet and capillary connection up to the stopcock. Fill the buret with the leveling water, and draw it through the other stopcock opening in such a manner that all gas bubbles are eliminated from the system. Draw the *Sample* into the buret. By raising the leveling bottle, force the measured specimen into the pipet. The absorption may be facilitated by rocking the pipet or by flowing the specimen between pipet and buret. Draw any residual gas into the buret, and measure its volume.
  Acceptance criteria: NMT 1.0 mL of gas remains (NLT 99.0%, by volume, of CO_2).

IMPURITIES
- **NITROGEN DIOXIDE**
  Sample: 550 ± 50 mL, obtained as directed in the *Analysis*
  Analysis: Arrange the container so that when its valve is opened, a portion of the liquid phase of the contents is released through a piece of tubing of sufficient length to allow all of the liquid to vaporize during passage through it, and to prevent frost from reaching the inlet of the detector tube. Release into the tubing a flow of liquid sufficient to provide 550 mL of the vaporized specimen plus any excess necessary to ensure adequate flushing of air from the system. Pass 550 ± 50 mL of this gas through a nitric

www.webofpharma.com

oxide–nitrogen dioxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.
Acceptance criteria: NMT 2.5 ppm

- **Limit of Ammonia**
Sample: 1050 ± 50 mL of the gas obtained as directed in the test for *Nitrogen Dioxide*
Analysis: Proceed with Carbon Dioxide as directed in the test for *Nitrogen Dioxide*, except pass 1050 ± 50 mL of this gas through an ammonia detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.
Acceptance criteria: NMT 0.0025%
- **Limit of Hydrogen Sulfide**
Sample: 1050 ± 50 mL, released from the vapor phase
Analysis: Pass 1050 ± 50 mL, released from the vapor phase, through a hydrogen sulfide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.
Acceptance criteria: NMT 1 ppm
- **Limit of Nitric Oxide**
Sample: 550 ± 50 mL, released from the vapor phase
Analysis: Pass 550 ± 50 mL, released from the vapor phase, through a nitric oxide–nitrogen dioxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.
Acceptance criteria: NMT 2.5 ppm
- **Carbon Monoxide**
Sample: 1050 ± 50 mL, released from the vapor phase of the contents of the container
Analysis: Pass 1050 ± 50 mL, released from the vapor phase of the contents of the container, through a carbon monoxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.
Acceptance criteria: NMT 0.001%
- **Sulfur Dioxide**
Sample: 1050 ± 50 mL, obtained as directed in the test for *Nitrogen Dioxide*
Analysis: Proceed with Carbon Dioxide as directed in the test for *Nitrogen Dioxide*, except to pass 1050 ± 50 mL through a sulfur dioxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.
Acceptance criteria: NMT 5 ppm

SPECIFIC TESTS

- **Water Determination**
Analysis: Flush the regulator that has been flushed with 5 L or more of the gas specimen. Pass 50 ± 5 L, released from the vapor phase, through a water vapor detector tube connected to the regulator with a minimum length of metal or polyethylene tubing. Measure the gas passing through the detector tube with a gas flowmeter set at a flow rate of 2 L/min.
Acceptance criteria: NMT 150 mg/m³

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in cylinders.

Urea C 13



Urea [^{13}C] 61.05
[58069-82-2.].

DEFINITION

Urea C 13 contains NLT 98.0% and NMT 102.0% of urea C 13 ($^{13}CH_4N_2O$).

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention times of the major peaks corresponding to the mass-to-charge (*m*/*z*) ratios of 61 and 63 in the *Sample solution* correspond to those of the *Standard solution*, as obtained in the test for *Isotopic Purity*.

ASSAY

- **Procedure**
Mobile phase: Acetonitrile, methanol, and water (89:10:1)
System suitability solution: 2.5 mg/mL of urea and 0.003 mg/mL of biuret in *Mobile phase*
Standard solution: 2 mg/mL of USP Urea C 13 RS in *Mobile phase*
Sample solution: 2 mg/mL of Urea C 13 in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 25-cm; 5-µm packing L8
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between urea and biuret, *System suitability solution*
Relative standard deviation: NMT 1%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the areas for the major peaks.
Calculate the percentage of urea C 13 ($^{13}CH_4N_2O$) in the portion of Urea C 13 taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Urea C 13 RS in the *Standard solution* (mg/mL)
C_U = concentration of Urea C 13 in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%
- **Limit of Biuret**
Standard solution: 0.033 mg/mL of biuret in water
Sample solution: 33.3 mg/mL of Urea C 13 in water
Analysis: Treat 3 mL of the *Sample solution* and 3 mL of the *Standard solution* separately as follows. To each solution add 3 mL of sodium hydroxide solution (10 in 100) and 3 drops of copper sulfate solution (0.5 in 100), and allow to stand for 5 min.
Acceptance criteria: NMT 0.1%; any reddish-violet color in the *Sample solution* is not more intense than that in the *Standard solution*.
- **Isotopic Purity**
Standard solution: 0.4 mg/mL of USP Urea C 13 RS in methanol
Sample solution: 0.4 mg/mL of Urea C 13 in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩ and *Mass Spectrometry* ⟨736⟩.)
Mode: GC

www.webofpharma.com

Detector: Mass spectrometer, electron energy 70 eV
Column: 0.25-mm × 15-m capillary; coated with a 0.1-µm film of phase G25 or G35
Temperatures
Injection port: 250°
Detector: 200°
Transfer line: 265°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	15	250	0

Carrier gas: Helium
Flow rate: 1 mL/min
Injection volume: 1 µL
Split ratio: 1:25
Analysis
Samples: *Sample solution* and *Standard solution*
[NOTE—The *Standard solution* chromatogram is also intended for use in *Identification* test *B.*]
Record the total ion chromatogram, and combine all of the mass spectra scans across the entire major peak. Record the peak intensities at mass-to-charge (*m/z*) ratios of 60, 61, 62, and 63.
Calculate the percentage of carbon that is C 13 in the portion of Urea C 13 taken:

$$\text{Result} = [(I_{61} + I_{63})/(I_{60} + I_{61} + I_{63})] \times 100$$

Calculate the percentage of oxygen that is O 18 in the portion of Urea C 13 taken:

$$\text{Result} = [(I_{62} + I_{63})/(I_{60} + I_{61} + I_{62} + I_{63})] \times 100$$

I_{60} = relative peak intensity at an *m/z* ratio of 60 in the *Sample solution*
I_{61} = relative peak intensity at an *m/z* ratio of 61 in the *Sample solution*
I_{62} = relative peak intensity at an *m/z* ratio of 62 in the *Sample solution*
I_{63} = relative peak intensity at an *m/z* ratio of 63 in the *Sample solution*

Acceptance criteria
C 13: NLT 99%
O 18: NMT 15%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Urea C 13 RS

Urea C 13 for Oral Solution

DEFINITION
Urea C 13 for Oral Solution is a dry powder prepared from Urea C 13. It contains NLT 90.0% and NMT 110.0% of the labeled amount of urea C 13 ($^{13}CH_4N_2O$). It contains no preservatives.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Mobile phase: Acetonitrile, methanol, and water (89:10:1)
System suitability solution: 2.5 mg/mL of urea and 0.003 mg/mL of biuret in *Mobile phase*
Standard solution: 2 mg/mL of USP Urea C 13 RS in *Mobile phase*
Sample solution: 2 mg/mL of urea C 13 from a portion of Urea C 13 for Oral Solution in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 25-cm; 5-µm packing L8
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between urea and biuret, *System suitability solution*
Relative standard deviation: NMT 1%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the areas for the major peaks.
Calculate the percentage of the labeled amount of urea C 13 ($^{13}CH_4N_2O$) in the portion of Urea C 13 for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Urea C 13 RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of urea C 13 in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩
Acceptance criteria
Total aerobic microbial count: NMT 10^3 cfu/g
Yeast count: NMT 10^2 cfu/g
Salmonella* species** andEscherichia coli*:** Absent
- **COMPLETENESS OF SOLUTION** ⟨641⟩
Sample solution: Nominally 100 mg/mL of urea C 13 from a portion of Urea C 13 for Oral Solution in carbon dioxide-free water
Acceptance criteria: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in sterile, well-closed containers. Store at 15°–30°.
- **LABELING:** Label it to indicate that the solution is to be discarded if particulate matter is visible after reconstitution.
[NOTE—It is to be reconstituted with sterile purified water.]
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Urea C 13 RS

www.webofpharma.com

Urea C 14 Capsules



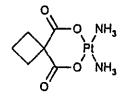
» Urea C 14 Capsules contain $^{14}CH_4N_2O$ in which a portion of the molecules are labeled with radioactive ^{14}C to provide 0.04 MBq (or 1 µCi) of radioactivity per capsule. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{14}C expressed as MBq (or µCi).

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
Expiration date—The expiration date is not later than two years from the date of manufacture.
Labeling—Label it to include the following: the amount of ^{14}C, expressed in MBq (or µCi) per Capsule at the time of calibration; the expiration date; the total radioactivity per container; and the statement, "Caution—Radioactive material."
Radionuclide identification ⟨821⟩—A solution of 1 or more Capsules in 1 N hydrochloric acid when tested using a liquid scintillation counter produces beta emission having a 49 keV mean and a 156 keV max.

Dissolution ⟨711⟩—
Medium: simulated gastric fluid TS; 500 mL.
Apparatus 1: 50 rpm.
Time: 10 minutes.
Procedure—Determine the background levels of ^{14}C with a 1-mL portion of the solution under test using a liquid scintillation counter.
Tolerances: not less than 80% *(Q)* of the labeled amount of ^{14}C is dissolved in 10 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Radionuclidic purity ⟨821⟩—Determine the radionuclidic purity of a solution of 1 or more Capsules in water using a liquid scintillation counter: not less than 99.9% of the radioactivity is present as C 14.

Radiochemical purity—
Adsorbent: 0.25-mm layer of chromatographic cellulose.
Test solution—Open 2 Capsules and place them in a suitable container, add 8 mL of methanol, and mix.
Reference solution: 40 mg of urea per mL, in water.
Application volume: 20 µL of the *Test solution* and 4 µL of the *Reference solution*.
Developing solvent system: n-butanol saturated with water.
Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. Locate the spots on the plate by spraying with Ehrlich's reagent. Determine the radioactivity distribution with a suitable radiation detector (see *Radioactivity* ⟨821⟩), and obtain the R_F value: the R_F value of the principal spot from the *Test solution* corresponds to that obtained from the *Reference solution*, and the radioactivity of the ^{14}C band is not less than 90% of the total radioactivity.
Assay for radioactivity ⟨821⟩—Prepare a solution of 1 or more Capsules in 1 N hydrochloric acid. Using a liquid scintillation counter, determine the radioactivity, in MBq (or mCi) per mL by use of a calibrated system.

Carboplatin



$C_6H_{12}N_2O_4Pt$ 371.25
Platinum, diammine[1,1-cyclobutanedicarboxylato(2-)-*O,O'*]-, (*SP*-4-2);
cis-Diammine(1,1-cyclobutanedicarboxylato)platinum [41575-94-4].

DEFINITION
Carboplatin contains NLT 98.0% and NMT 102.0% of carboplatin ($C_6H_{12}N_2O_4Pt$), calculated on the dried basis.
[**CAUTION**—Great care should be taken in handling Carboplatin because it is a suspected carcinogen.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile and water (87:13)
Standard solution: 1 mg/mL of USP Carboplatin RS in water. Use it within 2 h.
Sample solution: 1 mg/mL of Carboplatin in water. Use it within 2 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L8
Flow rate: 2.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 1.2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carboplatin ($C_6H_{12}N_2O_4Pt$) in the portion of Carboplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Carboplatin RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

OTHER COMPONENTS
- **PLATINUM CONTENT**

Sample: 0.2 g of Carboplatin, from *Loss on Drying*
Analysis: Ignite the *Sample* to constant weight at 800 ± 50°, and weigh the residue. The residue is platinum.
Calculate the platinum content in the portion of Carboplatin taken:

USP Monographs

www.webofpharma.com

$$\text{Result} = (W_U/W_S) \times 100$$

W_U = weight of platinum
W_S = weight of *Sample*

Acceptance criteria: 52.0%–53.0% on the dried basis

IMPURITIES

• **LIMIT OF 1,1-CYCLOBUTANEDICARBOXYLIC ACID**

Solution A: Dissolve 8.5 g of tetrabutylammonium hydrogen sulfate in 80 mL of water. Add 3.4 mL of phosphoric acid, and adjust with 10 N sodium hydroxide to a pH of 7.55.

Mobile phase: Acetonitrile, *Solution A*, and water (100:20:880)

Standard solution: 5 µg/mL of 1,1-cyclobutanedicarboxylic acid in *Mobile phase*

System suitability solution: 2.5 µg/mL of 1,1-cyclobutanedicarboxylic acid and 0.5 mg/mL of Carboplatin in *Mobile phase* prepared as follows. Mix 1.0 mL of *Standard solution* with 1.0 mL of *Standard solution* in the *Assay*.

Sample solution: 1 mg/mL of Carboplatin in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.0-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 100 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for carboplatin and 1,1-cyclobutanedicarboxylic acid are 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between the carboplatin and 1,1-cyclobutanedicarboxylic acid peaks

Relative standard deviation: NMT 10%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 1,1-cyclobutanedicarboxylic acid in the portion of Carboplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 1,1-cyclobutanedicarboxylic acid from the *Sample solution*
r_S = peak response of 1,1-cyclobutanedicarboxylic acid from the *Standard solution*
C_S = concentration of 1,1-cyclobutanedicarboxylic acid in the *Standard solution* (mg/mL)
C_U = concentration of Carboplatin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

• **ORGANIC IMPURITIES**

Mobile phase, Standard solution, Sample solution, and **System suitability:** Proceed as directed in the *Assay*.

Diluted standard solution: 2.5 µg/mL of USP Carboplatin RS in water, from the *Standard solution*

Chromatographic system: Proceed as directed in the *Assay*, and the run time is at least 2.5 times the retention time of the carboplatin peak.

Analysis

Samples: *Sample solution* and *Diluted standard solution*

Calculate the percentage of each impurity in the portion of Carboplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of carboplatin from the *Diluted standard solution*
C_S = concentration of USP Carboplatin RS in the *Diluted standard solution* (mg/mL)
C_U = concentration of Carboplatin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cisplatin[a]	0.3	0.25
Carboplatin	1.0	—
Any individual unspecified impurity	—	0.25
Total impurities	—	0.5

[a] *cis*-Diamminedichloroplatinum(II).

SPECIFIC TESTS

• **CRYSTALLINITY** ⟨695⟩: Meets the requirements

• **pH** ⟨791⟩

Sample solution: 10 mg/mL in water

Acceptance criteria: 5.0–7.0

• **LOSS ON DRYING** ⟨731⟩

Sample: 1 g

Analysis: Dry the *Sample* at 105° to constant weight.

Acceptance criteria: NMT 0.5%

• **TRANSMITTANCE**

Sample solution: 10 mg/mL of Carboplatin in water

Analysis: Determine the percent transmittance in 1-cm cells at a wavelength of 440 nm, using water as the blank.

Acceptance criteria: NLT 97%

• **WATER-INSOLUBLE MATTER**

Sample: 1 g

Analysis: Transfer the *Sample* to a 150-mL beaker. Add 100 mL of water, and dissolve by stirring with a stirring bar for 30 min. With the aid of suction, pass through a tared filtering crucible. Rinse the beaker with water, and transfer the rinsings to the crucible. Dry the crucible at 130 ± 10° to constant weight.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Carboplatin RS

Carboplatin for Injection

DEFINITION

Carboplatin for Injection is a sterile, lyophilized mixture of Carboplatin and Mannitol. It contains NLT 90.0% and NMT 110.0% of the labeled amount of carboplatin ($C_6H_{12}N_2O_4Pt$).

[**CAUTION**—Great care should be taken in handling Carboplatin because it is a suspected carcinogen.]

www.webofpharma.com

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution: 10 mg/mL of USP Carboplatin RS in water

Sample solution: Nominally equivalent to 10 mg/mL of carboplatin in water from the contents of one container

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 µL

Developing solvent system: Acetone and water (80:20)

Spray reagent: Add 5.6 g of stannous chloride to 10 mL of hydrochloric acid, and stir for 5 min. [Note—It is not necessary that all of the solids dissolve.] Add 90 mL of water and 1 g of potassium iodide, and stir. Prepare this solution fresh daily.

Analysis

Samples: *Standard solution* and *Sample solution*

Place the plate in a chromatographic chamber lined with filter paper and equilibrated for 2 h in *Developing solvent system*. Develop the chromatogram until the solvent front has moved 10 cm from the origin. Remove the plate from the chamber, and air-dry at room temperature for 2 h. Spray with the *Spray reagent*, and heat at 110° for 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in appearance and R_F value to that of the *Standard solution*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Mobile phase: Acetonitrile and water (87:13)

Standard solution: 1 mg/mL of USP Carboplatin RS. Use this solution within 2 h.

Sample solution: Nominally equivalent to 1 mg/mL of carboplatin from the contents of one container diluted with water. Complete chromatographic analysis of this solution within 2 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L8

Flow rate: 2.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 1.2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carboplatin ($C_6H_{12}N_2O_4Pt$) in the portion of Carboplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Carboplatin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of carboplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Uniformity of Dosage Units** ⟨905⟩**:** Meets the requirements

IMPURITIES

• **Limit of 1,1-Cyclobutanedicarboxylic Acid**

Solution A: Dissolve 8.5 g of tetrabutylammonium hydrogen sulfate in 80 mL of water. Add 3.4 mL of phosphoric acid, and adjust with 10 N sodium hydroxide to a pH of 7.55.

Mobile phase: Acetonitrile, *Solution A*, and water (100:20:880)

Standard solution A: 0.01 mg/mL of 1,1-cyclobutanedicarboxylic acid in *Mobile phase*

Standard solution B: 5 µg/mL of 1,1-cyclobutanedicarboxylic acid in *Mobile phase*

System suitability solution: 2.5 µg/mL of 1,1-cyclobutanedicarboxylic acid and 0.5 mg/mL of carboplatin prepared as follows. Mix 1.0 mL of *Standard solution B* with 1.0 mL of *Standard solution* in the *Assay*.

Sample solution: Nominally equivalent to 1 mg/mL of carboplatin from the contents of one container diluted with *Mobile phase*. Complete the chromatographic analysis of the solution within 2 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.0-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 100 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for carboplatin and 1,1-cyclobutanedicarboxylic acid are 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between the carboplatin and 1,1-cyclobutanedicarboxylic acid peaks

Relative standard deviation: NMT 10%

Analysis

Samples: *Standard solution A* and *Sample solution*

Calculate the percentage of 1,1-cyclobutanedicarboxylic acid in the portion of Carboplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 1,1-cyclobutanedicarboxylic acid from the *Sample solution*

r_S = peak response of 1,1-cyclobutanedicarboxylic acid from *Standard solution A*

C_S = concentration of 1,1-cyclobutanedicarboxylic acid in *Standard solution A* (mg/mL)

C_U = nominal concentration of carboplatin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

• **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 0.54 USP Endotoxin Units/mg of carboplatin

• **Sterility Tests** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements

• **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

• **pH** ⟨791⟩

Sample solution: Use Sterile Water for Injection, and constitute as directed in the labeling.

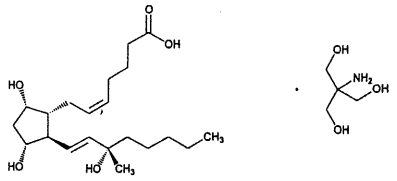
Acceptance criteria: 5.0–7.0

www.webofpharma.com

• **WATER DETERMINATION** ⟨921⟩, *Method I*
Analysis: Use anhydrous formamide as the extraction solvent. Introduce 50 mL of anhydrous formamide into the titration vessel, and titrate with the *Reagent* to the electrometric endpoint. Use the formamide thus dried to rinse a suitable glass syringe equipped with an 8-cm long, 22-gauge needle. Add the rinse back to the titration vessel, and titrate the vessel contents again, if necessary. Via the syringe, withdraw 5 mL of the formamide thus titrated and, through the closure of the container, expel the contents into the container. Shake the container to obtain a solution. With the same syringe, withdraw all of the contents of the container, and transfer to the titration vessel. Titrate to the endpoint, adjusting the feeding speed control to the lowest setting to avoid overtitration.
Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution,* and protect from light.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Carboplatin RS

Carboprost Tromethamine



$C_{21}H_{36}O_5 \cdot C_4H_{11}NO_3$ 489.64
Prosta-5,13-dien-1-oic acid, 9,11,15-trihydroxy-15-methyl-, (5*Z*, 9α,11α,13*E*,15*S*)-, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1);
(*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-[(*E*)-(3*S*)-3-hydroxy-3-methyl-1-octenyl]cyclopentyl]-5-heptenoic acid compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1);
(15*S*)-15-Methylprostaglandin $F_{2\alpha}$ tromethamine [58551-69-2].

DEFINITION
Carboprost Tromethamine contains NLT 95.0% and NMT 105.0% of carboprost tromethamine ($C_{21}H_{36}O_5 \cdot C_4H_{11}NO_3$), calculated on the dried basis.
[**CAUTION**—Great care should be taken to prevent inhaling particles of Carboprost Tromethamine and exposing the skin to it.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 0.02 M monobasic potassium phosphate
Mobile phase: Acetonitrile, methanol, and *Buffer* (20:30:50). Adjust with phosphoric acid to an apparent pH of 3.0.
Diluent: Acetonitrile, methanol, and water (20:30:50)
Standard solution: 1.0 mg/mL of USP Carboprost Tromethamine RS in *Diluent*
Sample solution: 1.0 mg/mL of Carboprost Tromethamine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4-mm × 15-cm; 3-µm packing L1
Column temperature: 30 ± 2°
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for the relative retention times of *trans*-carboprost and carboprost.]
Suitability requirements
Resolution: NLT 1.2 between *trans*-carboprost and carboprost
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carboprost tromethamine ($C_{21}H_{36}O_5 \cdot C_4H_{11}NO_3$) in the portion of Carboprost Tromethamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Carboprost Tromethamine RS in the *Standard solution* (mg/mL)
C_U = concentration of Carboprost Tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%
• **ORGANIC IMPURITIES**
Buffer, Mobile phase, and **Diluent:** Prepare as directed in the *Assay*.
Standard stock solution: 1.0 mg/mL of USP Carboprost Tromethamine RS in *Diluent*
System suitability solution: 0.1 mg/mL of 15-epicarboprost in the *Standard stock solution*
Standard solution: 0.025 mg/mL of USP Carboprost Tromethamine RS in *Diluent* from the *Standard stock solution*
Sensitivity solution: 0.001 mg/mL of USP Carboprost Tromethamine RS in *Diluent* from the *Standard stock solution*
Sample solution: 1 mg/mL of Carboprost Tromethamine in *Diluent*
Chromatographic system: Proceed as directed in the *Assay*, except use an *Injection volume* of 20 µL.
System suitability
Samples: *System suitability solution, Standard solution,* and *Sensitivity solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.0 between 15-epicarboprost and *trans*-carboprost; NLT 1.2 between *trans*-carboprost and carboprost, *System suitability solution*

www.webofpharma.com

Relative standard deviation: NMT 5.0% for carboprost, *Standard solution*
Signal-to-noise ratio: NLT 10 for carboprost, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Carboprost Tromethamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of carboprost tromethamine from the *Standard solution*
C_S = concentration of USP Carboprost Tromethamine RS in the *Standard solution* (mg/mL)
C_U = concentration of Carboprost Tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
15-Epicarboprost[a]	0.90	2.0
trans-Carboprost[b]	0.94	3.0
Carboprost	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	4.0

[a] (*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-[(*E*)-(3*R*)-3-hydroxy-3-methyloct-1-enyl]cyclopentyl]-5-heptenoic acid.
[b] (*E*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-[(*E*)-(3*S*)-3-hydroxy-3-methyloct-1-enyl]cyclopentyl]-5-heptenoic acid.

SPECIFIC TESTS
• **Optical Rotation,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL of Carboprost Tromethamine in alcohol
Acceptance criteria: +18° to +24°
• **Loss on Drying** ⟨731⟩
Analysis: Dry under vacuum at a pressure not exceeding 5 mm of mercury at 50° for 16 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers, and store in a freezer.
• **USP Reference Standards** ⟨11⟩
USP Carboprost Tromethamine RS

Carboprost Tromethamine Injection

DEFINITION
Carboprost Tromethamine Injection is a sterile solution of Carboprost Tromethamine in aqueous solution, which may also contain Benzyl Alcohol, Sodium Chloride, and Tromethamine. It contains NLT 90.0% and NMT 110.0% of the labeled amount of carboprost ($C_{21}H_{36}O_5$).

IDENTIFICATION
Change to read:
• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Extract the equivalent of 2.5 mg of carboprost tromethamine from a volume of Injection, with 1.5–2 times its volume of chloroform. Discard the chloroform layer, and acidify the aqueous layer with 3–5 drops of hydrochloric acid. Extract the acidified solution with an equivalent volume of chloroform. Filter the chloroform layer through a pledget of cotton, and concentrate it to a volume of less than 1 mL. Combine the resulting solution with 150–180 mg of potassium bromide. Dry the potassium bromide mixture in a vacuum overnight, and prepare a pellet from the dried mixture.
Acceptance criteria: Meets the requirements

ASSAY
• **Procedure**
Buffer: Dissolve 10.5 g of citric acid in 75 mL of water.Adjust with 5 N sodium hydroxide to a pH of 4.0, and dilute with water to 100 mL.
Mobile phase: Methylene chloride, 1,3-butanediol, and water (992: 7: 0.5)
Internal standard solution: 3 mg/mL of guaifenesin in *Mobile phase*
System suitability solution: Transfer 5 mg of USP Carboprost Tromethamine RS to a stoppered, 50-mL centrifuge tube. Add 20.0 mL of methylene chloride and 2 mL of *Buffer*. Shake the stoppered tube for 10 min, and centrifuge. Remove and discard the top (aqueous) layer, and transfer a 4.0-mL aliquot of the lower (methylene chloride) layer to a suitable vial. Evaporate with the aid of a stream of nitrogen to dryness. Add 100 µL of a freshly prepared solution of α-bromo-2′-acetonaphthone in acetonitrile (1 in 50). Swirl to wash down the sides of the vial. Add 50 µL of a freshly prepared solution of diisopropylethylamine in acetonitrile (1 in 100), swirl again, and place the vial in a suitable heating device maintained at 30°–35° for NLT 15 min. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 2.0 mL of *Internal standard solution*, mix, and pass the resulting solution through a filter of fine pore size. Protect the filtrate from light prior to injection to prevent degradation of the naphthacyl ester of carboprost.
Standard stock solution: Prepare a solution containing 332 µg/mL of USP Carboprost Tromethamine RS and 9 mg/mL of benzyl alcohol.
Standard solution: 266 µg/mL of USP Carboprost Tromethamine RS, prepared as follows. Transfer 2.0 mL of *Standard stock solution* into a stoppered centrifuge tube. Add 20.0 mL of methylene chloride and 1.0 mL of *Buffer*, shake the stoppered tube for 10 min, and centrifuge. Remove and discard the top (aqueous) layer, transfer an 8.0-mL aliquot of the lower (methylene chloride) layer to a suitable vial, and evaporate the solution with the aid of a stream of nitrogen. [Note—The residue does not evaporate to dryness because of the presence of benzyl alcohol.] Add 100 µL of a freshly prepared solution of α-bromo-2′-acetonaphthone in acetonitrile (1 in 50), and swirl to wash down the sides of the vial. Add 50 µL of a freshly prepared solution of diisopropylethylamine in acetonitrile (1 in 100). Swirl again, and place the vial in a suitable heating device maintained at 30°–35° for NLT 15 min. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 1.0 mL of *Internal standard solution*, mix, and pass the resulting solution through a filter of fine

USP Monographs

www.webofpharma.com

pore size. Protect the filtrate from light prior to injection to prevent degradation of the naphthacyl ester of carboprost.

Sample solution: Nominally 200 µg/mL of carboprost, prepared as follows. Pipet a volume of Injection, equivalent to 500 µg of carboprost, to a stoppered, 50-mL centrifuge tube. Add 20.0 mL of methylene chloride and 1.0 mL of *Buffer*, shake the stoppered tube for 10 min, and centrifuge. Remove and discard the top (aqueous) layer, transfer an 8.0-mL aliquot of the lower (methylene chloride) layer to a suitable vial, and evaporate the solution with the aid of a stream of nitrogen. [NOTE—The residue does not evaporate to dryness because of the presence of benzyl alcohol.] Add 100 µL of a freshly prepared solution of α-bromo-2′-acetonaphthone in acetonitrile (1 in 50), and swirl to wash down the sides of the vial. Add 50 µL of a freshly prepared solution of diisopropylethylamine in acetonitrile (1 in 100). Swirl again, and place the vial in a suitable heating device maintained at 30°–35° for NLT 15 min. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 1.0 mL of *Internal standard solution*, mix, and pass the resulting solution through a filter of fine pore size. Protect the filtrate from light prior to injection to prevent degradation of the naphthacyl ester of carboprost.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L3
Flow rate: 1.8 mL/min
Injection volume: 10 µL

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for guaifenesin and 2-naphthacyl ester of carboprost are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between guaifenesin and 2-naphthacyl ester of carboprost
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of carboprost ($C_{21}H_{36}O_5$) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of the 2-naphthacyl ester of carboprost to the internal standard of the *Sample solution*
R_S = peak response ratio of the 2-naphthacyl ester of carboprost to the internal standard of the *Standard solution*
C_S = concentration of USP Carboprost Tromethamine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of carboprost in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of carboprost, 368.51
M_{r2} = molecular weight of carboprost tromethamine, 489.64

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 714.3 USP Endotoxin Units/mg of carboprost tromethamine
- **pH** ⟨791⟩: 7.0–8.0
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and store in a refrigerator.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Carboprost Tromethamine RS

Carboxymethylcellulose Sodium

Cellulose carboxymethyl ether sodium salt [9004-32-4].

DEFINITION
Carboxymethylcellulose Sodium is the sodium salt of a polycarboxymethyl ether of cellulose. It contains NLT 6.5% and NMT 9.5% of sodium (Na), calculated on the dried basis.

IDENTIFICATION
- **A. PROCEDURE**
Sample solution: Add 1 g of powdered Carboxymethylcellulose Sodium to 50 mL of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced.
Analysis: To 1 mL of the *Sample solution*, diluted with an equal volume of water in a small test tube, add 5 drops of 1-naphthol TS. Incline the test tube, and carefully introduce down the side of the tube 2 mL of sulfuric acid so that it forms a lower layer.
Acceptance criteria: A red-purple color develops at the interface.
- **B. PROCEDURE**
Sample solution: Use the *Sample solution* from *Identification* test *A*.
Analysis: To 5 mL of the *Sample solution*, add an equal volume of barium chloride TS.
Acceptance criteria: A fine, white precipitate is formed.
- **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩: A portion of the *Sample solution* meets the requirements.
Sample solution: Use the *Sample solution* from *Identification* test *A*.

ASSAY
- **PROCEDURE**
Sample solution: Transfer to a beaker 500 mg of Carboxymethylcellulose Sodium, add 80 mL of glacial acetic acid, heat the mixture in a boiling water bath for 2 h, and cool to room temperature.
Analysis: Titrate the *Sample solution* with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 2.299 mg of Na.
Acceptance criteria: NLT 6.5% and NMT 9.5% of Na, on the dried basis

SPECIFIC TESTS
- **VISCOSITY—ROTATIONAL METHODS** ⟨912⟩
Analysis: Determine the viscosity in a water solution at the concentration stated on the label. Using undried Carboxymethylcellulose Sodium, weigh the amount that, on the dried basis, will provide 200 g of solution of the stated concentration. Add the substance in small amounts to 180 mL of stirred water contained in a tared, wide-mouth bottle, continue stirring rapidly until the powder is well wetted, add sufficient water to make the mixture weigh 200 g, and allow to stand, with occasional stirring, until solution is complete. Adjust the temperature to 25 ± 0.2°, and determine the viscosity, using a rotational type of viscometer, making certain that the system reaches equilibrium before taking the final reading.

www.webofpharma.com

Acceptance criteria: The viscosity of solutions of 2% or higher concentration is NLT 80.0% and NMT 120.0% of that stated on the label; the viscosity of solutions of less than 2% concentration is NLT 75.0% and NMT 140.0% of that stated on the label.

- **pH** ⟨791⟩: 6.5–8.5 in a solution (1 in 100)
- **Loss on Drying** ⟨731⟩: Dry a sample at 105° for 3 h: it loses NMT 10.0% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label it to indicate the viscosity in solutions of stated concentrations.

Carboxymethylcellulose Sodium Paste

DEFINITION

Carboxymethylcellulose Sodium Paste contains NLT 16.0% and NMT 17.0% of carboxymethylcellulose sodium.

IDENTIFICATION

- **A.**

Sample solution: Digest a quantity of Paste, equivalent to 1 g of carboxymethylcellulose sodium, with 50 mL of water until the solution is virtually complete. Filter.

Analysis: To 30 mL of the *Sample solution* add 3 mL of hydrochloric acid. Filter the solution, and save the filtrate for *Identification* test *C*.

Acceptance criteria: A white precipitate is formed.

- **B.**

Sample solution: The remainder of the *Sample solution* prepared in *Identification* test *A*

Analysis: To the *Sample solution* add an equal volume of barium chloride TS.

Acceptance criteria: A fine, white precipitate is formed.

- **C. Identification Tests—General,** *Sodium* ⟨191⟩: The filtrate from *Identification* test *A* meets the requirements of the tests.

ASSAY

- **Procedure**

Sample: 2 g

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N perchloric acid in dioxane VS

Endpoint detection: Potentiometric

Analysis: Transfer the *Sample* to a glass-stoppered, 250-mL conical flask. Add 75 mL of glacial acetic acid, attach a condenser, and reflux for 2 h. Cool, transfer the mixture to a 250-mL beaker with the aid of small volumes of glacial acetic acid. Titrate with *Titrant*. Each mL of 0.1 N perchloric acid is equivalent to 29.67 mg of carboxymethylcellulose sodium.

Acceptance criteria: 16.0%–17.0%

SPECIFIC TESTS

- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total bacterial count does not exceed 10^3 cfu/g, and the tests for *Salmonella* species and *Escherichia coli* are negative.
- **Consistency**

Apparatus: A penetrometer fitted with a polished cone-shaped metal plunger weighing 150 g, having a detachable steel tip of the following dimensions: the tip of the cone has an angle of 30°; the point of the tip is truncated to a diameter of 0.381 ± 0.025 mm; the base of the tip is 8.38 ± 0.05 mm in diameter; and the length of the tip is 14.94 ± 0.05 mm.

The remaining portion of the cone has an angle of 90°, is 28 mm in height, and has a maximum diameter at the base of 65 mm. The containers for the test are flat-bottom metal cylinders that are 100 ± 6 mm in diameter and NLT 65 mm in height. They are constructed of at least 1.6-mm (16-gauge) metal, and are provided with well-fitting, water-tight covers.

Sample: Paste

Analysis: Place the required number of containers in an oven, bring them and a quantity of the *Sample* to a temperature of 82 ± 2.5°, and pour the *Sample* into one or more of the containers, filling to within 6 mm of the rim. Cool to 25 ± 2.5° over a period of NLT 16 h, protected from drafts. Two h before the test, place the containers in a water bath at 25 ± 0.5°. If the room temperature is below 23.5° or above 26.5°, adjust the temperature of the cone to 25 ± 0.5° by placing it in the water bath.

Without disturbing the surface of the substance under test, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the test substance at a spot 25–38 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 s. Secure the plunger, and read the total penetration from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the test substance for each trial. Read the penetration to the nearest 0.1 mm.

Calculate the average of the three or more readings, and conduct further trials to a total of 10 if the individual results differ from the average by more than ±3%.

Acceptance criteria: The final average of the trials is 30.0–36.0 mm, indicating a consistency value of between 300 and 360.

- **Loss on Drying** ⟨731⟩

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, and avoid prolonged exposure to temperatures exceeding 30°.

Carboxymethylcellulose Sodium Tablets

» Carboxymethylcellulose Sodium Tablets contain an amount of sodium (Na) equivalent to not less than 6.5 percent and not more than 9.5 percent of the labeled amount of carboxymethylcellulose sodium.

Packaging and storage—Preserve in tight containers.

Identification—Digest a quantity of powdered Tablets, equivalent to about 1 g of carboxymethylcellulose sodium, with 50 mL of water until solution is virtually complete, and filter: the filtrate responds to the following tests.

A: To about 30 mL of the solution add 3 mL of hydrochloric acid: a white precipitate is formed.

B: To the remainder of the solution add an equal volume of barium chloride TS: a fine, white precipitate is formed.

C: The filtrate from *Identification* test *A* responds to the tests for *Sodium* ⟨191⟩.

Disintegration ⟨701⟩: 2 hours.

USP Monographs

www.webofpharma.com

Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 500 mg of carboxymethylcellulose sodium, add 80 mL of glacial acetic acid, heat the mixture on a steam bath for 2 hours, cool to room temperature, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 2.299 mg of Na.

Add the following:

▲Carboxymethylcellulose Sodium Compounded Intraperitoneal Solution, Veterinary

DEFINITION

Carboxymethylcellulose Sodium Compounded Intraperitoneal Solution, Veterinary contains NLT 80.0% and NMT 120.0% of the labeled amount of carboxymethylcellulose sodium ($C_8H_{15}NaO_8$). Prepare Carboxymethylcellulose Sodium Compounded Intraperitoneal Solution, Veterinary 10 mg/mL (1%) as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Carboxymethylcellulose Sodium[a]	5 g
Propylene Glycol	10 mL
Sterile Water for Injection, a sufficient quantity to make	500 mL

[a] High-viscosity carboxymethylcellulose sodium must be used.

Wet the *Carboxymethylcellulose Sodium* with the *Propylene Glycol* in a suitable container. Place 450 mL of *Sterile Water for Injection* in a calibrated container on a hot plate with a stir bar and heat to NMT 100° (do not allow to boil). Add the *Carboxymethylcellulose Sodium* and *Propylene Glycol* mixture to heated *Sterile Water for Injection* by pouring along the outer edge of the water level and not directly into the vortex created by the stir bar to avoid clumping. Continue heating and stirring to facilitate complete dissolution of the *Carboxymethylcellulose Sodium*. [NOTE—This may take 30–60 min to completely dissolve.] Once completely dissolved, bring to final volume with *Sterile Water for Injection*. Transfer to a 500-mL high-density polyethylene (HDPE) plastic single-dose container suitable for autoclaving and crimp seals tightly before autoclaving. Sterilize by autoclaving.

ASSAY

- **PROCEDURE**

Mobile phase: 0.052% (v/v) methanesulfonic acid
Diluent: 0.52% (v/v) methanesulfonic acid
Standard solution: 0.03 mg/mL of sodium prepared by mixing USP Sodium Chloride RS and water
Sample solution: Transfer 1 mL of Intraperitoneal Solution, Veterinary into a 25-mL volumetric flask and dilute with *Diluent* to volume.
[NOTE—The *Standard solution* and *Sample solution* are stable for only 24 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity
Column: 4.0-mm × 25-cm; packing L77
Column temperature: 15°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for sodium is about 3.3 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sodium (Na) in the portion of Intraperitoneal Solution, Veterinary:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of sodium from the *Sample solution*
r_S = peak response of sodium from the *Standard solution*
C_S = concentration of sodium in the *Standard solution* (mg/mL)
C_U = nominal concentration of sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 80.0%–120.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 6.7–7.7
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*: Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 1.75 USP Endotoxin Units/mL of carboxymethylcellulose sodium
- **VISCOSITY—ROTATIONAL METHODS** ⟨912⟩

Sample: 5 mL of Intraperitoneal Solution, Veterinary
Analysis: Determine at 25° by using a rotational viscometer.
Acceptance criteria: Between 600 and 1,200 centipoises

ADDITIONAL REQUIREMENTS

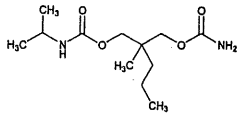
- **PACKAGING AND STORAGE:** Package in 500-mL autoclavable HDPE plastic containers. Store at controlled room temperature.
- **LABELING:** Label it to indicate to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** In the absence of performing and completing a sterility and endotoxins test, the storage conditions in *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩, *CSP Microbial Contamination Risk Levels, High-Risk Level CSPs* apply. After successful completion of sterility and endotoxin testing, NMT 60 days after the day on which it was compounded when stored at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sodium Chloride RS▲ (USP 1-May-2020)

USP Monographs

www.webofpharma.com

Carisoprodol



$C_{12}H_{24}N_2O_4$ 260.33

(±)-2-Methyl-2-propyl-1,3-propanediol carbamate isopropylcarbamate [78-44-4].

DEFINITION

Carisoprodol contains NLT 98.0% and NMT 102.0% of $C_{12}H_{24}N_2O_4$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak in the *Sample solution* corresponds to that in the *Standard solution* as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: Acetonitrile and water (50:50)
Solution A: Acetonitrile and water (25:75)
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	100	0
36	80	20
51	80	20
52	100	0
60	100	0

System suitability solution: 0.125 mg/mL each of USP Carisoprodol Related Compound A RS, USP Meprobamate RS, and USP Carisoprodol RS in *Diluent*
Standard solution: 2.5 mg/mL of USP Carisoprodol RS in *Diluent*
Sample solution: 2.5 mg/mL of Carisoprodol in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 15-cm; 4-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection size: 25 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between carisoprodol related compound A and meprobamate, *System suitability solution*
Tailing factor: NMT 2.5 for the carisoprodol peak, *Standard solution*
Relative standard deviation: NMT 2.0% for the carisoprodol peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carisoprodol ($C_{12}H_{24}N_2O_4$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carisoprodol from the *Sample solution*
r_S = peak response of carisoprodol from the *Standard solution*
C_S = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)
C_U = concentration of Carisoprodol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Diluent, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 10 µg/mL of USP Carisoprodol RS in *Diluent*
Sample solution: 50 mg/mL of Carisoprodol in *Diluent*. [NOTE—Sonication may be used to aid dissolution.]
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between carisoprodol related compound A and meprobamate, *System suitability solution*
Tailing factor: NMT 2.5 for the carisoprodol peak, *Standard solution*
Relative standard deviation: NMT 5.0% for the carisoprodol peak, 3 replicate injections of *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Identify the specified impurities using the relative retention times given in *Table 2*.
Calculate the percentage of each impurity in the portion of Carisoprodol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Sample solution*
r_S = peak response of carisoprodol from the *Standard solution*
C_S = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)
C_U = concentration of Carisoprodol in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Carisoprdol related compound A[a]	0.19	0.06	0.1

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Meprobamate	0.24	0.08	0.5
Carisoprodol mono-carbamate[b]	0.86	1.4	0.1
Carisoprodol	1.0	—	—
Any other unknown individual impurity	—	1.0	0.1
Total impurities	—	—	1.0

[a] 2-Hydroxymethyl-2-methylpentyl carbamate.
[b] *N*-Isopropyl-2-hydroxymethyl-2-methylpentyl carbamate.

SPECIFIC TESTS
- **Loss on Drying** ⟨731⟩: Dry a sample in vacuum at 60° for 3 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers at room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Carisoprodol RS
  USP Carisoprodol Related Compound A RS
  2-Hydroxymethyl-2-methylpentyl carbamate.
  $C_8H_{17}NO_3$ 175.23
  USP Meprobamate RS

Carisoprodol Tablets

DEFINITION
Carisoprodol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carisoprodol ($C_{12}H_{24}N_2O_4$).

IDENTIFICATION
- **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197A**▲ (CN 1-May-2020)
  Standard: Use a suitable portion of USP Carisoprodol RS.
  Sample: Powder a Tablet and use a suitable portion to prepare a specimen.
  Analysis
  Samples: *Standard* and *Sample*
  Compare the background corrected spectra in the range between 4000 and 400 cm^{-1}.
  Acceptance criteria: The spectrum obtained from the *Sample* shows bands at approximately 3445, 1689, 1604, 1525, 1410, 1244, 1072, and 780 cm^{-1}, similar to the spectrum from the *Standard*. [Note—Peak positions may vary slightly between instruments (±10 cm^{-1}). Other peaks may be present in the spectra that do not appear on the list.]

ASSAY
- **Procedure**
  Diluent: Acetonitrile and water (50:50)
  Mobile phase: Acetonitrile and water (25:75)
  System suitability solution: 0.1 mg/mL each of USP Carisoprodol Related Compound A RS, USP Meprobamate RS, and USP Carisoprodol RS in *Diluent*
  Standard solution: 2.5 mg/mL of USP Carisoprodol RS in *Diluent*
  Sample solution: Nominally 2.5 mg/mL of carisoprodol in *Diluent* prepared as follows. Transfer a quantity equivalent to the label claim of carisoprodol from powdered Tablets (NLT 20) to a suitable volumetric flask, and fill 50% of the flask volume with *Diluent*. Place in an ultrasonic bath for 30 min, and shake mechanically for 60 min. Dilute with *Diluent* to volume. Pass a portion of this solution through a suitable membrane filter, and use the filtrate as the *Sample solution*.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 200 nm
  Column: 4.6-mm × 15-cm; 4-µm packing L1
  Column temperature: 30°
  Flow rate: 1.5 mL/min
  Injection volume: 25 µL
  Run time: 1.5 times the retention time of carisoprodol
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [Note—See *Table 1* for the relative retention times.]
  Suitability requirements
  Resolution: NLT 1.5 between carisoprodol related compound A and meprobamate, *System suitability solution*
  Tailing factor: NMT 2.5 for the carisoprodol peak, *Standard solution*
  Relative standard deviation: NMT 2.0% for the carisoprodol peak, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of carisoprodol ($C_{12}H_{24}N_2O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carisoprodol from the *Sample solution*
r_S = peak response of carisoprodol from the *Standard solution*
C_S = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of carisoprodol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **Dissolution** ⟨711⟩
  Medium: 0.05 M phosphate buffer, pH 6.9 (see *Reagents, Indicators, and Solutions—Buffer Solutions*) containing 5 units of α-amylase per mL; 900 mL
  [Note—Use only freshly prepared solutions containing α-amylase; and equilibrate the *Medium* at 37° for NMT 1 h before beginning the *Dissolution* test.]
  Apparatus 2: 75 rpm
  Time: 60 min
  Mobile phase: Acetonitrile and water (40:60)
  System suitability solution: 2.4 mg/mL of 2-methyl-2-propyl-1,3-propanediol and 3.4 mg/mL of USP Carisoprodol RS in *Mobile phase*
  Standard solution: 0.4 mg/mL of USP Carisoprodol RS in *Medium*
  [Note—A volume of acetonitrile not exceeding 2% of the final volume of solution may be used to aid in dissolving carisoprodol.]
  Sample solution: Pass a portion of the solution under test through a suitable filter.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 3.9-mm × 30-cm; packing L1
Temperatures
Column: 30 ± 1°
Detector: 30 ± 1°
Flow rate: 2 mL/min
Injection volume: 150 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for 2-methyl-2-propyl-1,3-propanediol and carisoprodol are about 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between 2-methyl-2-propyl-1,3-propanediol and carisoprodol, *System suitability solution*
Relative standard deviation: NMT 2.0% for three replicate injections of the *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Record the peak responses, and measure the heights for the major peaks.
Calculate the percentage of the labeled amount of carisoprodol ($C_{12}H_{24}N_2O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of carisoprodol ($C_{12}H_{24}N_2O_4$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Diluent, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.01 mg/mL of USP Carisoprodol RS in *Diluent*
Sample solution: Nominally 10 mg/mL of carisoprodol in *Diluent* prepared as follows. Transfer a quantity equivalent to four times the label claim of carisoprodol from powdered Tablets (NLT 20 Tablets) to a suitable volumetric flask, and fill 50% of the flask volume with *Diluent*. Place in an ultrasonic bath for 30 min, and shake mechanically for 60 min. Dilute with *Diluent* to volume. Pass a portion of this solution through a suitable membrane filter, and use the filtrate as the *Sample solution*.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between carisoprodol related compound A and meprobamate, *System suitability solution*
Tailing factor: NMT 2.5 for the carisoprodol peak, *Standard solution*
Relative standard deviation: NMT 5.0% for the carisoprodol peak for three replicate injections of the *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Identify the specified impurities using the relative retention times given in *Table 1*.
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of carisoprodol from the *Standard solution*
C_S = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of carisoprodol in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Carisoprodol related compound A	0.19	0.06	0.75
Meprobamate	0.24	0.08	0.65
Carisoprodol monocarbamate[a]	0.86	1.4	0.20
Carisoprodol	1.0	—	—
Any other unknown degradation product	—	1.0	0.20
Total impurities	—	—	1.25

[a] 2-Hydroxymethyl-2-methylpentyl isopropylcarbamate.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Carisoprodol RS
USP Carisoprodol Related Compound A RS
2-Hydroxymethyl-2-methylpentyl carbamate.
$C_8H_{17}NO_3$ 175.23
USP Meprobamate RS

Carisoprodol and Aspirin Tablets

DEFINITION

Carisoprodol and Aspirin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of carisoprodol ($C_{12}H_{24}N_2O_4$) and aspirin ($C_9H_8O_4$).

IDENTIFICATION

• **A.** The retention times of aspirin and carisoprodol from the *Sample solution* correspond to those of *Standard solution A*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: Combine 5 mL of glacial acetic acid and 500 mL of water, and pass the mixture through a membrane filter of 0.5-µm or finer pore size. Use the filtrate.

Mobile phase: Methanol and *Buffer* (64:36)

Diluent: Acetonitrile, glacial acetic acid, and water (40:1:59)

Standard solution A: USP Reference Standards in *Diluent* as listed below and prepared as follows. Transfer 80 mg of USP Aspirin RS and 80*J* mg of USP Carisoprodol RS to a 25-mL volumetric flask. Add 15 mL of *Diluent*, swirl for 5 min, and sonicate for 25–30 s. Dilute with *Diluent* to volume.

Aspirin: 3.2 mg/mL of USP Aspirin RS

Carisoprodol: 3.2*J* mg/mL of USP Carisoprodol RS, where *J* is the ratio of the labeled amount, in mg, of carisoprodol to the labeled amount of aspirin

Standard solution B: 0.016 mg/mL of USP Salicylic Acid RS in *Diluent*

System suitability solution: 0.5 mg/mL of salicylic acid in *Standard solution A*

Sample solution: Nominally 3.25 mg/mL of aspirin prepared as follows. Finely powder NLT 20 Tablets. Transfer a portion of powder, equivalent to 325 mg of aspirin, to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and swirl for 5 min. Sonicate for 25–30 s, shake by mechanical means for 30 min, and dilute with *Diluent* to volume. Pass a portion of this solution through a membrane filter of 0.5-µm or finer pore size, and use the filtrate within 8 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector

Aspirin and carisoprodol: Refractive index

Salicylic acid: UV 313 nm

Column: 4.6-mm × 25-cm; packing L7

Temperatures

Refractive index detector: 30 ± 1°

Column: 30 ± 1°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *Standard solution A*, *Standard solution B*, and *System suitability solution*

[NOTE—The relative retention times for aspirin, salicylic acid, and carisoprodol are about 0.6, 0.7, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.2 between solvent and aspirin; NLT 1.5 between aspirin and salicylic acid, *System suitability solution* using the refractive index detector

Relative standard deviation: NMT 2.0% for *Standard solution A* using the refractive index detector; NMT 5.0% for *Standard solution B* at 313 nm

Analysis

Samples: *Standard solution A* and *Sample solution*

Calculate the percentages of the labeled amounts of aspirin ($C_9H_8O_4$) and carisoprodol ($C_{12}H_{24}N_2O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aspirin or carisoprodol from the *Sample solution*

r_S = peak response of aspirin or carisoprodol from *Standard solution A*

C_S = concentration of USP Aspirin RS or USP Carisoprodol RS in *Standard solution A* (mg/mL)

C_U = nominal concentration of aspirin or carisoprodol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amounts of carisoprodol ($C_{12}H_{24}N_2O_4$) and aspirin ($C_9H_8O_4$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 45 min

Buffer: Glacial acetic acid in water (1 in 50)

Mobile phase: Methanol and *Buffer* (51:49)

Standard solution: USP Reference Standards as listed below and prepared as follows. Transfer 90 mg of USP Aspirin RS and 90*J* mg of USP Carisoprodol RS to a 250-mL volumetric flask. Add 5 mL of acetonitrile, previously passed through a membrane filter of 0.5-µm or finer pore size, and swirl to dissolve. Dilute with water to volume.

Aspirin: 0.36 mg/mL of USP Aspirin RS

Carisoprodol: 0.36*J* mg/mL of USP Carisoprodol RS, where *J* is the ratio of the labeled amount, in mg, of carisoprodol to the labeled amount of aspirin

System suitability solution: 0.36 mg/mL of salicylic acid in the *Standard solution*

Sample solution: Filter a portion of the solution under test. Use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 3.9-mm × 30-cm; packing L1

Temperatures

Detector: 30 ± 1°

Column: 30 ± 1°

Flow rate: 2 mL/min

Injection volume: 300 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for aspirin and carisoprodol are 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between aspirin and salicylic acid; NLT 1.5 between carisoprodol and salicylic acid, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of aspirin ($C_9H_8O_4$) and carisoprodol ($C_{12}H_{24}N_2O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of aspirin or carisoprodol from the *Sample solution*

r_S = peak response of aspirin or carisoprodol from the *Standard solution*

C_S = concentration of USP Aspirin RS or USP Carisoprodol RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim of aspirin or carisoprodol (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amounts of aspirin ($C_9H_8O_4$) and carisoprodol ($C_{12}H_{24}N_2O_4$) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Content Uniformity* with respect to aspirin and to carisoprodol

USP Monographs

www.webofpharma.com

IMPURITIES

• **LIMIT OF FREE SALICYLIC ACID**

Mobile phase, Diluent, Standard solution B, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution B* and *Sample solution*

Calculate the percentage of free salicylic acid in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*

r_S = peak response of salicylic acid from *Standard solution B*

C_S = concentration of USP Salicylic Acid RS in *Standard solution B* (mg/mL)

C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0% of free salicylic acid

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Aspirin RS

USP Carisoprodol RS

USP Salicylic Acid RS

Carisoprodol, Aspirin, and Codeine Phosphate Tablets

DEFINITION

Carisoprodol, Aspirin, and Codeine Phosphate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of carisoprodol ($C_{12}H_{24}N_2O_4$), aspirin ($C_9H_8O_4$), and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$).

IDENTIFICATION

• **A.** The retention times of the aspirin, carisoprodol, and codeine phosphate peaks of the *Sample solutions* correspond to those of the *Standard solutions* obtained as directed in the *Assay* for *Aspirin and Carisoprodol* and the *Assay* for *Codeine Phosphate*.

ASSAY

• **ASPIRIN AND CARISOPRODOL**

Buffer: Combine 5 mL of glacial acetic acid and 500 mL of water, and pass the mixture through a membrane filter of 0.5-µm or finer pore size. Use the filtrate.

Mobile phase: Methanol and *Buffer* (64:36)

Diluent: Acetonitrile, glacial acetic acid, and water (40:1:59)

Standard solution A: USP Reference Standards in *Diluent* as listed below and prepared as follows. Transfer 80 mg of USP Aspirin RS and 80*J* mg of USP Carisoprodol RS to a 25-mL volumetric flask. Add 15 mL of *Diluent*, swirl for 5 min, and sonicate for 25–30 s. Dilute with *Diluent* to volume.

Aspirin: 3.2 mg/mL of USP Aspirin RS

Carisoprodol: 3.2*J* mg/mL of USP Carisoprodol RS, where *J* is the ratio of the labeled amount, in mg, of carisoprodol to the labeled amount of aspirin

Standard solution B: 0.016 mg/mL of USP Salicylic Acid RS in *Diluent*

System suitability solution: 0.5 mg/mL of salicylic acid in *Standard solution A*

Sample solution: Nominally 3.25 mg/mL of aspirin from NLT 20 Tablets prepared as follows. Finely powder NLT 20 Tablets. Transfer a portion of powder, equivalent to 325 mg of aspirin, to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and swirl for 5 min. Sonicate for 25–30 s, shake by mechanical means for 30 min, and dilute with *Diluent* to volume. Pass a portion of this solution through a membrane filter of 0.5-µm or finer pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector

Aspirin and carisoprodol: Refractive index

Salicylic acid: UV 313 nm

Column: 4.6-mm × 25-cm; packing L7

Temperatures

Refractive index detector: 30 ± 1°

Column: 30 ± 1°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *Standard solution A*, *Standard solution B*, and *System suitability solution*

[NOTE—The relative retention times for aspirin, salicylic acid, and carisoprodol are about 0.6, 0.7, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.2 between the solvent and aspirin peaks; NLT 1.5 between aspirin and salicylic acid, *System suitability solution* using the refractive index detector

Relative standard deviation: NMT 2.0% for *Standard solution A* using the refractive index detector; NMT 5.0% for *Standard solution B* at 313 nm

Analysis

Samples: *Standard solution A* and *Sample solution*

Calculate the percentages of the labeled amounts of aspirin ($C_9H_8O_4$) and carisoprodol ($C_{12}H_{24}N_2O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of aspirin or carisoprodol from the *Sample solution*

r_S = peak response of aspirin or carisoprodol from *Standard solution A*

C_S = concentration of USP Aspirin RS or USP Carisoprodol RS in the *Standard solution A* (mg/mL)

C_U = nominal concentration of aspirin or carisoprodol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amounts of aspirin ($C_9H_8O_4$) and carisoprodol ($C_{12}H_{24}N_2O_4$)

• **CODEINE PHOSPHATE**

Solution A: 3.7 g/L of docusate sodium in methanol

Solution B: 2 g/L of ammonium nitrate in water

Mobile phase: *Solution A* and *Solution B* (60:40) adjusted with glacial acetic acid to a pH of 3.3 ± 0.05

Diluent: Methanol and 0.01 N sulfuric acid (50:50)

System suitability solution: 0.16 mg/mL of USP Codeine Phosphate RS and 0.12 mg/mL of USP Codeine N-Oxide RS in *Diluent*

Standard solution: USP Reference Standards in *Diluent* as listed below. Swirl for 5 min, and sonicate for 25–30 s.

Codeine phosphate: 0.16 mg/mL of USP Codeine Phosphate RS

www.webofpharma.com

Aspirin: 0.16*f* mg/mL of USP Aspirin RS, where *f* is the ratio of the labeled amount, in mg, of aspirin to that of codeine phosphate

Sample solution: Nominally 0.16 mg/mL of codeine phosphate prepared as follows. Finely powder NLT 20 Tablets. Transfer an amount of powder equivalent to 16 mg of codeine phosphate to a 100-mL volumetric flask. Add 50 mL of *Diluent*, sonicate for 30 min, shake by mechanical means for 30 min, and dilute with *Diluent* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 50 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for codeine *N*-oxide and codeine phosphate are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.2 between codeine phosphate and codeine *N*-oxide, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of codeine phosphate hemihydrate, 406.37
M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37

Acceptance criteria: 90.0%–110.0% of the labeled amount of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 75 rpm
Time: 45 min

Procedure for aspirin and carisoprodol
Buffer: Glacial acetic acid in water (1 in 50)
Mobile phase: Methanol and *Buffer* (51:49)
Standard solution: USP Reference Standards as listed below and prepared as follows. Transfer 90 mg of USP Aspirin RS and 90*f* mg of USP Carisoprodol RS to a 250-mL volumetric flask. Add 5 mL of acetonitrile, previously passed through a membrane filter of 0.5-µm or finer pore size, and swirl to dissolve. Dilute with water to volume.
Aspirin: 0.36 mg/mL of USP Aspirin RS
Carisoprodol: 0.36*f* mg/mL of USP Carisoprodol RS, where *f* is the ratio of the labeled amount, in mg, of carisoprodol to the labeled amount of aspirin
System suitability solution: 0.36 mg/mL of salicylic acid in the *Standard solution*
Sample solution: Pass a portion of the solution under test through a suitable filter, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 3.9-mm × 30-cm; packing L1
Temperatures
Detector: 30 ± 1°
Column: 30 ± 1°
Flow rate: 2 mL/min
Injection volume: 300 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for aspirin and carisoprodol are 0.4 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between aspirin and salicylic acid; NLT 1.5 between carisoprodol and salicylic acid, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of aspirin ($C_9H_8O_4$) and carisoprodol ($C_{12}H_{24}N_2O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of aspirin or carisoprodol from the *Sample solution*
r_S = peak response of aspirin or carisoprodol from the *Standard solution*
C_S = concentration of USP Aspirin RS or USP Carisoprodol RS in the *Standard solution* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim of aspirin or carisoprodol (mg/Tablet)

Procedure for codeine phosphate
Buffer: 4.0 g/L of docusate sodium and 1.5 g/L of ammonium nitrate in water
Mobile phase: Acetonitrile and *Buffer* (45:55)
Standard solution: 0.018 mg/mL of USP Codeine Phosphate RS in water
Sample solution: Pass a portion of the solution under test through a suitable filter, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of codeine phosphate hemihydrate, 406.37
M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37
V = volume of the *Medium*, 900 mL
L = label claim of codeine phosphate (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amounts of aspirin ($C_9H_8O_4$), carisoprodol ($C_{12}H_{24}N_2O_4$), and codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) are dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements for *Content Uniformity* with respect to aspirin, carisoprodol, and codeine phosphate

IMPURITIES

• **Organic Impurities**

Limit of free salicylic acid

Mobile phase, Diluent, Standard solution B, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay* for *Aspirin and Carisoprodol.*

Analysis

Samples: *Standard solution B* and *Sample solution*

Calculate the percentage of free salicylic acid in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the *Sample solution*
r_S = peak response of salicylic acid from *Standard solution B*
C_S = concentration of USP Salicylic Acid RS in *Standard solution B* (mg/mL)
C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0% of free salicylic acid

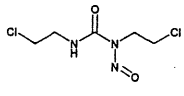
ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards** ⟨11⟩

USP Aspirin RS
USP Carisoprodol RS
USP Codeine N-Oxide RS
7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol *N*-oxide.
$C_{18}H_{21}NO_4$ 315.37
USP Codeine Phosphate RS
USP Salicylic Acid RS

Carmustine



$C_5H_9Cl_2N_3O_2$ 214.05

Urea, *N*,*N*′-bis(2-chloroethyl)-*N*-nitroso-; 1,3-Bis(2-chloroethyl)-1-nitrosourea [154-93-8].

DEFINITION

Carmustine contains NLT 98.0% and NMT 102.0% of $C_5H_9Cl_2N_3O_2$, calculated on the anhydrous and solvent-free basis.

[**Caution**—Use appropriate surgical gloves, arm covers, and a dust mask. Perform all work under a fume hood approved for testing cytotoxic agents when possible.]

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

Sample: Melt a small portion of the sample in a suitable container in a controlled water bath or oven, and set the temperature between 33° and 40°.

Standard: A similar preparation of USP Carmustine RS

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

• **Procedure**

[Note—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

Mobile phase: Acetonitrile and water (3:7)

Standard solution: 1.5 mg/mL of USP Carmustine RS in acetonitrile

Sample solution: 1.5 mg/mL of Carmustine in acetonitrile

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 200 nm

Refrigerated autosampler temperature: 4°–5°

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.9

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_5H_9Cl_2N_3O_2$ in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Carmustine RS in the *Standard solution* (mg/mL)
C_U = concentration of Carmustine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

Organic Impurities

• **Procedure 1: Limit of Ether-Insoluble Substances**

[Note—Perform in a well-ventilated fume hood.]

Analysis: Transfer 1.0 g of sample to a suitable container containing 10 mL of anhydrous ether, stir for 5 min, and immediately filter through a tared glass-filtering crucible of medium pore size. Wash the container with an additional 10 mL of ether, and filter through the same glass-filtering

USP Monographs

www.webofpharma.com

crucible. Dry the crucible at 105° for 1 h. Cool in a desiccator and weigh.

Acceptance criteria: The weight of the residue does not exceed 0.1%.

• **Procedure 2: Limit of Carmustine Related Compound A**

[NOTE—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Carmustine standard solution: Use the *Standard solution*, prepared as directed in the *Assay*.

Standard stock solution: 0.75 mg/mL of USP Carmustine Related Compound A RS in acetonitrile

Standard solution: 0.0075 mg/mL of USP Carmustine Related Compound A RS in acetonitrile, from the *Standard stock solution*

System suitability solution 1: 0.75 µg/mL of USP Carmustine Related Compound A RS in acetonitrile, from the *Standard solution*

System suitability solution 2: Transfer 5.0 mL of *Carmustine standard solution* and 10.0 mL of *Standard stock solution* into a 100-mL volumetric flask, and dilute with acetonitrile to volume. Transfer 5.0 mL of this solution into a 50-mL volumetric flask, and dilute with acetonitrile to volume.

System suitability

Samples: *Carmustine standard solution, System suitability solution 1,* and *System suitability solution 2*

[NOTE—The relative retention times for carmustine related compound A and carmustine are 0.3 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 10 between carmustine related compound A and carmustine, *System suitability solution 2*

Tailing factor: NMT 1.9, *Carmustine standard solution*

Relative standard deviation: NMT 5%, *System suitability solution 1*

Analysis

[NOTE—Run the *Sample solution* at least 1.5 times the retention time of carmustine.]

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of carmustine related compound A in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carmustine related compound A from the *Sample solution*

r_S = peak response of carmustine related compound A from the *Standard solution*

C_S = concentration of carmustine related compound A in the *Standard solution* (mg/mL)

C_U = concentration of Carmustine in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria

Carmustine related compound A: NMT 0.5%

Any unspecified impurity: NMT 0.1%

• **Procedure 3: Limit of 2-Chloroethylamine**

[NOTE—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

Standard solution 1 (0.2%): 1.2 mg/mL of 2-chloroethylamine monohydrochloride in methanol. [NOTE—1.2 mg/mL of 2-chloroethylamine monohydrochloride is equivalent to 0.8 mg/mL of 2-chloroethylamine.]

Standard solution 2 (0.1%): 0.4 mg/mL of USP Carmustine RS in methanol

Sample solution: 0.4 g/mL of Carmustine in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic plate (20-cm × 20-cm) coated with silica gel 60

Application volume: 1 µL

Developing solvent system 1: Ethyl acetate

Developing solvent system 2: Ethyl acetate and methanol (7:3)

Spray reagent 1: Diethylamine

Spray reagent 2: 0.1 N silver nitrate solution

Analysis

Samples: *Standard solution 1 (0.2%), Standard solution 2 (0.1%),* and *Sample solution*

Develop with *Developing solvent system 1* for 27 min, followed by air drying for 5 min. Develop again in *Developing solvent system 2* for 8 min, followed by air drying for 10 min. Spray the plate with *Spray reagent 1*, and heat the plate for 20 min in an oven at 100°. Allow the plates to cool to room temperature, and spray the plate with *Spray reagent 2*. Allow the plate to be exposed to UV light at 365 nm for 15 min. Examine the plate under UV light.

Acceptance criteria

2-Chloroethylamine: The spot for 2-chloroethylamine from the *Sample solution* is not more intense than the principal spot from *Standard solution 1 (0.2%)*.

Any unspecified impurity: Any spot if present in the chromatogram from the *Sample solution*, except the principal spot of carmustine and the spot of 2-chloroethylamine, is not more intense than the principal spot from *Standard solution 2 (0.1%)*.

• **Procedure 4: Limit of 2-Chloroethanol**

Standard solution: 0.02 mg/mL of 2-chloroethanol in acetonitrile

System suitability solution: 0.01 mg/mL of 2-chloroethanol in acetonitrile, diluted from the *Standard solution*

Sample solution: 10 mg/mL of Carmustine in acetonitrile. [NOTE—Prepare in low-actinic glassware, and keep refrigerated until use.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m × 0.53-mm column bonded with a 1-µm film of phase G16

Temperature

Injector: 90°

Detector: 260°

Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	6
40	30	80	14
80	30	200	3

Carrier gas: Helium

Flow rate: 7 mL/min

Injection size: 5 µL

www.webofpharma.com

System suitability
Sample: *System suitability solution*
Suitability requirements
Relative standard deviation: NMT 5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 2-chloroethanol in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 2-chloroethanol from the *Sample solution*
r_S = peak response of 2-chloroethanol from the *Standard solution*
C_S = concentration of 2-chloroethanol in the *Standard solution* (mg/mL)
C_U = concentration of Carmustine in the *Sample solution* (mg/mL)

Acceptance criteria
2-Chloroethanol: NMT 0.1%

• **Procedure 5: Limit of Acetaldehyde**
Standard solution: 10 µg/mL of acetaldehyde in acetonitrile
Sample solution: 10 mg/mL of Carmustine in acetonitrile. [NOTE—Prepare in low-actinic glassware, and keep refrigerated until use.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 30-m × 0.53-mm column bonded with a 5-µm film of phase G1
Temperature
Injector: 70°
Detector: 260°
Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	6
40	30	210	3

Injector split ratio: 15:1
Carrier gas: Helium
Flow rate: 3 mL/min
Injection size: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetaldehyde in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaldehyde from the *Sample solution*
r_S = peak response of acetaldehyde from the *Standard solution*
C_S = concentration of acetaldehyde in the *Standard solution* (mg/mL)
C_U = concentration of Carmustine in the *Sample solution* (mg/mL)

Acceptance criteria
Acetaldehyde: NMT 0.1%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature between 2° and 8°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Carmustine RS
USP Carmustine Related Compound A RS
1,3-Bis(2-chloroethyl) urea.
$C_5H_{10}Cl_2N_2O$ 185.05

Carmustine for Injection

DEFINITION
Carmustine for Injection is a sterile lyophilized preparation of carmustine. It contains NLT 90.0% and NMT 110.0% of the labeled amount of carmustine ($C_5H_9Cl_2N_3O_2$).
[CAUTION—Use appropriate surgical gloves, arm covers, and a dust mask. Perform all work under a fume hood approved for testing cytotoxic agents when possible.]

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)
Sample: Melt a small portion of the sample in a suitable container in a controlled water bath or oven, and set the temperature between 33° and 40°.
Standard: A similar preparation of USP Carmustine RS
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
[NOTE—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]
Mobile phase: See the gradient table below.

Time (min)	Water (%)	Acetonitrile (%)
0	90	10
2.5	90	10
7	40	60
8.5	90	10
10.5	90	10

Diluent: Acetonitrile and water (1:3)
Standard stock solution: 2.0 mg/mL of USP Carmustine RS in acetonitrile
Standard solution: 0.2 mg/mL of USP Carmustine RS in *Diluent*, from *Standard stock solution*
Impurity standard stock solution: 0.1 mg/mL of USP Carmustine Related Compound A RS in acetonitrile
System suitability solution: 0.2 mg/mL of USP Carmustine RS and 0.002 mg/mL of USP Carmustine Related Compound A RS in *Diluent*, from the *Standard stock solution* and *Impurity standard stock solution*, respectively

www.webofpharma.com

Sample stock solution: 2.0 mg/mL of carmustine in acetonitrile, from Carmustine for Injection. [NOTE—Allow test vials to warm to room temperature in a desiccator for 1 h.]
Sample solution: 0.2 mg/mL of carmustine in *Diluent*, from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Refrigerated autosampler temperature: 5°
Column: 4.6-mm × 7.5-cm; 3-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for carmustine related compound A and carmustine are 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between carmustine related compound A and carmustine
Tailing factor: NMT 1.5 for the carmustine related compound A and carmustine peaks
Relative standard deviation: NMT 2.0% for the carmustine related compound A and carmustine peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carmustine ($C_5H_9Cl_2N_3O_2$) in the portion of Carmustine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the *Sample solution*
r_S = peak area of the *Standard solution*
C_S = concentration of carmustine in the *Standard solution* (mg/mL)
C_U = nominal concentration of carmustine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES
ORGANIC IMPURITIES
• **Procedure: Limit of Carmustine Related Compound A**
Diluent, Impurity standard stock solution, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 0.002 mg/mL of USP Carmustine Related Compound A RS in *Diluent*, from the *Impurity standard stock solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carmustine related compound A in the portion of Carmustine for Injection taken:

$$\text{Result} = (r_U/r_S) \times [100 \times C_S/(C_U \times A)] \times 100$$

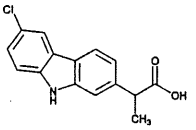
r_U = peak response of carmustine related compound A from the *Sample solution*
r_S = peak response of carmustine related compound A from the *Standard solution*
C_S = concentration of carmustine related compound A in the *Standard solution* (mg/mL)
C_U = nominal concentration of carmustine in the *Sample solution* (mg/mL)
A = assay of Carmustine for Injection, as a percentage

Acceptance criteria
Carmustine related compound A: NMT 1.0%

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.95 USP Endotoxin Unit/mg of carmustine
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
• **pH** ⟨791⟩**:** Between 4.0 and 6.8 in a constituted solution prepared as directed in the labeling
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution* at a temperature between 2° and 8°.
• **LABELING:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.
• **CONSTITUTED SOLUTION:** At time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Carmustine RS
Urea, *N,N'*-bis(2-chloroethyl)-*N*-nitroso-; 1,3-Bis(2-chloroethyl)-1-nitrosourea.
$C_5H_9Cl_2N_3O_2$ 214.05
USP Carmustine Related Compound A RS
1,3-Bis(2-chloroethyl) urea.
$C_5H_{10}Cl_2N_2O$ 185.05

Carprofen



$C_{15}H_{12}ClNO_2$ 273.71
9*H*-Carbazole-2-acetic acid, 6-chloro-α-methyl-, (±)-; (±)-6-Chloro-α-methylcarbazole-2-acetic acid [53716-49-7].

DEFINITION
Carprofen contains NLT 98.0% and NMT 102.0% of carprofen ($C_{15}H_{12}ClNO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)**:** [NOTE—Methods described in ⟨197K⟩ or ⟨197A⟩ may be used. If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in acetone, evaporate to dryness, and record new spectra using the residues.]
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

[NOTE—Protect all solutions containing carprofen from light.]

Mobile phase: Acetonitrile, methanol, glacial acetic acid, and water (40: 25: 0.2: 35)

System suitability stock solution: 16 µg/mL of USP Carprofen Related Compound A RS in *Mobile phase*. Sonicate if necessary.

Standard solution: 160 µg/mL of USP Carprofen RS in *Mobile phase*. Sonicate if necessary.

System suitability solution: 1.6 µg/mL of USP Carprofen Related Compound A RS and 16 µg/mL of USP Carprofen RS in *Mobile phase* prepared as follows. Mix 10 mL of *System suitability stock solution* and 10 mL of the *Standard solution* and dilute with *Mobile phase* to 100 mL.

Sample solution: 160 µg/mL of Carprofen in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between carprofen and carprofen related compound A

Tailing factor: NMT 2.0 for the carprofen peak

Relative standard deviation: NMT 2.0% for the carprofen peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of carprofen ($C_{15}H_{12}ClNO_2$) in the portion of Carprofen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Carprofen RS in the *Standard solution* (µg/mL)

C_U = concentration of Carprofen in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Carprofen taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual peak (other than the major peak of carprofen)

r_T = sum of all the peak responses

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carprofen related compound A (carbazole)	0.9	0.5
2-[1,1-Dimethoxy-2-hydroxypropyl]-6-chlorocarbazole	1.3	0.5
2-[2-Chloropropionyl]-6-chloro-9-acetylcarbazole	3.3	0.5
Any individual unspecified impurity	—	0.1
Total Impurities	—	1.0

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

• **LABELING:** Label it to indicate that it is intended for veterinary use only.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Carprofen RS

USP Carprofen Related Compound A RS

Carbazole.

$C_{12}H_9N$ 167.21

Carprofen Tablets

» Carprofen Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of carprofen ($C_{15}H_{12}ClNO_2$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**

USP Carprofen RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—

Reference specimen—Mix about 2 mg of USP Carprofen RS with 200 mg of potassium bromide, and grind thoroughly for 10 to 15 minutes. Compress the mixture into a clear pellet. Record the IR spectrum of the pellet immediately after preparation.

Test specimen—Grind into powder not fewer than 4 Tablets. Transfer the powder, equivalent to about 100 mg of carprofen, to a 125-mL separatory funnel. Add 30 mL of water and 3 drops of hydrochloric acid, and shake for about 5 minutes. Add about 30 mL of methylene chloride, and shake for another 5 minutes. Allow the phases to separate. Carefully drain and collect the lower methylene chloride layer through anhydrous sodium sulfate that is placed on a cotton pledget into a suitable container. Evaporate the methylene chloride on a

www.webofpharma.com

steam bath with the aid of a stream of nitrogen to dryness. Dry the residue in vacuum at 60° for about 30 minutes. Mix about 2 mg of the dried residue with 200 mg of potassium bromide, and grind thoroughly for 10 to 15 minutes. Compress the mixture into a clear pellet. Record the IR spectrum of the carprofen sample pellet immediately after preparation.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—

[NOTE—Use low-actinic volumetric flasks, dissolution vessels, and evaporation covers.]

Medium: 0.05 M phosphate buffer, pH 7.5 (prepared by dissolving 6.8 g of monobasic potassium phosphate in 600 mL of water, mixing, adding 18 mL of 2 N sodium hydroxide, mixing, diluting with water to 1000 mL, and adjusting with 0.2 N sodium hydroxide or 0.2 N hydrochloric acid to a pH of 7.50 ± 0.05); 900 mL, degassed.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{15}H_{12}ClNO_2$ dissolved by employing the following method.

Standard solution—

FOR TABLETS LABELED TO CONTAIN 25 MG—Transfer about 25 mg of USP Carprofen RS, accurately weighed, to a 900-mL volumetric flask. Slowly add 10 mL of methanol. Dilute with *Medium* to volume, and mix.

FOR TABLETS LABELED TO CONTAIN 75 MG—Transfer about 75 mg of USP Carprofen RS, accurately weighed, to a 900-mL volumetric flask. Slowly add 30 mL of methanol. Dilute with *Medium* to volume, and mix.

FOR TABLETS LABELED TO CONTAIN 100 MG—Transfer about 100 mg of USP Carprofen RS, accurately weighed, to a 900-mL volumetric flask. Slowly add 40 mL of methanol. Dilute with *Medium* to volume, and mix.

Test solution—Pass a portion of the solution under test through a suitable 0.45-µm filter.

System suitability solution—Determine the absorbance of the *Standard solution,* as directed for *Procedure,* five times: the relative standard deviation is not more than 2.0%.

Procedure—Determine the amount of $C_{15}H_{12}ClNO_2$ dissolved by measuring the absorbance of the *Test solution* in comparison with the appropriate *Standard solution* at the wavelength of maximum absorbance at about 300 nm, using a 0.5-cm cell for Tablets labeled to contain 25 mg, a 0.2-cm cell for Tablets labeled to contain 75 mg, and a 0.1-cm cell for Tablets labeled to contain 100 mg. Use *Medium* as the blank. Calculate the percentage of $C_{15}H_{12}ClNO_2$ dissolved by the formula:

$$\frac{A_U \times W_S \times 100}{A_S \times LC}$$

in which A_U and A_S are the absorbances obtained from the *Test solution* and the *Standard solution,* respectively; W_S is the weight, in mg, of USP Carprofen RS used to prepare the *Standard solution;* 100 is the conversion factor to percentage; and *LC* is the Tablet label claim, in mg.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{15}H_{12}ClNO_2$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements for *Content Uniformity.*

PROCEDURE FOR CONTENT UNIFORMITY— [NOTE—Use low-actinic glassware.]

Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay.*

Standard solution—Prepare as directed for the *Standard preparation* in the *Assay.*

Test solution—Transfer 10 Tablets individually to 10 separate volumetric flasks of a suitable calibrated volume such that an interim concentration of 0.5 mg per mL of *Mobile phase* can be prepared. To each flask, add *Mobile phase* to 80% of the calibrated volume, sonicate for 10 minutes, then stir for 10 minutes. Sonicate again for 10 minutes, and stir for another 10 minutes or until the Tablets are completely disintegrated. Cool to room temperature, dilute with *Mobile phase* to volume to obtain an interim concentration of 0.5 mg of carprofen per mL, and mix. Quantitatively transfer 5.0 mL of the individual solutions to 10 separate 50.0-mL volumetric flasks, dilute with *Mobile phase* to volume, and mix. Pass the solution through a polyvinylidenefluoride (PVDF) filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate. The final concentration is about 0.05 mg of carprofen per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay.* Calculate the percentage of the labeled content of $C_{15}H_{12}ClNO_2$ in the portion of Tablets taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which the terms are as defined therein.

Chromatographic purity—

Mobile phase—Proceed as directed in the *Assay.*

Standard solution— [NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of USP Carprofen RS in *Mobile phase* to obtain a solution having a known concentration of 0.05 µg of carprofen per mL.

Sensitivity solution— [NOTE—Use low-actinic glassware.] Quantitatively dilute the *Standard solution* with *Mobile phase* to obtain a solution containing about 0.005 µg of carprofen per mL.

Test solution—Use the *Assay preparation.*

Blank solution—Transfer an accurately weighed portion of the Tablet base, equivalent to the weight of 1 Tablet, to a volumetric flask of the same calibrated volume as that used to prepare the *Test solution.* To each flask add *Mobile phase* to 80% of the calibrated volume. Sonicate for 10 minutes, then stir for 10 minutes. Sonicate again for 10 minutes, and stir for another 10 minutes. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Quantitatively transfer 5.0 mL of the solution to a 50.0-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass the solution through a PVDF filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L7. The flow rate is about 1.0 mL per minute. Wash the column after each series of analyses with a mixture of acetonitrile and water (20:80) for 30 minutes; gradually change the composition of acetonitrile and water to 80:20 over 10 minutes; continue to wash at 80:20 for 30 minutes; gradually change the composition to 50:50 over 10 minutes; and continue to wash at 50:50 for another 30 minutes. Chromatograph the *Standard solution,* the *Sensitivity solution,* and the *Test solution,* and record the peak responses as directed for *Procedure:* for the *Standard solution,* the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%; for the *Sensitivity solution,* the carprofen peak should be defined and integratable; for the *Test solution,* the resolution, *R,* between carprofen and the nearest impurity peak is not less than 2.0. After every six injections of any solution, inject a *Standard solution* in duplicate. The ratio of the average response of the

www.webofpharma.com

duplicate injections to that obtained from the initial five replicate injections is 0.95 to 1.05.

Procedure—Inject a volume (about 50 µL) of the *Standard solution,* the *Test solution,* and the *Blank solution* into the chromatograph, record the chromatograms, and measure all the peak areas. Calculate the percentage of carprofen-related compounds in the portion of Tablets taken by the formula:

$$0.1(C_S/C_U)(r_i/r_S)$$

in which C_S is the concentration, in µg per mL, of carprofen in the *Standard solution;* C_U is the concentration, in mg per mL, of carprofen in the *Test solution;* r_i is the peak area of any peak other than carprofen obtained from the *Test solution;* and r_S is the peak area of carprofen obtained from the *Standard solution:* not more than 0.5% of any single impurity is found; and the sum of all impurities is not more than 2.0%. Disregard any peak also observed in the *Blank solution.*

Assay—

Mobile phase—Mix 500 mL of acetonitrile, 500 mL of water, and 1 mL of phosphoric acid. Degas before using. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation— [NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of USP Carprofen RS in *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

Assay preparation— [NOTE—Use low-actinic glassware.] Accurately weigh 20 Tablets, and calculate the average Tablet weight. Grind the Tablets into uniform powder. Transfer three accurately weighed portions of the powder, each equivalent to the weight of one Tablet, into three volumetric flasks of a suitable calibrated volume such that an interim concentration of 0.5 mg per mL of *Mobile phase* can be prepared. To each flask add *Mobile phase* to 80% of the calibrated volume, sonicate for 10 minutes, then stir for 10 minutes. Sonicate again for 10 minutes, and stir for another 10 minutes. Cool to room temperature, dilute with *Mobile phase* to volume to obtain an interim concentration of 0.5 mg of carprofen per mL, and mix. Quantitatively transfer 5.0 mL of the solution to a 50.0-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass the solution through a PVDF filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate. The final concentration is about 0.05 mg of carprofen per mL.

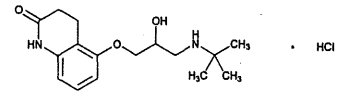
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Wash the column after each series of analyses with a mixture of acetonitrile and water (20:80) for 30 minutes; gradually change the composition of acetonitrile and water to 80:20 over 10 minutes; continue to wash at 80:20 for 30 minutes; gradually change the composition to 50:50 over 10 minutes; and continue to wash at 50:50 for another 30 minutes. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the column efficiency for carprofen is not less than 4000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for five replicate injections is not more than 2.0%. Inject the *Standard preparation* in duplicate after every 12 injections or fewer of any other solution. The ratio of the average area of the duplicate injections to that obtained from the initial five replicate injections is 0.95 to 1.05.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of the labeled content of carprofen ($C_{15}H_{12}ClNO_2$) in the portion of Tablets taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of USP Carprofen RS in the *Standard preparation* and carprofen in the *Assay preparation,* respectively; and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Carteolol Hydrochloride



$C_{16}H_{24}N_2O_3 \cdot HCl$ 328.83

2(1*H*)-Quinolinone, 5-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-3,4-dihydro-, monohydrochloride;
5-[3-(*tert*-Butylamino)-2-hydroxypropoxy]-3,4-dihydrocarbostyril monohydrochloride [51781-21-6].

DEFINITION

Carteolol Hydrochloride contains NLT 98.0% and NMT 101.5% of carteolol hydrochloride ($C_{16}H_{24}N_2O_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 10 µg/mL
Medium: Water
Acceptance criteria: Meets the requirements

- **C. CHLORIDE AND SULFATE,** *Chloride* ⟨191⟩

Sample solution: 20 mg/mL
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: 0.67 g/L of dibasic sodium phosphate prepared as follows. Dissolve 1.34 g of dibasic sodium phosphate in 1900 mL of water, adjust with 1 M phosphoric acid to a pH of 6.0 ± 0.05, and dilute with water to volume.
Mobile phase: Acetonitrile and *Buffer* (250:750) [NOTE—Increasing the proportion of pH 6.0 buffer increases resolution.]
Standard stock solution: 1 mg/mL of USP Carteolol Hydrochloride RS in water
Standard solution: 0.1 mg/mL of USP Carteolol Hydrochloride RS from *Standard stock solution* prepared as follows. Transfer 10 mL of *Standard stock solution* to a 100-mL volumetric flask containing 5 mL of acetonitrile, and dilute with water to volume.
System suitability stock solution: Dissolve 50 mg of *p*-acetotoluidide in a 100-mL volumetric flask in 50 mL of acetonitrile, and dilute with water to volume.
System suitability solution: 0.05 mg/mL of *p*-acetotoluidide and 0.1 mg/mL of USP Carteolol

USP Monographs

www.webofpharma.com

Hydrochloride RS in water from *System suitability stock solution* and *Standard stock solution*
Sample stock solution: 1 mg/mL of Carteolol Hydrochloride in water
Sample solution: 0.1 mg/mL of Carteolol Hydrochloride in water prepared as follows. Transfer 10.0 mL of *Sample stock solution* to a 100-mL volumetric flask containing 5 mL of acetonitrile, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 252 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for carteolol and *p*-acetotoluidide are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3 between carteolol and *p*-acetotoluidide, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carteolol hydrochloride ($C_{16}H_{24}N_2O_3 \cdot HCl$) in the portion of Carteolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Carteolol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Carteolol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **ARSENIC,** *Method II* ⟨211⟩**:** NMT 3 ppm
• **ORGANIC IMPURITIES**
Standard solution A: 0.5 mg/mL of USP Carteolol Hydrochloride RS in methanol
Standard solution B: Dilute 5.0 mL of *Standard solution A* with methanol to 50 mL.
Standard solution C: Dilute 5.0 mL of *Standard solution B* with methanol to 10 mL.
Sample solution: Transfer 250 mg of Carteolol Hydrochloride to a 10-mL volumetric flask, and dissolve in methanol, using heat or sonication if necessary to achieve dissolution. Dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, methanol, and ammonium hydroxide (50:20:1)
Analysis
Samples: *Standard solutions A, B,* and *C* and *Sample solution*
Line a chromatographic chamber with filter paper, and saturate the paper with the *Developing solvent system*. Allow the spots on the plate to dry, place the plate in the chamber, and develop until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, and allow to air-dry. Examine the plate under short-wavelength UV light.
Acceptance criteria
The R_F value of the principal spot from the *Sample solution* corresponds to that from *Standard solution A*.
Individual impurities: Compare the sizes and intensities of any spots other than the principal spot from the *Sample solution* with those of the principal spots from the *Standard solutions*: no spot exceeds in size or intensity the principal spot from *Standard solution B*, NMT 0.2%.
Total impurities: The sum of all the impurity spots is NMT 0.5%.

SPECIFIC TESTS
• **PH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 5.0–6.0
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Carteolol Hydrochloride RS

Carteolol Hydrochloride Ophthalmic Solution

» Carteolol Hydrochloride Ophthalmic Solution is a sterile, aqueous, isotonic solution of Carteolol Hydrochloride. It contains a suitable antimicrobial preservative. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{16}H_{24}N_2O_3 \cdot HCl$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Carteolol Hydrochloride RS

Identification—
A: Prepare a test solution by diluting a suitable volume of Ophthalmic Solution with water, if necessary, to obtain a solution containing about 1 mg of carteolol hydrochloride per mL. Separately apply 10 µL of the test solution and 10 µL of a Standard solution of USP Carteolol Hydrochloride RS in water containing about 1 mg per mL to the starting line of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry. Line a chromatographic chamber with filter paper, and saturate the paper with a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (50:20:1). Place the plate in the chamber, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow to air-dry. Examine the plate under short-wavelength UV light: the R_F value of the principal spot in the chromatogram obtained from the test solution corresponds to that in the chromatogram obtained from the Standard solution.
B: The retention time of the carteolol peak in the chromatogram of the *Assay preparation* obtained as directed

www.webofpharma.com

in the *Assay* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 6.0 and 8.0.

Assay—

pH 6.0 buffer, Mobile phase, Diluent, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Carteolol Hydrochloride.*

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of carteolol hydrochloride, to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a filter having a porosity of 0.5 µm or finer, discarding the first 2 mL of the filtrate, and use the filtrate as the *Assay preparation.*

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{16}H_{24}N_2O_3 \cdot HCl$ in each mL of the Ophthalmic Solution taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Carteolol Hydrochloride RS in the *Standard preparation;* V is the volume, in mL, of Ophthalmic Solution taken; and r_U and r_S are the carteolol peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Carteolol Hydrochloride Tablets

DEFINITION

Carteolol Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carteolol hydrochloride ($C_{16}H_{24}N_2O_3 \cdot HCl$).

IDENTIFICATION

• **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**

Buffer: 0.67 g/L of dibasic sodium phosphate prepared as follows. Dissolve 1.34 g of dibasic sodium phosphate in 1900 mL of water, adjust with 1 M phosphoric acid to a pH of 6.0 ± 0.05, and dilute with water to volume.

Mobile phase: Acetonitrile and *Buffer* (250:750)

[NOTE—Increasing the proportion of buffer increases resolution.]

Diluent: Methanol and *Buffer* (50:50)

Standard stock solution: 1 mg/mL of USP Carteolol Hydrochloride RS in water

Standard solution: 0.1 mg/mL of USP Carteolol Hydrochloride RS from *Standard stock solution* prepared as follows. Transfer 10 mL of *Standard stock solution* to a 100-mL volumetric flask containing 5 mL of acetonitrile, and dilute with water to volume.

System suitability stock solution: Dissolve 50 mg of *p*-acetotoluidide in a 100-mL volumetric flask in 50 mL of acetonitrile, and dilute with water to volume.

System suitability solution: 0.05 mg/mL of *p*-acetotoluidide and 0.1 mg/mL of USP Carteolol Hydrochloride RS in water from *System suitability stock solution* and *Standard stock solution*

Sample solution: Nominally 0.1 mg/mL of Carteolol Hydrochloride in *Diluent* prepared as follows. Transfer an amount equivalent to 10 mg of carteolol hydrochloride from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add 50 mL of *Diluent,* and shake by mechanical means for 1 h. Add 5 mL of acetonitrile, and dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 0.5-µm or finer pore size, discarding the first 2 mL of filtrate. Use the clear filtrate as the *Sample solution.*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 252 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for carteolol and *p*-acetotoluidide are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3 between carteolol and *p*-acetotoluidide, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carteolol hydrochloride ($C_{16}H_{24}N_2O_3 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of carteolol hydrochloride from the *Sample solution*

r_S = peak area of carteolol hydrochloride from the *Standard solution*

C_S = concentration of USP Carteolol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of carteolol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Buffer: Dissolve 2.0 g of monobasic potassium phosphate in water to make 1 L of solution.

Mobile phase: Acetonitrile and *Buffer* (400:600)

Standard solution: 1.1L µg/mL of USP Carteolol Hydrochloride RS, where L is the labeled amount, in mg, of carteolol hydrochloride per Tablet

Sample solution: Pass a portion of the solution under test through a filter of 1-µm or finer pore size, discarding the first 2 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 252 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2%

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of carteolol hydrochloride ($C_{16}H_{24}N_2O_3 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Carteolol Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of medium, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of carteolol hydrochloride ($C_{16}H_{24}N_2O_3 \cdot HCl$)
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Limit of Dehydrocarteolol Hydrochloride**
Buffer, Mobile phase, and **Diluent:** Proceed as directed in the *Assay*.
Standard solution: 1 µg/mL of USP Dehydrocarteolol Hydrochloride RS in *Diluent*
Sample solution: Transfer an amount nominally equivalent to 10 mg of carteolol hydrochloride from NLT 20 finely powdered Tablets to a 100-mL volumetric flask, add 50 mL of *Diluent*, and shake by mechanical means for 1 h. Dilute with *Diluent* to volume. Pass a portion of this solution through a filter of 0.5-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorometric, with excitation at 300 nm and a 418-nm emission filter
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dehydrocarteolol hydrochloride in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

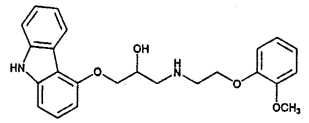
r_U = peak response of dehydrocarteolol from the *Sample solution*
r_S = peak response of dehydrocarteolol from the *Standard solution*
C_S = concentration of USP Dehydrocarteolol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of carteolol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Carteolol Hydrochloride RS
USP Dehydrocarteolol Hydrochloride RS
5-(3-*tert*-Butylamino-2-hydroxy)-propoxycarbostyril hydrochloride.
$C_{16}H_{22}N_2O_3 \cdot HCl$ 326.82

Carvedilol



$C_{24}H_{26}N_2O_4$ 406.47
2-Propanol, 1-(9*H*-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]-, (±)-;
(±)-1-(Carbazol-4-yloxy)-3-[[2-(*o*-methoxyphenoxy)eth yl] amino]-2-propanol [72956-09-3].

DEFINITION

Carvedilol contains NLT 98.0% and NMT 102.0% of $C_{24}H_{26}N_2O_4$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**
Buffer: 2.72 g/L of monobasic potassium phosphate. Adjust with dilute phosphoric acid to a pH of 2.0.
Mobile phase: Acetonitrile and *Buffer* (31:69)
System suitability solution: 0.05 mg/mL each of USP Carvedilol RS and USP Carvedilol Related Compound A RS in *Mobile phase*
Standard solution: 0.04 mg/mL of USP Carvedilol RS in *Mobile phase*
Sample solution: 0.04 mg/mL of Carvedilol in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 55°
Flow rate: 1 mL/min
Run time: 60 min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 4.0 between carvedilol and carvedilol related compound A
Tailing factor: NMT 1.5 for the carvedilol peak
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carvedilol ($C_{24}H_{26}N_2O_4$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carvedilol from the *Sample solution*
r_S = peak response of carvedilol from the *Standard solution*
C_S = concentration of carvedilol in the *Standard solution* (mg/mL)
C_U = concentration of Carvedilol in the *Sample solution* (mg/mL)

USP Monographs

www.webofpharma.com

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1% from 1 g
- **ORGANIC IMPURITIES, PROCEDURE 1:** [NOTE—On the basis of the impurities present, perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*. *Organic Impurities, Procedure 2* is recommended when carvedilol related compound F is a potential impurity.]

Buffer and **Mobile phase:** Prepare as directed in the *Assay*.
System suitability solution: 0.05 mg/mL each of USP Carvedilol RS and USP Carvedilol Related Compound C RS in *Mobile phase*
Standard solution: 1 µg/mL each of USP Carvedilol RS, USP Carvedilol Related Compound A RS, USP Carvedilol Related Compound B RS, USP Carvedilol Related Compound D RS, and USP Carvedilol Related Compound E RS, and 0.2 µg/mL of USP Carvedilol Related Compound C RS in *Mobile phase*
Sample solution: 1 mg/mL of Carvedilol in *Mobile phase*
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: Dual wavelength, UV 220 and 240 nm. Use 220 nm for quantitating carvedilol related compound E, and use 240 nm for carvedilol and all other related compounds.
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 55°
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 17 between carvedilol and carvedilol related compound C
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carvedilol related compound A, carvedilol related compound B, carvedilol related compound C, carvedilol related compound D, carvedilol related compound E, and any other individual impurity in the portion of Carvedilol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding related compound or any other impurity from the *Sample solution*
r_S = peak response of the corresponding related compound from the *Standard solution*. To calculate the percentage of any other individual impurity use the peak response of carvedilol.
C_S = concentration of the corresponding related compound in the *Standard solution* (mg/mL). To calculate the percentage of any other impurities for C_S, use the concentration of USP Carvedilol RS.
C_U = concentration of Carvedilol in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carvedilol related compound E[a]	0.35	0.1
Carvedilol related compound A[b]	0.52	0.1
Carvedilol bisalkylpyrocatechol derivative (if present)[c]	0.70	0.15
Carvedilol	1.0	—
Carvedilol related compound C[d]	3.6	0.02
Carvedilol related compound D[e]	5.0	0.1
Carvedilol related compound B[f]	8.5	0.1
Any other individual impurity	—	0.10
Total impurities	—	0.5[g]

[a] 2-(2-Methoxyphenoxy)ethyl amine.
[b] 1-(4-(2-Hydroxy-3-(2-(2-methoxyphenoxy)ethylamino)propoxy)-9*H*-carbazol-9-yl)-3-(2-(2-methoxyphenoxy)ethylamino) propan-2-ol.
[c] 3,3′-{2,2′-[1,2-Phenylenebis(oxy)]bis(ethane-2,1-diyl)}bis(azanediyl)bis(1-(9*H*-carbazol-4-yloxy)propan-2-ol).
[d] 1-(9*H*-Carbazol-4-yloxy)-3-(benzyl(2-(2-methoxyphenoxy)ethyl)amino) propan-2-ol.
[e] 4-(Oxiran-2-ylmethoxy)-9*H*-carbazole.
[f] 3,3′-(2-(2-Methoxyphenoxy)ethylazanediyl)bis(1-(9*H*-carbazol-4-yloxy) propan-2-ol).
[g] Disregard any impurity less than 0.01%.

- **ORGANIC IMPURITIES, PROCEDURE 2**

Solution A: Acetonitrile and trifluoroacetic acid (100: 0.1)
Solution B: Trifluoroacetic acid and water (0.1: 100)
Diluent: Acetonitrile, trifluoroacetic acid, and water (22: 0.1: 78)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	22	78
20	22	78
33	38	62
45	38	62
55	55	45
65	55	45
68	22	78
80	22	78

System suitability solution: 1.0 mg/mL of USP Carvedilol System Suitability Mixture RS in *Diluent*
Sample solution: 1 mg/mL of Carvedilol in *Diluent*
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L68
Column temperature: 30°
Flow rate: 1.4 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.8 between carvedilol and carvedilol related compound F
Analysis
Sample: *Sample solution*

www.webofpharma.com

Calculate the percentage of each impurity in the portion of Carvedilol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity in the *Sample solution*
r_T = sum of all the peak responses in the *Sample solution*

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carvedilol related compound A[a]	0.7	0.1
Carvedilol	1.0	—
Carvedilol related compound F[b]	1.2	0.1[c]
N-Isopropylcarvedilol[d]	1.6	0.1
Carvedilol related compound C[e]	1.8	0.02
Carvedilol related compound B[f]	2.1	0.1
Biscarbazole[g]	3	0.1
Any other individual impurity	—	0.1
Total impurities	—	0.5

[a] 1-(4-(2-Hydroxy-3-(2-(2-methoxyphenoxy)ethylamino)propoxy)-9*H*carbazol-9-yl)-3-(2-(2-methoxyphenoxy)ethylamino) propan-2-ol.
[b] 1-(2-(2-Methoxyphenoxy)ethylamino)-3-(6,7,8,9-tetrahydro-5*H*-carbazol-4-yloxy)propan-2-ol.
[c] This impurity is quantitated using the procedure under *Organic Impurities, Procedure 3: Carvedilol Related Compound F.*
[d] 1-(*H*-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]*N*-isopropylamino]-2-propanol.
[e] 1-(9*H*-Carbazol-4-yloxy)-3-(benzyl(2-(2-methoxyphenoxy)ethyl)amino)-propan-2-ol.
[f] 3,3′-(2-(2-Methoxyphenoxy)ethylazanediyl)bis(1-(9*H*-carbazol-4-yloxy) propan-2-ol).
[g] 1,3-Bis-(9*H*-carbazol-4-yloxy)-2-propanol.

- **ORGANIC IMPURITIES, PROCEDURE 3: CARVEDILOL RELATED COMPOUND F** (if present)

Solution A: Trifluoroacetic acid and water (0.5: 100)
Solution B: Methanol and trifluoroacetic acid (100: 0.5)
Diluent: Water and acetonitrile (1:1)
Mobile phase: *Solution A* and *Solution B* (65:35)
System suitability solution: 1.5 mg/mL of USP Carvedilol System Suitability Mixture RS in *Diluent*
Sample solution: 1.5 mg/mL of Carvedilol in *Diluent* prepared as follows. Initially add *Diluent* to fill about 80% of the total volume. Sonicate briefly to facilitate dissolution. Cool, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 30-mm; 3-µm packing L7
Column temperature: 40°
Flow rate: 2 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between carvedilol and carvedilol related compound F
Analysis
Sample: *Sample solution*

Calculate the percentage of carvedilol related compound F in the portion of the sample taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of carvedilol related compound F from the *Sample solution*
r_T = sum of the peak responses of carvedilol and carvedilol related compound F from the *Sample solution*
F = relative response factor, 1.1

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* by HPLC other than *Procedure 1* is used, then the labeling states the test with which the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Carvedilol RS
USP Carvedilol Related Compound A RS
1-(4-(2-Hydroxy-3-(2-(2-methoxy phenoxy)ethylamino) propoxy)-9*H*-carbazol-9-yl)-3-(2-(2-methoxyphenoxy) ethylamino) propan-2-ol.
$C_{36}H_{43}N_3O_7$ 629.74
USP Carvedilol Related Compound B RS
3,3′-(2-(2-Methoxyphenoxy)ethylazanediyl)bis(1-(9*H*-carbazol-4-yloxy)propan-2-ol).
$C_{39}H_{39}N_3O_6$ 645.74
USP Carvedilol Related Compound C RS
1-(9*H*-Carbazol-4-yloxy)-3-(benzyl(2-(2-methoxyphen oxy)ethyl)amino)propan-2-ol.
$C_{31}H_{32}N_2O_4$ 496.60
USP Carvedilol Related Compound D RS
4-(Oxiran-2-ylmethoxy)-9*H*-carbazole.
$C_{15}H_{13}NO_2$ 239.27
USP Carvedilol Related Compound E RS
2-(2-Methoxyphenoxy)ethyl amine.
$C_9H_{13}NO_2$ 167.21
USP Carvedilol System Suitability Mixture RS
Mixture of approximately 0.1% carvedilol related compound F (1-(2-(2-Methoxyphenoxy)ethylamino)-3-(2,3,4,9-tetrahydro-1*H*-carbazol-5-yloxy)propan-2-ol) in a matrix of carvedilol drug substance.

Carvedilol Tablets

DEFINITION
Carvedilol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carvedilol ($C_{24}H_{26}N_2O_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Wavelength range: 250–400 nm

www.webofpharma.com

Cell: 0.2 cm

Sample solution: 0.125 mg/mL of carvedilol prepared as follows. Place 10 Tablets in a 150-mL polypropylene tube, and disintegrate the Tablets in methanol (100 mL for the Tablet strengths 3.125, 6.25, and 25 mg, and 50 mL for the Tablet strength 12.5 mg) using a mechanical homogenizer. Transfer the homogenate to an appropriate volumetric flask, and dilute with methanol to volume. Pass through a suitable PTFE filter of 0.45-µm pore size.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 0.7 g of anhydrous monobasic potassium phosphate in 500 mL of water, and add 10 mL of triethylamine. Adjust with phosphoric acid to a pH of 3.0 ± 0.1.

Mobile phase: Dissolve 1.04 g of sodium dodecyl sulfate in 150 mL of *Buffer* in a 2-L volumetric flask, and sonicate. Add 720 mL of acetonitrile, and dilute with water to volume. Pass through a nylon 66 filter of 0.2-µm pore size.

Diluent: Methanol and 1 M hydrochloric acid (9:1)

Methanol solution: Methanol and water (1:1)

Standard solution: 0.0125 mg/mL of USP Carvedilol RS prepared as follows. Dissolve a quantity of USP Carvedilol RS in a mixture of *Diluent* and water (9:1), and sonicate until the solution is clear. Dilute with *Methanol solution* to obtain the required final concentration.

Sample stock solution: Transfer a portion of the powdered Tablets (NLT 20), equivalent to 25 mg of carvedilol, to a 100-mL volumetric flask. Add 10 mL of water, shake by hand, then add 70 mL of *Diluent*, and sonicate for 30 min. Shake on a mechanical shaker for about 30 min, and dilute with *Diluent* to volume to prepare a 0.25-mg/mL solution. Centrifuge an appropriate amount (about 50 mL) at 2000 rpm for 10 min.

Sample solution: 0.0125 mg/mL of carvedilol in *Methanol solution* from the *Sample stock solution*. Pass a portion of the solution through a suitable syringe filter of 0.45-µm pore size, discard the first 5 mL, and use the filtrate as the *Sample solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 50-mm; packing L7

Column temperature: 40°

Flow rate: 1 mL/min

Run time: 30 min

Injection size: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carvedilol ($C_{24}H_{26}N_2O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.7% (7 mL/L) of hydrochloric acid, adjusted with 50% (w/w) sodium hydroxide to a pH of 1.45 ± 0.2; 900 mL; deaerated

Apparatus 2: 50 rpm

Time: 30 min

Standard stock solution: Transfer about 7 mg of USP Carvedilol RS to a 250-mL volumetric flask. Add 5 mL of methanol, and sonicate until dissolved. Cool to room temperature, dilute with *Medium* to volume, and mix well.

Standard solution: On the basis of the label claim and using the *Standard stock solution*, prepare a solution of USP Carvedilol RS in *Medium* having an appropriate concentration (C_S), as shown in *Table 1*.

Table 1

Label Claim (mg)	C_S (mg/mL)
25	0.028
12.5	0.014
6.25	0.007
3.125	0.0035

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Analytical wavelengths: 285 and 380 nm

Path length: 1 cm

Blank: *Medium*

Analysis: Calculate the corrected absorbance of the *Standard solution* and the *Sample solution*:

$$A_{corr} = A_{285} - A_{380}$$

A_{corr} = corrected absorbance of the *Standard solution* or the *Sample solution*
A_{285} = absorbance of the *Standard solution* or the *Sample solution* at 285 nm
A_{380} = absorbance of the *Standard solution* at 380 nm

Calculate the percentage of carvedilol dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/L) \times 100$$

A_U = corrected absorbance from the *Sample solution*
A_S = corrected absorbance from the *Standard solution*
C_S = corrected concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of carvedilol ($C_{24}H_{26}N_2O_4$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Simulated gastric fluid without enzymes; 900 mL

Apparatus 2, Time, Standard stock solution, Standard solution, Sample solution, and **Analysis:** Proceed as directed in *Test 1*.

Tolerances: NLT 80% (Q) of the labeled amount of carvedilol ($C_{24}H_{26}N_2O_4$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: Simulated gastric fluid with pepsin, pH 1.45 (dissolve 12.0 g of sodium chloride and 19.2 g of purified pepsin (porcine origin, activity 800–2500 Units/mg of

USP Monographs

www.webofpharma.com

protein) in 18 mL of hydrochloric acid and sufficient water to make 6 L; adjust with hydrochloric acid to a pH of 1.45); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer: 2.72 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.0 ± 0.05.
Mobile phase: *Buffer* and acetonitrile (650:350)
Standard stock solution: 1.4 mg/mL of USP Carvedilol RS in methanol
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (*L*/900) mg/mL, where *L* is the Tablet label claim, in mg.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-mm; 5-µm packing L7
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3500 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis: Calculate the percentage of carvedilol dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of carvedilol ($C_{24}H_{26}N_2O_4$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩
Buffer, Mobile phase, Diluent, Methanol solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: 0.25 mg/mL of carvedilol prepared as follows. Place 1 Tablet into a volumetric flask of appropriate size, based on the label claim. Add water to the flask up to about 10% of volume, and shake by hand to disintegrate the Tablet. Fill the flask up to 75% of volume with *Diluent*, and sonicate for 30 min to obtain complete disintegration. Shake on a mechanical shaker for 30 min, allow to cool, and dilute with *Diluent* to volume. Centrifuge an appropriate amount of this solution for 10 min at 2400 rpm, and transfer 4 mL of supernatant into a 100-mL volumetric flask. Fill the flask to about 85% of volume with *Methanol solution*, and sonicate for 20 min, with intermittent shaking. Dilute with *Methanol solution* to volume, and pass through a suitable syringe filter of 0.45-µm pore size.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carvedilol ($C_{24}H_{26}N_2O_4$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES
• **Organic Impurities**
Buffer, Mobile phase, Diluent, Methanol solution, and **Sample stock solution:** Prepare as directed in the *Assay*.
Standard stock solution: Use the *Standard solution* from the *Assay*.
Standard solution: 1.25 µg/mL USP Carvedilol RS in a mixture of *Diluent* and water (1:1) from the *Standard stock solution*
Sample solution: Dilute with water to volume, 25 mL of the supernatant from the *Sample stock solution* in a 50-mL volumetric flask. Pass a portion of the solution through a suitable syringe filter of 0.45-µm pore size.
Chromatographic system: Proceed as directed in the *Assay*, except for *Injection size*.
Injection size: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of carvedilol from the *Standard solution*
C_S = concentration of USP Carvedilol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of carvedilol in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: NMT 0.2% (specified or unspecified)
Total impurities: NMT 1.0%
[Note—Disregard any peaks with a relative retention time less than or equal to 0.04 and peaks with less than 0.05% of the nominal carvedilol peak response in the *Sample solution*.]

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers protected from moisture. Store at controlled room temperature.
• **Labeling:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
• **USP Reference Standards** ⟨11⟩
USP Carvedilol RS

Casanthranol

» Casanthranol is obtained from Cascara Sagrada. It contains in each 100 g not less than 20.0 g of total hydroxyanthracene derivatives calculated on the dried basis, calculated as cascaroside A. Not less

www.webofpharma.com

than 80 percent of the total hydroxyanthracene derivatives consists of cascarosides, calculated as cascaroside A.

Packaging and storage—Preserve in tight, light-resistant containers, at a temperature not exceeding 30°.
Loss on drying ⟨731⟩—Dry it in vacuum at 80° for 16 hours: it loses not more than 10.0% of its weight.
Residue on ignition ⟨281⟩: not more than 4.0%.
Assay for total hydroxyanthracene derivatives—[NOTE 1—Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.6 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results. NOTE 2—Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed for *Volumetric Solutions* in the section *Reagents, Indicators, and Solutions*.]

Ferric chloride solution—Dissolve 100 g of ferric chloride in water to make 100 mL.

Assay solution—Mix a portion of Casanthranol, and transfer an accurately weighed quantity of about 500 mg to a 100-mL volumetric flask. Add about 30 mL of 70 percent alcohol, swirl to dissolve, dilute with 70 percent alcohol to volume, and mix. Quickly filter through soft, rapid-flow filter paper, taking precautions to minimize loss by evaporation.

Assay preparation—Pipet 10 mL of *Assay solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the combined water layers, with the aid of water, to a 50-mL volumetric flask, filtering through a small pledget of cotton, water-wet, dilute with water to volume, and mix.

Procedure—Pipet 10 mL of *Assay preparation* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 hours by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 minutes, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix. Evaporate a 20.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 1 in 200 solution of magnesium acetate in methanol. Determine the absorbance against methanol as a reference, in 1-cm cells at the wavelength of maximum absorbance at about 515 nm. Calculate the quantity, in mg, of total hydroxyanthracene derivatives in the portion of Casanthranol taken by the formula:

$$155A_U$$

in which A_U is the absorbance of the solution from the *Assay preparation*.

Assay for cascarosides—[NOTE 1—Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.7 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results. NOTE 2—Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed for *Volumetric Solutions* in the section *Reagents, Indicators, and Solutions*.]

Ferric chloride solution and *Assay solution*—Prepare as directed in the *Assay for total hydroxyanthracene derivatives*.

Assay preparation—Pipet 10 mL of *Assay solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined aqueous phase with 30 mL of clear, freshly prepared water-saturated ethyl acetate, and transfer the water layer to another separatory funnel. Repeat the extraction with two additional 30-mL portions of the freshly prepared water-saturated ethyl acetate. Add 5 mL of water to the combined ethyl acetate extracts, shake, allow the phases to separate, discard the ethyl acetate extracts, and add 30 mL of the freshly prepared water-saturated ethyl acetate to the water wash. Shake, allow the phases to separate, and discard the ethyl acetate phase. Transfer the combined aqueous phases, with the aid of water, to a 50-mL volumetric flask, filtering through a small pledget of cotton, water-wet, dilute with water to volume, and mix.

Procedure—Pipet 15 mL of *Assay preparation* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 hours by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 minutes, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix. Evaporate a 20.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 1 in 200 solution of magnesium acetate in methanol. Determine the absorbance, against methanol as a reference, in 1-cm cells at the wavelength of maximum absorbance at about 515 nm. Calculate the quantity, in mg, of cascarosides in the portion of Casanthranol taken by the formula:

$$103.5A_U$$

in which A_U is the absorbance of the solution from the *Assay preparation*.

Stimuli Article

www.webofpharma.com

Cascara Sagrada

DEFINITION

Cascara Sagrada is the dried bark of *Frangula purshiana* (DC.) A.Gray [syn. *Rhamnus purshiana* DC.] (Fam. Rhamnaceae). It yields NLT 7.0% of the total hydroxyanthracene derivatives, calculated as cascaroside A and calculated on the dried basis. NLT 60% of the total hydroxyanthracene derivatives consists of cascarosides, calculated as cascaroside A.

[NOTE—Collect Cascara Sagrada NLT 1 year before use.]

IDENTIFICATION

• **A.**

Sample: 100 mg of powdered Cascara Sagrada

Analysis: Add the *Sample* to 10 mL of hot water, shake the mixture occasionally until it is cold, filter, dilute the filtrate with water to 10 mL, and add 10 mL of 6 N ammonium hydroxide.

Acceptance criteria: An orange color is produced.

• **B.**

Sample: A portion of Cascara Sagrada

Analysis: Treat the *Sample* with 6 N ammonium hydroxide.

Acceptance criteria: It becomes red to reddish brown in color.

• **C.**

Sample: 100 mg of powdered Cascara Sagrada

Analysis: Macerate the *Sample* with 1 mL of alcohol, add 10 mL of water, boil the mixture, then cool, filter, and shake the filtrate with 10 mL of ether: a greenish-yellow ether layer separates. Shake 3 mL of the ether layer with 3 mL of 6 N ammonium hydroxide, and dilute the separated ammonia solution with 20 mL of water.

Acceptance criteria: A distinct orange-pink color remains.

COMPOSITION

• **CONTENT OF TOTAL HYDROXYANTHRACENE DERIVATIVES**

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.6 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this procedure, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions*.

Ferric chloride solution: 1 g/mL of ferric chloride in water

Sample stock solution: Add 1 g of Cascara Sagrada to 70 mL of boiling water, boil for several minutes, with stirring. Allow to cool, and transfer with the aid of water to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter through suitable filter paper.

Sample solution: Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the combined water layers, with the aid of water, to a 50-mL volumetric flask, dilute with water to volume, and mix.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Visible

Analytical wavelength: 515 nm

Cell: 1 cm

Blank: Methanol

Analysis

Sample: *Sample solution*

Pipet 15 mL of *Sample solution* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 h by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 min, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix. Evaporate a 15.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 5-mg/mL solution of magnesium acetate in methanol.

Calculate the weight, in mg, of total hydroxyanthracene derivatives (T_{HD}) in the portion of Cascara Sagrada taken:

$$T_{HD} = A_U \times F$$

A_U = absorbance of the *Sample solution*

F = conversion factor, 138. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A and the dilutions to prepare the solution for analysis.]

Calculate the percentage of total hydroxyanthracene derivatives, calculated as cascaroside A:

$$\text{Result} = (T_{HD}/W) \times 100$$

T_{HD} = weight of total hydroxyanthracene derivatives (mg)

W = weight of Cascara Sagrada taken to prepare the *Sample stock solution* (mg)

Acceptance criteria: NLT 7.0%, calculated as cascaroside A, on the dried basis

• **CONTENT OF CASCAROSIDES**

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.7 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this procedure, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions*.

Ferric chloride solution and **Sample stock solution:** Prepare as directed in the test for *Content of Total Hydroxyanthracene Derivatives*.

Sample solution: Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first

USP Monographs

www.webofpharma.com

separatory funnel. Extract the combined aqueous phase with 30 mL of clear, freshly prepared, water-saturated ethyl acetate, and transfer the water layer to another separatory funnel. Repeat the extraction with two additional 30-mL portions of the freshly prepared, water-saturated ethyl acetate. Add 5 mL of water to the combined ethyl acetate extracts, shake, allow the phases to separate, discard the ethyl acetate extracts, and add 30 mL of the freshly prepared, water-saturated ethyl acetate to the water wash. Shake, allow the phases to separate, and discard the ethyl acetate phase. Transfer the combined aqueous phases, with the aid of water, to a 50-mL volumetric flask. Dilute with water to volume.

Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Visible
Analytical wavelength: 515 nm
Cell: 1 cm
Blank: Methanol

Analysis
Proceed as directed for *Analysis* in *Content of Total Hydroxyanthracene Derivatives*, except to evaporate a 20.0-mL portion of the methylene chloride solution instead of 15.0 mL.
Sample: *Sample solution*
Determine the absorbance and calculate the percentage of cascarosides with respect to the content of total hydroxyanthracene derivatives in the portion of Cascara Sagrada taken:

$$\text{Result} = (A_U/T_{HD}) \times F \times 100$$

A_U = absorbance of the *Sample solution*
T_{HD} = weight of total hydroxyanthracene derivatives (mg)
F = conversion factor, 103.5. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A and the dilutions to prepare the solution for analysis.]

Acceptance criteria: NLT 60% of the total hydroxyanthracene derivatives consists of cascarosides, calculated as cascaroside A, on the dried basis.

CONTAMINANTS

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Pesticide Residue Analysis*: Meets the requirements

SPECIFIC TESTS

• **BOTANICAL CHARACTERISTICS**

Macroscopic
Cascara Sagrada: The bark is usually in the form of flattened or transversely curved pieces, occasionally in quills of variable length and from 1 to 5 mm in thickness. The outer surface is brown, purplish brown, or brownish red, longitudinally ridged, with or without grayish or whitish lichen patches, sometimes with numerous lenticels and occasionally with moss attached. The inner surface is longitudinally striate, light yellow, weak reddish brown, or moderate yellowish brown. The fracture is short with projections of phloem fiber bundles in the inner bark.
Powdered Cascara Sagrada: The powder is moderate yellowish brown to dusky yellowish orange.

Microscopic
Cascara Sagrada: The transverse section of the bark shows a yellowish-brown, purple, or reddish-brown cork of up to 10 or more rows of small cells; stone cells in yellowish, tangentially elongated groups of 20–50 cells in the cortex, pericycle, and outer phloem regions; phloem rays 1–4 cells wide, 15–25 cells deep, frequently diagonal or curved, forming converging groups; phloem fibers in small bundles, more or less surrounded by crystal fibers and located between the phloem rays; parenchyma with brown walls and containing starch grains and calcium oxalate crystals.
Powdered Cascara Sagrada: It shows numerous broken phloem fiber bundles with accompanying crystal fibers containing monoclinic prisms of calcium oxalate; stone cells more or less adherent, in small groups with thick, finely lamellated and porous walls; fragments of reddish-brown to yellow cork; masses of parenchyma and phloem ray cells colored reddish brown to orange upon the addition of a solution of an alkali; starch grains spheroidal, up to 8 µm in diameter; calcium oxalate in monoclinic prisms or rosette aggregates from 6 to 20 µm in diameter, occasionally up to 45 µm in diameter.

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Foreign Organic Matter*: NMT 4.0%

• **WATER DETERMINATION** ⟨921⟩, *Method III, Procedure for Articles of Botanical Origin*
Analysis: Dry at 105° for 5 h.
Acceptance criteria: NMT 12.0%

Cascara Sagrada Extract

DEFINITION

Cascara Sagrada Extract contains, in each 100 g, NLT 10.0 g and NMT 12.0 g of total hydroxyanthracene derivatives, of which NLT 50% consists of cascarosides, both calculated as cascaroside A.

Mix 900 g of Cascara Sagrada, in coarse powder, with 4000 mL of boiling water, and macerate the mixture for 3 h. Then transfer it to a percolator, allow it to drain, exhaust it by percolation, using boiling water as the menstruum, and collect 5000 mL of percolate. Evaporate the percolate to dryness, reduce the Extract to a fine powder, and, after assaying, add sufficient starch, dried at 100°, or other inert, nontoxic diluents to make the product contain, in each 100 g, 11 g of hydroxyanthracene derivatives. Mix the powders, and pass the Extract through a number 60 sieve.

ASSAY

• **CONTENT OF TOTAL HYDROXYANTHRACENE DERIVATIVES**

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.6 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.
Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions*.
Ferric chloride solution: 1 g/mL of ferric chloride in water
Sample stock solution: Transfer 1 g of Extract to a 100-mL volumetric flask. Add 60 mL of 70% alcohol, swirl or sonicate for 15–20 min several times, and allow to stand overnight. Sonicate or swirl for 10–15 min, dilute with 70% alcohol to volume, mix, and filter through suitable filter paper.
Sample solution: Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene

USP Monographs

www.webofpharma.com

chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the combined water layers, with the aid of water, to a 50-mL volumetric flask, and dilute with water to volume, and mix.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Visible

Analytical wavelength: 515 nm

Cell: 1 cm

Blank: Methanol

Analysis

Sample: *Sample solution*

Analysis: Pipet 10 mL of *Sample solution* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 h by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 min, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, and dilute with methylene chloride to volume. Evaporate a 20.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 5 mg/mL solution of magnesium acetate in methanol.

Calculate the quantity, in mg, of total hydroxyanthracene derivatives (T_{HD}) in the portion of Cascara Sagrada Extract taken:

$$T_{HD} = A_U \times F$$

A_U = absorbance of the *Sample solution*

F = conversion factor, 155.2. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A and the dilutions to prepare the solution for analysis.]

Calculate the percentage of total hydroxyanthracene derivatives in the portion of Cascara Sagrada Extract taken:

$$\text{Result} = (T_{HD}/W) \times 100$$

W = weight of Cascara Sagrada Extract taken to prepare the *Sample stock solution* (mg)

Acceptance criteria: 10.0%–12.0% of total hydroxyanthracene derivatives, calculated as cascaroside A

• **CONTENT OF CASCAROSIDES**

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.7 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions.*

Ferric chloride solution and **Sample stock solution:** Prepare as directed in the *Assay* for *Content of Total Hydroxyanthracene Derivatives.*

Sample solution: Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined aqueous phase with 30 mL of clear, freshly prepared, water-saturated ethyl acetate, and transfer the water layer to another separatory funnel. Repeat the extraction with two additional 30-mL portions of the freshly prepared, water-saturated ethyl acetate. Add 5 mL of water to the combined ethyl acetate extracts, shake, allow the phases to separate, discard the ethyl acetate extracts, and add 30 mL of the freshly prepared, water-saturated ethyl acetate to the water wash. Shake, allow the phases to separate, and discard the ethyl acetate phase. Transfer the combined aqueous phases, with the aid of water, to a 50-mL volumetric flask. Dilute with water to volume, and mix.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Visible

Analytical wavelength: 515 nm

Cell: 1 cm

Blank: Methanol

Analysis

Sample: *Sample solution*

Pipet 25 mL of the *Sample solution* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 h by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 min, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix. Evaporate a 20.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 5-mg/mL solution of magnesium acetate in methanol.

Determine the absorbance and calculate the percentage of cacarosides with respect to the content of total hydroxyanthracene derivatives in the portion of Cascara Sagrada Extract taken:

$$\text{Result} = (A_U/T_{HD}) \times F \times 100$$

A_U = absorbance of the *Sample solution*

T_{HD} = weight of total hydroxyanthracene derivatives (mg)

F = conversion factor, 62.06. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A and the dilutions to prepare the solution for analysis.]

Acceptance criteria: NLT 50% of total hydroxyanthracene derivatives calculated as cascaroside A

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature not exceeding 30°.

Cascara Tablets

DEFINITION

Cascara Tablets are prepared from Cascara Sagrada Extract. They contain NLT 9.35% and NMT 12.65% of total hydroxyanthracene derivatives, calculated as cascaroside A, in the labeled amount of Cascara Sagrada Extract. NLT 50% of the hydroxyanthracene derivatives are cascarosides, calculated as cascaroside A.

STRENGTH

- **CONTENT OF TOTAL HYDROXYANTHRACENE DERIVATIVES**

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.6 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions, Volumetric Solutions.*

Ferric chloride solution: 1 g/mL of ferric chloride in water

Sample stock solution: Weigh and finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 1 g of Cascara Sagrada Extract, to a 100-mL volumetric flask. Add 60 mL of 70% alcohol, swirl or sonicate for 15–20 min several times, and allow to stand overnight. Sonicate or swirl for 10–15 min, dilute with 70% alcohol to volume, mix, and filter through suitable filter paper.

Sample solution: Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the combined water layers, with the aid of water, to a 50-mL volumetric flask, dilute with water to volume, and mix.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Visible

Analytical wavelength: 515 nm

Cell: 1 cm

Blank: Methanol

Analysis

Sample: *Sample solution*

Pipet 10 mL of *Sample solution* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 h by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 min, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix.

Evaporate a 15.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 5-mg/mL solution of magnesium acetate in methanol.

Calculate the quantity, in mg, of total hydroxyanthracene derivatives (T_{HD}) in the portion of Cascara Sagrada Extract taken:

$$T_{HD} = A_U \times F$$

A_U = absorbance of the *Sample solution*

F = conversion factor, 206.9. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A, and the dilutions to prepare the solution for analysis.]

Calculate the percentage of total hydroxyanthracene derivatives in the nominal amount of Cascara Sagrada Extract taken:

$$\text{Result} = (T_{HD}/W) \times 100$$

W = nominal weight of Cascara Sagrada Extract in the portion of Tablets powder taken to prepare the *Sample stock solution* (mg)

Acceptance criteria: 9.35%–12.65% in the labeled amount of Cascara Sagrada Extract, calculated as cascaroside A

- **CONTENT OF CASCAROSIDES**

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.7 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions, Volumetric Solutions.*

Ferric chloride solution and **Sample stock solution:** Prepare as directed in *Content of Total Hydroxyanthracene Derivatives.*

Sample solution: Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the water layer to the first separatory funnel. Extract the combined aqueous phase with 30 mL of clear, freshly prepared, water-saturated ethyl acetate, and transfer the water layer to another separatory funnel. Repeat the extraction with two additional 30-mL portions of the freshly prepared, water-saturated ethyl acetate. Add 5 mL of water to the combined ethyl acetate extracts, shake, allow the phases to separate, discard the ethyl acetate extracts, and add 30 mL of the freshly prepared, water-saturated ethyl acetate to the water wash. Shake, allow the phases to separate, and discard the ethyl acetate phase. Transfer the combined aqueous phases, with the aid of water, to a 50-mL volumetric flask. Dilute with water to volume, and mix.

www.webofpharma.com

Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Visible
Analytical wavelength: 515 nm
Cell: 1 cm
Blank: Methanol
Analysis
Sample: *Sample solution*
Prepare as directed for *Analysis* in *Content of Total Hydroxyanthracene Derivatives*, except to pipet 20 mL of *Sample solution*.
Determine the absorbance, and calculate the percentage of cacarosides with respect to the content of total hydroxyanthracene derivatives in the nominal amount of Cascara Sagrada Extract in the portion of Tablets powder taken:

$$\text{Result} = (A_U/T_{HD}) \times F \times 100$$

A_U = absorbance of the *Sample solution*
T_{HD} = weight of total hydroxyanthracene derivatives (mg)
F = conversion factor, 103.5. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A, and the dilutions to prepare the solution for analysis.]

Acceptance criteria: NLT 50% of the content of total hydroxyanthracene derivatives are cascarosides, calculated as cascaroside A.

PERFORMANCE TESTS
- **DISINTEGRATION** ⟨701⟩**:** 60 min
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers; if the Tablets are coated, well-closed containers may be used.

Cascara Sagrada Fluidextract

DEFINITION
Prepare Cascara Sagrada Fluidextract as follows. To 1000 g of coarsely ground Cascara Sagrada add 3000 mL of boiling water, and allow to macerate in a suitable percolator for 2 h. Allow the percolation to proceed at a moderate rate, gradually adding boiling water until the drug is practically exhausted of its active principles. Evaporate the percolate on a water bath or in a vacuum still to NMT 800 mL. Cool, add 200 mL of alcohol and, if necessary, add sufficient water to make the product measure 1000 mL. Mix.

OTHER COMPONENTS
- **ALCOHOL DETERMINATION,** *Method I* ⟨611⟩**:** 18.0%–20.0% of C_2H_5OH

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

Aromatic Cascara Fluidextract

DEFINITION
Prepare Aromatic Cascara Fluidextract as follows.

Cascara Sagrada, as a very coarse powder	1000 g
Magnesium Oxide	120 g
Suitable sweetening agent(s)	
Suitable essential oils(s)	
Suitable flavoring agent(s)	
Alcohol	200 mL
Purified Water, a sufficient quantity, to make	1000 mL

Mix the Cascara Sagrada with *Magnesium Oxide*, moisten it uniformly with 2000 mL of boiling water, and set it aside in a shallow container for 48 h, stirring occasionally. Pack it in a percolator, and percolate with boiling water until the material is exhausted. Evaporate the percolate, at a temperature not exceeding 100°, to 750 mL, and at once dissolve in it the flavoring agent(s). When the liquid has cooled, add the *Alcohol*, in which the sweetening agent(s) and oils have been dissolved, and add sufficient water to make the Aromatic Fluidextract measure 1000 mL. Mix.

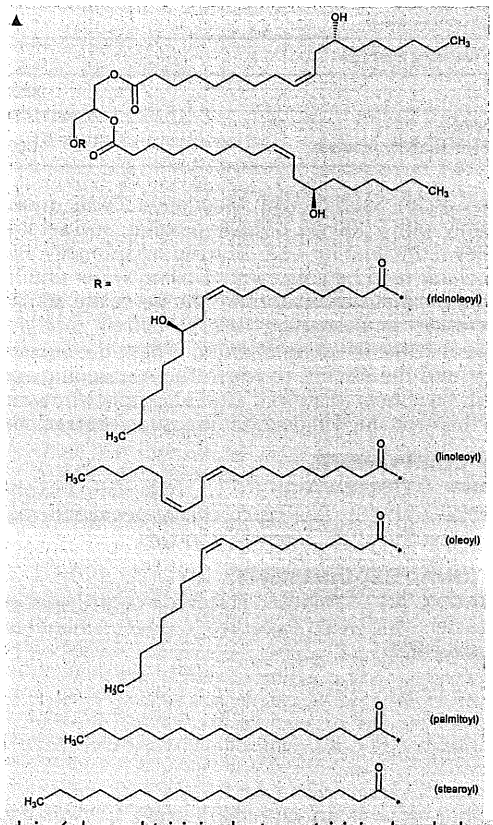
OTHER COMPONENTS
- **ALCOHOL DETERMINATION** ⟨611⟩**:** 18%–20% of C_2H_5OH, determined by the gas–liquid chromatographic method, using acetone as the internal standard

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

www.webofpharma.com

Castor Oil

Add the following:



Triricinolein (glyceryl triricinoleate or triricinoleoyl-glycerol) predominates [8001-79-4▲ 2S(USP41)].

Change to read:

DEFINITION

Castor Oil is the fixed oil obtained from the seed of *Ricinus communis* L. (Family Euphorbiaceae). ▲Castor Oil consists of NLT 90.0% of the triglyceride of ricinoleic acid.▲ 2S(USP41) It contains no added substances.

IDENTIFICATION

Add the following:

▲• A. Identity by Fatty Acid Composition

Diluent: *n*-Heptane

Standard solution 1: 0.2 mg/mL each of methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, methyl linolenate, methyl *cis*-11-eicosenoate, and methyl ricinoleate from, respectively, USP Methyl Palmitate RS, USP Methyl Stearate RS, USP Methyl Oleate RS, USP Methyl Linoleate RS, USP Methyl Linolenate RS, methyl *cis*-11-eicosenoate, and USP Methyl Ricinoleate RS in *Diluent*

Standard solution 2: 4 mg/mL each of methyl stearate and methyl ricinoleate from USP Methyl Stearate RS and USP Methyl Ricinoleate RS in *Diluent*

Sample solution: Transfer 140 mg of Castor Oil to a 10-mL screw-cap test tube, add 3.0 mL of *Diluent*, and mix well. Add 0.5 mL of 0.5 M sodium methoxide in methanol,[1] and mix with the sample. Allow the reaction to proceed at room temperature for 2 h. After 2 h, add 5 mL of water, and mix. Separate the organic layer (the upper layer), and remove the lower layer. Place an aliquot of the organic layer into an autosampler vial.

Chromatographic system

(See Chromatography ⟨621⟩, System Suitability.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 15-m fused silica capillary; bonded with a 0.25-µm layer of phase G7

Temperatures

Injection port: 240°

Detector: 250°

Column: See *Table 1* for the oven program.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)	Total Time (min)
80	0	80	1	1
80	30	140	0	3
140	3	150	0	6.3
150	1	155	0	11.3
155	2	165	0	16.3
165	3	220	10	45

Column mode: See *Table 2* for the pressure program.

Table 2

Pressure (psi)	Pressure Ramp (psi/min)	Hold Time (min)	Total Time (min)
10	0	16	16
4	5	9 or 0[a]	26.2 or 17.2
3	10	19 or 28	45

[a] If considerable discrimination of late-eluting compounds is observed, the hold time can be adjusted from 9 to 0 min. Thus the total time should be 17.2 min. The next step of the hold time should be 28 min.

Carrier gas: Hydrogen

Injection volume: 0.5 µL

Injection type: Split ratio, 60:1

Liner: Single taper, low-pressure drop with deactivated wool

Run time: 45 min

System suitability

Sample: *Standard solution 1*

[NOTE—See *Table 3* for relative retention times.]

Table 3

Component	Relative Retention Time
Methyl palmitate (C16:0)	0.62
Methyl stearate (C18:0)	0.98

[1] 0.5 M sodium methoxide in methanol is available from Sigma-Aldrich (www.sigmaaldrich.com), product #403067. Any other equivalent reagent can be used as well.

www.webofpharma.com

Table 3 *(continued)*

Component	Relative Retention Time
Methyl oleate (C18:1)	1.00
Methyl linoleate (C18:2)	1.08
Methyl linolenate (C18:3)	1.19
Methyl *cis*-11-eicosenoate (C20:1)	1.63
Methyl ricinoleate	2.68

Suitability requirements

Resolution: NLT 1.5 between the methyl stearate and methyl oleate peaks

Relative standard deviation: NMT 2.0% for the peak area ratio of methyl ricinoleate to methyl linoleate

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

The peak of methyl *cis*-11-octadecenoate, which is an isomer of methyl oleate, can be resolved from the methyl oleate peak with a resolution of about 1 and a relative retention time of 1.01 with respect to methyl oleate.

Calculate the relative response factor (*F*) for methyl ricinoleate:

$$F = (r_S/r_R) \times (C_R/C_S)$$

r_S = peak area of methyl stearate from *Standard solution 2*

r_R = peak area of methyl ricinoleate from *Standard solution 2*

C_R = concentration of USP Methyl Ricinoleate RS in *Standard solution 2* (mg/mL)

C_S = concentration of USP Methyl Stearate RS in *Standard solution 2* (mg/mL)

Correct the peak area of methyl ricinoleate in the *Sample solution* by multiplying by *F*.

Calculate the percentage of each fatty acid component in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual fatty acid methyl ester, except for the uncorrected peak area of methyl ricinoleate (or corrected peak area of methyl ricinoleate) in the *Sample solution*

r_T = sum of all the peak areas, excluding the solvent and methyl ricinoleate peaks and including the corrected peak area of methyl ricinoleate in the *Sample solution*

Acceptance criteria: Castor Oil exhibits the composition profile of fatty acids shown in *Table 4*.

Table 4

Component	Percentage (%)
Palmitic acid (C16:0)	≤2.0
Stearic acid (C18:0)	≤2.5
Oleic acid (C18:1)	2.5–6.0
Linoleic acid (C18:2)	2.5–7.0
Linolenic acid (C18:3)	≤1.0

Table 4 *(continued)*

Component	Percentage (%)
cis-11-Eicosenoic acid (C20:1)	≤1.0
Ricinoleic acid	85.0–92.0
cis-11-Octadecenoic acid or any other unidentified fatty acid	≤1.0 ▲2S(USP41)

Add the following:

▲• **B. Distinction from Most Other Fixed Oils:** It is only slightly soluble in solvent hexane (distinction from most other fixed oils), but it yields a clear liquid with an equal volume of alcohol (foreign fixed oils). ▲2S(USP41)

Add the following:

▲• **C. Identification of Refined Castor Oil or Virgin Castor Oil by Using Ultraviolet Absorption**

Sample solution: Dissolve 1.0 g of Castor Oil in alcohol, and dilute with alcohol to 100 mL.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 270 nm

Cell: 1 cm

Analysis: Determine the UV-Vis absorbance using the *Instrumental conditions*.

Acceptance criteria

Refined Castor Oil: The absorbance is within the range of 0.7–1.5.

Virgin Castor Oil: The absorbance is NMT 0.7. ▲2S(USP41)

ASSAY

Add the following:

▲• **Triglyceride Composition**

[Note—The fatty acid radicals are designated as linoleic (L), oleic (O), palmitic (P), ricinoleic (R), and stearic (S), and the common abbreviations for triglycerides used are as follows: triricinolein (glyceryl triricinoleate or triricinoleoyl-glycerol) (RRR), diricinoleoyl-linoleoyl-glycerol (RRL), diricinoleoyl-oleoyl-glycerol (RRO), diricinoleoyl-palmitoyl-glycerol (RRP), and diricinoleoyl-stearoyl-glycerol (RRS).]

Solution A: Methanol

Solution B: 2-Propanol

Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	50	50
23	0	100
25	100	0
35	100	0

Diluent: 2-Propanol

System suitability solution: 2.0 mg/mL of USP Castor Oil RS in *Diluent*

Sample solution: 2.0 mg/mL of Castor Oil in *Diluent*

USP Monographs

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Evaporative light-scattering
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 25°
Detector: 40°
Flow rate: 1.0 mL/min
Injection volume: 5 µL
Run time: 35 min
[NOTE—Depending on the different settings of the detector, the temperature and *Flow rate* can be adjusted as long as system suitability requirements are met.]
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 6* for relative retention times.]

Table 6

Component	Relative Retention Time
RRR	1.0
RRL	1.6
RRO[a]	1.8
RRS	2.0

[a] RRP coelutes with RRO, and the percentage of RRP is about 10 times less than that of RRO.

Suitability requirements
Resolution: NLT 5.0 between the RRR and RRL peaks; NLT 2.0 between the RRL and RRO peaks; NLT 3.0 between the RRO and RRS peaks
Tailing factor: 0.8–1.8 for the RRR peak
Relative standard deviation: NMT 2% for the RRR peak
Analysis
Samples: *System suitability solution* and *Sample solution*
Calculate the percentage of each of the triglycerides in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual triglyceride
r_T = sum of all the peak areas, excluding the solvent peak

Acceptance criteria: Castor Oil exhibits the composition profile of triglycerides shown in *Table 7*.

Table 7

Component	Percentage (%)
RRR	≥90.0
RRL	2.0–4.0
RRO	2.5–5.0
RRS	0.2–0.8 ▲2S(USP41)

IMPURITIES

Delete the following:

▲• **HEAVY METALS** ⟨231⟩, *Method II*: NMT 10 ppm ▲(Official 1-Jan-2018)

SPECIFIC TESTS
• **SPECIFIC GRAVITY** ⟨841⟩: 0.957–0.961

Delete the following:

▲• **DISTINCTION FROM MOST OTHER FIXED OILS:** It is only partly soluble in solvent hexane (distinction from most other fixed oils), but it yields a clear liquid with an equal volume of alcohol (foreign fixed oils). ▲2S(USP41)

Delete the following:

▲• **FATS AND FIXED OILS** ⟨401⟩, *Free Fatty Acids*: The free fatty acids in 10 g require NMT 3.5 mL of 0.10 N sodium hydroxide for neutralization. ▲2S(USP41)

Add the following:

▲• **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Acid Value*: NMT 2.0 ▲2S(USP41)

Change to read:

• **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Hydroxyl Value*
Free acid determination: ▲The acid value (*A*) is determined from *Fats and Fixed Oils* ⟨401⟩, *Procedures, Acid Value*. ▲2S(USP41)
Hydroxyl value determination
Sample: 2 g
Blank: 5.0 mL of a freshly prepared mixture of acetic anhydride and pyridine (1:3)
Titrimetric system
(See Titrimetry ⟨541⟩.)
Mode: Residual titration
Titrant: 0.5 N alcoholic potassium hydroxide VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a glass-stoppered, 250-mL conical flask. Add 5.0 mL of a freshly prepared mixture of acetic anhydride and pyridine (1:3), and swirl to mix. Connect the flask to a reflux condenser, and heat on a steam bath for 2 h. Add 10 mL of water through the condenser, swirl to mix, heat on a steam bath for an additional 10 min, and allow to cool to room temperature. Add through the condenser 15 mL of normal butyl alcohol that has been neutralized previously to phenolphthalein, remove the condenser, and wash the tip of the condenser and the sides of the flask with an additional 10 mL of neutralized normal butyl alcohol. Add 1 mL of phenolphthalein TS, and titrate with *Titrant* to a faint pink endpoint.
Calculate the hydroxyl value in the portion of Oil taken:

$$\text{▲Result} = (V_B - V_T) \times [(M_r \times N)/W] + A \text{▲ 2S(USP41)}$$

V_B = volume of *Titrant* consumed by the *Blank* (mL) ▲▲2S(USP41)
V_T = volume of *Titrant* consumed by the *Sample* in the hydroxyl value determination (mL)
M_r = milliequivalent weight of potassium hydroxide, 56.11 mg/mEq
N = actual normality of the *Titrant*
W = sample weight from the hydroxyl value determination (g)

www.webofpharma.com

▲*A* = acid value from the test for *Fats and Fixed Oils, Acid Value*▲ 2S(USP41)

Acceptance criteria: 160–168

Add the following:

▲• **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Peroxide Value*: NMT 10.0▲ 2S(USP41)

Delete the following:

▲• **FATS AND FIXED OILS,** *Iodine Value* ⟨401⟩: 83–88▲ 2S(USP41)

Delete the following:

▲• **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Saponification Value*: 176–182▲ 2S(USP41)

Add the following:

▲• **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Unsaponifiable Matter*: NMT 0.8%▲ 2S(USP41)

Add the following:

▲• **WATER DETERMINATION** ⟨921⟩: NMT 0.3%▲ 2S(USP41)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight containers, avoid exposure to excessive heat, ▲and protect from light.▲ 2S(USP41)

Add the following:

▲• **LABELING:** Where Castor Oil is intended for use in the manufacture of injectable dosage forms, it is so labeled. Where Castor Oil must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled.▲ 2S(USP41)

Add the following:

▲**OTHER REQUIREMENTS:** For Castor Oil intended for use in injectable dosage forms, which is specified in the *Labeling* section, the following specifications must be met:

• **WATER DETERMINATION** ⟨921⟩: NMT 0.2%
• **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Acid Value*: NMT 0.8
• **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Peroxide Value*: NMT 5.0
• **ULTRAVIOLET ABSORPTION**
Sample solution: Dissolve 1.0 g of Castor Oil in alcohol, and dilute with alcohol to 100 mL.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 270 nm
Cell: 1 cm
Analysis: Determine the UV-Vis absorbance using the *Instrumental conditions* described above.
Acceptance criteria: The absorbance is within the range of 0.7–1.5.
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: The level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Castor Oil is used can be met. Where the label states that Castor Oil must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Castor Oil is used can be met.
• **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Saponification Value*: 176–182▲ 2S(USP41)

Add the following:

▲• **USP REFERENCE STANDARDS** ⟨11⟩
USP Castor Oil RS
USP Methyl Linoleate RS
USP Methyl Linolenate RS
USP Methyl Oleate RS
USP Methyl Palmitate RS
USP Methyl Ricinoleate RS
USP Methyl Stearate RS▲ 2S(USP41)

Castor Oil Capsules

DEFINITION
Castor Oil Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of castor oil, calculated from the tests for *Weight Variation* and *Specific Gravity*.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)
Standard solution: 40 mg/mL of Castor Oil in chloroform
Sample solution: 40 mg/mL of the oil from Capsules in chloroform
Acceptance criteria: Meet the requirements

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

SPECIFIC TESTS
• **SPECIFIC GRAVITY** ⟨841⟩
Sample: Capsule contents
Acceptance criteria: 0.957–0.961
• **FATS AND FIXED OILS,** *Free Fatty Acids* ⟨401⟩: The free fatty acids in 10 g require NMT 3.5 mL of 0.10 N sodium hydroxide for neutralization.
• **FATS AND FIXED OILS,** *Hydroxyl Value* ⟨401⟩
Free acid determination: Determine the amount of free acid in the Capsule contents.
Sample: 10 g of Capsule contents
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.5 N alcoholic potassium hydroxide VS
Endpoint detection: Visual
Analysis 1: Transfer the *Sample* to a 250-mL conical flask, add 10 mL of pyridine that has been neutralized previously to phenolphthalein, and swirl to mix. Add 1 mL of phenolphthalein TS, and titrate with *Titrant* to a faint pink endpoint. Record the volume of *Titrant* consumed.
Hydroxyl value determination: Determine the hydroxyl value of the Capsule contents.
Sample: 2 g from the Capsule contents
Blank: 5.0 mL of a freshly prepared mixture of 1 volume of acetic anhydride and 3 volumes of pyridine

www.webofpharma.com

Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 0.5 N alcoholic potassium hydroxide VS
Endpoint detection: Visual

Analysis 2: Transfer the *Sample* to a glass-stoppered, 250-mL conical flask. Add 5.0 mL of a freshly prepared mixture of 1 volume of acetic anhydride and 3 volumes of pyridine, and swirl to mix. Connect the flask to a reflux condenser, and heat on a steam bath for 2 h. Add 10 mL of water through the condenser, swirl to mix, heat on a steam bath for an additional 10 min, and allow to cool to room temperature. Add through the condenser 15 mL of normal butyl alcohol that previously has been neutralized to phenolphthalein, remove the condenser, and wash the tip of the condenser and the sides of the flask with an additional 10 mL of neutralized normal butyl alcohol. Add 1 mL of phenolphthalein TS, and titrate with *Titrant* to a faint pink endpoint.

Calculate the hydroxyl value in the portion of Capsule contents taken:

$$\text{Result} = (M_r \times N/W) \times [B + (W \times A/C) - T]$$

M_r = milliequivalent weight of potassium hydroxide, 56.11 mg/mEq
N = actual normality of the *Titrant*
W = *Sample* weight for the hydroxyl determination (g)
B = *Titrant* volume consumed by the *Blank* (mL)
A = *Titrant* volume consumed by the *Sample* in the free acid determination (mL)
C = *Sample* weight for the free acid determination (g)
T = *Titrant* volume consumed by the *Sample* in the hydroxyl determination (mL)

Acceptance criteria: 160–168 in mg of KOH/g of Capsule content or hydroxyl value

• **Fats and Fixed Oils,** *Iodine Value* ⟨401⟩
Sample: Capsule contents
Acceptance criteria: 83–88

• **Fats and Fixed Oils,** *Saponification Value* ⟨401⟩
Sample: Capsule contents
Acceptance criteria: 176–182

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, preferably at controlled room temperature.

Castor Oil Emulsion

DEFINITION

Castor Oil Emulsion contains NLT 90.0% and NMT 120.0% of the labeled amount of Castor Oil.

IDENTIFICATION

• **A.**
Sample: 10 mL of Emulsion well shaken
Analysis: Transfer the *Sample* to a 125-mL separator. Add 10 mL of 1 N hydrochloric acid and 20 mL of solvent hexane. Shake vigorously for 2–3 min, allow the layers to separate, discard the aqueous phase, and filter the upper layer through anhydrous sodium sulfate into a small beaker. Evaporate the solvent on a steam bath, and to the residue add 1–2 drops of sulfuric acid.
Acceptance criteria: A red color indicates the presence of castor oil.

ASSAY

• **Procedure**
Internal standard solution: 12 mg/mL of di(2-ethylhexyl) phthalate in chloroform
Standard solution: Transfer 100 mg of castor oil to a 100-mL boiling flask equipped with a suitable reflux condenser connected by a ground-glass joint. Add 30 mL of a mixture of 300 mL of methanol and 3.7 mL of sulfuric acid, reflux in a water bath maintained at 75°–80° for 2.5 h, cool, and rinse down the condenser with 10 mL of water. Transfer the contents of the flask to a 125-mL separator with the aid of 10 mL of water. Rinse the condenser and the flask with 25 mL of solvent hexane, and transfer to the separator. Shake the separator for 2 min, and draw off the aqueous layer into a second 125-mL separator. Add 20 mL of solvent hexane to the second separator, shake for 2 min, discard the aqueous layer, and transfer the solvent hexane layer to the first separator with the aid of 10 mL of solvent hexane. Wash the combined extracts with three 5-mL portions of water, discarding the washings, and transfer the washed extract to a 125-mL conical flask through a funnel containing anhydrous sodium sulfate, with the aid of 25 mL of solvent hexane. Place the flask in a hot water bath, and evaporate with the aid of a current of air to dryness. To the residue add 10.0 mL of *Internal standard solution*, and mix until solution is complete.
Sample solution: Transfer an amount of Emulsion, well-shaken and nominally equivalent to 100 mg of castor oil, to a long-neck, round-bottom 100-mL boiling flask equipped with a suitable reflux condenser connected by a ground-glass joint. Prepare as directed in *Standard solution*, beginning with "Add 30 mL of a mixture of 300 mL of methanol and 3.7 mL of sulfuric acid".
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 1.8-m × 4-mm; packed with 4% liquid phase G25 on support S1
Column conditioning: Flush with helium for 2–5 min, then heat without further flushing at 250° for NLT 30 min, then cool to room temperature, and finally heat while helium is flowing through it at 250° for NLT 60 min.
Temperature
Column: 245°
Injector: 300°
Detector: 300°
Flow rate: Adjust to obtain a peak due to castor oil 5.5 min after introduction of the specimen and an internal standard peak 8 min after introduction of the specimen.
Carrier gas: Helium
Injection size: 5 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the heights of the peaks due to castor oil and the internal standard.
Calculate the percentage of castor oil in the portion of Emulsion taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times 100$$

R_U = ratio of the heights of the peaks due to castor oil and internal standard, *Sample solution*
R_S = ratio of the heights of the peaks due to castor oil and internal standard, *Standard solution*
W_S = weight of castor oil taken to prepare the *Standard solution* (mg)

www.webofpharma.com

W_U = nominal weight of castor oil in the amount of Emulsion taken to prepare the *Sample solution* (mg)

Acceptance criteria: 90.0%–120.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Aromatic Castor Oil

DEFINITION

Aromatic Castor Oil is Castor Oil containing suitable flavoring agents. It contains NLT 95.0% of castor oil.

ASSAY

- **PROCEDURE**

Internal standard solution: 12 mg/mL of di(2-ethylhexyl) phthalate in chloroform

Standard solution: Transfer 100 mg of Castor Oil to a 100-mL boiling flask equipped with a suitable reflux condenser connected by a ground-glass joint. Add 30 mL of a mixture of 300 mL of methanol and 3.7 mL of sulfuric acid, reflux in a water bath maintained at 75°–80° for 2.5 h, cool, and rinse down the condenser with 10 mL of water. Transfer the contents of the flask to a 125-mL separator with the aid of 10 mL of water. Rinse the condenser and the flask with 25 mL of solvent hexane, and transfer to the separator. Shake the separator for 2 min, and draw off the aqueous layer into a second 125-mL separator. Add 20 mL of solvent hexane to the second separator, shake for 2 min, discard the aqueous layer, and transfer the solvent hexane layer to the first separator with the aid of 10 mL of solvent hexane. Wash the combined extracts with three 5-mL portions of water, discarding the washings, and transfer the washed extract to a 125-mL conical flask, through a funnel containing anhydrous sodium sulfate, with the aid of 25 mL of solvent hexane. Place the flask in a hot water bath, and evaporate with the aid of a current of air to dryness. To the residue add 10.0 mL of *Internal standard solution*, and mix until solution is complete.

Sample solution: Transfer an amount of Aromatic Castor Oil, well-shaken and nominally equivalent to 100 mg of castor oil, to a long-neck, round-bottom 100-mL boiling flask equipped with a suitable reflux condenser connected by a ground-glass joint. Proceed as directed for the *Standard solution*, beginning with "Add 30 mL of a mixture of 300 mL of methanol and 3.7 mL of sulfuric acid...".

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 1.8-m × 4-mm column packed with 4% liquid phase G25 on support S1

Column conditioning: Flush with helium for 2–5 min, then heat without further flushing at 250° for NLT 30 min, then cool to room temperature, and finally heat while helium is flowing through it at 250° for NLT 60 min.

Temperature

Column: 245°

Injector: 300°

Detector: 300°

Flow rate: Adjust to obtain a peak due to castor oil 5.5 min after introduction of the specimen and an internal standard peak 8 min after introduction of the specimen.

Carrier gas: Helium

Injection size: 5 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the heights of the peaks due to castor oil and the *Internal standard solution*.

Calculate the percentage of castor oil in the portion of Aromatic Castor Oil taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times 100$$

R_U = ratio of the heights of the peaks due to castor oil and the internal standard, *Sample solution*

R_S = ratio of the heights of the peaks due to castor oil and the internal standard, *Standard solution*

W_S = weight of Castor Oil taken to prepare the *Standard solution* (mg)

W_U = nominal weight of castor oil in the sample of Aromatic Castor Oil taken to prepare the *Sample solution* (mg)

Acceptance criteria: NLT 95.0%

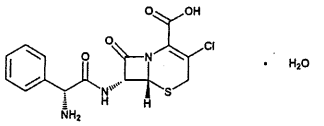
OTHER COMPONENTS

- **ALCOHOL DETERMINATION,** *Method I* ⟨611⟩**:** NMT 4.0% of C_2H_5OH

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Cefaclor



$C_{15}H_{14}ClN_3O_4S \cdot H_2O$ 385.82

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-chloro-8-oxo-, monohydrate, [6*R*-[6α,-7β(*R**)]]-;

(6*R*,7*R*)-7-[(*R*)-2-Amino-2-phenylacetamido]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate;

3-Chloro-7-D-(2-phenylglycinamido)-3-cephem-4-carboxylic acid monohydrate [70356-03-5].

Anhydrous 367.80

[53994-73-3].

DEFINITION

Cefaclor has a potency of NLT 950 µg/mg and NMT 1020 µg/mg of cefaclor ($C_{15}H_{14}ClN_3O_4S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of water and 10 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5 ± 0.1, and add 220 mL of methanol.

USP Monographs

www.webofpharma.com

System suitability solution: 0.3 mg/mL of USP Cefaclor RS and 0.3 mg/mL of USP Cefaclor Delta-3 Isomer RS in *Mobile phase*

Standard solution: 0.3 mg/mL of USP Cefaclor RS in *Mobile phase*. Sonicate briefly, if necessary, to achieve dissolution, and avoid heating the solution. Use within 8 h if stored at room temperature or within 20 h if stored at 5°.

Sample solution: 0.3 mg/mL of Cefaclor in *Mobile phase*. Sonicate briefly, if necessary, to achieve dissolution, and avoid heating the solution. Use within 8 h if stored at room temperature or within 20 h if stored at 5°.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for cefaclor and cefaclor delta-3 isomer are about 1.0 and 1.35, respectively.]
Suitability requirements
Resolution: NLT 2.5 between the cefaclor peak and the cefaclor delta-3 isomer peak
Tailing factor: NMT 1.5 for the cefaclor peak
Relative standard deviation: NMT 0.73% for the cefaclor peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the potency, in µg/mg, of cefaclor ($C_{15}H_{14}ClN_3O_4S$) in the portion of Cefaclor taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Cefaclor RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefaclor in the *Sample solution* (mg/mL)
P = designated potency of USP Cefaclor RS (µg/mg)

Acceptance criteria: 950–1020 µg/mg on the anhydrous basis

IMPURITIES

• **ORGANIC IMPURITIES**

Diluent: 2.4 g/L of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.5

Solution A: 6.9 g/L of monobasic sodium phosphate in water; adjusted with phosphoric acid to a pH of 4.0

Solution B: Acetonitrile and *Solution A* (45:55), degassing for NMT 2 min

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
30	75	25
45	0	100
55	0	100
60	95	5
70	95	5

Standard solution: 0.05 mg/mL of USP Cefaclor RS in *Diluent*. Sonicate briefly, if necessary, to dissolve, and avoid heating. Use within 18 h if stored at room temperature, or within 24 h when stored at 5°.

System suitability solution: 0.05 mg/mL of USP Cefaclor RS and 0.05 mg/mL of USP Cefaclor Delta-3 Isomer RS in *Diluent*

Sample solution: 5 mg/mL of Cefaclor in *Diluent*. Sonicate briefly, if necessary, to dissolve, and avoid heating. Use within 2 h when stored at room temperature or within 20 h when stored at 5°.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for cefaclor and cefaclor delta-3 isomer are 1.0 and 0.85, respectively.]
Suitability requirements
Resolution: NLT 2.0 between cefaclor delta-3 isomer and cefaclor
Tailing factor: NMT 1.2 for the cefaclor peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each cefaclor related compound in the portion of Cefaclor taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of an individual related compound from the *Sample solution*
r_S = peak response of the cefaclor peak from the *Standard solution*
C_S = concentration of USP Cefaclor RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefaclor in the *Sample solution* (mg/mL)
P = designated potency of USP Cefaclor RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria
Individual impurities: NMT 0.5% of any individual cefaclor related compound
Total impurities: NMT 2.0% of total cefaclor related compounds
In an acceptable determination, the difference between duplicate determinations of total cefaclor related compounds is NMT 0.2% absolute, or the variation from the mean of the two values is NMT 10%, whichever is greater.

SPECIFIC TESTS

• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
• **PH** ⟨791⟩
Sample: 25-mg/mL aqueous suspension
Acceptance criteria: 3.0–4.5
• **WATER DETERMINATION** ⟨921⟩, *Method I*: 3.0%–6.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cefaclor RS

www.webofpharma.com

USP Cefaclor Delta-3 Isomer RS
(6*R*,7*R*)-7-{[(2*R*)-(Aminophenylacetyl)]amino}-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
$C_{15}H_{14}ClN_3O_4S$ 367.80

Cefaclor Capsules

DEFINITION
Cefaclor Capsules contain NLT 90.0% and NMT 120.0% of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of water and 10 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5 ± 0.1, and add 220 mL of methanol.
System suitability solution: 0.3 mg/mL of USP Cefaclor RS and 0.3 mg/mL of USP Cefaclor Delta-3 Isomer RS in *Mobile phase*
Standard solution: 0.3 mg/mL of USP Cefaclor RS in *Mobile phase*. Sonicate briefly, if necessary, to achieve dissolution, and avoid heating the solution. Use within 8 h if stored at room temperature, or within 20 h if stored at 5°.
Sample solution: Nominally 0.3 mg/mL of cefaclor prepared as follows. Remove the contents of NLT 20 Capsules as completely as possible, and weigh. Mix the combined contents, and transfer a portion of the powder, nominally equivalent to 75 mg of cefaclor, to a 250-mL volumetric flask, and dilute with *Mobile phase* to volume. Sonicate, if necessary, to dissolve. Filter to obtain a clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for cefaclor and cefaclor delta-3 isomer are about 1.0 and 1.35, respectively.]
Suitability requirements
Resolution: NLT 2.5 between the cefaclor and cefaclor delta-3 isomer peaks
Tailing factor: NMT 1.5 for the cefaclor peak
Relative standard deviation: NMT 1.0% for the cefaclor peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cefaclor RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefaclor in the *Sample solution* (mg/mL)
P = designated potency of USP Cefaclor RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: A known concentration of USP Cefaclor RS in *Medium*
Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium*, if necessary
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Maximum absorbance at about 264 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Cefaclor RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
- **ORGANIC IMPURITIES**

Diluent: 2.4 g/L of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.5.
Solution A: 6.9 g/L of monobasic sodium phosphate adjusted with phosphoric acid to a pH of 4.0
Solution B: Acetonitrile and *Solution A* (45:55), degassed for NMT 2 min
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
30	75	25
45	0	100
55	0	100
60	95	5
70	95	5

Standard solution: 0.05 mg/mL of USP Cefaclor RS in *Diluent*. Sonicate briefly, if necessary, to dissolve, and avoid

www.webofpharma.com

heating. Use within 18 h if stored at room temperature, or within 24 h when stored at 5°.

System suitability solution: 0.05 mg/mL of USP Cefaclor RS and 0.05 mg/mL of USP Cefaclor Delta-3 Isomer RS in *Diluent*

Sample solution: Nominally 5 mg/mL of cefaclor from Capsules prepared as follows. Remove the contents of NLT 20 Capsules as completely as possible, and mix. Transfer a portion of the combined contents, containing nominally about 50 mg of cefaclor, to a 10-mL volumetric flask. Dissolve in *Diluent*, using brief sonication, if necessary, to achieve dissolution. Avoid heating. Dilute with *Diluent* to volume, mix, and filter. Use within 3 h if stored at room temperature, or within 20 h when stored at 5°.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for cefaclor delta-3 isomer and cefaclor are about 0.85 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between cefaclor delta-3 isomer and cefaclor

Tailing factor: NMT 1.2 for the cefaclor peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each related compound in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of an individual related compound from the *Sample solution*

r_S = peak response of the cefaclor peak from the *Standard solution*

C_S = concentration of USP Cefaclor RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cefaclor in the *Sample solution* (mg/mL)

P = potency of USP Cefaclor RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: The reporting level for impurities is 0.1%.

Individual impurities: NMT 0.5% of any individual cefaclor-related compound

Total impurities: NMT 2.0% of all cefaclor-related compounds

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Cefaclor RS

USP Cefaclor Delta-3 Isomer RS

(6*R*,7*R*)-7-{[(2*R*)-Aminophenylacetyl)]amino}-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.

$C_{15}H_{14}ClN_3O_4S$ 367.80

Cefaclor for Oral Suspension

DEFINITION

Cefaclor for Oral Suspension is a dry mixture of Cefaclor and one or more suitable buffers, colors, diluents, and flavors. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of water and 10 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5 ± 0.1, and add 220 mL of methanol.

System suitability solution: 0.3 mg/mL of USP Cefaclor RS and 0.3 mg/mL of USP Cefaclor Delta-3 Isomer RS in *Mobile phase*

Standard solution: 0.3 mg/mL of USP Cefaclor RS in *Mobile phase*. Sonicate briefly, if necessary, to achieve dissolution, and avoid heating the solution. Use within 8 h if stored at room temperature, or within 20 h if stored at 5°.

Sample stock solution: Constitute Cefaclor for Oral Suspension as directed in the labeling, freshly mixed and free from air bubbles.

Sample solution: Nominally 0.3 mg/mL of cefaclor from *Sample stock solution* diluted with *Mobile phase*. Sonicate if necessary to ensure complete dissolution of the cefaclor, and filter to obtain a clear solution.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for cefaclor and cefaclor delta-3 isomer are about 1.0 and 1.35, respectively.]

Suitability requirements

Resolution: NLT 2.5 between the cefaclor and cefaclor delta-3 isomer peaks

Tailing factor: NMT 1.5 for the cefaclor peak

Relative standard deviation: NMT 1.0% for the cefaclor peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$) in the portion of Cefaclor for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Cefaclor RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cefaclor in the *Sample solution* (mg/mL)

USP Monographs

www.webofpharma.com

P = designated potency of USP Cefaclor RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** For solid packaged in single-unit containers, meets the requirements

IMPURITIES
- **ORGANIC IMPURITIES**

Diluent: 2.4 g/L of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.5
Solution A: 6.9 g/L of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 4.0
Solution B: Acetonitrile and *Solution A* (45:55), degassing for NMT 2 min
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
30	75	25
45	0	100
55	0	100
60	95	5
70	95	5

Standard solution: 0.05 mg/mL of USP Cefaclor RS in *Diluent*. Sonicate briefly, if necessary, to dissolve, and avoid heating. Use within 18 h if stored at room temperature, or within 24 h when stored at 5°.
System suitability solution: 0.05 mg/mL of USP Cefaclor RS and 0.05 mg/mL of USP Cefaclor Delta-3 Isomer RS in *Diluent*
Sample stock solution: Constitute Cefaclor for Oral Suspension as directed in the labeling, freshly mixed and free from air bubbles.
Sample solution: Nominally 5 mg/mL of cefaclor from *Sample stock solution* diluted with *Diluent*. Sonicate if necessary, avoid heating to ensure complete dissolution of the cefaclor, and filter. Use within 3 h if stored at room temperature, or within 20 h when stored at 5°.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for cefaclor and cefaclor delta-3 isomer are 1.0 and 0.85, respectively.]
Suitability requirements
Resolution: NLT 2.0 between cefaclor delta-3 isomer and cefaclor
Tailing factor: NMT 1.2 for the cefaclor peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each related compound in the portion of Cefaclor for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of an individual related compound from the *Sample solution*
r_S = peak response of the cefaclor from the *Standard solution*
C_S = concentration of USP Cefaclor RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefaclor in the *Sample solution* (mg/mL)
P = potency of cefaclor in USP Cefaclor RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria
Individual impurities: NMT 1.0% of any individual cefaclor related compound
Total impurities: NMT 3.0% of all cefaclor related compounds. Disregard any peak less than 0.1%.

SPECIFIC TESTS
- **pH** ⟨791⟩

Sample: Suspension constituted as directed in the labeling
Acceptance criteria: 2.5–5.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Cefaclor RS
USP Cefaclor Delta-3 Isomer RS
(6*R*,7*R*)-7-{[(2*R*)-Aminophenylacetyl)]amino}-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
$C_{15}H_{14}ClN_3O_4S$ 367.80

Cefaclor Extended-Release Tablets

DEFINITION
Cefaclor Extended-Release Tablets contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of water and 10 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5 ± 0.1, and add 220 mL of methanol.
System suitability solution: 0.3 mg/mL of USP Cefaclor RS and 0.3 mg/mL of USP Cefaclor Delta-3 Isomer RS in *Mobile phase*
Standard solution: 0.3 mg/mL of USP Cefaclor RS in *Mobile phase*. Sonicate briefly, if necessary, to achieve dissolution, and avoid heating the solution. Use within 8 h if stored at room temperature, or within 20 h if stored at 5°.
Sample solution: Transfer a quantity of finely powdered Tablets (NLT 20), nominally equivalent to 75 mg of cefaclor, to a 250-mL volumetric flask, and dilute with *Mobile phase* to volume. Sonicate, if necessary, to dissolve. Filter to obtain a clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

www.webofpharma.com

Detector: UV 265 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for cefaclor and cefaclor delta-3 isomer are about 1.0 and 1.35, respectively.]
Suitability requirements
Resolution: NLT 2.5 between the cefaclor peak and the cefaclor delta-3 isomer peak
Tailing factor: NMT 1.5 for the cefaclor peak
Relative standard deviation: NMT 1.0% for the cefaclor peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cefaclor RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefaclor in the *Sample solution* (mg/mL)
P = designated potency of USP Cefaclor RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1 (10-mesh basket): 100 rpm
Time: 30, 60, and 240 min
Standard solution: 0.025 mg/mL of USP Cefaclor RS in *Medium*
Sample solution: Filtered portion of the solution under test, diluted with *Medium* to obtain a solution with an estimated concentration of 25 µg/mL of cefaclor
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Maximum absorbance at about 265 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Cefaclor RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: The percentages of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$) dissolved at the times specified conform to *Table 1*.

Table 1

Time (min)	Amount Dissolved (%)
30	5–30
60	20–50
240	NLT 80

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Diluent: 2.4 g/L of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.5
Solution A: 6.9 g/L of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 4.0
Solution B: Acetonitrile and *Solution A* (45:55), degassing for NMT 2 min
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	95	5
30	75	25
45	0	100
55	0	100
60	95	5
70	95	5

Standard solution: 0.05 mg/mL of USP Cefaclor RS in *Diluent*. Sonicate briefly, if necessary, to dissolve, and avoid heating. Use within 18 h if stored at room temperature, or within 24 h when stored at 5°.
System suitability solution: 0.05 mg/mL of USP Cefaclor RS and 0.05 mg/mL of USP Cefaclor Delta-3 Isomer RS in *Diluent*
Sample solution: Nominally 5 mg/mL of cefaclor from Tablets prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, containing nominally about 50 mg of cefaclor, to a 10-mL volumetric flask. Dissolve in *Diluent*, using brief sonication, if necessary, to achieve dissolution. Avoid heating. Dilute with *Diluent* to volume, mix, and filter. Use within 3 h if stored at room temperature, or within 20 h if stored at 5°.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for cefaclor and cefaclor delta-3 isomer are 1.0 and 0.85, respectively.]
Suitability requirements
Resolution: NLT 2.0 between cefaclor delta-3 isomer and cefaclor
Tailing factor: NMT 1.2 for the cefaclor peak
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the percentage of each related compound in the portion of Tablets taken:

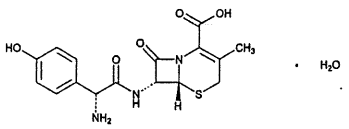
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of an individual related compound from the *Sample solution*
r_S = peak response of the cefaclor from the *Standard solution*
C_S = concentration of USP Cefaclor RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefaclor in the *Sample solution* (mg/mL)
P = potency of USP Cefaclor RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria
Individual impurities: NMT 0.6% of any individual cefaclor related compound
Total impurities: NMT 2.0% of all cefaclor related compounds. Disregard any peak less than 0.1%.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cefaclor RS
  USP Cefaclor Delta-3 Isomer RS
  (6*R*,7*R*)-7-{[(2*R*)-Aminophenylacetyl)]amino}-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
  $C_{15}H_{14}ClN_3O_4S$ 367.80

Cefadroxil



$C_{16}H_{17}N_3O_5S \cdot H_2O$ 381.40
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[amino(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-, monohydrate, [6*R*-[6α,7β (*R**)]]-;
(6*R*,7*R*)-7-[(*R*)-2-Amino-2-(*p*-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate [66592-87-8].
Hemihydrate 372.40
[119922-85-9].
Anhydrous 363.39
[50370-12-2].

DEFINITION
Cefadroxil has a potency equivalent to NLT 950 µg/mg and NMT 1050 µg/mg of cefadroxil ($C_{16}H_{17}N_3O_5S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Buffer: 2.7 g/L of monobasic potassium phosphate
  Mobile phase: Acetonitrile and *Buffer* (4:96)
  Standard solution: 50 µg/mL of USP Cefadroxil RS in *Mobile phase* prepared as follows. Transfer a suitable quantity of USP Cefadroxil RS to a suitable volumetric flask, dissolve in *Mobile phase* using 50% of the final volume, sonicate to dissolve, and dilute with *Mobile phase* to volume.
  Sample solution: 50 µg/mL of Cefadroxil in *Mobile phase* prepared as follows. Transfer a suitable quantity of Cefadroxil to a suitable volumetric flask, dissolve in *Mobile phase* using 50% of the final volume, sonicate to dissolve, and dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 0.73%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the quantity, in µg/mg, of cefadroxil ($C_{16}H_{17}N_3O_5S$) in the portion of Cefadroxil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cefadroxil RS in the *Standard solution* (µg/mL)
C_U = concentration of Cefadroxil in the *Sample solution* (µg/mL)
P = potency of cefadroxil in USP Cefadroxil RS (µg/mg)

Acceptance criteria: 950–1050 µg/mg on the anhydrous basis

IMPURITIES
- **ORGANIC IMPURITIES**
  Solution A: 50 mg/mL of sodium hydroxide
  Solution B: 4 g/L of monobasic sodium phosphate dihydrate adjusted with *Solution A* to a pH of 5.0
  Solution C: Acetonitrile and *Solution B* (1:1)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
35	85	15
50	40	60
60	0	100
61	100	0
70	100	0

Diluent: 3.5 g/L of monobasic potassium phosphate and 4.6 g/L of dibasic sodium phosphate anhydrous

www.webofpharma.com

System suitability stock solution 1: 0.5 mg/mL of USP Cefadroxil Related Compound D RS in *Diluent*. Sonicate as needed to dissolve.
System suitability stock solution 2: 0.5 mg/mL of USP Cefadroxil Related Compound I RS in *Diluent*. Sonicate as needed to dissolve.
System suitability solution: 10 µg/mL of cefadroxil related compound D from *System suitability stock solution 1*, 10 µg/mL of cefadroxil related compound I from *System suitability stock solution 2*, and 1 mg/mL of USP Cefadroxil System Suitability Mixture RS in *Solution B*. Store the sample in the refrigerator, and discard after 14 h.
Standard solution: 10 µg/mL of USP Cefadroxil RS in *Solution B*. Store the sample in the refrigerator, and discard after 14 h.
Sample solution: 1 mg/mL of Cefadroxil in *Solution B*. Store the sample in the refrigerator, and discard after 14 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Autosampler temperature: 6°
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between cefadroxil related compound D and cefadroxil related compound I, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Cefadroxil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of cefadroxil from the *Standard solution*
C_S = concentration of the USP Cefadroxil RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefadroxil in the *Sample solution* (mg/mL)
P = potency of cefadroxil in USP Cefadroxil RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*. The reporting threshold is 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amoxicillin related compound I[a]	0.16	0.5
Cefadroxil *R*-sulfoxide[b]	0.22	0.15
Cefadroxil *S*-sulfoxide[c]	0.41	0.15
Cefadroxil related compound B[d]	0.49	0.5
Dimethylformamide[e]	0.55	—
Cefadroxil related compound D[f]	0.71	0.5
Cefadroxil related compound I[g]	0.80	0.15
Diketopiperazine derivative[h]	0.87	0.5
Cefadroxil	1.0	—
N-Phenylglycyl delta-3 cefadroxil[i]	1.4	0.15
Cefadroxil ethyl homolog[j]	1.5	0.15
N-Phenylglycyl cefadroxil[k]	1.8	0.5
3-Hydroxy-4-methylthiophenone[l]	2.0	0.5
N-Ethoxycarbonyl 7-aminodesacetoxycephalosporanic acid[m]	2.1	0.15
O-Ethoxycarbonyl cefadroxil[n]	2.3	0.3
Cefadroxil dimer[o]	2.4	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

[a] D-Hydroxyphenylglycine; (*R*)-2-Amino-2-(4-hydroxyphenyl)acetic acid.
[b] (6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5*R*-oxide.
[c] (6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5*S*-oxide.
[d] 7-Aminodesacetoxycephalosporanic acid; (6*R*,7*R*)-7-Amino-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[e] This impurity is listed here for information only. It is not to be reported as an impurity in this test.
[f] L-Cefadroxil; (6*R*,7*R*)-7-[(*S*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[g] Delta-3 cefadroxil; (6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
[h] 3-(Aminomethylene)-6-(4-hydroxyphenyl)piperazine-2,5-dione.
[i] (6*R*,7*R*)-7-{(2*R*)-2-[2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
[j] (6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-ethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[k] (6*R*,7*R*)-7-{(2*R*)-2-[2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[l] 3-Hydroxy-4-methylthiophen-2(5*H*)-one.
[m] (6*R*,7*R*)-7-(Ethoxycarbonylamino)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[n] (6*R*,7*R*)-7-((*R*)-2-Amino-2-{4-[(ethoxycarbonyl)oxy]phenyl}acetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[o] (6*R*,7*R*)-7-[(*R*)-2-{(6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamido}-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

• **DIMETHYLANILINE** ⟨223⟩**:** Meets the requirements

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in water
Acceptance criteria: +165.0° to +178.0°
• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
• **PH** ⟨791⟩
Sample solution: 50 mg/mL suspension in water
Acceptance criteria: 4.0–6.0
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** 4.2%–6.0%, except where it is labeled as being in the hemihydrate form it is between 2.4% and 4.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** The hemihydrate form is so labeled.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cefadroxil RS

USP Monographs

www.webofpharma.com

USP Cefadroxil Related Compound D RS
(6*R*,7*R*)-7-[(*S*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
$C_{16}H_{17}N_3O_5S$ 363.39

USP Cefadroxil Related Compound I RS
(6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
$C_{16}H_{17}N_3O_5S$ 363.39

USP Cefadroxil System Suitability Mixture RS
This is a mixture of cefadroxil and *O*-ethoxycarbonyl cefadroxil [(6*R*,7*R*)-7-((*R*)-2-Amino-2-{4-[(ethoxycarbonyl)oxy]phenyl}acetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid].
$C_{19}H_{21}N_3O_7S$ 435.45

Cefadroxil Capsules

DEFINITION
Cefadroxil Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 10 N potassium hydroxide to a pH of 5.0.
Mobile phase: Acetonitrile and *Buffer* (40:960)
Standard solution: 1.06 mg/mL of USP Cefadroxil RS in *Buffer*. This solution contains the equivalent of 1 mg/mL of cefadroxil. Use this solution on the day prepared.
Sample solution: Remove the contents of NLT 10 Capsules as completely as possible, and weigh. Transfer a portion of the powder, nominally equivalent to 200 mg of cefadroxil, to a 200-mL volumetric flask. Dilute with *Buffer* to volume, and stir by mechanical means for 5 min. Use this solution on the day prepared.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cefadroxil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefadroxil in the *Sample solution* (mg/mL)
P = potency of cefadroxil in USP Cefadroxil RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Standard solution: USP Cefadroxil RS in *Medium* at a known concentration similar to that in the *Sample solution*
Sample solution: Sample per *Dissolution* ⟨711⟩. Suitably dilute with *Medium*, if necessary, and filter.
Analysis: Determine the amount of cefadroxil ($C_{16}H_{17}N_3O_5S$) dissolved from UV absorbances at 263 nm of the *Sample solution* in comparison to the *Standard solution*.
Tolerances: NLT 80% (*Q*) of the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
- **ORGANIC IMPURITIES**

Solution A: 50 mg/mL of sodium hydroxide
Solution B: 4 g/L of monobasic sodium phosphate dihydrate in water, adjusted with *Solution A* to a pH of 5.2
Solution C: Acetonitrile and *Solution B* (1:1)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
35	85	15
50	40	60
60	0	100
61	100	0
70	100	0

Diluent: 3.5 g/L of monobasic potassium phosphate and 4.6 g/L of dibasic sodium phosphate anhydrous
System suitability stock solution 1: 0.5 mg/mL of USP Cefadroxil Related Compound D RS in *Diluent*. Sonicate as needed to dissolve.
System suitability stock solution 2: 0.5 mg/mL of USP Cefadroxil Related Compound I RS in *Diluent*. Sonicate as needed to dissolve.
System suitability solution: 10 µg/mL of cefadroxil related compound D from *System suitability stock solution 1*, 10 µg/mL of cefadroxil related compound I from *System suitability stock solution 2*, and 1 mg/mL of USP Cefadroxil System Suitability Mixture RS in *Solution B*. Store refrigerated, and discard after 14 h.
Standard solution: 10 µg/mL of USP Cefadroxil RS in *Solution B*
Sample solution: Nominally, 1 mg/mL of cefadroxil in *Solution B* prepared as follows. Transfer the finely powdered contents of NLT 10 Capsules to a suitable volumetric flask, and add 50% of the final volume of *Solution B*. Sonicate with intermittent shaking. Cool to room temperature, dilute with *Solution B* to final volume, and mix. Centrifuge a

www.webofpharma.com

portion of this solution, and filter the supernatant solution. Store refrigerated, and discard after 14 h.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Autosampler temperature: 6°

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for cefadroxil related compound D, cefadroxil related compound I, and cefadroxil are about 0.71, 0.80, and 1.0, respectively.]

Suitability requirements
Resolution: NLT 1.5 between cefadroxil related compound D and cefadroxil related compound I, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of cefadroxil from the *Standard solution*
C_S = concentration of USP Cefadroxil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefadroxil in the *Sample solution* (mg/mL)
P = potency of cefadroxil in USP Cefadroxil RS (µg/mg)
F_1 = conversion factor, 0.001 mg/µg
F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound I[a]	0.16	1.3	0.5
Cefadroxil related compound B[b]	0.52	0.63	0.5
Cefadroxil related compound D[c, d]	0.71	—	—
Diketopiperazine derivative[e]	0.89	1.3	0.5
Cefadroxil	1.0	—	—
N-Phenylglycyl delta-3 cefadroxil[f]	1.4	1.5	0.15
N-Phenylglycyl cefadroxil[g, d]	1.8	—	—
3-Hydroxy-4-methylthiophenone[h]	1.9	0.40	0.5
N-Ethoxycarbonyl 7-aminodesacetoxycephalosporanic acid[i, d]	2.2	—	—
O-Ethoxycarbonyl cefadroxil[j, d]	2.4	—	—
Any individual, unspecified impurity	—	—	0.2
Total impurities	—	—	2.0

[a] D-Hydroxyphenylglycine; (*R*)-2-Amino-2-(4-hydroxyphenyl)acetic acid.
[b] 7-Aminodesacetoxycephalosporanic acid; (6*R*,7*R*)-7-Amino-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[c] L-Cefadroxil; (6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[d] Process impurities that are controlled in the drug substance are not to be reported, are not included in total impurities, and are listed here for information only.
[e] 3-(Aminomethylene)-6-(4-hydroxyphenyl)piperazine-2,5-dione.
[f] (6*R*,7*R*)-7-{(2*R*)-2-[2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
[g] (6*R*,7*R*)-7-{(2*R*)-2-[2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[h] 3-Hydroxy-4-methylthiophen-2(5*H*)-one.
[i] (6*R*,7*R*)-7-(Ethoxycarbonylamino)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[j] (6*R*,7*R*)-7-((*R*)-2-Amino-2-{4-[(ethoxycarbonyl)oxy]phenyl}acetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 7.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Capsules prepared using the hemihydrate form of Cefadroxil are so labeled.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cefadroxil RS
  USP Cefadroxil Related Compound D RS
  (6*R*,7*R*)-7-[(*S*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
  $C_{16}H_{17}N_3O_5S$ 363.39
  USP Cefadroxil Related Compound I RS
  (6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
  $C_{16}H_{17}N_3O_5S$ 363.39
  USP Cefadroxil System Suitability Mixture RS
  This is a mixture of cefadroxil and *O*-ethoxycarbonyl cefadroxil [(6*R*,7*R*)-7-((*R*)-2-Amino-2-{4-[(ethoxycarbonyl)oxy]phenyl}acetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid].
  $C_{19}H_{21}N_3O_7S$ 435.45

Cefadroxil for Oral Suspension

DEFINITION
Cefadroxil for Oral Suspension is a dry mixture of Cefadroxil and one or more suitable buffers, colors, diluents, and flavors. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of $C_{16}H_{17}N_3O_5S$.

IDENTIFICATION
- **THIN-LAYER CHROMATOGRAPHY**
  Standard solution: 2 mg/mL of USP Cefadroxil RS

USP Monographs

www.webofpharma.com

Sample solution: Constitute 1 container of Cefadroxil for Oral Suspension as directed in the labeling. Dilute a portion of the resulting suspension with water to a concentration of 2 mg/mL. Pass through a suitable filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of binder-free silica gel
Application volume: 20 µL
Pre-developing solvent system: *n*-Hexane and tetradecane (95:5)
Solution A: 1 in 15 solution of ninhydrin in acetone
Developing solvent system: 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and *Solution A* (60: 40: 1.5)
Spray reagent: 1 in 500 solution of ninhydrin in dehydrated alcohol. Protect this solution from light.

Analysis
Samples: *Standard solution* and *Sample solution*
Place the thin-layer chromatographic plate in a chamber containing the *Pre-developing solvent system* and allow the solvent front to move the length of the plate. Remove the plate from the chamber and allow the solvent to evaporate. Apply the *Sample solution* and *Standard solution* to the plate, allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Spray the plate with the *Spray reagent*, dry for 10 min at 110°, and examine the chromatogram.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**
Buffer: 6.86 g/L of monobasic potassium phosphate. Adjust with 10 N potassium hydroxide to a pH of 5.0.
Mobile phase: Acetonitrile and *Buffer* (40:960)
Standard solution: 1.06 mg/mL of USP Cefadroxil RS in *Buffer*. Use this solution on the day prepared. [NOTE—This solution contains the equivalent of 1 mg/mL of cefadroxil ($C_{16}H_{17}N_3O_5S$).]
Sample solution: Constitute a container of Cefadroxil for Oral Suspension as directed in the labeling. Dilute a portion of the resulting suspension with *Buffer* to prepare a solution containing nominally 1 mg/mL. Pass through a suitable filter of 0.8-µm or finer pore size, and use the filtrate. Use this solution on the day prepared.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor, k′: 2.0–3.5
Column efficiency: NLT 1800 theoretical plates
Tailing factor: NMT 2.2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$) in the portion of Cefadroxil for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of cefadroxil from the *Sample solution*
r_S = peak response of cefadroxil from the *Standard solution*
C_S = concentration of USP Cefadroxil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefadroxil in the *Sample solution* (mg/mL)
P = potency of cefadroxil in USP Cefadroxil RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 25 rpm
Time: 30 min
Standard solution: USP Cefadroxil RS in *Medium* at a known concentration
Sample solution: Transfer 5.0 mL of the constituted Cefadroxil for Oral Suspension (weighed) to the dissolution vessel.
Analysis: Determine the amount of cefadroxil dissolved by employing UV absorption at the wavelength of 263 nm on the *Sample solution* in comparison with the *Standard solution*.
Calculate the amount of cefadroxil dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/D) \times V$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor

Tolerances: NLT 75% (Q) of the labeled amount of cefadroxil is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
For solid packaged in single-unit containers: Meets the requirements
• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

SPECIFIC TESTS

• **PH** ⟨791⟩**:** 4.5–6.0, in the suspension constituted as directed in the labeling
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.0%, except where it is labeled as containing 100 mg of cefadroxil per mL after constitution, in which case the limit is NMT 3.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cefadroxil RS

Cefadroxil Tablets

DEFINITION

Cefadroxil Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$).

www.webofpharma.com

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 2 mg/mL of USP Cefadroxil RS

Sample solution: Nominally 2 mg/mL of cefadroxil from powdered Tablets dissolved in water and filtered

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Adsorbent: 0.25-mm layer of binder-free silica gel

Application volume: 20 µL

Pre-developing solvent solution: *n*-Hexane and tetradecane (95:5)

Solution A: 66.7-mg/mL solution of ninhydrin in acetone

Developing solvent system: 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and *Solution A* (60: 40: 1.5)

Spray reagent: 2-mg/mL solution of ninhydrin in dehydrated alcohol. Protect from light.

Analysis

Samples: *Standard solution* and *Sample solution*

Place the thin-layer chromatographic plate in a chamber containing the *Pre-developing solvent solution* to a depth of about 1 cm, and allow the solvent front to move the length of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. Apply the *Sample solution* and *Standard solution* to the plate, allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Spray the plate with the *Spray reagent*, dry for 10 min at 110°, and examine the chromatogram.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water, adjusted with 10 N potassium hydroxide to a pH of 5.0

Mobile phase: Acetonitrile and *Buffer* (40:960)

Standard solution: 1.06 mg/mL of USP Cefadroxil RS in *Buffer*. This solution contains nominally 1 mg/mL of cefadroxil. Use this solution on the day prepared.

Sample solution: Nominally 1 mg/mL of cefadroxil from finely powdered Tablets (NLT 10) in *Buffer*. Stir by mechanical means for 5 min. Use this solution on the day prepared.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor, k′: 2.0–3.5

Column efficiency: NLT 1800 theoretical plates

Tailing factor: NMT 2.2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Cefadroxil RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cefadroxil in the *Sample solution* (mg/mL)

P = potency of cefadroxil in USP Cefadroxil RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: Prepare a solution having a known concentration of USP Cefadroxil RS in *Medium*.

Sample solution: Sample per ⟨711⟩. Pass a portion of the solution under test through a suitable filter, and dilute with water if necessary.

Instrumental conditions

Mode: UV

Analytical wavelength: 263 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 75% (*Q*) of the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 8.0%

ADDITIONAL REQUIREMENTS

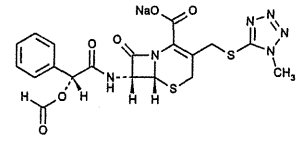
• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** The Tablets prepared using the hemihydrate form of cefadroxil are so labeled.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cefadroxil RS

Cefamandole Nafate



$C_{19}H_{17}N_6NaO_6S_2$ 512.49

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(formyloxy)phenylacetyl]amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-, monosodium salt, [6*R*-[6α,7β (*R**)]]-;

Sodium (6*R*,7*R*)-7-(*R*)-mandelamido-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate formate (ester) [42540-40-9].

DEFINITION

Cefamandole Nafate has a potency equivalent to NLT 810 µg/mg and NMT 1000 µg/mg of cefamandole ($C_{18}H_{18}N_6O_5S_2$), calculated on the anhydrous basis.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: 10% solution of triethylamine in water, adjusted with phosphoric acid to a pH of 2.5

Mobile phase: Acetonitrile and *Buffer* (25:75)

Standard solution: 0.5 mg/mL of USP Cefamandole Nafate RS in *Mobile phase*. Use this solution immediately after it is prepared.

System suitability solution: 0.05 mg/mL of USP Cefamandole Nafate RS in *Mobile phase*. Heat at 60° for 30 min.

Sample solution: 0.5 mg/mL of Cefamandole Nafate in *Mobile phase*. Use this solution immediately after it is prepared.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 7.0 between the two main peaks, *System suitability solution*

Relative standard deviation: NMT 0.8% for the cefamandole peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, in µg/mg, of cefamandole ($C_{18}H_{18}N_6O_5S_2$) in the portion of Cefamandole Nafate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times P \times F$$

r_U = sum of the peak responses of cefamandole and cefamandole nafate from the *Sample solution*

r_S = sum of the peak responses of cefamandole and cefamandole nafate from the *Standard solution*

C_S = concentration of USP Cefamandole Nafate RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

M_{r1} = molecular weight of cefamandole, 462.50

M_{r2} = molecular weight of cefamandole nafate, 512.49

P = potency of cefamandole nafate in USP Cefamandole Nafate RS (mg/mg)

F = conversion factor, 1000 µg/mg

Acceptance criteria: 810–1000 µg/mg on the anhydrous basis

SPECIFIC TESTS

• **PH** ⟨791⟩

Sample solution: 100 mg/mL

Acceptance criteria: 3.5–7.0

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.0%

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.15 USP Endotoxin Unit/mg of cefamandole, when the label states that Cefamandole Nafate is sterile or must be subjected to further processing during the preparation of injectable dosage forms

• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements for *Test for Sterility of the Product to Be Examined, Membrane Filtration* when the label states that Cefamandole Nafate is sterile

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cefamandole Nafate RS

Cefamandole Nafate for Injection

DEFINITION

Cefamandole Nafate for Injection is a sterile mixture of Cefamandole Nafate and one or more suitable buffers. It has a potency equivalent to NLT 810 µg/mg and NMT 1000 µg/mg of cefamandole ($C_{18}H_{18}N_6O_5S_2$), calculated on the anhydrous and sodium carbonate-free basis. It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of cefamandole ($C_{18}H_{18}N_6O_5S_2$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• **PROCEDURE**

Buffer: 10% solution of triethylamine in water, adjusted with phosphoric acid to a pH of 2.5

Mobile phase: Acetonitrile and *Buffer* (25:75)

Standard solution: 0.5 mg/mL of USP Cefamandole Nafate RS in *Mobile phase*. Use this solution immediately after it is prepared.

System suitability solution: 0.05 mg/mL of USP Cefamandole Nafate RS in *Mobile phase*. Heat at 60° for 30 min.

Sample solution: Equivalent to 0.5 mg/mL of cefamandole nafate from Cefamandole Nafate for Injection in *Mobile phase*. Use this solution immediately after it is prepared.

Sample solution 1 (where the article is represented as being in a single-dose container)**:** Equivalent to 0.5 mg/mL of cefamandole nafate from Cefamandole Nafate for Injection in *Mobile phase* prepared as follows. Constitute a container of Cefamandole Nafate for Injection in a volume of *Mobile phase* corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with *Mobile phase*.

Sample solution 2 (where the label states the quantity of cefamandole in a given volume of constituted solution)**:** Equivalent to 0.5 mg/mL of cefamandole nafate from Cefamandole Nafate for Injection in *Mobile phase* prepared as follows. Constitute Cefamandole Nafate for Injection in a volume of *Mobile phase* corresponding to the volume of solvent specified in the labeling. Dilute a volume of the constituted solution with *Mobile phase*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 7.0 between the two main peaks, *System suitability solution*

Relative standard deviation: NMT 0.8% for the cefamandole peak, *Standard solution*

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution 1* or *Sample solution 2*
Calculate the percentage of the labeled amount of cefamandole ($C_{18}H_{18}N_6O_5S_2$) in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times P \times 100$$

r_U = sum of the peak responses of cefamandole and cefamandole nafate from *Sample solution 1* or *Sample solution 2*
r_S = sum of the peak responses of cefamandole and cefamandole nafate from the *Standard solution*
C_S = concentration of USP Cefamandole Nafate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefamandole in *Sample solution 1* or in *Sample solution 2* (mg/mL)
M_{r1} = molecular weight of cefamandole, 462.50
M_{r2} = molecular weight of cefamandole nafate, 512.49
P = potency of USP Cefamandole Nafate RS (mg/mg)

Calculate the potency, in µg/mg, of cefamandole ($C_{18}H_{18}N_6O_5S_2$) in the portion of Cefamandole Nafate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times P \times F$$

r_U = sum of the peak responses of cefamandole and cefamandole nafate from *Sample solution 1* or *Sample solution 2*
r_S = sum of the peak responses of cefamandole and cefamandole nafate from the *Standard solution*
C_S = concentration of USP Cefamandole Nafate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefamandole in *Sample solution 1* or *Sample solution 2* (mg/mL)
M_{r1} = molecular weight of cefamandole, 462.50
M_{r2} = molecular weight of cefamandole nafate, 512.49
P = potency of USP Cefamandole Nafate RS (mg/mg)
F = conversion factor, 1000 µg/mg

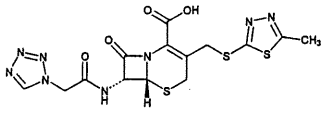
Acceptance criteria: 810–1000 µg/mg of $C_{18}H_{18}N_6O_5S_2$, calculated on the anhydrous and sodium carbonate-free basis; 90.0%–115.0% of the labeled amount of $C_{18}H_{18}N_6O_5S_2$

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity: Perform the *Assay* on individual containers using *Sample solution 1* or *Sample solution 2*, or both, as appropriate.
Acceptance criteria: Meets the requirements

SPECIFIC TESTS
• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.15 USP Endotoxin Unit/mg of cefamandole
• **STERILITY TESTS** ⟨71⟩**:** It meets the requirements for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
• **pH** ⟨791⟩
Sample solution: 100 mg/mL
Acceptance criteria: 6.0–8.0, determined after 30 min
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩ **:** Meets the requirements for small-volume injections
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 3.0%
• **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cefamandole Nafate RS

Cefazolin



$C_{14}H_{14}N_8O_4S_3$ 454.51
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-8-oxo-7-[[1*H*-tetrazol-1-yl)acetyl]amino]-(6*R-trans*); (6*R*,7*R*)-3-[[(5-Methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-8-oxo-7-[2-(1*H*-tetrazol-1-yl)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [25953-19-9].

DEFINITION
Cefazolin contains NLT 95.0% and NMT 103.0% of cefazolin ($C_{14}H_{14}N_8O_4S_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Protect all solutions containing cefazolin from light.
Buffer A: 0.9 g/L of anhydrous dibasic sodium phosphate and 1.3 g/L of citric acid monohydrate in water
Buffer B: 5.7 g/L of anhydrous dibasic sodium phosphate and 3.6 g/L of monobasic potassium phosphate in water
Mobile phase: Acetonitrile and *Buffer A* (10:90)
Standard solution: 50 µg/mL of USP Cefazolin RS in *Buffer B*
Sample solution: 50 µg/mL of Cefazolin in *Buffer B*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Tailing factor: NMT 1.5
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cefazolin ($C_{14}H_{14}N_8O_4S_3$) in the portion of Cefazolin taken:

Official Monographs

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of cefazolin from the *Sample solution*
r_S = peak response of cefazolin from the *Standard solution*
C_S = concentration of USP Cefazolin RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefazolin in the *Sample solution* (mg/mL)
P = potency of cefazolin in USP Cefazolin RS (mg/mg)

Acceptance criteria: 95.0%–103.0% on the anhydrous basis

IMPURITIES

• ORGANIC IMPURITIES

Protect all solutions containing cefazolin from light.
Solution A: 6.8 g/L of monobasic potassium phosphate in water
Solution B: 6.8 g/L of monobasic potassium phosphate adjusted with 10% sodium hydroxide to a pH of 6.8 before final dilution with water
Solution C: Acetonitrile and *Solution A* (1:1)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	98	2
7	98	2
15	85	15
30	80	20
35	80	20
45	50	50
50	50	50
55	98	2
65	98	2

Blank: Use *Solution B*.
System suitability stock solution: 2 mg/mL of USP Cefazolin RS in 0.05 M sodium hydroxide. Set the solution aside at room temperature for 5 min. [NOTE—The cefazolin epimer is formed upon treatment of cefazolin with sodium hydroxide.]
System suitability solution: *System suitability stock solution* and *Solution B* (1:24)
Standard solution: 25 µg/mL of USP Cefazolin RS in *Solution B*. Use this solution immediately after preparation.
Sample solution: 2.5 mg/mL of Cefazolin in *Solution B*. Use this solution immediately after preparation.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 and 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 8.0 between cefazolin and cefazolin epimer, 254 nm

Analysis
Samples: *Blank*, *Standard solution*, and *Sample solution*
Calculate the percentage of tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin taken:

$$\text{Result} = (r_{U(210)}/r_{S(254)}) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{U(210)}$ = peak response of tetrazolylacetic acid or tetrazolylacetamide acetal at 210 nm from the *Sample solution*
$r_{S(254)}$ = peak response of cefazolin at 254 nm from the *Standard solution*
C_S = concentration of USP Cefazolin RS in the *Standard solution* (mg/mL)
C_U = concentration of the Cefazolin in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Calculate the percentage of each impurity other than tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin taken:

$$\text{Result} = (r_{U(254)}/r_{S(254)}) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{U(254)}$ = peak response of each impurity other than tetrazolylacetic acid or tetrazolylacetamide acetal at 254 nm from the *Sample solution*
$r_{S(254)}$ = peak response of cefazolin at 254 nm from the *Standard solution*
C_S = concentration of USP Cefazolin RS in the *Standard solution* (mg/mL)
C_U = concentration of the Cefazolin in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks corresponding to those in the *Blank*.

Table 2

Name	Analytical Wavelength (nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tetrazolylacetic acid[a]	210	0.07	0.40	1.0
Tetrazolyl-acetamide acetal[b]	210	0.08	0.33	1.0
Cefazolin open-ring lactone[c,d] or Cefazolin 3-hydroxymethyl[e]	254	0.20	1.0	0.5
Methylthiadiazole thiol[f]	254	0.23	0.91	1.0
7-Aminocephalosporanic acid[g]	254	0.42	1.1	1.0
Cefazolin 3-methyl analog[h]	254	0.44	0.87	1.0
Cefazolin lactone[i]	254	0.50	0.85	1.0
Cefazolin acetoxy analog[j]	254	0.61	0.68	1.0
Cefazolin deacylated[k]	254	0.68	1.2	1.0
Cefazoloic acid isomers[l]	254	0.84	1.0	1.0
Cefazolin	254	1.0	—	—
Cefazolin epimer[m]	254	1.2	0.98	1.0
Cefazolin pivaloyl[n]	254	1.4	0.92	1.0

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Analytical Wavelength (nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	254	—	1.0	0.1
Total impurities	—	—	—	3.5

[a] 2-(1*H*-Tetrazol-1-yl)acetic acid.
[b] *N*-(2,2-Dihydroxyethyl)-2-(1*H*-tetrazol-1-yl)acetamide.
[c] The identification of this impurity is tentative. The names of the most likely compounds are listed in footnotes [d] and [e].
[d] (*R*)-2-[2-(1*H*-Tetrazol-1-yl)acetamido]-2-[(*R*)-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid.
[e] (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[f] 5-Methyl-1,3,4-thiadiazole-2-thiol (MMTD).
[g] (6*R*,7*R*)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ACA).
[h] (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[i] *N*-[(5a*R*,6*R*)-1,7-Dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl]-2-(1*H*-tetrazol-1-yl)acetamide.
[j] (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[k] (6*R*,7*R*)-7-Amino-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[l] Three isomers of this impurity may not be fully resolved by this method. The limit applies to the sum of the isomers, which are as follows: Cefazolin open-ring delta-3: (2*R*)-2-[(*R*)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxy)methyl]-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid. Cefazolin open-ring delta-2: (2*R*)-2-[(*R*)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxy)methyl]-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,4-dihydro-2*H*-1,3-thiazine-4-carboxylic acid. Cefazolin open-ring delta-4: (2*R*)-2-[(*R*)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxy)methyl]-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.
[m] (6*R*,7*S*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[n] (6*R*,7*R*)-3-((5-Methyl-1,3,4-thiadiazol-2-ylthio)methyl)-8-oxo-7-pivalamido-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 2.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cefazolin RS

Cefazolin Injection

» Cefazolin Injection is a sterile solution of Cefazolin and Sodium Bicarbonate in a diluent containing one or more suitable tonicity-adjusting agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cefazolin ($C_{14}H_{14}N_8O_4S_3$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Maintain in the frozen state.
Labeling—It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

USP Reference standards ⟨11⟩—
USP Cefazolin RS
Identification—The retention time of the major peak for cefazolin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.15 USP Endotoxin Unit per mg of cefazolin.
Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
pH ⟨791⟩: between 4.5 and 7.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Assay—
pH 3.6 Buffer—Dissolve 0.900 g of anhydrous dibasic sodium phosphate and 1.298 g of citric acid monohydrate in water to make 1000 mL.
pH 7.0 Buffer—Dissolve 5.68 g of anhydrous dibasic sodium phosphate and 3.63 g of monobasic potassium phosphate in water to make 1000 mL.
Mobile phase—Prepare a suitable mixture of *pH 3.6 Buffer* and acetonitrile (9:1). Pass through a membrane filter having a 10-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Transfer 750 mg of salicylic acid to a 100-mL volumetric flask, dissolve in 10 mL of methanol, dilute with *pH 7.0 Buffer* to volume, and mix.
Standard preparation—Transfer about 25 mg of USP Cefazolin RS, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *pH 7.0 Buffer* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.
Assay preparation—Allow 1 container of Injection to thaw, and mix. Transfer an accurately measured volume of the Injection, equivalent to about 50 mg of cefazolin, to a 50-mL volumetric flask, dilute with *pH 7.0 Buffer* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 30-cm column that contains 10-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*. The relative retention times are about 0.7 for salicylic acid and 1.0 for cefazolin; the resolution, *R*, between the analyte and internal standard peaks is not less than 4.0; the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cefazolin ($C_{14}H_{14}N_8O_4S_3$) in each mL of Injection taken by the formula:

$$(1000C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Cefazolin RS, calculated on the anhydrous basis, in the *Standard preparation*; V is the volume, in mL, of Injection taken; and R_U and R_S are the peak response ratios of cefazolin to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

USP Monographs

www.webofpharma.com

Cefazolin for Injection

DEFINITION

Cefazolin for Injection contains an amount of Cefazolin Sodium equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of ($C_{14}H_{14}N_8O_4S_3$).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Sample solution: 20 µg/mL in 0.1 M sodium bicarbonate
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Buffer A: 0.9 g/L of anhydrous dibasic sodium phosphate and 1.298 g/L of citric acid monohydrate in water. The pH of *Buffer A* is 3.6.

Buffer B: 5.68 g/L of anhydrous dibasic sodium phosphate and 3.63 g/L of monobasic potassium phosphate in water. The pH of *Buffer B* is 7.0.

Mobile phase: Acetonitrile and *Buffer A* (10:90). Pass through a membrane filter having a 10-µm or finer pore size.

Internal standard solution: 7.5 mg/mL of salicylic acid in methanol and *Buffer B* (10:90) prepared as follows. Transfer a suitable portion of salicylic acid to a suitable volumetric flask, dissolve first in methanol using 10% of the final volume, dilute with *Buffer B* to volume, and mix.

Standard stock solution: 1 mg/mL of USP Cefazolin RS in *Buffer B*

Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *Buffer B* to volume, and mix.

Sample stock solution 1 (where it is packaged for dispensing and is represented as being in a single-dose container): Nominally 1 mg/mL of cefazolin from Cefazolin for Injection prepared as follows. Constitute Cefazolin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with *Buffer B*.

Sample solution 1: Transfer 5.0 mL of the *Sample stock solution 1* to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *Buffer B* to volume, and mix.

Sample stock solution 2 (where the label states the quantity of cefazolin in a given volume of constituted solution): Nominally 1 mg/mL of cefazolin from Cefazolin for Injection prepared as follows. Constitute Cefazolin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Dilute an aliquot of the constituted solution with *Buffer B*.

Sample solution 2: Transfer 5.0 mL of the *Sample stock solution 2* to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *Buffer B* to volume, and mix.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.0-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for salicylic acid and cefazolin are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between the analyte and internal standard peaks
Column efficiency: NLT 1500 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution*, *Sample solution 1*, or *Sample solution 2*
Calculate the percentage of the labeled amount of cefazolin ($C_{14}H_{14}N_8O_4S_3$) in the container and in the volume of constituted solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P \times 100$$

R_U = peak response ratio of cefazolin to the internal standard of the *Sample solution*
R_S = peak response ratio of cefazolin to the internal standard of the *Standard solution*
C_S = concentration of USP Cefazolin RS, calculated on the anhydrous basis, in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefazolin in the *Sample solution* (mg/mL)
P = potency of cefazolin in USP Cefazolin RS (mg/mg)

Acceptance criteria: 90.0%–115.0%. Where the test for *Uniformity of Dosage Units* ⟨905⟩ has been performed using the *Analysis* for content uniformity, use the average of these determinations as the *Assay* value.

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  Procedure for content uniformity
  Perform the *Assay* on individual containers using *Sample solution 1* or *Sample solution 2*, or both, as appropriate.
  Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 55 mg/mL in 0.1 M sodium bicarbonate
  Acceptance criteria: –10° to –24°
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.15 USP Endotoxin Unit/mg of cefazolin
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **pH** ⟨791⟩
  Sample solution: 100 mg/mL of cefazolin
  Acceptance criteria: 4.0–6.0
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 6.0%
- **PARTICULATE MATTER IN INJECTION** ⟨788⟩: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*

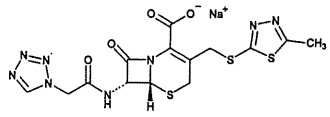
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*.

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cefazolin RS

Cefazolin Sodium



$C_{14}H_{13}N_8NaO_4S_3$ 476.49
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-8-oxo-7-[[(1*H*-tetrazol-1-yl)acetyl]amino]-, monosodium salt (6*R*-*trans*)-;
Monosodium (6*R*,7*R*)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-8-oxo-7-[2-(1*H*-tetrazol-1-yl)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate; [27164-46-1].

DEFINITION
Cefazolin Sodium has a potency equivalent to NLT 89.1% and NMT 110.1% of cefazolin sodium ($C_{14}H_{13}N_8NaO_4S_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
Solution A: Acetone and water (9:1)
Standard: USP Cefazolin RS
Sample: 150 mg
Analysis: Dissolve the *Sample* in 5 mL of water, add 0.5 mL of 2 N acetic acid, swirl, and allow to stand for 10 min in an ice bath. Filter the precipitate, and rinse with 1–2 mL of water. Dissolve in *Solution A*, evaporate the solvent almost to dryness, and dry in an oven at 60° for 30 min.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*: Meets the requirements

ASSAY
• **PROCEDURE**
Protect all solutions containing cefazolin from light.
Buffer A: 0.9 g/L of anhydrous dibasic sodium phosphate and 1.3 g/L of citric acid in water
Buffer B: 5.7 g/L of anhydrous dibasic sodium phosphate and 3.6 g/L of monobasic potassium phosphate in water
Mobile phase: Acetonitrile and *Buffer A* (1:9). Pass through a suitable filter.
Standard solution: 50 µg/mL of USP Cefazolin RS in *Buffer B*
Sample solution: 50 µg/mL of Cefazolin Sodium in *Buffer B*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cefazolin sodium ($C_{14}H_{13}N_8NaO_4S_3$) in the portion of Cefazolin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times P \times 100$$

r_U = peak response of cefazolin from the *Sample solution*
r_S = peak response of cefazolin from the *Standard solution*
C_S = concentration of USP Cefazolin RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefazolin Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of cefazolin sodium, 476.49
M_{r2} = molecular weight of cefazolin, 454.51
P = potency of USP Cefazolin RS (mg/mg)

Acceptance criteria: 89.1%–110.1% on the anhydrous basis

IMPURITIES
• **ORGANIC IMPURITIES**
Protect all solutions containing cefazolin from light.
Solution A: 6.8 g/L of monobasic potassium phosphate
Solution B: 6.8 g/L of monobasic potassium phosphate adjusted with 10% sodium hydroxide to a pH of 6.8 before final dilution
Solution C: Acetonitrile and *Solution A* (1:1)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	98	2
7	98	2
15	85	15
30	80	20
35	80	20
45	50	50
50	50	50
55	98	2
65	98	2

Blank: *Solution B*
System suitability stock solution: 2 mg/mL of USP Cefazolin RS in 0.05 M sodium hydroxide. Set the solution aside at room temperature for 5 min. [NOTE—The cefazolin epimer is formed upon treatment of cefazolin with sodium hydroxide.]
System suitability solution: *System suitability stock solution* and *Solution B* (1:24)
Standard solution: 25 µg/mL of USP Cefazolin RS in *Solution B*. Use this solution promptly after preparation.
Sample solution: 2.5 mg/mL of Cefazolin Sodium in *Solution B*. Use this solution promptly after preparation.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 and 254 nm

www.webofpharma.com

Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 8.0 between cefazolin and cefazolin epimer at 254 nm

Analysis
Samples: *Blank*, *Standard solution*, and *Sample solution*
Calculate the percentage of tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin Sodium taken:

$$\text{Result} = (r_{U(210)}/r_{S(254)}) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{U(210)}$ = peak response of tetrazolylacetic acid or tetrazolylacetamide acetal at 210 nm from the *Sample solution*
$r_{S(254)}$ = peak response of cefazolin at 254 nm from the *Standard solution*
C_S = concentration of USP Cefazolin RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefazolin Sodium in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Calculate the percentage of each impurity other than tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin Sodium taken:

$$\text{Result} = (r_{U(254)}/r_{S(254)}) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{U(254)}$ = peak response of each impurity other than tetrazolylacetic acid and tetrazolylacetamide acetal at 254 nm from the *Sample solution*
$r_{S(254)}$ = peak response of cefazolin at 254 nm from the *Standard solution*
C_S = concentration of USP Cefazolin RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefazolin Sodium in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks corresponding to those in the *Blank*.

Table 2

Name	Analytical Wavelength (nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tetrazolylacetic acid[a]	210	0.07	0.40	1.0
Tetrazolylacetamide acetal[b]	210	0.08	0.33	1.0
Cefazolin open-ring lactone[c, d] or cefazolin 3-hydroxymethyl[c, e]	254	0.20	1.0	0.5
Methylthiadiazole thiol[f]	254	0.23	0.91	1.0
7-Aminocephalosporanic acid[g]	254	0.42	1.1	1.0
Cefazolin 3-methyl analog[h]	254	0.44	0.87	1.0
Cefazolin lactone[i]	254	0.50	0.85	1.0
Cefazolin acetoxy analog[j]	254	0.61	0.68	1.0
Cefazolin deacylated[k]	254	0.68	1.2	1.0
Cefazoloic acid isomers[l]	254	0.84	1.0	1.0
Cefazolin	254	1.0	—	—
Cefazolin epimer[m]	254	1.2	0.98	1.0
Cefazolin pivaloyl[n]	254	1.4	0.92	1.0
Any individual unspecified impurity	254	—	1.0	0.1
Total impurities	—	—	—	3.5

[a] 2-(1*H*-Tetrazol-1-yl)acetic acid.
[b] *N*-(2,2-Dihydroxyethyl)-2-(1*H*-tetrazol-1-yl)acetamide.
[c] The identification of this impurity is tentative. The names of the most likely compounds are listed in footnotes [d] and [e].
[d] (*R*)-2-[2-(1*H*-Tetrazol-1-yl)acetamido]-2-[(*R*)-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid.
[e] (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[f] 5-Methyl-1,3,4-thiadiazole-2-thiol (MMTD).
[g] (6*R*,7*R*)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ACA).
[h] (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[i] *N*-[(5a*R*,6*R*)-1,7-Dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl]-2-(1*H*-tetrazol-1-yl)acetamide.
[j] (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[k] (6*R*,7*R*)-7-Amino-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[l] Three isomers of this impurity may not be fully resolved by this method. The limit applies to the sum of the isomers, which are as follows: Cefazolin open-ring delta-3: (2*R*)-2-{(*R*)-[2-(1*H*-tetrazol-1-yl)acetamido](carboxy)methyl}-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid. Cefazolin open-ring delta-2: (2*R*)-2-{(*R*)-[2-(1*H*-tetrazol-1-yl)acetamido](carboxy)methyl}-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,4-dihydro-2*H*-1,3-thiazine-4-carboxylic acid. Cefazolin open-ring delta-4: (2*R*)-2-{(*R*)-[2-(1*H*-tetrazol-1-yl)acetamido](carboxy)methyl}-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.
[m] (6*R*,7*S*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[n] (6*R*,7*R*)-3-((5-Methyl-1,3,4-thiadiazol-2-ylthio)methyl)-8-oxo-7-pivalamido-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

SPECIFIC TESTS

- **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 55 mg/mL, in 0.1 M sodium bicarbonate
  Acceptance criteria: −10° to −24°
- **pH** ⟨791⟩
  Sample solution: 100 mg/mL of cefazolin
  Acceptance criteria: 4.0–6.0
- **Water Determination** ⟨921⟩, *Method I*: NMT 6.0%
- **Sterility Tests** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Where the label states that Cefazolin Sodium is sterile, it meets the requirements.
- **Bacterial Endotoxins Test** ⟨85⟩: Where the label states that Cefazolin Sodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Units/mg of cefazolin.

ADDITIONAL REQUIREMENTS

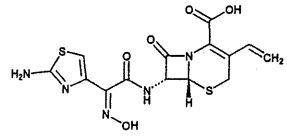
- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or

www.webofpharma.com

must be subjected to further processing during the preparation of injectable dosage forms.

• **USP Reference Standards** ⟨11⟩
USP Cefazolin RS

Cefdinir



$C_{14}H_{13}N_5O_5S_2$ 395.41

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)(hydroxyimino)acetyl]amino]-3-ethenyl-8-oxo-, [6*R*-[6α,7β(*Z*)]]-;
(–)-(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7²-(*Z*)-oxime [91832-40-5].

DEFINITION

Cefdinir contains NLT 940 µg/mg and NMT 1030 µg/mg of cefdinir ($C_{14}H_{13}N_5O_5S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Solution A: 14.2 g/L of anhydrous dibasic sodium phosphate

Solution B: 13.6 g/L of monobasic potassium phosphate

Solution C: Dilute tetramethylammonium hydroxide (10%) with water to obtain a 0.1% solution. Adjust with 10% phosphoric acid to a pH of 5.5.

Solution D: 37.2 mg/mL of edetate disodium

Buffer: Combine appropriate amounts of *Solution A* and *Solution B* (about 2:1) to obtain a solution with a pH of 7.0.

Mobile phase: Acetonitrile, methanol, *Solution C*, and *Solution D* (300:200:4500:2)

System suitability solution: 0.2 mg/mL of USP Cefdinir RS and 0.5 mg/mL of USP Cefdinir Related Compound A RS in *Buffer*

Standard solution: 0.2 mg/mL of USP Cefdinir RS in *Buffer*

Sample solution: 0.2 mg/mL of Cefdinir in *Buffer*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 5 µL

System suitability
Samples: *System suitability solution* and *Standard solution*.
USP Cefdinir Related Compound A RS should produce four peaks.
Tailing factor: NMT 1.5 for cefdinir, *System suitability solution*
Resolution: NLT 1.2 between the second peak of cefdinir related compound A and cefdinir, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of cefdinir ($C_{14}H_{13}N_5O_5S_2$) in the portion of Cefdinir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = purity of USP Cefdinir RS (µg/mg)

Acceptance criteria: 940–1030 µg/mg on the anhydrous basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.20%

• **Organic Impurities**

Solution A, Solution B, Solution C, Solution D, and **Buffer:** Prepare as directed in the *Assay*.

Solution E: To 1000 mL of *Solution C* add 0.4 mL of *Solution D*.

Solution F: Acetonitrile, methanol, *Solution C*, and *Solution D* (300: 200: 500: 0.4)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution E (%)	Solution F (%)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50
38	95	5
58	95	5

System suitability solution 1: 15 µg/mL of cefdinir from the *Sample solution*, diluted with *Solution C*

System suitability solution 2: 1.5 µg/mL of cefdinir from *System suitability solution 1*, diluted with *Solution C*

System suitability solution 3: 1.5 mg/mL of USP Cefdinir RS and 0.1 mg/mL of USP Cefdinir Related Compound A RS, dissolved initially in *Buffer* corresponding to 15% of the final volume, and diluted with *Solution C* to volume

Sample stock solution: 10 mg/mL of Cefdinir in *Buffer*

Sample solution: 1.5 mg/mL of cefdinir from the *Sample stock solution*, in *Solution C*. [Note—Prepare fresh immediately before use.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 10 µL

USP Monographs

www.webofpharma.com

System suitability
Samples: *System suitability solution 1*, *System suitability solution 2*, and *System suitability solution 3*. USP Cefdinir Related Compound A RS should produce four peaks.
Suitability requirements
Response ratio: The response of cefdinir from *System suitability solution 2* is between 7% and 13% of that from *System suitability solution 1*.
Resolution: NLT 1.5 between cefdinir and the third peak of USP Cefdinir Related Compound A RS, *System suitability solution 3*
Relative standard deviation: NMT 2.0% for cefdinir, *System suitability solution 3*
Analysis
Sample: *Sample solution*. Record the chromatogram for at least 1.8 times the retention time of the cefdinir peak. Calculate the percentage of each impurity in the portion of Cefdinir taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in *Buffer*, as obtained in the *Assay*
Acceptance criteria: −61° to −67° at 20°
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.0% for anhydrous; 4.0%–8.5% for hydrated forms. For this monograph, the term "hydrated forms" refers to several known forms of Cefdinir. Use a mixture of formamide and methanol (2:1) as the solvent.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cefdinir RS
USP Cefdinir Related Compound A RS
(2*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido]-2-[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid (three other stereoisomers are also present in this RS).
$C_{14}H_{15}N_5O_6S_2$ 413.43

Cefdinir Capsules

DEFINITION
Cefdinir Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$).

Table 2 (Cefdinir)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Thiazolylacetyl glycine oxime[a]	0.10	0.5
Thiazolylacetyl glycine oxime acetal[b]	0.12	0.5
3-Methyl cefdinir[c]	0.74	0.7
Cefdinir related compound A (cefdinir open ring lactone a)[d, e]	0.85	0.7
Cefdinir related compound A (cefdinir open ring lactone b)[d, e]	0.93	
Cefdinir related compound A (cefdinir open ring lactone c)[d, e]	1.11	
Cefdinir related compound A (cefdinir open ring lactone d)[d, e]	1.14	
Cefdinir lactone[f]	1.22	0.5
Cefdinir isoxazole analog[g]	1.36	0.5
E-Cefdinir[h]	1.51	0.7
Cefdinir decarboxy open ring lactone a[i, j]	1.61	0.5
Cefdinir decarboxy open ring lactone b[i, j]	1.64	
Any other individual, unidentified impurity	—	0.2
Total impurities	—	3.0

[a] 1*N*-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.
[b] (*Z*)-2-(2-Aminothiazol-4-yl)-*N*-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide.
[c] (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[d] 2(*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido)]-2-[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid.
[e] Cefdinir related compound A is a mixture of 4 isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported. The limit for the sum of the 4 isomers is 0.7%.
[f] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-{(3*RS*,5a*R*,6*R*)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl}acetamide.
[g] (6*R*,7*R*)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[h] (6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl-)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[i] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-{[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]methyl}acetamide.
[j] Cefdinir decarboxy open ring lactone is a mixture of 2 isomers labeled cefdinir decarboxy open ring lactones a and b. The sum of the values is reported. The limit for sum of the 2 isomers is 0.5%.

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Buffer: Prepare as directed in the *Assay*.
Standard solution: 10 µg/mL of USP Cefdinir RS in *Buffer*
Sample solution: Equivalent to 10 µg/mL of cefdinir from Capsules in *Buffer*. Filter before use.
Cell size: 1 cm
Blank: Use the *Buffer*.
Acceptance criteria: *Sample solution* maxima and minima occur at the same wavelengths as those in the *Standard solution*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer: 10.7 g/L of dibasic sodium phosphate and 3.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid or sodium hydroxide to a pH of 7.0 ± 0.05 before final dilution.
Solution A: 7 g/L of citric acid monohydrate. Adjust with phosphoric acid to a pH of 2.0 ± 0.05.
Mobile phase: Methanol, tetrahydrofuran, and *Solution A* (111:28:1000)
System suitability solution: 50 µg/mL of USP Cefdinir RS and 175 µg/mL of *m*-hydroxybenzoic acid in *Buffer*
Standard solution: 50 µg/mL of USP Cefdinir RS in *Buffer*
Sample solution: Equivalent to 50 µg/mL of cefdinir, from Capsule contents (NLT 20), in *Buffer*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Flow rate: 1.4 mL/min
Injection volume: 15 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: Greater than 3.0 between cefdinir and *m*-hydroxybenzoic acid, *System suitability solution*
Tailing factor: NMT 2.0 for cefdinir, *System suitability solution*
Relative standard deviation: NMT 1.0% for cefdinir, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cefdinir from the *Sample solution*
r_S = peak response of cefdinir from the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = nominal concentration of cefdinir in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 50 mM phosphate buffer, pH 6.8; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Detector: UV 290 nm
Cell length: 0.1-cm flow cell
Standard solution: 0.33 mg/mL of USP Cefdinir RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute with *Medium* to a concentration of about 0.33 mg/mL of cefdinir.
Blank: Dissolve 1 empty Capsule in 100 mL of *Medium*, and dilute to 900 mL. Filter if necessary.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution* (mL/mL)
L = label claim (mg/Capsule)

Tolerances: NLT 80% (*Q*) of the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: 14.2 g/L of anhydrous dibasic sodium phosphate
Solution B: 13.6 g/L of monobasic potassium phosphate
Buffer: Combine appropriate amounts of *Solution A* and *Solution B* (about 2:1) to obtain a solution with a pH of 7.0 ± 0.1.
Solution C: Dilute tetramethylammonium hydroxide (10% aqueous) with water to obtain a 0.1% solution. Adjust with dilute phosphoric acid (1 in 10) to a pH of 5.5 ± 0.1.
Solution D: 37.2 mg/mL of edetate disodium
Solution E: To 1000 mL of *Solution C* add 0.4 mL of *Solution D*.
Solution F: Acetonitrile, methanol, *Solution C*, and *Solution D* (150: 100: 250: 0.2)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution E (%)	Solution F (%)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50
38	95	5
58	95	5

System suitability stock solution 1: 40 µg/mL of USP Cefdinir Related Compound A RS in *Solution C*
System suitability stock solution 2: 40 µg/mL of USP Cefdinir Related Compound B RS in *Solution C*
System suitability solution: Transfer 37.5 mg of USP Cefdinir RS to a 25-mL volumetric flask. Add about 10 mL of *Buffer*. Add 5.0 mL each of *System suitability stock solution*

www.webofpharma.com

1 and *System suitability stock solution 2*, and dilute with *Solution C* to volume.

Standard stock solution: 750 µg/mL of USP Cefdinir RS in *Buffer*

Standard solution: 15 µg/mL of USP Cefdinir RS, from the *Standard stock solution*, in *Solution C*

Sample solution: Transfer an equivalent to 300 mg of cefdinir from Capsule contents (NLT 20) to a 200-mL volumetric flask. Dissolve in 30 mL of *Buffer*, and dilute with *Solution C* to volume to obtain a solution with a nominal concentration of about 1.5 mg/mL of cefdinir.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Temperatures

Autosampler: 4°

Column: 40°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between cefdinir and the third peak of USP Cefdinir Related Compound A RS, *System suitability solution*

Tailing factor: NMT 1.5 for cefdinir related compound B, *System suitability solution*

Relative standard deviation: NMT 2.0% for the cefdinir peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (100/F)$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of cefdinir in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Thiazolylacetyl glycine oxime[a]	0.10	1.0	0.5
Thiazolylacetyl glycine oxime acetal[b]	0.13	1.0	0.5
Cefdinir sulfoxide[c]	0.36	1.0	0.2
Cefdinir thiazine analog[d]	0.46	0.68	0.7
3-Methyl cefdinir[e]	0.75	1.0	0.7
Cefdinir impurity 1[f]	0.77	1.0	0.3
Cefdinir related compound A (cefdinir open ring lactone a)[g, h]	0.85	0.65	2.5
Cefdinir related compound A (cefdinir open ring lactone b)[g, h]	0.94	0.65	
Cefdinir related compound A (cefdinir open ring lactone c)[g, h]	1.11	0.65	
Cefdinir related compound A (cefdinir open ring lactone d)[g, h]	1.14	0.65	
7*S*-Cefdinir[i]	1.18	1.0	0.2
Cefdinir lactone[j]	1.23	1.0	1.0
Cefdinir related compound B[k]	1.28	1.0	0.2
Cefdinir isoxazole analog[l]	1.37	0.72	0.5
Cefdinir impurity 2[f]	1.44	1.0	0.5
Cefdinir glyoxalic analog[m]	1.49	1.0	0.2
E-Cefdinir[n]	1.51	1.0	1.2
Cefdinir decarboxy open ring lactone a[o, p]	1.62	1.0	1.0
Cefdinir decarboxy open ring lactone b[o, p]	1.64	1.0	
Cefdinir impurity 3[f]	1.82	1.0	0.2
Individual unidentified impurities	—	1.0	0.2
Total impurities	—	—	5.0

[a] *N*-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.

[b] (*Z*)-2-(2-Aminothiazol-4-yl)-*N*-(2,2-dihydroxyethyl)-2-(hydroxyimino) acetamide.

[c] (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-5,8-dioxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[d] (*R*,*Z*)-2-{(*R*)-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido](carboxy)methyl}-5-ethylidene-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

[e] (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[f] Cefdinir impurity 1, cefdinir impurity 2, and cefdinir impurity 3 are unidentified impurities.

[g] Cefdinir related compound A is a mixture of four isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported. The limit for the sum of the four isomers is 2.5%.

[h] 2(*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid.

[i] (6*R*,7*S*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[j] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-((3*RS*,5a*R*,6*R*)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl) acetamide.

[k] (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[l] (6*R*,7*R*)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[m] (6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-oxoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[n] (6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[o] Cefdinir decarboxy open ring lactone is a mixture of two isomers labeled cefdinir decarboxy open ring lactone a and b. The sum of the values is reported. The limit for the sum of the two isomers is 1.0%.

[p] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-{[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]methyl}acetamide.

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cefdinir RS
  USP Cefdinir Related Compound A RS
  (2*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido]-2-[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid (three other stereoisomers are also present in this RS).
  $C_{14}H_{15}N_5O_6S_2$ 413.43
  USP Cefdinir Related Compound B RS
  (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
  $C_{14}H_{13}N_4O_4S_2$ 365.41

Cefdinir for Oral Suspension

DEFINITION

Cefdinir for Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$). It may contain one or more suitable buffers, flavors, preservatives, stabilizing agents, sweeteners, and suspending agents.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 10.7 mg/mL of anhydrous dibasic sodium phosphate and 3.4 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid or sodium hydroxide to a pH of 7.0 ± 0.05 before final dilution.
Solution A: 7 mg/mL of citric acid monohydrate. Adjust with phosphoric acid to a pH of 2.0 ± 0.05.
Mobile phase: Methanol, tetrahydrofuran, and *Solution A* (111:28:1000)
System suitability solution: 50 µg/mL of USP Cefdinir RS and 175 µg/mL of *m*-hydroxybenzoic acid in *Buffer*
Standard solution: 50 µg/mL of USP Cefdinir RS in *Buffer*
Sample solution: Equivalent to 50 µg/mL of cefdinir from constituted Cefdinir for Oral Suspension in *Buffer*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Flow rate: 1.4 mL/min
Injection volume: 15 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between cefdinir and *m*-hydroxybenzoic acid, *System suitability solution*
Tailing factor: NMT 2.0 for cefdinir, *System suitability solution*
Relative standard deviation: NMT 1.0% for cefdinir, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$) in the portion of Cefdinir for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cefdinir from the *Sample solution*
r_S = peak response of cefdinir from the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = nominal concentration of cefdinir in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.05 M phosphate buffer, pH 6.8; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Detector: UV 290 nm
Standard solution: 0.14 mg/mL of USP Cefdinir RS in *Medium*
Sample solution: Transfer 5 mL, by weight, of the reconstituted Cefdinir for Oral Suspension into the vessel. After the appropriate time, withdraw a portion of the solution under test, and pass through a suitable filter of 0.45-µm pore size. Dilute a portion of each filtered sample with *Medium* as necessary to obtain a solution having a concentration of about 0.14 mg/mL of cefdinir.
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (d/W_U) \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
d = density of Cefdinir for Oral Suspension (mg/mL)
W_U = weight of reconstituted Cefdinir for Oral Suspension taken (mg)
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution* (mL/mL)
L = label claim (mg/mL)

Tolerances: NLT 80% (Q) of the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For single-unit containers
Acceptance criteria: Meets the requirements

- **DELIVERABLE VOLUME** ⟨698⟩

For multiple-unit containers
Acceptance criteria: Meets the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: 14.2 mg/mL of anhydrous dibasic sodium phosphate
Solution B: 13.6 mg/mL of monobasic potassium phosphate
Buffer: Combine appropriate amounts of *Solution A* and *Solution B* (about 2:1) to obtain a solution with a pH of 7.0 ± 0.1.
Solution C: Dilute tetramethylammonium hydroxide (10% aqueous) with water to obtain a 0.1% solution. Adjust with dilute phosphoric acid (1 in 10) to a pH of 5.5 ± 0.1.
Solution D: 37.2 mg/mL of edetate disodium
Solution E: To 1000 mL of *Solution C* add 0.4 mL of *Solution D*.
Solution F: Acetonitrile, methanol, *Solution C*, and *Solution D* (150: 100: 250: 0.2)

www.webofpharma.com

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution E (%)	Solution F (%)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50
38	95	5
58	95	5

System suitability stock solution 1: 40 µg/mL of USP Cefdinir Related Compound A RS in *Solution C*

System suitability stock solution 2: 40 µg/mL of USP Cefdinir Related Compound B RS in *Buffer*

System suitability solution: Transfer 37.5 mg of USP Cefdinir RS to a 25-mL volumetric flask, and add about 10 mL of *Buffer*. Add 5.0 mL each of *System suitability stock solution 1* and *System suitability stock solution 2*, and dilute with *Solution C* to volume.

Standard stock solution: 750 µg/mL of USP Cefdinir RS in *Buffer*

Standard solution: 15 µg/mL of USP Cefdinir RS from the *Standard stock solution* in *Solution C*

Sample solution: Transfer a quantity equivalent to 150 mg of cefdinir from the constituted Cefdinir for Oral Suspension to a 100-mL volumetric flask. Dissolve in 30 mL of *Buffer*, and dilute with *Solution C* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Temperatures

Autosampler: 4°

Column: 40°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between cefdinir and the third peak of USP Cefdinir Related Compound A RS, *System suitability solution*

Tailing factor: NMT 1.5 for cefdinir related compound B, *System suitability solution*

Relative standard deviation: NMT 2.0% for the cefdinir peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cefdinir for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (100/F)$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of cefdinir from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of cefdinir in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Thiazolylacetyl glycine oxime[a]	0.10	1.0	0.5
Thiazolylacetyl glycine oxime acetal[b]	0.13	1.0	0.6
Cefdinir sulfoxide[c]	0.36	1.0	0.2
Cefdinir thiazine analog[d]	0.46	0.68	0.3
3-Methyl cefdinir[e]	0.75	1.0	0.7
Cefdinir impurity 1[f]	0.77	1.0	0.2
Cefdinir related compound A (cefdinir open ring lactone a)[g, h]	0.85	0.65	3.3
Cefdinir related compound A (cefdinir open ring lactone b)[g, h]	0.94	0.65	
Cefdinir related compound A (cefdinir open ring lactone c)[g, h]	1.11	0.65	
Cefdinir related compound A (cefdinir open ring lactone d)[g, h]	1.14	0.65	
7*S*-Cefdinir[i]	1.18	1.0	0.2
Cefdinir lactone[j]	1.23	1.0	0.8
Cefdinir related compound B[k]	1.28	1.0	0.2
Cefdinir isoxazole analog[l]	1.37	0.72	0.5
Cefdinir impurity 2[f]	1.44	1.0	0.2
Cefdinir glyoxalic analog[m]	1.49	1.0	0.2
E-Cefdinir[n]	1.51	1.0	1.4
Cefdinir decarboxy open ring lactone a[o, p]	1.62	1.0	1.1
Cefdinir decarboxy open ring lactone b[o, p]	1.64	1.0	
Cefdinir impurity 3[f]	1.82	1.0	0.2
Individual unidentified impurities	—	1.0	0.2

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	6.2

[a] *N*-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.
[b] (*Z*)-2-(2-Aminothiazol-4-yl)-*N*-(2,2-dihydroxyethyl)-2-(hydroxyimino) acetamide.
[c] (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-5,8-dioxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[d] (*R*,*Z*)-2-[(*R*)-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido] (carboxy)methyl]-5-ethylidene-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.
[e] (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[f] Cefdinir impurity 1, cefdinir impurity 2, and cefdinir impurity 3 are unidentified impurities.
[g] Cefdinir related compound A is a mixture of four isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported; the limit for the sum of the four isomers is 3.3%.
[h] 2(*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2*RS*, 5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid.
[i] (6*R*,7*S*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[j] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-((3*RS*,5a*R*,6*R*)-3-methyl-1,7-dioxo-1,3,4,5*a*,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl) acetamide.
[k] (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[l] (6*R*,7*R*)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[m] (6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-oxoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[n] (6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[o] Cefdinir decarboxy open ring lactone is a mixture of two isomers labeled cefdinir decarboxy open ring lactone a and b. The sum of the values is reported; the limit for the sum of the two isomers is 1.1%.
[p] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-[[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]methyl)acetamide.

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.2–4.8

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Cefdinir RS
  USP Cefdinir Related Compound A RS
  (2*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido]-2-[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid (three other stereoisomers are also present in this RS).
  $C_{14}H_{15}N_5O_6S_2$ 413.43
  USP Cefdinir Related Compound B RS
  (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo](4.2.0)]oct-2-ene-2-carboxylic acid.
  $C_{14}H_{13}N_4O_4S_2$ 365.41

Cefepime for Injection

DEFINITION
Cefepime for Injection is a sterile mixture of Cefepime Hydrochloride and Arginine. It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of cefepime ($C_{19}H_{24}N_6O_5S_2$).

IDENTIFICATION
- **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩
  Standard solution: 20 mg/mL of arginine
  Sample solution: 40 mg/mL of Cefepime for Injection
  Developing solvent system: *n*-Propyl alcohol, ammonium hydroxide, and water (7:4:5)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Proceed as directed in *Thin-Layer Chromatographic Identification Test* ⟨201⟩, except to spray the plate with ninhydrin TS.
  Acceptance criteria: Arginine appears as a dark red spot. The intensity and the R_F value of the spot from the *Sample solution* correspond to those from the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**
  Solution A: 0.68 mg/mL of monobasic potassium phosphate in water
  Solution B: Acetonitrile and *Solution A* (1:9), adjusted with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0
  Solution C: Acetonitrile and *Solution A* (1:1), adjusted with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0
  Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
10	100	0
30	50	50
35	50	50
36	100	0
45	100	0

Standard solution: 1.4 mg/mL of USP Cefepime Hydrochloride RS in *Solution B*. [Note—Sonicate if necessary. Inject immediately or store in a refrigerator and use within 12 h.]
Sample solution: Constitute one container of Cefepime for Injection as directed on the label, and dilute using *Solution B* to 1 mg/mL of cefepime. [Note—For products that are designed for administration with a syringe, withdraw the entire withdrawable contents of the vial and transfer to a suitable volumetric flask. Dilute with *Solution B* to volume. For all other types, transfer the contents of the reconstituted vial quantitatively to a suitable volumetric flask, and dilute with *Solution B* to volume.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{19}H_{24}N_6O_5S_2$ in the portion of Cefepime for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cefepime Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefepime in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES
ORGANIC IMPURITIES
• Procedure 1: Limit of *N*-Methylpyrrolidine
Mobile phase: Acetonitrile and 0.01 N nitric acid (1:19)
Standard solution: 0.05 mg/mL of *N*-methylpyrrolidine in 0.002 N nitric acid
Sample solution: Equivalent to 5 mg/mL of cefepime hydrochloride in 0.002 N nitric acid. [NOTE—Inject this solution immediately.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity
Column: 4.0-mm x 25-cm; 5-µm packing L76
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 4.0%
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Record the chromatogram of the *Sample solution* for about 6 times the retention time of the *N*-methylpyrrolidine peak.]
Calculate the percentage of *N*-methylpyrrolidine in the portion of Cefepime for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *N*-methylpyrrolidine from the *Sample solution*
r_S = peak response of *N*-methylpyrrolidine from the *Standard solution*
C_S = concentration of *N*-methylpyrrolidine in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefepime in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%
• Procedure 2: Other Organic Impurities
Solution A, Solution B, Solution C, Mobile phase, Chromatographic system, and **Sample solution:** Prepare as directed for *Assay*.
System suitability solution: 1.4 mg/mL of USP Cefepime Hydrochloride RS and 15 µg/mL each of USP Cefepime Related Compound D RS and USP Cefepime Related Compound E RS in *Solution B*
System suitability
Sample: *System suitability solution*
Suitability requirements
[NOTE—See *Impurity Table 1* for the relative retention times.]
Resolution: NLT 2.0 between cefepime related compound E and cefepime related compound D
Tailing factor: NMT 1.5, cefepime
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Cefepime for Injection taken:

$$\text{Result} = (r_U/r_T) \times 1/F \times 100$$

r_U = peak response for each impurity
r_T = sum of the peak responses from the chromatogram
F = relative response factor from *Impurity Table 1*

Acceptance criteria
[NOTE—The reporting level is 0.2% for cefepime impurity C and 0.05% for all other related compounds.]
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 2.2%. [NOTE—Total impurities include *N*-methylpyrrolidine.]

Impurity Table 1

Name	Relative Retention Time (%)	Relative Response Factor	Acceptance Criteria, NMT (%)
Cefepime amine derivative[a, b] (cefepime related compound E)	0.4	—	—
Thiazolylglyoxalic methyloxime[a, c] (cefepime related compound D)	0.5	—	—
Thiazolyloxime acetaldehyde[d] (cefepime related compound C)	0.6	0.63	0.5
Cefepime dimer[a, e] (cefepime related compound F)	0.8	—	—
Cefepime	1.0	—	—
E-Cefepime[f] (cefepime related compound A)	2.7	0.71	0.5
Cefepime dioxime[a, g] (cefepime related compound B)	4.3	—	—
Any individual unspecified impurity	—	1.0	0.5

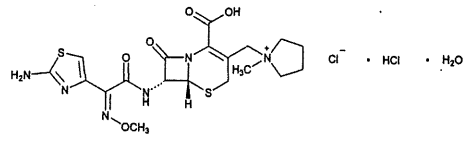
[a] These impurities are synthetic process impurities that are controlled in the drug substance. They are listed here for reference only.
[b] (6*R*,7*R*)-7-Amino-3-[(1-methylpyrrolidinium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.
[c] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.
[d] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)-*N*-(2-oxoethyl)acetamide.
[e] 1-{[[(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-{(6*R*,7*R*)-2-carboxy-3-[(1-methylpyrrolidinium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylcarbamoyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl}-1-methylpyrrolidinium chloride.
[f] 1-({(6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl}methyl)-1-methylpyrrolidinium chloride.
[g] 1-({(6*R*,7*R*)-7-[(*Z*)-2-{2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]thiazol-4-yl}-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl}methyl)-1-methylpyrrolidinium chloride.

www.webofpharma.com

SPECIFIC TESTS
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*: At the time of use, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.06 USP Endotoxin Unit/mg of cefepime
- **STERILITY TESTS** ⟨71⟩: Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **pH** ⟨791⟩: 4.0–6.0, in a solution containing 100 mg/mL of cefepime
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 4.0%
- **OTHER REQUIREMENTS:** Meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers as described under *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, and store in a refrigerator or at controlled room temperature. Store reconstituted solution in a refrigerator for NMT 7 days.
- **LABELING:** Label it to indicate that it is to be diluted with a suitable parenteral vehicle before intravenous infusion.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cefepime Hydrochloride RS
  USP Cefepime Related Compound D RS
  Thiazolylglyoxalic methyloxime; (*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetic acid.
  $C_6H_7N_3O_3S$ 201.20
  USP Cefepime Related Compound E RS
  Cefepime amine derivative; 1-{[(6*R*,7*R*)-7-amino-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl}-1-methylpyrrolidin-1-ium chloride.
  $C_{13}H_{20}ClN_3O_3S$ 333.83

Cefepime Hydrochloride



$C_{19}H_{25}ClN_6O_5S_2 \cdot HCl \cdot H_2O$ 571.50

Pyrrolidinium, 1-[[7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methyl-, chloride, monohydrochloride, monohydrate, [6*R*-[6α,7β(*Z*)]]-;
1-[[(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride, 7²-(*Z*)-(*O*-methyloxime), monohydrochloride, monohydrate [123171-59-5].

DEFINITION
Cefepime Hydrochloride contains the equivalent of NLT 825 µg/mg and NMT 911 µg/mg of cefepime ($C_{19}H_{24}N_6O_5S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **▲A.▲** (USP 1-May-2019) **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M▲** (CN 1-May-2020)
  Sample: Proceed as directed in the chapter, but do not dry.
  Acceptance criteria: Meets the requirements

Add the following:

▲• B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY
- **PROCEDURE**
  Solution A: 0.68 mg/mL of monobasic potassium phosphate in water
  Solution B: Acetonitrile and *Solution A* (1:9). Adjust with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0.
  Solution C: Acetonitrile and *Solution A* (1:1). Adjust with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0.
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
10	100	0
30	50	50
35	50	50
36	100	0
45	100	0

Standard solution: 1.4 mg/mL of USP Cefepime Hydrochloride RS in *Solution B*. [NOTE—Sonicate, if necessary. Inject immediately or store in a refrigerator and use within 12 h.]
Sample solution: 1.4 mg/mL of Cefepime Hydrochloride in *Solution B*. [NOTE—Sonicate, if necessary. Inject immediately or store in a refrigerator and use within 12 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg, of cefepime ($C_{19}H_{24}N_6O_5S_2$) in each milligram of Cefepime Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cefepime Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefepime Hydrochloride in the *Sample solution* (mg/mL)
P = content of cefepime in USP Cefepime Hydrochloride RS (µg/mg)

Acceptance criteria: 825–911 µg/mg on the anhydrous basis

USP Monographs

www.webofpharma.com

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

• **LIMIT OF *N*-METHYLPYRROLIDINE**

Mobile phase: Acetonitrile and 0.01 N nitric acid (1:19)

Standard solution: 0.05 mg/mL of *N*-methylpyrrolidine in 0.002 N nitric acid

Sample solution: Equivalent to 5 mg/mL of Cefepime Hydrochloride in 0.002 N nitric acid. [NOTE—Inject this solution immediately.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Conductivity

Column: 4.0-mm × 25-cm; 5-µm packing L76

Flow rate: 1 mL/min

Injection volume: 10 µL

▲Run time: NLT 6 times the retention time of *N*-methylpyrrolidine▲ (USP 1-May-2019)

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 4.0%

Analysis

Samples: *Standard solution* and *Sample solution* ▲▲ (USP 1-May-2019)

Calculate the percentage of *N*-methylpyrrolidine in the portion of Cefepime Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of *N*-methylpyrrolidine from the *Sample solution*

r_S = peak response of *N*-methylpyrrolidine from the *Standard solution*

C_S = concentration of *N*-methylpyrrolidine in the *Standard solution* (mg/mL)

C_U = concentration of Cefepime Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.3%

• **ORGANIC IMPURITIES**

Solution A, Solution B, Solution C, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 1.4 mg/mL of USP Cefepime Hydrochloride RS and 15 µg/mL each of USP Cefepime Related Compound D RS and USP Cefepime Related Compound E RS in *Solution B*

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between cefepime related compound E and cefepime related compound D

Tailing factor: NMT 1.5 for cefepime

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Cefepime Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. [NOTE—The reporting level is 0.2% for cefepime impurity C and 0.05% for all other related compounds.]

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cefepime amine derivative[a] (cefepime related compound E)	0.4	0.48	0.1
Thiazolylglyoxalic methyloxime[b] (cefepime related compound D)	0.5	1.0	0.1
Thiazolyloxime acetaldehyde[c] (cefepime related compound C)	0.6	0.63	0.3
Cefepime dimer[d] (cefepime related compound F)	0.8	1.0	0.2
Cefepime	1.0	—	—
E-Cefepime[e] (cefepime related compound A)	2.7	0.71	0.3
Cefepime dioxime[f] (cefepime related compound B)	4.3	0.71	0.2
Any individual unspecified impurity	—	1.0	0.1
Total impurities (excluding *N*-methylpyrrolidine)	—	—	1.0

[a] (6*R*,7*R*)-7-Amino-3-[(1-methylpyrrolidinium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

[b] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.

[c] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)-*N*-(2-oxoethyl)acetamide.

[d] 1-{[(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-{(6*R*,7*R*)-2-carboxy-3-[(1-methylpyrrolidinium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylcarbamoyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl}-1-methylpyrrolidinium chloride.

[e] 1-({(6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl}methyl)-1-methylpyrrolidinium chloride.

[f] 1-({(6*R*,7*R*)-7-[(*Z*)-2-{2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]thiazol-4-yl}-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl}methyl)-1-methylpyrrolidinium chloride.

SPECIFIC TESTS

Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲The level of bacterial endotoxins are such that the requirement under the relevant dosage form monograph(s) in which Cefepime Hydrochloride is used can be met. Where the label states that Cefepime Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Cefepime Hydrochloride is used can be met.▲ (USP 1-May-2019)

Change to read:

• **STERILITY TESTS** ⟨71⟩: Where the label states that Cefepime Hydrochloride is sterile, it meets the requirements.▲▲ (USP 1-May-2019)

• **CRYSTALLINITY** ⟨695⟩: Meets the requirements

• **WATER DETERMINATION** ⟨921⟩, *Method I*: 3.0%–4.5%

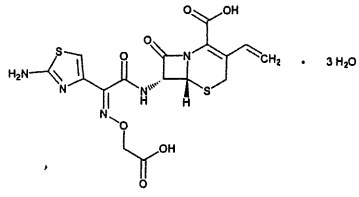
USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cefepime Hydrochloride RS
  USP Cefepime Related Compound D RS
  Thiazolylglyoxalic methyloxime;
  (*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.
  $C_6H_7N_3O_3S$ 201.20
  USP Cefepime Related Compound E RS
  Cefepime amine derivative;
  1-{[(6*R*,7*R*)-7-Amino-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl}-1-methylpyrrolidin-1-ium chloride.
  $C_{13}H_{20}ClN_3O_3S$ 333.83

Cefixime



$C_{16}H_{15}N_5O_7S_2 \cdot 3H_2O$ 507.50

$C_{16}H_{15}N_5O_7S_2$ (anhydrous) 453.46

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)[(carboxymethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-, trihydrate, [6*R*-[6α,7β(*Z*)]]-;
(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7²-(*Z*)-[*O*-(carboxymethyl)oxime]trihydrate [125110-14-7].
Anhydrous [79350-37-1].

DEFINITION

Cefixime contains the equivalent of NLT 950 µg/mg and NMT 1030 µg/mg of cefixime ($C_{16}H_{15}N_5O_7S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Dissolve 5 mg by trituration in 2 mL of methanol, and evaporate with the aid of gentle heat to dryness.
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
  Solution A: 25 mL of 0.4 M tetrabutylammonium hydroxide solution diluted with water to 1000 mL, and adjusted with 1.5 M phosphoric acid to a pH of 6.5
  Solution B: 13.6 g/L of monobasic potassium phosphate in water
  Solution C: 14.2 g/L of anhydrous dibasic sodium phosphate in water
  Buffer: Adjust an aliquot of *Solution C* with *Solution B* to a pH of 7.0.
  Mobile phase: Acetonitrile and *Solution A* (1:3)
  System suitability solution: 1 mg/mL of USP Cefixime RS in water. Heat this solution at 95° in an oil bath for 45 min, cool, and use promptly.
  Standard solution: 0.2 mg/mL of cefixime from USP Cefixime RS in *Buffer*. Use this solution promptly.
  Sample solution: 0.22 mg/mL of Cefixime in *Buffer*. Use this solution promptly.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 12.5-cm; 4-µm packing L1
  Column temperature: 40°
  Flow rate: Adjusted so that the retention time of cefixime is 10 min
  Injection volume: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for cefixime (*E*)-isomer and cefixime are about 0.9 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 2.0 between cefixime and cefixime (*E*)-isomer, *System suitability solution*
  Column efficiency: NLT 4000 theoretical plates, *Standard solution*
  Calculate as follows:

$$\text{Result} = 5.545(t/W_{h/2})^2$$

t = retention time
$W_{h/2}$ = peak width at half height

  Tailing factor: NLT 0.9 and NMT 2.0 for the analyte peak, *Standard solution*
  Calculate as follows:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$ = width of peak of 10% height
f = distance from the peak maximum to the leading edge of the peak measured at 10% of the peak height

  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the quantity, in µg/mg, of cefixime ($C_{16}H_{15}N_5O_7S_2$) in the portion of Cefixime taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cefixime RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefixime in the *Sample solution* (mg/mL)
P = potency of cefixime in USP Cefixime RS (mg/mg)
F = conversion factor, 1000 µg/mg

  Acceptance criteria: 950–1030 µg/mg on the anhydrous basis

www.webofpharma.com

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Solution C, Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Sample solution*

Calculate the percentage of each impurity in the portion of Cefixime taken:

$$\text{Result} = (r_U/r_S) \times P \times F \times 100$$

r_U = peak area for each impurity
r_S = cefixime peak area
P = potency of cefixime calculated in the *Assay* (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria

Individual impurities: NMT 1.0% of any individual impurity is found.

Total impurities: NMT 2.0%

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Diluent: 20-mg/mL solution of sodium bicarbonate

Sample solution: 10 mg/mL in *Diluent*

Acceptance criteria: –75° to –88°

• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements

• **PH** ⟨791⟩

Sample solution: 0.7 mg/mL of cefixime

Acceptance criteria: 2.6–4.1

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** 9.0%–12.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label to indicate that it is the trihydrate form. Where the quantity of Cefixime is indicated in the labeling of any preparation containing Cefixime, this shall be understood to be in terms of anhydrous cefixime ($C_{16}H_{15}N_5O_7S_2$).

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cefixime RS

Cefixime for Oral Suspension

DEFINITION

Cefixime for Oral Suspension is a dry mixture of Cefixime and one or more suitable diluents, flavors, preservatives, and suspending agents. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cefixime ($C_{16}H_{15}N_5O_7S_2$)/mL when constituted as directed in the labeling.

IDENTIFICATION

• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 0.4 M tetrabutylammonium hydroxide solution and water (1:39). Adjust with 1.5 M phosphoric acid to a pH of 6.5.

Solution B: 13.6 mg/mL of monobasic potassium phosphate

Solution C: 14.2 mg/mL of anhydrous dibasic sodium phosphate. Adjust a volume of this solution with a sufficient volume of *Solution B* to a pH of 7.0.

Mobile phase: Acetonitrile and *Solution A* (1:3)

System suitability solution: 1 mg/mL of USP Cefixime RS. [NOTE—Heat this solution at 95° in an oil bath for 45 min, cool, and use promptly.]

Standard solution: 0.2 mg/mL of USP Cefixime RS in *Solution C*. [NOTE—Use this solution promptly.]

Sample solution: Constitute Cefixime for Oral Suspension as directed in the labeling. Quantitatively dilute a suitable aliquot of the suspension, freshly mixed and free from air bubbles, with *Solution C* to obtain a solution having a nominal concentration of 0.2 mg of cefixime/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 12.5-cm; 4-µm packing L1

Temperature: 40°

Flow rate: Adjust flow rate so that the retention time of cefexime is about 10 min.

Injection size: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cefixime (*E*)-isomer and cefixime are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between cefixime and cefixime (*E*)-isomer, *System suitability solution*

Column efficiency: NLT 4000 theoretical plates, *Standard solution*. Use the following formula to calculate column efficiency:

$$\text{Result} = 5.545(t/W_{h/2})^2$$

Tailing factor: NLT 0.9 and NMT 2.0 for the analyte peak, *Standard solution*. Use the following formula to calculate tailing factor:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$ = peak width at 10% peak height

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{16}H_{15}N_5O_7S_2$ in the constituted suspension prepared from the Cefixime for Oral Suspension:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cefixime from the *Sample solution*
r_S = peak response of cefixime from the *Standard solution*
C_S = concentration of USP Cefixime RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefixime in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩ **FOR SOLIDS PACKAGED IN SINGLE-UNIT CONTAINERS:** Meets the requirements

• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

www.webofpharma.com

SPECIFIC TESTS
- **pH** ⟨791⟩: 2.5–4.5, in the suspension constituted as directed in the labeling

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label it to indicate that the cefixime contained therein is in the trihydrate form.
- **USP Reference Standards** ⟨11⟩
  USP Cefixime RS

Cefixime Tablets

DEFINITION
Cefixime Tablets contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of cefixime ($C_{16}H_{15}N_5O_7S_2$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**

Solution A: 25 mL of 0.4 M tetrabutylammonium hydroxide solution diluted with water to 1000 mL, and adjusted with 1.5 M phosphoric acid to a pH of 6.5
Solution B: 13.6 g/L of monobasic potassium phosphate in water
Solution C: 14.2 g/L of anhydrous dibasic sodium phosphate in water
Buffer: Adjust an aliquot of *Solution C* with *Solution B* to a pH of 7.0.
Mobile phase: Acetonitrile and *Solution A* (1:3)
System suitability solution: 1 mg/mL of USP Cefixime RS in water. Heat this solution at 95° in an oil bath for 45 min, cool, and use promptly.
Standard solution: 0.2 mg/mL of USP Cefixime RS in *Buffer*. Use this solution promptly.
Sample stock solution: Nominally 4 mg/mL of cefixime in *Buffer* from finely powdered Tablets (NLT 20). Sonicate as required, and centrifuge.
Sample solution: Nominally 0.2 mg/mL of cefixime from *Sample stock solution* in *Buffer*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 12.5-cm; 4-µm packing L1
Column temperature: 40°
Flow rate: Adjusted so that the retention time of cefixime is about 10 min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for cefixime (*E*)-isomer and cefixime are about 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between cefixime and cefixime (*E*)-isomer, *System suitability solution*
Column efficiency: NLT 4000 theoretical plates for the *Standard solution*
Calculate as follows:

$$\text{Result} = 5.545(t/W_{h/2})^2$$

t = retention time
$W_{h/2}$ = peak width at half height

Tailing factor: NLT 0.9 and NMT 2.0 for the analyte peak
Calculate as follows:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$ = width of peak of 10% height
f = distance from the peak maximum to the leading edge of the peak measured at 10% of the peak height

Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cefixime ($C_{16}H_{15}N_5O_7S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cefixime RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefixime in the *Sample solution* (mg/mL)
P = potency of cefixime in USP Cefixime RS (mg/mg)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **Dissolution** ⟨711⟩

Medium: 6.8 g/L of monobasic potassium phosphate in water, adjusted with 1 N sodium hydroxide to a pH of 7.2; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Detector: UV 288 nm
Standard solution: USP Cefixime RS in *Medium*. An amount of methanol not to exceed 0.1% of the total volume of the *Standard solution* may be used to bring the USP Cefixime RS into solution before dilution with *Medium*, and the solution may be sonicated to ensure complete dissolution of the USP Cefixime RS.
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.
Tolerances: NLT 75% (*Q*)

- **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

SPECIFIC TESTS
- **Water Determination,** *Method I* ⟨921⟩: NMT 10.0%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label the Tablets to indicate that the cefixime contained therein is in the trihydrate form.
- **USP Reference Standards** ⟨11⟩
  USP Cefixime RS

USP Monographs

www.webofpharma.com

Cefmenoxime for Injection

» Cefmenoxime for Injection contains an amount of Cefmenoxime Hydrochloride equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$). It may contain Sodium Carbonate.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Cefmenoxime Hydrochloride RS

Identification—

Change to read:

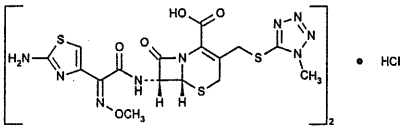
A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 25 µg per mL.
Medium: pH 6.8 buffer prepared as directed in the *Assay*— under *Cefmenoxime Hydrochloride.*
B: The retention time of the cefmenoxime peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay.*
Pyrogen ⟨151⟩—It meets the requirements, the test dose being 1.0 mL per kg of a solution of Cefmenoxime for Injection in sterile water for injection having a concentration of 60 mg of cefmenoxime per mL.
Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 6.4 and 7.9, in a solution containing the equivalent of 100 mg of cefmenoxime per mL.
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 1.5% of its weight.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Assay—
pH 6.8 buffer, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Cefmenoxime Hydrochloride.*
Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute a container of Cefmenoxime for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with water to obtain a solution containing the equivalent of about 1 mg of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$) per mL. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 80 µg of cefmenoxime per mL.
Assay preparation 2 (where the label states the quantity of cefmenoxime in a given volume of constituted solution)—Constitute a container of Cefmenoxime for Injection in a volume of water, accurately measured, equivalent to the volume of diluent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with water to obtain a solution containing about 1 mg of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$) per mL. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 80 µg of cefmenoxime per mL.
Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Cefmenoxime Hydrochloride.* Calculate the quantity, in mg, of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$) withdrawn from the container or in the portion of constituted solution taken by the formula:

$$1.6(L/D)(W_S P_S/1000)(R_U/R_S)$$

in which L is the labeled quantity, in mg, of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$) in the container or in the volume of constituted solution taken; D is the concentration, in µg of cefmenoxime per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the volume of constituted solution taken, respectively, and the extent of dilution; W_S is the weight, in mg, of USP Cefmenoxime Hydrochloride RS taken to prepare the *Standard preparation*; P_S is the designated cefmenoxime ($C_{16}H_{17}N_9O_5S_3$) content, in µg per mg, of USP Cefmenoxime Hydrochloride RS; and R_U and R_S are the response ratios of the cefmenoxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cefmenoxime Hydrochloride



$(C_{16}H_{17}N_9O_5S_3)_2 \cdot HCl$ 1059.58
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino] -3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-, hydrochloride (2:1), [6*R*-[6α,7β(*Z*)]]-.
(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)-thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 7²-(*Z*)-(*O*-methyloxime), hydrochloride (2:1) [75738-58-8].

» Cefmenoxime Hydrochloride contains the equivalent of not less than 869 µg and not more than 1015 µg of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Cefmenoxime Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 25 µg per mL.
Medium: pH 6.8 buffer (prepared as directed in the *Assay*).

USP Monographs

www.webofpharma.com

B: The retention time of the cefmenoxime peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

Crystallinity ⟨695⟩: meets the requirements.

Pyrogen ⟨151⟩—Where the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements, the test dose being 1.0 mL per kg of a solution in pyrogen-free sodium carbonate solution (prepared by dissolving 14.0 g of sodium carbonate, previously heated at 170° for not less than 4 hours, in 1000 mL of sterile water for injection) containing 60 mg per mL.

Sterility Tests ⟨71⟩—Where the label states that it is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to use *Fluid A* to each 100 mL of which has been added 2.0 g of sodium carbonate previously sterilized by heating at 180° for 2 hours.

Water Determination, *Method I* ⟨921⟩: not more than 1.5%, the *Test Preparation* being prepared as directed for a hygroscopic specimen, except to use 20 mL of a mixture of formamide (previously dried over anhydrous sodium sulfate for 24 hours) and methanol (2:1), instead of methanol, to dissolve the specimen, to use two 5-mL portions of the same formamide and methanol mixture to rinse the container, and to determine the water content of the formamide and methanol mixture.

Assay—

pH 6.8 buffer—Dissolve 6.4 g of monobasic potassium phosphate and 18.9 g of dibasic sodium phosphate in 750 mL of water, adjust with 1 N sodium hydroxide to a pH of 6.8 ± 0.1, dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a suitable mixture of water, acetonitrile, and glacial acetic acid (50:10:1). Filter through a suitable filter of 0.5 µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Prepare a solution of phthalimide in methanol containing 1.5 mg per mL.

Standard preparation—Transfer about 50 mg of USP Cefmenoxime Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of *pH 6.8 buffer*, and dissolve by swirling. Dilute with *Mobile phase* to volume, and mix. Transfer 4.0 mL of this solution to a second 50-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 80 µg of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$) per mL.

Assay preparation—Transfer about 50 mg of Cefmenoxime Hydrochloride, accurately weighed, to a 50-mL volumetric flask, add 10 mL of *pH 6.8 buffer*, and dissolve by swirling. Dilute with *Mobile phase* to volume, and mix. Transfer 4.0 mL of this solution to a second 50-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*:the resolution, *R*, between the phthalimide and the cefmenoxime peaks is not less than 2.3; the column efficiency, determined from the cefmenoxime peak, is not less than 1200 theoretical plates when calculated by the formula:

$$5.545(t_r/W_{h/2})^2$$

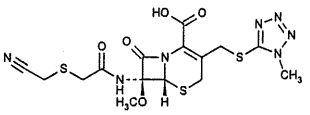
the tailing factor for the cefmenoxime peak is not more than 1.6; and the relative standard deviation of replicate injections is not more than 2.0%.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$) in each mg of the Cefmenoxime Hydrochloride taken by the formula:

$$(W_S P_S / W_U)(R_U / R_S)$$

in which W_S is the weight, in mg, of USP Cefmenoxime Hydrochloride RS taken to prepare the *Standard preparation*; P_S is the designated cefmenoxime ($C_{16}H_{17}N_9O_5S_3$) content, in µg per mg, of USP Cefmenoxime Hydrochloride RS; W_U is the weight, in mg, of Cefmenoxime Hydrochloride taken to prepare the *Assay preparation*, and R_U and R_S are the peak response ratios of the cefmenoxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cefmetazole



$C_{15}H_{17}N_7O_5S_3$ 471.53

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(cyanomethyl)thio]acetyl]amino]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-, (6*R*-*cis*)-.

(6*R*,7*S*)-7-[2-[(Cyanomethyl)thio]acetamido]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [56796-20-4].

» Cefmetazole contains not less than 970 µg and not more than 1030 µg of cefmetazole ($C_{15}H_{17}N_7O_5S_3$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Cefmetazole RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Water Determination, *Method I* ⟨921⟩: not more than 0.5%.

Assay—

Mobile phase—Dissolve 5.75 g of monobasic ammonium phosphate in 700 mL of water, add 3.2 mL of a 40% solution of tetrabutylammonium hydroxide, 280 mL of methanol, and 25 mL of tetrahydrofuran, and mix. Adjust with phosphoric

USP Monographs

www.webofpharma.com

acid to a pH of 4.5 ± 0.1, pass through a filter having a 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Cefmetazole RS in *Mobile phase* to obtain a solution having a known concentration of about 200 µg of cefmetazole ($C_{15}H_{17}N_7O_5S_3$) per mL. [NOTE—Use this solution within 10 minutes.]

Resolution solution—Prepare a solution of USP Cefmetazole RS in 0.01 N sodium hydroxide containing about 1 mg per mL. Heat at 95° for 10 minutes. To 1 mL of this solution add 2 mL of *Standard preparation*, and dilute with *Mobile phase* to obtain 20 mL of solution. This solution contains cefmetazole and cefmetazole lactone (resolution compound).

Assay preparation—Transfer about 20 mg of Cefmetazole, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. [NOTE—Use this solution within 10 minutes.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*:the resolution, *R*, between cefmetazole and cefmetazole lactone is not less than 3.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 1250 theoretical plates; the tailing factor is not less than 0.94 and not more than 1.6; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of cefmetazole ($C_{15}H_{17}N_7O_5S_3$) in each mg of Cefmetazole taken by the formula:

$$100(C/M)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of cefmetazole ($C_{15}H_{17}N_7O_5S_3$) in the *Standard preparation*; M is the quantity, in mg, of Cefmetazole taken to prepare the *Assay preparation*; and r_U and r_S are the cefmetazole peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cefmetazole Injection

» Cefmetazole Injection is a sterile isoosmotic solution of Cefmetazole and Sodium Citrate in Water for Injection. It contains one or more buffer substances and a tonicity-adjusting agent. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefmetazole ($C_{15}H_{17}N_7O_5S_3$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Maintain in the frozen state.

Labeling—It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes the conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

USP Reference standards ⟨11⟩—
USP Cefmetazole RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that observed in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.2 USP Endotoxin Unit per mg of cefmetazole.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to use water instead of *Fluid A*.

pH ⟨791⟩: between 4.2 and 6.2.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Assay—

Mobile phase, Resolution solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay*— under *Cefmetazole*.

Assay preparation—Allow the contents of a container of Injection to thaw, and mix the resultant solution. Transfer an accurately measured volume of this solution, equivalent to about 40 mg of cefmetazole, to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. [NOTE—Use this solution within 10 minutes.]

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Cefmetazole*. Calculate the quantity, in mg, of cefmetazole ($C_{15}H_{17}N_7O_5S_3$) in each mL of the Injection by the formula:

$$0.2(C/VM)(r_U/r_S)$$

in which V is the volume, in mL, of Injection taken to prepare the *Assay preparation*, and the other terms are as defined therein.

Cefmetazole for Injection

» Cefmetazole for Injection contains an amount of Cefmetazole Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefmetazole ($C_{15}H_{17}N_7O_5S_3$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.

USP Reference standards ⟨11⟩—
USP Cefmetazole RS

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.2 USP Endotoxin Unit per mg of cefmetazole.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It meets the requirements in the tests for *Identification, pH,* and *Water Determination, Method I 921:* under *Cefmetazole Sodium*. It meets also the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

www.webofpharma.com

Assay—

Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cefmetazole.*

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Cefmetazole for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Mobile phase* to obtain a solution containing about 0.2 mg of cefmetazole per mL. [NOTE—Use this solution within 10 minutes.]

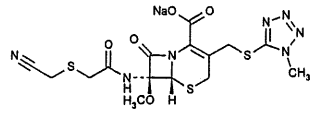
Assay preparation 2 (where the label states the quantity of cefmetazole in a given volume of constituted solution)—Constitute Cefmetazole for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Mobile phase* to obtain a solution containing about 0.2 mg of cefmetazole per mL. [NOTE—Use this solution within 10 minutes.]

Procedure—Proceed as directed in the *Assay* under *Cefmetazole.* Calculate the quantity, in mg, of cefmetazole ($C_{15}H_{17}N_7O_5S_3$) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C/1000)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of cefmetazole in the container, or in the volume of constituted solution taken; D is the concentration, in mg per mL, of cefmetazole ($C_{15}H_{17}N_7O_5S_3$) per mL, of *Assay preparation 1* or *Assay preparation 2,* based on the labeled quantity in the container or in the portion of constituted solution taken, respectively; C is the concentration, in µg per mL, of cefmetazole ($C_{15}H_{17}N_7O_5S_3$) in the *Standard preparation;* and r_U and r_S are the cefmetazole peak responses obtained from the relevant *Assay preparation* and the *Standard preparation,* respectively.

Cefmetazole Sodium



$C_{15}H_{16}N_7NaO_5S_3$ 493.52

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(cyanomethyl)thio]acetyl]amino]-7-methoxy-3- [[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-, monosodium salt, (6*R-cis*)-.

Sodium (6*R,7S*)-7-[2-[(cyanomethyl)thio]acetamido]-7-methoxy-3-[[(1-methyl-1 *H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [56796-39-5].

» Cefmetazole Sodium contains the equivalent of not less than 860 µg and not more than 1003 µg of cefmetazole ($C_{15}H_{17}N_7O_5S_3$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩—
USP Cefmetazole RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

pH ⟨791⟩: between 4.2 and 6.2, in a solution (1 in 10).

Water Determination, *Method I* ⟨921⟩: not more than 0.5%.

Other requirements—Where the label states that Cefmetazole Sodium is sterile, it meets the requirements in the tests for *Sterility Tests* ⟨71⟩ and for *Bacterial Endotoxins Test 85* — under *Cefmetazole for Injection.* Where the label states that Cefmetazole Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements in the test for *Bacterial Endotoxins Test 85* — under *Cefmetazole for Injection.*

Assay—

Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cefmetazole.*

Assay preparation—Transfer about 21 mg of Cefmetazole Sodium, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. [NOTE—Use this solution within 10 minutes.]

Procedure— Proceed as directed in the *Assay* under *Cefmetazole.* Calculate the quantity, in µg, of cefmetazole ($C_{15}H_{17}N_7O_5S_3$) per mg of Cefmetazole Sodium taken by the formula:

$$100(C/M)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of cefmetazole ($C_{15}H_{17}N_7O_5S_3$) in the *Standard preparation; M* is the quantity, in mg, of Cefmetazole Sodium taken to prepare the *Assay preparation;*and r_U and r_S are the cefmetazole peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cefonicid for Injection

» Cefonicid for Injection contains an amount of Cefonicid Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefonicid ($C_{18}H_{18}N_6O_8S_3$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*

USP Reference standards ⟨11⟩—
USP Cefonicid Sodium RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.35 USP Endotoxin Unit per mg of cefonicid.

www.webofpharma.com

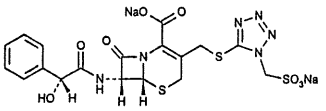
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It responds to the *Identification* tests and meets the requirements for *Specific rotation 781S:*, *pH 791:*, and *Water Determination, Method I 921:* under *Cefonicid Sodium.* It meets also the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
Mobile phase—Prepare a mixture of water, methanol, and 0.2 M monobasic ammonium phosphate (33:5:3). Pass through a filter having a 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Cefonicid Sodium RS in *Mobile phase* to obtain a solution having a known concentration of about 200 µg of cefonicid ($C_{18}H_{18}N_6O_8S_3$) per mL.
Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Cefonicid for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Mobile phase* to obtain a solution containing about 200 µg of cefonicid per mL.
Assay preparation 2 (where the label states the quantity of cefonicid in a given volume of constituted solution)—Constitute Cefonicid for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Mobile phase* to obtain a solution containing about 200 µg of cefonicid per mL.
Resolution solution—Dissolve a quantity of USP Cefonicid Sodium RS in *Mobile phase* to obtain a solution containing about 0.2 mg per mL. Heat on a steam bath for 30 minutes, and cool. This *Resolution solution* contains a mixture of cefonicid and desacetyl cefonicid.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the cefonicid peak and the desacetyl cefonicid peak is not less than 1.1; the column efficiency determined from the analyte peak is not less than 1500 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cefonicid ($C_{18}H_{18}N_6O_8S_3$) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of cefonicid in the container, or in the volume of constituted solution taken; D is the concentration, in µg per mL, of cefonicid in *Assay preparation 1* or *Assay preparation 2,* based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution; C is the concentration, in µg per mL, of cefonicid in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the relevant *Assay preparation* and the *Standard preparation,* respectively.

Cefonicid Sodium



$C_{18}H_{16}N_6Na_2O_8S_3$ 586.53
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(hydroxyphenylacetyl)amino]-8-oxo-3-[[[1-(sulfomethyl)-1*H*-tetrazol-5-yl]thio]methyl]disodium salt, [6*R*-[6α, 7β(*R**)]]-.
(6*R*,7*R*)-[7-[(*R*)-Mandelamido]-8-oxo-3-[[[1-(sulfomethyl)-1*H*-tetrazol-5-yl]thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, disodium salt [61270-78-8].

» Cefonicid Sodium contains the equivalent of not less than 832 µg and not more than 970 µg of cefonicid ($C_{18}H_{18}N_6O_8S_3$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Cefonicid Sodium RS

Identification—
A: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefonicid, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
B: It responds to the tests for *Sodium* ⟨191⟩.
Specific rotation ⟨781S⟩: between –37° and –47°.
Test solution: 10 mg per mL, in methanol.
pH ⟨791⟩: between 3.5 and 6.5, in a solution (1 in 20).
Water Determination, *Method I* ⟨921⟩: not more than 5.0%.
Other requirements—Where the label states that Cefonicid Sodium is sterile, it meets the requirements for *Sterility Tests 71* — and *Bacterial Endotoxins Test 85* — under *Cefonicid for Injection.* Where the label states that Cefonicid Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial Endotoxins Test 85* — under *Cefonicid for Injection.*

Assay—
Mobile phase—Prepare a mixture of water, methanol, and 0.2 M monobasic ammonium phosphate (33:5:2). Pass through a filter having a 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Cefonicid Sodium RS in *Mobile phase* to obtain a solution having a known concentration of about 200 µg of cefonicid ($C_{18}H_{18}N_6O_8S_3$) per mL.
Assay preparation—Transfer about 40 mg of Cefonicid Sodium, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

USP Monographs

www.webofpharma.com

Resolution solution—Dissolve a quantity of USP Cefonicid Sodium RS in *Mobile phase* to obtain a solution containing about 0.2 mg per mL. Heat on a steam bath for 30 minutes, and cool. This *Resolution solution* contains a mixture of cefonicid and desacetyl cefonicid.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution *R*, between the cefonicid and the desacetyl cefonicid peaks is not less that 1.1; the column efficiency determined from the analyte peak is not less than 1500 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of cefonicid ($C_{18}H_{18}N_6O_8S_3$) per mg of the Cefonicid Sodium taken by the formula:

$$200(C/M)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL of cefonicid ($C_{18}H_{18}N_6O_8S_3$) in the *Standard preparation*; *M* is the quantity, in mg, of Cefonicid Sodium taken to prepare the *Assay preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cefoperazone Injection

» Cefoperazone Injection is a sterile solution of Cefoperazone Sodium and a suitable osmolality adjusting substance in Water for Injection. It may contain a suitable buffer. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefoperazone ($C_{25}H_{27}N_9O_8S_2$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Maintain in the frozen state.
Labeling—It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
USP Reference standards ⟨11⟩—
USP Cefoperazone Dihydrate RS
Identification—The retention time of the major peak for cefoperazone in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.2 USP Endotoxin Unit per mg of cefoperazone.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
pH ⟨791⟩: between 4.5 and 6.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Assay—
Mobile phase, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Cefoperazone Sodium*.

Assay preparation—Quantitatively dilute an accurately measured volume of Injection with *Mobile phase* to obtain a solution containing about 0.16 mg of cefoperazone per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Cefoperazone Sodium*. Calculate the quantity, in mg, of cefoperazone ($C_{25}H_{27}N_9O_8S_2$), in the volume of Injection taken by the formula:

$$(L/D)(C)(r_U/r_S)$$

in which *L* is the labeled quantity, in mg, of cefoperazone ($C_{25}H_{27}N_9O_8S_2$), in the volume of Injection taken; *D* is the concentration, in mg of cefoperazone ($C_{25}H_{27}N_9O_8S_2$) per mL, of the *Assay preparation*, based on the labeled quantity in the portion of Injection taken and the extent of dilution; and the other terms are as defined therein.

Cefoperazone for Injection

» Cefoperazone for Injection contains an amount of Cefoperazone Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefoperazone ($C_{25}H_{27}N_9O_8S_2$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.
USP Reference standards ⟨11⟩—
USP Cefoperazone Dihydrate RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.20 USP Endotoxin Unit per mg of cefoperazone.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
pH ⟨791⟩: between 4.5 and 6.5, in a solution (1 in 4).
Water Determination, Method I ⟨921⟩: not more than 5.0%, except that where it is in the freeze-dried form, the limit is not more than 2.0%.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements for the *Identification*— tests under *Cefoperazone Sodium* and meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—
Mobile phase, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Cefoperazone Sodium*.

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Cefoperazone for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic

USP Monographs

www.webofpharma.com

needle and syringe, and quantitatively dilute with *Mobile phase* to obtain a solution containing about 0.16 mg of cefoperazone per mL.

Assay preparation 2 (where the label states the quantity of cefoperazone in a given volume of constituted solution)—Constitute Cefoperazone for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Mobile phase* to obtain a solution containing about 0.16 mg of cefoperazone per mL.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg of cefoperazone per mg, of the Cefoperazone for Injection taken by the formula:

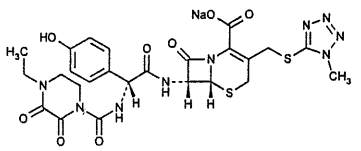
$$1000(C/M)(r_U/r_S)$$

in which C is the concentration, in mg of cefoperazone ($C_{25}H_{27}N_9O_8S_2$) per mL, of the *Standard preparation; M* is the concentration, in mg per mL, of the *Assay preparation* based on the weight of Cefoperazone for Injection taken and the extent of dilution; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of cefoperazone ($C_{25}H_{27}N_9O_8S_2$), withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of cefoperazone ($C_{25}H_{27}N_9O_8S_2$), in the container, or in the volume of constituted solution taken; and D is the concentration, in mg of cefoperazone ($C_{25}H_{27}N_9O_8S_2$) per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution; and the other terms are as defined therein.

Cefoperazone Sodium



$C_{25}H_{26}N_9NaO_8S_2$ 667.65

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino](4-hydroxyphenyl)acetyl]amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-, monosodium salt, [6*R*-[6α,7β(*R**)]]-.

Sodium (6*R*,7*R*)-7-[(*R*)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-(*p*-hydroxyphenyl)acetamido-3-[[(1-methyl-*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [62893-20-3].

» Cefoperazone Sodium contains the equivalent of not less than 870 µg and not more than 1015 µg of cefoperazone ($C_{25}H_{27}N_9O_8S_2$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩—
USP Cefoperazone Dihydrate RS

Identification—

A: The retention time of the major peak for cefoperazone in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

B: It responds to the tests for *Sodium* ⟨191⟩.

Crystallinity ⟨695⟩: meets the requirements.

pH ⟨791⟩: between 4.5 and 6.5, in a solution (1 in 4).

Water Determination, *Method I* ⟨921⟩: not more than 5.0%.

Other requirements—Where the label states that Cefoperazone Sodium is sterile, it meets the requirements for *Sterility Tests 71* — and *Bacterial Endotoxins Test 85* — under *Cefoperazone for Injection.* Where the label states that Cefoperazone Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial Endotoxins Test 85* — under *Cefoperazone for Injection.*

Assay—

Mobile phase—Place 14 mL of triethylamine and 5.7 mL of glacial acetic acid in a 100-mL volumetric flask, dilute with water to volume, and mix. Prepare a suitable mixture of water, acetonitrile, 1 N acetic acid, and this solution (876: 120: 2.8: 1.2). Filter through a membrane filter (1-µm or finer porosity), and degas.

Standard preparation—Dissolve a suitable quantity of USP Cefoperazone Dihydrate RS, accurately weighed, in *Mobile phase* to obtain a solution having a known concentration of about 0.16 mg of cefoperazone ($C_{25}H_{27}N_9O_8S_2$) per mL.

Assay preparation—Using a suitable quantity of Cefoperazone Sodium, accurately weighed, proceed as directed under *Standard preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed under *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%, and the tailing factor is not more than 1.5.

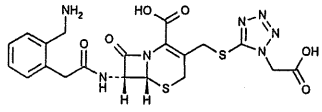
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg of cefoperazone per mg, of the Cefoperazone Sodium taken by the formula:

$$1000(C/M)(r_U/r_S)$$

in which C is the concentration, in mg of cefoperazone ($C_{25}H_{27}N_9O_8S_2$) per mL, of the *Standard preparation; M* is the concentration, in mg per mL, of the *Assay preparation* based on the weight of Cefoperazone Sodium taken and the extent of dilution; and r_U and r_S are the peak responses from the *Assay preparation* and the *Standard preparation,* respectively.

www.webofpharma.com

Ceforanide



$C_{20}H_{21}N_7O_6S_2$ 519.55

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[2-(aminomethyl)phenyl]acetyl]amino]-3-[[[1-(carboxymethyl)1*H*-tetrazol-5-yl]thio]methyl]-8-oxo-, (6*R-trans*)-.
(6*R,7R*)-7-[2-(α-Amino-*o*-tolyl)acetamido]-3-[[[1-(carboxymethyl)- 1*H*-tetrazol-5-yl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
7-[*o*-(Aminomethyl)phenylacetamido]-3-[[[1-(carboxymethyl)-1*H*-tetrazol-5-yl]thio]methyl]-3-cephem-4-carboxylic acid [60925-61-3].

» Ceforanide contains not less than 900 µg and not more than 1050 µg of ceforanide ($C_{20}H_{21}N_7O_6S_2$) per mg.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Ceforanide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The retention time of the major peak for ceforanide in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Bacterial Endotoxins Test ⟨85⟩ —Where the label states that Ceforanide is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains not more than 0.25 USP Endotoxin Unit per mg of ceforanide.

Sterility Tests ⟨71⟩ —Where the label states that Ceforanide is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined,* except to dissolve 6 g of Ceforanide in *Fluid A* to each 1000 mL of which has been added 10 g of sterile L-lysine, and to rinse the membrane with three 100-mL portions of *Fluid D* and one 100-mL portion of *Fluid A.*

pH ⟨791⟩: between 2.5 and 4.5, in a suspension containing 50 mg per mL.

Water Determination, *Method I* ⟨921⟩: not more than 5.0%.

Assay—

Mobile phase—Mix 18 mL of tetrabutylammonium hydroxide solution (1 in 10) and 8.6 mL of 11 N potassium hydroxide, and add the mixture to 700 mL of water. Add 200 mL of methanol, adjust with phosphoric acid to a pH of 7.0, and add water to obtain 1000 mL of solution, making adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). Filter, using a filter having a porosity of 1 µm or finer, and degas.

Standard preparation—Dissolve an accurately weighed quantity of USP Ceforanide RS in *Mobile phase* to obtain a solution having a known concentration of about 1 mg per mL. Use this solution within 5 minutes.

Assay preparation—Using a suitable quantity of Ceforanide, accurately weighed, proceed as directed under *Standard preparation.* Use this solution within 5 minutes.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains 5- to 10-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed under *Procedure:*the column efficiency determined from the analyte peak is not less than 1900 theoretical plates; the tailing factor for the analyte peak is not more than 1.2; the capacity factor, *k′,* is not less than 1.8 and not more than 5.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of $C_{20}H_{21}N_7O_6S_2$ in each mg of the Ceforanide taken by the formula:

$$(CP/M)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ceforanide RS in the *Standard preparation; P* is the potency, in µg per mg, of the USP Ceforanide RS; *M* is the concentration, in mg per mL, of the *Assay preparation,* based on the amount of Ceforanide taken and the extent of dilution; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ceforanide for Injection

» Ceforanide for Injection is a sterile mixture of Ceforanide and L-lysine. It contains not less than 900 µg and not more than 1050 µg of ceforanide ($C_{20}H_{21}N_7O_6S_2$) per mg on the L-lysine-free basis, and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{20}H_{21}N_7O_6S_2$.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Ceforanide RS

Identification—

A: The retention time of the major peak for L-lysine in the chromatogram of the *Test preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the test for *Content of L-lysine.*

B: The retention time of the major peak in ceforanide in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation;* as obtained in the *Assay.*

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.25 USP Endotoxin Unit per mg of ceforanide.

Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined,* except to constitute each container with 3 mL of *Fluid A* for each g of ceforanide contained therein, and to rinse the membrane with three 100-mL portions of *Fluid D* and one 100-mL portion of *Fluid A.*

Supplement 1 Official Monographs

www.webofpharma.com

pH ⟨791⟩: between 5.5 and 8.5, when constituted as directed in the labeling.
Water Determination, *Method I* ⟨921⟩: not more than 3.0%.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Content of L-lysine—

Mobile phase—Mix 62 volumes of methanol and 38 volumes of water, and adjust with glacial acetic acid to a pH of 3.0, making adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Stock standard solution—Transfer about 36 mg of L-lysine, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard preparation—Transfer 2.0 mL of *Stock standard solution* to a glass-stoppered, 10-mL volumetric flask, add 2.0 mL of a 1.4% solution of tris(hydroxymethyl)aminomethane and 3.0 mL of a 1.5% solution of 1-fluoro-2,4-dinitrobenzene in dehydrated alcohol, insert the stopper tightly, and mix. Heat at 50° in a water bath for 30 minutes. Remove the flask from the water bath, allow to cool, dilute with methanol to volume, and mix.

Test preparation—Transfer about 150 mg of Ceforanide for Injection, accurately weighed, to a 100-mL volumetric flask, add water to volume, and mix. Transfer 2.0 mL of the resulting solution to a glass-stoppered, 10-mL volumetric flask, and proceed as directed under *Standard preparation*, beginning with "add 2.0 mL of a 1.4% solution of tris(hydroxymethyl) aminomethane."

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5- to 10-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*:the column efficiency determined from the derivatized L-lysine peak is not less than 1500 theoretical plates; the tailing factor for the same peak is not more than 1.3, the resolution; *R*, between the derivatized L-lysine peak and the 1-fluoro-2,4-dinitrobenzene peak is not less than 4.5; the capacity factor, *k'*, is not less than 4 and not more than 6; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of L-lysine in the Ceforanide for Injection taken by the formula:

$$10(C/M)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of L-lysine in the *Stock standard solution*; *M* is the quantity, in mg, of Ceforanide for Injection taken; and r_U and r_S are the peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively. Use this percentage to calculate, on an L-lysine-free basis, the result from *Assay preparation 1* obtained as directed in the *Assay*.

Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—

Mobile phase , *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Ceforanide*.

Assay preparation 1—Dissolve a suitable quantity of Ceforanide for Injection, accurately weighed, in *Mobile phase*, and dilute quantitatively and stepwise with *Mobile phase* to obtain a solution having a concentration of about 1 mg of ceforanide per mL. Use this solution within 5 minutes.

Assay preparation 2 (where it is represented as being in a single-dose container)—Constitute Ceforanide for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively and stepwise with *Mobile phase* to obtain a solution containing about 1 mg of ceforanide per mL. Use this solution within 5 minutes.

Assay preparation 3 (where the label states the quantity of ceforanide in a given volume of constituted solution)—Constitute Ceforanide for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively and stepwise with *Mobile phase* to obtain a solution containing about 1 mg of ceforanide per mL. Use this solution within 5 minutes.

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Ceforanide*. Calculate the quantity, in µg, of ceforanide ($C_{20}H_{21}N_7O_6S_2$) in each mg of the Ceforanide for Injection taken by the formula:

$$(CP/M)(r_U/r_S)$$

in which *M* is the concentration, in mg per mL, of *Assay preparation 1* based on the weight of Ceforanide for Injection taken and the extent of dilution, and the other terms are as defined therein. Calculate the quantity, in mg, of $C_{20}H_{21}N_7O_6S_2$ withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(CP/1000)(r_U/r_S)$$

in which *L* is the labeled quantity, in mg, of ceforanide in the container, or in the volume of constituted solution taken; *D* is the concentration, in mg of ceforanide per mL, of *Assay preparation 2* or *Assay preparation 3*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively; and the extent of dilution, and the other terms are as defined therein.

Cefotaxime Injection

DEFINITION

Cefotaxime Injection is a sterile solution of Cefotaxime Sodium in Water for Injection. It contains one or more suitable buffers, and it may contain Dextrose or Sodium Chloride as a tonicity-adjusting agent. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of cefotaxime ($C_{16}H_{17}N_5O_7S_2$).

IDENTIFICATION

- **A.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 7.1 g/L of anhydrous dibasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 6.25

Solution A: Methanol and *Buffer* (14:86). Pass through a filter having a pore size of 0.5 µm or less, and degas before use.

Solution B: Methanol and *Buffer* (40:60). Pass through a filter having a pore size of 0.5 µm or less, and degas before use.

Mobile phase: See *Table 1*.

USP Monographs

www.webofpharma.com

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7	100	0
9	80	20
16	80	20
46	0	100
51	0	100
56	100	0

Standard solution: 0.8 mg/mL of USP Cefotaxime Sodium RS in *Solution A*. Use this solution promptly. It may be used within 24 h if stored in a refrigerator.
System suitability solution: Mix 1 mL of *Standard solution*, 7.0 mL of water, and 2.0 mL of methanol. Add 25 mg of sodium carbonate, mix, and allow to stand at room temperature for 10 min, with occasional swirling. Add 3 drops of glacial acetic acid and 1 mL of *Standard solution*.
Sensitivity solution: 1.6 µg/mL of USP Cefotaxime Sodium RS in *Solution A*
Sample solution: Nominally 0.8 mg/mL of cefotaxime,prepared as follows. Allow one container of Injection to thaw, and mix. Transfer an aliquot of the Injection to a suitable volumetric flask, and dilute with *Solution A* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution, System suitability solution*, and *Sensitivity solution*. [NOTE—The retention times for desacetylcefotaxime and cefotaxime in the *System suitability solution* are 3.5 min and 14 min, respectively. The retention time for cefotaxime in the *Standard solution* is 12–15 min.]
Suitability requirements
Sensitivity: The response of the cefotaxime peak from the *Sensitivity solution* is between 0.18% and 0.22% of the response of the cefotaxime peak of the *Standard solution*.
Resolution: NLT 20 between desacetylcefotaxime and cefotaxime, *System suitability solution*
Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cefotaxime ($C_{16}H_{17}N_5O_7S_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak area of the *Sample solution*
r_S = peak area of the *Standard solution*
C_S = concentration of USP Cefotaxime Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefotaxime in the *Sample solution* (mg/mL)
P = potency of cefotaxime in USP Cefotaxime Sodium RS (µg/mg)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase, Standard solution, System suitability solution, Sensitivity solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = r_U/(r_T + r_C) \times 100$$

r_U = peak area of each individual impurity
r_T = sum of all of the impurity peak areas
r_C = peak area of cefotaxime

Acceptance criteria
Disregard any impurity peak that is less than 0.1%.
Individual impurities: NMT 6.0%
Total impurities: NMT 10.0%

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.20 USP Endotoxin Unit/mg of cefotaxime
• **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
• **pH** ⟨791⟩: 5.0–7.5
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements for small-volume injections.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose containers, as described in *Packaging and Storage Requirements* ⟨659⟩. Maintain in the frozen state.
• **LABELING:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just before use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cefotaxime Sodium RS

Cefotaxime for Injection

DEFINITION
Cefotaxime for Injection contains an amount of Cefotaxime Sodium equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of cefotaxime ($C_{16}H_{17}N_5O_7S_2$).

IDENTIFICATION
Where the label indicates that there are no added substances:

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B. Identification Tests—General,** *Sodium* ⟨191⟩: Meets the requirements
Where the label indicates that there are added substances:
• **C.** The retention time of the major peak of the *Sample solution 1, 2, 3,* or *4* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: 7.1 g/L of anhydrous dibasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 6.25

Solution A: Methanol and *Buffer* (14:86). Pass through a filter having a pore size of 0.5 µm or finer, and degas before use.

Solution B: Methanol and *Buffer* (40:60). Pass through a filter having a pore size of 0.5 µm or finer, and degas before use.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7	100	0
9	80	20
16	80	20
46	0	100
51	0	100
56	100	0

Standard solution: 0.8 mg/mL of USP Cefotaxime Sodium RS in *Solution A*. Use this solution promptly. It may be used within 24 h if stored in a refrigerator.

System suitability solution: Mix 1 mL of *Standard solution*, 7.0 mL of water, and 2.0 mL of methanol. Add 25 mg of sodium carbonate, mix, and allow to stand at room temperature for 10 min, with occasional swirling. Add 3 drops of glacial acetic acid and 1 mL of *Standard solution*.

Sensitivity solution: 1.6 µg/mL of USP Cefotaxime Sodium RS in *Solution A*

Sample solution 1 (for use where the *Weight Variation* test is to be performed): 0.8 mg/mL of Cefotaxime for Injection in *Solution A*. Use this solution promptly. It may be used within 24 h if stored in a refrigerator.

Sample solution 2 (for use in assaying vials and infusion bottles packaged for dispensing): Nominally 0.8 mg/mL of cefotaxime, prepared as follows. Constitute one container of Cefotaxime for Injection with the smallest volume of diluent specified in the labeling. Invert the container, and withdraw all of the withdrawable contents of the container with a hypodermic needle and syringe. Transfer the contents of the syringe to a 100-mL volumetric flask, dilute with *Solution A* to volume, and mix. Do not rinse the syringe or container. Dilute a suitable aliquot of this solution with *Solution A*. Use this solution promptly. It may be used within 24 h if stored in a refrigerator.

Sample solution 3 (for use in assaying piggyback infusion bottles): Nominally 0.8 mg/mL of cefotaxime, prepared as follows. Constitute one container of Cefotaxime for Injection with the smallest volume of diluent recommended in the labeling, using the directions specified in the labeling. Invert the container, and withdraw all of the withdrawable contents of the container with a hypodermic needle and syringe. Transfer the contents of the syringe to a 100-mL volumetric flask, dilute with *Solution A* to volume, and mix. Do not rinse the syringe or container. Dilute a suitable aliquot of this solution with *Solution A*. Use this solution promptly. It may be used within 24 h if stored in a refrigerator.

Sample stock solution 4 (for use in assaying pharmacy bulk packages where the label states the quantity of cefotaxime in a given volume of constituted solution): Nominally 10 mg/mL of cefotaxime, prepared as follows. Constitute one container of Cefotaxime for Injection with the volume of diluent specified in the labeling. With a hypodermic needle and syringe, withdraw a suitable aliquot of the reconstituted product, transfer to a suitable volumetric flask, dilute with *Solution A* to volume, and mix. Do not rinse the syringe or container.

Sample solution 4: Nominally 0.8 mg/mL of cefotaxime from *Sample stock solution 4* in *Solution A*. Use this solution promptly. It may be used within 24 h if stored in a refrigerator.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution, System suitability solution*, and *Sensitivity solution*. [NOTE—The retention times for desacetylcefotaxime and cefotaxime in the *System suitability solution* are 3.5 min and 14 min, respectively. The retention time for cefotaxime in the *Standard solution* is 12–15 min.]

Suitability requirements

Sensitivity: The response of the cefotaxime peak from the *Sensitivity solution* is between 0.18% and 0.22% of the response of the cefotaxime peak of the *Standard solution*.

Resolution: NLT 20 between desacetylcefotaxime and cefotaxime, *System suitability solution*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Sample solution 1*, or *Sample solution 2*, or *Sample solution 3*, or *Sample solution 4*, and *Standard solution*

Calculate the percentage of the labeled amount of cefotaxime ($C_{16}H_{17}N_5O_7S_2$) withdrawn from the container, or in the portion of Cefotaxime for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak area of the *Sample solution*

r_S = peak area of the *Standard solution*

C_S = concentration of USP Cefotaxime Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cefotaxime in *Sample solution 1*, *2*, *3*, or *4* (mg/mL)

P = potency of cefotaxime in USP Cefotaxime Sodium RS (µg/mg)

Acceptance criteria: 90.0%–115.0%. Where the test for *Uniformity of Dosage Units* has been peformed using the *Procedure for Content Uniformity*, use the average of these determinations as the *Assay* value.

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase; Standard solution; System suitability solution; Sample solutions 1, 2, 3 or 4; Chromatographic system; and System suitability: Proceed as directed in the *Assay*.

USP Monographs

www.webofpharma.com

Analysis
Calculate the percentage of each impurity in the portion of Cefotaxime for Injection taken:

$$\text{Result} = r_U/(r_T + r_C) \times 100$$

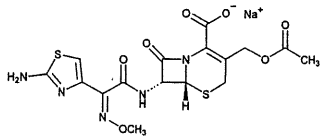
r_U = peak area of each individual impurity
r_T = sum of all of the impurity peak areas
r_C = peak area of cefotaxime

Acceptance criteria
Disregard any impurity peak that is less than 0.1%.
Individual impurities: NMT 6.0%
Total impurities: NMT 10.0%

SPECIFIC TESTS
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.20 USP Endotoxin Unit/mg of cefotaxime
- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** It meets the requirements for small-volume injections.
- **pH** ⟨791⟩
  Sample solution: 100-mg/mL solution
  Acceptance criteria: 4.5–6.5
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 100°–105° for 3 h.
  Acceptance criteria: NMT 3.0%
- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cefotaxime Sodium RS

Cefotaxime Sodium



$C_{16}H_{16}N_5NaO_7S_2$ 477.45

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyloxy)methyl]-7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-, monosodium salt, [6*R*-[6α,7β (*Z*)]]-;
Sodium (6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 7²-(*Z*)-(*O*-methyloxime), acetate (ester) [64485-93-4].

DEFINITION
Cefotaxime Sodium contains the equivalent of NLT 916 µg/mg and NMT 964 µg/mg of cefotaxime ($C_{16}H_{17}N_5O_7S_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*
- **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩**:** It meets the requirements.

ASSAY
- **PROCEDURE**
  Buffer: 7.1 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 6.25.
  Solution A: Methanol and *Buffer* (14:86)
  Solution B: Methanol and *Buffer* (40:60)
  Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7	100	0
9	80	20
16	80	20
45	0	100
50	0	100
55	100	0
65	100	0

System suitability stock solution: 0.08 mg/mL each of USP Cefetamet RS and USP Cefotaxime Related Compound E RS prepared as follows. Dissolve USP Cefetamet RS and USP Cefotaxime Related Compound E RS in the minimum volume of methanol. Sonicate, if necessary, and dilute with *Solution A* to volume.
System suitability solution: 8 µg/mL each of cefetamet and cefotaxime related compound E from *System suitability stock solution* in *Solution A.* Store refrigerated, and use within 24 h.
Standard solution: 0.8 mg/mL of USP Cefotaxime Sodium RS in *Solution A.* Store refrigerated, and use within 24 h.
Sensitivity solution: 1.6 µg/mL of USP Cefotaxime Sodium RS from the *Standard solution* in *Solution A.* Store refrigerated, and use within 24 h.
Sample solution: 0.8 mg/mL of Cefotaxime Sodium in *Solution A.* Store refrigerated, and use within 24 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 235 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Temperatures
Column: 30°
Autosampler: 4°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution, Standard solution,* and *Sensitivity solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between cefetamet and cefotaxime related compound E, *System suitability solution*

USP Monographs

www.webofpharma.com

Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Sensitivity: Calculate the peak response ratio:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak response from the *Sensitivity solution*
r_S = peak response from the *Standard solution*

Acceptance criteria: 0.18%–0.22%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of cefotaxime ($C_{16}H_{17}N_5O_7S_2$) in the portion of Cefotaxime Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cefotaxime Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefotaxime Sodium in the *Sample solution* (mg/mL)
P = potency of cefotaxime in USP Cefotaxime Sodium RS (µg/mg)

Acceptance criteria: 916–964 µg/mg on the dried basis

IMPURITIES

• ORGANIC IMPURITIES, PROCEDURE 1

Use *Organic Impurities, Procedure 1*, when the impurity profile includes *N*-formyl cefotaxime and cefotaxime dioxime.
Buffer, Solution A, Solution B, Mobile phase, System suitability solution, Sensitivity solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Cefotaxime Sodium taken:

$$\text{Result} = [r_U/(r_T + r_C)] \times 100$$

r_U = peak response of each individual impurity
r_T = sum of all of the impurity peak responses
r_C = peak response of cefotaxime

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deacetylcefotaxime[a]	0.26	1.0
Cefetamet[b]	0.52	1.0
Cefotaxime related compound E[c]	0.62	1.0
Cefotaxime	1.0	—
N-Formyl cefotaxime[d]	1.8	1.0
E-Cefotaxime[e]	2.2	1.0
Cefotaxime dimer[f]	2.3	1.0
Cefotaxime dioxime[g]	3.0	0.2
Any individual unspecified impurity	—	0.2
Total impurities	—	3.0

[a] (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[b] Deacetoxycefotaxime; (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[c] Deacetylcefotaxime lactone; (*Z*)-2-(2-Aminothiazol-4-yl)-*N*-[(5a*R*,6*R*)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl]-2-(methoxyimino)acetamide.
[d] (6*R*,7*R*)-3-(Acetoxymethyl)-7-[(*Z*)-2-(2-formamidothiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[e] (6*R*,7*R*)-3-(Acetoxymethyl)-7-[(*E*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino) acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[f] (6*R*,7*R*)-3-[(4-{(*Z*)-2-[(6*R*,7*R*)-3-(Acetoxymethyl)-2-carboxy-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylamino]-1-(methoxyimino)-2-oxoethyl}thiazol-2-ylamino)methyl]-7-[(*E*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[g] (6*R*,7*R*)-3-(Acetoxymethyl)-7-[(*Z*)-2-{2-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]thiazol-4-yl}-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

• ORGANIC IMPURITIES, PROCEDURE 2

Use *Organic Impurities, Procedure 2*, when the impurity profile includes thiazolylglyoxalic methyloxime, 7-aminocephalosporanic acid, cefotaxime open ring lactone, and bromoacetyl analog.
Buffer: 3.6 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 6.2.
Solution A: Acetonitrile and *Buffer* (2:98)
Solution B: Acetonitrile and *Buffer* (60:40)
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
25	80	20
40	60	40
55	0	100
60	0	100
65	100	0
75	100	0

Diluent: 4.6 g/L of anhydrous dibasic sodium phosphate and 3.5 g/L of monobasic potassium phosphate in water
System suitability stock solution: 0.1 mg/mL of USP Cefotaxime Related Compound E RS prepared as follows. Dissolve in acetonitrile and *Diluent*, using 20% and 40% respectively of the final volume, sonicate as needed to dissolve, and dilute with *Diluent* to volume.
System suitability solution: 10 µg/mL of cefotaxime related compound E from *System suitability stock solution* and 1 mg/mL of USP Cefotaxime Sodium RS in *Diluent*. Store refrigerated, and use within 2 h.
Standard solution: 10 µg/mL of USP Cefotaxime Sodium RS in *Diluent*. Store refrigerated, and use within 2 h.
Sample solution: 1 mg/mL of Cefotaxime Sodium in *Diluent*. Store refrigerated, and use within 2 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

www.webofpharma.com

Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
Autosampler temperature: 4°

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between cefotaxime and cefotaxime related compound E, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Cefotaxime Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of cefotaxime from the *Standard solution*
C_S = concentration of USP Cefotaxime Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefotaxime Sodium in the *Sample solution* (mg/mL)
P = potency of cefotaxime in USP Cefotaxime Sodium RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 4*. The reporting threshold is 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Thiazolylglyoxalic methyloxime[a]	0.13	0.15
7-Aminocephalosporanic acid[b]	0.41	0.15
Deacetylcefotaxime[c]	0.57	1.0
	0.60	0.15
Cefotaxime open ring lactone[d]	0.71	0.15
Cefetamet[e]	0.74	1.0
Cefotaxime	1.0	—
Cefotaxime related compound E[f]	1.08	1.0
Cefotaxime dimer[g]	1.26	1.0
E-Cefotaxime[h]	1.34	1.0
Bromoacetyl analog[i]	1.48	0.15
Any individual unspecified impurity	—	0.2
Total impurities	—	3.0

[a] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.
[b] (6*R*,7*R*)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[c] (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[d] (2*R*)-(*Z*)-2-[2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-(7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl)acetic acid. This system resolves two diasteroisomers.
[e] Deacetoxycefotaxime; (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[f] Deacetylcefotaxime lactone; (*Z*)-2-(2-Aminothiazol-4-yl)-*N*-[(5a*R*,6*R*)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl]-2-(methoxyimino)acetamide.
[g] (6*R*,7*R*)-3-[(4-{(*Z*)-2-[(6*R*,7*R*)-3-(Acetoxymethyl)-2-carboxy-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylamino]-1-(methoxyimino)-2-oxoethyl}thiazol-2-ylamino)methyl]-7-[(*E*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[h] (6*R*,7*R*)-3-(Acetoxymethyl)-7-[(*E*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[i] (6*R*,7*R*)-3-(Acetoxymethyl)-7-[(*Z*)-4-bromo-2-(methoxyimino)-3-oxobutanamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

SPECIFIC TESTS

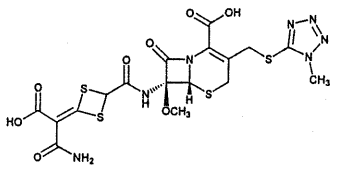
- **Optical Rotation,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL in water
  Acceptance criteria: +58° to +64°
- **pH** ⟨791⟩
  Sample solution: 100 mg/mL in water
  Acceptance criteria: 4.5–6.5
- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 100°–105° for 3 h.
  Acceptance criteria: NMT 3.0%
- **Sterility Tests** ⟨71⟩**:** Where the label states that Cefotaxime Sodium is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **Bacterial Endotoxins Test** ⟨85⟩**:** Where the label states that Cefotaxime Sodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.20 Endotoxin Units/mg of cefotaxime.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP Reference Standards** ⟨11⟩
  USP Cefetamet RS
  Deacetoxycefotaxime; (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
  $C_{14}H_{15}N_5O_5S_2$ 397.43
  USP Cefotaxime Related Compound E RS
  Deacetylcefotaxime lactone; (*Z*)-2-(2-Aminothiazol-4-yl)-*N*-[(5a*R*,6*R*)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl]-2-(methoxyimino)acetamide.
  $C_{14}H_{13}N_5O_5S_2$ 395.41
  USP Cefotaxime Sodium RS

www.webofpharma.com

Cefotetan



$C_{17}H_{17}N_7O_8S_4$ 575.62

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7- [[[4-(2-amino-1-carboxy-2-oxoethylidene)-1,3-dithietan-2-yl] carbonyl]amino]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)-thio]methyl]-8-oxo-, [6R-(6α,7α)]-;
(6*R*,7*S*)-4-[[2-Carboxy-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl]carbamoyl]-1,3-dithietane-$\Delta^{2,\alpha}$-malonamic acid;
(6*R*,7*S*)-7-[4-(Carbamoylcarboxymethylene)-1,3-dithietane-2-carboxamido]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl) thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [69712-56-7].

DEFINITION

Cefotetan contains NLT 950 µg/mg and NMT 1030 µg/mg of cefotetan ($C_{17}H_{17}N_7O_8S_4$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Protect the *Standard solution*, the *System suitability solution*, and the *Sample solution* from light, and use within 2 h.

Mobile phase: Methanol, acetonitrile, glacial acetic acid, and 0.1 M phosphoric acid (105:105:100:1700)

Standard solution: 0.2 mg/mL of USP Cefotetan RS in methanol, acetonitrile, and water (5:5:90), prepared as follows. Place a suitable quantity of USP Cefotetan RS in a suitable volumetric flask, and add methanol, using 5% of the final volume. Swirl for several min, and add acetonitrile, using 5% of the final volume. Swirl until dissolved, and dilute with water to volume.

System suitability solution: Transfer 10 mL of *Standard solution* to a glass-stoppered flask containing a few mg of magnesium carbonate. Sonicate for 10 min. If the solution is not turbid, add a few more mg of magnesium carbonate, and repeat the sonication. Pass the turbid solution through a filter of 0.5-µm or finer pore size. Use the clear filtrate.

Sample solution: 0.2 mg/mL of Cefotetan in methanol, acetonitrile, and water (5:5:90), prepared as follows. Place a suitable quantity of Cefotetan in a suitable volumetric flask, and add methanol, using 5% of the final volume. Swirl for several min, and add acetonitrile, using 5% of the final volume. Swirl until dissolved, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for cefotetan and cefotetan tautomer are 0.75 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between cefotetan and cefotetan tautomer, *System suitability solution*

Column efficiency: NLT 1500 theoretical plates, *Standard solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of cefotetan ($C_{17}H_{17}N_7O_8S_4$) in the portion of Cefotetan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cefotetan RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefotetan in the *Sample solution* (mg/mL)
P = potency of cefotetan in USP Cefotetan RS (µg/mg)

Acceptance criteria: 950–1030 µg/mg on the anhydrous basis

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩**:** Where the label states that Cefotetan is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*, except use *Fluid A* to each 1000 mL of which has been added 10 g of sodium bicarbonate before sterilization.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Cefotetan is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.17 USP Endotoxin Unit/mg of cefotetan.
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Cefotetan RS

Cefotetan Injection

DEFINITION

Cefotetan Injection is a sterile isoosmotic solution of Cefotetan and Sodium Bicarbonate in Water for Injection. It contains one or more buffer substances and a tonicity-adjusting agent. It contains NLT 90.0% and NMT 120.0% of the labeled amount of cefotetan ($C_{17}H_{17}N_7O_8S_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Protect the *Standard solution*, the *System suitability solution*, and the *Sample solution* from light. Use the *Standard solution* and the *System suitability solution* within 2 h. Use the *Sample solution* within 10 min.

Mobile phase: Methanol, acetonitrile, glacial acetic acid, and 0.1 M phosphoric acid (105:105:100:1700)

Standard solution: 0.2 mg/mL of USP Cefotetan RS in methanol, acetonitrile, and water (5:5:90), prepared as follows. Place a suitable quantity of USP Cefotetan RS in a suitable volumetric flask, and add methanol, using 5% of the final volume. Swirl for several min, and add acetonitrile, using 5% of the final volume. Swirl until dissolved, and dilute with water to volume.

System suitability solution: Transfer 10 mL of *Standard solution* to a glass-stoppered flask containing a few mg of magnesium carbonate. Sonicate for 10 min. If the solution is not turbid, add a few more mg of magnesium carbonate, and repeat the sonication. Pass the turbid solution through a filter of 0.5-µm or finer pore size. Use the clear filtrate.

Sample solution: 0.2 mg/mL of cefotetan in methanol, acetonitrile, and water (5:5:90) from Injection, prepared as follows. Allow the contents of a container of Injection to thaw, and mix the resultant solution. Transfer a suitable aliquot of this solution to a suitable volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cefotetan and cefotetan tautomer are 0.75 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between cefotetan and cefotetan tautomer, *System suitability solution*

Column efficiency: NLT 1500 theoretical plates, *Standard solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefotetan ($C_{17}H_{17}N_7O_8S_4$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Cefotetan RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cefotetan in the *Sample solution* (mg/mL)

P = potency of cefotetan in USP Cefotetan RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.17 USP Endotoxin Unit/mg of cefotetan

• **STERILITY TESTS** ⟨71⟩: Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **pH** ⟨791⟩: 4.0–6.5

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Maintain in the frozen state.

• **LABELING:** Meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just before use, describes the conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cefotetan RS

Cefotetan for Injection

DEFINITION

Cefotetan for Injection contains an amount of Cefotetan Disodium equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of cefotetan ($C_{17}H_{17}N_7O_8S_4$).

IDENTIFICATION

• **A.** The retention time of the major peak of the appropriate *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩: Meets the requirements

ASSAY

• **PROCEDURE**

[NOTE—Protect the *Standard solution*, the *System suitability solution*, *Sample solution A*, and *Sample solution B* from light, and use within 2 h.]

Solution A: Acetonitrile, methanol, and water (1:1:18)

Mobile phase: Acetonitrile, methanol, glacial acetic acid, and 0.1 M phosphoric acid (105:105:100:1700)

Standard solution: 20 mg of USP Cefotetan RS in a 100-mL volumetric flask. Add 5 mL of methanol, swirl for several min, add 5 mL of acetonitrile, and swirl until dissolved. Dilute with water to volume.

System suitability solution: 10 mL of *Standard solution* in a glass-stoppered flask containing a few mg of magnesium carbonate. Sonicate for 10 min. If the solution is not turbid, add a few more mg of magnesium carbonate, and repeat the sonication. Filter the turbid solution through a filter of 0.5-µm or finer pore size. Use the clear filtrate.

Sample solution A (where the package is represented as being in a single-dose container): Constitute Cefotetan for Injection as directed in the labeling. Withdraw all of the withdrawable contents, and quantitatively dilute with *Solution A* to obtain a solution containing the equivalent of 200 µg/mL of cefotetan.

Sample solution B (where the label states the quantity of cefotetan in a given volume of constituted solution): Constitute Cefotetan for Injection as directed in the labeling. Dilute an aliquot of the constituted solution with *Solution A* to obtain a solution containing the equivalent of 200 µg/mL of cefotetan.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

www.webofpharma.com

Flow rate: 2 mL/min
Injection size: 20 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for cefotetan and cefotetan tautomer are 0.75 and 1.0, respectively, *System suitability solution*.]
Suitability requirements
Resolution: NLT 2.0 between cefotetan and cefotetan tautomer, *System suitability solution*
Column efficiency: NLT 1500 theoretical plates, *Standard solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution*, and *Sample solution A* or *Sample solution B*
Calculate the percentage of $C_{17}H_{17}N_7O_8S_4$ withdrawn from the container, or in the portion of solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from *Sample solution A* or *Sample solution B*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cefotetan RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cefotetan in *Sample solution A* or *Sample solution B* (µg/mL)

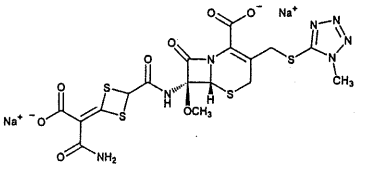
Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

SPECIFIC TESTS
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*: Meets the requirements at the time of use
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.17 USP Endotoxin Unit/mg of cefotetan
- **STERILITY TESTS** ⟨71⟩: Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **pH** ⟨791⟩: 4.0–6.5, in a solution 100 mg/mL
- **WATER DETERMINATION,** *Method Ic* ⟨921⟩: NMT 2.8%
- **OTHER REQUIREMENTS:** It meets the requirements under *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve as described under *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Cefotetan RS

Cefotetan Disodium



$C_{17}H_{15}N_7Na_2O_8S_4$ 619.58

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7- [[[4-(2-amino-1-carboxy-2-oxoethylidene)-1,3-dithietan-2-yl] carbonyl]amino]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl) thio]methyl]-8-oxo-, disodium salt, [6*R*-(6α,7α)]-;
(6*R*,7*S*)-4-[[2-Carboxy-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl]carbamoyl]-1,3-dithietane-$\Delta^{2,\alpha}$-malonamic acid, disodium salt;
(6*R*,7*S*)-7-[4-(Carbamoylcarboxymethylene)-1,3-dithietane-2-carboxamido]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl) thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, disodium salt [74356-00-6].

DEFINITION
Cefotetan Disodium contains the equivalent of NLT 830 µg/mg and NMT 970 µg/mg of cefotetan ($C_{17}H_{17}N_7O_8S_4$), calculated on the anhydrous basis.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩: Meets the requirements

ASSAY
- **PROCEDURE**
Protect the *Standard solution*, the *System suitability solution*, and the *Sample solution* from light, and use within 2 h.
Mobile phase: Methanol, acetonitrile, glacial acetic acid, and 0.1 M phosphoric acid (105:105:100:1700)
Standard solution: 0.2 mg/mL of USP Cefotetan RS in methanol, acetonitrile, and water (5:5:90), prepared as follows. Place a suitable quantity of USP Cefotetan RS in a suitable volumetric flask, and add methanol, using 5% of the final volume. Swirl for several min, and add acetonitrile, using 5% of the final volume. Swirl until dissolved, and dilute with water to volume.
System suitability solution: Transfer 10 mL of *Standard solution* to a glass-stoppered flask containing a few mg of magnesium carbonate. Sonicate for 10 min. If the solution is not turbid, add a few more mg of magnesium carbonate, and repeat the sonication. Pass the turbid solution through a filter of 0.5-µm or finer pore size. Use the clear filtrate.
Sample solution: 0.2 mg/mL of Cefotetan Disodium in methanol, acetonitrile, and water (5:5:90), prepared as follows. Place a suitable quantity of Cefotetan Disodium in a suitable volumetric flask, and add methanol, using 5% of the final volume. Swirl for several min, and add acetonitrile, using 5% of the final volume. Swirl until dissolved, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

www.webofpharma.com

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for cefotetan and cefotetan tautomer are 0.75 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between cefotetan and cefotetan tautomer, *System suitability solution*
Column efficiency: NLT 1500 theoretical plates, *Standard solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of cefotetan ($C_{17}H_{17}N_7O_8S_4$) in the portion of Cefotetan Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

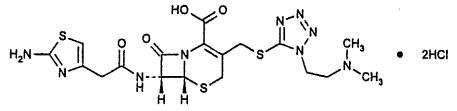
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cefotetan RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefotetan Disodium in the *Sample solution* (mg/mL)
P = potency of cefotetan in USP Cefotetan RS (µg/mg)

Acceptance criteria: 830–970 µg/mg on the anhydrous basis

SPECIFIC TESTS
- **pH** ⟨791⟩
Sample solution: 100 mg/mL
Acceptance criteria: 4.0–6.5
- **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 2.5%
- **STERILITY TESTS** ⟨71⟩**:** Where the label states that Cefotetan Disodium is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Cefotetan Disodium is sterile or it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.17 USP Endotoxin Unit/mg of cefotetan.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Cefotetan RS

Cefotiam Hydrochloride



$C_{18}H_{23}N_9O_4S_3 \cdot 2HCl$ 598.55

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7[[-(2-amino-4-thiazolyl)acetyl]-amino]-3-[[[1-[2-(dimethylamino) ethyl]-1*H*-tetrazol-5-yl]-thio]methyl]-8-oxo, hydrochloride, (6*R-trans*)-.
(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)acetamido]-3-[[[1-[2-(dimethylamino)ethyl]-1*H*-tetrazol-5-yl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid dihydrochloride.
7(*R*)-[2-(2-Amino-4-thiazolyl)acetamido]-3-[[[1-[2-dimethyl amino)ethyl]-1*H*-tetrazol-5-yl]thio]methyl]-3-cephem-4-carboxylic acid dihydrochloride [66309-69-1].

» Cefotiam Hydrochloride contains the equivalent of not less than 790 µg and not more than 925 µg of cefotiam ($C_{18}H_{23}N_9O_4S_3$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Cefotiam Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: water.
B: The retention time of the cefotiam peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay.*
Crystallinity ⟨695⟩: meets the requirements.
Pyrogen—Where the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements of the *Pyrogen Test* ⟨151⟩, the test dose being 1.0 mL per kg of a solution in pyrogen-free sodium carbonate solution (prepared by dissolving 25.6 g of sodium carbonate, previously heated at 170° for not less than 4 hours, in 1000 mL of Sterile Water for Injection) containing 40 mg per mL.
Sterility Tests ⟨71⟩ —Where the label states that it is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
Water Determination, *Method I* ⟨921⟩: not more than 7.0%, the *Test Preparation* being prepared as directed for a hygroscopic specimen, except to use a mixture of 20 mL of formamide (previously dried over anhydrous sodium sulfate for 24 hours) and methanol (2:1), instead of methanol, to dissolve the specimen, and to determine the water content of the formamide and methanol mixture.

Assay—
Mobile phase—Dissolve 13.1 g of ammonium sulfate in 850 mL of water, adjust with 2 N ammonium hydroxide to a pH of 6.5 ± 0.1, add 150 mL of acetonitrile, and mix. Filter through a suitable filter of 0.5 µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Cefotiam Hydrochloride RS, quantitatively in water to obtain a solution having a known concentration of about 1000 µg of cefotiam ($C_{18}H_{23}N_9O_4S_3$) per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with

USP Monographs

www.webofpharma.com

Mobile phase to volume, and mix. This solution contains the equivalent of about 50 µg of cefotiam ($C_{18}H_{23}N_9O_4S_3$) per mL. Use this solution without delay.

Assay preparation—Transfer about 60 mg of Cefotiam Hydrochloride, accurately weighed, to a 50-mL volumetric flask, add water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Use this solution without delay.

System suitability solution—Prepare a solution of USP Cefotiam Hydrochloride RS in water containing about 1 mg per mL. Heat this solution at 95° for 3 minutes, and cool. Transfer 1 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency, determined from the cefotiam peak, is not less than 1985 theoretical plates when calculated by the formula:

$$5.545(t_r/W_{h/2})^2$$

the tailing factor for the cefotiam peak is not more than 1.8, and the relative standard deviation for replicate injections is not more than 1.0%. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for de-tetrazol-cefotiam and 1.0 for cefotiam; and the resolution, *R*, between the de-tetrazol-cefotiam peak and the cefotiam peak is not less than 4.0.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of cefotiam ($C_{18}H_{23}N_9O_4S_3$) in each mg of the Cefotiam Hydrochloride taken by the formula:

$$1000(C/W)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of cefotiam ($C_{18}H_{23}N_9O_4S_3$) in the *Standard preparation*, based on the quantity of USP Cefotiam Hydrochloride RS taken to prepare the *Standard preparation*, the designated cefotiam ($C_{18}H_{23}N_9O_4S_3$) content, in µg per mg, of USP Cefotiam Hydrochloride RS, and the extent of dilution; *W* is the weight, in mg, of Cefotiam Hydrochloride taken to prepare the *Assay preparation*; and r_U and r_S are the cefotiam peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cefotiam for Injection

» Cefotiam for Injection contains an amount of Cefotiam Hydrochloride equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefotiam ($C_{18}H_{23}N_9O_4S_3$). It may contain Sodium Carbonate.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*

USP Reference standards ⟨11⟩—
USP Cefotiam Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: water.

B: The retention time of the cefotiam peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*; as obtained in the *Assay*.

Pyrogen—It meets the requirements of the *Pyrogen Test* ⟨151⟩, the test dose being 1.0 mL per kg of a solution prepared by diluting Cefotiam for Injection with Sterile Water for Injection to a concentration of 40 mg of cefotiam per mL.

Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 5.7 and 7.2, in a solution containing the equivalent of 100 mg of cefotiam per mL.

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 6.0% of its weight.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Assay—

Mobile phase , Standard preparation, System suitability solution, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Cefotiam Hydrochloride.*

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute a container of Cefotiam for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution containing the equivalent of about 1 mg of cefotiam ($C_{18}H_{23}N_9O_4S_3$) per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 50 µg of cefotiam per mL. Use this solution without delay.

Assay preparation 2 (where the label states the quantity of cefotiam in a given volume of constituted solution)—Constitute a container of Cefotiam for Injection in a volume of water, accurately measured, equivalent to the volume of diluent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with water to obtain a solution containing about 1 mg of cefotiam ($C_{18}H_{23}N_9O_4S_3$) per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 50 µg of cefotiam per mL. Use this solution without delay.

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Cefotiam Hydrochloride.* Calculate the quantity, in mg, of cefotiam ($C_{18}H_{23}N_9O_4S_3$) withdrawn from the container, or in the portion of constituted solution taken by the formula:

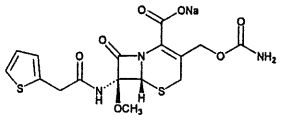
$$C(L/D)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of cefotiam ($C_{18}H_{23}N_9O_4S_3$) in the *Standard preparation,* based on the quantity of USP Cefotiam Hydrochloride RS taken to prepare the *Standard preparation,* the designated cefotiam

www.webofpharma.com

($C_{18}H_{23}N_9O_4S_3$) content, in µg per mg, of USP Cefotiam Hydrochloride RS, and the extent of dilution; *L* is the labeled quantity, in mg, of cefotiam ($C_{18}H_{23}N_9O_4S_3$) in the container, or in the volume of constituted solution taken; *D* is the concentration, in µg of cefotiam per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the volume of constituted solution taken, respectively, and the extent of dilution; and r_U and r_S are the cefotiam peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cefoxitin Sodium



$C_{16}H_{16}N_3NaO_7S_2$ 449.43

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[(aminocarbonyl)oxy]methyl]-7-methoxy-8-oxo-7-[(2-thienylacetyl)amino]-, sodium salt (*6R-cis*)-;

Sodium (*6R,7S*)-3-(hydroxymethyl)-7-methoxy-8-oxo-7-[2-(2-thienyl)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate carbamate (ester) [33564-30-6].

DEFINITION

Cefoxitin Sodium contains the equivalent of NLT 927 µg/mg and NMT 970 µg/mg of cefoxitin ($C_{16}H_{17}N_3O_7S_2$), corresponding to NLT 97.5% and NMT 102.0% of cefoxitin sodium ($C_{16}H_{16}N_3NaO_7S_2$), calculated on the anhydrous and acetone- and methanol-free basis.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy:* **197U▲** (CN 1-May-2020)

Buffer: 1.0 g/L of monobasic potassium phosphate and 1.8 g/L of anhydrous dibasic sodium phosphate in water

Sample solution: 20 µg/mL in *Buffer*

Acceptance criteria: Meets the requirements

- **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: Dissolve 1.0 g of monobasic potassium phosphate and 1.8 g of dibasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 7.1 ± 0.1, and dilute with water to 1 L. Pass through a membrane filter of 1-µm or finer pore size.

Mobile phase: Acetonitrile, water, and glacial acetic acid (160:840:10). Pass through a membrane filter of 1-µm or finer pore size.

Standard solution: 0.3 mg/mL of USP Cefoxitin RS in *Buffer.* Sonicate, if necessary, to dissolve. Use this solution within 5 h.

Sample solution: 0.3 mg/mL of Cefoxitin Sodium in *Buffer.* Use this solution within 5 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 5- to 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2800 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of cefoxitin ($C_{16}H_{17}N_3O_7S_2$) in the portion of Cefoxitin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of cefoxitin in USP Cefoxitin RS (mg/mg)

F = conversion factor, 1000 µg/mg

Acceptance criteria: 927–970 µg/mg of cefoxitin, corresponding to NLT 97.5% and NMT 102.0% of cefoxitin sodium, on the anhydrous and acetone- and methanol-free basis

IMPURITIES

- **LIMIT OF ACETONE AND METHANOL**

Standard stock solution 1: 0.5% v/v of acetone in water

Standard stock solution 2: 0.5% v/v of methanol in water

Standard solution: 0.050% v/v of acetone from *Standard stock solution 1* and 0.005% v/v of methanol from *Standard stock solution 2* in water

Sample solution: Transfer 5.0 g of Cefoxitin Sodium to a 50-mL volumetric flask, and dissolve in and dilute with water to volume. Transfer 3.0 mL of the resulting solution to a 15-mL centrifuge tube, cool in an ice-water bath for 2 min, and add 3.0 mL of 0.24 N hydrochloric acid while swirling vigorously. Centrifuge to obtain a clear solution.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: GC

Detector: Flame ionization

Columns

Precolumn: Packed with 60- to 80-mesh silane-treated glass beads

Analytical: 6.3-mm × 1.8-m glass; support S2

Temperatures

Columns: 110°

Injection port: 100°

Detector: 200°

Carrier gas: Nitrogen

Flow rate: 50 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 160 theoretical plates for acetone and NLT 200 theoretical plates for methanol

Tailing factor: NMT 1.3 for acetone and NMT 2.3 for methanol

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentages of acetone and methanol in the portion of Cefoxitin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times D$$

r_U = peak area of acetone or methanol in the *Sample solution*
r_S = peak area of acetone or methanol in the *Standard solution*
C_S = concentration of acetone or methanol in the *Standard solution* (% v/v)
C_U = concentration of Cefoxitin Sodium in the *Sample solution* (g/mL)
D = density of acetone or methanol at 20° (g/mL)

Acceptance criteria: NMT 0.7% of acetone and NMT 0.1% of methanol

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL in methanol
  Acceptance criteria: +206° to +214°, calculated on the anhydrous and acetone- and methanol-free basis
- **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
- **pH** ⟨791⟩
  Sample solution: 100 mg/mL
  Acceptance criteria: 4.2–7.0
- **WATER DETERMINATION,** *Method I* ⟨921⟩
  Analysis: Use a mixture of ethylene glycol and pyridine (3:1) in the titration vessel in place of methanol.
  Acceptance criteria: NMT 1.0%
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Cefoxitin Sodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.13 USP Endotoxin Unit/mg of cefoxitin.
- **STERILITY TESTS** ⟨71⟩**:** Where the label states that Cefoxitin Sodium is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a cold place.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cefoxitin RS

Cefoxitin Injection

DEFINITION

Cefoxitin Injection is a sterile solution of Cefoxitin Sodium and one or more suitable buffer substances in Water for Injection. It contains Dextrose or Sodium Chloride as a tonicity-adjusting agent. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cefoxitin ($C_{16}H_{17}N_3O_7S_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: Dissolve 1.0 g of monobasic potassium phosphate and 1.8 g of dibasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 7.1 ± 0.1, and dilute with water to 1 L. Pass through a membrane filter of 1-µm or finer pore size.
  Mobile phase: Acetonitrile, water, and glacial acetic acid (160:840:10). Pass through a membrane filter of 1-µm or finer pore size.
  Standard solution: 0.3 mg/mL of USP Cefoxitin RS in *Buffer*. Sonicate, if necessary, to dissolve. Use this solution within 5 h.
  Sample solution: 0.3 mg/mL of cefoxitin prepared as follows. Allow one container of Injection to thaw, and mix. Dilute an aliquot of Injection with *Buffer* to a suitable volume. Use this solution within 5 h.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 3.9-mm × 30-cm; 5- to 10-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 2800 theoretical plates
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 1.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of cefoxitin ($C_{16}H_{17}N_3O_7S_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of cefoxitin in the *Sample solution* (mg/mL)
P = potency of cefoxitin in USP Cefoxitin RS (mg/mg)

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.13 USP Endotoxin Unit/mg of cefoxitin
- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **pH** ⟨791⟩**:** 4.5–8.0
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** It meets the requirements for small-volume injections.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Maintain in the frozen state.
- **LABELING:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just before use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cefoxitin RS

www.webofpharma.com

Cefoxitin for Injection

DEFINITION

Cefoxitin for Injection contains Cefoxitin Sodium equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of cefoxitin ($C_{16}H_{17}N_3O_7S_2$).

ASSAY

- **PROCEDURE**

Buffer: Dissolve 1.0 g of monobasic potassium phosphate and 1.8 g of dibasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 7.1 ± 0.1, and dilute with water to 1 L. Pass through a membrane filter of 1-µm or finer pore size.

Mobile phase: Acetonitrile, water, and glacial acetic acid (160:840:10). Pass through a membrane filter of 1-µm or finer pore size.

Standard solution: 0.3 mg/mL of USP Cefoxitin RS in *Buffer*. Sonicate, if necessary, to dissolve. Use this solution within 5 h.

Sample solution 1 (where it is represented as being in a single-dose container): Nominally 0.3 mg/mL of cefoxitin prepared as follows. Constitute Cefoxitin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, transfer to a suitable volumetric flask, and dilute with water to volume. Use this solution within 5 h.

Sample solution 2 (where the label states the quantity of cefoxitin in a given volume of constituted solution): Nominally 0.3 mg/mL of cefoxitin prepared as follows. Constitute Cefoxitin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Transfer a suitable aliquot of the constituted solution to a suitable volumetric flask, and dilute with water to volume. Use this solution within 5 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 5- to 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2800 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Sample solution 1* or *Sample solution 2* and *Standard solution*

Calculate the percentage of the labeled amount of cefoxitin ($C_{16}H_{17}N_3O_7S_2$) in the portion of Cefoxitin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of cefoxitin in *Sample solution 1* or *Sample solution 2* (mg/mL)

P = potency of cefoxitin in USP Cefoxitin RS (mg/mg)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

SPECIFIC TESTS

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.13 USP Endotoxin Unit/mg of cefoxitin
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements for small-volume injections.
- **pH** ⟨791⟩

Sample solution: 100 mg/mL

Acceptance criteria: 4.2–7.0

- **WATER DETERMINATION,** *Method I* ⟨921⟩

Analysis: Use a mixture of ethylene glycol and pyridine (3:1) in the titration vessel in place of methanol.

Acceptance criteria: NMT 1.0%

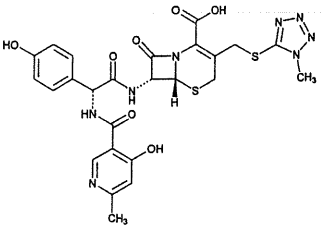
- **OTHER REQUIREMENTS:** It meets the requirements of the *Identification* tests in *Cefoxitin Sodium*. It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Cefoxitin RS

Cefpiramide



$C_{25}H_{24}N_8O_7S_2$ 612.64

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[[(4-hydroxy-6-methyl-3-pyridinyl)carbonyl]amino] (4-hydroxyphenyl)acetyl]amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo, [6*R*-[6α,7β (*R**)]]-;
(6*R*,7*R*)-7-[(*R*)-2-(4-Hydroxy-6-methylnicotinamido)-2-(*p*-hydroxyphenyl)acetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl) thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [70797-11-4].

DEFINITION

Cefpiramide contains NLT 974 µg/mg and NMT 1026 µg/mg of $C_{25}H_{24}N_8O_7S_2$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

www.webofpharma.com

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 1.36 g/L of monobasic potassium phosphate in water adjusted with 1 N sodium hydroxide to a pH of 6.8 ± 0.1 prior to final dilution

Mobile phase: Tetrahydrofuran, acetonitrile, methanol, and *Buffer* (40:40:40:880)

System suitability solution: 1 mg/mL of USP Cefpiramide RS in 0.01 N sodium hydroxide. Heat this solution at 95° for 10 min. Mix 1 mL of this solution with 19 mL of *Mobile phase*. This solution contains a mixture of cefpiramide and cefpiramide lactone.

Standard solution: 0.25 mg/mL of USP Cefpiramide RS in *Mobile phase*

Sample solution: 0.25 mg/mL of Cefpiramide in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.0-mm × 15- to 30-cm; 5- to 10-µm packing L7

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cefpiramide and cefpiramide lactone are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5 between cefpiramide lactone and cefpiramide, *System suitability solution*

Tailing factor: 0.95–1.4, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of cefpiramide ($C_{25}H_{24}N_8O_7S_2$) in each mg of the portion of Cefpiramide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Cefpiramide RS in the *Standard solution* (mg/mL)

C_U = concentration of Cefpiramide taken to prepare the *Sample solution* (mg/mL)

P = potency of cefpiramide in USP Cefpiramide RS (mg/mg)

F = conversion factor, 1000 µg/mg

Acceptance criteria: 974–1026 µg/mg on the anhydrous basis

IMPURITIES

• **ORGANIC IMPURITIES**

System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Buffer: 4.08 g/L of monobasic potassium phosphate in water, adjusted with 1 N sodium hydroxide to a pH of 7.5 ± 0.1 prior to final dilution

Mobile phase: Methanol and *Buffer* (250:750)

Standard stock solution: 0.15 mg/mL of sodium 5-mercapto-1-methyltetrazole and 0.25 mg/mL of USP Cefpiramide RS in *Buffer*

Standard solution: 3 µg/mL of sodium 5-mercapto-1-methyltetrazole and 5 µg/mL of USP Cefpiramide RS from the *Standard stock solution* in *Mobile phase*

Sample solution: 0.5 mg/mL of Cefpiramide in *Mobile phase*

***N*-Ethylmaleimide solution:** 40 mg/mL of *N*-ethylmaleimide in methanol

Test preparation: 10 mg/mL of sodium 5-mercapto-1-methyltetrazole in *N-Ethylmaleimide solution* in a stoppered centrifuge tube. Sonicate for 15 min.

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the water content of sodium 5-mercapto-1-methyltetrazole by the titrimetric method (see *Water Determination* ⟨921⟩), using 5.0 mL of the *Test preparation*.

Calculate the percentage of 5-mercapto-1-methyltetrazole in the portion of Cefpiramide taken:

$$\text{Result} = (r_U/r_S) \times (M_{r1}/M_{r2}) \times (C_S/C_U) \times F \times 100$$

r_U = peak response of 5-mercapto-1-methyltetrazole from the *Sample solution*

r_S = peak response of 5-mercapto-1-methyltetrazole from the *Standard solution*

M_{r1} = molecular weight of 5-mercapto-1-methyltetrazole, 115.14

M_{r2} = molecular weight of anhydrous sodium 5-mercapto-1-methyltetrazole, 138.13

C_S = concentration of sodium 5-mercapto-1-methyltetrazole, corrected for water, in the *Standard solution* (µg/mL)

C_U = concentration of Cefpiramide in the *Sample solution* (mg/mL)

F = conversion factor, 0.001 mg/µg

Calculate the percentage of each other impurity in the portion of Cefpiramide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of each other impurity from the *Sample solution*

r_S = peak response of cefpiramide from the *Standard solution*

C_S = concentration of USP Cefpiramide RS in the *Standard solution* (µg/mL)

C_U = concentration of Cefpiramide in the *Sample solution* (µg/mL)

P = potency of cefpiramide in USP Cefpiramide RS (µg/mg)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
5-Mercapto-1-methyltetrazole	0.20	0.7
Cefpiramide	1.0	—
Any other individual impurity	—	0.7
Total impurities	—	2.0

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL, in dimethylformamide

Acceptance criteria: −100° to −112°

• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements

USP Monographs

www.webofpharma.com

- **pH** ⟨791⟩
  Sample solution: 5-mg/mL suspension in water
  Acceptance criteria: 3.0–5.0
- **Water Determination,** *Method I* ⟨921⟩**:** NMT 9.0%
- **Pyrogen Test** ⟨151⟩
  Sample solution: 50 mg/mL of cefpiramide in Sterile Water for Injection
  Test dose: 1.0 mL/kg of the *Sample solution*
  Acceptance criteria: Where the label states that Cefpiramide is sterile, or it must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements.
- **Sterility Tests** ⟨71⟩**:** Where the label states that Cefpiramide is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP Reference Standards** ⟨11⟩
  USP Cefpiramide RS

Cefpiramide for Injection

DEFINITION
Cefpiramide for Injection contains NLT 90.0% and NMT 120.0% of the labeled amount of cefpiramide ($C_{25}H_{24}N_8O_7S_2$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **Procedure**
  Buffer: 1.36 g/L of monobasic potassium phosphate in water adjusted with 1 N sodium hydroxide to a pH of 6.8 ± 0.1 before final dilution
  Mobile phase: Tetrahydrofuran, acetonitrile, methanol, and *Buffer* (40:40:40:880)
  System suitability solution: 1 mg/mL of USP Cefpiramide RS in 0.01 N sodium hydroxide. Heat this solution at 95° for 10 min. Dilute 1 mL of this solution with *Mobile phase* to 20 mL. This solution contains a mixture of cefpiramide and cefpiramide lactone.
  Standard solution: 0.25 mg/mL of USP Cefpiramide RS in *Mobile phase*
  Sample solution 1 (where it is represented as being in a single-dose container): Constitute a container of Cefpiramide for Injection in a volume of water corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with *Mobile phase* to obtain a solution containing the nominal equivalent of 0.25 mg/mL of cefpiramide.
  Sample solution 2 (where the label states the quantity of cefpiramide in a given volume of constituted solution): Constitute a container of Cefpiramide for Injection in a volume of water equivalent to the volume of diluent specified in the labeling. Dilute the constituted solution with water to obtain a solution nominally containing 0.25 mg/mL of cefpiramide.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability.*)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.0-mm × 15- to 30-cm; 5- to 10-µm packing L7
  Flow rate: 1.5 mL/min
  Injection size: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [Note—The relative retention times for cefpiramide and cefpiramide lactone are 0.7 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 5 between cefpiramide lactone and cefpiramide, *System suitability solution*
  Tailing factor: 0.95–1.4, *Standard solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution 1* or *Sample solution 2*
  Calculate the percentage of cefpiramide ($C_{25}H_{24}N_8O_7S_2$) withdrawn from the container, or in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Cefpiramide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefpiramide in *Sample solution 1* or *Sample solution 2* (mg/mL)

  Acceptance criteria: 90.0%–120.0%

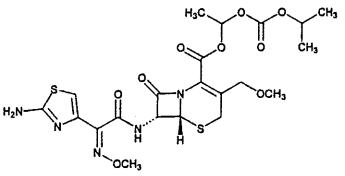
SPECIFIC TESTS
- **Pyrogen Test** ⟨151⟩
  Sample solution: 50 mg/mL of cefpiramide from Cefpiramide for Injection in Sterile Water for Injection
  Test dose: 1.0 mL/kg of the *Sample solution*
  Acceptance criteria: Meets the requirements
- **Sterility Tests** ⟨71⟩**:** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **pH** ⟨791⟩
  Sample solution: Equivalent to 100 mg/mL of cefpiramide from Cefpiramide for Injection
  Acceptance criteria: 6.0–8.0 in water
- **Water Determination,** *Method I* ⟨921⟩**:** NMT 3.0%
- **Particulate Matter in Injections** ⟨788⟩**:** It meets the requirements for small-volume injections.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩*, Injection Packaging, Packaging for constitution.*
- **USP Reference Standards** ⟨11⟩
  USP Cefpiramide RS

USP Monographs

www.webofpharma.com

Cefpodoxime Proxetil



$C_{21}H_{27}N_5O_9S_2$ 557.60

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-carboxylic acid, 7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-(methoxymethyl)-8-oxo-,1-[[(1-methylethoxy)carbonyl]oxy]ethyl ester, [6*R*-[6α,7β(*Z*)]]-.

(±)-1-Hydroxyethyl(+)-(6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-3-methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, 7²-(*Z*)-(*O*-methyloxime), isopropyl carbonate (ester) [87239-81-4].

» Cefpodoxime Proxetil contains the equivalent of not less than 690 µg and not more than 804 µg of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, at a temperature not exceeding 25°.

USP Reference standards ⟨11⟩—
USP Cefpodoxime Proxetil RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020)

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 15 µg per mL.
Medium: acetonitrile.

C: Dissolve 1 mg of it in 4 mL of water, add 1 mL of 1 N sulfuric acid while cooling in an ice bath, add 1 mL of a freshly prepared solution of sodium nitrite (1 in 100), allow to stand for 2 minutes, then add 1 mL of ammonium sulfamate solution (1 in 100). Allow to stand for 1 minute, and add 1 mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride TS: a red-purple color develops.

Specific rotation ⟨781S⟩: between +35.0° and +48.0°.
Test solution: 10 mg per mL, in methanol.

Water Determination, *Method I* ⟨921⟩: not more than 3.0%.

Residue on ignition ⟨281⟩: not more than 0.2%.

Isomer ratio—Using the chromatogram of the *Assay preparation* obtained in the *Assay,* calculate the ratio of the cefpodoxime proxetil *R*-epimer peak response to the sum of the peak responses of the cefpodoxime proxetil *S*-epimer peak and the cefpodoxime proxetil *R*-epimer peak: the ratio is between 0.5 and 0.6.

Chromatographic purity—
Solution A—Prepare filtered and degassed 0.02 M ammonium acetate.
Solution B—Use filtered and degassed acetonitrile.
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluent—Prepare a degassed mixture of water and acetonitrile (2:1).
System suitability solution—Dissolve a quantity of USP Cefpodoxime Proxetil RS in *Diluent* to obtain a solution containing about 10 µg per mL.
[NOTE—A volume of methanol not exceeding 10% of the total volume in the final solution may be used to facilitate dissolution.]
Test solution—Transfer about 50 mg of Cefpodoxime Proxetil, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, using sonication if necessary, dilute with *Diluent* to volume, and mix. This solution should be injected promptly, but may be analyzed within 24 hours when stored at 8°.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 260-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The column temperature is maintained at a constant temperature of about 30°. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	90	10	equilibration (10 minutes)
0–10	90→68	10→32	linear gradient
10–40	68	32	isocratic
40–80	68→50	32→50	linear gradient
80–85	50	50	isocratic
85–90	50→25	50→75	linear gradient
90–95	25	75	isocratic
95–100	25→90	75→10	linear gradient

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the retention time for cefpodoxime proxetil *R*-epimer is between 37 and 42 minutes; the relative retention times are about 0.9 for cefpodoxime proxetil *S*-epimer and 1.0 for cefpodoxime proxetil *R*-epimer; the resolution, *R,* between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 4.0; the column efficiency is not less than 19,000 theoretical plates determined from the cefpodoxime proxetil *R*-epimer peak; and the relative standard deviation for replicate injections determined from the sum of the areas of the cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer peaks is not more than 2.0%.
Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak areas. Calculate the percentage of each impurity in the portion of Cefpodoxime Proxetil taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak area for each impurity; and r_s is the sum of the areas of all the peaks: not more than 3.0% of any peak at a relative retention time of about 0.86 is found; not more than 1.0% for any peak at relative retention times of about 1.27, 1.39, and other individual peaks having relative retention times higher than 2.0 is found; not more than 0.5% of any other individual impurity is found; and not more than 6.0% of total impurities is found, impurity peaks of less than 0.05% being disregarded.

www.webofpharma.com

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 0.02 M ammonium acetate and acetonitrile (6:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a degassed mixture of water and acetonitrile (6:4).

Standard preparation—Transfer about 25 mg of USP Cefpodoxime Proxetil RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, mix, and pass through a filter having a 0.45-µm or finer porosity.

Assay preparation—Transfer about 50 mg of Cefpodoxime Proxetil, accurately weighed, to a 100-mL volumetric flask, dissolve in 10 mL of methanol, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, mix, and pass through a filter having a 0.45-µm or finer porosity.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 235-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 2 mL per minute. The column temperature is maintained at a constant temperature of about 30°. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.9 for cefpodoxime proxetil *S*-epimer and 1.0 for cefpodoxime proxetil *R*-epimer; the resolution, *R,* between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 2.5; the tailing factor for cefpodoxime proxetil *R*-epimer is not more than 1.5; and the relative standard deviation determined from the sum of the areas of the cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer peaks for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity in µg of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) in each mg of Cefpodoxime Proxetil taken by the formula:

$$2000(CP/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cefpodoxime Proxetil RS in the *Standard preparation; P* is the designated potency, in µg per mg, of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) in USP Cefpodoxime Proxetil RS; *W* is the weight, in mg, of Cefpodoxime Proxetil taken to prepare the *Assay preparation;* and r_U and r_S are the sums of the peak responses for cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cefpodoxime Proxetil for Oral Suspension

» Cefpodoxime Proxetil for Oral Suspension contains Cefpodoxime Proxetil and one or more buffers, suspending agents, sweeteners, flavorings, and preservatives. When constituted as directed in the labeling, it contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$).

Packaging and storage—Preserve in tight containers, at a temperature not exceeding 30°. Store the constituted Oral Suspension in a refrigerator.

USP Reference standards ⟨11⟩—
USP Cefpodoxime Proxetil RS

Identification—The retention times of the cefpodoxime proxetil *R*-epimer peak and the cefpodoxime proxetil *S*-epimer peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Uniformity of dosage units ⟨905⟩—
FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩: meets the requirements.

pH ⟨791⟩: between 4.0 and 5.5, in the suspension constituted as directed in the labeling.

Water Determination ⟨921⟩: not more than 1.5%.

Assay—

Mobile phase, Diluent, and *Chromatographic system*—Prepare as directed in the *Assay* under *Cefpodoxime Proxetil*

Standard preparation—Transfer about 30 mg of USP Cefpodoxime Proxetil RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass through a filter having a 0.45-µm or finer porosity.

Assay preparation—Constitute a container of Cefpodoxime Proxetil for Oral Suspension as directed in the labeling. Shake the resulting suspension thoroughly, and determine its density. Transfer an accurately weighed quantity of the suspension, equivalent to about 50 mg of cefpodoxime, to a 100-mL volumetric flask. Add 10 mL of water, and shake to disperse. Add 20 mL of acetonitrile, and sonicate for 15 minutes. Cool to room temperature, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, mix, and pass through a filter having a 0.45-µm or finer porosity.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) in the portion of Oral Suspension taken by the formula:

$$2CP(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cefpodoxime Proxetil RS in the *Standard preparation; P* is the designated potency, in µg per mg, of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) in USP Cefpodoxime Proxetil RS; and r_U and r_S are the sums of the peak responses for cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cefpodoxime Proxetil Tablets

» Cefpodoxime Proxetil Tablets contain an amount of Cefpodoxime Proxetil equivalent to not less than 90.0 percent and not more than 110.0 percent of

USP Monographs

www.webofpharma.com

the labeled amount of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$).

Packaging and storage—Preserve in tight containers, at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Cefpodoxime Proxetil RS

Identification—The retention times of the cefpodoxime proxetil *R*-epimer peak and the cefpodoxime proxetil *S*-epimer peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium—Dissolve 54.5 g of glycine and 42.6 g of sodium chloride in about 500 mL of water in a 1000-mL volumetric flask. Cautiously add, with swirling, 14.2 mL of hydrochloric acid, and allow to cool. Dilute with water to volume, and mix. Transfer 50 mL of this stock solution to a flask, and dilute with water to 900 mL to obtain a solution having a pH of 3.0 ± 0.1. [NOTE—If necessary, adjust the pH of the stock solution with 10 N sodium hydroxide so that when 50 mL is diluted with water to 900 mL the pH of the *Dissolution Medium* is 3.0 ± 0.1.]
Apparatus 2: 75 rpm.
Time: 30 minutes.
Procedure—Determine the amount of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) dissolved by employing UV absorption at about 259 nm on filtered portions of the solution under test in comparison with a Standard solution having a known concentration of USP Cefpodoxime Proxetil RS prepared by dissolving an accurately weighed portion in a small volume of methanol and diluting quantitatively with *Dissolution Medium.*
Tolerances—Not less than 70% *(Q)* of the labeled amount of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

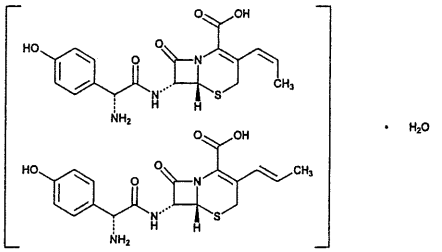
Water Determination ⟨921⟩: not more than 5.0%.

Assay—
Mobile phase, Diluent, and *Chromatographic system*—Prepare as directed in the *Assay* under *Cefpodoxime Proxetil.*
Standard preparation—Transfer about 30 mg of USP Cefpodoxime Proxetil RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass through a filter having a 0.45-µm or finer porosity.
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of cefpodoxime to a 100-mL volumetric flask. Dissolve in 40 mL of *Diluent,* sonicating for 5 minutes. Cool to room temperature, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, mix, and pass through a filter having a 0.45-µm or finer porosity.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) in the portion of Tablets taken by the formula:

$$2CP(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cefpodoxime Proxetil RS in the *Standard preparation; P* is the designated potency, in µg per mg, of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) in USP Cefpodoxime Proxetil RS; and r_U and r_S are the sums of the peak responses for cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cefprozil



$C_{18}H_{19}N_3O_5S \cdot H_2O$ 407.44
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[amino(4-hydroxyphenyl)acetyl]amino]-8-oxo-3-(1-propenyl)-, monohydrate, [6*R*-[6α,7β(*R**)]]-;
(6*R*,7*R*)-7-[(*R*)-2-Amino-2-(*p*-hydroxyphenyl)acetamido]-8-oxo-3-propenyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate [121123-17-9].
Anhydrous 389.43
[92665-29-7].

DEFINITION
Cefprozil contains NLT 900 µg/mg and NMT 1050 µg/mg of cefprozil ($C_{18}H_{19}N_3O_5S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention times of the cefprozil (*Z*)-isomer and cefprozil (*E*)-isomer peaks from the *Sample solution* correspond to those of the Standard solutions, as obtained in the *Assay.*

ASSAY
- **PROCEDURE**

Buffer: 11.5 g/L of monobasic ammonium phosphate in water. Adjust, if necessary, with phosphoric acid to a pH of 4.4.
Mobile phase: Acetonitrile and *Buffer* (100:900)
System suitability solution: 0.125 mg/mL each of USP Cefprozil (*Z*)-Isomer RS and USP Cefprozil (*E*)-Isomer RS in water. Use this solution within 6 h.
Standard solution 1: 0.25 mg/mL of USP Cefprozil (*Z*)-Isomer RS in water. Use this solution within 6 h.
Standard solution 2: 0.025 mg/mL of USP Cefprozil (*E*)-Isomer RS in water. Use this solution within 6 h.
Sample solution: 0.3 mg/mL of Cefprozil in water. Shake to dissolve. Use this solution within 6 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 30-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution 1*

www.webofpharma.com

[NOTE—The relative retention times for cefprozil (*Z*)-isomer and cefprozil (*E*)-isomer are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between cefprozil (*Z*)-isomer and cefprozil (*E*)-isomer, *System suitability solution*

Tailing factor: 0.9–1.1, *Standard solution 1*

Relative standard deviation: NMT 2.0%, *Standard solution 1*

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the amount (µg/mg) of cefprozil (*Z*)-isomer ($C_{18}H_{19}N_3O_5S$) in the portion of Cefprozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response of cefprozil (*Z*)-isomer from the *Sample solution*

r_S = peak response of cefprozil (*Z*)-isomer from *Standard solution 1*

C_S = concentration of USP Cefprozil (*Z*)-Isomer RS in *Standard solution 1* (mg/mL)

C_U = concentration of Cefprozil in the *Sample solution* (mg/mL)

P = potency of USP Cefprozil (*Z*)-Isomer RS (µg/mg)

Calculate the amount (µg/mg) of cefprozil (*E*)-isomer ($C_{18}H_{19}N_3O_5S$) in the portion of Cefprozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response of cefprozil (*E*)-isomer from the *Sample solution*

r_S = peak response of cefprozil (*E*)-isomer from *Standard solution 2*

C_S = concentration of USP Cefprozil (*E*)-Isomer RS in *Standard solution 2* (mg/mL)

C_U = concentration of Cefprozil in the *Sample solution* (mg/mL)

P = potency of USP Cefprozil (*E*)-Isomer RS (µg/mg)

Calculate the amount (µg/mg) of cefprozil ($C_{18}H_{19}N_3O_5S$) in the portion of Cefprozil taken by adding the values, in µg/mg, of the cefprozil (*Z*)-isomer and the cefprozil (*E*)-isomer.

Acceptance criteria: 900–1050 µg/mg on the anhydrous basis

IMPURITIES

• ORGANIC IMPURITIES, PROCEDURE 1

Use *Organic Impurities, Procedure 1* when the impurity profile includes *Z*-cefprozil open ring, *E*-cefprozil open ring, and cefprozil related compound K.

Solution A: 11.5 g/L of monobasic ammonium phosphate in water. Adjust, if necessary, with phosphoric acid or ammonium hydroxide to a pH of 4.4.

Solution B: Acetonitrile and *Solution A* (1:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	81	19
8	81	19
20	36	64
25	36	64
27	81	19
30	81	19

[NOTE—These gradient elution times are established on an HPLC system with a dwell volume of approximately 1.3 mL. The gradient elution times in *Table 1* can be adjusted as necessary to achieve the separation described.]

Standard stock solution: 0.25 mg/mL each of USP Cefprozil (*Z*)-Isomer RS, USP Amoxicillin Related Compound I RS, and USP Cefprozil Related Compound D RS in a mixture of 1 M hydrochloric acid and *Solution A*. Prepare the solution as follows. Dissolve USP Amoxicillin Related Compound I RS, USP Cefprozil (*Z*)-Isomer RS, and USP Cefprozil Related Compound D RS in 1 M hydrochloric acid, using 20% of the final volume. Dilute with *Solution A* to volume.

Sensitivity solution: 2.5 µg/mL each of cefprozil (*Z*)-isomer, amoxicillin related compound I, and cefprozil related compound D in *Solution A* from *Standard stock solution*. Store the solution at 4°, and use within 8 h.

Standard solution: 50 µg/mL each of cefprozil (*Z*)-isomer, amoxicillin related compound I, and cefprozil related compound D in *Solution A* from the *Standard stock solution*. Store the solution at 4°, and use within 12 h.

Sample solution: 5 mg/mL of Cefprozil in a mixture of 1 M hydrochloric acid and *Solution A*, prepared as follows. Dissolve the Cefprozil first in 1 M hydrochloric acid using 4% of the final volume, and then dilute with *Solution A* to volume. Store the solution at 4°, and use within 3 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Autosampler: 4°

Column: 40°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Sensitivity solution* and *Standard solution*

[NOTE—USP Cefprozil Related Compound D RS contains the (*Z*)- and (*E*)-isomers of cefprozil related compound D. See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.4 between the (*E*)-isomer of cefprozil related compound D and cefprozil (*Z*)-isomer, *Standard solution*

Relative standard deviation: NMT 10.0% for cefprozil, amoxicillin related compound I, and each isomer of cefprozil related compound D, *Standard solution*

Signal-to-noise ratio: NLT 10 for cefprozil, amoxicillin related compound I, and each isomer of cefprozil related compound D, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amoxicillin related compound I in the portion of Cefprozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of amoxicillin related compound I from the *Sample solution*

www.webofpharma.com

r_S = peak response of amoxicillin related compound I from the *Standard solution*
C_S = concentration of USP Amoxicillin Related Compound I RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefprozil in the *Sample solution* (mg/mL)
P = potency of amoxicillin related compound I in USP Amoxicillin Related Compound I RS (mg/mg)

Calculate the percentage of cefprozil related compound D in the portion of Cefprozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = sum of the responses for cefprozil related compound D (*Z*)-isomer and cefprozil related compound D (*E*)-isomer from the *Sample solution*
r_S = peak response of cefprozil related compound D (*Z*)-isomer from the *Standard solution*
C_S = concentration of USP Cefprozil Related Compound D RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefprozil in the *Sample solution* (mg/mL)
P = potency of cefprozil related compound D (*Z*)-isomer in USP Cefprozil Related Compound D RS (mg/mg)

Calculate the percentage of each of the other impurities in the portion of Cefprozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of cefprozil from the *Standard solution*
C_S = concentration of USP Cefprozil (*Z*)-Isomer RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefprozil in the *Sample solution* (mg/mL)
P = potency of USP Cefprozil (*Z*)-Isomer RS (mg/mg)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amoxicillin related compound I[a]	0.40	0.3
Cefadroxil	0.54	0.5
Hydroxyphenyldiketopiperazine[b]	0.61	0.3
Cefprozil related compound D (*Z*)-isomer[c, d]	0.69	0.3
Cefprozil related compound D (*E*)-isomer[e]	0.91	
O-Acyl cefprozil[f]	0.76	0.2
Cefprozil (*Z*)-isomer	1.0	—
Cefprozil (*E*)-isomer	1.37	—
Z-Cefprozil open ring[g]	1.74	0.2

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cefprozil related compound H (*Z*)-isomer[h, i]	1.95	0.2
Cefprozil related compound H (*E*)-isomer[j]	2.19	
E-Cefprozil open ring[k]	2.08	0.2
Cefprozil related compound K[l, m]	2.76	0.1
	2.86	0.1
	2.91	0.1
	3.01	0.1
Any individual unspecified impurity	—	0.1
Total impurities	—	2.0

[a] (*R*)-2-Amino-2-(4-hydroxyphenyl)acetic acid.
[b] 3-(Aminomethylene)-6-(4-hydroxyphenyl)piperazine-2,5-dione.
[c] 7-Amino-3-propenylcephalosporanic acid (*Z*-isomer); (6*R*,7*R*)-7-Amino-8-oxo-3-[(*Z*)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[d] The sum of the two isomers is reported. The limit for the sum is 0.3%.
[e] 7-Amino-3-propenylcephalosporanic acid (*E*-isomer); (6*R*,7*R*)-7-Amino-8-oxo-3-[(*E*)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[f] (6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-[(*R*)-2-amino-2-(4-hydroxyphenyl)acetoxy] phenyl)acetamido]-8-oxo-3-[(*Z*)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[g] (*R*)-2-((*R*)-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl)-5-[(*Z*)-prop-1-enyl]-3,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.
[h] *N*-Acyl cefprozil (*Z*-isomer); (6*R*,7*R*)-7-((*R*)-2-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido)-8-oxo-3-[(*Z*)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[i] The sum of the two isomers is reported. The limit for the sum is 0.2%.
[j] *N*-Acyl cefprozil (*E*-isomer); (6*R*,7*R*)-7-((*R*)-2-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido)-8-oxo-3-[(*E*)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[k] (*R*)-2-((*R*)-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl)-5-[(*E*)-prop-1-enyl]-3,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.
[l] Hydroxyphenyldiketopiperazine lactone; 3-(5-Ethyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl)-6-(4-hydroxyphenyl)piperazine-2,5-dione.
[m] The system resolves four isomers of cefprozil related compound K.

- **ORGANIC IMPURITIES, PROCEDURE 2**

Use *Organic Impurities, Procedure 2* when the impurity profile includes ethoxycarbonyl cefprozil, methoxycefadroxil, cefprozil delta-3 isomer, cefprozil amide, and cefprozil dimer.

Solution A: 4 g/L of monobasic sodium phosphate adjusted with dilute phosphoric acid (1 in 10) to a pH of 4.2 ± 0.05
Solution B: Acetonitrile and *Solution A* (1:1)
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	70	30
40	40	60
50	0	100
60	0	100
62	95	5
70	95	5

Diluent: 0.85 g/L of monobasic potassium phosphate and 1.16 g/L of anhydrous dibasic sodium phosphate in water

www.webofpharma.com

System suitability stock solution: 0.15 mg/mL of USP Cefadroxil RS and 0.75 mg/mL of USP Cefprozil Related Compound D RS, prepared as follows. Dissolve USP Cefadroxil RS in *Solution A*, using 20% of the final volume. Add USP Cefprozil Related Compound D RS, mix, and dilute with *Diluent* to volume.
System suitability solution: 15 µg/mL of USP Cefadroxil RS and 75 µg/mL of USP Cefprozil Related Compound D RS from the *System suitability stock solution* and 1.5 mg/mL of USP Cefprozil RS in *Solution A*
Standard solution: 15 µg/mL of USP Cefprozil RS in *Solution A*
Sample solution: 1.5 mg/mL of Cefprozil in *Solution A*. Refrigerate the solution, and use within 1 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 4°
Column: NMT 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between the (*Z*)-isomer of cefprozil related compound D and cefadroxil; NLT 1.5 between cefadroxil and the (*E*)-isomer of cefprozil related compound D, *System suitability solution*
Relative standard deviation: NMT 5.0% for the sum of the cefprozil (*Z*)-isomer and cefprozil (*E*)-isomer, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Cefprozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = sum of the responses for cefprozil (*Z*)-isomer and cefprozil (*E*)-isomer from the *Standard solution*
C_S = concentration of USP Cefprozil RS in the *Standard solution* (mg/mL)
C_U = concentration of Cefprozil in the *Sample solution* (mg/mL)
P = potency of USP Cefprozil RS (mg/mg)
F = relative response factor (see *Table 4*)

Acceptance criteria: See *Table 4*. The reporting threshold is 0.05%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound I[a]	0.17	1.5	0.15
Cefprozil related compound D (*Z*)-isomer[b]	0.57	0.56	0.30
Cefadroxil	0.62	1.1	1.0
Methoxycefadroxil[c]	0.65	0.44	0.15
Cefprozil related compound D (*E*)-isomer[d]	0.73	0.56	0.30
Cefprozil delta-3 isomer[e]	0.92	0.95	0.2
Cefprozil (*Z*)-isomer	1.0	—	—
Cefprozil (*E*)-isomer	1.17	—	—
Cefprozil related compound H[f]	1.33	0.93	0.15
Cefprozil amide[g]	1.46	0.90	0.15
Ethoxycarbonyl-cefprozil[h]	2.08	0.70	0.15
Cefprozil dimer[i]	2.21	0.90	0.2
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	2.00

[a] (*R*)-2-Amino-2-(4-hydroxyphenyl)acetic acid.
[b] 7-Amino-3-propenylcephalosporanic acid (*Z*-isomer); (6*R*,7*R*)-7-Amino-8-oxo-3-[(*Z*)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[c] (6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[d] 7-Amino-3-propenylcephalosporanic acid (*E*-isomer); (6*R*,7*R*)-7-Amino-8-oxo-3-[(*E*)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[e] (6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-8-oxo-3-[(*Z*)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
[f] *N*-Acyl cefprozil (*Z*-isomer); (6*R*,7*R*)-7-((*R*)-2-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido)-8-oxo-3-[(*Z*)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[g] (*R*)-2-{(6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-8-oxo-3-[(*Z*)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamido}-2-(4-hydroxyphenyl)acetic acid.
[h] (6*R*,7*R*)-7-{(*R*)-2-Amino-2-[4-(ethoxycarbonyloxy)phenyl]acetamido}-8-oxo-3-[(*Z*)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[i] (6*R*,7*R*)-7-[(*R*)-2-{(6*R*,7*R*)-7-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-8-oxo-3-[(*Z*)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamido}-2-(4-hydroxyphenyl)acetamido]-8-oxo-3-[(*Z*)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

SPECIFIC TESTS

- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **pH** ⟨791⟩
Sample solution: 5 mg/mL in water
Acceptance criteria: 3.5–6.5
- **WATER DETERMINATION** ⟨921⟩, *Method I*: 3.5%–6.5%
- **CEFPROZIL (*E*)-ISOMER RATIO**
Buffer, Mobile phase, System suitability solution, Standard solution 1, Standard solution 2, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*
Calculate the ratio of the cefprozil (*E*)-isomer to total cefprozil in the portion of Cefprozil taken:

$$\text{Result} = E/(E + Z)$$

E = amount of cefprozil (*E*)-isomer as determined in the *Assay* (µg/mg)
Z = amount of cefprozil (*Z*)-isomer as determined in the *Assay* (µg/mg)

USP Monographs

www.webofpharma.com

Acceptance criteria: The ratio is 0.06–0.11.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Amoxicillin Related Compound I RS
(*R*)-2-Amino-2-(4-hydroxyphenyl)acetic acid.
$C_8H_9NO_3$ 167.16
USP Cefadroxil RS
USP Cefprozil RS
USP Cefprozil (*E*)-Isomer RS
USP Cefprozil (*Z*)-Isomer RS
USP Cefprozil Related Compound D RS
7-Amino-3-propenylcephalosporanic acid;
(6*R*,7*R*)-7-Amino-8-oxo-3-[(*Z*)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
$C_{10}H_{12}N_2O_3S$ 240.28

Cefprozil for Oral Suspension

DEFINITION

Cefprozil for Oral Suspension is a dry mixture of Cefprozil and one or more suitable buffers, flavors, preservatives, suspending agents, and sweeteners. It contains NLT 90.0% and NMT 120.0% of the labeled amount of cefprozil ($C_{18}H_{19}N_3O_5S$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Acetone and 0.1 N hydrochloric acid (4:1)
Standard solution: 5 mg/mL of USP Cefprozil (*Z*)-Isomer RS in *Diluent*
Sample solution: Nominally 5 mg/mL of cefprozil in *Diluent* from Cefprozil for Oral Suspension. Shake for 5 min, and allow to settle. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Butyl alcohol, glacial acetic acid, and water (60:20:20)
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry, and develop the chromatogram in an equilibrated chamber with the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate, and allow it to air-dry in a hood. Place the dry plate in a chamber containing iodine vapors. Examine the plate, and locate the spots.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

- **B.** The retention times of the cefprozil (*Z*)-isomer and cefprozil (*E*)-isomer peaks of the *Sample solution* correspond to those of *Standard solution 1* and *Standard solution 2*, respectively, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 11.5 g/L of monobasic ammonium phosphate in water. Adjust, if necessary, with phosphoric acid to a pH of 4.4.
Mobile phase: Acetonitrile and *Buffer* (100:900). [NOTE—Decreasing the proportion of acetonitrile increases retention times and improves the resolution between the cefprozil isomer peaks.]
Standard solution 1: 0.25 mg/mL of USP Cefprozil (*Z*)-Isomer RS. Use this solution within 6 h.
Standard stock solution: 0.25 mg/mL of USP Cefprozil (*E*)-Isomer RS
Standard solution 2: 0.025 mg/mL of USP Cefprozil (*E*)-Isomer RS in water from the *Standard stock solution*. Use this solution within 6 h.
System suitability solution: A mixture of equal volumes of *Standard solution 1* and the *Standard stock solution*. Use this solution within 6 h.
Sample stock solution: Nominally 1 mg/mL of cefprozil in water from Cefprozil for Oral Suspension. Prepare as follows. Constitute one container of Cefprozil for Oral Suspension as directed in the labeling. Transfer a suitable aliquot, freshly mixed and free from air bubbles, to a volumetric flask, dilute with water to volume, and mix, sonicating briefly.
Sample solution: Nominally 0.3 mg/mL of cefprozil in water from the *Sample stock solution*. Pass a portion of this solution through a filter of 0.5-µm or finer pore size. Use this solution within 6 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution 1* and *System suitability solution*
[NOTE—The relative retention times for the cefprozil (*Z*)-isomer and the cefprozil (*E*)-isomer are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.5 between cefprozil (*Z*)-isomer and cefprozil (*E*)-isomer, *System suitability solution*
Column efficiency: NLT 2500 theoretical plates, cefprozil (*Z*)-isomer, *Standard solution 1*
Calculate as follows:

$$\text{Result} = (t_R/W_{h/2})^2 \times 5.545$$

t_R = retention time of cefprozil (*Z*)-isomer
$W_{h/2}$ = peak width at half-height

Tailing factor: 0.9–1.1, cefprozil (*Z*)-isomer, *Standard solution 1*
Calculate as follows:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$ = width of the peak at 10% height
f = distance from the peak maximum to the leading edge of the peak measured at 10% of the peak height

Relative standard deviation: NMT 2.0%, *Standard solution 1*
Analysis
Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*
Calculate the concentration, in mg/mL, of the cefprozil (*Z*)-isomer in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times P \times F$$

www.webofpharma.com

r_U = peak response of the cefprozil (*Z*)-isomer from the *Sample solution*
r_S = peak response of the cefprozil (*Z*)-isomer from *Standard solution 1*
C_S = concentration of USP Cefprozil (*Z*)-Isomer RS in *Standard solution 1* (mg/mL)
P = potency of the cefprozil (*Z*)-isomer in USP Cefprozil (*Z*)-Isomer RS (µg/mg)
F = correction factor, 0.001 mg/µg

Calculate the concentration, in mg/mL, of the cefprozil (*E*)-isomer in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times P \times F$$

r_U = peak response of the cefprozil (*E*)-isomer from the *Sample solution*
r_S = peak response of the cefprozil (*E*)-isomer from *Standard solution 2*
C_S = concentration of USP Cefprozil (*E*)-Isomer RS in *Standard solution 2* (mg/mL)
P = potency of the cefprozil (*E*)-isomer in USP Cefprozil (*E*)-Isomer RS (µg/mg)
F = correction factor, 0.001 mg/µg

Calculate the percentage of the labeled amount of cefprozil ($C_{18}H_{19}N_3O_5S$) in the portion of Cefprozil for Oral Suspension taken:

$$\text{Result} = [(C_Z + C_E)/C_U] \times 100$$

C_Z = concentration of the cefprozil (*Z*)-isomer in the *Sample solution* (mg/mL)
C_E = concentration of the cefprozil (*E*)-isomer in the *Sample solution* (mg/mL)
C_U = nominal concentration of cefprozil in the *Sample solution* (mg/mL)

Acceptance criteria: 90%–120.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
For solids packaged in single-unit containers: Meets the requirements
• **DELIVERABLE VOLUME** ⟨698⟩
For solids packaged in multiple-unit containers: Meets the requirements

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: Constitute Cefprozil for Oral Suspension as directed in the labeling.
Acceptance criteria: 4.0–6.0
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 3.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cefprozil (*E*)-Isomer RS
USP Cefprozil (*Z*)-Isomer RS

Cefprozil Tablets

DEFINITION

Cefprozil Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of cefprozil ($C_{18}H_{19}N_3O_5S$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**
Diluent: Acetone and 0.1 N hydrochloric acid (4:1)
Standard solution: 5 mg/mL of USP Cefprozil (*Z*)-Isomer RS in *Diluent*
Sample solution: Nominally 2.5 mg/mL of cefprozil from 1 Tablet in *Diluent*. Shake for 5 min, and allow the mixture to settle. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Butyl alcohol, glacial acetic acid, and water (60:20:20)
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry, and develop the chromatogram in an equilibrated chamber with the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate, and allow it to air-dry in a hood. Place the dry plate in a chamber containing iodine vapors. Examine the plate, and locate the spots.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.
• **B.** The retention times of the cefprozil (*Z*)-isomer and cefprozil (*E*)-isomer peaks of the *Sample solution* correspond to those of *Standard solution 1* and *Standard solution 2*, respectively, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer: 11.5 g/L of monobasic ammonium phosphate in water. Adjust, if necessary, with phosphoric acid to a pH of 4.4.
Mobile phase: Acetonitrile and *Buffer* (100:900). [NOTE—Decreasing the proportion of acetonitrile increases retention times and improves the resolution between the cefprozil isomer peaks.]
Standard solution 1: 0.25 mg/mL of USP Cefprozil (*Z*)-Isomer RS. Use this solution within 6 h.
Standard stock solution: 0.25 mg/mL of USP Cefprozil (*E*)-Isomer RS
Standard solution 2: 0.025 mg/mL of USP Cefprozil (*E*)-Isomer RS in water from the *Standard stock solution*. Use this solution within 6 h.
System suitability solution: A mixture of equal volumes of *Standard solution 1* and the *Standard stock solution*. Use this solution within 6 h.
Sample stock solution: Nominally 6 mg/mL of cefprozil in water from Tablets, prepared as follows. Transfer a suitable number of Tablets to a volumetric flask containing water. Allow the Tablets to disintegrate with the aid of swirling and sonication. Dilute with water to volume.
Sample solution: Nominally 0.3 mg/mL of cefprozil in water from the *Sample stock solution*. Pass a portion of this solution through a filter of 0.5-µm or finer pore size. Use this solution within 6 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution 1* and *System suitability solution*

USP Monographs

www.webofpharma.com

[NOTE—The relative retention times for cefprozil (*Z*)-isomer and cefprozil (*E*)-isomer are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between cefprozil (*Z*)-isomer and cefprozil (*E*)-isomer, *System suitability solution*

Column efficiency: NLT 2500 theoretical plates, cefprozil (*Z*)-isomer, *Standard solution 1*

Calculate as follows:

$$\text{Result} = (t_R/W_{h/2})^2 \times 5.545$$

t_R = retention time of cefprozil (*Z*)-isomer
$W_{h/2}$ = peak width at half-height

Tailing factor: 0.9–1.1, cefprozil (*Z*)-isomer, *Standard solution 1*

Calculate as follows:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$ = width of the peak at 10% height
f = distance from the peak maximum to the leading edge of the peak measured at 10% of the peak height

Relative standard deviation: NMT 2.0%, *Standard solution 1*

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the concentration, in mg/mL, of the cefprozil (*Z*)-isomer in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times P \times F$$

r_U = peak response of the cefprozil (*Z*)-isomer from the *Sample solution*
r_S = peak response of the cefprozil (*Z*)-isomer from *Standard solution 1*
C_S = concentration of USP Cefprozil (*Z*)-Isomer RS in *Standard solution 1* (mg/mL)
P = potency of the cefprozil (*Z*)-isomer in USP Cefprozil (*Z*)-Isomer RS (µg/mg)
F = correction factor, 0.001 mg/µg

Calculate the concentration, in mg/mL, of the cefprozil (*E*)-isomer in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times P \times F$$

r_U = peak response of the cefprozil (*E*)-isomer from the *Sample solution*
r_S = peak response of the cefprozil (*E*)-isomer from *Standard solution 2*
C_S = concentration of USP Cefprozil (*E*)-Isomer RS in *Standard solution 2* (mg/mL)
P = potency of the cefprozil (*E*)-isomer in USP Cefprozil (*E*)-Isomer RS (µg/mg)
F = correction factor, 0.001 mg/µg

Calculate the percentage of the labeled amount of cefprozil ($C_{18}H_{19}N_3O_5S$) in the portion of Tablets taken:

$$\text{Result} = [(C_Z + C_E)/C_U] \times 100$$

C_Z = concentration of the cefprozil (*Z*)-isomer in the *Sample solution* (mg/mL)
C_E = concentration of the cefprozil (*E*)-isomer in the *Sample solution* (mg/mL)
C_U = nominal concentration of cefprozil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Mobile phase, Standard solution 1, Standard solution 2, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Sample solution: Pass the solution under test through a suitable filter. Dilute, if necessary, to 0.3 mg/mL of cefprozil.

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the quantity, in mg, of cefprozil (*Z*)-isomer dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times P \times F$$

r_U = peak response of cefprozil (*Z*)-isomer from the *Sample solution*
r_S = peak response of cefprozil (*Z*)-isomer from *Standard solution 1*
C_S = concentration of USP Cefprozil (*Z*)-Isomer RS in *Standard solution 1* (mg/mL)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL
P = potency of the cefprozil (*Z*)-isomer in USP Cefprozil (*Z*)-Isomer RS (µg/mg)
F = correction factor, 0.001 mg/µg

Calculate the quantity, in mg, of cefprozil (*E*)-isomer dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times P \times F$$

r_U = peak response of cefprozil (*E*)-isomer from the *Sample solution*
r_S = peak response of cefprozil (*E*)-isomer from *Standard solution 2*
C_S = concentration of USP Cefprozil (*E*)-Isomer RS in *Standard solution 2* (mg/mL)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL
P = potency of the cefprozil (*E*)-isomer in USP Cefprozil (*E*)-Isomer RS (µg/mg)
F = correction factor, 0.001 mg/µg

Calculate the percentage of cefprozil ($C_{18}H_{19}N_3O_5S$) dissolved:

$$\text{Result} = (M_Z + M_E) \times 100/L$$

M_Z = quantity of cefprozil (*Z*)-isomer dissolved (mg)
M_E = quantity of cefprozil (*E*)-isomer dissolved (mg)
L = label claim of cefprozil (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of cefprozil ($C_{18}H_{19}N_3O_5S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 7.0%

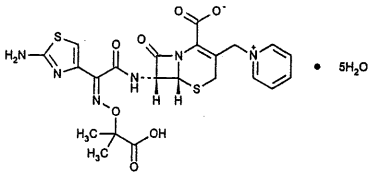
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

www.webofpharma.com

• **USP Reference Standards** ⟨11⟩
USP Cefprozil (*E*)-Isomer RS
USP Cefprozil (*Z*)-Isomer RS

Ceftazidime



$C_{22}H_{22}N_6O_7S_2 \cdot 5H_2O$ 636.65
Pyridinium, 1-[[7-[[(2-amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-, hydroxide, inner salt, pentahydrate, [6*R*[6α,7β(*Z*)]]-.
1-[[(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl] methyl]pyridinium hydroxide, inner salt, 7²-(*Z*)-[*O*(1-carboxy-1-methylethyl)oxime], pentahydrate [78439-06-2].
Anhydrous 546.59

» Ceftazidime contains not less than 95.0 percent and not more than 102.0 percent of $C_{22}H_{22}N_6O_7S_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.
USP Reference standards ⟨11⟩—
USP Ceftazidime, Delta-3-Isomer RS
USP Ceftazidime Pentahydrate RS
Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftazidime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Crystallinity ⟨695⟩: meets the requirements.
Sterility Tests ⟨71⟩ —Where the label states that it is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined,* except to use *Fluid A* to each 1000 mL of which has been added 10 g of sodium bicarbonate before sterilization.
pH ⟨791⟩: between 3.0 and 4.0, in a solution containing 5 mg per mL.
Loss on drying ⟨731⟩—Dry about 300 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses between 13.0% and 15.0% of its weight.
Other requirements—Where the label states that Ceftazidime is sterile or that it must be subjected to further processing during the preparation of injectable or other sterile dosage forms, it meets the requirements for *Bacterial endotoxins* under *Ceftazidime for Injection.*

Assay—
pH 7 Buffer—Dissolve 42.59 g of anhydrous dibasic sodium phosphate and 27.22 g of monobasic potassium phosphate in water to make 1000 mL of solution.

Mobile phase—Mix 40 mL of acetonitrile and 200 mL of *pH 7 Buffer,* and dilute with water to obtain 2000 mL of solution. Filter, using a filter having a porosity of 1 µm or finer, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer about 29 mg of USP Ceftazidime Pentahydrate RS, accurately weighed, to a 25-mL volumetric flask containing 2.5 mL of *pH 7 Buffer,* and shake until dissolved. Dilute with water to volume, and mix. [Note—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this stock solution to a 50-mL volumetric flask, dilute with water to volume, and mix. This solution contains about 100 µg of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) per mL.
Assay preparation—Transfer about 115 mg of Ceftazidime, accurately weighed, to a 100-mL volumetric flask containing 10.0 mL of *pH 7 Buffer,* and shake until dissolved. Dilute with water to volume, and mix. [Note—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.
Resolution solution—Prepare a solution of USP Ceftazidime, Delta-3-Isomer RS in *pH 7 Buffer* containing about 0.1 mg per mL. Immediately prior to chromatography, mix 1 mL of this solution with 8 mL of water and 1 mL of the stock solution used to prepare the *Standard preparation.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between ceftazidime and ceftazidime, delta-3-isomer is not less than 2.0. Chromatograph the *Standard preparation,* and record the responses as directed for *Procedure:* the tailing factor for the analyte peak is not less than 0.75 and not more than 1.5, and the relative standard deviation for replicate injections is not more than 1.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{22}H_{22}N_6O_7S_2$ in the portion of Ceftazidime taken by the formula:

$$C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ceftazidime Injection

» Ceftazidime Injection is a sterile isoosmotic solution of Ceftazidime in Water for Injection. It contains one or more suitable buffers and a tonicity-adjusting agent. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{22}H_{22}N_6O_7S_2$.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging.* Maintain in the frozen state.

USP 43 Monographs

www.webofpharma.com

Labeling—It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

USP Reference standards ⟨11⟩—
USP Ceftazidime, Delta-3-Isomer RS
USP Ceftazidime Pentahydrate RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftazidime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.1 USP Endotoxin Unit per mg of ceftazidime.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH ⟨791⟩: between 5.0 and 7.5.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Assay—

pH 7 Buffer, Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Ceftazidime*

Assay preparation—Allow a container of the Injection to thaw, and mix the solution. Transfer an accurately measured volume of the Injection, equivalent to about 50 mg of ceftazidime, to a 50-mL volumetric flask, dilute with *pH 7 buffer* to volume, and mix. Transfer 5.0 mL of this solution to a second 50-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Ceftazidime* Calculate the quantity, in mg, of $C_{22}H_{22}N_6O_7S_2$ in each mL of the Injection taken by the formula:

$$0.5(C/V)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) in the *Standard preparation; V* is the volume, in mL, of Injection taken; and r_U and r_S are the ceftazidime peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ceftazidime for Injection

» Ceftazidime for Injection is a sterile mixture of Sterile Ceftazidime and Sodium Carbonate or Arginine. It contains not less than 90.0 percent and not more than 105.0 percent of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) on the dried and sodium carbonate- or arginine-free basis, and not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ceftazidime ($C_{22}H_{22}N_6O_7S_2$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution,* protected from light.

USP Reference standards ⟨11⟩—
USP L-Arginine RS
USP Ceftazidime, Delta-3-Isomer RS
USP Ceftazidime Pentahydrate RS

Identification—

A: The chromatograms of the *Assay preparations* exhibit a major peak for ceftazidime, the retention time of which corresponds to that in the chromatogram of the *Standard preparation.*

B: It dissolves in 1 N hydrochloric acid with effervescence, evolving a colorless gas, which when passed into *calcium hydroxide TS* produces a white precipitate immediately.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.1 USP Endotoxin Unit per mg of ceftazidime.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩**:** between 5.0 and 7.5, in a solution constituted in the sealed container, taking care to relieve the pressure inside the container during constitution, containing 100 mg of ceftazidime per mL.

Loss on drying ⟨731⟩—Dry about 300 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 25° for 4 hours: where it contains arginine, it loses not more than 12.5% of its weight. Where it contains sodium carbonate, it loses not more than 13.5% of its weight. Where it contains arginine, use the percentage loss obtained, *m,* to calculate, on the dried and arginine-free basis, the result from *Assay preparation 1* obtained as directed in the *Assay.* Where it contains sodium carbonate, heat the residue in vacuum at a pressure not exceeding 5 mm of mercury at 100° an additional 3 hours, and calculate the total percentage of weight loss. Use this percentage, *m,* to calculate, on the dried and sodium carbonate-free basis, the result from *Assay preparation 1* obtained as directed in the *Assay.*

Particulate Matter in Injections ⟨788⟩ **:** meets the requirements for small-volume injections.

Sodium carbonate *(where present)*—

Potassium chloride solution—Dissolve 19.07 g of potassium chloride in water to make 1000 mL of solution.

Standard preparation—Dissolve a suitable quantity of sodium chloride, previously dried at 105° for 2 hours and accurately weighed, in water to obtain a solution having a known concentration of about 14 µg per mL. Transfer 10 mL of this solution to a 100-mL volumetric flask, add 10.0 mL of *Potassium chloride solution,* dilute with water to volume, and mix.

Test preparation—Use the stock solution used to prepare *Assay preparation 1* in the *Assay,* diluting it quantitatively, and stepwise if necessary, with water to obtain a solution containing about 12.5 µg of sodium carbonate per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add 10.0 mL of *Potassium chloride solution,* dilute with water to volume, and mix.

Blank solution—Transfer 10.0 mL of *Potassium chloride solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparation* and the *Test preparation* at the sodium emission line of 589.0 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a sodium hollow-cathode lamp and an air–acetylene flame, using the *Blank solution* as the blank. Calculate the percentage of sodium carbonate (Na_2CO_3) in the portion of Ceftazidime for Injection taken by the formula:

$$(105.99/116.88)(0.1C/M)(A_U/A_S)$$

in which 105.99 is the molecular weight of sodium carbonate; 116.88 is twice the molecular weight of sodium chloride; C is the concentration, in µg per mL, of sodium chloride in the *Standard preparation; M* is the quantity, in mg, of Ceftazidime

USP Monographs

www.webofpharma.com

for Injection in each mL of the *Test preparation,* based on the quantity taken to prepare the stock solution and the extent of dilution; and A_U and A_S are the absorbances of the *Test preparation* and the *Standard preparation,* respectively. Use this percentage to calculate, on the dried and sodium carbonate-free basis, the result from *Assay preparation 1* obtained as directed in the *Assay.*

Limit of pyridine—

Mobile phase—Mix 300 mL of acetonitrile and 100 mL of 0.25 M monobasic ammonium phosphate, dilute with water to obtain 1000 mL of solution, and adjust with ammonium hydroxide to a pH of 7.0 ± 0.1. Pass this solution through a filter having a 1-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

pH 7 Buffer—Dissolve 5.68 g of anhydrous dibasic sodium phosphate and 3.63 g of monobasic potassium phosphate in water to make 1000 mL of solution.

Standard solution—Transfer about 250 mg of pyridine, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix. Immediately prior to chromatography, transfer 2.0 mL of this solution to a 200-mL volumetric flask, dilute with *pH 7 Buffer* to volume, and mix. This solution contains about 25 µg of pyridine per mL.

Test solution—Transfer about 660 mg of Ceftazidime for Injection, just removed from its container and accurately weighed, to a 100-mL volumetric flask, promptly add *pH 7 buffer* to volume, and mix. Store this solution in a cool place, and use it within 1 hour.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.6 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor for the analyte peak is not more than 2.5; and the relative standard deviation for replicate injections is not more than 3%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the responses for the pyridine peaks. Calculate the percentage of pyridine in the portion of Ceftazidime for Injection taken by the formula:

$$10(C/W)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of pyridine in the *Standard solution; W* is the weight, in mg, of Ceftazidime for Injection taken; and r_U and r_S are the pyridine peak responses obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.4% of pyridine is found where it contains sodium carbonate; and not more than 0.3% where it contains arginine.

Content of arginine *(where present)*—

Mobile phase—Dissolve 1.15 g of monobasic ammonium phosphate in about 800 mL of water. Adjust with phosphoric acid to a pH of 2.0 ± 0.1, dilute with water to 1000 mL, and mix. Prepare a filtered and degassed mixture of acetonitrile and this solution (750:250). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve accurately weighed quantities of USP Ceftazidime Pentahydrate RS and USP L-Arginine RS in water to obtain a solution containing known concentrations of about 0.2 mg of each per mL.

Test preparation—Quantitatively dissolve an accurately weighed portion of Ceftazidime for Injection in water to obtain a solution having a concentration of about 0.2 mg of ceftazidime per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 206-nm detector, a 4.6-mm × 50-cm saturator pre-column containing packing L27, and a 4-mm × 25-cm analytical column containing packing L20. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the responses as directed for *Procedure:* the resolution, *R,* between the ceftazidime and the arginine peaks is not less than 6.0; and the tailing factor for the arginine peak is not more than 4.0.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of arginine ($C_6H_{14}N_4O_2$) in the Ceftazidime for Injection taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP L-Arginine RS in the *Standard preparation; C_U* is the concentration, in mg per mL, of Ceftazidime for Injection in the *Test preparation,* based on the weight, in mg, of Ceftazidime for Injection taken and the extent of dilution; and r_U and r_S are the arginine peak responses obtained from the *Test preparation* and the *Standard preparation,* respectively. Use this percentage to calculate, on the anhydrous and arginine-free basis, the assay result from *Assay preparation 1* obtained as directed in the *Assay.*

Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—

pH 7 buffer, Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Ceftazidime.*

Assay preparation 1—Transfer an accurately weighed quantity of Ceftazidime for Injection, equivalent to about 250 mg of ceftazidime ($C_{22}H_{22}N_6O_7S_2$), to a 250-mL volumetric flask, dilute with water to volume, and mix to obtain a stock solution. [NOTE—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

Assay preparation 2 (where it is represented as being in a single-dose container)—Constitute a container of Ceftazidime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution containing about 1 mg of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) per mL. [NOTE—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

Assay preparation 3 (where the label states the quantity of ceftazidime in a given volume of constituted solution)—Constitute a container of Ceftazidime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with water to obtain a solution containing about 1 mg of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) per mL. [NOTE—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Ceftazidime* Calculate the percentage of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) on the dried and sodium carbonate-free or

USP Monographs

www.webofpharma.com

arginine-free basis in the portion of Ceftazidime for Injection taken by the formula:

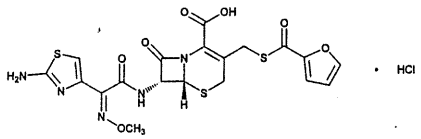
$$25{,}000\{C/[W(100 - m - s)]\}(r_U/r_S)$$

in which C is the concentration, in µg per mL, of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) in the *Standard preparation*; W is the quantity, in mg, of Ceftazidime for Injection taken to prepare *Assay preparation 1*; m is the total percentage of loss on drying; s is the percentage of sodium carbonate or arginine in the Ceftazidime for Injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) in the container, or in the volume of constituted solution taken; and D is the concentration, in µg, of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) per mL, of *Assay preparation 2* or *Assay preparation 3*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution.

Ceftiofur Hydrochloride



$C_{19}H_{17}N_5O_7S_3 \cdot HCl$ 560.02

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[(2-furanylcarbonyl)thio]methyl]-8-oxo-, monohydrochloride, [6*R*-[6α,7β(*Z*)]]-;

(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-3-(mercaptomethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7²-(*Z*)-(*O*-methyloxime), 2-furoate (ester), monohydrochloride [103980-44-5].

DEFINITION

Ceftiofur Hydrochloride contains NLT 844 µg/mg and NMT 956 µg/mg of ceftiofur ($C_{19}H_{17}N_5O_7S_3$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Solution A: Tetrabutylammonium hydroxide, 40% in water

Solution B: Dissolve 3.85 g of ammonium acetate and 13.5 mL of *Solution A* in 700 mL of water. Adjust with glacial acetic acid to a pH of 6.7.

Mobile phase: Mix 700 mL of *Solution B* with 200 mL of methanol and 110 mL of tetrahydrofuran.

Diluent: 0.05 M ammonium acetate

Standard solution: 0.16 mg/mL of USP Ceftiofur Hydrochloride RS prepared as follows. Dissolve USP Ceftiofur Hydrochloride RS in methanol using about 4% of the final volume and dilute with *Diluent* to volume.

Sample solution: 0.16 mg/mL of Ceftiofur Hydrochloride prepared as follows. Dissolve Ceftiofur Hydrochloride in methanol using about 4% of the final volume and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ceftiofur ($C_{19}H_{17}N_5O_7S_3$) in the portion of Ceftiofur Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ceftiofur Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Ceftiofur Hydrochloride in the *Sample solution* (mg/mL)

P = potency of ceftiofur in USP Ceftiofur Hydrochloride RS (µg/mg)

Acceptance criteria: 844–956 µg/mg on the anhydrous and solvent-free basis

IMPURITIES

- **Low Molecular Weight Impurities**

Solution A: Acetonitrile, trifluoroacetic acid, and water (50:1:950)

Solution B: Acetonitrile, trifluoroacetic acid, and water (800:1:200)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
35	60	40
50	0	100
55	0	100
60	100	0
75	100	0

Diluent: Acetonitrile and water (1:1)

System suitability solution: 0.1 mg/mL of USP Ceftiofur System Suitability Mixture RS in *Diluent*. Sonicate as needed to dissolve. Inject within 20 min of preparation.

www.webofpharma.com

Peak identification solution: 15 µg/mL of USP Cefotaxime Sodium RS in *Diluent*
Sample stock solution: 3 mg/mL of Ceftiofur Hydrochloride in *Diluent*
Sample solution: 0.3 mg/mL of Ceftiofur Hydrochloride from *Sample stock solution* in water. Inject the *Sample solution* within 20 min of preparation.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for ceftiofur delta-3 isomer and ceftiofur are 0.98 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between ceftiofur delta-3 isomer and ceftiofur
Analysis
Samples: *Sample solution* and *Peak identification solution*
[NOTE—The elution order of the *N*-deacyl ceftiofur and cefotaxime peaks may be reversed depending on the column used. Determine the location of the cefotaxime peak by using the *Peak identification solution*.]
Calculate the percentage of each impurity in the portion of Ceftiofur Hydrochloride taken:

$$\text{Result} = \{r_U/[r_S + (\Sigma r_U/F)]\} \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of ceftiofur from the *Sample solution*
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05% of the total corrected peak area.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Aminothiazolyl oxime ethyl ester[a]	0.03	1.6	0.5
7-Aminocephalosporanic acid[b]	0.10	0.77	0.5
2-Furoic acid[c]	0.33	2.5	0.5
N-Deacyl ceftiofur[d]	0.70	0.61	0.5
Cefotaxime[e]	0.71	1.0	0.5
Ceftiofur delta-3 isomer[f]	0.98	1.0	0.5
Ceftiofur	1.0	—	—
Ceftiofur *E*-isomer[g]	1.08	1.0	3.6
Dihydrothiophenyl thioester[h]	1.2	1.0	0.5
Ceftiofur amide dimer[i]	1.3	1.0	0.8
N-Trityl ceftiofur oxime[j]	1.5	0.48	0.5
Any other individual impurity	—	1.0	0.5
Total impurities	—	—	6.0

[a] (*Z*)-Ethyl 2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetate.
[b] (6*R*,7*R*)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid; 7-ACA.
[c] Furan-2-carboxylic acid.
[d] (6*R*,7*R*)-7-Amino-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[e] (6*R*,7*R*)-3-(Acetoxymethyl)-7-((*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[f] (6*R*,7*R*)-7-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
[g] (6*R*,7*R*)-7-((*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[h] *S*-(4-Hydroxy-5-oxo-2,5-dihydrothiophen-3-yl)methyl furan-2-carbothioate.
[i] (6*R*,7*S*)-7-[(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(furan-2-carbonylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-enecarboxamido)-3-[(furan-2-carbonylthio)methyl]-8-oxo-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[j] (*Z*)-2-(Methoxyimino)-2-(2-(tritylamino)thiazol-4-yl)acetic acid.

Change to read:

- **HIGH MOLECULAR WEIGHT IMPURITIES (CEFTIOFUR POLYMERS)**

To minimize leaching of plastic components, avoid contact between the *Mobile phase* and plastics during all steps including during the preparation of the *Mobile phase*, the *System suitability solution*, and the *Sample solution*, and when filling the injection vial.
Solution A: 45% of potassium hydroxide in water
Solution B: 0.68 g/L of monobasic potassium phosphate. Adjust with *Solution A* to a pH of 7.5.
Mobile phase: 10 g/L of electrophoresis grade sodium dodecyl sulfate in *Solution A*. Stir or slightly heat to dissolve.
Blank: Use the *Mobile phase*.
System suitability solution: 0.15 mg/mL of USP Ceftiofur System Suitability Mixture RS in *Mobile phase*. Sonicate if necessary to dissolve. Inject within 20 min of preparation.
Sample solution: 0.15 mg/mL of Ceftiofur Hydrochloride in *Mobile phase*. Inject within 20 min of preparation.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.0-mm × 25-cm; 5-µm packing L20
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of the ceftiofur peak
System suitability
Sample: *System suitability solution*
[NOTE—Adjust the chromatographic system so that the retention time of ceftiofur is NLT 2.5 min. The relative retention times of ceftiofur *E*-isomer and ceftiofur are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.0 between ceftiofur and ceftiofur *E*-isomer

USP Monographs

www.webofpharma.com

Analysis
Samples: *Blank* and *Sample solution*
Calculate the percentage of high molecular weight impurities in the portion of Ceftiofur Hydrochloride taken:

$$\text{Result} = \{100 \times (r_U/F)/[(r_U/F) + r_C + r_A]\} - T$$

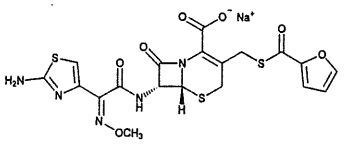
r_U = sum of the responses of all peaks that elute prior to ceftiofur from the *Sample solution*, corrected for the *Blank* if necessary
F = relative response factor, 0.8
r_C = peak response of ceftiofur from the *Sample solution*▲ (ERR 1-Jun-2019)
r_A = sum of the responses of all peaks that elute after ceftiofur from the *Sample solution*▲ (ERR 1-Jun-2019)
T = total impurities from *Table 2*

Acceptance criteria
Total high molecular weight impurities: NMT 3.9%

SPECIFIC TESTS
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 5 mg/mL in dimethylformamide
Acceptance criteria: −115° to −127° on the anhydrous and solvent-free basis
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Ceftiofur Hydrochloride is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 1.0 USP Endotoxin Unit/mg of ceftiofur hydrochloride.
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 6.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a freezer.
- **LABELING:** Label it to indicate that it is intended for veterinary use only. Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Cefotaxime Sodium RS
USP Ceftiofur Hydrochloride RS
USP Ceftiofur System Suitability Mixture RS
This is a mixture of ceftiofur, ceftiofur delta-3 isomer, ceftiofur *E*-isomer, and other impurities.
Ceftiofur delta-3 isomer
(6*R*,7*R*)-7-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
$C_{19}H_{17}N_5O_7S_3$ 523.56
Ceftiofur *E*-isomer
(6*R*,7*R*)-7-((*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
$C_{19}H_{17}N_5O_7S_3$ 523.56
USP Endotoxin RS

Ceftiofur Sodium



$C_{19}H_{16}N_5NaO_7S_3$ 545.54
$C_{19}H_{16}N_5NaO_7S_3 \cdot H_2O$ 563.55
$C_{19}H_{16}N_5NaO_7S_3 \cdot 3H_2O$ 599.59
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[(2-furanylcarbonyl)thio]methyl]-8-oxo-, monosodium salt, [6*R*-[6α,7β(*Z*)]]-;
Sodium (6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-3-(mercaptomethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, 7²-(*Z*)-(*O*-methyloxime), 2-furoate (ester) [104010-37-9].

DEFINITION
Ceftiofur Sodium is the sodium salt of ceftiofur. It is anhydrous or contains one or three molecules of water of hydration. It has a potency equivalent to NLT 862 µg/mg and NMT 970 µg/mg of ceftiofur ($C_{19}H_{17}N_5O_7S_3$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Solution A: Tetrabutylammonium hydroxide, 40% in water
Solution B: Dissolve 3.85 g of ammonium acetate and 13.5 mL of *Solution A* in 700 mL of water. Adjust with glacial acetic acid to a pH of 6.7.
Mobile phase: Mix 700 mL of *Solution B* with 200 mL of methanol and 110 mL of tetrahydrofuran.
Diluent: 0.05 M ammonium acetate
Standard solution: 0.16 mg/mL of USP Ceftiofur Sodium Trihydrate RS in *Diluent*
Sample solution: 0.16 mg/mL of Ceftiofur Sodium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of ceftiofur ($C_{19}H_{17}N_5O_7S_3$) in the portion of Ceftiofur Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

USP Monographs

www.webofpharma.com

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ceftiofur Sodium Trihydrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Ceftiofur Sodium in the *Sample solution*, corrected for water (mg/mL)
P = potency of ceftiofur in USP Ceftiofur Sodium Trihydrate RS (µg/mg)

Acceptance criteria: 862–970 µg/mg on the anhydrous and solvent-free basis

IMPURITIES

• Low Molecular Weight Impurities

Solution A: Water and trifluoroacetic acid (1000:1)
Solution B: Acetonitrile and trifluoroacetic acid (1000:1)
Solution C: *Solution A* and *Solution B* (950:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	0	100
15	18	82
50	18	82
110	60	40
111	0	100
120	0	100

Buffer: 3.5 g/L of sodium phosphate dibasic dihydrate, adjusted with phosphoric acid to a pH of 8.0
Diluent: Acetonitrile and *Buffer* (40:60)
System suitability solution: 1 mg/mL of USP Ceftiofur System Suitability Mixture RS in *Diluent*. Sonicate as needed to dissolve.
Peak identification solution: 15 µg/mL of USP Cefotaxime Sodium RS in *Diluent*
Standard solution: 0.005 mg/mL of USP Ceftiofur Sodium Trihydrate RS in *Diluent*
Sample solution: 1 mg/mL of Ceftiofur Sodium in *Diluent*. Inject within 20 min of preparation.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL
Run time: 3.5 times the retention time of ceftiofur
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for ceftiofur delta-3 isomer and ceftiofur are 0.93 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between ceftiofur delta-3 isomer and ceftiofur, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Sample solution* and *Peak identification solution*
[NOTE—The elution order of the *N*-deacyl ceftiofur and cefotaxime peaks may be reversed depending on the column used. Determine the location of the cefotaxime peak by using the *Peak identification solution*.]

Calculate the percentage of each impurity in the portion of Ceftiofur Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of ceftiofur from the *Standard solution*
C_S = concentration of USP Ceftiofur Sodium Trihydrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Ceftiofur Sodium in the *Sample solution*, corrected for water (mg/mL)
P = potency of ceftiofur in USP Ceftiofur Sodium Trihydrate RS (µg/mg)
F_1 = conversion factor, 0.001 mg/µg
F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Aminothiazolyl oxime[a]	0.07	1.5	0.5
2-Furoic acid[b]	0.21	2.4	0.5
Thiofuroic acid[c]	0.30	0.5	0.5
Ceftiofur thiolactone[d]	0.43	1.0	0.5
N-Deacyl ceftiofur[e]	0.45	1.0	0.5
Cefotaxime (if present)[f, g]	0.47	1.0	0.5
Ceftiofur mercaptan[h]	0.52	1.0	0.5
Ceftiofur disulfide dimer[i]	0.85	0.75	0.5
Ceftiofur delta-3 isomer[j]	0.93	1.0	0.5
Ceftiofur	1.0	—	—
Ceftiofur *E*-isomer[k]	1.4	1.0	0.5
Ceftiofur dioxime[l]	2.1	1.0	0.5
N-Furoyl ceftiofur (if present)[g, m]	2.3	1.0	0.5
Difuroyl disulfide[n]	2.4	1.0	0.5
Any other individual impurity	—	1.0	0.5

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	2.2

[a] (*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.
[b] Furan-2-carboxylic acid.
[c] Furan-2-carbothioic acid.
[d] (*Z*)-2-(2-Aminothiazol-4-yl)-*N*-((5a*R*,6*R*)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]thieno[3,4-*d*][1,3]thiazin-6-yl)-2-(methoxyimino) acetamide.
[e] (6*R*,7*R*)-7-Amino-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[f] (6*R*,7*R*)-3-(Acetoxymethyl)-7-((*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino) acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[g] If possible from the manufacturing process.
[h] (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-(mercaptomethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[i] (6*R*,6′*R*,7*R*,7′*R*,*Z*)-3,3′-[Dithiobis(methylene)]bis{7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid}.
[j] (6*R*,7*R*)-7-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
[k] (6*R*,7*R*)-7-((*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[l] (6*R*,7*R*)-7-{(*Z*)-2-(2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino) acetamido]thiazol-4-yl)-2-(methoxyimino)acetamido}-3-[[(furan-2-carbonyl) thio]methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[m] (6*R*,7*R*)-3-{[(Furan-2-carbonyl)thio]methyl}-7-{(*Z*)-2-[2-(furan-2-carboxamido)thiazol-4-yl]-2-(methoxyimino)acetamido}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[n] Difuran-2-oyl disulfide.

Change to read:

- **HIGH MOLECULAR WEIGHT IMPURITIES (CEFTIOFUR POLYMERS)**

[NOTE—Perform this test if this impurity is possible from the manufacturing process.]

To minimize leaching of plastic components, avoid contact between the *Mobile phase* and plastics during all steps including during preparation of the *Mobile phase*, the *System suitability solution*, and the *Sample solution*, and when filling the injection vial.

Solution A: 0.68 g/L of monobasic potassium phosphate. Adjust with 45% potassium hydroxide solution to a pH of 7.5.

Mobile phase: 10 g/L of electrophoresis grade sodium dodecyl sulfate in *Solution A*. Stir or slightly heat to dissolve.

Blank: Use the *Mobile phase*.

System suitability solution: 0.15 mg/mL each of USP Ceftiofur System Suitability Mixture RS in *Mobile phase*. Sonicate if necessary to dissolve. Inject within 20 min of preparation.

Sample solution: 0.15 mg/mL of Ceftiofur Sodium in *Mobile phase*. Inject within 20 min of preparation.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.0-mm × 25-cm; 5-µm packing L20
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of the ceftiofur peak

System suitability
Sample: *System suitability solution*

[NOTE—Adjust the chromatographic system so that the retention time of ceftiofur is NLT 2.5 min. The relative retention times of ceftiofur *E*-isomer and ceftiofur are 0.9 and 1.0, respectively.]

Suitability requirements
Resolution: NLT 1.0 between ceftiofur and ceftiofur *E*-isomer

Analysis
Samples: *Blank* and *Sample solution*
Calculate the percentage of high molecular weight impurities in the portion of Ceftiofur Sodium taken:

$$\text{Result} = \{100 \times (r_U/F)/[(r_U/F) + r_C + r_A]\} - T$$

r_U = sum of the responses of all peaks that elute prior to ceftiofur from the *Sample solution*, corrected for the *Blank* if necessary
F = relative response factor, 0.8
r_C = peak response of ceftiofur from the *Sample solution*▲ ▲ (ERR 1-Jun-2019)
r_A = sum of the responses of all peaks that elute after ceftiofur from the *Sample solution*▲ ▲ (ERR 1-Jun-2019)
T = total impurities from *Table 2*

Acceptance criteria
Total high molecular weight impurities: NMT 11.0%

- **RESIDUAL SOLVENTS** ⟨467⟩

Acceptance criteria
Tetrahydrofuran (if present): NMT 0.7%
Acetone (if present): NMT 3.5%
[NOTE—For the *Acceptance criteria* for any other residual solvents, see *Residual Solvents* ⟨467⟩.]

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: 50 mg/mL
Acceptance criteria: 5.0–7.5

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 10 mg/mL in water
Acceptance criteria: −60.0° to −74.0°

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Ceftiofur Sodium is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 1.0 USP Endotoxin Unit/mg of ceftiofur sodium.
- **WATER DETERMINATION** ⟨921⟩, *Method I*

Acceptance criteria
Where it is labeled as anhydrous: NMT 2.0%
Where it is labeled as the monohydrate: NMT 4.0%
Where it is labeled as the trihydrate: NMT 10.0%

ADDITIONAL REQUIREMENTS

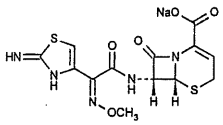
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature except where it is labeled as monohydrate, in which case it should be stored in the freezer.
- **LABELING:** Label it to indicate that it is intended for veterinary use only and to indicate whether it is anhydrous, monohydrate, or trihydrate. Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Cefotaxime Sodium RS
USP Ceftiofur Sodium Trihydrate RS
USP Ceftiofur System Suitability Mixture RS
This is a mixture of ceftiofur, ceftiofur delta-3 isomer, ceftiofur *E*-isomer, and other impurities.
Ceftiofur delta-3 isomer

www.webofpharma.com

(6*R*,7*R*)-7-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
$C_{19}H_{17}N_5O_7S_3$ 523.56
Ceftiofur *E*-isomer
(6*R*,7*R*)-7-((*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
$C_{19}H_{17}N_5O_7S_3$ 523.56
USP Endotoxin RS

Ceftizoxime Sodium



$C_{13}H_{12}N_5NaO_5S_2$ 405.38
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(2,3-dihydro-2-imino-4-thiazoly)(methoxyimino)acetyl]amino]-8-oxomonosodium salt, [6*R*-[6α,7β(*Z*)]]-.
Sodium (6*R*,7*R*)-7-[2-(2-imino-4-thiazolin-4-yl)glyoxylamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 7[2]-(*Z*)-(*O*-methyloxime) [68401-82-1].

» Ceftizoxime Sodium contains the equivalent of not less than 850 µg and not more than 995 µg of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Ceftizoxime RS

Identification—
A: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftizoxime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.
B: It responds to the tests for *Sodium* ⟨191⟩.
Crystallinity ⟨695⟩: meets the requirements.
pH ⟨791⟩: between 6.0 and 8.0, in a solution (1 in 10).
Water Determination, *Method I* ⟨921⟩: not more than 8.5%.
Other requirements—Where the label states that Ceftizoxime Sodium is sterile, it meets the requirements for *Sterility Tests 71* — and *Bacterial Endotoxins Test 85* — under *Ceftizoxime for Injection*. Where the label states that Ceftizoxime Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial Endotoxins Test 85* — under *Ceftizoxime for Injection*.

Assay—
pH 3.6 Buffer—Dissolve 1.42 g of citric acid monohydrate and 1.73 g of dibasic sodium phosphate in water to obtain 1000 mL of solution.

pH 7.0 Buffer—Dissolve 3.63 g of monobasic potassium phosphate and 10.73 g of dibasic sodium phosphate in water to obtain 1000 mL of solution.
Mobile phase—Prepare a mixture of *pH 3.6 Buffer* and acetonitrile (about 9:1). Filter through a filter (1 µm or finer porosity), and degas. Adjust the composition, if necessary, to meet the performance requirements under *Chromatographic system*.
Internal standard solution—Dissolve 1.2 g of salicylic acid in 10 mL of methanol, and dilute with *pH 7.0 Buffer* to obtain 200 mL of solution.
Standard preparation—Dissolve a suitable quantity of USP Ceftizoxime RS, accurately weighed, in *pH 7.0 Buffer* to obtain a solution having a known concentration of about 1 mg of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$) per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix. This *Standard preparation* contains about 0.02 mg of ceftizoxime per mL.
Assay preparation—Using a suitable quantity of Ceftizoxime Sodium, accurately weighed, proceed as directed under *Standard preparation*.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 30-cm column that contains 5- to 10-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the column efficiency determined from the analyte peak is not less than 2000 theoretical plates; the tailing factor for the analyte peak is not more than 2, the resolution; *R,* between the analyte and internal standard peaks is not less than 4; and the relative standard deviation for replicate injections is not more than 2%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for ceftizoxime and 1.0 for salicylic acid. Calculate the quantity, in µg, of ceftizoxime per mg of the Ceftizoxime Sodium taken by the formula:

$$1000(C/M)(R_U/R_S)$$

in which C is the concentration, in mg of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$) per mL, of the *Standard preparation; M* is the concentration, in mg per mL, of the *Assay preparation* based on the weight of Ceftizoxime Sodium taken and the extent of dilution; and R_U and R_S are the peak response ratios of the ceftizoxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ceftizoxime Injection

» Ceftizoxime Injection is a sterile solution of Ceftizoxime Sodium in a diluent containing one or more tonicity-adjusting agents in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Maintain in the frozen state.

USP Monographs

www.webofpharma.com

Labeling—It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

USP Reference standards ⟨11⟩—
USP Ceftizoxime RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftizoxime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.10 USP Endotoxin Unit per mg of ceftizoxime.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH ⟨791⟩: between 5.5 and 8.0.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Assay—

pH 3.6 Buffer , *pH 7.0 Buffer*, *Mobile phase*, *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Ceftizoxime Sodium*.

Assay preparation—Allow 1 container of Injection to thaw, and mix. Transfer an accurately measured volume of the Injection, equivalent to about 40 mg of ceftizoxime, to a 100-mL volumetric flask, dilute with *pH 7.0 Buffer* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* —under *Ceftizoxime Sodium*. Calculate the quantity, in mg, of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$) in each mL of the Injection taken by the formula:

$$2000(C/V)(R_U/R_S)$$

in which V is the volume, in mL, of Injection taken, and the other terms are as defined therein.

Ceftizoxime for Injection

» Ceftizoxime for Injection contains an amount of Ceftizoxime Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.

USP Reference standards ⟨11⟩—
USP Ceftizoxime RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.10 USP Endotoxin Unit per mg of ceftizoxime.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It responds to the *Identification* tests and meets the requirements for *Crystallinity*, *pH*, and *Water Determination, Method I 921:* under *Ceftizoxime Sodium*. It meets also the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—

pH 3.6 Buffer , *pH 7.0 Buffer*, *Mobile phase*, *Internal standard solution*, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Ceftizoxime Sodium*.

Standard preparation—Dissolve a suitable quantity of USP Ceftizoxime RS, accurately weighed, in *pH 7.0 Buffer* to obtain a solution having a known concentration of about 1 mg of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$) per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix. This *Standard preparation* contains about 0.02 mg of ceftizoxime per mL.

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Ceftizoxime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *pH 7.0 Buffer* to obtain a solution containing about 1 mg of ceftizoxime per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.

Assay preparation 2 (where the label states the quantity of ceftizoxime in a given volume of constituted solution)—Constitute Ceftizoxime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *pH 7.0 Buffer* to obtain a solution containing about 1 mg of ceftizoxime per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.

Procedure—Proceed with Ceftizoxime for Injection as directed for *Procedure* in the *Assay*— under *Ceftizoxime Sodium*. Calculate the quantity, in mg, of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C)(R_U/R_S)$$

in which L is the labeled quantity, in mg of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$), in the container, or in the volume of constituted solution taken, and D is the concentration, in mg of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$) per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively; and the extent of dilution, C is the concentration, in mg of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$) per mL, of the *Standard preparation*; and R_U and R_S are the peak response ratios of the ceftizoxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ceftriaxone Injection

» Ceftriaxone Injection is a sterile solution of Ceftriaxone Sodium in a diluent containing one or more tonicity-adjusting agents in Water for

www.webofpharma.com

Injection. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Maintain in the frozen state.
Labeling—It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
USP Reference standards ⟨11⟩—
USP Ceftriaxone Sodium RS
USP Ceftriaxone Sodium E-Isomer RS
Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftriaxone, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.20 USP Endotoxin Unit per mg of ceftriaxone.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
pH ⟨791⟩: between 6.0 and 8.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Assay—
pH 7.0 Buffer—Dissolve 13.6 g of dibasic potassium phosphate and 4.0 g of monobasic potassium phosphate in water to obtain 1000 mL of solution. Adjust this solution with phosphoric acid or 10 N potassium hydroxide to a pH of 7.0 ± 0.1.
pH 5.0 Buffer—Dissolve 25.8 g of sodium citrate in 500 mL of water, adjust with citric acid solution (1 in 5) to a pH of 5.0 ± 0.1, and dilute with water to a volume of 1000 mL.
Mobile phase—Dissolve 3.2 g of tetraheptylammonium bromide in 400 mL of acetonitrile, add 44 mL of *pH 7.0 Buffer* and 4 mL of *pH 5.0 Buffer*, and add water to make 1000 mL. Pass through a membrane filter of 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Ceftriaxone Sodium RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL. Use this solution promptly after preparation.
Resolution solution—Dissolve a suitable quantity of USP Ceftriaxone Sodium E-Isomer RS in *Standard preparation*, and dilute with *Mobile phase* to obtain a solution containing about 160 µg of USP Ceftriaxone Sodium E-Isomer RS per mL and 160 µg of USP Ceftriaxone Sodium RS per mL. Use this solution promptly after preparation.
Assay preparation—Allow 1 container of Injection to thaw, and mix. Transfer an accurately measured volume of the Injection, equivalent to about 40 mg of ceftriaxone, to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Use this solution promptly after preparation.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 270-nm detector and a 4.0-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*. The resolution, *R*, between the ceftriaxone *E*-isomer and ceftriaxone peaks is not less than 3. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*. The column efficiency determined from the analyte peak is not less than 1500 theoretical plates, the tailing factor for the analyte is not more than 2, and the relative standard deviation for replicate injections is not more than 2%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$) in each mL of Injection taken by the formula:

$$200(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Ceftriaxone Sodium RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and r_U and r_S are the ceftriaxone peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ceftriaxone for Injection

DEFINITION
Ceftriaxone for Injection contains an amount of Ceftriaxone Sodium equivalent to NLT 776 µg/mg of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$), calculated on the anhydrous basis, and the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Protect solutions containing ceftriaxone sodium from light.
Solution A: 9 g/L of monobasic potassium phosphate in water
Solution B: 24 g/L of dibasic sodium phosphate, dodecahydrate in water
Solution C: 20 g/L of citric acid in water. Adjust with 10 N sodium hydroxide TS to a pH of 5.0 prior to dilution.
Buffer: Combine 389 mL of *Solution A* and 611 mL of *Solution B*. Adjust with 10 N sodium hydroxide TS or phosphoric acid to a pH of 7.0.
Mobile phase: Dissolve 2.0 g each of tetradecylammonium bromide and tetraheptylammonium bromide in a mixture of 440 mL of water, 55 mL of *Buffer*, 5.0 mL of *Solution C*, and 500 mL of acetonitrile.
System suitability solution: 50 µg/mL each of USP Ceftriaxone Sodium RS and USP Ceftriaxone Sodium E-Isomer RS in *Mobile phase*
Standard solution: 0.3 mg/mL of USP Ceftriaxone Sodium RS in *Mobile phase*
Sample solution 1: Nominally 0.3 mg/mL of ceftriaxone from Ceftriaxone for Injection in *Mobile phase*
Sample solution 2 (where it is represented as being in a single-dose container): Nominally 0.3 mg/mL of ceftriaxone in *Mobile phase* prepared as follows. Constitute Ceftriaxone for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents using a suitable hypodermic needle and syringe, and transfer to

USP Monographs

www.webofpharma.com

a suitable volumetric flask. Dilute with *Mobile phase* to volume.

Sample solution 3 (where the label states the quantity of ceftriaxone in a given volume of constituted solution): Nominally 0.3 mg/mL of ceftriaxone in *Mobile phase* prepared as follows. Constitute Ceftriaxone for Injection in a volume of water corresponding to the volume of solvent specified in the labeling, and dilute with *Mobile phase* to final volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for ceftriaxone and ceftriaxone *E*-isomer are 1.0 and 1.4, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the ceftriaxone and ceftriaxone *E*-isomer peaks, *System suitability solution*
Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 0.7%, *Standard solution*

Analysis
Samples: *Standard solution*, *Sample solution 1*, and *Sample solution 2* or *Sample solution 3*
Calculate the quantity, in µg/mg, of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$) in the portion of Ceftriaxone for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response of ceftriaxone from *Sample solution 1*
r_S = peak response of ceftriaxone from the *Standard solution*
C_S = concentration of USP Ceftriaxone Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ceftriaxone in *Sample solution 1* (mg/mL)
P = potency of ceftriaxone in USP Ceftriaxone Sodium RS (µg/mg)

Acceptance criteria: NLT 776 µg/mg on the anhydrous basis
Calculate the percentage of the labeled amount of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$) in the portion of Ceftriaxone for Injection withdrawn from the container or in the portion of the constituted solution:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of ceftriaxone from *Sample solution 2* or *Sample solution 3*
r_S = peak response of ceftriaxone from the *Standard solution*
C_S = concentration of USP Ceftriaxone Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ceftriaxone in *Sample solution 2* or *Sample solution 3* (mg/mL)
P = potency of ceftriaxone in USP Ceftriaxone Sodium RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0% of the labeled amount of ceftriaxone

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Protect solutions containing ceftriaxone sodium from light.
Solution A, Solution B, Solution C, Buffer, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 3 µg/mL of USP Ceftriaxone Sodium RS in *Mobile phase*
Sample solution: Nominally 0.3 mg/mL of ceftriaxone from Ceftriaxone for Injection in *Mobile phase*
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for ceftriaxone and ceftriaxone *E*-isomer are listed in *Table 1*.]
Suitability requirements
Resolution: NLT 3.0 between the ceftriaxone and ceftriaxone *E*-isomer
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Ceftriaxone for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of ceftriaxone from the *Standard solution*
C_S = concentration of USP Ceftriaxone Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ceftriaxone in the *Sample solution* (mg/mL)
P = potency of ceftriaxone in USP Ceftriaxone Sodium RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 1*. Disregard any peak below 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deacetylcefotaxime lactone[a]	0.20	0.5
7-Aminocephalosporanic acid[b]	0.34	—
Ceftriaxone triazine analog[c]	0.62	1.0
Ceftriaxone benzothiazolyl oxime[d]	0.72	0.2
Deacyl ceftriaxone[e]	0.78	1.0
Ceftriaxone	1.0	—
Ceftriaxone-3-ene isomer[f]	1.3	0.3
Ceftriaxone *E*-isomer[g]	1.4	1.0
Any individual unspecified impurity	—	0.2

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	5.0

[a] (*Z*)-2-(2-Aminothiazol-4-yl)-*N*-[(5a*R*,6*R*)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl]-2-(methoxyimino) acetamide.
[b] Process impurities that are controlled in the drug substance are not to be reported, are not included in total impurities, and are listed here for information only.
[c] 3-Mercapto-2-methyl-1,2-dihydro-1,2,4-triazine-5,6-dione.
[d] (*Z*)-*S*-Benzothiazol-2-yl 2-(2-aminothiazol-4-yl)-2-(methoxyimino) thioacetate.
[e] (6*R*,7*R*)-7-Amino-3-[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[f] (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.
[g] (6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

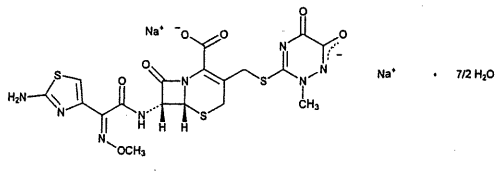
SPECIFIC TESTS

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions.*
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.20 USP Endotoxin Units/mg of ceftriaxone
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** It meets the requirements.
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
- **pH** ⟨791⟩
  Sample solution: 100 mg/mL
  Acceptance criteria: 6.0–8.0
- **WATER DETERMINATION** ⟨921⟩, *Method I***:** 8.0%–11.0%
- **OTHER REQUIREMENTS:** It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution,* and protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ceftriaxone Sodium RS
  USP Ceftriaxone Sodium E-Isomer RS
  (6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, disodium salt.
  $C_{18}H_{16}N_8Na_2O_7S_3$ 598.53

Ceftriaxone Sodium



$C_{18}H_{16}N_8Na_2O_7S_3 \cdot 3½H_2O$ 661.60

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-3-[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl) thio]methyl]-, disodium salt, [6*R*-[6α,7β(*Z*)]]-, hydrate, (2:7); (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-*as*-triazin-3-yl) thio]methyl]-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid, 7²-(*Z*)-(*O*-methyloxime), disodium salt, hemiseptahydrate [104376-79-6].
Anhydrous 598.56

DEFINITION

Ceftriaxone Sodium contains the equivalent of NLT 795 µg/mg of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*

ASSAY

- **PROCEDURE**

Protect solutions containing ceftriaxone sodium from light.
Solution A: 9 g/L of monobasic potassium phosphate in water
Solution B: 24 g/L of dibasic sodium phosphate, dodecahydrate in water
Solution C: 20 g/L of citric acid in water. Adjust with 10 N sodium hydroxide to a pH of 5.0 prior to final dilution.
Buffer: Combine 389 mL of *Solution A* and 611 mL of *Solution B.* Adjust with 10 N sodium hydroxide TS or phosphoric acid to a pH of 7.0.
Mobile phase: Dissolve 2.0 g each of tetradecylammonium bromide and tetraheptylammonium bromide in a mixture of 440 mL of water, 55 mL of *Buffer,* 5.0 mL of *Solution C,* and 500 mL of acetonitrile.
System suitability solution: 50 µg/mL of USP Ceftriaxone Sodium RS and 50 µg/mL of USP Ceftriaxone Sodium E-Isomer RS in *Mobile phase*
Standard solution: 0.3 mg/mL of USP Ceftriaxone Sodium RS in *Mobile phase*
Sample solution: 0.3 mg/mL of Ceftriaxone Sodium in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for ceftriaxone and ceftriaxone *E*-isomer are 1.0 and 1.4, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the ceftriaxone and ceftriaxone *E*-isomer peaks, *System suitability solution*
Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 0.7%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the quantity, in µg/mg, of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$) in the portion of Ceftriaxone Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response of ceftriaxone from the *Sample solution*
r_S = peak response of ceftriaxone from the *Standard solution*
C_S = concentration of USP Ceftriaxone Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Ceftriaxone Sodium in the *Sample solution* (mg/mL)
P = potency of ceftriaxone in USP Ceftriaxone Sodium RS (µg/mg)

Acceptance criteria: NLT 795 µg/mg on the anhydrous basis

IMPURITIES

• **ORGANIC IMPURITIES**

Protect solutions containing ceftriaxone sodium from light.

Solution A, Solution B, Solution C, Buffer, Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 3 µg/mL of USP Ceftriaxone Sodium RS in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for ceftriaxone and ceftriaxone *E*-isomer are listed in *Table 1*.]

Suitability requirements

Resolution: NLT 3.0 between the ceftriaxone *E*-isomer and ceftriaxone peaks, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each individual impurity in the portion of Ceftriaxone Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of ceftriaxone from the *Standard solution*
C_S = concentration of USP Ceftriaxone Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Ceftriaxone Sodium in the *Sample solution* (mg/mL)
P = potency of ceftriaxone in USP Ceftriaxone Sodium RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 1*. Disregard any peak below 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deacetylcefotaxime lactone[a]	0.20	0.5
7-Aminocephalosporanic acid[b,c] (if present)	0.34	0.5
Ceftriaxone triazine analog[d]	0.62	1.0
Ceftriaxone benzothiazolyloxime[e]	0.72	0.2
Deacyl ceftriaxone[f]	0.78	0.5
Ceftriaxone	1.0	—
Ceftriaxone 3-ene isomer[g]	1.3	0.3
Ceftriaxone *E*-isomer[h]	1.4	0.5
Any individual unspecified impurity	—	0.2
Total impurities	—	2.5

[a] (*Z*)-2-(2-Aminothiazol-4-yl)-*N*-{(5a*R*,6*R*)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl}-2-(methoxyimino) acetamide.

[b] 7-ACA; (6*R*,7*R*)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid.

[c] To be reported if present in the impurity profile.

[d] 3-Mercapto-2-methyl-1,2-dihydro-1,2,4-triazine-5,6-dione.

[e] (*Z*)-*S*-Benzothiazol-2-yl 2-(2-aminothiazol-4-yl)-2-(methoxyimino) thioacetate.

[f] (6*R*,7*R*)-7-Amino-3-{[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[g] (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-{[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.

[h] (6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-{[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

SPECIFIC TESTS

• **CRYSTALLINITY** ⟨695⟩: Meets the requirements

• **pH** ⟨791⟩

Sample solution: 100 mg/mL

Acceptance criteria: 6.0–8.0

• **WATER DETERMINATION** ⟨921⟩, *Method I*: 8.0%–11.0%

• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Where the label states that it is sterile, it meets the requirements.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.20 USP Endotoxin Units/mg of ceftriaxone.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.

• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ceftriaxone Sodium RS

USP Ceftriaxone Sodium E-Isomer RS

(6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-{[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, disodium salt.

$C_{18}H_{16}N_8Na_2O_7S_3$ 598.53

www.webofpharma.com

Cefuroxime Injection

» Cefuroxime Injection is a sterile isoosmotic solution of Cefuroxime Sodium in Water for Injection. It contains one or more suitable buffers and a tonicity-adjusting agent. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefuroxime ($C_{16}H_{16}N_4O_8S$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Maintain in the frozen state.
Labeling—It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
USP Reference standards ⟨11⟩—
USP Cefuroxime Sodium RS
Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefuroxime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.10 USP Endotoxin Unit per mg of cefuroxime.
Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
pH ⟨791⟩: between 5.0 and 7.5.
Particulate Matter in Injections ⟨788⟩ : meets the requirements for small-volume injections.
Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩.

Assay—
pH 3.4 Acetate buffer, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Cefuroxime Sodium*
Assay preparation—Allow a container of Injection to thaw, and mix the solution. Transfer an accurately measured volume of the Injection, equivalent to about 50 mg of cefuroxime, to a 50-mL volumetric flask, dilute with water to volume, and mix. Immediately transfer 5.0 mL of this solution to a second 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Cefuroxime Sodium* Calculate the quantity, in mg, of cefuroxime ($C_{16}H_{16}N_4O_8S$) in each mL of the Injection taken by the formula:

$$1000(C/V)(R_U/R_S)$$

in which V is the volume, in mL, of Injection taken, and the other terms are as defined therein.

Cefuroxime for Injection

» Cefuroxime for Injection contains an amount of Cefuroxime Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefuroxime ($C_{16}H_{16}N_4O_8S$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.
USP Reference standards ⟨11⟩—
USP Cefuroxime Sodium RS
Constituted solution—At the time of use, the constituted solution for intravenous administration prepared from Cefuroxime for Injection meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.10 USP Endotoxin Unit per mg of cefuroxime.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
Uniformity of dosage units ⟨905⟩: meets the requirements.
Procedure for content uniformity—Perform the *Assay* on individual containers using *Assay preparation 1* or *Assay preparation 2*, or both, as appropriate.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements of the tests for *Identification, pH,* and *Water Determination, Method I 921:* under *Cefuroxime Sodium*. It meets also the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—
pH 3.4 Acetate buffer, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay*— under *Cefuroxime Sodium*.
Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Cefuroxime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution containing about 1 mg of cefuroxime per mL. Immediately transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix.
Assay preparation 2 (where the label states the quantity of cefuroxime in a given volume of constituted solution or suspension)—Constitute Cefuroxime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution or suspension quantitatively with water to obtain a solution containing about 1 mg of cefuroxime per mL. Immediately transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Cefuroxime Sodium*. Calculate the quantity, in mg, of cefuroxime ($C_{16}H_{16}N_4O_8S$) withdrawn from the container, or in the portion of constituted solution or suspension taken by the formula:

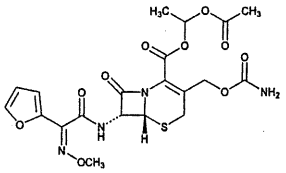
$$(L/D)(C)(R_U/R_S)$$

in which L is the labeled quantity, in mg of cefuroxime ($C_{16}H_{16}N_4O_8S$), in the container, or in the volume of constituted solution or suspension taken; D is the concentration, in mg of cefuroxime ($C_{16}H_{16}N_4O_8S$) per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution or suspension taken, respectively, and the extent of dilution; C is the concentration, in mg of cefuroxime ($C_{16}H_{16}N_4O_8S$) per mL, of the *Standard preparation;* and R_U and

www.webofpharma.com

R_S are the peak response ratios of cefuroxime to the internal standard obtained from the *Assay preparation* and the *Standard preparation,* respectively. Where the test for *Uniformity of dosage units* has been performed using the *Procedure for content uniformity,* use the average of these determinations as the *Assay* value.

Cefuroxime Axetil



$C_{20}H_{22}N_4O_{10}S$ 510.47

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[(aminocarbonyl)oxy]methyl]-7-[[2-furanyl(methoxyimino) acetyl]amino]-8-oxo-, 1-(acetyloxy)ethyl ester, [6*R*-[6α, 7β(*Z*)]]-;

(*RS*)-1-Hydroxyethyl (6*R*,7*R*)-7-[2-(2-furyl)glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, 7²-(*Z*)-(*O*-methyloxime), 1-acetate 3-carbamate [64544-07-6].

DEFINITION

Cefuroxime Axetil is a mixture of the diastereoisomers of cefuroxime axetil ($C_{20}H_{22}N_4O_{10}S$). It contains the equivalent of NLT 745 µg/mg and NMT 875 µg/mg of cefuroxime ($C_{16}H_{16}N_4O_8S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)**:** Meets the requirements
- **B.** The retention times of cefuroxime axetil diastereoisomers A and B of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 23 g/L of monobasic ammonium phosphate

Mobile phase: Methanol and *Solution A* (38:62)

System suitability stock solution: 0.1 mg/mL of USP Cefuroxime Axetil Delta-3 Isomers RS in methanol

System suitability solution: 10 µg/mL of cefuroxime axetil delta-3 isomers from *System suitability stock solution* and 0.2 mg/mL of USP Cefuroxime Axetil RS in *Mobile phase*

Standard solution: 0.2 mg/mL of USP Cefuroxime Axetil RS in *Mobile phase*. Protect the solution from light, refrigerate, and use on the day prepared.

Sample solution: 0.2 mg/mL of Cefuroxime Axetil in *Mobile phase*. Protect the solution from light, refrigerate, and use on the day prepared.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; 5-µm packing L13

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between cefuroxime axetil diastereoisomers A and B; NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*

Relative standard deviation: NMT 2.0% for the sum of cefuroxime axetil diastereoisomers A and B, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of cefuroxime ($C_{16}H_{16}N_4O_8S$) in the portion of Cefuroxime Axetil taken:

$$\text{Result} = (r_U/r_T) \times (C_S/C_U) \times P$$

r_U = sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Sample solution*

r_T = sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Standard solution*

C_S = concentration of USP Cefuroxime Axetil RS in the *Standard solution* (mg/mL)

C_U = concentration of Cefuroxime Axetil in the *Sample solution* (mg/mL)

P = potency of cefuroxime, on the anhydrous basis, in USP Cefuroxime Axetil RS (µg/mg)

Acceptance criteria: 745–875 µg/mg on the anhydrous basis

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Peak identification solution: 30 µg/mL of USP Cefuroxime Axetil E-Isomers RS in *Mobile phase*

Reference solution: 2 µg/mL of USP Cefuroxime Axetil RS in *Mobile phase*. Protect the solution from light, refrigerate, and use on the day prepared.

System suitability

Samples: *System suitability solution* and *Peak identification solution*

[NOTE—See *Table 1* for relative retention times. Use the *Peak identification solution* to identify the locations of the cefuroxime axetil *E*-isomers.]

Suitability requirements

Resolution: NLT 1.5 between cefuroxime axetil diastereoisomers A and B; NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*

Analysis

Samples: *Sample solution* and *Reference solution*

Calculate the percentage of each impurity in the portion of Cefuroxime Axetil taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_T = sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Sample solution*

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05 times the sum of the responses of the two major peaks from the *Reference solution*.

www.webofpharma.com

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methoxyiminofuranyl acetic acid[a]	0.24	0.30
Cefuroxime[b]	0.30	0.5
Cefuroxime lactone[c]	0.42	0.30
Cefuroxime axetil diastereoisomer B	0.9	—
Cefuroxime axetil diastereoisomer A	1.0	—
Cefuroxime axetil delta-3 isomers[d, e]	1.2	1.20
Cefuroxime axetil *E*-isomers[d, f]	1.7	1.0
	2.1	
Cefuroxime axetil dimer[g, h]	2.5	0.5
	3.4	
	3.8	
Any other individual impurity	—	0.30
Total impurities	—	3.0

[a] (*Z*)-2-(Furan-2-yl)-2-(methoxyimino)acetic acid.
[b] (6*R*,7*R*)-3-[(Carbamoyloxy)methyl]-7-[(*Z*)-2-(furan-2-yl)-2-(methoxyimino) acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[c] (*Z*)-*N*-((5a*R*,6*R*)-1,7-Dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*] [1,3]thiazin-6-yl)-2-(furan-2-yl)-2-(methoxyimino)acetamide.
[d] The system may resolve two isomers. The limit is for the sum of the two isomers.
[e] (1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate.
[f] (1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*E*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.
[g] The system may resolve three isomers. The limit is for the sum of the three isomers.
[h] (6*R*,6′*R*,7*R*,7′*R*,*Z*)-Oxybis(ethane-1,1-diyl)bis[3-[(carbamoyloxy)methyl]-7-[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate].

SPECIFIC TESTS

- **CRYSTALLINITY** ⟨695⟩: Particles that do not show birefringence or exhibit extinction positions are amorphous, and particles that show birefringence and exhibit extinction positions are crystalline.
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.5%
- **DIASTEREOISOMER RATIO**

Solution A, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the ratio of cefuroxime axetil diastereoisomer A to the sum of cefuroxime axetil diastereoisomers A and B:

$$\text{Result} = r_A/r_T$$

r_A = peak response of cefuroxime axetil diastereoisomer A
r_T = sum of the peak responses of cefuroxime axetil diastereoisomers A and B

Acceptance criteria: 0.48–0.55

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate whether it is amorphous or crystalline.

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Cefuroxime Axetil RS
USP Cefuroxime Axetil Delta-3 Isomers RS
(1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate.
$C_{20}H_{22}N_4O_{10}S$ 510.47
USP Cefuroxime Axetil E-Isomers RS
(1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*E*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.
$C_{20}H_{22}N_4O_{10}S$ 510.47

Cefuroxime Axetil for Oral Suspension

DEFINITION

Cefuroxime Axetil for Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of cefuroxime ($C_{16}H_{16}N_4O_8S$).

IDENTIFICATION

- The retention times of the major peaks for cefuroxime axetil diastereoisomers A and B of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 23 g/L of monobasic ammonium phosphate in water
Mobile phase: Methanol and *Solution A* (19:31)
System suitability stock solution A: 1.2 mg/mL of USP Cefuroxime Axetil RS in methanol
System suitability stock solution B: 0.16 mg/mL of USP Cefuroxime Axetil Delta-3-Isomers RS in methanol
System suitability solution: Transfer 10.0 mL of *System suitability stock solution A* to a 50-mL volumetric flask. Add 5.0 mL of methanol and 3.8 mL of *System suitability stock solution B*. Dilute with *Solution A* to volume.
Standard stock solution: 1.2 mg/mL of USP Cefuroxime Axetil RS in methanol. [NOTE—Use this solution promptly.]
Standard solution: Transfer 10.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add 8.8 mL of methanol, and dilute with *Solution A* to volume. [NOTE—Use this *Standard solution* promptly, or refrigerate and use on the day prepared.]
Sample stock solution: Equivalent to 2.5 mg/mL of cefuroxime, from constituted Oral Suspension, in methanol. Pass through a suitable filter. [NOTE—Constitute as directed on the label. To a suitable aliquot, freshly prepared and free of bubbles, add a suitable volume of methanol, shake by mechanical means for 10 min, dilute to volume with methanol, and mix.]
Sample solution: Transfer 5.0 mL of the filtered *Sample stock solution* to a 50-mL volumetric flask. Add 13.8 mL of methanol, and dilute with *Solution A* to volume. [NOTE—Protect the *Sample solution* from light and use promptly, or refrigerate and use on the day prepared.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 25-cm; 5-µm packing L13
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*

www.webofpharma.com

[NOTE—The relative retention times for acetanilide, cefuroxime axetil diastereoisomer B, cefuroxime axetil diastereoisomer A, and cefuroxime axetil delta-3 isomers are 0.4, 0.8, 0.9, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between cefuroxime axetil diastereoisomer A and B; NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*

Column efficiency: NLT 3000 theoretical plates when measured using the cefuroxime axetil diastereoisomer A peak, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{16}H_{16}N_4O_8S$ in the Cefuroxime Axetil for Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P \times F \times [1 - (K/100)] \times 100$$

R_U = sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Sample solution*

R_S = sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of cefuroxime axetil in the *Sample solution* (mg/mL)

P = potency of cefuroxime in anhydrous USP Cefuroxime Axetil RS (µg/mg)

F = unit conversion factor, 0.001 mg/µg

K = water content of USP Cefuroxime Axetil RS (%)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.07 M of pH 7.0 phosphate buffer (dissolve 3.7 mg/mL of monobasic sodium phosphate and 5.7 mg/mL of anhydrous dibasic sodium phosphate in water); 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Analysis: Test 5.0 mL of constituted Cefuroxime Axetil for Oral Suspension equivalent to 125 or 250 mg of cefuroxime. Determine the amount of cefuroxime equivalent dissolved by using UV absorption at the wavelength of maximum absorbance at 280 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Cefuroxime Axetil RS in the same *Medium*.

Tolerances: NLT 60% (Q) of the labeled amount of $C_{16}H_{16}N_4O_8S$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For solid packaged in single-unit containers: Constitute Cefuroxime Axetil for Oral Suspension as directed in the labeling. Mix, and allow the container to drain into a beaker for 5 s. Withdraw and assay 5.0 mL of the Oral Suspension from the beaker, or the total amount if it is less than 5 mL. It meets the requirements.

• **DELIVERABLE VOLUME** ⟨698⟩

For solid packaged in multiple-unit containers: Constitute Cefuroxime Axetil for Oral Suspension as directed in the labeling. It meets the requirements.

SPECIFIC TESTS

• **PH** ⟨791⟩**:** 3.5–7.0, in the solution constituted as directed in the labeling

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 6.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cefuroxime Axetil RS

USP Cefuroxime Axetil Delta-3-Isomers RS

Cefuroxime Axetil Tablets

DEFINITION

Cefuroxime Axetil Tablets contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of cefuroxime ($C_{16}H_{16}N_4O_8S$).

IDENTIFICATION

• **A.** The retention times of the peaks of cefuroxime axetil diastereoisomers A and B of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 23 g/L of monobasic ammonium phosphate in water

Mobile phase: Methanol and *Solution A* (38:62)

Buffer: 23 g/L of monobasic ammonium phosphate in water, adjusted with phosphoric acid to a pH of 2.4

System suitability stock solution: 0.1 mg/mL of USP Cefuroxime Axetil Delta-3 Isomers RS in methanol

System suitability solution: 10 µg/mL of cefuroxime axetil delta-3 isomers from *System suitability stock solution* and 0.3 mg/mL of USP Cefuroxime Axetil RS in *Mobile phase*

Standard solution: 0.3 mg/mL of cefuroxime axetil in *Mobile phase*. Protect the solution from light, refrigerate, and use on the day prepared.

Sample stock solution: Nominally 5 mg/mL of cefuroxime from finely powdered Tablets (NLT 5), prepared as follows. Transfer a suitable portion of the powder to a volumetric flask. Disperse in *Buffer*, using 5% of the final volume. Sonicate if necessary. Add methanol to fill the volumetric flask to about half its capacity and shake by mechanical means for about 10 min. Dilute with methanol to volume, and filter.

Sample solution: Nominally 0.25 mg/mL of cefuroxime from the *Sample stock solution* in *Mobile phase*. Protect the solution from light, refrigerate, and use on the day prepared.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; 5-µm packing L13

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 4* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between cefuroxime axetil diastereoisomers A and B; NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*

Relative standard deviation: NMT 2.0% for the sum of cefuroxime axetil diastereoisomers A and B, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of the labeled amount of cefuroxime ($C_{16}H_{16}N_4O_8S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (C_S/C_U) \times P \times F \times 100$$

r_U = sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Sample solution*
r_T = sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Standard solution*
C_S = concentration of USP Cefuroxime Axetil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cefuroxime in the *Sample solution* (mg/mL)
P = potency of cefuroxime, on the anhydrous basis, in USP Cefuroxime Axetil RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.07 N hydrochloric acid; 900 mL
Apparatus 2: 55 rpm
Times: 15 and 45 min
Standard solution: 10–20 µg/mL of cefuroxime from USP Cefuroxime Axetil RS in *Medium*
Sample solution: Pass portions of the solution under test through a suitable filter and dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 278 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage (Q) of the labeled amount of cefuroxime ($C_{16}H_{16}N_4O_8S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times P \times F \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Cefuroxime Axetil RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
P = potency of cefuroxime, on the anhydrous basis, in USP Cefuroxime Axetil RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria
For Tablets labeled to contain nominally 500 mg of cefuroxime: See *Table 1*.

Table 1

Time (min)	Amount Dissolved (%)
15	NLT 50
45	NLT 70

For all other Tablets: See *Table 2*.

Table 2

Time (min)	Amount Dissolved (%)
15	NLT 60
45	NLT 75

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium, Times, and **Analysis:** Proceed as directed in *Test 1*.
Apparatus 2: 100 rpm
Acceptance criteria: See *Table 3*.

Table 3

Time (min)	Amount Dissolved (%)
15	NLT 60
45	NLT 75

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Mobile phase, Buffer, System suitability solution, and **Sample solution:** Proceed as directed in the *Assay*.
Peak identification solution: 30 µg/mL of USP Cefuroxime Axetil E-Isomers RS in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 25-cm; 5-µm packing L13
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Peak identification solution*
[NOTE—See *Table 4* for the relative retention times. The *Peak identification solution* is used to identify the locations of the cefuroxime axetil *E*-isomers.]
Suitability requirements
Resolution: NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Sample solution*

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methoxyiminofuranyl acetic acid[a,b]	0.34	—

www.webofpharma.com

Table 4 *(continued)*

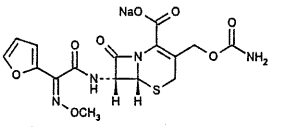
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cefuroxime[c]	0.41	0.70
Cefuroxime lactone[d]	0.52	0.30
Cefuroxime axetil diastereoisomer B	0.90	—
Cefuroxime axetil diastereoisomer A	1.0	—
Cefuroxime axetil delta-3 isomers[e,f]	1.1	1.20
Cefuroxime axetil *E*-isomers[e,g]	1.6	1.0
	1.9	
Cefuroxime axetil dimer[a,h,i]	2.6	—
	3.1	
	3.6	
Any other individual impurity	—	0.30
Total impurities	—	3.0

[a] Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total impurities.
[b] (*Z*)-2-(Furan-2-yl)-2-(methoxyimino)acetic acid.
[c] (6*R*,7*R*)-3-[(Carbamoyloxy)methyl]-7-[(*Z*)-2-(furan-2-yl)-2-(methoxyimino) acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
[d] (*Z*)-*N*-((5a*R*,6*R*)-1,7-Dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*] [1,3]thiazin-6-yl)-2-(furan-2-yl)-2-(methoxyimino)acetamide.
[e] The system may resolve two isomers. The limit is for the sum of the two isomers.
[f] (1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate.
[g] (1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*E*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.
[h] The system may resolve three isomers.
[i] (6*R*,6′*R*,7*R*,7′*R*,*Z*)-Oxybis(ethane-1,1-diyl)bis{3-[(carbamoyloxy)methyl]-7-[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate}.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** The labeling indicates whether the Tablets contain amorphous or crystalline Cefuroxime Axetil. If Tablets contain a mixture of amorphous and crystalline Cefuroxime Axetil, label to indicate the percentage of each contained therein. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cefuroxime Axetil RS
  USP Cefuroxime Axetil Delta-3 Isomers RS
  (1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate.
  $C_{20}H_{22}N_4O_{10}S$ 510.47
  USP Cefuroxime Axetil E-Isomers RS
  (1*RS*,6*R*,7*R*)-1-Acetoxyethyl 3-[(carbamoyloxy)methyl]-7-[(*E*)-2-(furan-2-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.
  $C_{20}H_{22}N_4O_{10}S$ 510.47

Cefuroxime Sodium



$C_{16}H_{15}N_4NaO_8S$ 446.37

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[(aminocarbonyl)oxy]methyl]-7-[[2-furanyl(methoxyimino) acetyl]amino]-8-oxo-, monosodium salt [6*R*-[6α,7β(*Z*)]]-.
Sodium (6*R*,7*R*)-7-[2-(2-furyl)glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, 7 [2] -(*Z*)-(*O*-methyloxime), carbamate (ester) [56238-63-2].

» Cefuroxime Sodium contains the equivalent of not less than 855 µg and not more than 1000 µg of cefuroxime ($C_{16}H_{16}N_4O_8S$), calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Cefuroxime Sodium RS

Identification—
A: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefuroxime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.
B: It responds to the tests for *Sodium* ⟨191⟩.
pH ⟨791⟩: between 6.0 and 8.5, in a solution (1 in 10).
Water Determination, *Method I* ⟨921⟩: not more than 3.5%.
Other requirements—Where the label states that Cefuroxime Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cefuroxime for Injection* Where the label states that Cefuroxime Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cefuroxime for Injection*

Assay—
pH 3.4 acetate buffer—Transfer 50 mL of 0.1 M sodium acetate to a 1000-mL volumetric flask, dilute with 0.1 N acetic acid to volume, and mix.
Mobile phase—Prepare a suitable mixture of *pH 3.4 acetate buffer* and acetonitrile (about 10:1). Filter through a membrane filter (1 µm or finer porosity), and degas.
Internal standard solution—Prepare a solution of orcinol in water containing 1.5 mg per mL.
Standard preparation—Dissolve a suitable quantity of USP Cefuroxime Sodium RS, accurately weighed, in water to obtain a solution having a known concentration of about 1 mg of cefuroxime ($C_{16}H_{16}N_4O_8S$) per mL. Immediately transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix. This *Standard preparation* contains about 0.05 mg of cefuroxime per mL.
Assay preparation—Using a suitable quantity of Cefuroxime Sodium, accurately weighed, proceed as directed in the first sentence under *Standard preparation*. Immediately transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add

www.webofpharma.com

20.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L15. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the column efficiency determined from the analyte peak is not less than 1300 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; the resolution, *R*, between the analyte and internal standard peaks is not less than 3.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for cefuroxime and 1.0 for orcinol. Calculate the quantity, in µg, of cefuroxime per mg of the Cefuroxime Sodium taken by the formula:

$$1000(C/M)(R_U/R_S)$$

in which C is the concentration, in mg of cefuroxime ($C_{16}H_{16}N_4O_8S$) per mL, in the *Standard preparation;* M is the concentration, in mg per mL, in the *Assay preparation* based on the weight of Cefuroxime Sodium taken and the extent of dilution; and R_U and R_S are the peak response ratios of the cefuroxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Celecoxib

$C_{17}H_{14}F_3N_3O_2S$ 381.4

4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl] benzenesulfonamide;

p-[5-*p*-Tolyl-3-(trifluoromethyl)pyrazol-1-yl] benzenesulfonamide [169590-42-5].

DEFINITION

Celecoxib contains NLT 98.0% and NMT 102.0% of $C_{17}H_{14}F_3N_3O_2S$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K or 197M▲ (CN 1-May-2020)
  [NOTE—If the spectra obtained show differences, dissolve the substance to be examined and the Reference Standard separately in isopropyl alcohol, evaporate to dryness, and record the new spectra.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 2.7 g/L of monobasic potassium phosphate adjusted with phosphoric acid to a pH of 3.0 ± 0.2

Mobile phase: Methanol, acetonitrile, and *Buffer* (3:1:6)

Diluent: Methanol and water (3:1)

System suitability solution: 0.5 mg/mL of USP Celecoxib RS and 2.4 µg/mL each of USP Celecoxib Related Compound A RS and USP Celecoxib Related Compound B RS in *Diluent*

Standard solution: 0.5 mg/mL of USP Celecoxib RS in *Diluent*

Sample solution: 0.5 mg/mL of Celecoxib in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-µm packing L11

Column temperature: 60°

Flow rate: 1.5 mL/min

Injection size: 25 µL

Run time: About 1.5 times the celecoxib peak elution

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.8 between celecoxib related compound A and celecoxib and NLT 1.8 between celecoxib and celecoxib related compound B, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{17}H_{14}F_3N_3O_2S$ in the portion of Celecoxib taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

INORGANIC IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%, using a platinum crucible

ORGANIC IMPURITIES

- **Procedure**

Buffer, Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.5 µg/mL of USP Celecoxib RS in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.8 between celecoxib related compound A and celecoxib and NLT 1.8 between celecoxib and celecoxib related compound B, *System suitability solution*

Signal-to-noise ratio: NLT 20, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Celecoxib taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity in the *Sample solution*

r_S = peak response of celecoxib in the *Standard solution*

C_S = concentration of celecoxib in the *Standard solution* (mg/mL)

www.webofpharma.com

C_U = concentration of Celecoxib in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurities: See *Table 1*. [NOTE—Disregard any impurity peak less than 0.05%.]

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Celecoxib related compound A[a]	0.9	0.4
Celecoxib	1.0	—
Celecoxib related compound B[b]	1.1	0.10
Individual unspecified impurity	—	0.10
Total impurities	—	0.5

[a] 4-[5-(3-Methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl] benzenesulfonamide.
[b] 4-[3-(4-Methylphenyl)-5-(trifluoromethyl)-1*H*-pyrazol-1-yl] benzenesulfonamide.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%, using a 400-mg sample

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and moisture. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Celecoxib RS
p-[5-*p*-Tolyl-3-(trifluoromethyl)pyrazol-1-yl] benzenesulfonamide.
$C_{17}H_{14}F_3N_3O_2S$ 381.4

USP Celecoxib Related Compound A RS
4-[5-(3-Methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide.
$C_{17}H_{14}F_3N_3O_2S$ 381.4

USP Celecoxib Related Compound B RS
4-[3-(4-Methylphenyl)-5-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide.
$C_{17}H_{14}F_3N_3O_2S$ 381.4

Oxidized Cellulose

DEFINITION

Oxidized Cellulose contains NLT 16.0% and NMT 24.0% of carboxyl groups (COOH), calculated on the dried basis. It is sterile.

IDENTIFICATION

• **A.**

Sample solution: 200 mg in 10 mL of 0.25 N sodium hydroxide

Analysis 1: Shake the *Sample solution* for 1 min. Add 10 mL of water, and shake.

Acceptance criteria 1: The *Sample solution* shows no more than a slight haze and is substantially free from fibers and foreign particles.

Analysis 2: Allow the resulting solution to stand for 10 min.

Acceptance criteria 2: Any swollen fibers initially present are no longer visible.

Analysis 3: Acidify the resulting solution with 3 N hydrochloric acid.

Acceptance criteria 3: A flocculent white precipitate is formed.

ASSAY

• **PROCEDURE**

Solution A: 20 mg/mL of calcium acetate

Sample: 500 mg, previously dried under vacuum over phosphorus pentoxide for 18 h

Blank: 50.0 mL of *Solution A*

Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N sodium hydroxide VS
Endpoint detection: Visual

Analysis: Place the *Sample* in a 125-mL conical flask. Add 50.0 mL of *Solution A*, swirl until the sample is completely covered, allow the mixture to stand for 30 min, then add phenolphthalein TS. Titrate the solution with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 4.502 mg of carboxyl groups (COOH).

Acceptance criteria: 16.0%–24.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.15%

• **LIMIT OF NITROGEN**

Solution A: 40 mg/mL of boric acid

Solution B: Methyl red TS and bromocresol green TS (1:4)

Sample: 1 g, previously dried under vacuum over phosphorus pentoxide for 18 h

Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.02 N sulfuric acid VS
Endpoint detection: Visual

Analysis: Place a 125-mL conical flask, containing 30 mL of *Solution A* and 6 drops of *Solution B*, beneath the condenser of the distillation apparatus so that the tip of the condenser is well below the surface of the resulting solution. To a 500-mL Kjeldahl flask, add the *Sample*, and add 1 g of Devarda's alloy, 100 mL of recently boiled water, a small lump of paraffin, and 100 mL of 1 N sodium hydroxide. Connect the Kjeldahl flask to the condenser by a suitable trap bulb. Heat the mixture in the flask until 45–50 mL of distillate has collected in the receiver. Rinse the condenser, and titrate the resulting solution with *Titrant* to a pale pink endpoint. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 0.2801 mg of nitrogen.

Acceptance criteria: NMT 0.5%

• **LIMIT OF FORMALDEHYDE**

Solution A: Formaldehyde in water (1 in 40,000)

Standard: 0.50 mL of *Solution A*

Sample: 500 mg

Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Vis
Analytical wavelength: 570 nm
Blank: Mixture of 0.5 mL of water and 10 mL of chromotropic acid TS

Analysis: Transfer the *Sample* to a 500-mL iodine flask. Add 250 mL of water, and allow to stand for NLT 2 h with intermittent shaking. Pipet 0.50 mL each of the supernatant from the resulting solution and the *Standard* into two separate glass-stoppered test tubes. To each test tube add 10 mL of chromotropic acid TS. Stopper the tubes loosely, and heat in a boiling water bath for 30 min. Cool, and determine the absorbance of each solution against the *Blank*.

USP Monographs

www.webofpharma.com

Acceptance criteria: 0.5%; the absorbance of the *Sample* is NMT the *Standard*.

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩

Sample: 250 mg

Analysis: Proceed as directed in the chapter, adding 0.5 mL of 0.1 N sodium hydroxide to the portions of media used.

Acceptance criteria: Meets the requirements

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum over phosphorus pentoxide for 18 h.

Acceptance criteria: NMT 15.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, protected from direct sunlight. Store in a cold place.
- **LABELING:** The package bears a statement to the effect that the sterility of Oxidized Cellulose cannot be guaranteed if the package bears evidence of damage, or if the package has been previously opened. Oxidized Cellulose meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Oxidized Regenerated Cellulose

DEFINITION

Oxidized Regenerated Cellulose contains NLT 18.0% and NMT 24.0% of carboxyl groups (COOH), calculated on the dried basis. It is sterile.

IDENTIFICATION

- **A.**

Sample solution: 200 mg in 10 mL of 0.25 N sodium hydroxide

Analysis 1: Shake the *Sample solution* for 1 min. Add 10 mL of water, and shake.

Acceptance criteria 1: The *Sample solution* shows no more than a slight haze and is substantially free from fibers and foreign particles.

Analysis 2: Allow the resulting solution to stand for 10 min.

Acceptance criteria 2: Any swollen fibers initially present are no longer visible.

Analysis 3: Acidify the resulting solution with 3 N hydrochloric acid.

Acceptance criteria 3: A flocculent white precipitate is formed.

ASSAY

- **PROCEDURE**

Sample: 1 g of Oxidized Regenerated Cellulose, previously dried at 90° for 2 h

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N hydrochloric acid VS

Endpoint detection: Visual

Analysis: Place the *Sample* in a 250-mL conical flask, add 10 mL of 0.5 N sodium hydroxide VS, swirl to dissolve, and add 100 mL of water. Immediately titrate with *Titrant* to a phenolphthalein endpoint. Perform a blank determination, and note the difference in volumes required. Each mL of the difference in volumes of 0.1 N hydrochloric acid consumed is equivalent to 4.50 mg of carboxyl groups (COOH).

Acceptance criteria: 18.0%–24.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.15%
- **LIMIT OF NITROGEN**

Solution A: 40 mg/mL of boric acid

Solution B: Methyl red TS and bromocresol green TS (1:4)

Sample: 1 g, previously dried

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.02 N sulfuric acid VS

Endpoint detection: Visual

Analysis: Place a 125-mL conical flask, containing 30 mL of *Solution A* and 6 drops of *Solution B*, beneath the condenser of the distillation apparatus so that the tip of the condenser is well below the surface of the resulting solution. To a 500-mL Kjeldahl flask add the *Sample*, and add 1 g of Devarda's alloy, 100 mL of recently boiled water, a small lump of paraffin, and 100 mL of 1 N sodium hydroxide. Connect the Kjeldahl flask to the condenser by a suitable trap bulb. Heat the mixture in the flask until 45–50 mL of distillate has collected in the receiver. Rinse the condenser, and titrate the resulting solution with *Titrant* to a pale pink endpoint that persists for 30 s. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 0.2801 mg of nitrogen.

Acceptance criteria: NMT 0.5%

- **LIMIT OF FORMALDEHYDE**

Solution A: Formaldehyde in water (1 in 40,000)

Standard: 0.50 mL of *Solution A*

Sample: 500 mg

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Vis

Analytical wavelength: 570 nm

Blank: Mixture of 0.5 mL of water and 10 mL of chromotropic acid TS

Analysis: Transfer the *Sample* to a 500-mL iodine flask. Add 250 mL of water, and allow to stand for NLT 2 h with intermittent shaking. Pipet 0.50 mL each of the supernatant from the resulting solution and the *Standard* into two separate glass-stoppered test tubes. To each test tube add 10 mL of chromotropic acid TS. Stopper the tubes loosely, and heat in a boiling water bath for 30 min. Cool, and determine the absorbance of each solution against the *Blank*.

Acceptance criteria: 0.5% CH_2O; the absorbance of the *Sample* is NMT the *Standard*.

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩

Sample: 250 mg

Analysis: Proceed as directed in the chapter, adding 0.5 mL of 0.1 N sodium hydroxide to the portions of media used.

Acceptance criteria: Meets the requirements

- **LOSS ON DRYING** ⟨731⟩

Sample: 150 mg

Analysis: Dry at 90° for 2 h.

Acceptance criteria: NMT 15.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, protected from direct sunlight. Store at controlled room temperature.
- **LABELING:** The package bears a statement to the effect that the sterility of Oxidized Regenerated Cellulose cannot be guaranteed if the package bears evidence of damage, or if the package has been previously opened. Oxidized Regenerated Cellulose meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

USP Monographs

www.webofpharma.com

Cellulose Sodium Phosphate

» Cellulose Sodium Phosphate is prepared by phosphorylation of alpha cellulose. It has an inorganic bound phosphate content of not less than 31.0 percent and not more than 36.0 percent, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

pH ⟨791⟩—Place 3 g of it in a 100-mL beaker, add 60 mL of water, and stir occasionally for 5 minutes. Filter through a sintered-glass crucible. The pH of the filtrate is between 6.0 and 9.0.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 10.0% of its weight.

Nitrogen Determination ⟨461⟩, *Method I*: not more than 1.0%.

Calcium binding capacity—

Standard calcium solution—Transfer about 0.33 g of dried calcium carbonate, primary standard grade, accurately weighed, to a 250-mL beaker with the aid of a few mL of water, and dilute with water to about 50 mL. Carefully and dropwise add 2 N hydrochloric acid until all of the solid dissolves, and add 2 drops in excess. Heat the solution to boiling, and boil for 5 minutes. Cool the solution, and transfer to a 1000-mL volumetric flask. Dilute with water to volume, and mix. Calculate the molarity, *M*, of the solution taken by the formula:

$$g/100.09$$

in which *g* is the weight, in g, of calcium carbonate taken.

Standard edetate disodium titrant—Dissolve 10 g of edetate disodium in 100 mL of water. Slowly add alcohol until the first permanent precipitate is formed. Filter, and discard the solid. Add an equal volume of alcohol to the filtrate. Filter the resulting precipitate, discard the filtrate, and wash the residue on the filter, first with acetone, then with ethyl ether. Dry at 80° for 4 days at about 50% relative humidity. Transfer about 3.72 g of this purified edetate disodium, accurately weighed, to a 1000-mL volumetric flask, and dissolve with water. Dilute with water to volume, and mix. Calculate the molarity, M_S, of the solution taken by the formula:

$$w/372.24$$

in which *w* is the weight, in g, of the purified edetate disodium taken.

Procedure—Transfer 0.15 ± 0.02 g of Cellulose Sodium Phosphate, accurately weighed, to a 250-mL beaker. Add 150.0 mL of *Standard calcium solution*, and stir the mixture for 5 minutes on a magnetic stirrer. Filter, discarding the first few mL of the filtrate. To 50.0 mL of the filtrate add about 50 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue. Titrate with *Standard edetate disodium titrant* to a permanent deep blue color, and designate the number of mL consumed as V_S. Calculate the calcium binding capacity of the undried Cellulose Sodium Phosphate, in mmol per g, by the formula:

$$(150M - 3V_S M_S)/W$$

in which *W* is the weight, in g, of Cellulose Sodium Phosphate taken; and the other terms are as defined above. The calcium binding capacity, calculated on the dried basis, is not less than 1.8 mmol per g.

Free phosphate—

Standard preparation—Prepare as directed under *Inorganic bound phosphate*.

Test preparation—Transfer about 2 g of Cellulose Sodium Phosphate, accurately weighed, to a 250-mL beaker. Add 100 mL of water, accurately measured, stir, allow to stand for 5 minutes, stir again, and filter through moderately retentive filter paper, collecting the filtrate in a dry flask.

Procedure—Transfer 2.0 mL of the *Standard preparation* and 5.0 mL of the *Test preparation* to separate 100-mL volumetric flasks. Proceed as directed in the *Procedure* under *Inorganic bound phosphate*, beginning with "Treat each of these." Calculate the percentage of free phosphate taken by the formula:

$$(4000/W)(A_U/A_S)$$

in which *W* is the weight, in mg, of undried Cellulose Sodium Phosphate taken: not more than 3.5%, calculated on the dried basis, is found.

Sodium content—

Standard stock solution—Dissolve 508.5 mg of sodium chloride, previously dried at 105° for 2 hours, in 100 mL of water, transfer to a 1000-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains 200 µg of sodium.

Standard preparations—Transfer 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks, dilute each with water to volume, and mix.

Test preparation—Dissolve about 250 mg of Cellulose Sodium Phosphate, accurately weighed, in 10 mL of a mixture of 20 mL of perchloric acid and 15 mL of nitric acid. Heat cautiously to the production of dense, white fumes, cool, transfer to a 1000-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the emission of the *Test preparation* and of each *Standard preparation* at the sodium emission line of 589 nm, with a suitable flame photometer. Plot the emissions of the *Standard preparations* versus their concentration of sodium, and draw a straight line best fitting the four plotted points. From the graph so obtained determine the concentration of sodium in the *Test preparation*. Calculate the percentage of sodium in the undried Cellulose Sodium Phosphate. The content of sodium, calculated on the dried basis, is not less than 9.5% and not more than 13.0%.

Inorganic bound phosphate—

Standard preparation—Transfer 358.2 mg of monobasic potassium phosphate, primary standard grade, to a 250-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Each mL of this solution contains 1.0 mg of phosphate.

Test preparation—Transfer about 250 mg of Cellulose Sodium Phosphate, accurately weighed, to a 250-mL conical flask. Rinse to the bottom with a few mL of water. Add 10 mL of a mixture of 20 mL of perchloric acid and 15 mL of nitric acid. Heat cautiously to the production of dense, white fumes, cool the clear, almost colorless, solution, and transfer to a 100-mL volumetric flask with the aid of water. Dilute with water to volume, and mix.

Procedure—Transfer 2.0-mL portions of the *Standard preparation* and the *Test preparation* to separate 100-mL volumetric flasks. Treat each of these and a third flask, providing the blank, as follows: Add 10 mL of 5 N nitric acid, 10.0 mL of ammonium vanadate TS, and about 60 mL of water. Swirl, and add 10.0 mL of a freshly prepared solution of 2.5 g of ammonium molybdate in 50 mL of warm water. Dilute with water to volume, and mix. Concomitantly

USP Monographs

www.webofpharma.com

determine the absorbances, A_U and A_S, of the solutions from the *Standard preparation* and the *Test preparation*, respectively, at 400 nm with a suitable spectrophotometer, using the reagent blank to set the instrument. Calculate the percentage of total phosphate taken by the formula:

$$(10{,}000/W)(A_U/A_S)$$

in which W is the weight, in mg, of Cellulose Sodium Phosphate taken. Calculate the percentage of inorganic bound phosphate in the undried Cellulose Sodium Phosphate by subtracting from this result the percentage of *Free phosphate*.

Cellulose Sodium Phosphate for Oral Suspension

» Cellulose Sodium Phosphate for Oral Suspension contains Cellulose Sodium Phosphate. It has an inorganic bound phosphate content of not less than 28.0 percent and not more than 36.0 percent calculated on the dried basis.

Packaging and storage—Preserve in tight containers. Store in a refrigerator.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 10.0% of its weight.

Calcium binding capacity—

Standard calcium solution and *Standard edetate disodium titrant*—Proceed as directed for *Calcium binding capacity* under *Cellulose Sodium Phosphate*.

Procedure—Transfer an accurately weighed amount of Oral Suspension, equivalent to about 0.15 g of cellulose sodium phosphate, to a 250-mL beaker. Proceed as directed for *Procedure* in *Calcium binding capacity* under *Cellulose Sodium Phosphate*, beginning with "Add 150.0 mL of *Standard calcium solution*." Calculate the calcium binding capacity, in mmol per g, of the portion of undried Cellulose Sodium Phosphate for Oral Suspension taken by the formula:

$$(150M_S - 3V_SM_S)/W$$

in which W is the weight, in g, of Cellulose Sodium Phosphate for Oral Suspension taken; and the other terms are as defined therein: not less than 1.8 mmol per g, calculated on the dried basis, is found.

Free phosphate—

Standard preparation—Proceed as directed for *Free phosphate* under *Cellulose Sodium Phosphate*.

Test preparation—Transfer an accurately weighed amount of Cellulose Sodium Phosphate for Oral Suspension, equivalent to about 2 g of cellulose sodium phosphate, to a 250-mL beaker. Proceed as directed for *Free phosphate* under *Cellulose Sodium Phosphate*, beginning with "Add 100 mL of water."

Procedure—Proceed as directed for *Free phosphate* under *Cellulose Sodium Phosphate*. Calculate the percentage of free phosphate in the portion of Cellulose Sodium Phosphate for Oral Suspension taken by the formula:

$$(4000/W)(A_U/A_S)$$

in which W is the weight, in mg, of undried Cellulose Sodium Phosphate for Oral Suspension taken; and the other terms are as defined therein: not more than 6.0%, calculated on the dried basis, is found.

Inorganic bound phosphate—

Standard preparation—Proceed as directed for *Inorganic bound phosphate* under *Cellulose Sodium Phosphate*.

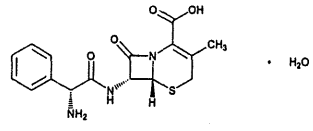
Test preparation—Transfer an accurately weighed amount of Cellulose Sodium Phosphate for Oral Suspension, equivalent to about 250 mg of cellulose sodium phosphate, to a 250-mL conical flask. Proceed as directed for *Test preparation* in *Inorganic bound phosphate* under *Cellulose Sodium Phosphate*, beginning with "Rinse to the bottom."

Procedure—Proceed as directed for *Inorganic bound phosphate* under *Cellulose Sodium Phosphate*. Calculate the percentage of total phosphate in the portion of Cellulose Sodium Phosphate for Oral Suspension taken by the formula:

$$(10{,}000/W)(A_U/A_S)$$

in which W is the weight, in mg, of Cellulose Sodium Phosphate for Oral Suspension taken; and the other terms are as defined therein. Calculate the percentage of inorganic bound phosphate in the portion of undried Cellulose Sodium Phosphate for Oral Suspension by subtracting from this result the percentage of free phosphate.

Cephalexin



$C_{16}H_{17}N_3O_4S \cdot H_2O$ 365.40

$C_{16}H_{17}N_3O_4S$ 347.40

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-methyl-8-oxo-, monohydrate, [6*R*-[6α,7β (*R**)]]-;
(6*R*,7*R*)-7-[(*R*)-2-Amino-2-phenylacetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate [23325-78-2].
Anhydrous [15686-71-2].

DEFINITION

Cephalexin has a potency of NLT 950 µg/mg and NMT 1030 µg/mg of $C_{16}H_{17}N_3O_4S$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: 0.985 g/L of sodium-1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of 3.0 ± 0.1

Standard stock solution: 1 mg/mL of USP Cephalexin RS in water

Standard solution: 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*

www.webofpharma.com

Sample stock solution: 1 mg/mL of Cephalexin in water
Sample solution: 0.4 mg/mL of Cephalexin in *Mobile phase* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1 of low acidity
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg, of cephalexin ($C_{16}H_{17}N_3O_4S$) per mg of the Cephalexin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cephalexin RS in the *Standard solution* (mg/mL)
C_U = concentration of Cephalexin in the *Sample solution* (mg/mL)
P = potency of cephalexin in USP Cephalexin RS (µg/mg)

Acceptance criteria: 950–1030 µg/mg on the anhydrous basis

IMPURITIES

ORGANIC IMPURITIES

• **Procedure 1**
Solution A: Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 1000 mL of water and 15 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5 ± 0.1.
Solution B: Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 300 mL of water and 15 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5 ± 0.1, and add 350 mL of acetonitrile and 350 mL of methanol.
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
1	100	0
33.3	0	100
34.3	0	100

Diluent: 18 mg/mL of monobasic potassium phosphate in water
Standard solutions: 0.08 mg/mL and 0.16 mg/mL of cephalexin ($C_{16}H_{17}N_3O_4S$) from USP Cephalexin RS in *Diluent*, taking into account the stated potency of the USP Cephalexin RS
Sample solution: 5 mg/mL of Cephalexin in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1 of low acidity
Flow rate: 1 mL/min
Injection size: 20 µL
Analysis
Samples: *Standard solutions* and *Sample solution*
Plot the responses of the cephalexin peaks from the *Standard solutions* versus their concentrations, calculated on the anhydrous basis, in mg/mL, and draw a straight line through the two points and zero. From the line and the peak responses of the *Sample solution*, determine the concentration, I, in mg/mL, of each cephalexin-related substance of the *Sample solution* other than the cephalexin peak.
Calculate the percentage of each cephalexin-related substance:

$$\text{Result} = I/C \times 100$$

I = concentration of each cephalexin-related substance in the *Sample solution* as determined from the calibration curve (mg/mL)
C = concentration of cephalexin from the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: NMT 1.0% of any individual cephalexin-related substance
Total impurities: NMT 5.0%
• **Procedure 2: Dimethylaniline** ⟨223⟩: Meets the requirement

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩: +149° to +158°
Sample solution: 5 mg/mL, in pH 4.4 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)
• **CRYSTALLINITY** ⟨695⟩: Meets the requirements
• **pH** ⟨791⟩: 3.0–5.5, in an aqueous suspension containing 50 mg/mL
• **WATER DETERMINATION,** *Method I* ⟨921⟩: 4.0%–8.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cephalexin RS

Cephalexin Capsules

DEFINITION

Cephalexin Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cephalexin ($C_{16}H_{17}N_3O_4S$).

IDENTIFICATION

• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Mobile phase: 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of 3.0 ± 0.1
Standard stock solution: 1 mg/mL of USP Cephalexin RS in water
Standard solution: 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*
Sample stock solution: Equivalent to 1 mg/mL of cephalexin from combined contents of NLT 20 Capsules in

www.webofpharma.com

water. Sonicate, if necessary, to dissolve the cephalexin. Filter, if necessary, to obtain a clear solution.
Sample solution: 0.4 mg/mL of cephalexin in *Mobile phase* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1 of low acidity
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cephalexin ($C_{16}H_{17}N_3O_4S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cephalexin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cephalexin in the *Sample solution* (mg/mL)
P = potency of cephalexin in USP Cephalexin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Standard solution: 20 µg/mL of USP Cephalexin RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration of about 20 µg/mL.
Spectrometric conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 262 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 80% (Q) of the labeled amount of cephalexin ($C_{16}H_{17}N_3O_4S$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Cephalexin RS

Cephalexin for Oral Suspension

DEFINITION
Cephalexin for Oral Suspension is a dry mixture of Cephalexin and one or more suitable buffers, colors, diluents, and flavors. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of $C_{16}H_{17}N_3O_4S$ per mL when constituted as directed in the labeling.

IDENTIFICATION
• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of 3.0 ± 0.1
Standard stock solution: 1 mg/mL of USP Cephalexin RS in water
Standard solution: Mix 10.0 mL of *Standard stock solution* with 15.0 mL of *Mobile phase*.
Sample stock solution: Nominally equivalent to 1 mg/mL of cephalexin from Oral Suspension, constituted as directed in the labeling, freshly mixed and free from air bubbles. Sonicate, if necessary, to assure complete dissolution of the cephalexin. Filter, if necessary, to obtain a clear solution.
Sample solution: Mix 10.0 mL of *Sample stock solution* and 15.0 mL of *Mobile phase*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1 of low acidity
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cephalexin ($C_{16}H_{17}N_3O_4S$) in each mL of the constituted Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cephalexin RS in the *Standard stock solution* (mg/mL)
C_U = nominal concentration of cephalexin from the *Sample stock solution* (mg/mL)
P = potency of USP Cephalexin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
• **Uniformity of Dosage Units** ⟨905⟩ For solid packaged in single-unit containers: meets the requirements
• **Deliverable Volume** ⟨698⟩: Meets the requirements

SPECIFIC TESTS
• **pH** ⟨791⟩: 3.0–6.0, constituted as directed in the labeling

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Cephalexin RS

USP Monographs

www.webofpharma.com

Cephalexin Tablets

DEFINITION
Cephalexin Tablets are prepared from Cephalexin or Cephalexin Hydrochloride. They contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cephalexin ($C_{16}H_{17}N_3O_4S$).

IDENTIFICATION
• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170). Adjust with phosphoric acid to a pH of 3.0 ± 0.1.
Standard stock solution: 1 mg/mL of USP Cephalexin RS in water
Standard solution: 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*
Sample stock solution: Equivalent to 1 mg/mL of cephalexin from combined contents of powdered Tablets (NLT 20) in water. Sonicate, if necessary, to assure complete dissolution of the cephalexin. Filter, if necessary, to obtain a clear solution.
Sample solution: 0.4 mg/mL of cephalexin in *Mobile phase* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1 of low acidity
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cephalexin ($C_{16}H_{17}N_3O_4S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cephalexin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cephalexin in the *Sample solution* (mg/mL)
P = potency of cephalexin in USP Cephalexin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
For Cephalexin
Medium: Water; 900 mL
Apparatus 1: Use 40-mesh cloth and 100 rpm
Time: 30 min
Standard solution: 20 µg/mL of USP Cephalexin RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute, if necessary, with *Medium* to a concentration that is similar to the *Standard solution*.
Spectrometric conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 262 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 80% (Q) of the labeled amount of cephalexin ($C_{16}H_{17}N_3O_4S$) is dissolved.
For Cephalexin hydrochloride
Medium, Standard solution, Sample solution, Spectrometric conditions, and **Analysis:** Proceed as directed *For Cephalexin*.
Apparatus 1: Use 10-mesh cloth and 150 rpm.
Time: 45 min
Tolerances: NLT 75% (Q) of the labeled amount of cephalexin ($C_{16}H_{17}N_3O_4S$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** The label states whether the Tablets contain Cephalexin or Cephalexin Hydrochloride.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cephalexin RS

Cephalexin Tablets for Oral Suspension

DEFINITION
Cephalexin Tablets for Oral Suspension contain NLT 90.0% and NMT 110.0% of the labeled amount of cephalexin ($C_{16}H_{17}N_3O_4S$).

IDENTIFICATION
• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of 3.0 ± 0.1
Standard stock solution: 1 mg/mL of USP Cephalexin RS in water
Standard solution: 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*
Sample stock solution: Nominally equivalent to 1 mg/mL of cephalexin from combined contents of NLT 20 powdered Tablets for Oral Suspension in water. Pass a portion of the solution through a filter having a 1-µm or finer pore size.
Sample solution: 0.4 mg/mL of cephalexin in *Mobile phase* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1 of low acidity
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*

www.webofpharma.com

Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cephalexin ($C_{16}H_{17}N_3O_4S$) in each Tablet for Oral Suspension:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cephalexin RS in the *Standard stock solution* (mg/mL)
C_U = nominal concentration of cephalexin in the *Sample stock solution* (mg/mL)
P = potency of cephalexin in USP Cephalexin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISINTEGRATION** ⟨701⟩**:** Tablets for Oral Suspension disintegrate in 3 min, using water at 20 ± 5°.
- **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: Use 40-mesh cloth and 100 rpm.
Time: 30 min
Standard solution: 20 µg/mL of USP Cephalexin RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration of about 20 µg/mL.
Spectrometric conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 262 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 80% (Q) of the labeled amount of cephalexin ($C_{16}H_{17}N_3O_4S$) is dissolved.
- **DISPERSION FINENESS:** Place 2 Tablets for Oral Suspension in 100 mL of water, and stir until completely dispersed. A smooth dispersion is obtained that passes through a No. 25 sieve.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Cephalexin RS

Cephalexin Hydrochloride

$C_{16}H_{17}N_3O_4S \cdot HCl \cdot H_2O$ 401.87
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-methyl-8-oxo-, monohydrochloride, monohydrate, [6*R*-[6α,7β (*R**)]]-;
(6*R*,7*R*)-7-[(2*R*)-2-Amino-2-phenylacetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monohydrochloride, monohydrate;
7-(D-2-Amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid hydrochloride monohydrate [105879-42-3].

DEFINITION
Cephalexin Hydrochloride contains the equivalent of NLT 800 µg/mg and NMT 880 µg/mg of cephalexin ($C_{16}H_{17}N_3O_4S$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** 10 mg/mL meets the requirements

ASSAY
- **PROCEDURE**
Mobile phase: 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of 3.0 ± 0.1
Standard stock solution: 1 mg/mL of USP Cephalexin RS in water
Standard solution: 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*
Sample stock solution: 1.15 mg/mL of Cephalexin Hydrochloride in water
Sample solution: 0.4 mg/mL of cephalexin in *Mobile phase* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1 of low acidity
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg, of cephalexin ($C_{16}H_{17}N_3O_4S$) in each mg of Cephalexin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cephalexin RS in the *Standard stock solution* (mg/mL)
C_U = concentration of Cephalexin Hydrochloride from the *Sample stock solution* (mg/mL)
P = potency of cephalexin in USP Cephalexin RS (µg/mg)

Acceptance criteria: 800–880 µg/mg

IMPURITIES
ORGANIC IMPURITIES
- **Procedure 1**
Solution A: 1 g of sodium 1-pentanesulfonate in a mixture of 1000 mL of water and 15 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5 ± 0.1.
Solution B: 1 g of sodium 1-pentanesulfonate in a mixture of 300 mL of water and 15 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5 ± 0.1, and add 350 mL of acetonitrile and 350 mL of methanol.
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0

www.webofpharma.com

Time (min)	Solution A (%)	Solution B (%)
1	100	0
33.3	0	100
34.3	0	100

Diluent: 18 mg/mL of monobasic potassium phosphate in water
Standard solutions: 0.08 mg/mL and 0.16 mg/mL of cephalexin ($C_{16}H_{17}N_3O_4S$) from USP Cephalexin RS in *Diluent*, taking into account the stated potency of the USP Cephalexin RS
Sample solution: 6 mg/mL of Cephalexin Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1 of low acidity
Flow rate: 1 mL/min
Injection size: 20 µL
Analysis
Samples: *Standard solutions* and *Sample solution*
Plot the responses of the cephalexin peaks of the *Standard solutions* versus their concentrations, calculated on the anhydrous basis, in mg/mL, and draw a straight line through the two points and zero. From the line and the peak responses of the *Sample solution*, determine the concentration, I, in mg/mL, of each cephalexin-related substance from the *Sample solution* other than the cephalexin peak.
Calculate the percentage of each cephalexin-related substance represented by each peak of the *Sample solution*, other than the cephalexin peak.

$$\text{Result} = (I/C) \times 100$$

I = concentration of each cephalexin-related substance other than cephalexin in the *Sample solution* (mg/mL)
C = concentration of cephalexin from the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: NMT 1.0% of any individual cephalexin-related substance is found.
Total impurities: NMT 5.0%
• **Procedure 2: Dimethylaniline** ⟨223⟩: Meets the requirement

SPECIFIC TESTS
• **CRYSTALLINITY** ⟨695⟩: Meets the requirements
• **pH** ⟨791⟩: 1.5–3.0, in a solution containing 10 mg/mL
• **WATER DETERMINATION,** *Method I* ⟨921⟩: 3.0%–6.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cephalexin RS

Cephalothin Injection

» Cephalothin Injection contains an amount of Cephalothin Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cephalothin ($C_{16}H_{16}N_2O_6S_2$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Maintain in the frozen state.
Labeling—It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
USP Reference standards ⟨11⟩—
USP Cephalothin Sodium RS
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.13 USP Endotoxin Unit per mg of cephalothin.
pH ⟨791⟩: between 6.0 and 8.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It responds to the *Identification* test *A* under *Cephalothin Sodium* and meets the requirements for *Sterility* under *Cephalothin for Injection*.

Assay—
Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay*—under *Cephalothin Sodium*.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of cephalothin ($C_{16}H_{16}N_2O_6S_2$), to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Cephalothin Sodium*. Calculate the quantity, in µg, of cephalothin ($C_{16}H_{16}N_2O_6S_2$) in each mL of the Injection taken by the formula:

$$100(CP/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cephalothin Sodium RS, in the *Standard preparation*; P is the assigned potency, in µg of cephalothin ($C_{16}H_{16}N_2O_6S_2$) per mg, of USP Cephalothin Sodium RS; V is the volume, in mL, of Injection taken to prepare the *Assay preparation*; and r_U and r_S are the cephalothin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cephalothin for Injection

» Cephalothin for Injection contains an amount of Cephalothin Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cephalothin ($C_{16}H_{16}N_2O_6S_2$). It may contain Sodium Bicarbonate.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.
USP Reference standards ⟨11⟩—
USP Cephalothin Sodium RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.
Specific rotation ⟨781S⟩: between +124° and +134°, calculated on the dried and sodium bicarbonate-free basis.

www.webofpharma.com

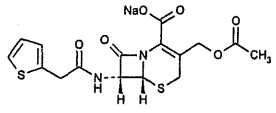
Test solution: a known amount of specimen, equivalent to about 50 mg of cephalothin, per mL, in water.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.13 USP Endotoxin Unit per mg of cephalothin.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
Uniformity of dosage units ⟨905⟩: meets the requirements.
Procedure for content uniformity—Perform the *Assay* on individual containers using *Assay preparation 1* or *Assay preparation 2*, or both, as appropriate.
pH ⟨791⟩: between 6.0 and 8.5, in the solution constituted as directed in the labeling.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Content of sodium bicarbonate *(if present)*—Dissolve about 1 g of it, accurately weighed, in 50 mL of water. Add methyl orange TS, and titrate with 0.1 N sulfuric acid VS. Each mL of 0.1 N sulfuric acid is equivalent to 8.401 mg of $NaHCO_3$. Calculate the percentage of sodium bicarbonate, and use the value obtained to calculate the *Specific rotation* on the dried and sodium bicarbonate-free basis.
Other requirements—It meets the requirements for *Identification* test *A* and *Loss on drying* under *Cephalothin Sodium*. It meets also the requirements *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
Mobile phase, Resolution solution, and Chromatographic system—Proceed as directed in the *Assay* under *Cephalothin Sodium*
Standard preparation—Dissolve a suitable quantity of USP Cephalothin Sodium RS, accurately weighed, in *Mobile phase* to obtain a solution having a known concentration of about 1 mg of cephalothin per mL.
Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Cephalothin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Mobile phase* to obtain a solution having a concentration of about 1 mg of cephalothin per mL.
Assay preparation 2 (where the label states the quantity of cephalothin in a given volume of constituted solution)—Constitute 1 container of Cephalothin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured portion of the constituted solution quantitatively with *Mobile phase* to obtain a solution having a concentration of about 1 mg of cephalothin per mL.
Procedure—Proceed as directed in the *Assay* under *Cephalothin Sodium* Calculate the quantity, in mg, of cephalothin ($C_{16}H_{16}N_2O_6S_2$) in the container, and in the portion of constituted solution taken by the formula:

$$(L/D)(CP/1000)(r_U/r_S)$$

in which L is the labeled quantity of cephalothin in the container, or in the volume of constituted solution taken; D is the concentration, in mg per mL, of cephalothin in *Assay preparation 1* or in *Assay preparation 2*, on the basis of the labeled quantity in the container, or in the portion of constituted solution taken, respectively, and the extent of dilution; and the other terms are as defined therein. Where the test for *Uniformity of dosage units* has been performed using the *Procedure for content uniformity*, use the average of these determinations as the *Assay* value.

Cephalothin Sodium



$C_{16}H_{15}N_2NaO_6S_2$ 418.42
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyloxy)methyl]-8-oxo-7-[(2-thienylacetyl)amino]-, monosodium salt, (6*R-trans*)-.
Monosodium (6*R*,7*R*)-3-(hydroxymethyl)-8-oxo-7-[2-(2-thienyl)-acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate acetate (ester) [58-71-9].

» Cephalothin Sodium contains the equivalent of not less than 850 µg of cephalothin ($C_{16}H_{16}N_2O_6S_2$) per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Cephalothin Sodium RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 25 µg per mL.
Medium: water.
B: It responds to the tests for *Sodium* ⟨191⟩.
Specific rotation ⟨781S⟩: between +124° and +134°.
Test solution: a known amount of specimen, equivalent to about 50 mg of cephalothin, per mL, in water.
Crystallinity ⟨695⟩: meets the requirements.
pH ⟨791⟩: between 4.5 and 7.0, in a solution containing 250 mg per mL.
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 1.5% of its weight.

Chromatographic purity—
Mobile phase , *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.
Standard solution—Use the *Standard preparation*, prepared as directed in the *Assay*, transfer 1.0 mL to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Test solution—Use the *Assay preparation* prepared as directed in the *Assay*.
Procedure—Proceed as directed for the *Procedure* in the *Assay*, except to inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* and to continue the chromatography of the *Test solution* for at least 4 times the retention time of the main cephalothin peak. The area of any peak in the chromatogram obtained from the *Test solution*, except the main peak, is not greater than the area of the main peak in the chromatogram obtained from the *Standard solution* (1.0%), and the sum of the areas of any such peaks is not greater than 3 times the area of the main peak in the chromatogram obtained from the *Standard solution* (3.0%). [NOTE—Any peak in the chromatogram obtained from the *Test solution* with an area less than one-tenth that of the main peak

USP Monographs

www.webofpharma.com

in the chromatogram obtained from the *Standard solution* is disregarded.]

Other requirements—Where the label states that Cephalothin Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial Endotoxins Test 85* — under *Cephalothin for Injection*. Where the label states that *Cephalothin Sodium* must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial Endotoxins Test 85* — under *Cephalothin for Injection*.

Assay—

Mobile phase—Dissolve 17 g of sodium acetate in 790 mL of water, add 0.6 mL of glacial acetic acid, and if necessary adjust with 0.1 N sodium hydroxide or glacial acetic acid to a pH of 5.9 ± 0.1. Add 150 mL of acetonitrile and 70 mL of alcohol, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Cephalothin Sodium RS quantitatively in *Mobile phase* to obtain a solution having a known concentration of about 1 mg per mL.

Resolution solution—Heat a 5-mL portion of the *Standard preparation* in a water bath at 90° for 10 minutes. Cool the solution, and immediately inject a portion of it into the chromatograph as directed under *Chromatographic system*.

Assay preparation—Transfer about 25 mg of Cephalothin Sodium, accurately weighed, to a 25-mL volumetric flask, add about 15 mL of *Mobile phase*, swirl to dissolve, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5 µm packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure:* the resolution between the two principal peaks is not less than 9.0. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the tailing factor is not more than 1.8, and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of cephalothin ($C_{16}H_{16}N_2O_6S_2$) in each mg of Cephalothin Sodium taken by the formula:

$$25(CP/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cephalothin Sodium RS in the *Standard preparation*; P is the assigned potency, in µg of cephalothin per mg, of USP Cephalothin Sodium RS; W is the quantity, in mg, of Cephalothin Sodium taken to prepare the *Assay preparation*; and r_U and r_S are the cephalothin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cephapirin for Injection

» Cephapirin for Injection contains an amount of Cephapirin Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cephapirin.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.

USP Reference standards ⟨11⟩—
USP Cephapirin Sodium RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.17 USP Endotoxin Unit per mg of cephapirin.

Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It responds to the *Identification* tests and meets the requirements for *Crystallinity, pH,* and *Water* under *Cephapirin Sodium*. It meets also the requirements for *Uniformity of Dosage Units* ⟨905⟩ and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water, dimethylformamide, glacial acetic acid, and 11.7 N potassium hydroxide (1834:160:4:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). Increase the proportion of dimethylformamide to decrease the retention time of cephapirin.

Resolution solution—Prepare a solution of Cephapirin Sodium in pH 2.0 hydrochloric acid buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) containing about 1 mg per mL. Place 10 mL of this solution in a test tube, and heat at 95° for 10 minutes, accurately timed. Promptly cool the tube in an ice water bath. Dilute 5 mL of the cooled solution with *Mobile phase* to obtain 50 mL of *Resolution solution*.

Standard preparation—Transfer about 21 mg of USP Cephapirin Sodium RS, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.2 mg of cephapirin per mL.

Assay preparation 1 (where it is packaged for dispensing and is represented as being in a single-dose container)—Constitute Cephapirin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing the equivalent of about 0.2 mg of cephapirin per mL.

Assay preparation 2 (where the label states the quantity of cephapirin in a given volume of constituted solution)—Constitute Cephapirin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing the equivalent of about 0.2 mg of cephapirin per mL. [NOTE—Use the *Standard preparation* and the *Assay preparation* within 1 hour.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the resolution, R, between the cephapirin peak and the peak having a retention time of about 0.9 relative to

www.webofpharma.com

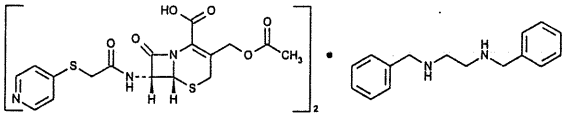
that of cephapirin is not less than 0.9. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.9 for cephapirin lactone and 1.0 for cephapirin; the column efficiency determined from the cephapirin peak is not less than 1200 theoretical plates; the tailing factor for the cephapirin peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the appropriate *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephapirin ($C_{17}H_{17}N_3O_6S_2$) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(CP/1000)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of cephapirin in the single-dose container, or in the volume of constituted solution taken; D is the concentration, in mg per mL, of cephapirin in *Assay preparation 1* or in *Assay preparation 2,* on the basis of the labeled quantity in the container, or in the portion of constituted solution taken, respectively, and the extent of dilution; C is the concentration, in mg per mL, of USP Cephapirin Sodium RS in the *Standard preparation; P* is the potency, in µg of cephapirin per mg, of USP Cephapirin Sodium RS; and r_U and r_S are the cephapirin peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cephapirin Benzathine



$(C_{17}H_{17}N_3O_6S_2)_2 \cdot C_{16}H_{20}N_2$ 1087.27

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyl-oxy)methyl]-8-oxo-7-[[(4-pyridinylthio)ace tyl] amino]-, (6*R-trans*)-, compd. with *N,N′*-bis(phenylmethyl)-1,2-ethanediamine (2:1).

(6*R,7R*)-3-(hydroxymethyl)-8-oxo-7-[2-(4-pyridylthio) acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid compound with *N,N′*-dibenzylethylenediamine (2:1) [97468-37-6].

» Cephapirin Benzathine contains the equivalent of not less than 715 µg and not more than 820 µg of cephapirin ($C_{17}H_{17}N_3O_6S_2$) per mg.

Packaging and storage—Preserve in well-closed containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—
USP Cephapirin Benzathine RS
USP Cephapirin Sodium RS

Change to read:

▲ ***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: 197K.▲ (CN 1-May-2020)

Crystallinity ⟨695⟩: meets the requirements.

pH ⟨791⟩: between 4.0 and 7.0, in a suspension (1 in 10).

Water Determination, *Method I* ⟨921⟩: not more than 5.0%.

Benzathine content—Using about 1 g of Cephapirin Benzathine, accurately weighed, proceed as directed in the test for *Benzathine content* under *Penicillin G Benzathine:* between 20.0% and 24.0% of benzathine ($C_{16}H_{20}N_2$), calculated on the anhydrous basis, is found.

Assay—

Solution A—Transfer about 26.2 mL of acetic acid and about 99.12 g of potassium acetate to a 4-L volumetric flask. Add 2000 mL of water, and mix to dissolve. Dilute with water to volume, and pass through a 0.45-µm nylon filter.

Solution B—Use acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Extraction solution: a mixture of 400 mL of acetic acid and 600 mL of water.

Dilution buffer—Dissolve about 205 g of potassium acetate in about 800 mL of water. Adjust with acetic acid to a pH of 7.5 to 8.2. Dilute with water to 1000 mL, and pass through a 0.45-µm nylon filter.

10% Acetic acid solution—Add about 10.0 mL of acetic acid to a 100-mL volumetric flask. Mix, and dilute with water to volume.

System suitability solution—Dissolve an accurately weighed quantity of USP Cephapirin Sodium RS in *10% Acetic acid solution* to prepare a solution containing a known concentration of about 2.0 mg per mL. Heat the solution at 50° for 12 to 18 hours.

Standard preparation—In duplicate, accurately weigh about 50 mg of USP Cephapirin Sodium RS, and transfer into a 25-mL volumetric flask. Add about 2.5 mL of *Extraction solution* and about 15.0 mL of *Dilution buffer,* and agitate to dissolve. Add 7.0 mL of acetonitrile, and mix well. Allow the solution to return to room temperature, and dilute with water to volume.

Assay preparation—In duplicate, weigh about 60 mg of Cephapirin Benzathine, and transfer into a 25-mL volumetric flask. Add about 2.5 mL of *Extraction solution* and 15.0 mL of *Dilution buffer,* and mix to dissolve. Add 7.0 mL of acetonitrile, and mix. Allow the flask to return to room temperature, and dilute with water to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 260-nm detector, a 3.2-mm × 15-mm guard column that contains 7-µm packing L1 and a 3.9-mm × 15-cm analytical column that contains 4-µm packing L1. The flow rate is about 2.0 mL per minute, and the columns are heated to 40°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–6	91.5	8.5	isocratic
6–10	91.5→80.0	8.5→20.0	linear
10–12	80.0	20.0	isocratic
12	80.0→91.5	20.0→8.5	return to initial
12–21	91.5	8.5	re-equilibration

Chromatograph the *System suitability solution* and the *Standard preparation,* and record the peak heights and valleys as directed for *Procedure.* Using the results from the *System suitability solution,* calculate the percentage of the height of the valley taken by the formula:

$$100(r_V/r_I)$$

www.webofpharma.com

in which r_V is the height of the valley between cephapirin and any impurity; and r_i is the impurity peak height. The percentage of the height of the valley is not more than 25% for the impurity peaks adjacent to the cephapirin peak.

[NOTE—The *System suitability solution* is acceptable as long as the cephapirin peak is larger than the two peaks on either side of the cephapirin peak.]

The relative standard deviation for replicate injections of the *Standard preparation* is not more than 3.0%.

Procedure—Separately inject equal volumes (about 2 µL) of the duplicate *Standard preparation* and the duplicate *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of $C_{17}H_{17}N_3O_6S_2$ in each mg of Cephapirin Benzathine taken by the formula:

$$P(W_S/W_U)(V_U/V_S)(r_U/r_S)$$

in which P is the assigned potency, in µg of cephapirin per mg, of USP Cephapirin Sodium RS; W_S and W_U are the quantities of USP Cephapirin Sodium RS and Cephapirin Benzathine, in mg, used to prepare the *Standard preparation* and the *Assay preparation*, respectively; V_S and V_U are the final volumes, in mL, of the *Standard preparation* and the *Assay preparation*, respectively; and r_U and r_S are the average peak areas of the cephapirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cephapirin Benzathine Intramammary Infusion

» Cephapirin Benzathine Intramammary Infusion is a suspension of Cephapirin Benzathine in a suitable vegetable oil vehicle. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled quantity of cephapirin ($C_{17}H_{17}N_3O_6S_2$). It contains a suitable dispersing agent.

Packaging and storage—Preserve in well-closed unit-dose disposable syringes at controlled room temperature.
Labeling—Label Intramammary Infusion to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Cephapirin Benzathine RS
USP Cephapirin Sodium RS

Change to read:

▲ ***Spectroscopic Identification Tests*** ⟨ 197 ⟩ , *Infrared Spectroscopy* : 197K▲ (CN 1-May-2020)—Prepare the test specimen as follows. Transfer the contents of 1 syringe of Intramammary Infusion to a 50-mL centrifuge tube, add 25 mL of toluene, mix for about 1 minute, and centrifuge. Remove and discard the toluene layer without disturbing the residue in the centrifuge tube. Wash the residue with two 25-mL portions of toluene. Dry the residue in vacuum at 60°, and use the dried residue as the test specimen. Mix the dried residue with 9 parts of potassium bromide, and record the IR spectrum, using the diffuse reflectance technique: the IR absorption spectrum so obtained corresponds to that of a similar dispersion of USP Cephapirin Benzathine RS in potassium bromide.

Water Determination, *Method I* ⟨921⟩ : not more than 1.0%, 10 mL of Intramammary Infusion being tested.

Assay—

Solution A, Solution B, Mobile phase, Extraction solution, Dilution buffer, 10% Acetic acid solution, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cephapirin Benzathine.*

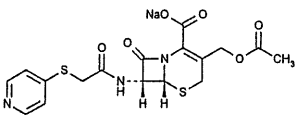
Assay preparation—Express the entire contents of a syringe of the Intramammary Infusion into a centrifuge tube. For each mL of Intramammary Infusion, add 1.0 *n*-heptane and 1.5 mL of *Extraction solution,* cap, and vortex at high speed for 5 minutes. Centrifuge for 5 minutes at a sufficient speed to break the emulsion. Remove the aqueous layer, and pass through a 0.45-µm nylon filter, discarding the first 0.5 mL. Transfer 2.5 mL of the filtered aqueous phase into a 25-mL volumetric flask that contains a solution composed of 15.0 mL of *Dilution buffer* and 7.0 mL of acetonitrile. Add water to volume, and mix well to obtain a single phase.

Procedure—Separately inject equal volumes (about 2 µL) of the duplicate *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the quantity, in mg, of cephapirin ($C_{17}H_{17}N_3O_6S_2$) in each syringe of Intramammary Infusion taken by the formula:

$$15PW(V_U/V_S)(r_U/r_S)$$

in which P is the assigned potency, in µg cephapirin per mg, of USP Cephapirin Sodium RS; W is the quantity of USP Cephapirin Sodium RS, in mg, used to prepare the *Standard preparation;* V_S is the final volume, in mL, of the *Standard preparation;* V_U is the entire volume of Intramammary Infusion, in mL, in one syringe; and r_U and r_S are the peak area and the average peak area of the cephapirin peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cephapirin Sodium



$C_{17}H_{16}N_3NaO_6S_2$ 445.45
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyl-oxy)methyl]-8-oxo-7-[[(4-pyridinylthio)ace tyl] amino]-, monosodium salt, (6*R-trans*)-.
Monosodium (6*R,7R*)-3-(hydroxymethyl)-8-oxo-7-[2-(4-pyridylthio)acetamido]-5-thia-1-azabicyclo-[4.2.0] oct-2-ene-2-carboxylate acetate (ester) [24356-60-3].

» Cephapirin Sodium has a potency equivalent to not less than 855 µg and not more than 1000 µg of cephapirin ($C_{17}H_{17}N_3O_6S_2$) per mg.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Cephapirin Sodium RS

USP Monographs

www.webofpharma.com

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: It responds to the tests for *Sodium* ⟨191⟩.

Crystallinity ⟨695⟩**:** meets the requirements.

pH ⟨791⟩**:** between 6.5 and 8.5, in a solution containing 10 mg of cephapirin per mL.

Water Determination, *Method I* ⟨921⟩**:** not more than 2.0%.

Other requirements—Where the label states that Cephapirin Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cephapirin for Injection* Where the label states that Cephapirin Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cephapirin for Injection.*

Assay—

Solution A, Solution B, Mobile phase, Extraction solution, Dilution buffer, 10% Acetic acid solution, System suitability solution, Standard preparation, and *Chromatographic system—*Proceed as directed in the *Assay* under *Cephapirin Benzathine*

*Assay preparation—*In duplicate, weigh about 50 mg of Cephapirin Sodium, and transfer into a 25-mL volumetric flask. Add about 2.5 mL of *Extraction solution* and 15.0 mL of *Dilution buffer,* and mix to dissolve. Add 7.0 mL of acetonitrile, and mix. Allow the flask to return to room temperature, and dilute with water to volume.

*Procedure—*Separately inject equal volumes (about 2 µL) of the duplicate *Standard preparation* and the duplicate *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of cephapirin ($C_{17}H_{17}N_3O_6S_2$) in each mg of Cephapirin Sodium taken by the formula:

$$P(W_S/W_U)(V_U/V_S)(r_U/r_S)$$

in which P is the assigned potency, in µg of cephapirin per mg, of USP Cephapirin Sodium RS; W_S and W_U are the quantities of USP Cephapirin Sodium RS and Cephapirin Sodium, in mg, used to prepare the *Standard preparation* and the *Assay preparation,* respectively; V_U and V_S are the final volumes, in mL, of the *Assay preparation* and the *Standard preparation,* respectively; and r_U and r_S are the average peak areas of the cephapirin peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cephapirin Sodium Intramammary Infusion

» Cephapirin Sodium Intramammary Infusion is a suspension of Cephapirin Sodium in a suitable vegetable oil vehicle. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled quantity of cephapirin ($C_{17}H_{17}N_3O_6S_2$). It contains a suitable dispersing agent.

Packaging and storage—Preserve in well-closed unit-dose disposable syringes at controlled room temperature.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩**—**
USP Cephapirin Sodium RS

Change to read:

▲ ***Spectroscopic Identification Tests*** ⟨ 197 ⟩ **:** *Infrared Spectroscopy* **:** 197K▲ (CN 1-May-2020)—Prepare the test specimen as follows. Transfer the contents of 1 syringe of Intramammary Infusion to a 50-mL centrifuge tube, add 25 mL of toluene, mix for about 1 minute, and centrifuge. Remove and discard the toluene layer without disturbing the residue in the centrifuge tube. Wash the residue with two 25-mL portions of toluene. Dry the residue in vacuum at 60°, and use the dried residue as the test specimen. Mix the dried residue with 9 parts of potassium bromide, and record the IR spectrum, using the diffuse reflectance technique: the IR absorption spectrum so obtained corresponds to that of a similar dispersion of USP Cephapirin Sodium RS in potassium bromide.

Water Determination, *Method I* ⟨921⟩ **:** not more than 1.0%, 10 mL of Intramammary Infusion being tested.

Assay—

Solution A, Solution B, Mobile phase, Extraction solution, Dilution buffer, 10% Acetic acid solution, System suitability solution, Standard preparation, and *Chromatographic system—*Proceed as directed in the *Assay* under *Cephapirin Benzathine.*

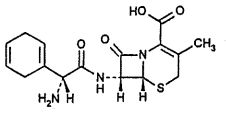
*Assay preparation—*Express the entire contents of a syringe of the Intramammary Infusion into a centrifuge tube. For each mL of Intramammary Infusion, add 1.0 *n*-heptane and 1.0 mL of *Extraction solution,* cap, and mix on a vortex mixer at high speed for 5 minutes. Centrifuge for 5 minutes at a speed sufficient to break the emulsion. Remove the aqueous layer, and pass through a 0.45-µm nylon filter, discarding the first 0.5 mL. Transfer 2.5 mL of the filtered aqueous phase into a 25-mL volumetric flask that contains a solution composed of 15.0 mL of *Dilution buffer* and 7.0 mL of acetonitrile. Add water to volume, and mix well to obtain a single phase.

*Procedure—*Separately inject equal volumes (about 2 µL) of the duplicate *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the quantity, in mg, of cephapirin ($C_{17}H_{17}N_3O_6S_2$) in each syringe of Intramammary Infusion taken by the formula:

$$10PW(V_U/V_S)(r_U/r_S)$$

in which P is the assigned potency, in µg of cephapirin per mg, of USP Cephapirin Sodium RS; W is the quantity of USP Cephapirin Sodium RS, in mg, used to prepare the *Standard preparation;* V_S is the final volume, in mL, of the *Standard preparation;* V_U is the entire volume of Intramammary Infusion, in mL, in one syringe; and r_U and r_S are the peak area and the average peak area of the cephapirin peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cephradine



$C_{16}H_{19}N_3O_4S$ 349.40

www.webofpharma.com

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(amino-1,4-cyclohexadien-1-ylacetyl)amino]-3-methyl-8-oxo-, [6*R*-[6α,7β(*R**)]]-.
(6*R*,7*R*)-7-[(*R*)-2-Amino-2-(1,4-cyclohexadien-1-yl) acetamido]-3- methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [38821-53-3].
Monohydrate 367.43
[31828-50-9 (non-stoichiometric hydrate)].
Dihydrate 385.44
[58456-86-3].

» Cephradine has a potency of not less than 900 µg and not more than 1050 µg of total cephalosporins per mg, calculated as the sum of cephradine ($C_{16}H_{19}N_3O_4S$) and cephalexin ($C_{16}H_{17}N_3O_4S$), calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
Labeling—Where it is the dihydrate form, the label so indicates. Where the quantity of cephradine is indicated in the labeling of any preparation containing Cephradine, this shall be understood to be in terms of anhydrous cephradine ($C_{16}H_{19}N_3O_4S$). Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Cephradine RS
USP Cephalexin RS

Change to read:

▲ ***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: 197K.▲ (CN 1-May-2020)
Crystallinity ⟨695⟩: meets the requirements.
pH ⟨791⟩: between 3.5 and 6.0, in a solution containing 10 mg per mL.
Water Determination, *Method I* ⟨921⟩: not more than 6.0%, except that if it is the dihydrate form, the limit is between 8.5%and 10.5%.
Limit of cephalexin—Using the chromatogram of the *Assay preparation* obtained in the *Assay*, calculate the percentage of cephalexin ($C_{16}H_{17}N_3O_4S$) in the portion of Cephradine taken by the formula:

$$100(r_{U\,x}/r_U)$$

in which $r_{U\,x}$ is the cephalexin peak response in the chromatogram obtained from the *Assay preparation*, and r_U is the sum of the cephalexin and cephradine peak responses in the chromatogram obtained from the *Assay preparation:* not more than 5.0%, calculated on the anhydrous basis, is found.
Other requirements—Where the label states that Cephradine is sterile, it meets the requirements for *Sterility Tests 71* — and *Bacterial Endotoxins Test 85* — under *Cephradine for Injection*. Where the label states that Cephradine must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial Endotoxins Test 85* — under *Cephradine for Injection*.

Assay—
Mobile phase—Prepare a mixture of water, methanol, 0.5 M sodium acetate, and 0.7 N acetic acid (782:200:15:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). Filter the solution through a filter of 1 µm or finer porosity, and degas before use.

Standard preparation—Dissolve an accurately weighed quantity of USP Cephradine RS quantitatively in *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL.
Resolution solution—Prepare a solution in *Mobile phase* containing in each mL about 0.5 mg of USP Cephradine RS and 0.5 mg of USP Cephalexin RS.
Assay preparation—Transfer about 50 mg of Cephradine, accurately weighed, to a 100-mL volumetric flask, add about 30 mL of *Mobile phase*, and sonicate. Dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure:*the relative retention times are about 0.8 for cephalexin and 1.0 for cephradine; and the resolution, *R*, between the cephalexin peak and the cephradine peak is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of total cephalosporins (sum of cephradine and cephalexin) in each mg of the Cephradine taken by the formula:

$$100(CP/M)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Cephradine RS in the *Standard preparation*; *P* is the designated potency, in µg per mg, of USP Cephradine RS; *M* is the quantity, in mg, of Cephradine taken to prepare the *Assay preparation*; and r_U and r_S are the sums of the cephradine and cephalexin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cephradine Capsules

» Cephradine Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cephradine, calculated as the sum of cephradine ($C_{16}H_{19}N_3O_4S$) and cephalexin ($C_{16}H_{17}N_3O_4S$).

Packaging and storage—Preserve in tight containers.
Labeling—The quantity of cephradine stated in the labeling is in terms of anhydrous cephradine ($C_{16}H_{19}N_3O_4S$).
USP Reference standards ⟨11⟩—
USP Cephradine RS
USP Cephalexin RS
Identification—Mix the contents of 1 Capsule with water to obtain a solution having a concentration of about 3 mg of cephradine per mL, and filter *(test solution)*. Place a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of binder-free silica gel in a chamber containing a mixture of *n*-hexane and tetradecane (95:5) to a depth of about 1 cm, allow the solvent front to move the length of the plate, remove the plate from the chamber, and allow the solvent to evaporate. On this plate apply 10 µL each of the *test solution* and a *Standard solution* containing 3 mg of USP Cephradine RS per mL. Allow the spots

USP Monographs

www.webofpharma.com

to dry, and develop the chromatogram in a solvent system consisting of a mixture of 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and a 1 in 15 solution of ninhydrin in acetone (60: 40: 1.5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, dry the plate for 10 minutes at 110°, and examine the chromatogram: the R_F value of the principal spot obtained from the *test solution* corresponds to that obtained from the *Standard solution.*

Dissolution ⟨711⟩—
Medium: 0.12 N hydrochloric acid; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{16}H_{19}N_3O_4S$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 255 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Cephradine RS in the same medium.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{16}H_{19}N_3O_4S$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, of the mixed contents of 4 Capsules in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: the Capsule contents lose not more than 7.0% of their weight.

Assay—
Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cephradine.*
Assay preparation—Transfer, as completely as possible, the contents of not fewer than 20 Capsules to a suitable tared container, determine the average weight per Capsule, and mix the combined contents. Transfer an accurately weighed portion of the powder, equivalent to about 125 mg of cephradine, to a 250-mL volumetric flask, add 50 mL of *Mobile phase*, sonicate for about 15 minutes, and shake by mechanical means for about 10 minutes. Dilute with *Mobile phase* to volume, and mix. Filter a portion of this mixture through a filter having a porosity of 0.5 µm or finer, discarding the first 5 mL of the filtrate. Use the filtrate as the *Assay preparation.*
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard cephradine preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephradine (sum of cephradine and cephalexin) in the portion of Capsules taken by the formula:

$$0.25CP(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cephradine RS in the *Standard preparation*; P is the designated potency, in µg per mg, of USP Cephradine RS; and r_U and r_S are the sums of the cephradine and cephalexin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cephradine for Injection

» Cephradine for Injection contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cephradine, calculated as the sum of cephradine ($C_{16}H_{19}N_3O_4S$) and cephalexin ($C_{16}H_{17}N_3O_4S$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Cephradine RS
USP Cephalexin RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
Identification—Dilute the contents of 1 container of Cephradine for Injection with water to obtain a test solution containing about 3 mg of cephradine per mL. Proceed as directed in the *Identification*— test under *Cephradine Capsules*, beginning with "Place a suitable thin-layer chromatographic plate": the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.20 USP Endotoxin Unit per mg of cephradine.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 8.0 and 9.6, in a solution containing 10 mg per mL.
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay*— under *Cephradine.*
Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Cephradine for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Mobile phase* to obtain a solution containing about 0.5 mg of cephradine per mL.
Assay preparation 2 (where the label states the quantity of cephradine in a given volume of constituted solution)—Constitute Cephradine for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Mobile phase* to obtain a solution containing about 0.5 mg of cephradine per mL.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and *Assay preparation 1* or *Assay preparation 2* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephradine (sum of

USP Monographs

www.webofpharma.com

cephradine and cephalexin) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(CP)(L/1000D)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cephradine RS in the *Standard preparation*; P is the designated potency, in µg per mg, of USP Cephradine RS; L is the labeled quantity, in mg of cephradine, in the container taken to prepare *Assay preparation 1*, or in the volume of constituted solution taken to prepare *Assay preparation 2*; D is the concentration, in mg of cephradine per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution; and r_U and r_S are the sums of the cephradine and cephalexin peak responses obtained from *Assay preparation 1* or *Assay preparation 2* and the *Standard preparation*, respectively.

Cephradine for Oral Suspension

» Cephradine for Oral Suspension is a dry mixture of Cephradine and one or more suitable buffers, colors, diluents, and flavors. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of cephradine, calculated as the sum of cephradine ($C_{16}H_{19}N_3O_4S$) and cephalexin ($C_{16}H_{17}N_3O_4S$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Cephradine RS
USP Cephalexin RS
Identification—Constitute 1 container of Cephradine for Oral Suspension as directed in the labeling. Mix a portion of the resulting suspension with water to obtain a concentration of about 3 mg of cephradine per mL, and filter *(test solution)*. Proceed as directed in the *Identification* test under *Cephradine Capsules*, beginning with "Place a suitable thin-layer chromatographic plate": the R_F value of the principal spot obtained from the *test solution* corresponds to that obtained from the *Standard solution*.

Uniformity of dosage units ⟨905⟩—
[F] *OR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS:* meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 3.5 and 6.0, in the suspension constituted as directed in the labeling.
Water Determination, *Method I* ⟨921⟩: not more than 1.5%.

Assay—
Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cephradine*.
Assay preparation—Constitute Cephradine for Oral Suspension as directed in the labeling. Dilute an accurately measured volume of the suspension so obtained, freshly mixed and free from air bubbles, quantitatively with *Mobile phase* to obtain a solution containing about 0.5 mg of cephradine per mL. Filter a portion of this mixture through a filter having a porosity of 0.5 µm or finer, discarding the first 5 mL of the filtrate. Use the filtrate as the *Assay preparation*.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephradine (sum of cephradine and cephalexin) in each mL of constituted Oral Suspension taken by the formula:

$$(CP)(L/1000D)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cephradine RS in the *Standard preparation*; P is the designated potency, in µg per mg, of USP Cephradine RS; L is the labeled quantity, in mg of cephradine, in each mL of the constituted Oral Suspension; D is the concentration, in mg of cephradine per mL, of the *Assay preparation*, based on the labeled quantity of cephradine per mL of constituted Oral Suspension and the extent of dilution; and r_U and r_S are the sums of the cephradine and cephalexin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cephradine Tablets

» Cephradine Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cephradine, calculated as the sum of cephradine ($C_{16}H_{19}N_3O_4S$) and cephalexin ($C_{16}H_{17}N_3O_4S$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Cephradine RS
USP Cephalexin RS
Identification—Mix a quantity of finely powdered Tablets with water to obtain a concentration of about 3 mg of cephradine per mL, and filter *(Test solution)*. Proceed as directed in the *Identification* test under *Cephradine Capsules* beginning with "Place a suitable thin-layer chromatographic plate": the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the *Standard solution*.

Dissolution ⟨711⟩—
Medium: 0.12 N hydrochloric acid; 900 mL.
Apparatus 2: 75 rpm.
Time: 60 minutes.
Procedure—Determine the amount of $C_{16}H_{19}N_3O_4S$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 255 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Cephradine RS in the same medium.
Tolerances—Not less than 85% *(Q)* of the labeled amount of $C_{16}H_{19}N_3O_4S$ is dissolved in 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 6.0%.

Assay—
Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cephradine*.
Assay preparation—Place not less than 5 Tablets in a high-speed glass blender jar containing an accurately measured volume of water, sufficient to yield a concentration of not less than 5 mg of cephradine per mL, and blend for 4 ± 1 minutes. Dilute an accurately measured volume of this stock solution quantitatively and stepwise with *Mobile phase*

www.webofpharma.com

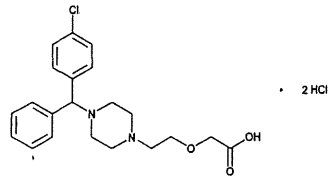
to obtain an *Assay preparation* containing about 0.5 mg of cephradine per mL.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephradine (sum of cephradine and cephalexin) in each Tablet taken by the formula:

$$(CP)(L/1000D)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cephradine RS in the *Standard preparation*; P is the designated potency, in µg per mg, of USP Cephradine RS; L is the labeled quantity, in mg of cephradine, in each Tablet; D is the concentration, in mg of cephradine per mL, of the *Assay preparation*, based on the labeled quantity per Tablet, the number of Tablets taken, and the extent of dilution; and r_U and r_S are the sums of the cephradine and cephalexin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cetirizine Hydrochloride



$C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ 461.81

(±)-[2-[4-[(4-Chlorophenyl)phenylmethyl]-1-piperazinyl] ethoxy]acetic acid, dihydrochloride;
(±)-[2-[4-(*p*-Chloro-α-phenylbenzyl)-1-piperazinyl]eth oxy] acetic acid, dihydrochloride [83881-52-1].

DEFINITION

Cetirizine Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TEST—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, water, and 1 M sulfuric acid (93: 6.6: 0.4)
Standard solution: 0.5 mg/mL USP Cetirizine Hydrochloride RS in *Mobile phase*
Sample solution: 0.5 mg/mL Cetirizine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ in the portion of Cetirizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Cetirizine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.2%

ORGANIC IMPURITIES

[NOTE—It is recommended that *Test 2* be performed if either cetirizine ethanol (2-{4-[(4-chloro phenyl) phenylmethyl]piperazin-1-yl}ethanol) or cetirizine acetic acid (2-{4-[(4-chloro phenyl)phenylmethyl] piperazin-1-yl}acetic acid) may be present in the test substance.]

- **Procedure 1**

Mobile phase and **Sample solution:** Proceed as directed in the *Assay*.
System suitability solution: 4 µg/mL each of USP Cetirizine Hydrochloride RS and USP Cetirizine Related Compound A RS in *Mobile phase*
Standard solution: 0.5 µg/mL of USP Cetirizine Hydrochloride RS in *Mobile phase*
Chromatographic system: Prepare as directed in the *Assay*. (See *Chromatography* ⟨621⟩, *System Suitability*.)
Run time: Three times the retention time of cetirizine
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Tailing factor: NMT 2.0 for cetirizine, *System suitability solution*
Resolution: NLT 2.0 between cetirizine and cetirizine related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0% cetirizine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Cetirizine hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for cetirizine from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Cetirizine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1* for values)

Acceptance criteria: See *Impurity Table 1*.

USP Monographs

www.webofpharma.com

Total impurities: NMT 0.3%. [NOTE—Disregard peaks below 0.02%.]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-CBH[a]	0.3	1.4	0.1
Dimer[b]	0.5	1.8	0.1
2-Chlorocetirizine[c]	0.85	0.49	0.1
Cetirizine related compound A[d]	0.9	0.95	0.1
Cetirizine	1.0	—	—
Deschlorocetirizine[e]	1.4	0.45	0.1
CBHP[f]	1.45	1.6	0.1
Any individual unspecified impurity	—	1.0	0.1

[a] 4-Chlorobenzhydrol.
[b] 1,4-Bis[(4-chlorophenyl)phenylmethyl]piperazine.
[c] 2-[2-[4-[(2-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.
[d] 2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid, ethyl ester (cetirizine ethyl ester).
[e] 2-[2-[4-(Diphenylmethyl]piperazin-1-yl]ethoxy]acetic acid.
[f] 1-[(4-Chlorophenyl)phenylmethyl]piperazine.

• **Procedure 2**

Solution A: 2 g/L tetrabutyl ammonium hydrogen sulfate and 3 g/L of monobasic sodium phosphate monohydrate in water. Adjust with 1 N sodium hydroxide to a pH of 2.8 ± 0.05.
Solution B: Methanol
Buffer: 1.4 g/L monobasic sodium phosphate monohydrate and 2.7 g/L of dibasic sodium phosphate heptahydrate. Adjust with either 1 N sodium hydroxide or 10% phosphoric acid to a pH of 6.9 ± 0.1.
Diluent: Acetonitrile and *Buffer* (1:1)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	58	42	1.2
40	58	42	1.2
68	20	80	1.5
108	20	80	1.5
110	58	42	1.2
120	58	42	1.2

Standard solution: 2 µg/mL of USP Cetirizine Hydrochloride RS in *Diluent*
Sample solution: 2 mg/mL cetirizine hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 232 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Column efficiency: NLT 6000 theoretical plates
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Cetirizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for cetirizine from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Cetirizine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 2* for values)

Acceptance criteria: See *Impurity Table 2*.
Total impurities: NMT 0.3%. [NOTE—Disregard peaks below 0.05%.]

Impurity Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Deschlorocetirizine[a]	0.35	0.56	0.1
Cetirizine ethanol[b]	0.53	1.2	0.1
CBHP[c]	0.66	1.3	0.1
2-Chlorocetirizine[d]	0.70	0.52	0.1
Cetirizine methyl ester[e]	0.81	0.96	0.1
3-Chlorocetirizine[f]	0.87	0.52	0.1
Cetirizine	1.0	—	—
Cetirizine acetic acid[g]	1.15	0.97	0.1
Cetirizine N-oxide[h]	1.25	0.81	0.1
4-CBH[i]	1.55	1.2	0.1
4-Chlorobenzophenone[j]	1.66	0.50	0.1
Cetirizine dimer[k]	2.48	1.4	0.1
Any individual unspecified impurity	—	1.0	0.10

[a] 2-{2-[4-(Diphenylmethyl)piperazin-1-yl]ethoxy}acetic acid.
[b] 2-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}ethanol.
[c] 1-[(4-Chlorophenyl)phenylmethyl]piperazine.
[d] 2-(2-{4-[(2-Chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy)acetic acid.
[e] Methyl 2-(2-{4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy)acetate.
[f] 2-[2-[4-[(3-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.
[g] 2-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}acetic acid.
[h] 2-(2-{4-[(4-Chlorophenyl)(phenyl)methyl]piperazin-1-yl}ethoxy)acetic acid *N*[1]-oxide.
[i] 4-Chlorobenzhydrol.
[j] (4-Chlorophenyl)phenylmethanone.
[k] 1,4-Bis[(4-chlorophenyl)phenylmethyl]piperazine.

SPECIFIC TESTS

• **pH** ⟨791⟩: 1.2-1.8, in an aqueous solution 1 in 20
• **Loss on Drying** ⟨731⟩: Dry a sample at 105° to a constant weight: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, protected from light and moisture. Store at room temperature.

www.webofpharma.com

- **LABELING:** Label it to indicate with which impurity procedures the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cetirizine Hydrochloride RS
  USP Cetirizine Related Compound A RS
  (*RS*)-2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid ethyl ester dihyrochloride.
  $C_{23}H_{29}ClN_2O_3 \cdot 2HCl$ 489.86

Cetirizine Hydrochloride Oral Solution

DEFINITION
Cetirizine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY
- **PROCEDURE**

Solution A: Acetonitrile
Solution B: 1.36 g/L of monobasic potassium phosphate in water. Adjust with a 2% solution of phosphoric acid in water to a pH of 3.5 ± 0.05.
Diluent: Acetonitrile and water (3:7)
Mobile phase: See the gradient table below.

Time (min)	*Solution A* (%)	*Solution B* (%)
0	5	95
15	5	95
22	25	75
35	25	75
40	5	95
50	5	95

Standard stock solution: 5 mg/mL of USP Cetirizine Hydrochloride RS in water
Standard solution: 0.1 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*, from the *Standard stock solution*
Sample solution: Transfer an amount of Oral Solution to a suitable volumetric flask to obtain a nominal concentration of 0.1 mg/mL of cetirizine hydrochloride. Dissolve in 60% of the flask volume of *Diluent* by swirling. Sonicate 3 min, and dilute with *Diluent* to volume. Pass through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 233 nm
Column: 4.6-mm × 25-cm; 5-µm packing L10
Column temperature: 50°
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

IMPURITIES
ORGANIC IMPURITIES
- **Procedure**

Solution A: Transfer 50 mL of water to a 100-mL volumetric flask, add 5.5 mL of sulfuric acid, and dilute with water to volume.
Mobile phase: Acetonitrile, water, and *Solution A* (965:33:1)
Diluent: Acetonitrile and water (7:13)
Standard solution: 6 µg/mL of USP Cetirizine Hydrochloride RS in *Diluent*
Sample solution: 0.6 mg/mL of cetirizine hydrochloride in *Diluent*. Transfer an amount of Oral Solution to a suitable volumetric flask, dissolve in *Diluent*, sonicate for 10 min, and dilute with *Diluent* to volume. Pass through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Column temperature: 30°
Flow rate: 2 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 10,000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for cetirizine from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Impurity Table 1*.
Total impurities: NMT 0.8%

USP Monographs

www.webofpharma.com

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cetirizine acetic acid[a]	0.69	P[b]
2-Chlorocetirizine[c]	0.83	P
Cetirizine	1.00	—
Cetirizineethanol[d]	1.30	P
Ethoxycetirizine[e]	1.38	P
CBHP[f]	1.52	P
Propylene glycol ester of cetirizine (diastereomer 1)[g]	1.53	0.2
Propylene glycol ester of cetirizine (diastereomer 2)[g]	1.61	0.2
Deschlorocetirizine[h]	1.65	P
Glyceryl ester of cetirizine[i]	2.20	0.5
Any individual unspecified impurity	—	0.2

[a] 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]acetic acid.
[b] P = Process impurity. Provided for information only; the content is not calculated and not reported. The content is controlled in the drug substance monograph.
[c] 2-[2-[4-[(2-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.
[d] 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol.
[e] 2-[2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy] ethoxy] acetic acid (ethoxycetirizine).
[f] 1-[(4-Chlorophenyl)phenylmethyl]piperazine.
[g] 2-Hydroxypropyl 2-(2-{4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl} ethoxy)acetate.
[h] 2-(2-[4-(Diphenylmethyl)piperazin-1-yl]ethoxy]acetic acid.
[i] 2,3-Dihydroxypropyl 2-(2-{4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl} ethoxy)acetate.

SPECIFIC TESTS

- **pH** ⟨791⟩: 4.0–5.1
- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total aerobic microbial count does not exceed 100 cfu/mL, and the total combined molds and yeasts count does not exceed 10 cfu/mL. It meets the requirements of the tests for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, and protect from light. Store at controlled room temperature or in a cold place.
- **USP Reference Standards** ⟨11⟩
  USP Cetirizine Hydrochloride RS

Cetirizine Hydrochloride Tablets

DEFINITION

Cetirizine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Solution A: 2 N sulfuric acid and water (2:33)
Buffer: 2.9 mL/L of phosphoric acid in water
Mobile phase: Acetonitrile and *Buffer* (3:7)
Diluent: Acetonitrile, *Solution A*, and water (100:1:100)
Standard solution: 0.2 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*
Sample solution: 0.2 mg/mL of cetirizine hydrochloride in *Diluent* from NLT 20 powdered Tablets. [Note—Sonicate, if necessary.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: 1.3 times the retention time of cetirizine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Dissolution** ⟨711⟩

Test 1
Medium: Water; 900 mL, degassed
Apparatus 2: 50 rpm
Time: 30 min
Buffer: 2.9 mL/L of phosphoric acid in water
Mobile phase: Acetonitrile and *Buffer* (2:3)
Standard solution: 11 µg/mL of USP Cetirizine Hydrochloride RS in water. This solution can be stored for 48 h at room temperature.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: 1.3 times the retention time of cetirizine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) dissolved:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Buffer: 0.4 g/L of 1-heptane sulfonic acid sodium salt
Mobile phase: Acetonitrile and *Buffer* (50:50). Adjust with 0.1 N sulfuric acid to a pH of 3.5.
Standard solution: 11 µg/mL of USP Cetirizine Hydrochloride RS in *Medium*
Sample solution: Pass a 20-mL portion of the solution under test through a nylon filter of 0.45-µm pore size. Discard the first 10 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 50 µL
Run time: 1.6 times the retention time of cetirizine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: (*L*/900) mg/mL of USP Cetirizine Hydrochloride RS in water, where *L* is the label claim of cetirizine hydrochloride, in mg/Tablet
Sample solution: Centrifuge a portion of the solution under test for NLT 15 min at 3000 rpm.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: UV 231 nm
Blank: *Medium*
Path length: 1 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A: 2 N sulfuric acid and water (2:33)
Buffer: 3.4 g/L of tetrabutyl ammonium hydrogen sulfate in water
Diluent: Acetonitrile, *Solution A*, and water (910:27:63)
Mobile phase: Acetonitrile, *Solution A*, and *Buffer* (93:5:2)
Standard solution: 1.5 µg/mL of USP Cetirizine Hydrochloride RS in *Diluent*
Sample solution: 0.5 mg/mL of cetirizine hydrochloride in *Diluent* from NLT 20 powdered Tablets. [Note—Sonicate, if necessary.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.0-mm × 25-cm; 5-µm packing L3
Flow rate: 0.8 mL/min
Injection volume: 20 µL
Run time: 2.5 times the retention time of cetirizine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of cetirizine from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

USP Monographs

www.webofpharma.com

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cetirizine lactose ester[a]	0.56	1.0	0.5
Cetirizine	1.0	—	—
Cetirizine ethanol[b]	1.67	1.2	0.4
Any unspecified degradation product	—	—	0.2
Total impurities	—	—	1

[a] 6-*O*-[2-(2-{4-[(4-Chlorophenyl)(phenyl)methyl]piperazin-1-yl}ethoxy)acetyl]-β-D-galactopyranosyl-(1→4)β-D-glucopyranose.
[b] 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store below 30°.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cetirizine Hydrochloride RS

Cetirizine Hydrochloride Orally Disintegrating Tablets

DEFINITION

Cetirizine Hydrochloride Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer A: Dissolve 13.2 g of sodium 1-decanesulfonate in 2760 mL of water. Add 5.5 g of monobasic sodium phosphate and 1.2 mL of phosphoric acid. Pass through a suitable filter.
Buffer B: Dissolve 13.2 g of sodium 1-decanesulfonate in 2000 mL of water. Add 5.5 g of monobasic sodium phosphate and 1.2 mL of phosphoric acid. Pass through a suitable filter.
Solution A: Acetonitrile and *Buffer A* (31:69)
Solution B: Acetonitrile and *Buffer B* (50:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35.0	0	100
35.1	100	0
45.0	100	0

Diluent: Acetonitrile and 0.01 N hydrochloric acid (20:80)
System suitability stock solution: 0.04 mg/mL of USP Meclizine Related Compound A RS, prepared as follows. Transfer a quantity of the compound into a suitable volumetric flask. Add 5% of the flask volume of acetonitrile to dissolve the components. Sonicate if needed. Dilute with *Diluent* to volume.
System suitability solution: 0.4 mg/mL of USP Cetirizine Hydrochloride RS and 1.2 µg/mL of USP Meclizine Related Compound A RS, prepared as follows. In a 100-mL volumetric flask, dissolve 40 mg of USP Cetirizine Hydrochloride RS in *Diluent*, and then pipet 3.0 mL of the *System suitability stock solution*. Dilute with *Diluent* to volume.
Standard solution: 0.4 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.4 mg/mL of cetirizine hydrochloride, prepared as follows. Transfer 20 Tablets into a 500-mL volumetric flask, add 375 mL of *Diluent*, and stir for at least 30 min. Dilute with *Diluent* to volume and pass a portion of this solution through a suitable filter (GHP or PVDF), discarding the first 1 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm. For *Identification* test *B*, use a diode array detector in the range of 210–300 nm.
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—Identify the components on the basis of their relative retention times, as shown in *Table 2*.]
Suitability requirements
Resolution: NLT 1.5 between cetirizine and meclizine related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩**:** NMT 30 s
- **DISSOLUTION** ⟨711⟩

Medium: pH 6.5 phosphate buffer (4.77 g/L monobasic potassium phosphate and 2.62 g/L of dibasic potassium phosphate); 900 mL, degassed by USP procedure
Apparatus 2: 50 rpm
Time: 15 min
Buffer: 2.7 g/L of monobasic potassium phosphate in water. Add 0.6 mL/L of phosphoric acid.
Mobile phase: Acetonitrile and *Buffer* (33:67)
Diluent: Acetonitrile and 0.01 N hydrochloric acid (20:80)

www.webofpharma.com

Standard stock solution: 0.1 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*
Standard solution: 0.01 mg/mL of USP Cetirizine Hydrochloride RS from *Standard stock solution*, in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 1–2 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 2.5 mL/min
Injection volume: 100 µL
Run time: 2.7 times the retention time of cetirizine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times 1/L \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer A, Buffer B, Solution A, Solution B, Mobile phase, and **Diluent:** Proceed as directed in the *Assay*.
System suitability stock solution: 0.04 mg/mL each of USP Cetirizine Related Compound G RS, USP Cetirizine Related Compound B RS, USP 4-Chlorobenzophenone RS, USP Meclizine Related Compound A RS, and USP Cetirizine Related Compound A RS, and 0.06 mg/mL each of USP Hydroxyzine Related Compound A RS and USP Cetirizine Related Compound C RS, prepared as follows. Transfer a quantity of each compound into a suitable volumetric flask. Add 5% of the flask volume of acetonitrile to dissolve the compounds. Sonicate as needed. Dilute with *Diluent* to volume.
System suitability solution: 0.4 mg/mL of USP Cetirizine Hydrochloride RS; 1.2 µg/mL each of USP Cetirizine Related Compound G RS, USP Cetirizine Related Compound B RS, USP 4-Chlorobenzophenone RS, USP Meclizine Related Compound A RS, and USP Cetirizine Related Compound A RS; and 1.8 µg/mL each of USP Hydroxyzine Related Compound A RS and USP Cetirizine Related Compound C RS, prepared as follows. In a 100-mL volumetric flask, dissolve 40 mg of USP Cetirizine Hydrochloride RS in *Diluent*, and then pipet 3.0 mL of the *System suitability stock solution*. Dilute with *Diluent* to volume.
Standard stock solution: 0.04 mg/mL each of USP Cetirizine Hydrochloride RS, USP Cetirizine Related Compound G RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS, prepared as follows. Transfer a quantity of each compound into a suitable volumetric flask. Add 5% of the flask volume of acetonitrile to dissolve the compounds. Sonicate if needed. Dilute with *Diluent* to volume.
Standard solution: 0.8 µg/mL each of USP Cetirizine Hydrochloride RS, USP Cetirizine Related Compound G RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS from *Standard stock solution*, in *Diluent*
Sample solution: Nominally 400 µg/mL of cetirizine hydrochloride, prepared as follows. Transfer 20 Tablets into a 500-mL volumetric flask, add 375 mL of *Diluent*, and stir for at least 30 min. Dilute with *Diluent* to volume and pass a portion of this solution through a suitable filter (GHP or PVDF), discarding the first 1 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm and 260 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between cetirizine and meclizine related compound A, *System suitability solution*
Relative standard deviation: NMT 10.0% for each component, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Chromatograph the *System suitability solution*, and identify the components on the basis of their relative retention times, as shown in *Table 2*. Use the peak response at 260 nm for 4-chlorobenzophenone. Use 230 nm for all other degradation products.
Calculate the percentage of cetirizine related compound G, hydroxyzine related compound A, and 4-chlorobenzophenone in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cetirizine related compound G, hydroxyzine related compound A, or 4-chlorobenzophenone from the *Sample solution*
r_S = peak response of the appropriate USP Reference Standard from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (µg/mL)
C_U = nominal concentration of cetirizine hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of all other degradation products in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other degradation product from the *Sample solution*
r_S = peak response of cetirizine from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cetirizine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

www.webofpharma.com

Table 2

Compound	Relative Retention Time	Wavelength (nm)	Acceptance Criteria, NMT (%)
Cetirizine related compound B	0.88	230	0.2
Meclizine related compound A	0.95	230	0.2
Cetirizine	1.00	230	—
Bromocetirizine	1.07	—	[a]
Cetirizine related compound C	1.09	—	[a]
Cetirizine related compound G	1.15	230	0.2
Hydroxyzine related compound A	1.23	230	0.2
4-Chlorobenzophenone	1.45	260	0.2
Cetirizine related compound A	1.53	230	0.2
Any individual unspecified degradation product	—	230	0.2
Total degradation products	—	—	0.8

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cetirizine Hydrochloride RS

USP Cetirizine Related Compound A RS

▲(RS)-2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid ethyl ester oxalate.

$C_{23}H_{29}ClN_2O_3 \cdot C_2H_2O_4$ 506.98▲ (ERR 1-Jun-2019)

USP Cetirizine Related Compound B RS

2-(4-Benzhydrylpiperazin-1-yl)ethan-1-ol.

$C_{19}H_{24}N_2O$ 296.41

USP Cetirizine Related Compound C RS

2-(2-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy)acetamide.

$C_{21}H_{26}ClN_3O_2$ 387.90

USP Cetirizine Related Compound G RS

2-{4-[(4-Chlorophenyl)phenylmethyl)piperazin-1-yl}ethanol.

$C_{19}H_{23}ClN_2O$ 330.85

USP 4-Chlorobenzophenone RS

(4-Chlorophenyl)phenylmethanone.

$C_{13}H_9ClO$ 216.66

USP Hydroxyzine Related Compound A RS

1-[(4-Chlorophenyl)phenylmethyl]piperazine.

$C_{17}H_{19}ClN_2$ 286.80

USP Meclizine Related Compound A RS

4-Chlorobenzhydrol.

$C_{13}H_{11}ClO$ 218.68

Cetirizine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets

DEFINITION

Cetirizine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

IDENTIFICATION

A. The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **CETIRIZINE HYDROCHLORIDE**

Buffer: 3.5 g/L of monobasic ammonium phosphate and 1.0 g/L of tetrabutylammonium bisulfate in water. Adjust with phosphoric acid to a pH of 2.5.

Diluent: Methanol and *Buffer* (2:3)

Solution A: Acetonitrile, methanol, and *Buffer* (9:2:29)

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
27.0	100	0
30.0	0	100
30.1	100	0
35.0	100	0

Standard stock solution: 0.5 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*. [NOTE—Sonicate to dissolve.]

Standard solution: 0.025 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent* from the *Standard stock solution*

Sample solution: 0.025 mg/mL of cetirizine hydrochloride (from NMT 10 finely powdered Tablets) prepared as follows. Dissolve the Tablets first in methanol, using 22.5% of the final flask volume. Sonicate for NLT 20 min with vigorous swirling every 5 min. To the solution add a volume of *Buffer* equal to 26% of the final flask volume. Allow the solution to equilibrate to room temperature. Dilute with *Diluent* to volume. Pass a portion through a membrane filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Temperatures

Column: 30°

Autosampler: 5°

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cetirizine from the *Sample solution*
r_S = peak response of cetirizine from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

• **PSEUDOEPHEDRINE HYDROCHLORIDE**

Buffer: 0.8 g/L of ammonium acetate in water. To 1 L of the solution add 1.0 mL of triethylamine. Adjust with glacial acetic acid to a pH of 4.5.

Mobile phase: Acetonitrile and *Buffer* (3:7)

Standard solution: 0.5 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Mobile phase*. [NOTE—Sonicate to dissolve.]

Sample stock solution: 2.4 mg/mL of pseudoephedrine hydrochloride (from 5 finely powdered Tablets) prepared as follows. Dissolve the crushed Tablets first in acetonitrile, using 24% of the final flask volume. Sonicate for NLT 15 min. To the solution add a volume of *Buffer* equal to 56% of the final flask volume. Sonicate for NLT 15 min. Shake the flask for NLT 10 min. Allow the solution to equilibrate to room temperature. Dilute with *Mobile phase* to volume. Centrifuge a portion for 15 min to obtain a clear supernatant.

Sample solution: 0.5 mg/mL of pseudoephedrine hydrochloride in *Mobile phase* from the *Sample stock solution*. Pass the solution through a membrane filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L9
Flow rate: 1.5 mL/min
Injection volume: 25 µL
Run time: 2 times the retention time of pseudoephedrine

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pseudoephedrine from the *Sample solution*
r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1
Medium: 0.1 N hydrochloric acid; 500 mL, deaerated
Apparatus 1: 100 rpm
Time: 30 min for cetirizine hydrochloride and 30 min (used only for adjustments in the calculations); 1, 2, and 6 h for pseudoephedrine hydrochloride
Buffer: 0.77 g/L of ammonium acetate in water. To 1 L of the solution add 1.0 mL of triethylamine. Adjust with glacial acetic acid to a pH of 4.5 ± 0.05.
Mobile phase: Acetonitrile and *Buffer* (3:7)
Standard stock solution: 0.5 mg/mL of USP Cetirizine Hydrochloride RS in water
Standard solution: 0.24 mg/mL of USP Pseudoephedrine Hydrochloride RS and 0.01 mg/mL of USP Cetirizine Hydrochloride RS in *Medium* from the *Standard stock solution*
Sample solution: At the times specified, withdraw 5 mL of the solution under test, and pass through a suitable filter of 0.45-µm pore size, discarding the first few mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV, 230 nm for cetirizine hydrochloride, 254 nm for pseudoephedrine hydrochloride
Column: 4.6-mm × 15-cm; 5-µm packing L9
Flow rate: 1.5 mL/min
Injection volume: 25 µL
Run time: 2 times the retention time of pseudoephedrine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both cetirizine and pseudoephedrine
Relative standard deviation: NMT 2.0% for both cetirizine and pseudoephedrine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cetirizine hydrochloride dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of cetirizine from the *Sample solution*
r_S = peak response of cetirizine from the *Standard solution*
C_S = concentration of cetirizine hydrochloride in the *Standard solution* (mg/mL)
L = label claim for cetirizine hydrochloride (mg/Tablet)
V = volume of *Medium*, 500 mL

Calculate the percentage of pseudoephedrine hydrochloride dissolved at each time point:

$$Q_{30} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_1 = (Q_{30} \times 5/500) + [(r_U/r_S) \times (C_S/L) \times 495 \times 100]$$

$$Q_2 = (Q_{30} \times 5/500) + (Q_1 \times 5/495) + [(r_U/r_S) \times (C_S/L) \times 490 \times 100]$$

$$Q_6 = (Q_{30} \times 5/500) + (Q_1 \times 5/495) + (Q_2 \times 5/490) + [(r_U/r_S) \times (C_S/L) \times 485 \times 100]$$

r_U = peak response of pseudoephedrine from the *Sample solution*

USP Monographs

www.webofpharma.com

r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of pseudoephedrine hydrochloride in the *Standard solution* (mg/mL)
L = label claim for pseudoephedrine hydrochloride (mg/Tablet)
V = initial volume of *Medium*, 500 mL

Tolerances

Cetirizine hydrochloride: NLT 80% (Q) of the labeled amount of cetirizine hydrochloride is dissolved in 30 min.

Pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$): See *Table 2*.

Table 2

Time (h)	Amount Dissolved
1	30%–50%
2	50%–70%
6	NLT 80%

The percentages of the labeled amount of pseudoephedrine hydrochloride dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.1 N HCl; 500 mL

Apparatus 1: 100 rpm

Time: 30 min for cetirizine hydrochloride and 30 min (used only for adjustments in the calculations); 1, 2, 4 and 8 h for pseudoephedrine hydrochloride

Buffer: 6.8 g/L of sodium acetate and 16.2 g/L of sodium 1-octanesulfonate

Mobile phase: Methanol and *Buffer* (50:50). Adjust with glacial acetic acid to a pH of 5.5.

Standard solution: 0.01 mg/mL of USP Cetirizine Hydrochloride RS and 0.24 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Medium*

Sample solution: Pass a 5-mL portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 4.6-mm × 10-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 2 mL/min

Injection volume: 100 µL

System suitability

[NOTE—The relative retention times for pseudoephedrine and cetirizine are 1.0 and 2.9, respectively.]

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates for both pseudoephedrine and cetirizine

Tailing factor: NMT 2.0 for both pseudoephedrine and cetirizine

Relative standard deviation: NMT 2.0% for both pseudoephedrine and cetirizine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of cetirizine from the *Sample solution*
r_S = peak response of cetirizine from the *Standard solution*
C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Calculate the concentration (C_i) of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the sample withdrawn from the vessel at each time point (i) shown in *Table 3*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of pseudoephedrine from the *Sample solution*
r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amounts (Q_i) of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at each time point (i) shown in *Table 3*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_S)]] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times [V - (3 \times V_S)]] + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_5 = \{[C_5 \times [V - (4 \times V_S)]] + [(C_4 + C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of pseudoephedrine hydrochloride in the portion of sample withdrawn at time point (i) (mg/mL)
V = volume of the *Medium* (500 mL)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)
L = label claim for pseudoephedrine hydrochloride (mg/Tablet)

Tolerances

Cetirizine hydrochloride: NLT 75% (Q) of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) is dissolved.

Pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$): See *Table 3*.

Table 3

Time Point (i)	Time (h)	Amount Dissolved
1	0.5	—
2	1	30%–50%
3	2	50%–70%
4	4	70%–90%
5	8	NLT 80%

www.webofpharma.com

The percentages of the labeled amount of pseudoephedrine hydrochloride dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Cetirizine Hydrochloride Related Compounds**

Buffer, Diluent, Solution A, and **Solution B:** Proceed as directed in the *Assay* for *Cetirizine hydrochloride.*

Mobile phase: See *Table 4.*

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	100	0
27	100	0
45	60	40
65	60	40
65.1	100	0
75	100	0

Standard stock solution: 0.5 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent.* [Note—Sonicate to dissolve.]

Standard solution: 1 µg/mL of USP Cetirizine Hydrochloride RS in *Diluent* from the *Standard stock solution*

Sample stock solution: 0.5 mg/mL of cetirizine hydrochloride (from NMT 10 finely powdered Tablets) prepared as follows. Dissolve the Tablets first in methanol, using 70% of the final flask volume. Sonicate for 15 min, and then shake for 15 min. Allow the solution to cool to room temperature, and dilute with methanol to volume. Centrifuge a portion for 10 min.

Sample solution: 0.2 mg/mL of cetirizine hydrochloride in *Buffer* from the *Sample stock solution.* Pass a portion through a suitable membrane filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Temperatures

Column: 30°

Autosampler: 5°

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1300 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the individual impurity from the *Sample solution*

r_S = peak response of cetirizine from the *Standard solution*

C_S = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 5*)

Acceptance criteria: See *Table 5.*

[Note—Disregard any peak less than 0.05% of the main peak.]

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cetirizineethanol[a]	0.54	1.4	0.3
Chlorobenzhydryl piperazine (CBHP)[b]	0.57	1.5	0.3
Cetirizine	1.0	—	—
Cetirizine acetic acid[c]	1.30	1.1	0.3
Cetirizine *N*-oxide[d]	1.47	1.2	0.3
Any unspecified degradation product	—	1.0	0.2
Total impurities	—	—	0.8

[a] 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol.
[b] 1-[(4-Chlorophenyl)phenylmethyl]piperazine.
[c] 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]acetic acid.
[d] 2-[4-[(4-Chlorophenyl)phenylmethyl]-1-oxide-1-piperazinyl]ethoxy]acetic acid.

• **Pseudoephedrine Hydrochloride Related Compounds**

Buffer: 11.2 g/L of sodium perchlorate monohydrate in water. Adjust with hydrochloric acid to a pH of 2.7.

Solution A: Methanol and *Buffer* (3:17)

Solution B: Methanol and *Buffer* (1:1)

Diluent: *Solution A*

Mobile phase: See *Table 6.*

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
35	28	72

Standard stock solution: 0.48 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*

Standard solution: 4.8 µg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent* from the *Standard stock solution*

System suitability stock solution: 49 µg/mL of ephedrine in *Diluent* from USP Ephedrine Sulfate RS

System suitability solution: 1.96 µg/mL of ephedrine and 0.46 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Standard stock solution* from the *System suitability stock solution* and the *Standard stock solution,* respectively

Sample stock solution: 2.4 mg/mL of pseudoephedrine hydrochloride (from NMT 25 finely powdered Tablets) prepared as follows. Dissolve the Tablets first in methanol, using 75% of the final flask volume. Sonicate for NLT 15 min, and then shake for 15 min. Allow the solution to cool to room temperature, and dilute with methanol to volume. Centrifuge a portion for 10 min.

Sample solution: 0.48 mg/mL of pseudoephedrine hydrochloride in *Diluent* from the *Sample stock solution.* Pass a portion through a suitable membrane filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

USP Monographs

www.webofpharma.com

Detector: UV 212 nm
Column: 4.6-mm × 25-cm; 4-µm packing L11
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 30 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.3 between ephedrine and pseudoephedrine, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the individual impurity from the *Sample solution*
r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 7*)

Acceptance criteria: See *Table 7*.
[NOTE—Disregard any peak less than 0.05% of the main peak.]

Table 7

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ephedrine[a, b]	0.95	—	—
Pseudoephedrine	1.0	—	—
Methcathinone[c]	1.1	1.1	0.2
Any unspecified degradation product	—	1.0	0.2
Total pseudoephedrine related impurities	—	—	0.5

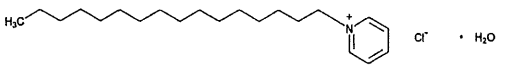
[a] [*R*-(*R**,*S**)]-α-[1-(Methylamino)ethyl]-benzenemethanol.
[b] For system suitability and identification purposes only.
[c] 2-Methylamino-1-phenylpropan-1-one.

Sum of cetirizine and pseudoephedrine related impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Cetirizine Hydrochloride RS
USP Ephedrine Sulfate RS
USP Pseudoephedrine Hydrochloride RS

Cetylpyridinium Chloride



$C_{21}H_{38}ClN \cdot H_2O$ 358.00
$C_{21}H_{38}ClN$ 339.99
Pyridinium, 1-hexadecyl-, chloride, monohydrate; 1-Hexadecylpyridinium chloride monohydrate [6004-24-6]. Anhydrous [123-03-5].

DEFINITION

Cetylpyridinium Chloride contains NLT 98.0% and NMT 102.0% of cetylpyridinium chloride ($C_{21}H_{38}ClN$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*
Sample solution: 2 mg/mL in water
Acceptance criteria: A 10-mL portion of the *Sample solution* meets the requirements of test *A*, except that when silver nitrate TS is added, turbidity is produced rather than a curdy white precipitate.

ASSAY

- **PROCEDURE**
Use 0.1% trifluoroacetic acid–rinsed glassware and silanized vials for all solutions containing cetylpyridinium chloride, as cetylpyridinium may react with the surface.
Solution A: Trifluoroacetic acid and water (1:999)
Solution B: Acetonitrile and trifluoroacetic acid (999:1)
Mobile phase: *Solution A* and *Solution B* (62.5: 37.5)
Standard solution: 0.25 mg/mL of USP Cetylpyridinium Chloride RS in *Solution A*
Sample solution: 0.25 mg/mL of Cetylpyridinium Chloride in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 258 nm
Column: 2.1-mm × 10-cm; 5-µm packing L78
Column temperature: 40°
Flow rate: 0.6 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cetylpyridinium chloride ($C_{21}H_{38}ClN$) in the portion of Cetylpyridinium Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cetylpyridinium Chloride RS in the *Standard solution* (mg/mL)

USP Monographs

www.webofpharma.com

C_U = concentration of Cetylpyridinium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2% on the anhydrous basis

• **ORGANIC IMPURITIES**

Use 0.1% trifluoroacetic acid–rinsed glassware and silanized vials for all solutions containing cetylpyridinium chloride, as cetylpyridinium may react with the surface.

Solution A, Solution B, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 2.5 µg/mL of USP Cetylpyridinium Chloride RS in *Solution A*

Sample solution: 2.5 mg/mL of Cetylpyridinium Chloride in *Solution A*

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each unspecified impurity in the portion of Cetylpyridinium Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response of cetylpyridinium from the *Standard solution*

C_S = concentration of USP Cetylpyridinium Chloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Cetylpyridinium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.04%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cetylpyridinium chloride	1.0	—
Any unspecified impurity	—	0.1 (Postponed indefinitely)
Total impurities	—	1.0

SPECIFIC TESTS

• **ACIDITY**

Sample: 500 mg

Analysis: Dissolve *Sample* in 50 mL of water, add phenolphthalein TS, and titrate with 0.020 N sodium hydroxide.

Acceptance criteria: NMT 2.5 mL is required for neutralization.

• **WATER DETERMINATION** ⟨921⟩, *Method I*: 4.5%–5.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cetylpyridinium Chloride RS

Cetylpyridinium Chloride Lozenges

DEFINITION

Cetylpyridinium Chloride Lozenges contain NLT 90.0% and NMT 125.0% of the labeled amount of cetylpyridinium chloride ($C_{21}H_{38}ClN \cdot H_2O$) in a suitable molded base.

IDENTIFICATION

• **A.**

Eluting solvent: Alcohol and 1.2 N hydrochloric acid (7:3)

Chromatographic column: Pack a pledget of fine glass wool in the base of a 10-mm × 200-mm chromatographic tube. Add styrene–divinylbenzene cation-exchange resin (strong acid form) to form a uniform column 12 cm in height, and top the column with a pledget of fine glass wool.

Standard solution: 5 µg/mL of USP Cetylpyridinium Chloride RS in *Eluting solvent*

Sample solution: Dissolve nominally 500 µg of cetylpyridinium chloride from NLT 20 finely powdered Lozenges in 50 mL of water. Immediately transfer this solution to the *Chromatographic column*, and discard the eluate. Wash the column, successively, with 200 mL of water, 100 mL of alcohol, 100 mL of water, and 100 mL of 3 N hydrochloric acid. Discard the washings. Elute the column with 80 mL of *Eluting solvent*. Collect the eluate in a 100-mL volumetric flask, and dilute with the *Eluting solvent* to volume.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Wavelength range: 225–300 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*.

ASSAY

• **PROCEDURE**

0.004 M sodium lauryl sulfate: Dissolve 1.15 g of sodium lauryl sulfate in 500 mL of water. Add 2 mL of sulfuric acid, and dilute with water to 1000 mL.

Standardization of 0.004 M sodium lauryl sulfate: Determine the molarity of the solution as follows. To a glass-stoppered 100-mL cylinder transfer 10.0 mL of 0.004 M cetylpyridinium chloride (1.432 mg/mL of USP Cetylpyridinium Chloride RS). Add 5 mL of 2 N sulfuric acid, 20 mL of chloroform, and 1 mL of methyl yellow TS. Titrate with the sodium lauryl sulfate solution with frequent vigorous shaking until the chloroform layer acquires the first permanent orange-pink color.

Calculate the molarity, and restandardize before each use. [NOTE—Sulfuric acid is included in this solution to inhibit precipitate formation. If a precipitate forms under storage, discard the solution, and prepare and standardize a fresh solution of *0.004 M sodium lauryl sulfate*.]

Sample solution: Nominally 0.1 mg/mL of cetylpyridinium chloride prepared as follows. Dissolve an accurately determined number of Lozenges (about 100) in about 400 mL of water in a 500-mL volumetric flask, and dilute with water to volume. Transfer a measured aliquot of this solution, equivalent to about 10 mg of cetylpyridinium chloride, to a glass-stoppered, 100-mL cylinder. Add 5 mL of 2 N sulfuric acid, 20 mL of chloroform, and 1 mL of methyl yellow TS. Insert the stopper, and shake until the chloroform layer develops a bright yellow color.

Analysis: Titrate with *0.004 M sodium lauryl sulfate*, shaking thoroughly after each addition, until the chloroform layer develops the first permanent orange-pink color. Each mL of

USP Monographs

www.webofpharma.com

0.004 M sodium lauryl sulfate is equivalent to 1.432 mg of cetylpyridinium chloride ($C_{21}H_{38}ClN \cdot H_2O$).
Acceptance criteria: 90.0%–125.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cetylpyridinium Chloride RS

Cetylpyridinium Chloride Topical Solution

DEFINITION
Cetylpyridinium Chloride Topical Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of cetylpyridinium chloride ($C_{21}H_{38}ClN \cdot H_2O$).

IDENTIFICATION
- **A.**
  Standard solution: 40 µg/mL of USP Cetylpyridinium Chloride RS in water
  Sample solution: 40 µg/mL of cetylpyridinium chloride from Topical Solution diluted with water
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*.
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*
  Sample solution: Evaporate on a steam bath a volume of Topical Solution equivalent to 500 mg of cetylpyridinium chloride from Topical Solution to one-half of its original volume.
  Acceptance criteria: The *Sample solution* meets the requirements of test *A*, except that when silver nitrate TS is added, turbidity is produced rather than a curdy white precipitate.

ASSAY
- **PROCEDURE**
  Sample solution: Add a volume of Topical Solution nominally equivalent to 150 mg of cetylpyridinium chloride to a glass-stoppered, 500-mL graduated cylinder. Add 10 mL of chloroform, 0.4 mL of bromophenol blue solution (1 in 2000), and 5 mL of a freshly prepared solution of sodium bicarbonate (4.2 in 1000).
  Analysis: Titrate the *Sample solution* with 0.02 M sodium tetraphenylboron VS until the blue color disappears from the chloroform layer. Add the last portions of the sodium tetraphenylboron solution dropwise, agitating vigorously after each addition. Each mL of 0.02 M sodium tetraphenylboron is equivalent to 7.160 mg of cetylpyridinium chloride ($C_{21}H_{38}ClN \cdot H_2O$).
  Acceptance criteria: 95.0%–105.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cetylpyridinium Chloride RS

Activated Charcoal

DEFINITION
Activated Charcoal is the residue from the destructive distillation of various organic materials, treated to increase its adsorptive power.

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩
  Sample: 0.50 g
  Acceptance criteria: NMT 4.0%
- **ACID-SOLUBLE SUBSTANCES**
  Sample: 1.0 g
  Analysis: Boil the *Sample* with a mixture of 20 mL of water and 5 mL of hydrochloric acid for 5 min. Filter into a tared porcelain crucible, and wash the residue with 10 mL of hot water, adding the washing to the filtrate. To the combined filtrate and washing add 1 mL of sulfuric acid. Evaporate to dryness, and ignite to constant weight.
  Acceptance criteria: The residue weighs NMT 35 mg (NMT 3.5%).
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Sample solution: A 10-mL portion of the filtrate obtained in the test for *Reaction*
  Acceptance criteria: The *Sample solution* shows no more chloride than is contained in 1.5 mL of 0.020 N hydrochloric acid (NMT 0.2%).
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Sample solution: A 10-mL portion of the filtrate obtained in the test for *Reaction*
  Acceptance criteria: The *Sample solution* shows no more sulfate than is contained in 1.0 mL of 0.020 N sulfuric acid (NMT 0.2%).
- **SULFIDE**
  Sample: 0.50 g
  Analysis: Place the *Sample* in a small conical flask. Add 20 mL of water and 5 mL of hydrochloric acid, and boil gently.
  Acceptance criteria: The escaping vapors do not darken paper moistened with lead acetate TS.
- **CYANOGEN COMPOUNDS**
  Sample: 5 g
  Analysis: Place the *Sample*, 50 mL of water, and 2 g of tartaric acid in a distilling flask connected to a condenser provided with a tightly fitting adapter, the end of which dips below the surface of a mixture of 2 mL of 1 N sodium hydroxide and 10 mL of water, contained in a small flask surrounded by ice. Heat the mixture in the distilling flask to boiling, and distill about 25 mL. Dilute the distillate with water to 50 mL, and mix. To 25 mL of the diluted distillate add 12 drops of ferrous sulfate TS, heat the mixture almost to boiling, cool, and add 1 mL of hydrochloric acid.
  Acceptance criteria: No blue color is produced.
- **UNCARBONIZED CONSTITUENTS**
  Sample: 0.25 g
  Analysis: Boil the *Sample* with 10 mL of 1 N sodium hydroxide for 5 s, and filter.
  Acceptance criteria: The filtrate is colorless.

SPECIFIC TESTS
- **ADSORPTIVE POWER**
  Alkaloids
  Sample: 1 g, previously dried at 120° for 4 h
  Analysis: Shake the *Sample* with a solution of 100 mg of strychnine sulfate in 50 mL of water for 5 min, and filter through a dry filter, rejecting the first 10 mL of the filtrate. To a 10-mL portion of the subsequent filtrate add 1 drop of hydrochloric acid and 5 drops of mercuric–potassium iodide TS.
  Acceptance criteria: No turbidity is produced.

www.webofpharma.com

Dyes
Sample: 250 mg
Analysis: Pipet 50 mL of methylene blue solution (1 in 1000) into each of two glass-stoppered, 100-mL flasks. Add the *Sample* to one of the flasks, insert the stopper in the flask, and shake for 5 min. Filter the contents of each flask through a dry filter, rejecting the first 20 mL of each filtrate. Pipet 25-mL portions of the remaining filtrates into two 250-mL volumetric flasks. Add to each flask 50 mL of sodium acetate solution (1 in 10), mix, and add from a buret 35.0 mL of 0.1 N iodine VS, swirling the mixture during the addition. Insert the stoppers in the flasks, and allow them to stand for 50 min, shaking them vigorously at 10-min intervals. Dilute each mixture with water to volume, mix, allow to stand for 10 min, and filter through dry filters, rejecting the first 30 mL of each filtrate. Titrate the excess iodine in a 100-mL aliquot of each subsequent filtrate with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Calculate the mL of 0.1 N iodine consumed in each titration.
Acceptance criteria: The difference between the two volumes is NLT 0.7 mL.

- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli.*
- **Reaction**

Sample: 3.0 g
Analysis: Boil the *Sample* with 60 mL of water for 5 min. Allow to cool, restore the original volume by the addition of water, and filter.
Acceptance criteria: The filtrate is colorless and is neutral to litmus.

- **Loss on Drying** ⟨731⟩: Dry a sample at 120° for 4 h: it loses NMT 15.0% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Chloral Hydrate



$C_2H_3Cl_3O_2$ 165.40
1,1-Ethanediol, 2,2,2-trichloro-.
Chloral hydrate [302-17-0].

» Chloral Hydrate contains not less than 99.5 percent and not more than 102.5 percent of $C_2H_3Cl_3O_2$.

Packaging and storage—Preserve in tight containers.
Identification—Transfer to a 125-mL conical flask a portion of a solution in water equivalent to about 1 mg of chloral hydrate, and add water to bring the volume to about 10 mL. Add 10 mL of 1-ethylquinaldinium iodide solution (15 in 1000), which has been filtered through a 0.45-µm filter. Add 60 mL of isopropyl alcohol, 5 mL of an aqueous 0.1 M monoethanolamine solution, and 15 mL of water. Mix, and heat in a water bath at 60° for 15 minutes: a blue color develops.
Acidity—A 1 in 20 solution in alcohol does not at once redden moistened blue litmus paper.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chloride ⟨221⟩—To a 1 in 10 solution in alcohol add a few drops of silver nitrate TS: any opalescence produced does not exceed that of a control containing 0.10 mL of 0.020 N hydrochloric acid (0.007%).
Readily carbonizable substances ⟨271⟩—Shake 500 mg, at intervals of 5 minutes during 1 hour, with 5 mL of sulfuric acid in a glass-stoppered cylinder that previously has been rinsed with sulfuric acid, and transfer the mixture to a comparison vessel: the mixture has no more color than *Matching Fluid P.*
Assay—Dissolve about 4 g of Chloral Hydrate, accurately weighed, in 10 mL of water, add 30.0 mL of 1 N sodium hydroxide VS, and allow the mixture to stand for 2 minutes. Add a few drops of phenolphthalein TS, and titrate the residual alkali at once with 1 N sulfuric acid VS. Each mL of 1 N sodium hydroxide corresponds to 165.4 mg of $C_2H_3Cl_3O_2$.

Chloral Hydrate Capsules

» Chloral Hydrate Capsules contain not less than 95.0 percent and not more than 110.0 percent of the labeled amount of $C_2H_3Cl_3O_2$.

Packaging and storage—Preserve in tight containers, preferably at controlled room temperature.
Identification—The contents of the Capsules respond to the *Identification*— test under *Chloral Hydrate.*

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Time: 15 minutes.
Procedure—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each capsule shell to rupture.
Tolerances—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay—Place a counted number of Capsules, equivalent to about 2.5 g of chloral hydrate, in a glass-stoppered, 250-mL flask, add 25 mL of water, insert the stopper in the flask, and heat on a steam bath with frequent swirling until the Capsules are dissolved. Cool to room temperature, add 25 mL of neutralized alcohol and 20.0 mL of 1 N sodium hydroxide VS, mix, and allow the mixture to stand for 4 minutes. Add phenolphthalein TS, and titrate the excess alkali with 1 N sulfuric acid VS. Each mL of 1 N sodium hydroxide is equivalent to 165.4 mg of $C_2H_3Cl_3O_2$.

Chloral Hydrate Oral Solution

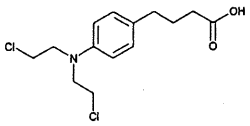
» Chloral Hydrate Oral Solution contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of chloral hydrate ($C_2H_3Cl_3O_2$).

www.webofpharma.com

Packaging and storage—Preserve in tight, light-resistant containers.
Identification—It meets the requirements for the *Identification* test under *Chloral Hydrate*
Assay—Transfer 25.0 mL of Oral Solution to a 250-mL conical flask with the aid of several portions of water. Add 30.0 mL of 1 N sodium hydroxide VS, and mix. After the mixture has stood for 2 minutes, add 5 drops of phenolphthalein TS, and immediately titrate the excess sodium hydroxide with 1 N sulfuric acid VS. Designate the volume of 1 N sodium hydroxide VS consumed as *A*. Transfer 5.0 mL of Oral Solution to a second 250-mL conical flask with the aid of several portions of water. Add 10 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Designate the volume of 0.1 N sodium hydroxide VS consumed as *B*. Calculate the weight, in mg, of chloral hydrate ($C_2H_3Cl_3O_2$) in the amount of Oral Solution taken by the first titration by the formula:

$$165.4(A - 0.5B).$$

Chlorambucil



$C_{14}H_{19}Cl_2NO_2$ 304.21
Benzenebutanoic acid, 4-[bis(2-chloroethyl)amino]-;
4-[*p*-[Bis(2-chloroethyl)amino]phenyl]butyric acid [305-03-3].

DEFINITION
Chlorambucil contains NLT 98.0% and NMT 101.0% of chlorambucil ($C_{14}H_{19}Cl_2NO_2$), calculated on the anhydrous basis.
[**CAUTION**—Great care should be taken to prevent inhaling particles of Chlorambucil and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)
  Sample solution: 8 mg/mL in carbon disulfide
  Cell: 1 mm
  Acceptance criteria: Meets the requirements
- **B.**
  Sample solution: Dissolve 50 mg of Chlorambucil in 5 mL of acetone, and dilute with water to 10 mL.
  Analysis: To the *Sample solution* add 1 drop of 2 N sulfuric acid, then add 4 drops of silver nitrate TS.
  Acceptance criteria: No opalescence is observed immediately (absence of chloride ion). Warm the solution on a steam bath: opalescence develops (presence of ionizable chlorine).

ASSAY
- **PROCEDURE**
  Sample: 200 mg of Chlorambucil
  Titrimetric system
  Mode: Direct titration
  Titrant: 0.1 N sodium hydroxide VS
  Endpoint detection: Visual
  Analysis: Dissolve the *Sample* in 10 mL of acetone, add 10 mL of water, and titrate with *Titrant*, using phenolphthalein TS as the indicator. Each mL of 0.1 N sodium hydroxide is equivalent to 30.42 mg of chlorambucil ($C_{14}H_{19}Cl_2NO_2$).
  Acceptance criteria: 98.0%–101.0% on the anhydrous basis

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 65°–69°
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Chlorambucil RS

Add the following:

▲Chlorambucil Compounded Oral Suspension

DEFINITION
Chlorambucil Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorambucil ($C_{14}H_{19}Cl_2NO_2$).
Prepare Chlorambucil Compounded Oral Suspension 2 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

Chlorambucil tablets,[a] equivalent to	200 mg of chlorambucil
Vehicle: a 1:1 mixture of Ora Plus[b] and Ora-Sweet,[b] a sufficient quantity to make	100 mL

[a] Chlorambucil 2-mg tablets, GlaxoSmithKline, Research Triangle Park, NC.
[b] Perrigo, Allegan, MI.

Place the *Chlorambucil tablets* into a suitable container and triturate to a fine powder. Add a small amount of *Vehicle* to form a smooth paste. Add a sufficient amount of *Vehicle* to make the contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY
- **PROCEDURE**
  Solution A: 0.2% (v/v) glacial acetic acid in water
  Mobile phase: Methanol and *Solution A* (65:35)
  Standard solution: 0.2 mg/mL of USP Chlorambucil RS in methanol
  Sample solution: Transfer 1.0 mL of Oral Suspension into a 10-mL volumetric flask, add methanol to volume, and filter. [NOTE—The *Standard solution* and the *Sample solution* must be used within 6 h of preparation.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 258 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Temperatures
  Autosampler: 15°
  Column: 40°
  Flow rate: 2.0 mL/min

USP Monographs

www.webofpharma.com

Injection volume: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for chlorambucil is about 3.6 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of chlorambucil ($C_{14}H_{19}Cl_2NO_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chlorambucil from the *Sample solution*
r_S = peak response of chlorambucil from the *Standard solution*
C_S = concentration of USP Chlorambucil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of chlorambucil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 3.5–4.5

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator.
• **BEYOND-USE DATE:** NMT 14 days after the day on which it was compounded when stored in a refrigerator
• **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Chlorambucil RS▲ (USP 1-May-2020)

Chlorambucil Tablets

DEFINITION
Chlorambucil Tablets contain NLT 85.0% and NMT 110.0% of the labeled amount of chlorambucil ($C_{14}H_{19}Cl_2NO_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S▲** (CN 1-May-2020)
Sample solution: Shake a quantity of finely powdered Tablets, equivalent to 16 mg of chlorambucil, with 20 mL of carbon disulfide. Filter, evaporate to dryness, and dissolve the residue in 2 mL of carbon disulfide.
Cell: 1 mm
Acceptance criteria: Meet the requirements

ASSAY
• **PROCEDURE**
Mobile phase: Mix 500 mL of alcohol with 1.0 mL of glacial acetic acid. Dilute with water to 1 L. Degas the solution at a pressure of approximately 250 mm of mercury for 2 min. [NOTE—The alcohol concentration may be varied to meet system suitability requirements and to provide a suitable elution time for chlorambucil.]
Internal standard solution: 0.4 mg/mL of USP Propylparaben RS in alcohol
Standard stock solution: 1 mg/mL of USP Chlorambucil RS in alcohol
Standard solution: 0.02 mg/mL of USP Chlorambucil RS in alcohol prepared as follows. Transfer 2.0 mL of the *Standard stock solution* into a 100-mL volumetric flask containing 50 mL of alcohol and, while gently swirling, add 5.0 mL of 0.1 N hydrochloric acid and 2.0 mL of *Internal standard solution*. Dilute with alcohol to volume.
Sample solution: Nominally 0.02 mg/mL of chlorambucil in alcohol prepared as follows. Transfer finely powdered Tablets (NLT 20), equivalent to 2 mg of chlorambucil, into a 100-mL volumetric flask containing 50 mL of alcohol and, while gently swirling, add 5.0 mL of 0.1 N hydrochloric acid and 2.0 mL of *Internal standard solution*. Sonicate for 5 min, and dilute with alcohol to volume. Filter through a medium pore size, sintered-glass filtering funnel, maintaining reduced pressure for the minimum necessary time to avoid solvent loss from evaporation.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 25- or 30-cm × 2-mm; 5- to 10-µm packing L1
Flow rate: Capable of giving the required *Resolution* in *Suitability requirements* and a suitable elution time
Injection volume: 10–12 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between the propylparaben and chlorambucil peaks
Relative standard deviation: NMT 2.0% for 6–8 injections, for the peak response ratio of chlorambucil to propylparaben
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of chlorambucil ($C_{14}H_{19}Cl_2NO_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of chlorambucil to propylparaben from the *Sample solution*
R_S = peak response ratio of chlorambucil to propylparaben from the *Standard solution*
C_S = concentration of USP Chlorambucil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of chlorambucil in the *Sample solution* (mg/mL)

Acceptance criteria: 85.0%–110.0%

PERFORMANCE TESTS
• **DISINTEGRATION** ⟨701⟩
Analysis: Place 1 Tablet in each of the six tubes of the basket, and if the Tablet has a soluble external coating, immerse the basket in water at room temperature for 5 min. Operate the apparatus, using simulated gastric fluid TS maintained at 37 ± 2° as the immersion fluid. After 30 min of operation in simulated gastric fluid TS, lift the basket from the fluid, and observe the Tablets. If the Tablets have not disintegrated completely, substitute simulated intestinal fluid TS maintained at 37 ± 2° as the immersion fluid, and continue the test for a total period of time equal to 45 min, including previous exposure to water and simulated gastric

USP Monographs

www.webofpharma.com

fluid TS. Lift the basket from the fluid, and observe the Tablets.

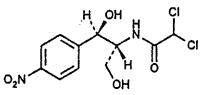
Acceptance criteria: All of the Tablets have disintegrated completely. If 1 or 2 Tablets fail to disintegrate completely, repeat the test on 12 additional Tablets: NLT 16 of the total of 18 Tablets tested disintegrate completely.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve coated Tablets in well-closed containers. Preserve uncoated Tablets in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Chlorambucil RS
  USP Propylparaben RS

Chloramphenicol



$C_{11}H_{12}Cl_2N_2O_5$ 323.13

Acetamide, 2,2-dichloro-*N*-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]-, [*R*-(*R**,*R**)]-.

D-*threo*-(−)-2,2-Dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide [56-75-7].

» Chloramphenicol contains not less than 97.0 percent and not more than 103.0 percent of $C_{11}H_{12}Cl_2N_2O_5$.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.

USP Reference standards ⟨11⟩—
USP Chloramphenicol RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Melting range ⟨741⟩: between 149° and 153°.

Specific rotation ⟨781S⟩: between +17.0° and +20.0°.
Test solution: 50 mg, undried, per mL, in dehydrated alcohol.

Crystallinity ⟨695⟩: meets the requirements.

Bacterial Endotoxins Test ⟨85⟩ —Where Chloramphenicol is intended for use in preparing injectable dosage forms, it contains not more than 0.2 USP Endotoxin Unit per mg of chloramphenicol.

Sterility Tests ⟨71⟩ —Where the label states that Chloramphenicol is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined,* except to use 1 g of solid specimen.

pH ⟨791⟩: between 4.5 and 7.5, in an aqueous suspension containing 25 mg per mL.

Chromatographic purity—Dissolve an accurately weighed quantity of Chloramphenicol in methanol to obtain a test solution containing 10 mg per mL. Prepare a solution of USP Chloramphenicol RS in methanol containing 10 mg per mL (*Standard solution A*). Dilute portions of *Standard solution A* quantitatively with methanol to obtain *Standard solution B* containing 100 µg per mL and *Standard solution C* containing 50 µg per mL. Apply separate 20-µL portions of the test solution and *Standard solutions B* and *C* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and glacial acetic acid (79:14:7) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and examine under short-wavelength UV light: any spot other than the principal spot obtained from the test solution does not exceed in size or intensity the principal spot obtained from *Standard solution B* (1%), and the sum of the impurities represented by all of the spots other than the principal spot, based on a comparison of the intensities of such spots with the intensities of the principal spots obtained from *Standard solutions B* and *C,* does not exceed 2%.

Assay—

Mobile phase—Prepare a suitable filtered mixture of water, methanol, and glacial acetic acid (55: 45: 0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 80 µg per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation.*

Assay preparation—Transfer about 200 mg of Chloramphenicol, accurately weighed, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 4.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 10-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed under *Procedure:* the column efficiency determined from the analyte peak is not less than 1800 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—[NOTE—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in the portion of Chloramphenicol taken by the formula:

$$2.5C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Chloramphenicol RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

www.webofpharma.com

Chloramphenicol Capsules

» Chloramphenicol Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Chloramphenicol RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 1: 100 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{11}H_{12}Cl_2N_2O_5$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 278 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Chloramphenicol RS in the same *Medium*.
Tolerances—Not less than 85% *(Q)* of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*— under *Chloramphenicol*.
Standard preparation—Transfer about 25 mg of USP Chloramphenicol RS, accurately weighed, to a 200-mL volumetric flask, add 10 mL of water, and heat on a steam bath until completely dissolved. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.
Assay preparation—Transfer an accurately counted number of Chloramphenicol Capsules, equivalent to about 2500 mg of chloramphenicol, to a 1000-mL volumetric flask, add 100 mL of water, and heat on a steam bath until the Capsules have disintegrated. Add 300 mL of water, and heat on a steam bath for 20 minutes, with occasional mixing. Cool to room temperature, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.
Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Chloramphenicol*. Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in each Capsule taken by the formula:

$$20(C/N)(r_U/r_S)$$

in which N is the number of Capsules taken, and the other terms are as defined therein.

Chloramphenicol Cream

» Chloramphenicol Cream contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$.

Packaging and storage—Preserve in collapsible tubes or in tight containers.
USP Reference standards ⟨11⟩—
USP Chloramphenicol RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.
Minimum fill ⟨755⟩: meets the requirements.

Assay—
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*— under *Chloramphenicol*.
Standard preparation—Transfer about 40 mg of USP Chloramphenicol RS, accurately weighed, to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.
Assay preparation—Transfer an accurately weighed quantity of Chloramphenicol Cream, equivalent to about 40 mg of chloramphenicol, to a 100-mL volumetric flask, add about 80 mL of methanol, and sonicate for about 10 minutes. Cool to room temperature, dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.
Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Chloramphenicol*. Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in the portion of Cream taken by the formula:

$$0.5C(r_U/r_S)$$

in which the terms are as defined therein.

Chloramphenicol Injection

» Chloramphenicol Injection is a sterile solution of Chloramphenicol in one or more suitable solvents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$. It may contain suitable buffers.

Packaging and storage—Preserve in single-dose or in multiple-dose containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Chloramphenicol RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.2 USP Endotoxin Unit per mg of chloramphenicol.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 1 mL from each container being transferred directly to the membrane filter.
pH ⟨791⟩: between 5.0 and 8.0, in a solution diluted with water (1:1).

www.webofpharma.com

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Chloramphenicol.*

Assay preparation—Transfer an accurately measured volume of Chloramphenicol Injection, equivalent to about 200 mg of chloramphenicol, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 4.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter this solution through a 0.5-µm or finer porosity filter.

Procedure—Proceed as directed for *Procedure*— in the *Assay* — under *Chloramphenicol.* Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in each mL of the Injection taken by the formula:

$$2.5(C/V)(r_U/r_S)$$

in which V is the volume, in mL, of Injection taken, and the other terms are as defined therein.

Chloramphenicol Ophthalmic Ointment

DEFINITION

Chloramphenicol Ophthalmic Ointment contains NLT 90.0% and NMT 130.0% of the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, glacial acetic acid, and water (450:1:550)

Standard stock solution: 0.25 mg/mL of USP Chloramphenicol RS in methanol

Standard solution: 0.1 mg/mL of USP Chloramphenicol RS from the *Standard stock solution* in *Mobile phase.* Pass through a suitable filter, and use the clear filtrate.

Sample stock solution: Nominally 0.25 mg/mL of chloramphenicol prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 25 mg of chloramphenicol to a suitable conical flask. Add 20 mL of cyclohexane, mix, and sonicate for 2 min. Add 60 mL of methanol. Filter this mixture, collecting the filtrate in a 100-mL volumetric flask. Wash the filter with methanol, collecting the washings in the volumetric flask. Dilute with methanol to volume. Transfer 50.0 mL of the resulting solution to a suitable round-bottom flask, and evaporate to dryness by rotating the flask under vacuum in a water bath at 35°. Dissolve the residue in 50.0 mL of methanol.

Sample solution: Nominally 0.1 mg/mL of chloramphenicol from the *Sample stock solution* in *Mobile phase.* Pass through a suitable filter, and use the clear filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 10-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak height from the *Sample solution*

r_S = peak height from the *Standard solution*

C_S = concentration of USP Chloramphenicol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of chloramphenicol in the *Sample solution* (mg/mL)

P = potency of chloramphenicol in USP Chloramphenicol RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–130.0%

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Chloramphenicol RS

Chloramphenicol Ophthalmic Solution

» Chloramphenicol Ophthalmic Solution is a sterile solution of Chloramphenicol. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$.

Packaging and storage—Preserve in tight containers, and store in a refrigerator until dispensed. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

Labeling—The labeling states that there is a 21-day beyond-use period after dispensing.

USP Reference standards ⟨11⟩—

USP Chloramphenicol RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay.*

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 7.0 and 7.5, except that in the case of Ophthalmic Solution that is unbuffered or is labeled for veterinary use it is between 3.0 and 6.0.

www.webofpharma.com

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*— under *Chloramphenicol.*

Standard preparation—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 100 µg per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation.*

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 50 mg of chloramphenicol, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation.*

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Chloramphenicol.* Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in each mL of the Ophthalmic Solution taken by the formula:

$$0.5(C/V)(r_U/r_S)$$

in which V is the volume, in mL, of Ophthalmic Solution taken, and the other terms are as defined therein.

Chloramphenicol for Ophthalmic Solution

» Chloramphenicol for Ophthalmic Solution is a sterile, dry mixture of Chloramphenicol with or without one or more suitable buffers, diluents, and preservatives. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$, when constituted as directed.

Packaging and storage—Preserve in tight containers.
Labeling—If packaged in combination with a container of solvent, label it with a warning that it is not for injection.
USP Reference standards ⟨11⟩—
USP Chloramphenicol RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay.*
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 7.1 and 7.5, in an aqueous solution containing 5 mg of chloramphenicol per mL.

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*— under *Chloramphenicol.*

Standard preparation—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 100 µg per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation.*

Assay preparation—Transfer the contents of 1 container of Chloramphenicol for Ophthalmic Solution to a suitable container with the aid of *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of about 100 µg of chloramphenicol per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation.*

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Chloramphenicol.* Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in the container of Chloramphenicol for Ophthalmic Solution taken by the formula:

$$(L/D)C(r_U/r_S)$$

in which L is the labeled quantity, in mg, of chloramphenicol in the container, D is the concentration, in µg per mL, of chloramphenicol in the *Assay preparation,* based on the labeled quantity and the extent of dilution, and the other terms are as defined therein.

Chloramphenicol Oral Solution

» Chloramphenicol Oral Solution is a solution of Chloramphenicol in a suitable solvent. It contains one or more suitable buffers and preservatives. It has a potency of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$.

Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate that it is for veterinary use only and that it is not to be used in animals raised for food production.
USP Reference standards ⟨11⟩—
USP Chloramphenicol RS
Identification—Prepare a *Test solution* containing 20 µg per mL chloramphenicol from Oral Solution diluted with water. The ultraviolet absorption spectrum of the *Test solution* exhibits maxima and minima only at the same wavelength as that of a similar solution of USP Chloramphenicol RS, concomitantly measured.
pH ⟨791⟩: between 5.0 and 8.5, when diluted with an equal volume of water.
Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Oral Solution diluted quantitatively and stepwise with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Add the following:

▲Chloramphenicol Compounded Oral Suspension, Veterinary

DEFINITION

Chloramphenicol Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 120.0% of the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$).

USP Monographs

www.webofpharma.com

Prepare Chloramphenicol Compounded Oral Suspension, Veterinary 200 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

Chloramphenicol tablets,[a] equivalent to	20 g of chloramphenicol
Purified Water	30 mL
Ora-Blend,[b] a sufficient quantity to make	100 mL

[a] Chloramphenicol 1-g tablets, Bimeda, Inc., Le Seuer, MN.
[b] Perrigo, Allegan, MI.

Place the *Chloramphenicol tablets* in a suitable container and cover with *Purified Water*. Allow the tablets to soak for 15–20 min. Add 30 mL of *Ora-Blend* and triturate to a smooth paste. Add a sufficient amount of *Ora-Blend* to make the contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the remainder of the *Ora-Blend*. Add sufficient *Ora-Blend* to bring to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: Mix 500 mL of methanol and 600 mL of water. Add 1 mL of glacial acetic acid.

Solution B: Add 0.5 mL of glacial acetic acid to 500 mL of methanol.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
6	100	0
10	40	60
10	100	0
15	100	0

Standard solution: Transfer 20 mg of USP Chloramphenicol RS into a 200-mL volumetric flask. Add approximately 160 mL of *Solution A*, sonicate for 5 min, then dilute with *Solution A* to volume.

Sample solution: Transfer 1 mL of Oral Suspension, Veterinary into a 200-mL volumetric flask. Add approximately 160 mL of *Solution A*, sonicate for 15 min, then dilute with *Solution A* to volume. Transfer 1 mL of this solution into a 10-mL volumetric flask. Dilute with *Solution A* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Temperatures

Autosampler: 4°

Column: 30°

Flow rate: 1.0 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for chloramphenicol is about 6.3 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chloramphenicol from the *Sample solution*

r_S = peak response of chloramphenicol from the *Standard solution*

C_S = concentration of USP Chloramphenicol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of chloramphenicol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.7–5.7

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored in a refrigerator or at controlled room temperature

• **LABELING:** Label it to indicate that it is for veterinary use only. Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Chloramphenicol RS▲ (USP 1-May-2020)

Chloramphenicol Otic Solution

» Chloramphenicol Otic Solution is a sterile solution of Chloramphenicol in a suitable solvent. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Chloramphenicol RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH ⟨791⟩: between 4.0 and 8.0, when diluted with an equal volume of water.

Water Determination, Method I ⟨921⟩: not more than 2.0%.

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*— under *Chloramphenicol*.

www.webofpharma.com

Standard preparation—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 100 µg per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

Assay preparation—Transfer an accurately measured volume of Otic Solution, equivalent to about 50 mg of chloramphenicol, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Chloramphenicol*. Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in each mL of the Otic Solution taken by the formula:

$$0.5(C/V)(r_U/r_S)$$

in which V is the volume, in mL, of Otic Solution taken, and the other terms are as defined therein.

Chloramphenicol Tablets

» Chloramphenicol Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$.

Packaging and storage—Preserve in tight containers.
Labeling—Label Tablets to indicate that they are for veterinary use only and are not to be used in animals raised for food production.
USP Reference standards ⟨11⟩—
USP Chloramphenicol RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.
Disintegration ⟨701⟩: 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*— under *Chloramphenicol*.

Standard preparation—Transfer about 25 mg of USP Chloramphenicol RS, accurately weighed, to a 200-mL volumetric flask, add 10 mL of water, and heat on a steam bath until completely dissolved. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of chloramphenicol, to a 200-mL volumetric flask, add 80 mL of water, and heat on a steam bath for 20 minutes, with occasional mixing. Cool to room temperature, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Chloramphenicol*. Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in the portion of Tablets taken by the formula:

$$4C(r_U/r_S)$$

in which the terms are as defined therein.

Chloramphenicol and Hydrocortisone Acetate for Ophthalmic Suspension

» Chloramphenicol and Hydrocortisone Acetate for Ophthalmic Suspension is a sterile, dry mixture of Chloramphenicol and Hydrocortisone Acetate with or without one or more suitable buffers, diluents, and preservatives. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$), and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$), when constituted as directed.

Labeling—If packaged in combination with a container of solvent, label it with a warning that it is not for injection.
USP Reference standards ⟨11⟩—
USP Chloramphenicol RS
USP Hydrocortisone Acetate RS
Identification—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
pH ⟨791⟩: between 7.1 and 7.5, in an aqueous suspension containing 5 mg of chloramphenicol per mL.

Assay—
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*— under *Chloramphenicol*.

Standard preparation—Transfer about 37.5 mg of USP Chloramphenicol RS and 37.5J mg of USP Hydrocortisone Acetate RS, both accurately weighed, J being the ratio of the labeled amount, in mg, of hydrocortisone acetate to the labeled amount, in mg, of chloramphenicol in the Chloramphenicol and Hydrocortisone Acetate for Ophthalmic Solution, to a 100-mL volumetric flask, add 15 mL of water and 75 mL of methanol, sonicate for a few seconds, dilute with methanol to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

Assay preparation—Transfer the contents of an accurately counted number of containers of Chloramphenicol and Hydrocortisone Acetate for Ophthalmic Solution, equivalent to about 37.5 mg of chloramphenicol, to a 100-mL volumetric flask with the aid of 5 mL of water for each 12.5 mg of chloramphenicol contained therein. Wash each container with methanol, and add the washings to the volumetric flask. Dilute with methanol to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with

USP Monographs

www.webofpharma.com

Mobile phase to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Chloramphenicol*. Calculate the quantity, in mg, of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) in each container taken by the formula:

$$0.5(C/N)(r_U/r_S)$$

in which N is the number of containers taken, and the other terms are as defined therein. Calculate the quantity, in mg, of hydrocortisone acetate ($C_{23}H_{32}O_6$) in each container taken by the formula:

$$500(C/N)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone Acetate RS in the *Standard preparation*, N is the number of containers taken, and r_U and r_S are the hydrocortisone acetate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Chloramphenicol and Polymyxin B Sulfate Ophthalmic Ointment

DEFINITION

Chloramphenicol and Polymyxin B Sulfate Ophthalmic Ointment contains NLT 90.0% and NMT 120.0% of the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) and NLT 90.0% and NMT 125.0% of the labeled amount of polymyxin B.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **CHLORAMPHENICOL**

Mobile phase: Methanol, glacial acetic acid, and water (450:1:550)

Standard stock solution: 0.25 mg/mL of USP Chloramphenicol RS in methanol

Standard solution: 0.1 mg/mL of USP Chloramphenicol RS from the *Standard stock solution* in *Mobile phase*. Pass through a suitable filter, and use the clear filtrate.

Sample stock solution: Nominally 0.25 mg/mL of chloramphenicol prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 25 mg of chloramphenicol to a suitable conical flask. Add 20 mL of cyclohexane, mix, and sonicate for 2 min. Add 60 mL of methanol. Filter this mixture, collecting the filtrate in a 100-mL volumetric flask. Wash the filter with methanol, collecting the washings in the volumetric flask. Dilute with methanol to volume. Transfer 50.0 mL of the resulting solution to a suitable round-bottom flask, and evaporate to dryness by rotating the flask under vacuum in a water bath at 35°. Dissolve the residue in 50.0 mL of methanol.

Sample solution: Nominally 0.1 mg/mL of chloramphenicol from the *Sample stock solution* in *Mobile phase*. Pass through a suitable filter, and use the clear filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak height from the *Sample solution*
r_S = peak height from the *Standard solution*
C_S = concentration of USP Chloramphenicol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of chloramphenicol in the *Sample solution* (mg/mL)
P = potency of chloramphenicol in USP Chloramphenicol RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

- **POLYMYXIN B**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Ophthalmic Ointment containing nominally 5000 Polymyxin B Units with 50 mL of ether in a separator. Extract with four 20-mL portions of *Buffer B.6*. Combine the aqueous extracts in a 100-mL volumetric flask, and dilute with *Buffer B.6* to volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard.
Acceptance criteria: 90.0%–125.0%

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Chloramphenicol RS
USP Polymyxin B Sulfate RS

Chloramphenicol Palmitate

$C_{27}H_{42}Cl_2N_2O_6$ 561.54

Hexadecanoic acid, 2-[(2,2-dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propyl ester, [*R*-(*R**,*R**)]-.
D-*threo*-(−)-2,2-Dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide α-palmitate [530-43-8].

» Chloramphenicol Palmitate has a potency equivalent to not less than 555 µg and not more than 595 µg of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) per mg.

USP Monographs

www.webofpharma.com

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Chloramphenicol Palmitate RS
Identification—The retention time of the chloramphenicol palmitate peak in the chromatogram of the *Assay preparation* corresponds to that of the chloramphenicol palmitate peak in the chromatogram of the *Standard preparation* as obtained in the *Assay*.
Melting range ⟨741⟩: between 87° and 95°.
Specific rotation ⟨781S⟩: between +21° and +25°.
Test solution: 50 mg, undried, per mL, in dehydrated alcohol.
Crystallinity ⟨695⟩: meets the requirements.
Loss on drying ⟨731⟩—Dry it to constant weight over phosphorus pentoxide in vacuum at a pressure not exceeding 5 mm of mercury: it loses not more than 0.5% of its weight.
Acidity—Dissolve 1.0 g by heating at 35° with 5 mL of a 1:1 mixture of 80 percent alcohol and ether, previously neutralized using phenolphthalein TS. Titrate with 0.1 N sodium hydroxide VS, using phenolphthalein TS, until on gentle shaking a pink color persists for not less than 30 seconds: not more than 0.4 mL is consumed.
Free chloramphenicol—Dissolve 1.0 g in 80 mL of xylene with the aid of gentle warming. Cool, and extract with three 15-mL portions of water, combining the aqueous extracts and discarding the xylene. Dilute the combined aqueous extracts with water to 50 mL, extract with 10 mL of toluene, allow to separate, and discard the toluene. Centrifuge a portion of the aqueous solution, and determine the absorbance of the clear solution at the wavelength of maximum absorbance at about 278 nm, using a suitable spectrophotometer, and using as a reagent blank to set the instrument to zero the solution obtained by the same procedure without the specimen: the absorbance is not more than 0.268 (0.045%).

Assay—
Mobile phase—Prepare a suitable degassed mixture of methanol, water, and glacial acetic acid (172:27:1).
Standard preparation—Transfer about 65 mg of USP Chloramphenicol Palmitate RS to a 50-mL volumetric flask, add about 40 mL of methanol and 1 mL of glacial acetic acid, and sonicate for a few minutes. Dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Assay preparation—Using about 65 mg of Chloramphenicol Palmitate, accurately weighed, prepare as directed under *Standard preparation*.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains 10-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the column efficiency determined from the analyte peak is not less than 2400 theoretical plates, and the relative standard deviation for replicate injections is not more than 0.5%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) equivalent in each mg of specimen taken by the formula:

$$(W_S/W_U)(P_S)(r_U/r_S)$$

in which W_S and W_U are the quantities, in mg, of USP Chloramphenicol Palmitate RS and Chloramphenicol Palmitate taken, respectively; P_S is the designated chloramphenicol equivalent, in µg per mg, of USP Chloramphenicol Palmitate RS; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Chloramphenicol Palmitate Oral Suspension

» Chloramphenicol Palmitate Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$). It contains one or more suitable buffers, colors, flavors, preservatives, and suspending agents.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Chloramphenicol Palmitate RS
USP Chloramphenicol Palmitate Polymorph A RS
USP Chloramphenicol Palmitate Nonpolymorph A RS
Identification—The retention time of the chloramphenicol palmitate peak in the chromatogram of the *Assay preparation* corresponds to that of the chloramphenicol palmitate peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Uniformity of dosage units ⟨905⟩—
FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 4.5 and 7.0.

Limit of polymorph A—
Standard preparation—Prepare a dry mixture of 1 part by weight of USP Chloramphenicol Palmitate Polymorph A RS and 9 parts by weight of USP Chloramphenicol Palmitate Nonpolymorph A RS. Prepare a 1 in 3 mineral oil dispersion of this mixture, and place a portion of it between two sodium chloride plates, taking care not to allow air bubbles to form.
Test preparation—Place 20 mL of previously mixed Oral Suspension in a 50-mL centrifuge tube, add 20 mL of water, and mix. Centrifuge, and discard the supernatant. Add 20 mL of water to the residue in the centrifuge tube, mix, centrifuge, and discard the supernatant. Repeat this washing two times. Dry the residue in vacuum over silica gel for not less than 14 hours. Prepare a 1 in 3 mineral oil dispersion of the dried residue, and place a portion of it between two sodium chloride plates, taking care not to allow air bubbles to form.
Procedure—Concomitantly record the absorption spectra of the *Standard preparation* and the *Test preparation* from about 11 µm to about 13 µm, with a suitable IR absorption spectrophotometer, using an empty cell to set the instrument to 100 percent transmittance. Adjust the cell thickness of the *Standard preparation* and of the *Test preparation* so that transmittances of 20% to 30% are obtained at 12.3 µm. On each spectrum, draw a straight baseline between the absorption minima at wavelengths of about 11.3 µm and 12.65 µm. Draw straight lines, perpendicular to the wavelength scale, at the wavelengths of maximum absorption at about 11.65 µm and 11.86 µm, intersecting both the baseline and the spectrum. Determine the absorbance ratio:

$$(A_{11.65a} - A_{11.65b})/(A_{11.86a} - A_{11.86b})$$

USP Monographs

www.webofpharma.com

in which the parenthetic expressions are the differences in absorbance values obtained at the wavelengths indicated by the subscripts for the spectrum (*a*) and at the point of intersection of the perpendicular line with the baseline (*b*). The absorbance ratio of the *Test preparation* is greater than that of the *Standard preparation*, corresponding to not more than 10% of polymorph A.

Assay—

Mobile phase , *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol Palmitate*.

Assay preparation—Transfer an accurately measured volume of Oral Suspension, well-shaken and free from air bubbles, equivalent to about 160 mg of chloramphenicol, to a 200-mL volumetric flask containing about 20 mL of methanol, add 4 mL of glacial acetic acid, dilute with methanol to volume, and mix. Filter about 20 mL of this solution through glass-fiber filter paper. Transfer 10.0 mL of the filtrate to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol Palmitate*. Calculate the quantity, in mg, of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) equivalent in each mL of Oral Suspension taken by the formula:

$$0.004(W_S/V)(P_S)(r_U/r_S)$$

in which V is the volume, in mL, of Oral Suspension taken, and the other terms are as defined therein.

Chloramphenicol Sodium Succinate

$C_{15}H_{15}Cl_2N_2NaO_8$ 445.18

Butanedioic acid, mono[2-[(2,2-dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propyl] ester, monosodium salt, [*R*-(*R**,*R**)]-.

D-*threo*-(−)-2,2-Dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide α-(sodium succinate) [982-57-0].

» Chloramphenicol Sodium Succinate has a potency equivalent to not less than 650 µg and not more than 765 µg of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) per mg.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.

USP Reference standards ⟨11⟩—
USP Chloramphenicol RS

Identification—The *Assay preparation* exhibits an absorption maximum at a wavelength of about 276 nm, as obtained in the *Assay*.

Specific rotation ⟨781S⟩: between +5.0° and +8.0°.
Test solution: 50 mg per mL.

pH ⟨791⟩: between 6.4 and 7.0, in a solution containing the equivalent of 250 mg of chloramphenicol per mL.

Water Determination, *Method I* ⟨921⟩: not more than 5.0%.

Limit of free chloramphenicol—

Mobile phase—Prepare a filtered and degassed mixture of 0.05 M monobasic ammonium phosphate, previously adjusted with 10% (v/v) phosphoric acid to a pH of 2.5 ± 0.1, and methanol (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase* to obtain a solution having a known concentration of about 6 µg per mL. Pass this solution through a filter having a 0.5-µm or finer porosity, and use the filtrate.

Test solution—Transfer about 33 mg of Chloramphenicol Sodium Succinate, accurately weighed, to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 10-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*:the column efficiency determined from the two major peaks, chloramphenicol-1-succinate and chloramphenicol-3-succinate, is not less than 1750 theoretical plates; the resolution, *R*, between the two peaks is not less than 2.0; and the tailing factor is not more than 1.2. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the free chloramphenicol peaks. Calculate the percentage of free chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) in the portion of Chloramphenicol Sodium Succinate taken by the formula:

$$5000(C/WQ)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Chloramphenicol RS in the *Standard solution*; W is the quantity, in mg, of Chloramphenicol Sodium Succinate taken to prepare the *Test solution*; Q is the quantity, in µg, of chloramphenicol in each mg of Chloramphenicol Sodium Succinate taken, as obtained in the *Assay*;and r_U and r_S are the peak areas obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 2.0% is found.

Other requirements—Where the label states that Chloramphenicol Sodium Succinate is sterile, it meets the requirements for *Sterility Tests 71* — and *Bacterial Endotoxins Test 85* — under *Chloramphenicol Sodium Succinate for Injection*. Where the label states that Chloramphenicol Sodium Succinate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial Endotoxins Test 85* — under *Chloramphenicol Sodium Succinate for Injection*.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 20 µg per mL.

Assay preparation—Dissolve an accurately weighed quantity of Chloramphenicol Sodium Succinate in water, and dilute quantitatively with water to obtain a solution having a concentration equivalent to about 20 µg of chloramphenicol per mL.

Procedure—Concomitantly determine the absorbance of the *Standard preparation*, at the wavelength of maximum absorbance at about 278 nm, and the absorbance of the *Assay preparation*, at the wavelength of maximum absorbance at about 276 nm, in 1-cm cells, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in µg, of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) in each

www.webofpharma.com

mg of Chloramphenicol Sodium Succinate taken by the formula:

$$(CP/W)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Chloramphenicol RS in the *Standard preparation*; P is the potency, in µg per mg, of USP Chloramphenicol RS; W is the weight, in µg, of Chloramphenicol Sodium Succinate taken in each mL of the *Assay preparation*; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Chloramphenicol Sodium Succinate for Injection

» Chloramphenicol Sodium Succinate for Injection contains an amount of Chloramphenicol Sodium Succinate equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*

USP Reference standards ⟨11⟩—
USP Chloramphenicol RS

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.2 USP Endotoxin Unit per mg of chloramphenicol.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Limit of free chloramphenicol—

Mobile phase, Standard solution, and *Chromatographic system*—Proceed as directed in the test for *Limit of free chloramphenicol*— under *Chloramphenicol Sodium Succinate.*

Test solution—Dissolve the contents of 1 container in a volume of *Mobile phase* to obtain a solution containing the equivalent of about 100 mg of chloramphenicol per mL. Dilute this solution quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing the equivalent of about 0.5 mg of chloramphenicol per mL. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity, and use the filtrate.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the free chloramphenicol peaks. Calculate the percentage of free chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) in the specimen taken by the formula:

$$0.1(C/D)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Chloramphenicol RS in the *Standard solution*; D is the concentration, in mg per mL, of chloramphenicol equivalent in the *Test solution*, based on the labeled quantity in the container and the extent of dilution; and r_U and r_S are the chloramphenicol peak areas obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 2.0% is found.

Other requirements—It meets the requirements of the tests for *Identification—, Specific rotation 781S:, pH 791:,* and *Water Determination, Method I 921:* under *Chloramphenicol Sodium Succinate.*

Assay—

Standard preparation—Proceed as directed in the *Assay*— under *Chloramphenicol Sodium Succinate.*

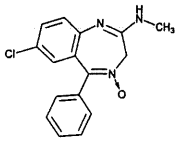
Assay preparation—Constitute 1 container of Chloramphenicol Sodium Succinate for Injection as directed in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with water to obtain a solution having a concentration of about 20 µg of chloramphenicol per mL.

Procedure—Proceed as directed in the *Assay*— under *Chloramphenicol Sodium Succinate.* Calculate the quantity, in mg, of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$), in each mL of the constituted Chloramphenicol Sodium Succinate for Injection taken by the formula:

$$(L/D)(CP/1000)(A_U/A_S)$$

in which L is the labeled quantity, in mg, of chloramphenicol in each mL of constituted solution; D is the concentration, in µg per mL, of chloramphenicol in the *Assay preparation*, on the basis of the labeled quantity of chloramphenicol in each mL of constituted solution and the extent of dilution; C is the concentration, in µg per mL, of USP Chloramphenicol RS in the *Standard preparation*; P is the potency, in µg per mg, of USP Chloramphenicol RS; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Chlordiazepoxide



$C_{16}H_{14}ClN_3O$ 299.75

3*H*-1,4-Benzodiazepin-2-amine, 7-chloro-*N*-methyl-5-phenyl-, 4-oxide;
7-Chloro-2-(methylamino)-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide [58-25-3].

DEFINITION

Chlordiazepoxide contains NLT 98.0% and NMT 102.0% of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*
- **C.**

Solution A: 1 mg/mL of sodium nitrite in water
Solution B: 5 mg/mL of ammonium sulfamate in water
Solution C: 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in water
Sample: 20 mg of Chlordiazepoxide

www.webofpharma.com

Analysis: Add 5 mL of hydrochloric acid and 10 mL of water to the *Sample,* and heat to boiling to effect hydrolysis. Allow the solution to cool. Add 2 mL of *Solution A,* shake, add 1 mL of *Solution B,* shake for 2 min, and add 1 mL of *Solution C.*

Acceptance criteria: A reddish-violet color is produced.

ASSAY

• **PROCEDURE**

Use low-actinic glassware.

Mobile phase: Methanol and water (60:40)

Standard solution: 0.2 mg/mL of USP Chlordiazepoxide RS in *Mobile phase*

Sample stock solution: 2 mg/mL of Chlordiazepoxide in *Mobile phase* prepared as follows. Transfer a suitable portion of Chlordiazepoxide to an appropriate volumetric flask, and dissolve in *Mobile phase.* Sonicate for 5 min, and dilute with *Mobile phase* to volume. Pass through a membrane filter of 0.5-µm or finer pore size.

Sample solution: 0.2 mg/mL of Chlordiazepoxide from *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3600 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) in the portion of Chlordiazepoxide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Chlordiazepoxide RS in the *Standard solution* (mg/mL)

C_U = concentration of Chlordiazepoxide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **ORGANIC IMPURITIES**

Standard solution A: 100 µg/mL of USP Chlordiazepoxide Related Compound A RS in acetone

Standard solution B: 10 µg/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone

Sample solution: Transfer 50.0 mg of Chlordiazepoxide to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volumes

Standard solution A and **Standard solution B:** 10 µL

Sample solution: 50 µL

Developing solvent system: Ethyl acetate

Spray reagent A: 2 N sulfuric acid

Spray reagent B: 1 mg/mL of sodium nitrite in water

Spray reagent C: 5 mg/mL of ammonium sulfamate in water

Spray reagent D: 1 mg/mL of *N*-(1-naphthyl) ethylenediamine dihydrochloride in water

Analysis

Samples: *Standard solution A, Standard solution B,* and *Sample solution*

Develop the chromatogram in a chromatographic chamber (not previously saturated with the *Developing solvent system*) using the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent A,* drying at 105° for 15 min, and then spraying in succession with *Spray reagent B, Spray reagent C,* and *Spray reagent D.*

Acceptance criteria: Any spots from the *Sample solution* are not greater in size or intensity than the spots at the respective R_F values produced by the *Standard solutions,* corresponding to NMT 0.1% of chlordiazepoxide related compound A, and NMT 0.01% of 2-amino-5-chlorobenzophenone.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP 2-Amino-5-chlorobenzophenone RS

$C_{13}H_{10}ClNO$ 231.68

USP Chlordiazepoxide RS

USP Chlordiazepoxide Related Compound A RS

7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.

$C_{15}H_{11}ClN_2O_2$ 286.72

Chlordiazepoxide Tablets

DEFINITION

Chlordiazepoxide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

• **B.**

Solution A: 1 mg/mL of sodium nitrite in water

Solution B: 5 mg/mL of ammonium sulfamate in water

Solution C: 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in water

Sample: Nominally 20 mg of chlordiazepoxide from a portion of finely powdered Tablets

Analysis: Add 5 mL of hydrochloric acid and 10 mL of water to the *Sample,* and heat to boiling to effect hydrolysis. Allow the solution to cool. Add 2 mL of *Solution A,* shake, add 1 mL of *Solution B,* shake for 2 min, and add 1 mL of *Solution C.*

Acceptance criteria: A reddish-violet color is produced.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Use low-actinic glassware.

Mobile phase: Methanol and water (60:40)

Standard solution: 0.2 mg/mL of USP Chlordiazepoxide RS in *Mobile phase*

Sample solution: Nominally 0.2 mg/mL of chlordiazepoxide from NLT 20 Tablets prepared as follows. Finely powder NLT 20 Tablets, and transfer a portion of powder equivalent to 5 mg of chlordiazepoxide to a 25-mL volumetric flask. Add 20 mL of *Mobile phase*, sonicate for 5 min, dilute with *Mobile phase* to volume, and pass through a membrane filter of 5-µm pore size. Discard the first 5 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3600 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Chlordiazepoxide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of chlordiazepoxide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Simulated gastric fluid TS, prepared without pepsin; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: USP Chlordiazepoxide RS in *Medium*

Sample solution: Pass a portion of solution under test through a suitable filter. Dilute with *Medium*, if necessary.

Instrumental conditions

Mode: UV

Analytical wavelength: The wavelength of maximum absorbance at about 309 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) dissolved.

Tolerances: NLT 85% (*Q*) of the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Standard solution A: 1 mg/mL of USP Chlordiazepoxide Related Compound A RS in acetone

Standard solution B: 100 µg/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone

Sample solution: Transfer a portion of finely powdered Tablets equivalent to 25 mg of chlordiazepoxide to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volumes

Standard solution A: 20 µL

Standard solution B: 5 µL

Sample solution: 50 µL

Developing solvent system: Ethyl acetate

Spray reagent A: 2 N sulfuric acid

Spray reagent B: 1 mg/mL of sodium nitrite in water

Spray reagent C: 5 mg/mL of ammonium sulfamate in water

Spray reagent D: 1 mg/mL of *N*-(1-naphthyl) ethylenediamine dihydrochloride in water

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in a chromatographic chamber (not previously saturated with the *Developing solvent system*) in *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent A*, drying at 105° for 15 min, and then spraying in succession with *Spray reagent B*, *Spray reagent C*, and *Spray reagent D*.

Acceptance criteria: Any spots from the *Sample solution* are not greater in size or intensity than the spots at the respective R_F values produced by the *Standard solutions*, corresponding to NMT 4.0% of chlordiazepoxide related compound A, and NMT 0.1% of 2-amino-5-chlorobenzophenone.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP 2-Amino-5-chlorobenzophenone RS

$C_{13}H_{10}ClNO$ 231.68

USP Chlordiazepoxide RS

USP Chlordiazepoxide Related Compound A RS

7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.

$C_{15}H_{11}ClN_2O_2$ 286.72

Chlordiazepoxide and Amitriptyline Hydrochloride Tablets

DEFINITION

Chlordiazepoxide and Amitriptyline Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) and an amount of amitriptyline hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of amitriptyline ($C_{20}H_{23}N$).

www.webofpharma.com

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Use low-actinic glassware.

Buffer: Combine 10.5 mL of 0.20 N sodium hydroxide and 100 mL of *Solution A*.

Solution A: 0.04 M acetic acid, 0.04 M phosphoric acid, and 0.04 M boric acid prepared as follows. Dissolve 2.402 g of glacial acetic acid, 4.612 g of phosphoric acid, and 2.473 g of boric acid in sufficient water to obtain a 1-L solution.

Diluent: Methanol, tetrahydrofuran, and *Buffer* (10:40:50)

Mobile phase: 0.01 M sodium lauryl sulfate in *Diluent*

Standard solution: 0.1 mg/mL of USP Chlordiazepoxide RS and 0.28 mg/mL of USP Amitriptyline Hydrochloride RS in *Diluent*

Sample stock solution: Nominally 1 mg/mL of chlordiazepoxide and nominally 2.5 mg/mL of amitriptyline from NLT 20 Tablets prepared as follows. Finely powder NLT 20 Tablets. Transfer a portion of the powder equivalent to 50 mg of chlordiazepoxide and 125 mg of amitriptyline, to a 50-mL volumetric flask. Add *Diluent* to volume, sonicate to disperse the mixture, and allow undissolved particles to settle.

Sample solution: Nominally 0.1 mg/mL of chlordiazepoxide and nominally 0.25 mg/mL of amitriptyline from *Sample stock solution* in *Diluent*. Pass a portion of this solution through a filter of 0.5-µm or finer pore size. Use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention times for chlordiazepoxide and amitriptyline are about 5 and 7 min, respectively.]

Suitability requirements

Resolution: NLT 2.0 between chlordiazepoxide and amitriptyline

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chlordiazepoxide from the *Sample solution*

r_S = peak response of chlordiazepoxide from the *Standard solution*

C_S = concentration of USP Chlordiazepoxide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of chlordiazepoxide in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of amitriptyline ($C_{20}H_{23}N$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of amitriptyline from the *Sample solution*

r_S = peak response of amitriptyline from the *Standard solution*

C_S = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of amitriptyline in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of amitriptyline, 277.40

M_{r2} = molecular weight of amitriptyline hydrochloride, 313.86

Acceptance criteria: 90.0%–110.0% each of the labeled amounts of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) and amitriptyline ($C_{20}H_{23}N$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Simulated gastric fluid TS, prepared without pepsin; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution A: USP Chlordiazepoxide RS in *Medium*

Standard solution B: USP Amitriptyline Hydrochloride RS in *Medium*

Sample solution: Pass a portion of solution under test through a suitable filter. Dilute with *Medium*, if necessary.

Instrumental conditions

Mode: UV

Analytical wavelengths: 239 and 309 nm

Blank: *Medium*

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Sample solution*, and *Blank*

Calculate the percentage of the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times C_{SA} \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*, 309 nm

A_S = absorbance of *Standard solution A*, 309 nm

C_{SA} = concentration of USP Chlordiazepoxide RS in *Standard solution A* (mg/mL)

D = dilution factor of the *Sample solution*, if needed

V = volume of *Medium*, 900 mL

L = label claim of chlordiazepoxide (mg/Tablet)

Calculate the absorbance of amitriptyline in the *Sample solution* at 239 nm (A_x):

$$\text{Result} = A_{U239} - \{A_{U309} \times [(C_{S309} \times A_{S239})/(C_{S239} \times A_{S309})]\}$$

A_{U239} = absorbance of the *Sample solution*, 239 nm

A_{U309} = absorbance of the *Sample solution*, 309 nm

C_{S309} = concentration of chlordiazepoxide from *Standard solution A*, 309 nm

A_{S239} = absorbance of *Standard solution A*, 239 nm

C_{S239} = concentration of chlordiazepoxide from *Standard solution A*, 239 nm

A_{S309} = absorbance of *Standard solution A*, 309 nm

Calculate the percentage of the labeled amount of amitriptyline ($C_{20}H_{23}N$) in the portion of Tablets taken:

$$\text{Result} = (A_X/A_S) \times C_S \times D \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

A_X = absorbance of the *Sample solution* determined from the previous equation

www.webofpharma.com

A_S = absorbance of amitriptyline from *Standard solution B*, 239 nm
C_S = concentration of USP Amitriptyline Hydrochloride RS in *Standard solution B* (mg/mL)
D = dilution factor of the *Sample solution*, if needed
M_{r1} = molecular weight of amitriptyline, 277.40
M_{r2} = molecular weight of amitriptyline hydrochloride, 313.86
V = volume of *Medium*, 900 mL
L = label claim of amitriptyline (mg/Tablet)

[NOTE—All of the chlordiazepoxide measurements may be made with either a single *Standard solution* or two separate *Standard solutions*.]

Tolerances: NLT 85% (*Q*) of the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), and an amount of amitriptyline hydrochloride equivalent to NLT 85% (*Q*) of the labeled amount of amitriptyline ($C_{20}H_{23}N$) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS,** *Content Uniformity* ⟨905⟩**:** Meet the requirements for both chlordiazepoxide and amitriptyline

IMPURITIES

• **ORGANIC IMPURITIES**

Standard solution A: 1 mg/mL of USP Chlordiazepoxide Related Compound A RS in acetone

Standard solution B: 50 µg/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone

Sample solution: Transfer a portion of finely powdered Tablets equivalent to 25 mg of chlordiazepoxide to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volumes
Standard solution A: 20 µL
Standard solution B: 10 µL
Sample solution: 50 µL
Developing solvent system: Ethyl acetate
Spray reagent A: 2 N sulfuric acid
Spray reagent B: 1 mg/mL of sodium nitrite in water
Spray reagent C: 5 mg/mL of ammonium sulfamate in water
Spray reagent D: 1 mg/mL of *N*-(1-naphthyl) ethylenediamine dihydrochloride in water

Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Develop the chromatogram in a chromatographic chamber (not previously saturated with the *Developing solvent system*) using the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent A*, drying at 105° for 15 min, and then spraying in succession with *Spray reagent B*, *Spray reagent C*, and *Spray reagent D*.

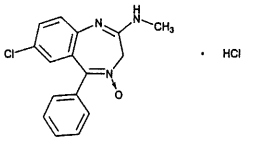
Acceptance criteria: Any spots from the *Sample solution* are not greater in size or intensity than the spots at the respective R_F values produced by the *Standard solutions*, corresponding to NMT 4.0% of chlordiazepoxide related compound A, and NMT 0.1% of 2-amino-5-chlorobenzophenone.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP 2-Amino-5-chlorobenzophenone RS
$C_{13}H_{10}ClNO$ 231.68
USP Amitriptyline Hydrochloride RS
USP Chlordiazepoxide RS
USP Chlordiazepoxide Related Compound A RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.
$C_{15}H_{11}ClN_2O_2$ 286.72

Chlordiazepoxide Hydrochloride



$C_{16}H_{14}ClN_3O \cdot HCl$ 336.22
3*H*-1,4-Benzodiazepin-2-amine, 7-chloro-*N*-methyl-5-phenyl-, 4-oxide, monohydrochloride;
7-Chloro-2-(methylamino)-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide monohydrochloride [438-41-5].

DEFINITION

Chlordiazepoxide Hydrochloride contains NLT 98.0% and NMT 102.0% of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C.**
Solution A: 1 mg/mL of sodium nitrite in water
Solution B: 5 mg/mL ammonium sulfamate in water
Solution C: 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in water
Sample: 20 mg of Chlordiazepoxide Hydrochloride
Analysis: Add 5 mL of hydrochloric acid and 10 mL of water to the *Sample*, and heat to boiling to effect hydrolysis. Allow the solution to cool. Add 2 mL of *Solution A*, 1 mL of *Solution B*, and 1 mL of *Solution C*.
Acceptance criteria: A reddish-violet color is produced.

ASSAY

• **PROCEDURE**
Use low-actinic glassware.
Mobile phase: Methanol and water (60:40)
Standard solution: 0.2 mg/mL of USP Chlordiazepoxide Hydrochloride RS in *Mobile phase*
Sample stock solution: 2 mg/mL of Chlordiazepoxide Hydrochloride in *Mobile phase* prepared as follows. Transfer a suitable portion of Chlordiazepoxide Hydrochloride to an appropriate volumetric flask, and dissolve in *Mobile phase*. Sonicate for 5 min, and dilute with *Mobile phase* to volume. Pass through a membrane filter of 0.5-µm or finer pore size.
Sample solution: 0.2 mg/mL of Chlordiazepoxide Hydrochloride from *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

USP Monographs

www.webofpharma.com

Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3600 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) in the portion of Chlordiazepoxide Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Chlordiazepoxide Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Chlordiazepoxide Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **Residue on Ignition** ⟨281⟩: NMT 0.1%
• **Organic Impurities**
Standard solution A: 100 µg/mL of USP Chlordiazepoxide Related Compound A RS in acetone
Standard solution B: 10 µg/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone
Sample solution: Transfer 50.0 mg of Chlordiazepoxide Hydrochloride to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.
Chromatographic system
(See Chromatography ⟨621⟩.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volumes
Standard solution A and **Standard solution B:** 10 µL
Sample solution: 50 µL
Developing solvent system: Ethyl acetate
Spray reagent A: 2 N sulfuric acid
Spray reagent B: 1 mg/mL of sodium nitrite in water
Spray reagent C: 5 mg/mL of ammonium sulfamate in water
Spray reagent D: 1 mg/mL of *N*-(1-naphthyl) ethylenediamine dihydrochloride in water
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Develop the chromatogram in a chromatographic chamber (not previously saturated with the *Developing solvent system*) using the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent A*, drying at 105° for 15 min, and then spraying in succession with *Spray reagent B*, *Spray reagent C*, and *Spray reagent D*.
Acceptance criteria: Any spots from the *Sample solution* are not greater in size or intensity than the spots at the respective R_F values produced by the *Standard solutions*, corresponding to NMT 0.1% of chlordiazepoxide related compound A, and NMT 0.01% of 2-amino-5-chlorobenzophenone.

SPECIFIC TESTS
• **Melting Range or Temperature,** *Class I* ⟨741⟩: 212°–218°, with decomposition
• **Loss on Drying** ⟨731⟩
Analysis: Dry under vacuum over phosphorus pentoxide at 60° for 4 h.
Acceptance criteria: NMT 0.5%
• **Sterility Tests** ⟨71⟩: Where the label states that Chlordiazepoxide Hydrochloride is sterile, it meets the requirements
• **Bacterial Endotoxins Test** ⟨85⟩: Where the label states that Chlordiazepoxide Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 3.57 USP Endotoxin Units/mg of Chlordiazepoxide Hydrochloride.
• **Other Requirements:** Where the label states that Chlordiazepoxide Hydrochloride is sterile, it meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
• **USP Reference Standards** ⟨11⟩
USP 2-Amino-5-chlorobenzophenone RS
$C_{13}H_{10}ClNO$ 231.68
USP Chlordiazepoxide Hydrochloride RS
USP Chlordiazepoxide Related Compound A RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.
$C_{15}H_{11}ClN_2O_2$ 286.72

Chlordiazepoxide Hydrochloride Capsules

DEFINITION
Chlordiazepoxide Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$).

IDENTIFICATION
• **A.**
Diluent: Sulfuric acid in dehydrated alcohol (1 in 360)
Sample stock solution: Nominally 0.6 mg/mL of chlordiazepoxide hydrochloride from Capsule powder prepared as follows. Transfer a portion of the Capsule powder, equivalent to 60 mg of chlordiazepoxide hydrochloride, to a 100-mL flask. Add methanol to volume, and filter, discarding the first 15 mL of the filtrate. Use the clear filtrate.
Sample solution: Nominally 6 µg/mL of chlordiazepoxide hydrochloride from *Sample stock solution* in *Diluent*
Instrumental conditions
Mode: UV
Cell: 1 cm
Blank: *Diluent*
Analysis
Sample: *Sample solution*

USP Monographs

www.webofpharma.com

Acceptance criteria: The *Sample solution* exhibits maxima at 245 ± 2 nm and 311 ± 2 nm, and the ratio A_{245}/A_{311} is between 2.90 and 3.45.

• **B.**

Solution A: 1 mg/mL of sodium nitrite in water
Solution B: 5 mg/mL of ammonium sulfamate in water
Solution C: 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in water
Sample: A portion of the contents of Capsules
Analysis: Add 5 mL of hydrochloric acid and 10 mL of water to the *Sample*, and heat to boiling to effect hydrolysis. Allow the solution to cool. Add 2 mL of *Solution A*, 1 mL of *Solution B*, and 1 mL of *Solution C*.
Acceptance criteria: A reddish-violet color is produced.

ASSAY

• **PROCEDURE**

Use low-actinic glassware.
Mobile phase: Methanol and water (60:40)
Standard solution: 0.2 mg/mL of USP Chlordiazepoxide Hydrochloride RS in *Mobile phase*
Sample solution: Nominally 0.2 mg/mL of chlordiazepoxide hydrochloride from the contents of Capsules prepared as follows. Combine the contents of NLT 20 Capsules in a suitable container, and transfer a portion of the contents, equivalent to 5 mg of chlordiazepoxide hydrochloride, to a 25-mL volumetric flask. Add 20 mL of *Mobile phase*, sonicate for 5 min, dilute with *Mobile phase* to volume, and pass through a membrane filter of 5-µm pore size. Discard the first 5 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3600 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Chlordiazepoxide Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of chlordiazepoxide hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Standard solution: USP Chlordiazepoxide Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: The wavelength of maximum absorbance at about 245 nm
Cell: 1 cm
Blank: Remove the contents of 12 Capsules as completely as possible with the aid of a current of air. Dissolve the empty Capsule shells in 900 mL of *Medium*. Pass a portion of the resulting solution through a suitable filter. Dilute with *Medium*, if necessary, for consistency with the treatment of the *Sample solution*.
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Determine the percentage of the labeled amount of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) dissolved.
Tolerances: NLT 85% (*Q*) of the labeled amount of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity
Use low-actinic glassware.
Diluent: 0.1 N hydrochloric acid
Standard solution: 6 µg/mL of USP Chlordiazepoxide Hydrochloride RS in *Diluent*
Sample stock solution: Transfer the contents of 1 Capsule to a 200-mL volumetric flask, dissolve in water, and dilute with water to volume. Filter and discard the first 20 mL of the filtrate.
Sample solution: Nominally 6 µg/mL of chlordiazepoxide hydrochloride from *Sample stock solution* in *Diluent*
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: The wavelength of maximum absorbance at about 245 nm
Cell: 1 cm
Blank: *Diluent*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution* using the *Blank*.
Calculate the percentage of the labeled amount of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Chlordiazepoxide Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of chlordiazepoxide hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Standard solution A: 1 mg/mL of USP Chlordiazepoxide Related Compound A RS in acetone
Standard solution B: 0.05 mg/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone
Sample solution: Transfer a portion of Capsule contents equivalent to 25 mg of chlordiazepoxide hydrochloride to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.

USP Monographs

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volumes
Standard solution A: 15 µL
Standard solution B: 10 µL
Sample solution: 50 µL
Developing solvent system: Ethyl acetate
Spray reagent A: 2 N sulfuric acid
Spray reagent B: 1 mg/mL of sodium nitrite in water
Spray reagent C: 5 mg/mL of ammonium sulfamate in water
Spray reagent D: 1 mg/mL of *N*-(1-naphthyl) ethylenediamine dihydrochloride in water
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Develop the chromatogram in a chromatographic chamber (not previously saturated with the *Developing solvent system*) using the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent A*, drying at 105° for 15 min, and then spraying in succession with *Spray reagent B*, *Spray reagent C*, and *Spray reagent D*.
Acceptance criteria: Any spots from the *Sample solution* are not greater in size or intensity than the spots at the respective R_F values produced by the *Standard solutions*, corresponding to NMT 3.0% of chlordiazepoxide related compound A, and NMT 0.1% of 2-amino-5-chlorobenzophenone.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP 2-Amino-5-chlorobenzophenone RS
$C_{13}H_{10}ClNO$ 231.68
USP Chlordiazepoxide Hydrochloride RS
USP Chlordiazepoxide Related Compound A RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.
$C_{15}H_{11}ClN_2O_2$ 286.72

Chlordiazepoxide Hydrochloride and Clidinium Bromide Capsules

DEFINITION

Chlordiazepoxide Hydrochloride and Clidinium Bromide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) and clidinium bromide ($C_{22}H_{26}BrNO_3$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
[NOTE—Use low-actinic glassware.]
Buffer: Dissolve 1.92 g of sodium 1-pentanesulfonate in 900 mL of water in a 1-L volumetric flask. Adjust with 1 N sulfuric acid to a pH of 3.8 ± 0.1. Dilute with water to volume.
Mobile phase: Methanol, tetrahydrofuran, and *Buffer* (6:24:70)
Diluent: Methanol and water (1:1)
Standard solution: 0.1 mg/mL of USP Chlordiazepoxide Hydrochloride RS and 0.05 mg/mL of USP Clidinium Bromide RS in *Diluent*
Sample solution: Weigh the contents of NLT 20 Capsules, and calculate the average weight per Capsule. Mix the combined contents of the Capsules, and transfer an amount equivalent to about 5 mg of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) to a 50-mL volumetric flask. Add about 25 mL of *Diluent*, sonicate for 5 min, and shake by mechanical means for 10 min. Dilute with *Diluent* to volume, and filter, discarding the first 20 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 212 nm
Column: 8-mm × 10-cm; packing L1
Flow rate: 3 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for clidinium bromide and chlordiazepoxide hydrochloride are about 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 5.0 between the clidinium bromide and chlordiazepoxide hydrochloride peaks
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chlordiazepoxide hydrochloride from the *Sample solution*
r_S = peak response of chlordiazepoxide hydrochloride from the *Standard solution*
C_S = concentration of USP Chlordiazepoxide Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of chlordiazepoxide hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of clidinium bromide ($C_{22}H_{26}BrNO_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clidinium bromide from the *Sample solution*
r_S = peak response of clidinium bromide from the *Standard solution*
C_S = concentration of USP Clidinium Bromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clidinium bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 100 rpm

www.webofpharma.com

Time: 30 min
Buffer: Dissolve 1.92 g of sodium 1-pentanesulfonate in 900 mL of water in a 1-L volumetric flask. Adjust with dilute sulfuric acid to a pH of 3.8 ± 0.1. Dilute with water to volume.
Mobile phase: Methanol, tetrahydrofuran, and *Buffer* (6:18:75)
Standard solution: Prepare a solution having known concentrations of USP Chlordiazepoxide Hydrochloride RS and USP Clidinium Bromide RS in *Medium*.
Sample solution: Pass a portion of the solution under test through a suitable filter. Combine equal volumes of the filtered solutions and use the pooled sample for the analysis. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*, if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 212 nm
Column: 4-mm × 25-cm; packing L1
Flow rate: 2 mL/min
Injection size: 100 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for clidinium bromide and chlordiazepoxide hydrochloride are about 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 5.0 between the clidinium bromide and chlordiazepoxide hydrochloride peaks
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the average percentage of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) or clidinium bromide ($C_{22}H_{26}BrNO_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of chlordiazepoxide hydrochloride or clidinium bromide from the *Sample solution*
r_S = peak response of chlordiazepoxide hydrochloride or clidinium bromide from the *Standard solution*
C_S = concentration of USP Chlordiazepoxide Hydrochloride RS or USP Clidinium Bromide RS in the *Standard solution* (mg/mL)
L = chlordiazepoxide hydrochloride or clidinium bromide label claim (mg)
V = volume of *Medium* (mL), 900

Tolerances: NLT 75% (Q) each of the labeled amounts of chlordiazepoxide hydrochloride ($C_{16}H_{14}ClN_3O \cdot HCl$) and clidinium bromide ($C_{22}H_{26}BrNO_3$) are dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **LIMIT OF CHLORDIAZEPOXIDE RELATED COMPOUND A AND 2-AMINO-5-CHLOROBENZOPHENONE**
Standard solution A: 1 mg/mL of USP Chlordiazepoxide Related Compound A RS in acetone
Standard solution B: 50 µg/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone
Sample solution: Transfer an amount equivalent to 25 mg of chlordiazepoxide hydrochloride from Capsule contents to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 50 µL for the *Sample solution*, 15 µL for *Standard solution A*, and 10 µL for *Standard solution B*
Developing solvent system: Ethyl acetate
Spray reagent: 2 N sulfuric acid
Analysis
Samples: *Standard solutions* and *Sample solution*
Proceed as directed in the chapter. Develop the chromatogram in a chromatographic chamber (not previously saturated with the developing solvent) in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent*. Dry at 105° for 15 min, and then spray in succession with sodium nitrite solution (1 in 1000), ammonium sulfamate solution (1 in 200), and *N*-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000).
Acceptance criteria: Any spots from the *Sample solution* are not greater in size or intensity than the spots at the respective R_F values produced by the *Standard solutions*, corresponding to NMT 3.0% of chlordiazepoxide related compound A and to NMT 0.1% of 2-amino-5-chlorobenzophenone.

• **LIMIT OF CLIDINIUM BROMIDE RELATED COMPOUND A**
Extracting solvent mixture: Dehydrated alcohol and cyclohexane (1:1)
Identification solution: Dissolve 50 mg of USP Clidinium Bromide RS in 1 mL of 0.1 N methanolic hydrochloric acid. To this solution add 20 µL of a solution of 25 mg/mL of USP Clidinium Bromide Related Compound A RS in methanol. Prepare this solution at the time of use.
Standard solution: 50 mg/mL of USP Clidinium Bromide RS in 0.1 N methanolic hydrochloric acid. [NOTE—Prepare this solution at the time of use.]
Sample solution: Empty a number of Capsules, equivalent to 25 mg of clidinium bromide, into a glass-stoppered centrifuge tube, and add 5 mL of the *Extracting solvent mixture*. Heat the tube gently, with shaking, to 50°, centrifuge, and decant the clear supernatant into a second tube. Repeat the addition of *Extracting solvent mixture* twice, heating, centrifuging, and decanting as before, and combine the three extracts in a single tube. Gently heating, evaporate the combined extracts under a stream of nitrogen to dryness. Dissolve the residue in 0.5 mL of methanol.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: Acetone, methanol, water, and hydrochloric acid (70:20:5:5)
Spray reagent: Dissolve 850 mg of bismuth subnitrate in a mixture of 10 mL of glacial acetic acid and 40 mL of water. In a separate container, dissolve 20 g of potassium iodide in 50 mL of water. Mix the two solutions, and dilute with dilute sulfuric acid (1 in 10) to 500 mL. Add 7.5 ± 2.5 g of iodine, and mix until solution is complete.
Chromatographic plates: Predevelop suitable thin-layer chromatographic plates by placing in a chromatographic chamber saturated with *Developing solvent system*, and allow the *Developing solvent system* to move 15 cm. Remove the plates from the chamber, dry at 105° for 15 min, and cool.
Analysis
Samples: *Identification solution*, *Standard solution*, and *Sample solution*

www.webofpharma.com

Proceed as directed in the chapter. Place the plates in an unsaturated chromatographic chamber containing freshly prepared *Developing solvent system*, and develop the chromatogram until the solvent front has moved 15 cm. Remove the plates, and dry at 105° for 10 min. Cool to room temperature, and spray with *Spray reagent*. Any spot in the chromatogram of the *Sample solution* occurring at an R_F value of 0.4 is not greater in size or intensity than the corresponding spot in the chromatogram of the *Identification solution*; and the *Standard solution* shows no spot at the R_F value corresponding to that of clidinium bromide related compound A.

Acceptance criteria: NMT 1.0% of clidinium bromide related compound A

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP 2-Amino-5-chlorobenzophenone RS
2-Amino-5-chlorobenzophenone.
$C_{13}H_{10}ClNO$ 231.68

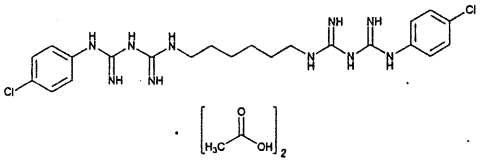
USP Chlordiazepoxide Hydrochloride RS

USP Chlordiazepoxide Related Compound A RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide.
$C_{15}H_{11}ClN_2O_2$ 286.72

USP Clidinium Bromide RS

USP Clidinium Bromide Related Compound A RS
3-Hydroxy-1-methylquinuclidinium bromide.
$C_8H_{16}BrNO$ 222.13

Chlorhexidine Acetate



$C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$ 625.55

2,4,11,13-Tetraazatetradecanediimidamide, *N*,*N*″-bis(4-chlorophenyl)-3,12-diimino-, diacetate; 1,1′-Hexamethylenebis[5-(*p*-chlorophenyl)biguanide] diacetate [56-95-1].

DEFINITION

Chlorhexidine Acetate contains NLT 98.0% and NMT 102.0% of chlorhexidine acetate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**

Solution A: 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Prepare a mixture of acetonitrile and this solution (3:7).

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

System suitability solution: 50 µg/mL of USP Chlorhexidine Acetate RS and 1 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

Standard solution: 50 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*

Sample solution: 50 µg/mL of Chlorhexidine Acetate in *Solution A*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 25-cm; base-deactivated 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 50 µL

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 3 between chlorhexidine and *p*-chloroaniline
Relative standard deviation: NMT 0.73% for chlorhexidine and NMT 5.0% for *p*-chloroaniline

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of chlorhexidine acetate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$) in the portion of Chlorhexidine Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chlorhexidine from the *Sample solution*
r_S = peak response of chlorhexidine from the *Standard solution*
C_S = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)
C_U = concentration of Chlorhexidine Acetate in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.15%
- **ORGANIC IMPURITIES**

Store the *System suitability solution*, the *Sample solution*, and the *Diluted sample solution* at a temperature of NMT 12°.
Solution A: 0.1% (v/v) Trifluoroacetic acid in acetonitrile
Solution B: 0.1% (v/v) Trifluoroacetic acid in water
Solution C: *Solution A* and *Solution B* (20:80)
Solution D: *Solution A* and *Solution B* (90:10)

USP Monographs

www.webofpharma.com

Mobile phase: See *Table 2*. Return to original conditions, and equilibrate the system.

Table 2

Time (min)	Solution C (%)	Solution D (%)
0	100	0
2	100	0
32	80	20
37	80	20
47	70	30
54	70	30

System suitability solution: 5.0 mg/mL of USP Chlorhexidine System Suitability Mixture RS in *Solution C*. See *Table 3* for relative retention times of the main components of the mixture.

Table 3

Components of USP Chlorhexidine System Suitability Mixture RS	Relative Retention Time
Chlorhexidine oxazinone analog	0.23
Chlorhexidine amine	0.25
Chlorhexidine guanidine	0.35
Chlorhexidine urea	0.36
p-Chlorophenyl urea	0.5
Chlorhexidine nitrile	0.6
Chlorhexidine dimer	0.85
o-Chlorhexidine	0.90
Specified unidentified impurity 2	0.91
Chlorhexidine glucityl triazine	0.96
Chlorhexidine	1.0
Oxochlorhexidine	1.4

Sample solution: 1.4 mg/mL of Chlorhexidine Acetate in *Solution C*

Diluted sample solution: Dilute 1.0 mL of *Sample solution* with *Solution C* to 100.0 mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; base-deactivated 5-µm packing L1
Temperatures
Column: 30°
Autosampler: NMT 12°
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Peak-to-valley ratio: NLT 2.0 between chlorhexidine urea and chlorhexidine guanidine

Analysis
Samples: *Sample solution* and *Diluted sample solution*
Calculate the percentage of each impurity in the portion of Chlorhexidine Acetate taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of chlorhexidine from the *Diluted sample solution*
D = dilution factor used to prepare the *Diluted sample solution*, 0.01

Acceptance criteria: See *Table 4*. The reporting level for impurities is 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorhexidine guanidine	0.35	0.15
Chlorhexidine nitrile	0.6	0.15
Chlorhexidine dimer	0.85	0.15
o-Chlorhexidine and specified unidentified impurity 2[a]	0.90–0.91	0.3[a]
Chlorhexidine	1.0	—
Oxochlorhexidine	1.4	0.3
Any individual unspecified impurity	—	0.10
Total impurities	—	0.7

[a] If present, o-chlorhexidine and specified unidentified impurity 2 may not be completely resolved by the method. These peaks are integrated together to determine conformance.

• **LIMIT OF *p*-CHLOROANILINE**
Solution A, Solution B, Mobile phase, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 1.0 µg/mL of USP *p*-Chloroaniline RS in *Solution A*
Sample solution: 2.0 mg/mL of Chlorhexidine Acetate in *Solution A*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount, in ppm, of *p*-chloroaniline in the portion of Chlorhexidine Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 0.001 \times 10^6$$

r_U = peak response of *p*-chloroaniline from the *Sample solution*
r_S = peak response of *p*-chloroaniline from the *Standard solution*
C_S = concentration of *p*-chloroaniline in the *Standard solution* (µg/mL)
C_U = concentration of Chlorhexidine Acetate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 500 ppm

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 3.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Chlorhexidine Acetate RS
USP Chlorhexidine System Suitability Mixture RS

www.webofpharma.com

The mixture contains chlorhexidine and the following impurities (other impurities may also be present):
Chlorhexidine oxazinone analog;
(5*R*,6*S*)-2-[(4-Chlorophenyl)amino]-5-hydroxy-6-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-5,6-dihydro-4*H*-1,3-oxazin-4-one.
$C_{13}H_{15}ClN_2O_6$ 330.72
Chlorhexidine amine;
1-(6-Aminohexyl)-5-(4-chlorophenyl)biguanide.
$C_{14}H_{23}ClN_6$ 310.83
Chlorhexidine guanidine;
1-[6-(Carbamimidoylamino)hexyl]-5-(4-chlorophenyl) biguanide.
$C_{15}H_{25}ClN_8$ 352.87
Chlorhexidine urea;
N-{[6-({[(4-Chlorophenyl)carbamimidoyl] carbamimidoyl}amino)hexyl]carbamimidoyl}urea.
$C_{16}H_{26}ClN_9O$ 395.89
p-Chlorophenyl urea;
1-(4-Chlorophenyl)urea.
$C_7H_7ClN_2O$ 170.60
Chlorhexidine nitrile;
1-(4-Chlorophenyl)-5-[6-[(cyanocarbamimidoyl) amino] hexyl]biguanide.
$C_{16}H_{24}ClN_9$ 377.88
Chlorhexidine dimer;
1,5-Bis[5-(4-chlorophenyl)biguanidylhexyl]biguanide.
$C_{30}H_{47}Cl_2N_{15}$ 688.70
o-Chlorhexidine;
1-(2-Chlorophenyl)-5-[6-({[(4-chloro phenyl) carbamimidoyl]carbamimidoyl}amino) hexyl] biguanide.
$C_{22}H_{30}Cl_2N_{10}$ 505.45
Specified unidentified impurity 2;
Chlorhexidine glucityl triazine;
1-(4-Chlorophenyl)-5-[6-({4-[(4-chlorophenyl)amino]-6-[(1*S*,2*R*,3*R*,4*R*)-1,2,3,4,5-pentahydroxypentyl]-1,3,5-triazin-2-yl}amino)hexyl]biguanide.
$C_{28}H_{38}Cl_2N_{10}O_5$ 665.57
Oxochlorhexidine;
N-(4-Chlorophenyl)-*N*′-{[6-({[(4-chloro phenyl) carbamimidoyl]carbamimidoyl}amino) hexyl] carbamimidoyl}urea.
$C_{22}H_{29}Cl_2N_9O$ 506.43
USP *p*-Chloroaniline RS

Chlorhexidine Acetate Topical Solution

DEFINITION
Chlorhexidine Acetate Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorhexidine acetate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$).

IDENTIFICATION
- **A.** The retention time of the major peak for chlorhexidine from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Acetate*: Meets the requirements of test *A*

Sample: Evaporate or dilute a volume of Topical Solution containing the equivalent of about 5 mg of chlorhexidine acetate to about 5 mL.

ASSAY
- **PROCEDURE**

Solution A: Dissolve 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Prepare a mixture of the resulting solution and acetonitrile (70:30).
Solution B: Acetonitrile
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

System suitability solution: 50 µg/mL of USP Chlorhexidine Acetate RS and 1 µg/mL of USP *p*-Chloroaniline RS in *Solution A*
Standard solution: 40 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*
Sample solution: Nominally 40 µg/mL of chlorhexidine acetate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 20 mg of chlorhexidine acetate, to a 100-mL volumetric flask, and dilute with methanol to volume. Further dilute a 10-mL portion of this solution with *Solution A* to 50 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—The approximate relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 3.0 between chlorhexidine and *p*-chloroaniline
Relative standard deviation: NMT 2.0% for the chlorhexidine peak, NMT 5.0% for the *p*-chloroaniline peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$ in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of chlorhexidine from the *Sample solution*
r_S = peak area of chlorhexidine from the *Standard solution*
C_S = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of chlorhexidine acetate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

www.webofpharma.com

IMPURITIES

ORGANIC IMPURITIES

• **Procedure: Limit of *p*-Chloroaniline**

Solution A, Solution B, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 1.0 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

Sample solution: Nominally 2.0 mg/mL of chlorhexidine acetate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 200 mg of chlorhexidine acetate, to a 100-mL volumetric flask, and dilute with *Solution A* to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The *p*-chloroaniline peak area from the *Sample solution* is NMT the *p*-chloroaniline peak area from the *Standard solution* (NMT 500 ppm, calculated with reference to the nominal content of chlorhexidine acetate).

SPECIFIC TESTS

• **pH** ⟨791⟩: 5.0–7.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.

• **LABELING:** Label it to indicate that it is for veterinary use only.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Chlorhexidine Acetate RS

USP *p*-Chloroaniline RS

Chlorhexidine Gluconate Oral Rinse

» Chlorhexidine Gluconate Oral Rinse is prepared from Chlorhexidine Gluconate Solution. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$).

Packaging and storage—Preserve in tight containers, protected from light, at controlled room temperature.

Labeling—Oral Rinse intended solely for veterinary use is so labeled. Oral Rinse intended for human use is labeled to indicate it is to be expectorated and not swallowed after rinsing.

USP Reference standards ⟨11⟩—

USP Chlorhexidine Acetate RS

USP *p*-Chloroaniline RS

USP Potassium Gluconate RS

Identification—

A: The retention time of the major peak for chlorhexidine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

B: To a volume of Oral Rinse, equivalent to about 10 mg of chlorhexidine gluconate, add 5 mL of a solution of cetyltrimethylammonium bromide (1 in 100), 1 mL of 10 N sodium hydroxide, and 1 mL of bromine TS: a deep red color is produced.

C: Use undiluted Oral Rinse as the test solution and prepare a Standard solution of USP Potassium Gluconate RS in water containing 0.6 mg/mL. Apply separate 15-µL portions of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel, and allow to dry. Develop the chromatogram in a solvent system consisting of a mixture of alcohol, water, ammonium hydroxide, and ethyl acetate (50:30:10:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 minutes. Allow to cool and spray with a spray reagent prepared as follows. Dissolve 2.5 g of ammonium molybdate in about 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 N sulfuric acid to volume, and mix. Heat the plate at 110° for about 10 minutes: the principal spot obtained from the test solution corresponds in color, size, and R_F value to that obtained from the Standard solution.

pH ⟨791⟩**:** between 5.0 and 7.0.

Limit of *p*-chloroaniline—

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Chlorhexidine Gluconate Solution*

Standard solutions—Prepare as directed for *Standard solutions* in the test for *Limit of p-chloroaniline* under *Chlorhexidine Gluconate Solution*

Test solution—Transfer 10.0 mL of Oral Rinse to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Procedure—Proceed as directed in the test for *Limit of p-chloroaniline* under *Chlorhexidine Gluconate Solution.* Calculate the quantity, in µg per mL, of *p*-chloroaniline in the Oral Rinse taken by the formula:

$$2.5C.$$

The limit is 3.0 µg per mL.

Content of alcohol—

Internal standard solution—Dilute 25 mL of *n*-propyl alcohol with water to 500 mL.

Standard solution—Transfer about 0.25 g of dehydrated alcohol, accurately weighed, to a 28-mL screw capped vial containing about 3 mL of water. Add 5.0 mL of *Internal standard solution,* and dilute with water to almost fill the vial. Cap the vial, and using a vortex mixer, mix for 15 seconds.

Test solution—Transfer about 2.5 g of Oral Rinse, accurately weighed, to a 28-mL screw-capped vial. Add 5.0 mL of *Internal standard solution,* and dilute with water to almost fill the vial. Cap the vial, and using a vortex mixer, mix for 15 seconds.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m column, the internal wall of which is coated with a 1.5-µm film of liquid phase G27. The column is maintained at about 150° between periods of use. The injection port is equipped with a split injection port with a split ratio of 10:1. The injection port and the detector block temperatures are maintained at about 250° and 275°, respectively. At the time of use the initial column temperature is maintained at about 35° until the alcohol peaks elute, then is increased at a rate of 30° per minute to a final temperature of about 225°. The carrier gas is helium. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are 1.0 for alcohol and about 1.5 for *n*-propyl alcohol; the resolution, *R,* between alcohol and *n*-propyl alcohol is not less than 2; the tailing factor for the alcohol peak is not more than 3.0; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 0.5 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the

www.webofpharma.com

responses for the major peaks. Calculate the percentage of alcohol (C_2H_5OH) in the Oral Rinse taken by the formula:

$$(W_S/W_U)(R_U/R_S)$$

in which W_S is the weight, in g, of dehydrated alcohol taken to prepare the *Standard solution;* W_U is the weight, in g, of Oral Rinse taken to prepare the *Test solution;* and R_U and R_S are the peak response ratios of alcohol to *n*-propyl alcohol obtained from the *Test solution* and the *Standard solution,* respectively: between 90.0% and 115.0% of the labeled amount of alcohol (C_2H_5OH) is found.

Assay—

Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Chlorhexidine Gluconate Solution*

Assay preparation—Transfer 5.0 mL of Oral Rinse to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Procedure—Proceed as directed in the *Assay* under *Chlorhexidine Gluconate Solution* Calculate the percentage (w/v) of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$) in the portion of Oral Rinse taken by the formula:

$$(897.76/625.55)(C/500)(r_U/r_S)$$

in which the terms are as defined therein.

Chlorhexidine Gluconate Solution

$C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ 897.76

2,4,11,13-Tetraazatetradecanediimidamide, *N,N″*-bis(4-chlorophenyl)-3,12-diimino-, di-D-gluconate; 1,1′-Hexamethylenebis[5-(*p*-chlorophenyl)biguanide] di-D-gluconate [18472-51-0].

DEFINITION

Chlorhexidine Gluconate Solution is an aqueous solution of chlorhexidine gluconate. It contains NLT 19.0% and NMT 21.0% of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$) (w/v).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Standard solution: 5 mg/mL of USP Chlorhexidine RS in 70% alcohol. Recrystallize this solution, and dry the crystals at 105° for 1 h.

Sample solution: To 1 mL of Chlorhexidine Gluconate Solution add 40 mL of water, and cool in ice. Add 10 N sodium hydroxide, dropwise with stirring, until the solution produces a red color on thiazol yellow paper, and add 1 mL in excess. Filter, wash the precipitate with water until the washings are free from alkali, recrystallize the residue from 70% alcohol, and dry the crystals at 105° for 1 h.

Acceptance criteria: Meets the requirements

• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 20 mg/mL of USP Potassium Gluconate RS

Sample solution: Dilute 10 mL of Chlorhexidine Gluconate Solution with water to 50 mL. This solution contains 40 mg/mL of chlorhexidine gluconate.

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (5:1:1:3)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram in a solvent system until the solvent front has moved 10 cm from the point of spotting. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Solution A: Dissolve 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Mix the resulting solution with acetonitrile (70:30).

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

System suitability solution: 50 µg/mL of USP Chlorhexidine Acetate RS and 1 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

Standard solution: 50 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*

Sample stock solution: Transfer 5.0 mL of Chlorhexidine Gluconate Solution to a 250-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 250-mL volumetric flask, and dilute with *Solution A*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 25-cm; base-deactivated 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *System suitability solution*

USP Monographs

www.webofpharma.com

[NOTE—The relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 3.0 between chlorhexidine and *p*-chloroaniline

Relative standard deviation: NMT 0.73% for chlorhexidine and NMT 5.0% for *p*-chloroaniline

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage (w/v) of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$) in the portion of Chlorhexidine Gluconate Solution taken:

$$\text{Result} = (r_U/r_S) \times (0.25 \times C_S) \times (M_{r1}/M_{r2})$$

r_U = peak response of chlorhexidine from the *Sample solution*

r_S = peak response of chlorhexidine from the *Standard solution*

C_S = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)

M_{r1} = molecular weight of chlorhexidine gluconate, 897.76

M_{r2} = molecular weight of chlorhexidine acetate, 625.55

Acceptance criteria: 19.0%–21.0% (w/v)

IMPURITIES

• ORGANIC IMPURITIES

Store the *System suitability solution*, the *Sample solution*, and the *Diluted sample solution* at a temperature of NMT 12°.

Solution A: 0.1% (v/v) Trifluoroacetic acid in acetonitrile

Solution B: 0.1% (v/v) Trifluoroacetic acid in water

Solution C: *Solution A* and *Solution B* (20:80)

Solution D: *Solution A* and *Solution B* (90:10)

Mobile phase: See *Table 2*. Return to original conditions, and equilibrate the system.

Table 2

Time (min)	Solution C (%)	Solution D (%)
0	100	0
2	100	0
32	80	20
37	80	20
47	70	30
54	70	30

System suitability solution: 5.0 mg/mL of USP Chlorhexidine System Suitability Mixture RS in *Solution C*

Sample solution: Dilute 1.0 mL of Chlorhexidine Gluconate Solution with *Solution C* to 100.0 mL.

Diluted sample solution: Dilute 1.0 mL of *Sample solution* with *Solution C* to 100.0 mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; base-deactivated 5-µm packing L1

Temperatures

Column: 30°

Autosampler: NMT 12°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Peak-to-valley ratio: NLT 2.0 between chlorhexidine urea and chlorhexidine guanidine

Resolution: NLT 3.0 between chlorhexidine oxazinone analog and chlorhexidine amine

Analysis

Samples: *Sample solution* and *Diluted sample solution*

Calculate the percentage of each impurity in the portion of Chlorhexidine Gluconate Solution taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of chlorhexidine from the *Diluted sample solution*

D = dilution factor used to prepare the *Diluted sample solution*, 0.01

Acceptance criteria: See *Table 3*. The reporting level for impurities is 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorhexidine oxazinone analog	0.23	0.2
Specified unidentified impurity 1	0.24	0.2
Chlorhexidine amine	0.25	0.3
Chlorhexidine guanidine	0.35	1.0
Chlorhexidine urea	0.36	0.2
p-Chlorophenyl urea	0.5	0.2
Chlorhexidine nitrile	0.6	0.4
Chlorhexidine dimer	0.85	0.5
o-Chlorhexidine and specified unidentified impurity 2	0.90–0.91	0.4[a]
Chlorhexidine glucityl triazine	0.96	0.4
Chlorhexidine	1.0	—
Oxochlorhexidine	1.4	0.4
Any individual unspecified impurity	—	0.10
Total impurities	—	3.0

[a] If present, o-chlorhexidine and specified unidentified impurity 2 may not be completely resolved by the method. These peaks are integrated together to determine conformance.

• LIMIT OF *p*-CHLOROANILINE

Solution A, Solution B, Mobile phase, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Diluent: 27.6 g of monobasic sodium phosphate in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL.

Standard solution: 1.0 µg/mL of USP *p*-Chloroaniline RS in *Diluent*

Sample stock solution: Transfer 5.0 mL of Chlorhexidine Gluconate Solution to a 100-mL volumetric flask, and dilute with water to volume.

USP Monographs

www.webofpharma.com

Sample solution: Transfer 10.0 mL of *Sample stock solution* to a 250-mL volumetric flask, and dilute with *Diluent* to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount, in µg/mL, of *p*-chloroaniline in the portion of Chlorhexidine Gluconate Solution taken:

$$\text{Result} = (r_U/r_S) \times D \times C_S$$

r_U = peak response of *p*-chloroaniline from the *Sample solution*

r_S = peak response of *p*-chloroaniline from the *Standard solution*

D = dilution factor used to prepare the *Sample solution*, 500

C_S = concentration of *p*-chloroaniline in the *Standard solution* (µg/mL)

Acceptance criteria: NMT 500 µg/mL

SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩: 1.06–1.07
- **PH** ⟨791⟩: 5.5–7.0, when diluted 1 in 20 with water

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Chlorhexidine RS

USP Chlorhexidine Acetate RS

USP Chlorhexidine System Suitability Mixture RS

The mixture contains chlorhexidine and the following impurities (other impurities may also be present):

Chlorhexidine oxazinone analog;
(5*R*,6*S*)-2-[(4-Chlorophenyl)amino]-5-hydroxy-6-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-5,6-dihydro-4*H*-1,3-oxazin-4-one.
$C_{13}H_{15}ClN_2O_6$ 330.72

Chlorhexidine amine;
1-(6-Aminohexyl)-5-(4-chlorophenyl)biguanide.
$C_{14}H_{23}ClN_6$ 310.83

Chlorhexidine guanidine;
1-[6-(Carbamimidoylamino)hexyl]-5-(4-chlorophenyl) biguanide.
$C_{15}H_{25}ClN_8$ 352.87

Chlorhexidine urea;
N-{[6-({[(4-Chlorophenyl)carbamimidoyl] carbamimidoyl}amino)hexyl]carbamimidoyl}urea.
$C_{16}H_{26}ClN_9O$ 395.89

p-Chlorophenyl urea;
1-(4-Chlorophenyl)urea.
$C_7H_7ClN_2O$ 170.60

Chlorhexidine nitrile;
1-(4-Chlorophenyl)-5-[6-[(cyanocarbamimidoyl)amino] hexyl]biguanide.
$C_{16}H_{24}ClN_9$ 377.88

Chlorhexidine dimer;
1,5-Bis[5-(4-chlorophenyl)biguanidylhexyl]biguanide.
$C_{30}H_{47}Cl_2N_{15}$ 688.70

o-Chlorhexidine;
1-(2-Chlorophenyl)-5-[6-({[(4-chloro phenyl) carbamimidoyl]carbamimidoyl} amino)hexyl] biguanide.
$C_{22}H_{30}Cl_2N_{10}$ 505.45

Specified unidentified impurity 2;
Chlorhexidine glucityl triazine;
1-(4-Chlorophenyl)-5-[6-({4-[(4-chlorophenyl)amino]-6-[(1*S*,2*R*,3*R*,4*R*)-1,2,3,4,5-pentahydroxypentyl]-1,3,5-triazin-2-yl}amino)hexyl]biguanide.
$C_{28}H_{38}Cl_2N_{10}O_5$ 665.57

Oxochlorhexidine;
N-(4-Chlorophenyl)-*N*′-{[6-({[(4-chloro phenyl) carbamimidoyl]carbamimidoyl}amino) hexyl] carbamimidoyl}urea.
$C_{22}H_{29}Cl_2N_9O$ 506.43

USP *p*-Chloroaniline RS

USP Potassium Gluconate RS

Chlorhexidine Gluconate Topical Solution

DEFINITION

Chlorhexidine Gluconate Topical Solution is prepared from Chlorhexidine Gluconate Solution. It contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$).

IDENTIFICATION

- **A.** The retention time of the major peak for chlorhexidine from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: Nominally 20 mg/mL of chlorhexidine gluconate from the Topical Solution

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (5:1:1:3)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram in a solvent system until the solvent front has moved 10 cm from the point of spotting. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for 10 min.

Acceptance criteria: The principal spot from the *Sample solution* corresponds in color, size, and R_F value to that from the *Standard solution*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Prepare a mixture of the resulting solution and acetonitrile (70:30).

Solution B: Acetonitrile

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55

www.webofpharma.com

Time (min)	Solution A (%)	Solution B (%)
15	45	55
16	100	0
21	100	0

System suitability solution: 50 µg/mL of USP Chlorhexidine Acetate RS and 1 µg/mL of USP *p*-Chloroaniline RS in *Solution A*
Standard solution: 50 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*.
Sample solution: Nominally about 80 µg/mL of chlorhexidine gluconate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 40 mg of chlorhexidine gluconate, to a 100-mL volumetric flask, and dilute with methanol to volume. Further dilute a 10-mL portion of this solution with *Solution A* to 50 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—The approximate relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 3.0 between chlorhexidine and *p*-chloroaniline
Relative standard deviation: NMT 2.0% for the chlorhexidine peak, NMT 5.0% for the *p*-chloroaniline peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of chlorhexidine from the *Sample solution*
r_S = peak area of chlorhexidine from the *Standard solution*
C_S = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of chlorhexidine gluconate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of chlorhexidine gluconate, 897.76
M_{r2} = molecular weight of chlorhexidine acetate, 625.55

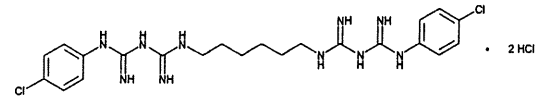
Acceptance criteria: 90.0%–110.0%

IMPURITIES
ORGANIC IMPURITIES
• **Procedure: Limit of *p*-Chloroaniline**
Solution A, Solution B, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1.0 µg/mL of USP *p*-Chloroaniline RS in *Solution A*
Sample solution: Nominally 0.4 mg/mL of chlorhexidine gluconate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 40 mg of chlorhexidine gluconate, to a 100-mL volumetric flask, and dilute with *Solution A* to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria
The *p*-chloroaniline peak area from the *Sample solution* is NMT the *p*-chloroaniline peak area from the *Standard solution* (equivalent to NMT 500 ppm in the portion of Chlorhexidine Gluconate Solution used to prepare the Topical Solution).

SPECIFIC TESTS
• **pH** ⟨791⟩: 5.0–7.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Chlorhexidine Acetate RS
USP *p*-Chloroaniline RS
USP Potassium Gluconate RS

Chlorhexidine Hydrochloride



$C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$ 578.37
2,4,11,13-Tetraazatetradecanediimidamide, *N*,*N*″-bis(4-chlorophenyl)-3,12-diimino-, dihydrochloride;
1,1′-Hexamethylenebis[5-(*p*-chlorophenyl)biguanide] dihydrochloride [3697-42-5].

DEFINITION
Chlorhexidine Hydrochloride contains NLT 98.0% and NMT 102.0% of chlorhexidine hydrochloride ($C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Dissolve 0.3 g in 10 mL of 6 N hydrochloric acid. Add 40 mL of water, filter if necessary, and cool the solution in ice. Add 10 N sodium hydroxide, dropwise with stirring, until the solution is alkaline to thiazol yellow paper, and add 1 mL in excess. Filter, wash the precipitate with water until the washings are free from alkali, recrystallize the residue from 70% alcohol, and dry the crystals at 105° for 1 h.
Standard: 5 mg/mL of USP Chlorhexidine RS in 70% alcohol. Recrystallize this solution, and dry the crystals at 105° for 1 h.
Acceptance criteria: Meets the requirements
• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY
• **PROCEDURE**
Solution A: 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to

www.webofpharma.com

2000 mL. Prepare a mixture of acetonitrile and this solution (3:7).
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

System suitability solution: 50 µg/mL of USP Chlorhexidine Acetate RS and 1 µg/mL of USP *p*-Chloroaniline RS in *Solution A*
Standard solution: 50 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*
Sample solution: 50 µg/mL of Chlorhexidine Hydrochloride in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 25-cm; base-deactivated 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 3 between chlorhexidine and *p*-chloroaniline
Relative standard deviation: NMT 0.73% for chlorhexidine and NMT 5.0% for *p*-chloroaniline
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of chlorhexidine hydrochloride ($C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$) in the portion of Chlorhexidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of chlorhexidine from the *Sample solution*
r_S = peak response of chlorhexidine from the *Standard solution*
C_S = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)
C_U = concentration of Chlorhexidine Hydrochloride in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of chlorhexidine hydrochloride, 578.37
M_{r2} = molecular weight of chlorhexidine acetate, 625.55

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **ORGANIC IMPURITIES**
Store the *System suitability solution*, the *Sample solution*, and the *Diluted sample solution* at a temperature of NMT 12°.
Solution A: 0.1% (v/v) Trifluoroacetic acid in acetonitrile
Solution B: 0.1% (v/v) Trifluoroacetic acid in water
Solution C: *Solution A* and *Solution B* (20:80)
Solution D: *Solution A* and *Solution B* (90:10)
Mobile phase: See *Table 2*. Return to original conditions, and equilibrate the system.

Table 2

Time (min)	Solution C (%)	Solution D (%)
0	100	0
2	100	0
32	80	20
37	80	20
47	70	30
54	70	30

System suitability solution: 5.0 mg/mL of USP Chlorhexidine System Suitability Mixture RS in *Solution C*. See *Table 3* for the relative retention times of the main components of the mixture.

Table 3

Components of USP Chlorhexidine System Suitability Mixture RS	Relative Retention Time
Chlorhexidine oxazinone analog	0.23
Chlorhexidine amine	0.25
Chlorhexidine guanidine	0.35
Chlorhexidine urea	0.36
p-Chlorophenyl urea	0.5
Chlorhexidine nitrile	0.6
Chlorhexidine dimer	0.85
o-Chlorhexidine	0.90
Specified unidentified impurity 2	0.91
Chlorhexidine glucityl triazine	0.96
Chlorhexidine	1.0
Oxochlorhexidine	1.4

Sample solution: 1.3 mg/mL of Chlorhexidine Hydrochloride in *Solution C*
Diluted sample solution: Dilute 1.0 mL of *Sample solution* with *Solution C* to 100.0 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; base-deactivated 5-µm packing L1
Temperatures
Column: 30°
Autosampler: NMT 12°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*

www.webofpharma.com

Suitability requirements
Peak-to-valley ratio: NLT 2.0 between chlorhexidine urea and chlorhexidine guanidine
Analysis
Samples: *Sample solution* and *Diluted sample solution*
Calculate the percentage of each impurity in the portion of Chlorhexidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of chlorhexidine from the *Diluted sample solution*
D = dilution factor used to prepare the *Diluted sample solution*, 0.01

Acceptance criteria: See *Table 4*. The reporting level for impurities is 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorhexidine guanidine	0.35	0.15
Chlorhexidine nitrile	0.6	0.15
Chlorhexidine dimer	0.85	0.2
o-Chlorhexidine and specified unidentified impurity 2	0.90–0.91	0.4[a]
Chlorhexidine	1.0	—
Oxochlorhexidine	1.4	0.4
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

[a] If present, *o*-chlorhexidine and specified unidentified impurity 2 may not be completely resolved by the method. These peaks are integrated together to determine conformance.

• **LIMIT OF *p*-CHLOROANILINE**
Solution A, Solution B, Mobile phase, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 1.0 µg/mL of USP *p*-Chloroaniline RS in *Solution A*
Sample solution: 2.0 mg/mL of Chlorhexidine Hydrochloride in *Solution A*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount, in ppm, of *p*-chloroaniline in the portion of Chlorhexidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 0.001 \times 10^6$$

r_U = peak response of *p*-chloroaniline from the *Sample solution*
r_S = peak response of *p*-chloroaniline from the *Standard solution*
C_S = concentration of *p*-chloroaniline in the *Standard solution* (µg/mL)
C_U = concentration of Chlorhexidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 500 ppm

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Chlorhexidine RS
USP Chlorhexidine Acetate RS
USP Chlorhexidine System Suitability Mixture RS
The mixture contains chlorhexidine and the following impurities (other impurities may also be present):
Chlorhexidine oxazinone analog;
(5*R*,6*S*)-2-[(4-Chlorophenyl)amino]-5-hydroxy-6-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-5,6-dihydro-4*H*-1,3-oxazin-4-one.
$C_{13}H_{15}ClN_2O_6$ 330.72
Chlorhexidine amine;
1-(6-Aminohexyl)-5-(4-chlorophenyl)biguanide.
$C_{14}H_{23}ClN_6$ 310.83
Chlorhexidine guanidine;
1-[6-(Carbamimidoylamino)hexyl]-5-(4-chlorophenyl) biguanide.
$C_{15}H_{25}ClN_8$ 352.87
Chlorhexidine urea;
N-{[6-({[(4-Chlorophenyl)carbamimidoyl] carbamimidoyl}amino)hexyl]carbamimidoyl}urea.
$C_{16}H_{26}ClN_9O$ 395.89
p-Chlorophenyl urea;
1-(4-Chlorophenyl)urea.
$C_7H_7ClN_2O$ 170.60
Chlorhexidine nitrile;
1-(4-Chlorophenyl)-5-[6-[(cyanocarbamimidoyl)amino] hexyl]biguanide.
$C_{16}H_{24}ClN_9$ 377.88
Chlorhexidine dimer;
1,5-Bis[5-(4-chlorophenyl)biguanidylhexyl]biguanide.
$C_{30}H_{47}Cl_2N_{15}$ 688.70
o-Chlorhexidine;
1-(2-Chlorophenyl)-5-[6-({[(4-chloro phenyl) carbamimidoyl]carbamimidoyl} amino)hexyl] biguanide.
$C_{22}H_{30}Cl_2N_{10}$ 505.45
Specified unidentified impurity 2;
Chlorhexidine glucityl triazine;
1-(4-Chlorophenyl)-5-[6-({4-[(4-chlorophenyl)amino]-6-[(1*S*,2*R*,3*R*,4*R*)-1,2,3,4,5-pentahydroxypentyl]-1,3,5-triazin-2-yl}amino)hexyl]biguanide.
$C_{28}H_{38}Cl_2N_{10}O_5$ 665.57
Oxochlorhexidine;
N-(4-Chlorophenyl)-*N*′-{[6-({[(4-chloro phenyl) carbamimidoyl]carbamimidoyl} amino)hexyl] carbamimidoyl}urea.
$C_{22}H_{29}Cl_2N_9O$ 506.43
USP *p*-Chloroaniline RS

Chlorophyllin Copper Complex Sodium

DEFINITION
Chlorophyllin Copper Complex Sodium contains sodium salts of copper-chelated chlorophyll derivatives. It contains no artificial coloring.

IDENTIFICATION
• **A. ULTRAVIOLET-VISIBLE SPECTROSCOPY** ⟨857⟩(in the visible region)
Sample solution: 10 µg/mL

www.webofpharma.com

Medium: pH 7.5 phosphate buffer, prepared by mixing 0.15 M dibasic sodium phosphate and 0.15 M monobasic potassium phosphate (21:4)
Acceptance criteria: The ratio of A_{405}/A_{630} is 3.0–3.9.

OTHER COMPONENTS

• **CONTENT OF TOTAL COPPER**
Stock solution 1: 1000 µg/mL of copper. Transfer 1.000 g of copper to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, and dilute with 0.2 N nitric acid to volume. [NOTE—Store in a polyethylene bottle.]
Stock solution 2: 10 µg/mL of copper. Transfer 5.0 mL of *Stock solution 1* into a 500-mL volumetric flask, and dilute with water to volume.
Standard solutions: Transfer 5.0, 10.0, 15.0, and 20.0 mL, respectively, of *Stock solution 2* to separate 100-mL volumetric flasks, and dilute the contents of each flask with water to volume. These *Standard solutions* contain 0.5, 1.0, 1.5, and 2.0 µg/mL of copper, respectively.
Sample solution: Transfer 100 mg of previously dried Chlorophyllin Copper Complex Sodium to a Kjeldahl flask. Add 2.0 mL of sulfuric acid, 1.0 mL of nitric acid, and 1.0 mL of hydrogen peroxide, and carefully heat under a fume hood until a light green color is obtained. [NOTE—If the solution has any hint of a brown tint, continue to add 0.5-mL portions of nitric acid until a green color is obtained.] Cool, transfer the contents quantitatively to a 1000-mL volumetric flask with several portions of water, dilute the contents of the flask with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, and dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Lamp: Copper hollow-cathode
Flame: Air–acetylene
Analytical wavelength: Copper emission line of 324.8 nm
Blank: Water
Analysis
Samples: *Standard solutions* and *Sample solution*
Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of copper, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, *C*, in µg/mL, of copper in the *Sample solution*.
Calculate the percentage of copper in the portion of Chlorophyllin Copper Complex Sodium taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

C = concentration of the *Sample solution* determined from the graph (µg/mL)
W = weight of Chlorophyllin Copper Complex Sodium taken to prepare the *Sample solution* (mg)
V = final volume of *Sample solution*, 5000 mL
F = conversion factor, 1000 µg/mg

Acceptance criteria: NLT 4.25% on the dried basis

• **CONTENT OF CHELATED COPPER**
Analysis: Calculate the percentage of chelated copper in the portion of Chlorophyllin Copper Complex Sodium taken by subtracting the percentage of ionic copper found in the test for *Limit of Ionic Copper* from the percentage of total copper found in the test for *Content of Total Copper*.
Acceptance criteria: NLT 4.0% on the dried basis

• **CONTENT OF SODIUM**
Standard stock solution: 100 µg/mL of sodium. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer to a 1000-mL volumetric flask, and dilute with water to volume.
Standard solutions: Transfer to each of four 100-mL volumetric flasks 10 mL of a nonionic wetting agent solution (1 in 500). To each flask add, respectively, 2.5, 5.0, 10.0, and 15.0 mL of the *Standard stock solution*, and dilute with water to volume. These *Standard solutions* contain 2.5, 5.0, 10.0, and 15.0 µg/mL of sodium, respectively.
Blank: Transfer 10 mL of a nonionic wetting agent solution (1 in 500) into a 100-mL volumetric flask, and dilute with water to volume.
Sample solution: Transfer 100 mg of Chlorophyllin Copper Complex Sodium to a 1000-mL volumetric flask. Add 100 mL of a solution of nonionic wetting agent (1 in 500) and 400 mL of water, and shake by mechanical means for 5 min. Dilute with water to volume.
Analysis
Samples: *Standard solutions* and *Sample solution*
Set the flame photometer for maximum transmission at a wavelength of 589 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage of transmittance of the other *Standard solutions*, and plot the percentage of transmittance versus the concentration, in µg/mL, of sodium. Read the percentage of transmittance of the *Sample solution*, and from the graph read the concentration, *C*, in µg/mL, of sodium in the *Sample solution*.
Calculate the percentage of sodium in the portion of Chlorophyllin Copper Complex Sodium taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

C = concentration of the *Sample solution* determined from the graph (µg/mL)
W = weight of Chlorophyllin Copper Complex Sodium taken to prepare the *Sample solution* (mg)
V = volume of *Sample solution*, 1000 mL
F = conversion factor, 1000 µg/mg

Acceptance criteria: 5%–7% on the dried basis

• **NITROGEN DETERMINATION,** *Method I* ⟨461⟩**:** NLT 4.0%

IMPURITIES

• **LIMIT OF IONIC COPPER**
Standard solutions: Prepare as directed in the test for *Content of Total Copper*.
Sample solution: Transfer 100 mg of Chlorophyllin Copper Complex Sodium to a 150-mL conical flask. Add 75 mL of water, and shake by mechanical means for 3 min. Adjust with 1 N hydrochloric acid to a pH of 3.0, transfer the suspension thus obtained to a 100-mL volumetric flask, and dilute with water to volume. Filter this suspension, discarding the first 10 mL of the filtrate. Use the clear filtrate for analysis.
Analysis
Samples: *Standard solutions* and *Sample solution*
Proceed as directed in the test for *Content of Total Copper*.
Calculate the percentage of ionic copper in the portion of Chlorophyllin Copper Complex Sodium taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

C = concentration of the *Sample solution* determined from the graph (µg/mL)

USP Monographs

www.webofpharma.com

W = weight of Chlorophyllin Copper Complex Sodium taken to prepare the *Sample solution* (in mg on the dried basis)
V = volume of *Sample solution*, 100 mL
F = conversion factor, 1000 µg/mg

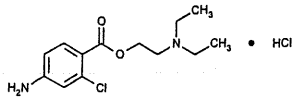
Acceptance criteria: NMT 0.25% on the dried basis
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 30% on the dried basis
- **ARSENIC,** *Method II* ⟨211⟩: NMT 3 ppm
- **LEAD** ⟨251⟩: NMT 10 ppm
- **IRON** ⟨241⟩: NMT 0.50%

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the tests for absence of *Escherichia coli* and *Salmonella* species.
- **pH** ⟨791⟩: 9.5–10.7, in a solution (1 in 100)
- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 2 h: it loses NMT 5% of its weight.
- **TEST FOR FLUORESCENCE**

Sample solution: 10 mg/mL
Analysis: Apply 10 µL of *Sample solution* on filter paper, allow to dry, and examine the area of application under long-wavelength UV light through a red optical filter.
Acceptance criteria: No fluorescence is visible.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Chloroprocaine Hydrochloride



$C_{13}H_{19}ClN_2O_2 \cdot HCl$ 307.22
Benzoic acid, 4-amino-2-chloro-, 2-(diethylamino)ethyl ester, monohydrochloride.
2-(Diethylamino)ethyl 4-amino-2-chlorobenzoate monohydrochloride [3858-89-7].

» Chloroprocaine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{13}H_{19}ClN_2O_2 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Chloroprocaine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: pH 4.5 buffer solution [prepared by dissolving 13.61 g of monobasic potassium phosphate in 750 mL of water, adjusting to a pH of 4.5 ± 0.1 with potassium hydroxide solution (1 in 180) and diluting with water to 1000 mL]. Absorptivities at 290 nm, calculated on the dried basis, do not differ by more than 3.0%.
C: It meets the requirements of the tests for *Chloride* ⟨191⟩.
Melting range, *Class I* ⟨741⟩: between 173° and 176°.
Acidity—Dissolve 1.0 g in 25 mL of water, add 2 drops of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 1.8 mL is required to produce a yellow color.
Loss on drying ⟨731⟩—Dry about 500 mg, accurately weighed, at 105° for 2 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.

Related compounds—
4-Amino-2-chlorobenzoic acid—Using the chromatograms obtained as directed for the *Assay*, calculate the percentage of 4-amino-2-chlorobenzoic acid ($C_7H_6ClNO_2$) in the Chloroprocaine Hydrochloride taken by the formula:

$$10{,}000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of 4-amino-2-chlorobenzoic acid in the *Standard preparation*; W is the quantity, in mg, of Chloroprocaine Hydrochloride taken to prepare the *Assay preparation*; and r_U and r_S are the 4-amino-2-chlorobenzoic acid peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 0.625% is found.

Assay—
Mobile phase—Dissolve 800 mg of sodium 1-heptanesulfonate in 740 mL of water, add 200 mL of acetonitrile, 50 mL of methanol, and 10 mL of glacial acetic acid, and mix. Filter and degas this solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard 4-amino-2-chlorobenzoic acid solution—Dissolve an accurately weighed quantity of recrystallized 4-amino-2-chlorobenzoic acid in methanol to obtain a solution having a known concentration of about 0.2 mg per mL.
Standard preparation—Transfer about 50 mg of USP Chloroprocaine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask containing 5.0 mL of *Standard 4-amino-2-chlorobenzoic acid solution*, add 15 mL of methanol, swirl to dissolve, dilute with water to volume, and mix. This solution contains about 1 mg of USP Chloroprocaine Hydrochloride RS and 0.02 mg of 4-amino-2-chlorobenzoic acid per mL.
System suitability solution—Mix equal volumes of *Standard 4-amino-2-chlorobenzoic acid solution* and *Standard preparation*.
Assay preparation—Transfer about 100 mg of Chloroprocaine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in 40 mL of methanol, dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 278-nm detector and a 3.9-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the responses as directed for *Procedure*:the relative retention times are about 0.35 for 4-amino-2-chlorobenzoic acid and 1.0 for chloroprocaine; and the resolution, R, between 4-amino-2-chlorobenzoic acid and chloroprocaine is not less than 5.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative standard

USP Monographs

www.webofpharma.com

deviation determined from chloroprocaine obtained from replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for 4-amino-2-chlorobenzoic acid and chloroprocaine. Calculate the quantity, in mg, of $C_{13}H_{19}ClN_2O_2 \cdot HCl$ in the portion of Chloroprocaine Hydrochloride taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Chloroprocaine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the chloroprocaine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Chloroprocaine Hydrochloride Injection

» Chloroprocaine Hydrochloride Injection is a sterile solution of Chloroprocaine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of chloroprocaine hydrochloride ($C_{13}H_{19}ClN_2O_2 \cdot HCl$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

USP Reference standards ⟨11⟩—
USP Chloroprocaine Hydrochloride RS

Identification—Dissolve 60 mg of USP Chloroprocaine Hydrochloride RS in 10 mL of water in a 60-mL separator, and in a second 60-mL separator mix a volume of Injection, equivalent to 60 mg of chloroprocaine hydrochloride, with sufficient water to obtain 10 mL of solution. Add 5 mL of dilute ammonium hydroxide (4 in 10) to each, mix, and immediately extract each with four 10-mL portions of chloroform, passing the extracts from the Reference Standard and the test specimen through cotton filters into separate 50-mL volumetric flasks. Dilute each with chloroform to volume, and mix. Add a mixture of chloroform and methanol (4:1) to a suitable chromatographic chamber arranged for thin-layer chromatography (see *Chromatography* ⟨621⟩), cover the chamber, and allow the system to equilibrate for 15 minutes. Apply separately 10-µL portions of the chloroform solutions obtained from the Reference Standard and the test specimen to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution (R_F about 0.40). After viewing, spray the chromatogram with iodoplatinate TS: violet-blue colored spots, characteristic of tertiary nitrogen compounds, are visible.

pH ⟨791⟩: between 2.7 and 4.0.

Related compounds—

4-Amino-2-chlorobenzoic acid—Using the chromatograms obtained as directed for the *Assay*, calculate the percentage of 4-amino-2-chlorobenzoic acid ($C_7H_6ClNO_2$) in the chloroprocaine hydrochloride contained in the Injection taken by the formula:

$$10{,}000(C/W_a)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of 4-amino-2-chlorobenzoic acid in the *Standard preparation;* W_a is the quantity, in mg, of chloroprocaine hydrochloride in the portion of Injection taken to prepare the *Assay preparation,* determined as directed in the *Assay preparation;* and r_U and r_S are the 4-amino-2-chlorobenzoic acid peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively: not more than 3.0% is found.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase, Standard 4-amino-2-chlorobenzoic acid solution, Standard preparation, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay*—under *Chloroprocaine Hydrochloride.*

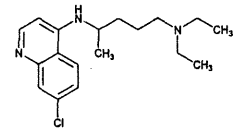
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of chloroprocaine hydrochloride, to a 100-mL volumetric flask, add 40 mL of methanol, dilute with water to volume, and mix.

Procedure—Proceed as directed in the *Assay*— under *Chloroprocaine Hydrochloride.* Calculate the quantity, in mg, of chloroprocaine hydrochloride ($C_{13}H_{19}ClN_2O_2 \cdot HCl$) in each mL of the Injection taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which V is the volume, in mL, of Injection taken, and the other terms are as defined therein.

Chloroquine



$C_{18}H_{26}ClN_3$ 319.87

1,4-Pentanediamine, N^4-(7-chloro-4-quinolinyl)-N^1,N^1-diethyl-;
7-Chloro-4-[[4-(diethylamino)-1-methylbutyl]amino]quinoline [54-05-7].

DEFINITION

Chloroquine contains NLT 98.0% and NMT 102.0% of chloroquine ($C_{18}H_{26}ClN_3$), calculated on the dried basis.

IDENTIFICATION

• **A. Identification—Organic Nitrogenous Bases** ⟨181⟩

Standard solution: Proceed as directed in the chapter except use chloroform instead of carbon disulfide in the test.

Sample solution: 8.75 mg/mL of chloroquine prepared as follows. Dissolve 35 mg of Chloroquine in 4 mL of chloroform. Pass through a dry filter.

Analysis: Determine the absorption of the *Standard solution* and *Sample solution* without delay in 1-mm cells between 7 and 15 µm, using chloroform in a matched cell as a blank.

www.webofpharma.com

Acceptance criteria: The IR absorption spectrum of the *Sample solution* so obtained exhibits maxima only at the same wavelengths as that of the *Standard solution*.

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Medium: Dilute hydrochloric acid (1 in 1000)
Sample solution: 10 µg/mL
Acceptance criteria: The ratio A_{343}/A_{329} is 1.00–1.15.

ASSAY
• **PROCEDURE**
Sample solution: Dissolve 250 mg of Chloroquine in 50 mL of glacial acetic acid. Add crystal violet TS.
Analysis: Titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 15.99 mg of chloroquine ($C_{18}H_{26}ClN_3$).
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.2%

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE ⟨741⟩:** 87°–92°
• **LOSS ON DRYING ⟨731⟩**
Analysis: Dry a sample at 105° for 2 h.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
• **USP REFERENCE STANDARDS ⟨11⟩**
USP Chloroquine Phosphate RS

Chloroquine Hydrochloride Injection

$C_{18}H_{26}ClN_3 \cdot 2HCl$ 392.79
1,4-Pentanediamine, N^4-(7-chloro-4-quinolinyl)-N^1,N^1-diethyl-, dihydrochloride;
7-(Chloro-4-[[4-diethylamino)-1-methylbutyl]amino] quinoline dihydrochloride [3545-67-3].

DEFINITION
Chloroquine Hydrochloride Injection is a sterile solution of Chloroquine in Water for Injection prepared with the aid of Hydrochloric Acid. It contains NLT 47.5 mg and NMT 52.5 mg of chloroquine hydrochloride ($C_{18}H_{26}ClN_3 \cdot 2HCl$) in each mL.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Sample solution: Prepare as directed in the *Assay*.
Standard solution: 7.5 µg/mL of USP Chloroquine Phosphate RS prepared similarly to the *Sample solution*
Instrumental conditions
Mode: UV
Wavelength range: 329–343 nm
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the absorbance ratio:

$$\text{Result} = A_{343}/A_{329}$$

A_{343} = absorbance of the *Sample solution* at 343 nm
A_{329} = absorbance of the *Sample solution* at 329 nm

Acceptance criteria: The peak maxima and minima of the *Sample solution* correspond to those of the *Standard solution*; the absorbance ratio is 1.00–1.15.
• **B.**
Sample solution: 20 mL of a 1-mg/mL solution of chloroquine hydrochloride from Injection, diluted with water
Analysis 1: To the *Sample solution* add 5 mL of trinitrophenol TS.
Acceptance criteria 1: A yellow precipitate is formed.
Analysis 2: Filter, wash the precipitate with water until the last washing is colorless, and dry over silica gel.
[**CAUTION**—Picrates may explode.]
Acceptance criteria 2: The precipitate melts at 205°–210°.
• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY
• **PROCEDURE**
Diluent 1: Dilute hydrochloric acid (1 in 100)
Diluent 2: Dilute hydrochloric acid (1 in 1000)
Standard solution: 10 µg/mL of USP Chloroquine Phosphate RS in *Diluent 2*
Sample stock solution: 150 µg/mL of chloroquine hydrochloride prepared as follows. Tranfer a volume of Injection, nominally 150 mg of chloroquine hydrochloride, to a 1-L volumetric flask, and dilute with water to volume.
Sample solution: 7.5 µg/mL of chloroquine hydrochloride prepared as follows. Transfer 5.0 mL of the *Sample stock solution* to a 100.0-mL volumetric flask, add 10.0 mL of *Diluent 1*, and dilute with water to volume.
Instrumental conditions
Mode: UV
Analytical wavelength: Maxima at about 343 nm
Blank: Water
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, in mg/mL, of chloroquine hydrochloride ($C_{18}H_{26}ClN_3 \cdot 2HCl$) in the portion of Injection taken:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times (V/V_i) \times (M_{r1}/M_{r2})$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Chloroquine Phosphate RS in the *Standard solution* (mg/mL)
D = dilution factor for the preparation of the *Sample solution*, 20
V = volume of the *Sample stock solution*, 1000 mL
V_i = volume of Injection taken for the *Sample stock solution* (mL)
M_{r1} = molecular weight of chloroquine hydrochloride, 392.79
M_{r2} = molecular weight of chloroquine phosphate, 515.86

Acceptance criteria: 47.5–52.5 mg/mL

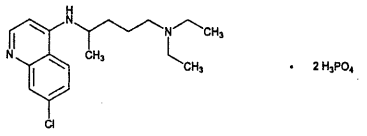
SPECIFIC TESTS
• **pH ⟨791⟩:** 5.5–6.5
• **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** It contains NMT 0.7 USP Endotoxin Unit/mg of chloroquine hydrochloride.
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Chloroquine Phosphate RS

Chloroquine Phosphate



$C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ 515.86

1,4-Pentanediamine, N^4-(7-chloro-4-quinolinyl)-N^1,N^1-diethyl-, phosphate (1:2);
7-Chloro-4-[[4-(diethylamino)-1-methylbutyl]amino] quinoline phosphate (1:2) [50-63-5].

DEFINITION
Chloroquine Phosphate contains NLT 98.0% and NMT 102.0% of chloroquine phosphate ($C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Medium: Dilute hydrochloric acid (1 in 1000)
  Sample solution: 10 µg/mL
  Ratio: A_{343}/A_{329}, 1.00–1.15
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Buffer: 1.4 g/L of anhydrous dibasic sodium phosphate in water. Adjust with 10% phosphoric acid to a pH of 3.0.
  Mobile phase: 0.4% triethylamine in methanol and *Buffer* (70:30)
  System suitability solution: 2.0 µg/mL each of USP Chloroquine Phosphate RS, USP Phenol RS, USP Hydroxychloroquine Sulfate RS, USP Chloroquine Related Compound A RS, USP Chloroquine Related Compound D RS, USP Chloroquine Related Compound E RS, and USP Chloroquine Related Compound G RS in *Mobile phase*
  Standard solution: 0.3 mg/mL of USP Chloroquine Phosphate RS in *Mobile phase*. Sonicate to dissolve if necessary.
  Sample solution: 0.3 mg/mL of Chloroquine Phosphate in *Mobile phase*. Sonicate to dissolve if necessary.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 260 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—See *Table 1* for the corresponding relative retention times.]
  Suitability requirements
  Resolution: NLT 2.0 between chloroquine and chloroquine related compound A, *System suitability solution*
  Tailing factor: NMT 2.0 for chloroquine, *Standard solution*
  Relative standard deviation: NMT 0.7% for chloroquine, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of chloroquine phosphate ($C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$) in the portion of Chloroquine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Chloroquine Phosphate RS in the *Standard solution* (mg/mL)
C_U = concentration of Chloroquine Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **ORGANIC IMPURITIES**
  Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
  Standard solution: Use the *System suitability solution*.
  Sample solution: 2 mg/mL of Chloroquine Phosphate in *Mobile phase*
  System suitability
  Sample: *System suitability solution*
  [NOTE—See *Table 1* for the corresponding relative retention times.]
  Suitability requirements
  Resolution: NLT 2.0 between chloroquine and chloroquine related compound A and NLT 2 between adjacent impurities
  Tailing factor: NMT 2.0 for peaks corresponding to chloroquine phosphate, phenol, hydroxychloroquine sulfate, chloroquine related compound A, chloroquine related compound D, chloroquine related compound E, and chloroquine related compound G
  Relative standard deviation: NMT 5.0% for chloroquine phosphate, phenol, hydroxychloroquine sulfate, chloroquine related compound A, chloroquine related compound D, chloroquine related compound E, and chloroquine related compound G
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of each specified impurity, other than chloroquine related compound G, in the portion of Chloroquine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

www.webofpharma.com

C_U = concentration of Chloroquine Phosphate in the *Sample solution* (mg/mL)

Calculate the percentage of chloroquine related compound G and any other unspecified impurity in the portion of Chloroquine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chloroquine related compound G or any other impurity from the *Sample solution*
r_S = peak response of USP Chloroquine Phosphate RS from the *Standard solution*
C_S = concentration of USP Chloroquine Phosphate RS in the *Standard solution* (mg/mL)
C_U = concentration of Chloroquine Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phenol	0.2	0.1
Chloroquine related compound G	0.27	0.1
Chloroquine related compound D	0.42	0.50
Hydroxychloroquine sulfate	0.49	0.1
Chloroquine related compound A	0.73	0.1
Chloroquine phosphate	1.0	—
Chloroquine related compound E	1.5	0.1
Any other individual impurity	—	0.10
Total impurities	—	2.0

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample at 105° for 16 h.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Chloroquine Phosphate RS
USP Chloroquine Related Compound A RS
4,7-Dichloroquinoline.
$C_9H_5Cl_2N$ 198.05
USP Chloroquine Related Compound D RS
Monoethyl chloroquine;
7-Chloro-4-{[4-(ethylamino)-1-methylbutyl]amino} quinoline.
$C_{16}H_{22}ClN_3$ 291.82
USP Chloroquine Related Compound E RS
5-Chloroquine isomer;
N^4-(5-Chloroquinolin-4-yl)-N^1,N^1-diethylpentane-1,4-diamine oxalate.
$C_{18}H_{26}ClN_3 \cdot C_2H_2O_4$ 409.91
USP Chloroquine Related Compound G RS
4-[(7-Chloroquinolin-4-yl)amino]-*N*,*N*-diethylpentan-1-amine oxide sulfate.
$C_{18}H_{26}Cl_3NO \cdot H_2SO_4$ 433.95
USP Hydroxychloroquine Sulfate RS
USP Phenol RS

Chloroquine Phosphate Compounded Oral Suspension

DEFINITION

Chloroquine Phosphate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of chloroquine phosphate ($C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$).
Prepare Chloroquine Phosphate Compounded Oral Suspension 15 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Chloroquine Phosphate tablets[a] equivalent to	1.5 g of chloroquine phosphate
Vehicle: a 1:1 mixture of Ora-Sweet[b] and Ora-Plus[b], a sufficient quantity to make	100 mL

[a] Aralen 500-mg tablets, Sanofi-Winthrop, NY.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Chloroquine Phosphate tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a chloroquine phosphate liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY

• **Procedure**
Buffer solution: 20 mM 1-heptanesulfonic acid adjusted to a pH of 3.4
Mobile phase: Acetonitrile and *Buffer solution* (34:66). Filter and degas.
Standard solution: 150 µg/mL of USP Chloroquine Phosphate RS in *Mobile phase*
Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of the Oral Suspension into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 340 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[Note—The retention time for chloroquine phosphate is about 9.4 min.]
Suitability requirements
Relative standard deviation: NMT 2% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of chloroquine phosphate ($C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Chloroquine Phosphate RS in the *Standard solution* (µg/mL)

USP Monographs

www.webofpharma.com

C_U = nominal concentration of chloroquine phosphate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.0–5.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored in a refrigerator or at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Chloroquine Phosphate RS

Chloroquine Phosphate Tablets

DEFINITION
Chloroquine Phosphate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of chloroquine phosphate ($C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Standard solution: 7.5 µg/mL of USP Chloroquine Phosphate RS in water
  Sample solution: Nominally 7.5 µg/mL of chloroquine phosphate from a filtered solution of finely powdered Tablets in water
  Instrumental conditions
  Mode: UV
  Wavelength range: 329–343 nm
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the absorbance ratio:

$$\text{Result} = A_{343}/A_{329}$$

A_{343} = absorbance of the *Sample solution* at 343 nm
A_{329} = absorbance of the *Sample solution* at 329 nm

  Acceptance criteria: The peak maxima and minima of the *Sample solution* correspond to those of the *Standard solution*; the absorbance ratio is 1.00–1.15.
- **B.**
  Sample solution: 20 mL of a filtered 1-mg/mL chloroquine phosphate solution from finely powdered Tablets in water
  Analysis 1: To the *Sample solution* add 5 mL of trinitrophenol TS.
  Acceptance criteria 1: A yellow precipitate is formed.
  Analysis 2: Filter, wash the precipitate with water until the last washing is colorless, and dry over silica gel. [**CAUTION**—Picrates may explode.]
  Acceptance criteria 2: The precipitate melts at 205°–210°.
- **C.** The retention time of the chloroquine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Buffer: 6.8 g/L of monobasic potassium phosphate in water. Add 1.0 mL of perchloric acid to each 1 L of solution, adjust with phosphoric acid to a pH of 2.5, and pass through a filter of 0.45-µm pore size.
  Mobile phase: Methanol and *Buffer* (22:78)
  System suitability solution: 0.15 mg/mL of USP Amodiaquine Hydrochloride RS and 0.15 mg/mL of USP Chloroquine Phosphate RS in water
  Standard solution: 0.15 mg/mL of USP Chloroquine Phosphate RS in water
  Sample solution: Nominally 0.15 mg/mL of chloroquine phosphate in water prepared as follows. Transfer nominally 7.5 mg of chloroquine phosphate from finely powdered Tablets (NLT 20) to a 50-mL volumetric flask, and dissolve in and dilute with water to volume. Sonicate for 20 min. Pass 10 mL through a nylon filter of 0.2-µm pore size, discarding the first 4 mL, and use 2 mL for the *Analysis*.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 224 nm
  Column: 4.6-mm × 10-cm; 5-µm packing L1
  Flow rate: 1.2 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for chloroquine phosphate and amodiaquine hydrochloride are 1.0 and 1.3, respectively.]
  Suitability requirements
  Resolution: NLT 1.5 between amodiaquine hydrochloride and chloroquine phosphate
  Tailing factor: NMT 1.5 for the amodiaquine and chloroquine peaks
  Relative standard deviation: NMT 2.0% for the amodiaquine and chloroquine peaks
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of chloroquine phosphate ($C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Chloroquine Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of chloroquine phosphate in the *Sample solution* (mg/mL)

  Acceptance criteria: 93.0%–107.0%

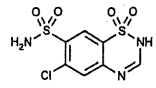
PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: Water; 900 mL
  Apparatus 2: 100 rpm
  Time: 45 min
  Detector: UV
  Analytical wavelength: 343 nm
  Standard solution: USP Chloroquine Phosphate RS in *Medium*
  Sample solution: Dilute with *Medium* to a concentration that is similar to the *Standard solution*.
  Tolerances: NLT 75% (*Q*) of the labeled amount of chloroquine phosphate ($C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$) is dissolved.

www.webofpharma.com

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amodiaquine Hydrochloride RS
  USP Chloroquine Phosphate RS

Chlorothiazide



$C_7H_6ClN_3O_4S_2$ 295.72
2 *H*-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-, 1,1-dioxide.
6-Chloro-2 *H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [58-94-6].

» Chlorothiazide contains not less than 98.0 percent and not more than 102.0 percent of $C_7H_6ClN_3O_4S_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73
USP Chlorothiazide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M.*▲ (CN 1-May-2020) Previously dried at 105° for 1 hour.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: sodium hydroxide solution (1 in 250).
Absorptivities at 292 nm, calculated on the dried basis, do not differ by more than 3.0%.
Loss on drying ⟨731⟩—Dry it at 105° for 1 hour: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chloride—Dissolve 1.00 g in a mixture of 10 mL of water and 10 mL of sodium hydroxide solution (1 in 10). Cool in an ice bath, and add 20 mL of water and 5 mL of nitric acid. A flocculent, white precipitate is formed. Titrate potentiometrically with 0.050 N silver nitrate, using a silver–silver chloride electrode system: not more than 0.28 mL is required (0.05%).
Selenium ⟨291⟩: 0.003%.

Related compounds—
Mobile phase, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay.*

Standard solution—Dissolve an accurately weighed quantity of USP Benzothiadiazine Related Compound A RS in *Mobile phase* to obtain a solution having a known concentration of about 1.5 µg per mL.
Test solution—Proceed as directed for *Assay preparation* in the *Assay.*
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.9 for benzothiadiazine related compound A and 1.0 for chlorothiazide. Calculate the quantity, in mg, of benzothiadiazine related compound A in the portion of Chlorothiazide taken by the formula:

$$0.2C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Benzothiadiazine Related Compound A RS in the *Standard solution;* and r_U and r_S are the peak responses of benzothiadiazine related compound A obtained from the *Test solution* and the *Standard solution,* respectively: not more than 1.0% is present.

Assay—
Mobile phase—Prepare a suitable degassed mixture of 0.1 M monobasic sodium phosphate and acetonitrile (9:1), adjust with phosphoric acid to a pH of 3.0 ± 0.1, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Resolution solution—Dissolve quantities of USP Reference Standards in *Mobile phase* to obtain solutions having known concentrations of about 0.15 mg per mL of USP Chlorothiazide RS and about 1.5 µg per mL of USP Benzothiadiazine Related Compound A RS.
Standard preparation— [NOTE—A volume of acetonitrile not exceeding 10% of the total volume of solution may be used to dissolve the reference standard.] Dissolve an accurately weighed quantity of USP Chlorothiazide RS in *Mobile phase* to obtain a solution having a known concentration of about 0.15 mg per mL.
Assay preparation—Transfer about 30 mg of Chlorothiazide, accurately weighed, to a 200-mL volumetric flask, dissolve in a small volume of acetonitrile, not exceeding 10% of the total volume of the solution, dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 1.5%. Chromatograph the *Resolution solution:* the resolution, *R,* between benzothiadiazine related compound A and chlorothiazide is not less than 3.5.
Procedure— [NOTE—The *Standard preparation* and the *Assay preparation* should be injected immediately upon preparation.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major chlorothiazide peaks. The relative retention times are about 0.9 for benzothiadiazine related compound A and 1.0 for chlorothiazide. Calculate the quantity, in mg, of $C_7H_6ClN_3O_4S_2$ in the portion of Chlorothiazide taken by the formula:

$$200C(r_U/r_S)$$

USP Monographs

www.webofpharma.com

in which C is the concentration, in mg per mL, of USP Chlorothiazide RS in the *Standard preparation;* and r_U and r_S are the peak responses of chlorothiazide obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Add the following:

▲Chlorothiazide Compounded Oral Suspension

DEFINITION

Chlorothiazide Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$).

Prepare Chlorothiazide Compounded Oral Suspension 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Chlorothiazide tablets,[a] equivalent to	5 g of chlorothiazide
Ora-Blend,[b] a sufficient quantity to make	100 mL

[a] Chlorothiazide 500-mg tablets, Mylan Pharmaceuticals, Inc., Morgantown, WV.
[b] Perrigo Pharmaceuticals, Allegan, MI.

Place the *Chlorothiazide tablets* in a suitable container and triturate to a fine powder. Add a small amount of *Ora-Blend* and mix well to form a smooth paste. Add a sufficient amount of *Ora-Blend* to make the contents pourable. Transfer the contents stepwise and quantitatively into a calibrated container using the remainder of the *Ora-Blend.* Add sufficient *Ora-Blend* to bring to final volume. Mix well.

ASSAY

• **PROCEDURE**

Solution A: 92.5 mM monobasic sodium phosphate monohydrate adjusted with phosphoric acid to a pH of 2.9

Mobile phase: Methanol and *Solution A* (20:80)

Standard solution: Transfer 20 mg of USP Chlorothiazide RS to a 200-mL volumetric flask and dissolve in about 20 mL of methanol. Add water to bring to final volume.

Sample solution: Transfer 1.0 mL of Oral Suspension into a 50-mL volumetric flask, and add methanol to volume. Transfer 1.0 mL of the resultant solution into a 10-mL volumetric flask, and add water to bring to final volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 2.0 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for chlorothiazide is about 4.4 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chlorothiazide from the *Sample solution*

r_S = peak response of chlorothiazide from the *Standard solution*

C_S = concentration of USP Chlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of chlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 3.9–4.9

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date.*

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Chlorothiazide RS▲ (USP 1-May-2019)

Chlorothiazide Oral Suspension

DEFINITION

Chlorothiazide Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**

Solution A: 0.1% formic acid in water

Solution B: Methanol

Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	94	6
4	94	6
14	80	20
18	40	60
25	40	60
26	94	6
30	94	6

www.webofpharma.com

Diluent: Acetonitrile and *Solution A* (30:70)
Standard solution: 0.1 mg/mL of USP Chlorothiazide RS in *Diluent*
Sample stock solution: 0.5 mg/mL of chlorothiazide in *Diluent* prepared as follows. Transfer a suitable amount of Oral Suspension, equivalent to 25 mg of chlorothiazide, to a 50-mL volumetric flask. Add 40 mL of *Diluent* and sonicate to dissolve. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.
Sample solution: Nominally 0.1 mg/mL of chlorothiazide in *Diluent*, from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm. For *Identification* test *B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 5°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chlorothiazide from the *Sample solution*
r_S = peak response of chlorothiazide from the *Standard solution*
C_S = concentration of USP Chlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of chlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Uniformity of Dosage Units** ⟨905⟩
For single-unit containers
Acceptance criteria: Meets the requirements
• **Deliverable Volume** ⟨698⟩
For multiple-unit containers
Acceptance criteria: Meets the requirements

IMPURITIES

• **Organic Impurities**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 10 µg/mL of USP Benzothiadiazine Related Compound A RS and 2 µg/mL of USP Chlorothiazide RS in *Diluent*
Sample solution: Nominally 1 mg/mL of chlorothiazide in *Diluent* prepared as follows. Transfer a suitable amount of Oral Suspension, equivalent to 25 mg of chlorothiazide, to a 25-mL volumetric flask. Add 20 mL of *Diluent* and sonicate to dissolve. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.
System suitability
Sample: *Standard solution*
[Note—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between the benzothiadiazine related compound A and chlorothiazide peaks
Relative standard deviation: NMT 5.0% for chlorothiazide
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual unspecified degradation product in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of chlorothiazide from the *Standard solution*
C_S = concentration of USP Chlorothiazide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of chlorothiazide in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A[a]	0.7	—
Chlorothiazide	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	2.0

[a] Not included in the total degradation products.

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.2–4.0

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Protect from freezing and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73
USP Chlorothiazide RS

Chlorothiazide Tablets

DEFINITION

Chlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: 0.1% formic acid in water
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	94	6
4	94	6
14	80	20
18	40	60
25	40	60
26	94	6
30	94	6

Diluent: Acetonitrile and *Solution A* (30:70)
Standard solution: 0.1 mg/mL of USP Chlorothiazide RS in *Diluent*
Sample stock solution: 0.5 mg/mL of chlorothiazide in *Diluent* prepared as follows. Transfer a suitable amount of finely powdered Tablets (NLT 20), equivalent to 25 mg of chlorothiazide, to a 50-mL volumetric flask. Add 40 mL of *Diluent* and sonicate. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.
Sample solution: Nominally 0.1 mg/mL of chlorothiazide in *Diluent* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm. For *Identification* test *B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 5°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chlorothiazide from the *Sample solution*
r_S = peak response of chlorothiazide from the *Standard solution*
C_S = concentration of USP Chlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of chlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.05 M pH 8.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL
Apparatus 2: 75 rpm
Time: 60 min
Standard solution: Known concentration of USP Chlorothiazide RS in *Medium*
Sample solution: Filtered portions of the solution under test, suitably diluted with *Medium* to a concentration that is similar to the *Standard solution*
Instrumental conditions
Mode: UV
Analytical wavelength: 294 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 75% (*Q*) of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 10 µg/mL of USP Benzothiadiazine Related Compound A RS and 2 µg/mL of USP Chlorothiazide RS in *Diluent*
Sample solution: Nominally 1 mg/mL of chlorothiazide in *Diluent* prepared as follows. Transfer a suitable amount of finely powdered Tablets (NLT 20) to an appropriate volumetric flask. Add *Diluent* to 80% of the final flask volume and sonicate. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between the benzothiadiazine related compound A and chlorothiazide peaks
Relative standard deviation: NMT 5.0% for chlorothiazide
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of chlorothiazide from the *Standard solution*
C_S = concentration of USP Chlorothiazide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of chlorothiazide in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A[a]	0.7	—
Chlorothiazide	1.0	—

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified degradation product	—	0.2
Total degradation products	—	2.0

[a] Not included in the total degradation products.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzothiadiazine Related Compound A RS
  4-Amino-6-chloro-1,3-benzenedisulfonamide.
  $C_6H_8ClN_3O_4S_2$ 285.73
  USP Chlorothiazide RS

Chlorothiazide Sodium for Injection

$C_7H_5ClN_3NaO_4S_2$ 317.71
2*H*-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-, 1,1-dioxide, monosodium salt.
6-Chloro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide monosodium salt [7085-44-1].

» Chlorothiazide Sodium for Injection is a sterile, freeze-dried mixture of Chlorothiazide Sodium (prepared by the neutralization of Chlorothiazide with the aid of Sodium Hydroxide) and Mannitol. It contains chlorothiazide sodium ($C_7H_5ClN_3NaO_4S_2$) equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of chlorothiazide ($C_7H_6ClN_3O_4S_2$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Chlorothiazide RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

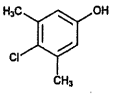
Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: sodium hydroxide solution (1 in 250).
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.3 USP Endotoxin Unit per mg of chlorothiazide sodium.
Uniformity of dosage units ⟨905⟩: meets the requirements.
pH ⟨791⟩: between 9.2 and 10.0, in a solution prepared as directed in the labeling.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer an accurately weighed portion of Chlorothiazide Sodium for Injection, equivalent to about 500 mg of chlorothiazide, to a 1000-mL volumetric flask, add sodium hydroxide solution (1 in 250) to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with sodium hydroxide solution (1 in 250) to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Chlorothiazide RS in the same medium having a known concentration of about 10 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 292 nm, with a suitable spectrophotometer, using sodium hydroxide solution (1 in 250) as the blank. Calculate the quantity, in mg, of $C_7H_6ClN_3O_4S_2$ in the portion of Chlorothiazide Sodium for Injection taken by the formula:

$$50C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Chlorothiazide RS in the Standard solution; and A_U and A_S are the absorbances of the assay solution and the Standard solution, respectively.

Chloroxylenol



C_8H_9ClO 156.61
Phenol, 4-chloro-3,5-dimethyl-;
4-Chloro-3,5-xylenol [88-04-0].

DEFINITION
Chloroxylenol contains NLT 98.5% of chloroxylenol (C_8H_9ClO).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

- **PROCEDURE**
  Internal standard solution: 4 mg/mL of USP Parachlorophenol RS in toluene
  Standard solution: 1 mg/mL of USP Chloroxylenol RS prepared as follows. Transfer 10 mg of USP Chloroxylenol RS to a 10.0-mL volumetric flask, add 2.0 mL of the *Internal standard solution*, and dilute with toluene to volume.
  Sample solution: 1 mg/mL of Chloroxylenol prepared as follows. Transfer 10 mg of Chloroxylenol to a 10.0 mL volumetric flask, add 2.0 mL of the *Internal standard solution*, and dilute with toluene to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: GC
  Detector: Flame ionization
  Column: 0.32-mm × 30-m; coated with a 0.50-µm film of phase G42
  Temperatures
  Injection port: 250°
  Detector: 250°
  Column: See *Table 1.*

www.webofpharma.com

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
105	0	105	1
105	6	190	8

Carrier gas: Helium
Flow rate: 2.4 mL/min
Injection volume: 2 µL
Split ratio: 25:1
Run time: 23 min

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between the parachlorophenol peak and the chloroxylenol peak
Tailing factor: NMT 1.5 for the chloroxylenol peak
Relative standard deviation: NMT 1.5%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of chloroxylenol (C_8H_9ClO) in the portion of Chloroxylenol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of the chloroxylenol peak to the parachlorophenol peak from the *Sample solution*
R_S = peak area ratio of the chloroxylenol peak to the parachlorophenol peak from the *Standard solution*
C_S = concentration of USP Chloroxylenol RS in the *Standard solution* (mg/mL)
C_U = concentration of Chloroxylenol in the *Sample solution* (mg/mL)

Acceptance criteria: NLT 98.5%

IMPURITIES

• **Residue on Ignition** ⟨281⟩**:** NMT 0.1%

• **Iron** ⟨241⟩
Sample: 0.10 g
Analysis: Transfer the *Sample* to a suitable crucible, add 5 drops of sulfuric acid, and ignite at a low heat until thoroughly ashed. Add 10 drops of sulfuric acid to the carbonized mass, and heat cautiously until white fumes are no longer evolved. Ignite, preferably in a muffle furnace, at 500°–600°, until the carbon is completely burned off. Cool, add 4 mL of 6 N hydrochloric acid, cover, digest on a steam bath for 15 min, uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 min. Dilute with water to 25 mL. Filter, if necessary. Rinse the crucible and the filter with 10 mL of water, combining the filtrate and rinsing in a 50-mL color-comparison tube; add 2 mL of hydrochloric acid; dilute with water to 47 mL; and mix.
Acceptance criteria: NMT 0.01%

• **Limit of Tetrachloroethylene**
Internal standard stock solution: 20 µL/mL of 1-butanol in methanol
Internal standard solution: 2 µL/mL of 1-butanol in methanol from *Internal standard stock solution*
Tetrachloroethylene standard stock solution: 20 µL/mL of tetrachloroethylene in methanol
Tetrachloroethylene standard solution: 2 µL/mL of tetrachloroethylene in methanol from *Tetrachloroethylene standard stock solution*
Standard solution: 0.4 µL/mL each of 1-butanol and tetrachloroethylene in methanol from *Internal standard solution* and *Tetrachloroethylene standard solution*, respectively, prepared as follows. Combine 5 mL each of *Internal standard solution* and *Tetrachloroethylene standard solution* in a 25-mL volumetric flask, dilute with methanol to volume, and mix.
Sample solution: 160 mg/mL of chloroxylenol and 0.4 µL/mL of 1-butanol in methanol prepared as follows. Weigh 4 g of chloroxylenol in a 25-mL volumetric flask, combine with 5 mL of *Internal standard solution*, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m; 1.0-µm film of phase G14 or G16
Carrier gas: Hydrogen
Temperatures
Injector: 240°
Detector: 240°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	0	70	2
70	35	210	5

Flow rate: 12.8 mL/min
Injection volume: 0.5 µL
Split ratio: 20:1

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for tetrachloroethylene and 1-butanol are about 1.0 and 1.9, respectively.]
Suitability requirements
Resolution: NLT 1.5 between tetrachloroethylene and the solvent front of methanol
Tailing factor: NM T 1.2 for the tetrachloroethylene and 1-butanol peaks
Relative standard deviation: NMT 8.0% for the ratio of the 1-butanol to the tetrachloroethylene peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of tetrachloroethylene in the portion of Chloroxylenol taken by comparing the peak response ratio of tetrachloroethylene to the internal standard from the *Standard solution* to that of the peak response ratio of tetrachloroethylene to the internal standard from the *Sample solution*:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times d \times F \times 100$$

R_U = peak response ratio of tetrachloroethylene to 1-butanol from the *Sample solution*
R_S = peak response ratio of tetrachloroethylene to 1-butanol from the *Standard solution*
C_S = concentration of tetrachloroethylene in the *Standard solution* (mL/mL)
C_U = concentration of Chloroxylenol in the *Sample solution* (mg/mL)
d = density of tetrachloroethylene, 1.623 g/mL
F = conversion factor, 1 mg/0.001 g

USP Monographs

www.webofpharma.com

Acceptance criteria: NMT 0.4% of tetrachloroethylene

• **ORGANIC IMPURITIES**

Standard solution: 0.02 mg/mL each of 3,5-dimethylphenol and USP Chloroxylenol Related Compound A RS in toluene

Sample solution: 10.0 mg/mL of Chloroxylenol in toluene

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Sample: *Standard solution*

[NOTE—For relative retention times, see *Table 3*.]

Suitability requirements

Resolution: NLT 8 between 3,5-dimethylphenol and chloroxylenol related compound A

Relative standard deviation: NMT 3%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of 3,5-dimethylphenol ($C_8H_{10}O$) and chloroxylenol related compound A (C_8H_9ClO) in the portion of Chloroxylenol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the appropriate analyte from the *Sample solution*

r_S = peak response of the 3,5-dimethylphenol or chloroxylenol related compound A from the *Standard solution*

C_S = concentration of 3,5-dimethylphenol or USP Chloroxylenol Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Chloroxylenol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_T = sum of all the peak responses

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
3,5-Dimethylphenol	0.58	0.2
Chloroxylenol related compound A	0.64	0.2
Chloroxylenol	1.0	—
Any individual impurity	—	0.5
Total impurities	—	1.5

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

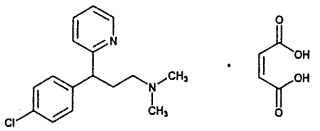
• **USP REFERENCE STANDARDS** ⟨11⟩

USP Chloroxylenol RS

USP Chloroxylenol Related Compound A RS
2-Chloro-3,5-dimethylphenol.

USP Parachlorophenol RS

Chlorpheniramine Maleate



$C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ 390.86

2-Pyridinepropanamine, γ-(4-chlorophenyl)-*N*,*N*-dimethyl-, (*Z*)-2-butenedioate (1:1);

2-[*p*-Chloro-α-[2-(dimethylamino)ethyl]benzyl] pyridine maleate (1:1) [113-92-8].

DEFINITION

Chlorpheniramine Maleate contains NLT 98.0% and NMT 102.0% of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention times of the maleic acid and chlorpheniramine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 5.44 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile

Diluent: Acetonitrile and *Solution A* (5:95)

System suitability stock solution: 0.02 mg/mL each of USP Pheniramine Maleate RS, USP Chlorpheniramine Related Compound B RS, and USP Chlorpheniramine Related Compound C RS in *Diluent*. Sonicate for 1 min.

System suitability solution: 0.5 mg/mL of USP Chlorpheniramine Maleate RS and 2 µg/mL each of USP Pheniramine Maleate RS, USP Chlorpheniramine Related Compound B RS, and USP Chlorpheniramine Related Compound C RS in *Diluent*, prepared as follows. Transfer 5.0 mg of USP Chlorpheniramine Maleate RS to a 10-mL volumetric flask, add 5 mL of *Diluent* and 1.0 mL of the *System suitability stock solution*, and dilute with *Diluent* to volume. Sonicate for 1 min.

Standard solution: 0.5 mg/mL of USP Chlorpheniramine Maleate RS in *Diluent*. Sonicate for 1 min.

Sample solution: 0.5 mg/mL of Chlorpheniramine Maleate in *Diluent*. Sonicate for 1 min.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
1	95	5
20	70	30
30	70	30
31	95	5
40	95	5

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

USP Monographs

www.webofpharma.com

Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
[NOTE—The relative retention times of maleic acid, chlorpheniramine related compound C, and chlorpheniramine are 0.18, 0.94 and 1.0, respectively.]
Resolution: NLT 1.5 between chlorpheniramine related compound C and chlorpheniramine; and NLT 2.0 between chlorpheniramine related compound B and pheniramine, *System suitability solution*
Tailing factor: NMT 2.0 for chlorpheniramine, *Standard solution*
Relative standard deviation: NMT 0.73% for chlorpheniramine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in the portion of Chlorpheniramine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of chlorpheniramine from the *Sample solution*
r_S = peak response of chlorpheniramine from the *Standard solution*
C_S = concentration of USP Chlorpheniramine Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of Chlorpheniramine Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%
• **ORGANIC IMPURITIES**
Solution A, Solution B, Diluent, System suitability solution, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1.4 µg/mL of USP Chlorpheniramine Maleate RS in *Diluent*, equivalent to 1.0 µg/mL of chlorpheniramine. Sonicate for 1 min.
Sensitivity solution: 0.28 µg/mL of USP Chlorpheniramine Maleate RS in *Diluent*
Sample solution: 0.5 mg/mL of Chlorpheniramine Maleate in *Diluent*. Sonicate for 1 min.
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between chlorpheniramine related compound C and chlorpheniramine; and NLT 2.0 between the chlorpheniramine related compound B and pheniramine, *System suitability solution*
Relative standard deviation: NMT 5.0% for chlorpheniramine, *Standard solution*
Signal-to-noise ratio: NLT 10 for chlorpheniramine, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Chlorpheniramine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of chlorpheniramine from the *Standard solution*
C_S = concentration of chlorpheniramine from the *Standard solution* (mg/mL)
C_U = concentration of Chlorpheniramine Maleate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks having areas less than 0.05% that of chlorpheniramine.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Maleic acid[a]	0.18	—	—
Diamine analog[b]	0.37	0.73	0.2
Chlorpheniramine related compound B[c]	0.49	0.77	0.1
Pheniramine[d]	0.57	—	—
Chlorpheniramine related compound C[e]	0.97	1.0	0.1
Chlorpheniramine	1.0	—	—
Chlorpheniramine nitrile[f]	1.19	1.0	0.1
Any other unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] Salt counter ion is included in the table for identification purposes only.
[b] 2-(4-Chlorophenyl)-4-(dimethylamino)-2-[2-(dimethylamino)ethyl] butanenitrile.
[c] Di(pyridin-2-yl)amine.
[d] Used only to establish the system suitability.
[e] 3-(4-Chlorophenyl)-*N*-methyl-3-(pyridin-2-yl)propan-1-amine.
[f] 2-(4-Chlorophenyl)-4-(dimethylamino)-2-(pyridin-2-yl)butanenitrile.

SPECIFIC TESTS

• **OPTICAL ROTATION** ⟨781⟩
Sample: 100 mg/mL in water at 20°
Acceptance criteria: –0.10° to +0.10°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Chlorpheniramine Maleate RS
USP Chlorpheniramine Related Compound B RS
Di(pyridin-2-yl)amine.
$C_{10}H_9N_3$ 171.20
USP Chlorpheniramine Related Compound C RS
▲3-(4-Chlorophenyl)-*N*-methyl-3-(pyridin-2-yl)propan-1-amine maleate.
$C_{15}H_{17}ClN_2 \cdot C_4H_4O_4$ 376.83▲ (ERR 1-Dec-2017)
USP Pheniramine Maleate RS

USP Monographs

www.webofpharma.com

Chlorpheniramine Maleate Extended-Release Capsules

» Chlorpheniramine Maleate Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$).

Packaging and storage—Preserve in tight containers.
Labeling—Label the Capsules to indicate the *Dissolution Test* with which the product complies.
USP Reference standards ⟨11⟩—
USP Chlorpheniramine Maleate RS

Identification—
A: The retention time of the chlorpheniramine peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
B: Transfer the contents of 1 Capsule to a 10-mL volumetric flask, add 5 mL of methanol, and insert the stopper into the flask. Sonicate this solution for 10 minutes, dilute with water to volume, mix, and filter. Apply separately 10 µL of this solution and 10 µL of a solution of USP Chlorpheniramine Maleate RS in a mixture of methanol and water (1:1) containing about 1.2 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate, methanol, and ammonium hydroxide (100:5:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength UV light: the R_F value of the principal spot observed in the chromatogram of the solution under test corresponds to that obtained from the Standard solution.

Dissolution ⟨711⟩—
TEST 1—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1.*
Medium: water; 500 mL.
Apparatus 1: 100 rpm.
Times: 1.5, 6.0, and 10.0 hours.
Procedure—Determine the amount of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ dissolved by employing the method set forth in the *Assay,* using a filtered portion of the solution under test in comparison with a Standard solution having a known concentration of USP Chlorpheniramine Maleate RS in the same medium.
Tolerances—The percentages of the labeled amount of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ dissolved at the times specified conform to *Acceptance Table 2.*

Time (hours)	Amount dissolved
1.5	between 15% and 40%
6.0	between 50% and 80%
10.0	not less than 70%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.* Proceed as directed for *Procedure* for *Method B* under *Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms.*
Medium—Prepare as directed under *Method B,* except use 900 mL of media. Operate the apparatus for 1 hour in the *Acid Stage* and use the acceptance criteria given under *Tolerances.* Operate the apparatus for 6 hours in the *Buffer Stage,* except to use 900 mL of simulated intestinal fluid TS without enzyme, and use the acceptance criteria given under *Tolerances.*
Apparatus 2: 50 rpm.
Times: 1.0 hour, 3.0 hours, 7.0 hours.
Procedure—Proceed as directed in *Test 1.*
Tolerances—The percentages of the labeled amount of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ dissolved at the times specified conform to *Acceptance Table 2.*

Time (hours)	Amount dissolved
1.0	between 30% and 60%
3.0	between 55% and 85%
7.0	not less than 70%

Uniformity of dosage units ⟨905⟩—meet the requirements.

Assay—
Mobile phase—Dissolve 2.0 g of sodium perchlorate in 350 mL of water. Add 650 mL of methanol and 2.0 mL of triethylamine, and mix. Filter, and degas this solution prior to use. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in dilute hydrochloric acid (1 in 100) to obtain a solution having a known concentration of about 0.12 mg per mL.
Assay preparation—Weigh and mix the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the mixture, equivalent to about 120 mg of chlorpheniramine maleate, to a 200-mL volumetric flask. Add about 100 mL of dilute hydrochloric acid (1 in 100), bring to a boil on a hot plate, and continue boiling moderately for 5 minutes. Cool, dilute with dilute hydrochloric acid (1 in 100) to volume, mix, and filter. Transfer 10.0 mL of the filtrate to a 50-mL volumetric flask, dilute with dilute hydrochloric acid (1 in 100) to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 261-nm detector and a 3.9-mm × 15-cm column that contains 10-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 900 theoretical plates, the tailing factor is not greater than 2.0, and the relative standard deviation for replicate injections is not more than 1.5%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in the portion of Capsules taken by the formula:

$$(1000C)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

www.webofpharma.com

Chlorpheniramine Maleate Injection

» Chlorpheniramine Maleate Injection is a sterile solution of Chlorpheniramine Maleate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Chlorpheniramine Maleate RS

Identification—
A: Dilute a volume of Injection, equivalent to about 50 mg of chlorpheniramine maleate, with dilute hydrochloric acid (1 in 1000) to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Transfer the liquid to a separator." The Injection meets the requirements of the test.
B: Evaporate a volume of Injection, equivalent to about 25 mg of chlorpheniramine maleate, on a steam bath to dryness, and dry the residue at 105° for 1 hour: it melts between 128° and 135°.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 8.8 USP Endotoxin Units per mg of chlorpheniramine maleate.
pH ⟨791⟩: between 4.0 and 5.2.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Proceed with Injection as directed under *Salts of Organic Nitrogenous Bases* ⟨501⟩, to prepare the solution employed for the determination of the absorbance, A_U, at 264 nm. For the determination of A_S, dissolve about 25 mg of USP Chlorpheniramine Maleate RS, accurately weighed, in 20 mL of dilute sulfuric acid (1 in 350), and treat this solution the same as the portion of Injection being assayed. Calculate the quantity, in mg, of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ in each mL of the Injection taken by the formula:

$$(C/V)(A_U/A_S)$$

in which C is the weight, in mg, of USP Chlorpheniramine Maleate RS in the *Standard preparation,* and V is the volume, in mL, of Injection taken.

Chlorpheniramine Maleate Oral Solution

» Chlorpheniramine Maleate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Chlorpheniramine Maleate RS

Identification—
A: Evaporate the remaining extract from the *Assay* on a steam bath to a small volume, then transfer it to a smaller, more suitable vessel, and evaporate just to the point where hexane vapors are no longer perceptible. Transfer the oily residue, with the aid of four 3-mL portions of dimethylformamide, to a suitable glass-stoppered graduated cylinder, dilute with dimethylformamide to 15.0 mL, and mix: the optical rotation of the solution so obtained, in a 100-mm tube, after correcting for the blank, is not more than +0.01° (*distinction from dexchlorpheniramine maleate*).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: the solution employed for measurement of absorbance in the *Assay*.
Alcohol Determination ⟨611⟩ (***if present***) ⟨611⟩: between 6.0% and 8.0% of C_2H_5OH.
Assay—Transfer 10 mL of Oral Solution, accurately measured, to a separator. Transfer about 40 mg of USP Chlorpheniramine Maleate RS, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, mix, and pipet 10 mL of this Standard solution into a separator similar to that containing the Oral Solution. Treat each solution as follows. Add 10 mL of sodium hydroxide solution (1 in 10), and extract with two 50-mL portions of solvent hexane. Combine the extracts in a second separator, wash with 10 mL of sodium hydroxide solution (1 in 250), and discard the washing. Extract the hexane solution with two 40-mL portions of dilute hydrochloric acid (1 in 100), collect the extracts in a 100-mL volumetric flask, add the same dilute acid to volume, and mix. Wash 50-mL portions of each solution, and of dilute hydrochloric acid (1 in 100), respectively, with three 30-mL portions of chloroform and then with 50 mL of solvent hexane, and discard the washings. Filter the acid phases through paper, discarding the first few mL of each filtrate, and determine the absorbances of the solutions obtained from the Oral Solution and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 264 nm, with a suitable spectrophotometer, using the extracted acid as the blank. Calculate the quantity, in µg, of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in each mL of the Oral Solution taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Chlorpheniramine Maleate RS in the Standard solution; and A_U and A_S are the absorbances of the solutions from the Oral Solution and the Standard solution, respectively.

Chlorpheniramine Maleate Tablets

» Chlorpheniramine Maleate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Chlorpheniramine Maleate RS
Identification—Disperse a portion of powdered Tablets, equivalent to about 25 mg of chlorpheniramine maleate, in about 20 mL of dilute hydrochloric acid (1 in 100). Dissolve about 25 mg of USP Chlorpheniramine Maleate RS in 20 mL of dilute hydrochloric acid (1 in 100). Treat each solution as follows. Render alkaline, to a pH of about 11, with sodium

www.webofpharma.com

hydroxide solution (1 in 10). Extract with two 50-mL portions of solvent hexane, collect the extracts in a beaker, and evaporate to dryness. Prepare a mineral oil dispersion of the residue so obtained and determine the IR absorption spectrum of the preparation in the region between 2 µm and 12 µm: the spectrum of the test preparation exhibits maxima only at the same wavelengths as that of the Standard preparation.

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 265 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Chlorpheniramine Maleate RS in the same *Medium*.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay—Using a portion of powdered Tablets equivalent to 4 mg of chlorpheniramine maleate, proceed as directed under *Salts of Organic Nitrogenous Bases* ⟨501⟩, but using dilute hydrochloric acid (1 in 100) instead of the dilute sulfuric acid (1 in 350), and dilute sulfuric acid (1 in 70), and using solvent hexane instead of the ether, and diluting 10 mL of the *Assay preparation* with dilute hydrochloric acid (1 in 100) to 25.0 mL to prepare the solution employed for the determination of the absorbance, A_U, at 264 nm. For the determination of A_S, prepare a solution containing about 40 mg of USP Chlorpheniramine Maleate RS, accurately weighed, in 200.0 mL of dilute hydrochloric acid (1 in 100), and treat 20.0 mL of this solution the same as the solution in dilute hydrochloric acid (1 in 100) of the portion of Tablets taken. Calculate the quantity, in mg, of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ in the portion of Tablets taken by the formula:

$$C(A_U/A_S)$$

in which C is the weight, in mg, of USP Chlorpheniramine Maleate RS in the 20.0-mL portion of the *Standard preparation*.

Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Capsules

» Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.
Labeling—The labeling indicates the *Dissolution Test* with which the product complies.
USP Reference standards ⟨11⟩—
USP Chlorpheniramine Maleate RS
USP Pseudoephedrine Hydrochloride RS

Identification—
A: The retention time of the major peak for chlorpheniramine maleate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for chlorpheniramine maleate*.
B: The retention time of the major peak for pseudoephedrine hydrochloride in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for pseudoephedrine hydrochloride*.

Dissolution ⟨711⟩—
TEST 1—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Times: 3, 6, and 12 hours.
Procedure—Determine the amounts of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved by employing the methods set forth in the *Assay for chlorpheniramine maleate* and the *Assay for pseudoephedrine hydrochloride*, respectively.
Tolerances—The percentages of the labeled amounts of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ and $C_{10}H_{15}NO \cdot HCl$ dissolved at the specified times conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
3	between 20% and 50%
6	between 45% and 75%
12	not less than 75%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium 1: simulated gastric fluid TS, prepared without pepsin; 900 mL.
Medium 2: simulated intestinal fluid TS, prepared without pancreatin; 900 mL.
Apparatus 2: 50 rpm.
Time for Medium 1: 1.5 hours.
Times for Medium 2: 3 and 6 hours.
Procedure—Determine the amounts of chlorpheniramine maleate ($C_{16}N_{19}ClN_2 \cdot C_4H_4O_4$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved by employing the methods set forth in the *Assay for chlorpheniramine maleate* and the *Assay for pseudoephedrine hydrochloride*, respectively, using Standard solutions having known concentrations of the relevant USP Reference Standard in the appropriate *Medium*.
Tolerances—The percentages of the labeled amounts of $C_{16}N_{19}ClN_2 \cdot C_4H_4O_4$ and $C_{10}H_{15}NO \cdot HCl$ dissolved at the specified times conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved (*Medium 1*)	Amount dissolved (*Medium 2*)
1.5	between 15% and 40%	
3.0		between 35% and 75%
6.0		not less than 50%

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay for chlorpheniramine maleate—
Mobile phase—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.15 g of triethylamine hydrochloride, 0.25 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid

www.webofpharma.com

in each 100 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with a phosphoric acid solution (1 in 1000) to obtain a solution having a known concentration of about 8 µg per mL.

Assay preparation—Transfer not fewer than 10 Capsules to a suitable container. Add 100 mL of water and 10 mL of a phosphoric acid solution (1 in 20), and heat gently until the Capsules are fully dispersed. Cool to room temperature, and transfer an accurately measured volume of the solution, equivalent to about 0.8 mg of chlorpheniramine maleate, to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter.

System suitability solution—Mix 1 part of the *Standard preparation* prepared above with 1 part of the *Standard preparation,* prepared as directed in the *Assay for pseudoephedrine hydrochloride.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Inject about 20 µL of the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between pseudoephedrine and chlorpheniramine is not less than 2.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor for the chlorpheniramine peak is not greater than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in the portion of Capsules taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation;* and r_U and r_S are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for pseudoephedrine hydrochloride—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for chlorpheniramine maleate.*

Standard preparation—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer about 1.0 mL of this solution to a 25-mL volumetric flask, dilute with 0.1% phosphoric acid to volume, and mix.

System suitability solution—Mix 1 part of the *Standard preparation* prepared above with 1 part of the *Standard preparation* prepared as directed in the *Assay for chlorpheniramine maleate.*

Assay preparation—Transfer not fewer than 10 Capsules to a suitable container. Add 100 mL of water and 10 mL of a phosphoric acid solution (1 in 20), and heat gently until the Capsules are fully dispersed. Cool to room temperature, and transfer an accurately measured volume of the solution, equivalent to about 12 mg of pseudoephedrine hydrochloride, to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Capsules taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Oral Solution

» Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Chlorpheniramine Maleate RS
USP Pseudoephedrine Hydrochloride RS

Identification—

A: The retention time of the major peak for chlorpheniramine maleate in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation,* as obtained in the *Assay for chlorpheniramine maleate.*

B: The retention time of the major peak for pseudoephedrine hydrochloride in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* in the *Assay for pseudoephedrine hydrochloride.*

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Assay for chlorpheniramine maleate—

Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay for chlorpheniramine maleate* under *Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Capsules.*

Standard preparation—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 1 mg per mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, add 80 mL of *Mobile phase,* dilute with water to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 1 mg of chlorpheniramine maleate, to a 100-mL volumetric flask. Add about 80 mL of *Mobile phase,* dilute with water to volume, mix, and filter.

www.webofpharma.com

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in the portion of Oral Solution taken by the formula:

$$(100C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in that *Standard preparation; V* is the volume, in mL, of Oral Solution taken for the *Assay preparation;* and r_U and r_S are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for pseudoephedrine hydrochloride—

Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay for chlorpheniramine maleate* under *Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Capsules.*

Standard preparation—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 1.5 mg per mL. Transfer about 1.0 mL of this solution to a 10-mL volumetric flask, add 8 mL of *Mobile phase,* dilute with water to volume, and mix.

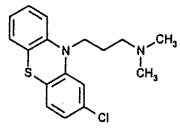
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 15 mg of pseudoephedrine hydrochloride, to a 100-mL volumetric flask. Add 80 mL of *Mobile phase,* dilute with water to volume, mix, and filter.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Oral Solution taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation; V* is the volume, in mL, of Oral Solution taken for the *Assay preparation;* and r_U and r_S are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Chlorpromazine



$C_{17}H_{19}ClN_2S$ 318.86

10*H*-Phenothiazine-10-propanamine, 2-chloro-*N*,*N*-dimethyl-.

2-Chloro-10-[3-(dimethylamino)propyl]phenothiazine [50-53-3].

» Chlorpromazine contains not less than 98.0 percent and not more than 101.0 percent of $C_{17}H_{19}ClN_2S$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

A: The IR absorption spectrum of a 1 in 100 solution in carbon disulfide, in a 1.0-mm cell between 7 µm and 15 µm, exhibits maxima only at the same wavelengths as that of a solution prepared by dissolving 55 mg of USP Chlorpromazine Hydrochloride RS in 3 mL of 1 N sodium hydroxide and extracting the resulting solution with 5.0 mL of carbon disulfide.

B: The principal spot found in the test for *Other alkylated phenothiazines* corresponds in R_F to the spot from the *Standard solution.*

Loss on drying ⟨731⟩—Dry it in vacuum at room temperature for 3 hours: it loses not more than 1.0% of its weight.

Other alkylated phenothiazines—Dissolve 45.0 mg in 10 mL of methanol. Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS in methanol to obtain a concentration of 5 mg per mL *(Standard solution),* and dilute it quantitatively and stepwise with methanol to obtain a concentration of 25 µg per mL (*Diluted standard solution*). Apply separately 10 µL of each of the three solutions to the starting line of a thin-layer chromatographic plate coated with chromatographic silica gel mixture. Develop the chromatogram, using as the solvent system a freshly prepared mixture of equal volumes of ether and ethyl acetate saturated with ammonium hydroxide, until the solvent front has moved about 10 cm from the origin. Remove the plate from the chamber, and air-dry for 20 minutes. View under short-wavelength UV light: the area and intensity of any spot, other than the principal spot, from the solution of Chlorpromazine are not greater than those of the spot from the *Diluted standard solution* (0.5%).

Assay—Place about 750 mg of Chlorpromazine, accurately weighed, in a 250-mL conical flask, and dissolve in 25 mL of glacial acetic acid, warming gently on a steam bath to effect solution. Cool, add crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.89 mg of $C_{17}H_{19}ClN_2S$.

Chlorpromazine Suppositories

» Chlorpromazine Suppositories contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{19}ClN_2S$.

Packaging and storage—Preserve in well-closed, light-resistant containers, at controlled room temperature.

USP Reference standards ⟨11⟩—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions

www.webofpharma.com

containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]
Identification—Suppositories respond to *Identification* test *B* under *Chlorpromazine*.
Other alkylated phenothiazines—Transfer a portion of Suppositories, equivalent to 45 mg of chlorpromazine, to a stoppered centrifuge tube, add 10 mL of methanol, shake vigorously to disperse the solid, warming gently if necessary, and centrifuge. Proceed as directed in the test for *Other alkylated phenothiazines*— under *Chlorpromazine*, beginning with "Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS." The area and intensity of any spot, other than the principal spot, from the solution from the Suppositories are not greater than those of the spot from the *Diluted standard solution* (0.5%).
Assay—Place not fewer than 10 Suppositories in a 250-mL beaker, reduce the mass to the consistency of a paste by crushing with a spatula, and mix. Weigh accurately a portion of the mass, equivalent to about 50 mg of chlorpromazine, place in a beaker, and dissolve in about 40 mL of ether. Transfer to a 250-mL separator with the aid of three 25-mL portions of ether, and extract with four 75-mL portions of 0.1 N hydrochloric acid, collecting the aqueous extracts in a 500-mL volumetric flask. Add 0.1 N hydrochloric acid to volume, and mix. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Dissolve an accurately weighed quantity of USP Chlorpromazine Hydrochloride RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 5.5 µg of chlorpromazine hydrochloride per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 254 nm and at 277 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of chlorpromazine ($C_{17}H_{19}ClN_2S$) in the portion of Suppositories taken by the formula:

$$10(0.897C)(A_{254} - A_{277})_U/(A_{254} - A_{277})_S$$

in which 0.897 is the ratio of the molecular weight of chlorpromazine to that of chlorpromazine hydrochloride; C is the concentration, in µg per mL, of USP Chlorpromazine Hydrochloride RS in the Standard solution; and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Suppositories (U) and the Standard solution (S), respectively.

Chlorpromazine Hydrochloride

$C_{17}H_{19}ClN_2S \cdot HCl$ 355.33
10*H*-Phenothiazine-10-propanamine, 2-chloro-*N*,*N*-dimethyl-, monohydrochloride.
2-Chloro-10-[3-(dimethylamino)propyl]phenothiazine monohydrochloride [69-09-0].

» Chlorpromazine Hydrochloride contains not less than 98.0 percent and not more than 101.5 percent of $C_{17}H_{19}ClN_2S \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Chlorpromazine Hydrochloride RS
[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: The principal spot found in the test for *Other alkylated phenothiazines* corresponds in R_F to the spot from the *Standard solution*.
C: A solution (1 in 10) responds to the tests for *Chloride* ⟨191⟩.
Melting range ⟨741⟩: between 195° and 198°.
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Other alkylated phenothiazines—Dissolve 50 mg, previously dried, in methanol to make 10 mL, and mix. Proceed as directed in the test for *Other alkylated phenothiazines*— under *Chlorpromazine*, beginning with "Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS." The area and intensity of any spot, other than the principal spot, from the solution of Chlorpromazine Hydrochloride are not greater than those of the spot from the *Diluted standard solution* (0.5%).
Assay—Transfer to a beaker about 700 mg of Chlorpromazine Hydrochloride, accurately weighed, and dissolve in 75 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 35.53 mg of $C_{17}H_{19}ClN_2S \cdot HCl$.

Chlorpromazine Hydrochloride Oral Concentrate

» Chlorpromazine Hydrochloride Oral Concentrate contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{19}ClN_2S \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Label it to indicate that it must be diluted prior to administration.
USP Reference standards ⟨11⟩—
USP Chlorpromazine Hydrochloride RS
[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—
A: It responds to *Identification* test *A* under *Chlorpromazine Hydrochloride Syrup*.
B: Dilute a portion of the Oral Concentrate with an equal volume of water: the resulting solution responds to the tests for *Chloride* ⟨191⟩.

USP Monographs

www.webofpharma.com

Microbial enumeration tests ⟨61⟩ and ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for the absence of *Escherichia coli.*

pH ⟨791⟩: between 2.3 and 4.1.

Limit of chlorpromazine sulfoxide—Proceed as directed in the test for *Limit of chlorpromazine sulfoxide*— under *Chlorpromazine Hydrochloride Syrup.*

Assay—Transfer an accurately measured volume of Oral Concentrate, previously diluted if necessary, equivalent to about 10 mg of chlorpromazine hydrochloride, to a 50-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Proceed as directed in the *Assay*— under *Chlorpromazine Hydrochloride Injection*, beginning with "Pipet 10 mL of the solution." Calculate the quantity, in mg, of $C_{17}H_{19}ClN_2S \cdot HCl$ in each mL of the Oral Concentrate taken by the formula:

$$1.25C(A_{254} - A_{277})_U / V(A_{254} - A_{277})_S$$

in which C is the concentration, in µg per mL, of USP Chlorpromazine Hydrochloride RS in the Standard solution; V is the volume, in mL, of Oral Concentrate taken; and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Oral Concentrate (U) and the Standard solution (S), respectively.

Chlorpromazine Hydrochloride Injection

» Chlorpromazine Hydrochloride Injection is a sterile solution of Chlorpromazine Hydrochloride in Water for Injection. It contains, in each mL, not less than 23.75 mg and not more than 26.25 mg of $C_{17}H_{19}ClN_2S \cdot HCl$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

A: Transfer a volume of Injection, equivalent to about 25 mg of chlorpromazine hydrochloride, to a 10-mL volumetric flask, dilute with methanol to volume, and mix (test solution). Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS in dilute methanol (9 in 10) to obtain a Standard solution having a known concentration of 2.5 mg per mL. Apply separately 5-µL portions of each of the two solutions to the starting line of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a freshly prepared mixture of equal volumes of ether and ethyl acetate saturated with ammonium hydroxide until the solvent front has moved about 10 cm from the origin. Remove the plate from the developing chamber, air-dry for 20 minutes, then view under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

B: It responds to the tests for *Chloride* ⟨191⟩.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 6.9 USP Endotoxin Units per mg of chlorpromazine hydrochloride.

pH ⟨791⟩: between 3.4 and 5.4.

Limit of chlorpromazine sulfoxide— [NOTE—Conduct this test without exposure to daylight, and with the minimum necessary exposure to artificial light.]

Test preparation—Pipet 4 mL of the test solution prepared with methanol as directed in *Identification* test *A* into a 10-mL volumetric flask, dilute with methanol to volume, and mix.

Standard preparation—Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS in methanol to obtain a solution having a concentration of 50 µg per mL.

Procedure—Apply separate 10-µL portions of the *Standard preparation* and the *Test preparation* to the starting line of a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Dry the applied solutions with the aid of a stream of nitrogen. Develop the chromatogram, using as the solvent system a freshly prepared mixture of equal volumes of ether and ethyl acetate saturated with ammonium hydroxide, until the solvent front has moved about 13 cm from the origin. Remove the plate from the chamber, and air-dry for 30 minutes. Examine under short-wavelength UV light: the area and intensity of the only other spot in the test specimen chromatogram, other than the principal spot, are not greater than those of the spot from the *Standard preparation* (5.0%).

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of chlorpromazine hydrochloride, to a 500-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Pipet 10 mL of the solution into a 250-mL separator, add about 20 mL of water, render alkaline with ammonium hydroxide, and extract with four 25-mL portions of ether. Extract the combined ether extracts with four 25-mL portions of 0.1 N hydrochloric acid, collecting the aqueous extracts in a 250-mL volumetric flask. Aerate to remove residual ether, add 0.1 N hydrochloric acid to volume, and mix. Dissolve a suitable quantity, accurately weighed, of USP Chlorpromazine Hydrochloride RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same acid to obtain a Standard solution having a known concentration of about 8 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 254 nm and at 277 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{17}H_{19}ClN_2S \cdot HCl$ in each mL of the Injection taken by the formula:

$$12.5C(A_{254} - A_{277})_U / V(A_{254} - A_{277})_S$$

in which C is the concentration, in µg per mL, of USP Chlorpromazine Hydrochloride RS in the Standard solution, V is the volume, in mL, of Injection taken, and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Injection (U) and the Standard solution (S), respectively.

Chlorpromazine Hydrochloride Syrup

» Chlorpromazine Hydrochloride Syrup contains, in each 100 mL, not less than 190 mg and not more than 210 mg of $C_{17}H_{19}ClN_2S \cdot HCl$.

www.webofpharma.com

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

A: Transfer a volume of it, equivalent to about 20 mg of chlorpromazine hydrochloride, to a 125-mL separator. Add 10 mL of water, 2 mL of sodium hydroxide solution (1 in 2), and mix. Extract with three 30-mL portions of ether. Filter the combined ether extracts through anhydrous sodium sulfate. With the aid of a stream of nitrogen evaporate the ether to about 5 mL. Quantitatively transfer the solution to a 40-mL centrifuge tube. Evaporate with a stream of nitrogen and mild heat to dryness. Dissolve the residue in 100 mL of methanol to obtain the Test solution. Separately apply 15 µL of this Test solution and 15 µL of a Standard solution, containing 0.2 mg of USP Chlorpromazine Hydrochloride RS per mL of methanol, to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram in a chamber containing a freshly prepared mixture of ethyl acetate that has been saturated with ammonium hydroxide, ether, and methanol (75:25:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and spray with Iodoplatinate reagent prepared by dissolving 100 mg of platinic chloride in 10 mL of 0.1 N hydrochloric acid, adding 25 mL of potassium iodide solution (1 in 25), 0.5 mL of formic acid, and diluting with water to 100 mL: the R_F value of the principal spot from the test solution corresponds to that obtained from the Standard solution.

B: Dilute a portion of the Syrup with an equal volume of water: the resulting solution responds to the tests for *Chloride* ⟨191⟩.

Limit of chlorpromazine sulfoxide—

Chlorpromazine sulfoxide standard solution—Transfer 5 mL of a solution in dilute hydrochloric acid (1 in 100) of USP Chlorpromazine Hydrochloride RS containing 10.6 mg per mL to a 50-mL volumetric flask. Add 2 mL of 30% hydrogen peroxide and heat at 60° for 10 minutes. Cool, dilute with 1 M sodium bisulfite to volume, and mix. Transfer 10.0 mL to a 60-mL separator, add 2 mL of sodium hydroxide solution (1 in 2), and mix. Extract with three 30-mL portions of ether. Filter the extracts through ether-wetted anhydrous sodium sulfate into a 250-mL conical flask. Cautiously evaporate the extracts to dryness. Dissolve the residue in 10.0 mL of methanol, and filter if necessary. Each mL of this solution contains 1 mg of chlorpromazine sulfoxide.

Procedure—Transfer an accurately measured volume of the Syrup, equivalent to about 20 mg of chlorpromazine hydrochloride, to a 125-mL separator. Add 10 mL of water and 2 mL of sodium hydroxide solution (1 in 2), and mix. Extract with three 30-mL portions of ether. Filter the combined ether extracts through anhydrous sodium sulfate. With the aid of a stream of nitrogen evaporate the ether to about 5 mL. Quantitatively transfer the solution to a 40-mL centrifuge tube. Evaporate with a stream of nitrogen and mild heat to dryness. Dissolve the residue in 1.0 mL of methanol to obtain the Test solution. Separately apply 15 µL of this Test solution and 15 µL of a *Chlorpromazine sulfoxide standard solution* to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram in a chamber containing a freshly prepared mixture of ethyl acetate that has been saturated with ammonium hydroxide, ether, and methanol (75:25:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and spray with Iodoplatinate reagent prepared by dissolving 100 mg of platinic chloride in 10 mL of 0.1 N hydrochloric acid, adding 25 mL of potassium iodide solution (1 in 25) and 0.5 mL of formic acid, and diluting with water to 100 mL. The chromatogram from the Test solution may exhibit a secondary spot whose R_F value corresponds to, and whose size and intensity are not greater than, those of the spot from the *Chlorpromazine sulfoxide standard solution* (5.0%).

Assay—Transfer an accurately measured volume of Syrup, equivalent to about 10 mg of chlorpromazine hydrochloride, to a 50-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Proceed as directed in the *Assay*— under *Chlorpromazine Hydrochloride Injection*, beginning with "Pipet 10 mL of the solution." Calculate the quantity, in mg, of $C_{17}H_{19}ClN_2S \cdot HCl$ in each mL of the Syrup taken by the formula:

$$1.25C(A_{254} - A_{277})_U / V(A_{254} - A_{277})_S$$

in which C is the concentration, in µg per mL, of USP Chlorpromazine Hydrochloride RS in the Standard solution; V is the volume, in mL, of Syrup taken; and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Syrup (U) and the Standard solution (S), respectively.

Chlorpromazine Hydrochloride Tablets

» Chlorpromazine Hydrochloride Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{17}H_{19}ClN_2S \cdot HCl$.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—

A: Tablets respond to *Identification* test *B* under *Chlorpromazine Hydrochloride*.

B: Digest a quantity of powdered Tablets, equivalent to about 25 mg of chlorpromazine hydrochloride, with 25 mL of water, and filter: the solution so obtained responds to *Identification* test *C* under *Chlorpromazine Hydrochloride*.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 1: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{17}H_{19}ClN_2S \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 254 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Chlorpromazine Hydrochloride RS in the same medium.

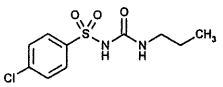
www.webofpharma.com

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{17}H_{19}ClN_2S \cdot HCl$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Other alkylated phenothiazines—Transfer a portion of finely powdered Tablets, equivalent to 50 mg of chlorpromazine hydrochloride, to a stoppered centrifuge tube, add 10 mL of methanol, shake vigorously, and centrifuge (remove any sugar coating by prior washing with water). Proceed as directed in the test for *Other alkylated phenothiazines*— under *Chlorpromazine*, beginning with "Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS." The area and intensity of any spot, other than the principal spot, from the solution from the Tablets are not greater than those of the spot from the *Diluted standard solution* (0.5%).
Assay—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of chlorpromazine hydrochloride, to a 500-mL volumetric flask. Add about 200 mL of water and 5 mL of hydrochloric acid, insert the stopper, and shake for about 10 minutes. Dilute with water to volume, and mix. Filter a portion of the solution, discarding the first 50 mL of the filtrate. Treat 10.0 mL of the filtrate as directed in the *Assay*— under *Chlorpromazine Hydrochloride Injection*, beginning with "Pipet 10 mL of the solution." Calculate the quantity, in mg, of $C_{17}H_{19}ClN_2S \cdot HCl$ in the portion of Tablets taken by the formula:

$$12.5C(A_{254} - A_{277})_U/(A_{254} - A_{277})_S$$

in which C is the concentration, in µg per mL, of USP Chlorpromazine Hydrochloride RS in the Standard solution, and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Tablets (U) and the Standard solution (S), respectively.

Chlorpropamide



$C_{10}H_{13}ClN_2O_3S$ 276.74
Benzenesulfonamide, 4-chloro-*N*-[(propylamino)carbonyl]-. 1-[(*p*-Chlorophenyl)sulfonyl]-3-propylurea [94-20-2].

» Chlorpropamide contains not less than 97.0 percent and not more than 103.0 percent of $C_{10}H_{13}ClN_2O_3S$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Chlorpropamide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩*: Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: It responds to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩. Prepare the test solution by dissolving an accurately weighed quantity of Chlorpropamide in acetone to obtain a solution containing 1 mg per mL. Develop the chromatogram in a solvent system consisting of a mixture of methylene chloride, methanol, cyclohexane, and ammonium hydroxide (100:50:30:10).
Melting range ⟨741⟩: between 126° and 129°.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 2 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.4%.
Selenium ⟨291⟩: 0.003%.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of equal volumes of acetonitrile and dilute glacial acetic acid (1 in 100). [NOTE—Do not exceed 50% of acetonitrile.] Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Chlorpropamide RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.
Assay preparation—Transfer about 50 mg of Chlorpropamide, accurately weighed, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 10 mL of this solution to a second 100-mL volumetric flask, add *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the tailing factor for the analyte peak is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{10}H_{13}ClN_2O_3S$ in the portion of Chlorpropamide taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpropamide RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Chlorpropamide Tablets

» Chlorpropamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{10}H_{13}ClN_2O_3S$.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Chlorpropamide RS
Identification—Shake a quantity of finely powdered Tablets, equivalent to about 100 mg of chlorpropamide, with 20 mL of 1 N hydrochloric acid, and extract with 50 mL of chloroform. Filter the chloroform through chloroform-washed cotton into a suitable beaker, and evaporate the chloroform on a steam bath with the aid of a current of dry air to dryness.

USP Monographs

www.webofpharma.com

Dry the residue at 105° for 1 hour: the residue so obtained responds to the *Identification* tests under *Chlorpropamide.*

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 60 minutes.
Procedure—Determine the amount of $C_{10}H_{13}ClN_2O_3S$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 230 nm of filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid in comparison with a Standard solution having a known concentration of USP Chlorpropamide RS in 0.1 N hydrochloric acid. [NOTE—A volume of alcohol not exceeding 10% of the final volume of the Standard solution may be used to dissolve the USP Chlorpropamide RS.]
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{10}H_{13}ClN_2O_3S$ is dissolved in 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase , *Standard preparation,* and *Chromatographic system*—Proceed as directed in the *Assay* under *Chlorpropamide.*
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of chlorpropamide, to a 100-mL volumetric flask. Add *Mobile phase* to volume, mix, and filter, discarding the first 10 mL of the filtrate. Pipet 10 mL of the filtrate into a second 100-mL volumetric flask, add *Mobile phase* to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Chlorpropamide.* Calculate the quantity, in mg, of $C_{10}H_{13}ClN_2O_3S$ in the portion of Tablets taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpropamide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Chlortetracycline Bisulfate

» Chlortetracycline Bisulfate has a potency equivalent to not less than 760 µg of chlortetracycline hydrochloride ($C_{22}H_{23}ClN_2O_8 \cdot HCl$) per mg, calculated on the dried and butyl alcohol-free basis.

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Label it to indicate that it is intended for veterinary use only.
USP Reference standards ⟨11⟩—
USP Chlortetracycline Hydrochloride RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Ultraviolet-Visible Spectroscopy:* ***197U***▲ (CN 1-May-2020)—
Solution: 40 µg per mL.
Medium: 0.1 N hydrochloric acid.
Absorptivity at 368 nm, calculated on the dried and butyl alcohol-free basis, is not less than 83.0% and not more than 95.0% of that of the USP Chlortetracycline Hydrochloride RS, the potency of the Reference Standard being taken into account.
Crystallinity ⟨695⟩: meets the requirements.
Loss on drying ⟨731⟩—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.
Sulfate content—Transfer about 1 g, accurately weighed, to a 250-mL beaker, and dissolve in about 100 mL of water. Neutralize the solution with 7.5 N ammonium hydroxide to litmus paper, and warm. Filter, and wash the filter with warm water. Neutralize the filtrate with 6 N hydrochloric acid to litmus, and add an additional 4 mL of 6 N hydrochloric acid. Heat the solution to boiling, and add, with constant stirring, sufficient boiling barium chloride TS to precipitate all of the sulfate. Add an additional 2 mL of barium chloride TS, and digest on a steam bath for 1 hour. Filter the mixture through ashless filter paper, transferring the residue quantitatively to the filter, and wash the residue with hot water until no precipitate is obtained when 1 mL of silver nitrate TS is added to 5 mL of washing. Transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents to constant weight. Perform a blank determination concurrently with the test specimen determination, and subtract the weight of residue obtained from that obtained in the test specimen determination to obtain the weight of residue attributable to the sulfate content of the specimen: not less than 15.0% is found, calculated on the dried and butyl alcohol-free basis.

Butyl alcohol—
Ceric ammonium nitrate solution—Dissolve 20 g of ceric ammonium nitrate in 4 N nitric acid to obtain 100 mL of solution.
Standard preparations—Transfer about 3 g of butyl alcohol, accurately weighed, to a 1000-mL volumetric flask containing 800 mL of water, shake to dissolve, dilute with water to volume, and mix (*Standard preparation 1*). Transfer 10.0 mL of *Standard preparation 1* and 1 drop of dimethicone to a 50-mL distilling flask equipped with a condenser and an extension that reaches into a collecting tube maintained in an ice-water bath. Distill slowly, and collect about 8 mL of distillate. Warm the distillate to ambient temperature, and transfer with the aid of water to a 10-mL volumetric flask. Dilute with water to volume, and mix (*Standard preparation 2*).
Test preparation—Transfer an accurately weighed specimen, equivalent to about 30 mg of butyl alcohol, to a 50-mL distilling flask equipped with a condenser and an extension that reaches into a collecting tube maintained in an ice bath. Add 25 mL of water and 1 drop of dimethicone to the distilling flask. Distill slowly, and collect about 8 mL of the distillate. Warm the distillate to ambient temperature, and transfer with the aid of water to a 10-mL volumetric flask. Dilute with water to volume, and mix.
Procedure—To four separate test tubes add, respectively, 5.0 mL of *Standard preparation 1*, 5.0 mL of *Standard preparation 2*, 5.0 mL of *Test preparation*, and 5.0 mL of water to provide a blank. To each add 2.0 mL of *Ceric ammonium nitrate solution*, and mix. Concomitantly determine the absorbances of the solutions from the *Standard preparations* and the *Test preparation* at the wavelength of maximum absorbance at about 475 nm, with a suitable spectrophotometer, using the blank to set the instrument to zero. In a suitable determination, the absorbance of the solution from *Standard preparation 2* is not less than 98.0% of the absorbance of the solution from *Standard preparation 1*. Calculate the percentage of butyl alcohol in the specimen taken by the formula:

$$1000(W_S/W_U)(A_U/A_S)$$

www.webofpharma.com

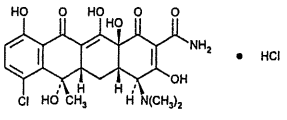
in which W_S is the weight, in g, of butyl alcohol taken to prepare *Standard preparation 1*, W_U is the weight, in mg, of specimen taken, and A_U and A_S are the absorbances of the solutions from the *Test preparation* and *Standard preparation 2*, respectively: not more than 15.0% is found.
Assay—Proceed with Chlortetracycline Bisulfate as directed for chlortetracycline under *Antibiotics—Microbial Assays* ⟨81⟩.

Chlortetracycline and Sulfamethazine Bisulfates Soluble Powder

» Chlortetracycline and Sulfamethazine Bisulfates Soluble Powder is a dry mixture of Chlortetracycline Bisulfate and Sulfamethazine Bisulfate and one or more suitable buffers and diluents. It contains the equivalent of not less than 85.0 percent and not more than 125.0 percent of the labeled amounts of chlortetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) and sulfamethazine ($C_{12}H_{14}N_4O_2S$).

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Label it to indicate that it is intended for veterinary use only.
USP Reference standards ⟨11⟩—
USP Chlortetracycline Hydrochloride RS
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.
Assay for chlortetracycline hydrochloride—Proceed as directed for chlortetracycline under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed quantity of Powder, equivalent to about 100 mg of chlortetracycline hydrochloride, dissolved in an accurately measured volume of 0.01 N hydrochloric acid to obtain a stock solution having a convenient concentration. Dilute an accurately measured volume of this stock solution quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.
Assay for sulfamethazine—Proceed as directed under *Nitrite Titration* ⟨451⟩, using an accurately weighed quantity of Powder, equivalent to about 500 mg of sulfamethazine. Each mL of 0.1 M sodium nitrite is equivalent to 27.83 mg of sulfamethazine ($C_{12}H_{14}N_4O_2S$).

Chlortetracycline Hydrochloride



$C_{22}H_{23}ClN_2O_8 \cdot HCl$ 515.34
2-Naphthacenecarboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride [4*S*-(4α,4aα,5aα,6β,12aα)]-.
7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [64-72-2].

» Chlortetracycline Hydrochloride has a potency of not less than 900 µg of $C_{22}H_{23}ClN_2O_8 \cdot HCl$ per mg. [NOTE—Chlortetracycline Hydrochloride labeled solely for use in preparing oral veterinary dosage forms has a potency of not less than 820 µg of $C_{22}H_{23}ClN_2O_8 \cdot HCl$ per mg.]

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.
USP Reference standards ⟨11⟩—
USP Chlortetracycline Hydrochloride RS
USP Oxytetracycline RS
USP Tetracycline Hydrochloride RS

Identification—
A: Proceed as directed for *Method II* under *Identification—Tetracyclines* ⟨193⟩, using a methanol solution containing 0.5 mg per mL as the *Test solution* and a methanol solution containing in each mL 0.5 mg of USP Chlortetracycline Hydrochloride RS, 0.5 mg of USP Oxytetracycline RS, and 0.5 mg of USP Tetracycline Hydrochloride RS as the *Resolution solution*.
B: A solution (1 in 20) meets the requirements of the tests for *Chloride* ⟨191⟩.
Specific rotation ⟨781S⟩: between –235° and –250°.
Test solution: 5 mg per mL, in water, that has been allowed to stand in the dark for 30 minutes.
Crystallinity ⟨695⟩: meets the requirements.
Sterility Tests ⟨71⟩ —Where the label states that Chlortetracycline Hydrochloride is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 6 g of specimen aseptically dissolved in 200 mL of *Fluid D* being used.
pH ⟨791⟩: between 2.3 and 3.3, in a solution containing 10 mg per mL.
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.
Assay—Proceed with Chlortetracycline Hydrochloride as directed under *Antibiotics—Microbial Assays* ⟨81⟩.

Chlortetracycline Hydrochloride Ointment

» Chlortetracycline Hydrochloride Ointment contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of $C_{22}H_{23}ClN_2O_8 \cdot HCl$ in a suitable ointment base.

Packaging and storage—Preserve in collapsible tubes or in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Chlortetracycline Hydrochloride RS
Minimum fill ⟨755⟩: meets the requirements.

USP Monographs

www.webofpharma.com

Water Determination, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed quantity of Ointment, equivalent to about 30 mg of chlortetracycline hydrochloride, shaken in a separator with about 50 mL of ether, and extracted with four 20-mL portions of 0.01 N hydrochloric acid. Combine the aqueous extracts in a 100-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Dilute this stock solution quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration assumed to be equal to the medium dose level of the Standard.

Chlortetracycline Hydrochloride Ophthalmic Ointment

DEFINITION
Chlortetracycline Hydrochloride Ophthalmic Ointment contains NLT 90.0% and NMT 125.0% of the labeled amount of chlortetracycline hydrochloride ($C_{22}H_{23}ClN_2O_8 \cdot HCl$).

ASSAY
• **PROCEDURE**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Shake a portion of Ophthalmic Ointment containing nominally 10 mg of chlortetracycline hydrochloride in a separator with 50 mL of ether, and extract with four 20-mL portions of 0.01 N hydrochloric acid. Combine the aqueous extracts in a 100-mL volumetric flask, and dilute with 0.01 N hydrochloric acid to volume.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with water to obtain a *Test Dilution* having a chlortetracycline hydrochloride concentration that is nominally equivalent to the median level of the standard.
Acceptance criteria: 90.0%–125.0%

SPECIFIC TESTS
• **STERILITY TESTS** ⟨71⟩: Meets the requirements
• **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Chlortetracycline Hydrochloride RS

Chlortetracycline Hydrochloride Soluble Powder

» Chlortetracycline Hydrochloride Soluble Powder contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of $C_{22}H_{23}ClN_2O_8 \cdot HCl$.

Packaging and storage—Preserve in tight containers, protected from light.
Labeling—Label it to indicate that it is intended for oral veterinary use only.
USP Reference standards ⟨11⟩—
USP Chlortetracycline Hydrochloride RS
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

Assay—
Assay preparation 1 (where it is labeled on a weight basis)—Dissolve about 3 g of Powder in an accurately measured volume of 0.01 N hydrochloric acid sufficient to obtain a solution containing not less than 1000 µg of chlortetracycline hydrochloride ($C_{22}H_{23}ClN_2O_8 \cdot HCl$) per mL.
Assay preparation 2 (where the label states the amount of chlortetracycline in the immediate container)—Transfer the contents of 1 container of Powder to an accurately measured volume of 0.01 N hydrochloric acid sufficient to obtain a solution containing not less than 1000 µg of chlortetracycline hydrochloride ($C_{22}H_{23}ClN_2O_8 \cdot HCl$) per mL.
Procedure—Proceed with Powder as directed for chlortetracycline under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Chlortetracycline Hydrochloride Tablets

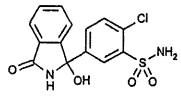
» Chlortetracycline Hydrochloride Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{22}H_{23}ClN_2O_8 \cdot HCl$.

Packaging and storage—Preserve in tight containers, protected from light.
Labeling—Label the Tablets to indicate that they are intended for veterinary use only.
USP Reference standards ⟨11⟩—
USP Chlortetracycline Hydrochloride RS
USP Oxytetracycline RS
USP Tetracycline Hydrochloride RS
Identification—Shake a suitable quantity of finely ground Tablet powder with methanol to obtain a solution containing about 0.5 mg of chlortetracycline hydrochloride per mL, and filter. Using the filtrate so obtained as the *Test solution*, and a methanol solution containing in each mL 0.5 mg of USP Chlortetracycline Hydrochloride RS, 0.5 mg of USP Oxytetracycline RS, and 0.5 mg of USP Tetracycline Hydrochloride RS as the *Resolution solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* ⟨193⟩.
Disintegration ⟨701⟩: 1 hour, simulated gastric fluid TS being used as the test medium in place of water.
Uniformity of dosage units ⟨905⟩: meet the requirements for *Weight Variation*.
Water Determination, *Method I* ⟨921⟩: not more than 3.0%, or where the Tablets have a diameter of greater than 15 mm, not more than 6.0%, a quantity of finely ground Tablet powder, accurately weighed, being used.
Assay—Transfer not less than 5 Tablets to a high-speed glass blender jar containing an accurately measured volume of 0.01 N hydrochloric acid, so that after blending for 3 to 5 minutes

www.webofpharma.com

a solution of convenient concentration is obtained. Proceed as directed for chlortetracycline under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this solution diluted quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Chlorthalidone



$C_{14}H_{11}ClN_2O_4S$ 338.77

Benzenesulfonamide, 2-chloro-5-(2,3-dihydro-1-hydroxy-3-oxo-1*H*-isoindol-1-yl)-.

2-Chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl)benzenesulfonamide [77-36-1].

» Chlorthalidone contains not less than 98.0 percent and not more than 102.0 percent of $C_{14}H_{11}ClN_2O_4S$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Chlorthalidone RS
USP Chlorthalidone Related Compound A RS
4′-Chloro-3′-sulfamoyl-2-benzophenone carboxylic acid.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020)

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 100 µg per mL.
Medium: 2 N hydrochloric acid in methanol (1 in 50).
Absorptivities at 275 nm, calculated on the dried basis, do not differ by more than 4.0%.

C: Dissolve about 50 mg in 3 mL of sulfuric acid: an intense yellow color develops.

Loss on drying ⟨731⟩—Dry about 2 g, accurately weighed, at 105° for 4 hours: it loses not more than 0.4% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chloride ⟨221⟩—Shake 1.0 g with 40 mL of water for 5 minutes, and filter through chloride-free filter paper previously rinsed with water: the filtrate shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.035%).

Limit of chlorthalidone related compound A—Proceed as directed in the *Assay,* except to calculate the percentage of chlorthalidone related compound A in the portion of Chlorthalidone taken by the formula:

$$0.1(C_R/C_T)(R_U/R_S)$$

in which C_R is the concentration, in µg per mL, of USP Chlorthalidone Related Compound A RS in the *Standard preparation;* C_T is the concentration, in mg per mL, of Chlorthalidone in the *Assay preparation;* and R_U and R_S are the peak response ratios of chlorthalidone related compound A to the internal standard obtained from the *Assay preparation* and the *Standard preparation,* respectively: not more than 1.0% is present. [NOTE—USP Chlorthalidone Related Compound A RS is 4′-chloro-3′-sulfamoyl-2-benzophenone carboxylic acid (CCA).]

Assay—

Mobile phase—Prepare a suitable degassed mixture of 0.01 M dibasic ammonium phosphate and methanol (3:2), adjust dropwise with phosphoric acid to a pH of 5.5 ± 0.1, and filter.

Internal standard solution—Prepare a solution of 2,7-naphthalenediol in methanol having a concentration of about 1.0 mg per mL.

Chlorthalidone related compound A solution—Prepare a solution of USP Chlorthalidone Related Compound A RS in methanol having a known concentration of about 5 µg per mL.

Standard preparation—Prepare a solution of USP Chlorthalidone RS in methanol having a known concentration of about 1 mg per mL. Pipet 5 mL of this solution into a 50-mL volumetric flask containing 5.0 mL of *Internal standard solution* and 10.0 mL of *Chlorthalidone related compound A solution.* Dilute with water to volume, and mix. This solution contains about 0.1 mg of chlorthalidone and about 1 µg of chlorthalidone related compound A per mL.

Assay preparation—Transfer about 50 mg of Chlorthalidone, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Pipet 5 mL of this solution into a 50-mL volumetric flask containing 5.0 mL of *Internal standard solution* and 10.0 mL of methanol. Dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph five replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.5 for chlorthalidone related compound A, 0.8 for chlorthalidone, and 1.0 for the internal standard; the resolution, *R,* between chlorthalidone and chlorthalidone related compound A, and between chlorthalidone and the internal standard is not less than 1.5; the tailing factor for chlorthalidone and chlorthalidone related compound A is not more than 2.0; and the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{14}H_{11}ClN_2O_4S$ in the portion of Chlorthalidone taken by the formula:

$$500C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Chlorthalidone RS in the *Standard preparation;* and R_U and R_S are the peak response ratios of chlorthalidone to the internal standard obtained from the *Assay preparation* and the *Standard preparation,* respectively.

USP Monographs

www.webofpharma.com

Chlorthalidone Tablets

» Chlorthalidone Tablets contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of $C_{14}H_{11}ClN_2O_4S$.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Chlorthalidone RS

Identification—
A: Digest a quantity of powdered Tablets, equivalent to about 100 mg of chlorthalidone, with 10 mL of acetone on a steam bath for about 5 minutes. Filter the solution into a 50-mL beaker, add 20 mL of water, and boil on the steam bath for about 5 minutes, passing a gentle current of air above the solution to remove the acetone. Cool in an ice bath, filter, and dry the crystals at 105° for 4 hours: the crystals so obtained respond to *Identification* test *A* under *Chlorthalidone*.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

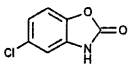
Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 75 rpm.
Time: 60 minutes.
Standard preparation—Dissolve an accurately weighed quantity of USP Chlorthalidone RS in methanol to obtain a solution having a known concentration of about 5 mg per mL.
Procedure—Determine the amount of $C_{14}H_{11}ClN_2O_4S$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 275 nm of filtered portions of the solution under test, suitably diluted with water, in comparison with a quantitative dilution in water of the *Standard preparation* having a known concentration of USP Chlorthalidone RS comparable to the concentration of the solution under test.
Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{14}H_{11}ClN_2O_4S$ is dissolved in 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase and *Internal standard solution*—Prepare as directed in the *Assay*— under *Chlorthalidone*.
Standard preparation—Prepare as directed in the *Assay*— under *Chlorthalidone*, except to substitute 10.0 mL of methanol for the *CCA solution*.
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of Chlorthalidone, to a 100-mL volumetric flask. Dissolve in about 50 mL of methanol, shake for 30 minutes, dilute with methanol to volume, and mix. Transfer about 30 mL of this solution to a 50-mL centrifuge tube, and centrifuge for 10 minutes. Pipet 5 mL of the clear supernatant into a 50-mL volumetric flask containing 5.0 mL of *Internal standard solution* and 10.0 mL of methanol. Dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 2.0%, and the resolution factor between chlorthalidone and the internal standard is not less than 1.5. The tailing factors for the chlorthalidone and internal standard peaks are not more than 2.0.
Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.8 for chlorthalidone and 1.0 for the internal standard. Calculate the quantity, in mg, of $C_{14}H_{11}ClN_2O_4S$ in the portion of Tablets taken by the formula:

$$1000C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Chlorthalidone RS in the *Standard preparation*; and R_U and R_S are the peak response ratios of chlorthalidone to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Chlorzoxazone



$C_7H_4ClNO_2$ 169.57
2(3*H*)-Benzoxazolone, 5-chloro-.
5-Chloro-2-benzoxazolinone [95-25-0].

» Chlorzoxazone contains not less than 98.0 percent and not more than 102.0 percent of $C_7H_4ClNO_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Chlorzoxazone RS
USP Chlorzoxazone Related Compound A RS
2-Amino-4-chlorophenol.
C_6H_6ClNO 143.57

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020) previously dried.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: methanol.
Melting range ⟨741⟩: between 189° and 194°.
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.15%.
Chromatographic purity—Prepare a *Test solution* in methanol containing 20 mg per mL. Dissolve a suitable quantity of USP Chlorzoxazone Related Compound A RS (2-Amino-4-chlorophenol) in methanol to obtain a solution containing 100 µg per mL (*Standard solution A*). Dissolve a suitable quantity of *p*-chlorophenol in methanol to obtain a solution containing 50 µg per mL (*Standard solution B*). Apply separate 10 µL portions of the three solutions to the starting line of a suitable thin-layer chromatographic plate (see

www.webofpharma.com

Chromatography ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of hexane and dioxane (63:37) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: any spot obtained from the *Test solution*, other than one corresponding to chlorzoxazone, does not exceed, in size or intensity, the principal spot obtained from *Standard solution A*, corresponding to not more than 0.5% of any individual impurity. Expose the plate to iodine vapors in a closed chamber, and locate the spots: any spot obtained from the *Test solution*, other than one corresponding to chlorzoxazone, does not exceed, in size or intensity, the principal spot obtained from *Standard solution B*, corresponding to not more than 0.25% of any individual impurity.

Chlorine content—Dissolve about 300 mg, accurately weighed, in 10 mL of alcohol in a suitable flask. Add 3.5 g of Raney's nickel-aluminum catalyst, and connect to a suitable reflux condenser. Chill the flask in an ice bath, and add through the condenser 75 mL of 2.5 N sodium hydroxide. When the reaction has subsided, remove the ice bath. After 10 minutes, heat the flask gently, gradually increasing the heat until the mixture refluxes rapidly. After 90 minutes from the time of the addition of the alkali, discontinue heating, cool, and rinse the condenser with water, collecting the rinsings in the flask. Transfer the liquid to a 200-mL volumetric flask, wash the residue with water, and add the washing to the volumetric flask. Dilute with water to volume, and mix. Transfer 100.0 mL of this solution to a beaker, neutralize, then acidify (using congo red as the indicator) by adding nitric acid dropwise with mixing. Titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using silver and calomel electrodes. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl: the content of Cl, calculated on the dried basis, is between 20.6% and 21.2%.

Assay—Transfer about 50 mg of Chlorzoxazone, accurately weighed, to a 100-mL volumetric flask. Dissolve in methanol, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Chlorzoxazone RS in methanol at a concentration of about 20 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 282 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_7H_4ClNO_2$ in the Chlorzoxazone taken by the formula:

$$2.5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Chlorzoxazone RS in the Standard solution; and A_U and A_S are the absorbances of the solution of Chlorzoxazone and the Standard solution, respectively.

Chlorzoxazone Tablets

» Chlorzoxazone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_7H_4ClNO_2$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Chlorzoxazone RS

Identification—

A: Disperse a portion of powdered Tablets, equivalent to about 100 mg of chlorzoxazone, in 100 mL of methanol, shake for 15 minutes, and filter. Transfer 2.0 mL of the filtrate to a 100-mL volumetric flask, dilute with methanol to volume, and mix: the UV absorption spectrum of this solution exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Chlorzoxazone RS, concomitantly measured.

B: The chromatogram of the *Assay preparation* obtained in the *Assay* exhibits a major peak for chlorzoxazone, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained in the *Assay*.

Dissolution ⟨711⟩— [NOTE—Use 2-liter vessels for this test.]

Medium: pH 6.8 phosphate buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 1800 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Procedure—Determine the amount of $C_7H_4ClNO_2$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 284 nm on filtered portions of the solution under test, diluted with *Medium*, if necessary, in comparison with a Standard solution of USP Chlorzoxazone RS in the same *Medium*.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_7H_4ClNO_2$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

1% Acetic acid solution—Dilute 10 mL of glacial acetic acid with water to make 1000 mL of solution.

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (70:30:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Prepare a solution of phenacetin in acetonitrile containing about 1.25 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Chlorzoxazone RS quantitatively in *Mobile phase* to obtain a stock solution having a known concentration of about 1.25 mg per mL. Transfer 5.0 mL of this stock solution to a 50-mL volumetric flask containing 10.0 mL of *Internal standard solution*, dilute with *1% Acetic acid solution* to volume, and mix.

Resolution solution—Prepare a solution of *p*-chlorophenol in acetonitrile containing about 8.5 mg per mL. Transfer 1 mL of this solution to a 50-mL volumetric flask containing 4 mL of the stock solution used to prepare the *Standard preparation* and 10 mL of *Internal standard solution*, dilute with *1% Acetic acid solution* to volume, and mix.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 125 mg of chlorzoxazone, to a 100-mL volumetric flask, add about 70 mL of acetonitrile, and shake by mechanical means for about 30 minutes. Dilute with acetonitrile to volume, and mix. Filter a portion of this solution, discarding the first 10 mL of the filtrate. Transfer 5.0 mL of the clear filtrate to a 50-mL volumetric flask containing 10.0 mL of *Internal standard solution*, dilute with *1% Acetic acid solution* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column containing packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as

www.webofpharma.com

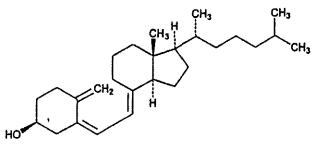
directed under *Procedure*:the relative retention times are about 0.7 for phenacetin, 1.0 for chlorzoxazone, and 1.2 for *p*-chlorophenol; and the resolution, *R*, between the chlorzoxazone peak and the *p*-chlorophenol peak is not less than 2.0. Chromatograph the *Standard preparation*, and record the responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_7H_4ClNO_2$ in the portion of Tablets taken by the formula:

$$1000C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Chlorzoxazone RS in the *Standard preparation*; and R_U and R_S are the peak response ratios of the chlorzoxazone peak to the phenacetin peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cholecalciferol



$C_{27}H_{44}O$ 384.64

9,10-Secocholesta-5,7,10(19)-trien-3-ol, (3β,5*Z*,7*E*)-; Cholecalciferol [67-97-0].

DEFINITION

Cholecalciferol contains NLT 97.0% and NMT 103.0% of cholecalciferol ($C_{27}H_{44}O$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (USP 1-May-2020)
Wavelength range: 2–12 µm
Acceptance criteria: Meets the requirements in the chapter

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (USP 1-May-2020)
Analytical wavelength: 265 nm
Sample solution: 10 µg/mL in alcohol
Acceptance criteria: Meets the requirements in the chapter. Absorptivities do not differ by more than 3.0%.

Change to read:

• **C.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

Delete the following:

▲• **D. THIN-LAYER CHROMATOGRAPHY**
[NOTE—For the *Standard solution* and the *Sample solution*, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and use the solutions immediately.]
Diluent: 10 mg/mL of squalane in chloroform
Standard solution: 50 mg/mL of USP Cholecalciferol RS in *Diluent*
Sample solution: 50 mg/mL of Cholecalciferol in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Cyclohexane and diethyl ether (1:1)
Spray reagent: 20 mg/mL of acetyl chloride in antimony trichloride TS
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Perform the development and subsequent operations in the dark.]
Place the plate in a chamber containing and equilibrated with *Developing solvent system*. Develop until the solvent front has moved about 15 cm above the line of application. Remove the plate, allow the solvent to evaporate, and spray with *Spray reagent*.
Acceptance criteria: The *Sample solution* shows a yellowish-orange area (cholecalciferol) having the same R_F value as the area of the *Standard solution* and may show below the cholecalciferol area a violet area, attributed to 7-dehydrocholesterol.▲ (USP 1-May-2020)

ASSAY

Change to read:

• **PROCEDURE**
▲▲ (USP 1-May-2020)
Mobile phase: *n*-Amyl alcohol in ▲hexane, solvent, chromatographic▲ (USP 1-May-2020) (3 in 1000)
System suitability solution: 250 mg of USP Vitamin D Assay System Suitability RS in 10 mL of a mixture of toluene and *Mobile phase* (1:1). Heat this solution, under reflux, at 90° for 45 min, and cool. [NOTE—This solution contains cholecalciferol, precholecalciferol, and *trans*-cholecalciferol.]
[NOTE—For the stock solutions, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and prepare the solutions fresh daily.]
Standard stock solution: 0.6 mg/mL of USP Cholecalciferol RS in toluene
Standard solution: 120 µg/mL of USP Cholecalciferol RS in *Mobile phase*, prepared from *Standard stock solution*
Sample stock solution: 0.6 mg/mL of Cholecalciferol in toluene
Sample solution: 120 µg/mL of Cholecalciferol in *Mobile phase*, prepared from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L3
▲Flow rate: 1.5 mL/min▲ (USP 1-May-2020)
Injection volume: 5–10 µL

www.webofpharma.com

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for precholecalciferol, *trans*-cholecalciferol, and cholecalciferol are 0.4, 0.5, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.0 between *trans*-cholecalciferol and precholecalciferol
Relative standard deviation: NMT 2.0% for the peak response of cholecalciferol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cholecalciferol ($C_{27}H_{44}O$) in the portion of Cholecalciferol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cholecalciferol RS in the *Standard solution* (µg/mL)
C_U = concentration of Cholecalciferol in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0%

SPECIFIC TESTS
• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 5 mg/mL in alcohol. [NOTE—Prepare and use the solution without delay. Use Cholecalciferol from a container opened not longer than 30 min.]
Acceptance criteria: +105° to +112°

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in hermetically sealed containers under nitrogen, and store in a cool place protected from light.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cholecalciferol RS
USP Vitamin D Assay System Suitability RS

Cholecalciferol Capsules

DEFINITION
Cholecalciferol Capsules contain a solution of Cholecalciferol in an edible oil or other suitable vehicle. Cholecalciferol Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$).

IDENTIFICATION
• **A.** The retention time of the major peak of cholecalciferol of the *Sample solution* corresponds to that of *Standard solution A*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**
[NOTE—Use low-actinic glassware throughout this procedure.]
Mobile phase: *n*-Hexane and isopropyl alcohol (99:1)
System suitability solution: 250 mg of USP Vitamin D Assay System Suitability RS in 10 mL of *n*-hexane. Heat this solution under reflux, at 60° for 1 h, and cool. [NOTE—This solution contains cholecalciferol, precholecalciferol, and *trans*-cholecalciferol.]
Standard stock solution: 50 µg/mL of USP Cholecalciferol RS in *n*-hexane. [NOTE—Prepare solution fresh daily.]
Standard solution A: 5 µg/mL of USP Cholecalciferol RS in *n*-hexane from the *Standard stock solution*
Standard solution B: Transfer a 5-mL volume of the *Standard stock solution* to a container having a polytef-lined screw cap. Displace the air with nitrogen and heat at 60° for 1 h under a nitrogen atmosphere, and cool. Quantitatively transfer the solution to a 50-mL volumetric flask, and dilute with *n*-hexane to volume.
Sample solution: Weigh NLT 30 Capsules in a tared weighing bottle. With a sharp blade or by other appropriate means, carefully open the Capsules, without loss of the shell material, and transfer as much as possible of the combined Capsule contents to a suitable container. Remove any adhering substance from the emptied Capsules and shell remains by washing with several small portions of *n*-hexane. Discard the washings, and allow the empty Capsules and shell remains to dry in a current of dry air until the odor of *n*-hexane is no longer perceptible. Weigh the empty Capsules and shell remains in the original tared weighing bottle, and calculate the average net weight per Capsule by difference. Dissolve a portion of the combined Capsule contents in *n*-hexane to prepare a cholecalciferol solution with a nominal concentration of 5 µg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 15-cm; 3-µm packing L8
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for precholecalciferol, *trans*-cholecalciferol, and cholecalciferol are 0.5, 0.6, and 1.0, respectively.]
Suitability requirements
Resolution: NLT ▲1.0▲ (USP 1-Aug-2019) between *trans*-cholecalciferol and precholecalciferol
Relative standard deviation: NMT 2.0% for cholecalciferol
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Cholecalciferol response factor
Calculate the *Cholecalciferol response factor* (F_C):

$$F_C = C_S/r_S$$

C_S = concentration of USP Cholecalciferol RS in *Standard solution A* (µg/mL)
r_S = peak area of cholecalciferol from *Standard solution A*

Precholecalciferol response factor
Calculate the concentration of cholecalciferol (C'_S), in µg/mL, in *Standard solution B*:

$$C'_S = F_C \times r'_S$$

F_C = *Cholecalciferol response factor*, as previously determined
r'_S = peak area of cholecalciferol from *Standard solution B*

Calculate the concentration of precholecalciferol (C'_{pre}), in µg/mL, in *Standard solution B*:

USP Monographs

www.webofpharma.com

$$C'_{pre} = C_S - C'_S$$

C_S = concentration of USP Cholecalciferol RS in *Standard solution A* (µg/mL)
C'_S = concentration of cholecalciferol in *Standard solution B* (µg/mL)

Calculate the *Precholecalciferol response factor* (F_{pre}):

$$F_{pre} = C'_{pre}/r_p$$

C'_{pre} = concentration of precholecalciferol in *Standard solution B* (µg/mL)
r_p = peak response of precholecalciferol from *Standard solution B*

Vitamin D content

Calculate the percentage of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$) in the portion of Capsules taken:

$$\text{Result} = \{[(F_C \times r_C) + (F_{pre} \times r_{pre})]/C_U\} \times 100$$

F_C = *Cholecalciferol response factor*, as previously determined
r_C = peak area of cholecalciferol from the *Sample solution*
F_{pre} = *Precholecalciferol response factor*, as previously determined
r_{pre} = peak area of precholecalciferol from the *Sample solution*
C_U = nominal concentration of cholecalciferol in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩
Buffer solution: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate and 1.66 mL of glacial acetic acid with water to obtain a 1000-mL solution having a pH of 4.5 ± 0.05
Immersion fluid: *Buffer solution*
Time: 45 min
Acceptance criteria: Meet the requirements
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Change to read:

• **LABELING:** Label the Capsules to indicate the content of cholecalciferol in ▲micrograms. Expression of the amount of cholecalciferol in terms of units may be added in parentheses after the mass units.[1] ▲ (USP 1-Aug-2019)
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cholecalciferol RS
USP Vitamin D Assay System Suitability RS

[1] Where articles are labeled in terms of units in addition to the required labeling, the relationship of the USP Units or International Units (IU) to mass units is as follows: 1 µg of cholecalciferol or ergocalciferol = 40 USP Units or IU.

Cholecalciferol Solution

DEFINITION

Cholecalciferol Solution is a solution of Cholecalciferol in an edible vegetable oil, in Polysorbate 80, or in Propylene Glycol. It contains NLT 90.0% and NMT 120.0% of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$).

ASSAY

• **PROCEDURE**
Mobile phase: Hexane and pentanol (997:3)
Standard stock solution: Dissolve USP Cholecalciferol RS in toluene, and dilute with *Mobile phase* to 50 µg/mL. [NOTE—Prepare this solution fresh daily.]
Standard solution A: 5 µg/mL from *Standard stock solution* in *Mobile phase*. [NOTE—Store at a temperature not above 0°.]
Standard solution B: Transfer 5.0 mL of *Standard stock solution* to a round-bottom flask fitted with a reflux condenser. Displace the air with nitrogen, and reflux for 1 h in a water bath under a nitrogen atmosphere to obtain a solution containing cholecalciferol and precholecalciferol. Cool, transfer the solution with the aid of several portions of *Mobile phase* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: Equivalent to 5 µg/mL of cholecalciferol in *Mobile phase* from an accurately measured volume of Cholecalciferol Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: 2 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution B*
[NOTE—The relative retention times for precholecalciferol and cholecalciferol are about 0.4 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.0 between the precholecalciferol peak and the cholecalciferol peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Cholecalciferol response factor
Calculate the cholecalciferol response factor, F_C:

$$F_C = C_S/r_S$$

C_S = concentration of USP Cholecalciferol RS in *Standard solution A* (µg/mL)
r_S = peak area of cholecalciferol from *Standard solution A*

Pre-cholecalciferol response factor
Calculate the concentration, C'_S, in µg/mL, of cholecalciferol in *Standard solution B*:

$$C'_S = F_C \times r'_S$$

F_C = response factor for cholecalciferol
r'_S = peak area of cholecalciferol from *Standard solution B*

Calculate the concentration, C'_{pre}, in µg/mL, of pre-cholecalciferol:

USP Monographs

www.webofpharma.com

$$C'_{pre} = C_S - C'_S$$

C_S = concentration of USP Cholecalciferol RS in *Standard solution A* (µg/mL)
C'_S = concentration of cholecalciferol in *Standard solution B* (µg/mL)

Calculate the response factor, F_{pre}, for pre-cholecalciferol:

$$F_{pre} = C'_{pre}/r_p$$

C'_{pre} = concentration of pre-cholecalciferol (µg/mL)
r_p = peak response of pre-cholecalciferol from *Standard solution B*

Content of vitamin D

Calculate the percentage of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$) in the portion of the Cholecalciferol Solution taken:

$$\text{Result} = \{[(F_C \times r_C) + (F_{pre} \times r_{pre})]/C_U\} \times 100$$

F_C = response factor for cholecalciferol
r_C = peak area of cholecalciferol from the *Sample solution*
F_{pre} = response factor for pre-cholecalciferol
r_{pre} = peak area of pre-cholecalciferol from the *Sample solution*
C_U = nominal concentration of cholecalciferol in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–120.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label it to indicate the concentration, in mg/mL, of cholecalciferol. Label it also to state that it is to be used for manufacturing only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cholecalciferol RS

Cholecalciferol Tablets

DEFINITION

Cholecalciferol Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of cholecalciferol ($C_{27}H_{44}O$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Use amber, low-actinic glassware.]

Diluent: Water, dimethylformamide, and isopropyl alcohol (5:10:35, v/v)
Mobile phase: Acetonitrile and water (70:30)
Standard stock solution: 0.5 mg/mL of USP Cholecalciferol RS in isopropyl alcohol
Ergocalciferol stock solution: 0.5 mg/mL of USP Ergocalciferol RS in isopropyl alcohol
System suitability solution: Combine equal volumes of *Standard stock solution* and *Ergocalciferol stock solution* and dilute with *Diluent* to obtain a concentration of about 1 µg/mL each of cholecalciferol and ergocalciferol.
Standard solution: 1 µg/mL of USP Cholecalciferol RS in *Diluent* from the *Standard stock solution*, prepared accurately
Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to 50 µg of cholecalciferol, into a 50-mL volumetric flask, add 5 mL of water and sonicate for 10 min with intermittent shaking. Add 10 mL of dimethylformamide, sonicate for 15 min with intermittent shaking, cool to room temperature, and dilute with isopropyl alcohol to volume. Mix well and pass through a 0.45-µm nylon filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 15-cm; 2.7-µm packing L96
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for cholecalciferol and ergocalciferol are 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between the cholecalciferol and ergocalciferol peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cholecalciferol ($C_{27}H_{44}O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of cholecalciferol from the *Sample solution*
r_S = peak area of cholecalciferol from the *Standard solution*
C_S = concentration of cholecalciferol in the *Standard solution* (µg/mL)
C_U = nominal concentration of cholecalciferol in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

[NOTE—Perform this test under light conditions that minimize photodegradation.]

Medium: 0.1% (w/v) octoxynol-9 in water; 500 mL
Apparatus 2: 75 rpm
Time: 30 min
Mobile phase: Acetonitrile and water (70:30)
Standard stock solution: 0.5 mg/mL of USP Cholecalciferol RS in isopropyl alcohol
Standard solution: Dilute *Standard stock solution* with *Medium* to obtain the concentration equivalent to that expected in the *Sample solution*.
Sample solution: Withdraw a portion of the solution under test, pass through a 0.45-µm PVDF filter, and use the pooled sample as the test specimen.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 15-cm; 2.7-µm packing L96
Column temperature: 40°

USP Monographs

www.webofpharma.com

Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cholecalciferol dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times V/L) \times 100$$

r_U = peak area of cholecalciferol from the *Sample solution*
r_S = peak area of cholecalciferol from the *Standard solution*
C_S = concentration of cholecalciferol in the *Standard solution* (µg/mL)
V = volume of *Medium*, 500 mL
L = label claim of cholecalciferol (µg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of cholecalciferol is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **Labeling:** Label the Tablets to indicate the content of cholecalciferol in µg.
• **USP Reference Standards** ⟨11⟩
USP Cholecalciferol RS
USP Ergocalciferol RS

Cholestyramine Resin

Cholestyramine.
Cholestyramine [11041-12-6].

» Cholestyramine Resin is a strongly basic anion-exchange resin in the chloride form, consisting of styrene-divinylbenzene copolymer with quaternary ammonium functional groups. Each g exchanges not less than 1.8 g and not more than 2.2 g of sodium glycocholate, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Cholestyramine Resin RS

Change to read:

Identification—▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
pH ⟨791⟩: between 4.0 and 6.0, in a slurry (1 in 100).
Loss on drying ⟨731⟩—Dry over phosphorus pentoxide at a pressure not exceeding 50 mm of mercury at 70° for 16 hours: it loses not more than 12.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Dialyzable quaternary amines—
pH 9.2 Buffer—Transfer 3.80 g of sodium borate decahydrate to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.
Bromothymol blue solution—Transfer 150 mg of bromothymol blue and 405 mg of sodium carbonate to a 100-mL volumetric flask, dilute with water to volume, and mix.
Standard solution—Take 1 mL of 60% benzyltrimethylammonium chloride solution, accurately pipeted, and dilute quantitatively, and stepwise, with water to obtain a stock solution having a concentration of 0.2 ± 0.01 mg per mL [NOTE—Prepare this solution fresh] . Cut a 20- to 25-cm piece of cellulose dialysis tubing* having a molecular weight cut-off that falls within the 6,000 to 14,000 range and a dry flat width of 5 to 9 cm, and place it in water to hydrate until pliable, appropriately sealing one end. Pipet 5 mL of the stock solution into the tubing, add 5 mL of water, appropriately seal the open end, place the tube in a suitable vessel containing 100 mL of water so that it is completely immersed in the water, and stir the fluid for 16 hours to effect dialysis.
Test solution—Cut a 20- to 25-cm piece of cellulose dialysis tubing* having a molecular weight cut-off that falls within the 6,000 to 14,000 range and a dry flat width of 5 to 9 cm, and place it in water to hydrate until pliable, appropriately sealing one end. Weigh 2 ± 0.01 g of Cholestyramine Resin, and carefully transfer the specimen into the tubing, taking care to ensure that none adheres to the upper walls of the tubing. Add 10 mL of water to the contents of the tube, appropriately seal the open end, and place the tube in a suitable vessel containing 100 mL of water so that it is completely immersed in the water. Stir the fluid for 16 hours to effect dialysis.
Procedure—Pipet the following into each of three separators: Separator 1—5 mL of *Standard solution,* 5 mL of *pH 9.2 Buffer,* 1 mL of *Bromothymol blue solution,* and 10 mL of chloroform; Separator 2—5 mL of *Test solution,* 5 mL of *pH 9.2 Buffer,* 1 mL of *Bromothymol blue solution,* and 10 mL of chloroform; Separator 3—5 mL of water, 5 mL of *pH 9.2 Buffer,* 1 mL of *Bromothymol blue solution,* and 10 mL of chloroform. Shake each separator vigorously for 1 minute, allow the phases to separate until the chloroform phase is clear, and collect the chloroform extracts in separate 25-mL volumetric flasks. Repeat the extraction process with a second 10-mL portion of chloroform, and combine with the previous extracts. Dilute each solution with chloroform to volume, if necessary, and mix. Concomitantly determine the absorbances of the *Test solution* and the *Standard solution* at the wavelength of maximum absorbance at about 420 nm, with a suitable spectrophotometer, using the solution from Separator 3 as the blank: the absorbance of the *Test solution* does not exceed that of the *Standard solution* (0.05% as benzyltrimethylammonium chloride).
Chloride content—To about 750 mg of Cholestyramine Resin, accurately weighed, add 100 mL of water and 50 mg of potassium nitrate. Add, with stirring, 2 mL of nitric acid, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, and using a silver-glass electrode system. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Not less than 13.0% and not more than 17.0% of Cl, calculated on the dried basis, is found.

Exchange capacity—
Mobile phase—Prepare a filtered and degassed mixture of 0.08 M monobasic potassium phosphate and acetonitrile (65:35). Adjust with phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

*A suitable tubing is Spectra/Por 1, Item # 132665, available from Spectrum Laboratories, Inc. (www.spectrapor.com), or equivalent.

USP Monographs

www.webofpharma.com

Potassium phosphate buffer—Transfer about 4 g of monobasic potassium phosphate and about 12 g of dibasic potassium phosphate to a 1-liter volumetric flask. Dissolve in and dilute with water to volume, and mix.

Sodium glycocholate solution—Transfer about 15 g of sodium glycocholate to a 500-mL volumetric flask, and dissolve in and dilute with *Potassium phosphate buffer* to volume.

Reference solution—Pipet 4.0 mL of *Sodium glycocholate solution* into a 100-mL volumetric flask, and dilute with water to volume.

Standard solution—Transfer about 100 mg of USP Cholestyramine Resin RS, accurately weighed, to a 25-mL conical flask. Pipet 15.0 mL of *Sodium glycocholate solution* into the flask, and stir by mechanical means for 2 hours. Transfer the contents to a centrifuge tube, and centrifuge for 15 minutes. Transfer 5.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with water to volume.

System suitability solution—Prepare a solution in water containing, in each mL, about 0.6 mg of sodium glycocholate and about 0.3 mg of taurodeoxycholic acid.

Test solution—Transfer about 100 mg of anhydrous Cholestyramine Resin, accurately weighed, to a 25-mL conical flask. Pipet 15.0 mL of *Sodium glycocholate solution* into the flask, and stir by mechanical means for 2 hours. Transfer the contents to a centrifuge tube, and centrifuge for 15 minutes. Transfer 5.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with water to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between sodium glycocholate and taurodeoxycholic acid is not less than 1.5. Chromatograph the *Reference solution*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Reference solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of sodium glycocholate absorbed on each g of the Resin taken by the formula:

$$M(2.5r_R - r_U)W_S/(2.5r_R - r_S)W_U$$

in which M is the stated value, in mg, of sodium glycocholate absorbed per g of USP Cholestyramine Resin RS; r_R, r_U, and r_S are the peak responses obtained from the *Reference solution*, the *Test solution*, and the *Standard solution*, respectively; W_U is the weight, in mg, of Cholestyramine Resin, calculated on the dried basis, taken to prepare the *Test solution;* and W_S is the weight, in mg, of USP Cholestyramine Resin RS taken to prepare the *Standard solution.*

Cholestyramine for Oral Suspension

» Cholestyramine for Oral Suspension is a mixture of Cholestyramine Resin with suitable excipients and coloring and flavoring agents. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of dried cholestyramine resin.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Cholestyramine Resin RS

Identification—Transfer a quantity of Cholestyramine for Oral Suspension, equivalent to about 500 mg of dried cholestyramine resin, to a suitable flask, add 100 mL of 0.1 N hydrochloric acid, stir to suspend the solid, and heat on a steam bath for 10 minutes. Filter, wash the residue with three 50-mL portions of water, and dry at 70° and at a pressure not exceeding 50 mm of mercury for 16 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Cholestyramine Resin RS.

Uniformity of dosage units ⟨905⟩: meets the requirements for *Weight Variation.*

Assay—

Mobile phase, Potassium phosphate buffer, Sodium glycocholate solution, Reference solution, Standard solution, System suitability solution, and *Chromatographic system*—Proceed as directed in the test for *Exchange capacity* under *Cholestyramine Resin.*

Test solution—Transfer an accurately weighed portion of Cholestyramine for Oral Suspension, equivalent to about 100 mg of cholestyramine resin, to a 25-mL conical flask. Pipet 15.0 mL of *Sodium glycocholate solution* into the flask, and stir by mechanical means for 2 hours. Transfer the contents to a centrifuge tube, and centrifuge for 15 minutes. Transfer 5.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with water to volume.

Procedure—Separately inject equal volumes (about 50 µL) of the *Reference solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cholestyramine resin per mg of Cholestyramine for Oral Suspension taken by the formula:

$$[M(2.5r_R - r_U)W_S] / [(2.5r_R - r_S)W_UQ]$$

in which M is the stated value, in mg, of sodium glycocholate absorbed per g of USP Cholestyramine Resin RS; r_R, r_U, and r_S are the peak responses obtained from the *Reference solution*, the *Test solution*, and the *Standard solution*, respectively; W_S is the weight, in mg, of USP Cholestyramine Resin RS taken to prepare the *Standard solution;* W_U is the weight, in mg, of Cholestyramine for Oral Suspension taken to prepare the *Test solution;* and Q is the quantity of sodium glycocholate absorbed per g of dried cholestyramine resin, as obtained in the test for *Exchange capacity* under *Cholestyramine Resin.*

Chromic Chloride

$CrCl_3 \cdot 6H_2O$ 266.45
Chromium chloride ($CrCl_3$) hexahydrate;
Chromium(3+) chloride hexahydrate [10060-12-5].

$CrCl_3$ 158.36
Anhydrous [10025-73-7].

DEFINITION

Chromic Chloride contains NLT 98.0% and NMT 101.0% of $CrCl_3 \cdot 6H_2O$.

www.webofpharma.com

IDENTIFICATION

• **A.**

Analysis: To 5 mL of a solution (1 in 250) in a test tube, add 1 mL of 5 N sodium hydroxide and 10 drops of 30% hydrogen peroxide, and heat gently for about 2 min.

Acceptance criteria: A yellow color develops.

• **B.**

Analysis: To 5 mL of a solution (1 in 250) in a test tube, add 5 drops of silver nitrate TS.

Acceptance criteria: A white, curdy precipitate that is insoluble in nitric acid is formed.

ASSAY

• **PROCEDURE**

Sample solution: Dissolve 0.4 g of Chromic Chloride in 100 mL of water contained in a glass-stoppered, 500-mL conical flask. Add 5 mL of 5 N sodium hydroxide, and mix. Pipet slowly 4 mL of 30% hydrogen peroxide into the flask, and boil the solution for 5 min. Cool the solution slightly, and add 5 mL of nickel sulfate solution (1 in 20). Boil the solution until no more oxygen is evolved. Cool, and add 2 N sulfuric acid dropwise until the color of the solution changes from yellow to orange. Add to the flask a freshly prepared solution of 4 g of potassium iodide and 2 g of sodium bicarbonate in 100 mL of water, then add 6 mL of hydrochloric acid. Immediately insert the stopper in the flask, and allow to stand in the dark for 10 min. Rinse the stopper and the sides of the flask with a few mL of water.

Analysis: Titrate the liberated iodine with 0.1 N sodium thiosulfate VS to an orange color. Add 3 mL of starch TS, and continue the titration to a blue-green endpoint. Each mL of 0.1 N sodium thiosulfate is equivalent to 8.882 mg of chromium chloride hexahydrate ($CrCl_3 \cdot 6H_2O$).

Acceptance criteria: 98.0%–101.0%

IMPURITIES

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Sample solution: Dissolve 2.0 g of Chromic Chloride in 10 mL of water. Add 1 mL of 3 N hydrochloric acid, and filter if necessary to obtain a clear solution. Wash the filter with two 5-mL portions of water, and dilute with water to 40 mL.

Control solution: Prepare as directed in the *Sample solution*, but use 1.0 g of the substance under test. After the filtration step, add 0.10 mL of 0.020 N sulfuric acid.

Analysis: To each solution add 3 mL of barium chloride TS, mix, and allow to stand overnight. Decant most of the supernatants, without disturbing the precipitates, but leaving twice the volume of liquid in the *Control solution* as in the *Sample solution*. Dilute each solution with water to 25 mL, and sonicate for 1 min.

Acceptance criteria: Any turbidity in the *Sample solution* does not exceed that in the *Control solution* (0.01%).

• **IRON** ⟨241⟩

Test preparation: Dissolve 1.0 g of Chromic Chloride in 100 mL of water. Transfer 10 mL of this solution to a 100-mL color comparison tube. Dilute with water to 45 mL, add 2 mL of hydrochloric acid, and mix.

Analysis: Proceed as directed for *Procedure*, except add 15 mL of butyl alcohol to the *Test preparation* and the *Standard Preparation* at the same time that the *Ammonium Thiocyanate Solution* is added. Shake for 30 s, and allow the layers to separate.

Acceptance criteria: The color in the upper butyl alcohol layer from the *Test preparation* is not darker than that from the *Standard Preparation* (NMT 0.01%).

SPECIFIC TESTS

• **INSOLUBLE MATTER**

Sample: 10 g

Analysis: Transfer the *Sample* to a 250-mL beaker. Add 100 mL of water, cover the beaker, and heat to boiling. Digest the hot solution on a steam bath for 30 min, and filter through a tared filtering crucible of fine porosity. Rinse the beaker with hot water, passing the rinsings through the filter, and wash the filter with hot water until the last washing is colorless. Dry the filter at 105°.

Acceptance criteria: The weight of the residue does not exceed 1 mg (0.01%).

• **SUBSTANCES NOT PRECIPITATED BY AMMONIUM HYDROXIDE**

Sample: 2.0 g

Analysis: Dissolve the *Sample* in 80 mL of water, heat the solution to boiling, and add a slight excess of ammonium hydroxide. Continue heating to remove the excess ammonia. Cool, dilute with water to 100.0 mL, and mix. Pass through a retentive filter, and transfer 50.0 mL of the clear filtrate to an evaporating dish that previously has been ignited and tared. Add 0.5 mL of sulfuric acid to the filtrate, and evaporate on a steam bath to dryness. Heat gently to remove the excess acid, and ignite gently.

Acceptance criteria: The weight of the residue does not exceed 2.0 mg (0.20% as sulfate).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Chromic Chloride Injection

» Chromic Chloride Injection is a sterile solution of Chromic Chloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chromium (Cr).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.

Labeling—Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.

Identification—The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 360 nm when tested as directed for *Procedure* in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 16.70 USP Endotoxin Units per µg of chromium.

pH ⟨791⟩: between 1.5 and 2.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Sodium chloride solution—Dissolve 54 g of sodium chloride in water, dilute with water to 2000 mL, and mix.

Chromium stock solution—Transfer 2.829 g of potassium dichromate, accurately weighed, to a 1000-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. This solution contains 1000 µg of chromium per mL. Store in a polyethylene bottle.

Standard preparations—Pipet 10 mL of the *Chromium stock solution* into a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL and 20.0 mL, respectively, of this stock solution to separate 100-mL volumetric flasks, and transfer 15.0 mL and 20.0 mL, respectively, of the stock solution to separate 50-mL volumetric flasks. Add 20 mL of *Sodium chloride solution* to each 100-mL volumetric flask, and 10 mL of *Sodium chloride solution* to each 50-mL volumetric flask, dilute the contents of each flask with water to volume,

www.webofpharma.com

and mix. These *Standard preparations* contain, respectively, 1.0, 2.0, 3.0, and 4.0 µg of chromium per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 60 µg of chromium, to a 25-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the Injection, calculate the amount, in mg, of sodium chloride in the volume of Injection taken, and add sufficient *Sodium chloride solution* to bring the total sodium chloride content of the flask to 135 mg. Dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the chromium emission line of 357.9 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a chromium hollow-cathode lamp and an air–acetylene flame, using a 1:5 dilution of the *Sodium chloride solution* as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of chromium, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, in µg per mL, of chromium in the *Assay preparation*. Calculate the quantity, in µg, of chromium in each mL of the Injection taken by the formula:

$$25C/V$$

in which C is the concentration, in µg per mL, of chromium in the *Assay preparation*, and V is the volume, in mL, of Injection taken.

Sodium Chromate Cr 51 Injection

Chromic acid ($H_2{}^{51}CrO_4$), disodium salt.
Disodium chromate ($Na_2{}^{51}CrO_4$) [7775-11-3].

» Sodium Chromate Cr 51 Injection is a sterile solution of radioactive chromium (^{51}Cr) processed in the form of sodium chromate in Water for Injection. For those uses where an isotonic solution is required, Sodium Chloride may be added in appropriate amounts as provided under *Injections and Implanted Drug Products* ⟨1⟩. Chromium 51 is produced by the neutron bombardment of enriched chromium 50.

Sodium Chromate Cr 51 Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{51}Cr as sodium chromate expressed in megabecquerels (millicuries) per mL at the time indicated in the labeling. The sodium chromate content is not less than 90.0 percent and not more than 110.0 percent of the labeled amount. The specific activity is not less than 370 megabecquerels (10 millicuries) per mg of sodium chromate at the end of the expiry period. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose or in multiple-dose containers.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of sodium chromate expressed in µg per mL; the amount of ^{51}Cr as sodium chromate expressed as total megabecquerels (millicuries) and as megabecquerels (millicuries) per mL at the time of calibration; a statement to indicate whether the contents are intended for diagnostic or therapeutic use; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay and the quantity of chromium, and also indicates that the radioactive half-life of ^{51}Cr is 27.8 days.

Radionuclide identification (see *Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of ^{51}Cr of known purity that exhibits a photopeak having an energy of 0.320 MeV.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than $175/V$ USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

pH ⟨791⟩: between 7.5 and 8.5.

Radiochemical purity—Place a volume of Injection, appropriately diluted so that it provides a count rate of about 20,000 counts per minute, about 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* ⟨621⟩), and immediately develop with a mixture of 5 parts of water, 2 parts of dilute alcohol (9.5 in 10), and 1 part of ammonium hydroxide. Air-dry the chromatogram, and determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. The radioactivity of the chromate band is not less than 90.0% of the total radioactivity. The R_F value for the chromate band falls within ±10% of the value found for a known sodium chromate specimen when determined under identical conditions.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩; not subject to *Container content*.

Assay for sodium chromate—

Standard stock preparation—Dissolve 3.735 g of potassium chromate in 1000 mL of water to obtain a solution having a known concentration of 1.0 mg per mL of chromium.

Standard preparation—Pipet 0.25, 0.50, 0.75, 0.100, 0.125, and 0.150 mL of the *Standard stock preparation*, accurately measured, into separate 100-mL volumetric flasks. To each flask add 0.42 mL of 0.1 N sodium bicarbonate, and dilute with water to volume to obtain solutions having final concentrations of 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 µg of chromium per mL.

Assay preparation—Use the Injection.

Blank preparation—Transfer 0.42 mL of 0.1 N sodium bicarbonate to a 100-mL volumetric flask, and dilute with water to volume.

Procedure—Concomitantly determine the absorbances of the *Assay preparation*, the *Standard preparations*, and the *Blank preparation* at the chromium emission line at 357.7 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a chromium hollow-cathode lamp and an air–acetylene (fuel-rich) flame using water to set the instrument to zero. Plot the absorbances of the *Standard preparations* and the *Blank preparation* versus concentration, in µg per mL, of chromium, and perform a regression analysis. A suitable standard curve will have an intercept between –0.002 and +0.002, and a regression coefficient of not less than 0.99. Using the standard curve so obtained, determine the concentration, C, in µg per mL, of

www.webofpharma.com

chromium in the Injection taken. Calculate the quantity of sodium chromate, in µg per mL, by the formula:

$$3.115C$$

in which 3.115 is the conversion factor.

Assay for radioactivity—Using a suitable counting assembly, determine the radioactivity, in MBq (µCi) per mL, of Injection by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

Chymotrypsin

Chymotrypsin [9004-07-3].

DEFINITION

Chymotrypsin is a proteolytic enzyme crystallized from an extract of the pancreas gland of the ox, *Bos taurus* L. (Fam. Bovidae). It contains NLT 1000 USP Chymotrypsin Units/mg, calculated on the dried basis, and NLT 90.0% and NMT 110.0% of the labeled potency, as determined by the *Assay*.

ASSAY

• **PROCEDURE**

Monobasic potassium phosphate solution: 9.08 mg/mL of monobasic potassium phosphate in water

Dibasic sodium phosphate solution: 9.46 mg/mL of anhydrous dibasic sodium phosphate in water

Phosphate buffer: Mix 38.9 mL of *Monobasic potassium phosphate solution* and 61.1 mL of *Dibasic sodium phosphate solution*. If necessary, adjust by the dropwise addition of *Dibasic sodium phosphate solution* to a pH of 7.0.

Substrate solution: Dissolve 23.7 mg of *N*-acetyl-L-tyrosine ethyl ester, suitable for use in assaying Chymotrypsin, in 50 mL of *Phosphate buffer*, with warming. When the solution is cool, dilute with additional *Phosphate buffer* to 100 mL. [NOTE—*Substrate solution* may be stored in the frozen state and used after thawing, but it is important to freeze it immediately after preparation.]

Sample solution: Dissolve a quantity of Chymotrypsin in 0.0012 N hydrochloric acid to yield a solution containing 12–16 USP Chymotrypsin Units/mL. The dilution is correct if, during the conduct of the *Assay*, there is a change in absorbance of between 0.008 and 0.012 in each 30-s interval.

Blank solution: Mix 0.2 mL of 0.0012 N hydrochloric acid and 3 mL of water.

Analysis

Samples: *Substrate solution*, *Sample solution*, and *Blank solution*

[NOTE—Determine the suitability of the substrate and check the adjustment of the spectrophotometer by performing the *Analysis* using USP Chymotrypsin RS in place of the *Sample solution*.]

Conduct the *Assay* in a suitable spectrophotometer equipped to maintain a temperature of 25 ± 1.0° in the cell compartment. Determine the temperature in the reaction cell before and after the absorbance measurement to ensure that the temperature does not change by more than 1.0°. Pipet 3.0 mL of *Blank solution* into a 1-cm cell. Place the cell in the spectrophotometer, and adjust the instrument so that the absorbance will read 0.00 at 237 nm. Pipet 0.2 mL of *Sample solution* into another 1-cm cell, add 3 mL of *Substrate solution*, and place the cell in the spectrophotometer. [NOTE—Carefully follow this order of addition, and begin timing the reaction from the addition of the *Substrate solution*.] Read the absorbance at 30-s intervals for NLT 5 min. Repeat the procedure on the same dilution at least once. Absolute absorbance values are less important than a constant rate of absorbance change. If the rate of change fails to remain constant for NLT 3 min, repeat the test and, if necessary, use a lower concentration. The duplicate determination of the *Sample solution* matches the first determination, of the same dilution, in rate of absorbance change.

Determine the average absorbance change per minute, using only the values within the 3-min portion of the curve where the rate of absorbance change is constant. Plot a curve of absorbance against time. One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075/min under the conditions specified in the *Assay*.

Calculate the number of USP Chymotrypsin Units/mg in the portion of Chymotrypsin taken:

$$\text{Result} = (A_2 - A_1)/(T \times W \times F)$$

A_2 = absorbance straight-line initial reading
A_1 = absorbance straight-line final reading
T = time elapsed between the initial and final readings (min)
W = weight of Chymotrypsin in the volume of solution used in determining the absorbance (mg)
F = Chymotrypsin activity conversion factor, 0.0075/min

Acceptance criteria: NLT 1000 USP Chymotrypsin Units/mg on the dried basis; 90.0%–110.0% of the labeled potency

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 2.5%

• **LIMIT OF TRYPSIN**

Tris buffer: Dissolve 294 mg of calcium chloride in 40 mL of 0.20 M tris(hydroxymethyl)aminomethane. Adjust with 1 N hydrochloric acid to a pH of 8.1, and dilute with water to 100 mL.

Substrate solution: Transfer 98.5 mg of *p*-toluenesulfonyl-L-arginine methyl ester hydrochloride, suitable for use in assaying trypsin, to a 25-mL volumetric flask. Add 5 mL of *Tris buffer*, and swirl until the substrate dissolves. Add 0.25 mL of methyl red–methylene blue TS, and dilute with water to volume.

Sample solution: 10 mg/mL of Chymotrypsin in water

Analysis

[NOTE—Determine the suitability of the substrate by performing the *Analysis* using the appropriate amount of USP Trypsin Crystallized RS in place of the *Sample solution*.]

By means of a micropipet, transfer 50 µL of *Sample solution* to a depression on a white spot plate. Add 0.2 mL of *Substrate solution*.

Acceptance criteria: No purple color develops within 3 min (NMT 1% of trypsin).

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the tests for absence of *Pseudomonas aeruginosa*, *Salmonella* species, and *Staphylococcus aureus*.

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid exposure to excessive heat.

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Chymotrypsin RS
USP Trypsin Crystallized RS

Chymotrypsin for Ophthalmic Solution

DEFINITION
Chymotrypsin for Ophthalmic Solution is sterile Chymotrypsin. When constituted as directed in the labeling, it yields a solution containing NLT 80.0% and NMT 120.0% of the labeled potency.

IDENTIFICATION
• **A.**
Monobasic potassium phosphate solution: 9.08 mg/mL of monobasic potassium phosphate in water
Dibasic sodium phosphate solution: 9.46 mg/mL of anhydrous dibasic sodium phosphate in water
Phosphate buffer: Mix 38.9 mL of *Monobasic potassium phosphate solution* and 61.1 mL of *Dibasic sodium phosphate solution*. If necessary, adjust by the dropwise addition of *Dibasic sodium phosphate solution* to a pH of 7.0.
Substrate solution: Transfer 237.0 mg of *N*-acetyl-L-tyrosine ethyl ester, suitable for use in assaying chymotrypsin, to a 100-mL volumetric flask, add 2 mL of alcohol, and swirl until solution is effected. Add 20 mL of *Phosphate buffer*, 1 mL of methyl red–methylene blue TS, and dilute with water to volume. If necessary, adjust by the dropwise addition of *Monobasic potassium phosphate solution* to a pH of 7.0.
Sample solution: Dissolve the contents of 1 vial of Chymotrypsin for Ophthalmic Solution in 1 mL of saline TS.
Analysis: Transfer 0.2 mL of the *Sample solution* to a suitable dish, and add 0.2 mL of the *Substrate solution*.
Acceptance criteria: A purple color is produced within 3 min.
[NOTE—This is distinct from trypsin, which produces no purple color within 3 min.]

ASSAY
• **PROCEDURE**
Monobasic potassium phosphate solution: 9.08 mg/mL of monobasic potassium phosphate in water
Dibasic sodium phosphate solution: 9.46 mg/mL of anhydrous dibasic sodium phosphate in water
Phosphate buffer: *Monobasic potassium phosphate solution* and *Dibasic sodium phosphate solution* (38.9: 61.1). If necessary, adjust by the dropwise addition of *Dibasic sodium phosphate solution* to a pH of 7.0.
Substrate solution: Dissolve 23.7 mg of *N*-acetyl-L-tyrosine ethyl ester, suitable for use in assaying chymotrypsin, in 50 mL of *Phosphate buffer*, with warming. When the solution is cool, dilute with additional *Phosphate buffer* to 100 mL. [NOTE—*Substrate solution* may be stored in the frozen state and used after thawing, but it is important to freeze it immediately after preparation.]
Sample stock solution: Dissolve the contents of 1 vial of Chymotrypsin for Ophthalmic Solution in 5.0 mL of 0.0012 N hydrochloric acid.
Sample solution: Dilute a volume (V_2, in mL) of the *Sample stock solution*, equivalent to 300 USP Chymotrypsin Units, with 0.0012 N hydrochloric acid to 25.0 mL. The dilution is correct if, during the conduct of the *Assay*, there is a change in absorbance of between 0.008 and 0.012 in each 30-s interval.
Blank solution: Mix 0.2 mL of 0.0012 N hydrochloric acid and 3 mL of water.

Analysis
Samples: *Substrate solution*, *Sample stock solution*, *Sample solution*, and *Blank solution*
[NOTE—Determine the suitability of the substrate and check the adjustment of the spectrophotometer by performing the *Analysis* using USP Chymotrypsin RS in place of the *Sample solution*.]
Conduct the *Assay* in a suitable spectrophotometer equipped to maintain a temperature of 25 ± 1.0° in the cell compartment. Determine the temperature in the reaction cell before and after the absorbance measurement to ensure that the temperature does not change by more than 1.0°. Pipet 3.0 mL of *Blank solution* into a 1-cm cell. Place the cell in the spectrophotometer, and adjust the instrument so that the absorbance will read 0.00 at 237 nm. Pipet 0.2 mL of *Sample solution* into another 1-cm cell, add 3 mL of *Substrate solution*, and place the cell in the spectrophotometer. [NOTE—Carefully follow this order of addition, and begin timing the reaction from the addition of the *Substrate solution*.] Read the absorbance at 30-s intervals for NLT 5 min. Repeat the procedure on the same dilution at least once. Absolute absorbance values are less important than a constant rate of absorbance change. If the rate of change fails to remain constant for NLT 3 min, repeat the test and, if necessary, use a lower concentration. The duplicate determination at the same dilution matches the first determination in rate of absorbance change.
Determine the average absorbance change per minute, using only the values within the 3-min portion of the curve where the rate of absorbance change is constant. Plot a curve of absorbance against time. One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075/min under the conditions specified in the *Assay*.
Calculate the percentage of the labeled potency of USP Chymotrypsin Units in a vial:

$$\text{Result} = [F_1 \times (V_1/V_2) \times (A_2 - A_1)]/(T \times F_2 \times F_3)$$

F_1 = total USP Chymotrypsin Units in the *Sample solution*, 300
V_1 = volume of the *Sample stock solution*, 5 mL
V_2 = volume as defined in the *Sample solution* (mL)
A_2 = absorbance straight-line initial reading
A_1 = absorbance straight-line final reading
T = time elapsed between the initial and final readings (min)
F_2 = number of USP Chymotrypsin Units in the solution on which the absorbance was determined, 2.4
F_3 = chymotrypsin activity conversion factor, 0.0075/min

Acceptance criteria: 80.0%–120.0% of the labeled potency

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Analysis: Assay 10 individual units as directed in the *Assay*, and calculate the average of the 10 results.
Acceptance criteria: Meets the requirements of the chapter and the average is 80.0%–120.0% of the labeled potency. The contents of NMT 2 vials deviate by more than 10% from the average content. The contents of none of the vials deviate by more than 15% from the average.

IMPURITIES
• **LIMIT OF TRYPSIN**
Tris buffer: Dissolve 294 mg of calcium chloride in 40 mL of 0.20 M tris(hydroxymethyl)aminomethane. Adjust with

www.webofpharma.com

1 N hydrochloric acid to a pH of 8.1, and dilute with water to 100 mL.

Substrate solution: Transfer 98.5 mg of *p*-toluenesulfonyl-L-arginine methyl ester hydrochloride, suitable for use in assaying trypsin, to a 25-mL volumetric flask. Add 5 mL of *Tris buffer*, and swirl until the substrate dissolves. Add 0.25 mL of methyl red–methylene blue TS, and dilute with water to volume.

Sample solution: 10 mg/mL of Chymotrypsin for Ophthalmic Solution

Analysis

[NOTE—Determine the suitability of the substrate by performing the *Analysis* using the appropriate amount of USP Trypsin Crystallized RS in place of the *Sample solution*.]

By means of a micropipet, transfer 50 µL of the *Sample solution* to a depression on a white spot plate. Add 0.2 mL of the *Substrate solution*.

Acceptance criteria: No purple color develops within 3 min (NMT 1% of trypsin).

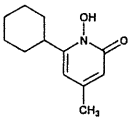
SPECIFIC TESTS

- **pH** ⟨791⟩: 4.3–8.7, in the solution constituted as directed in the labeling
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **COMPLETENESS OF SOLUTION** ⟨641⟩: It dissolves in the solvent and in the concentration recommended in the labeling to yield a clear solution.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Chymotrypsin RS
  USP Trypsin Crystallized RS

Ciclopirox



$C_{12}H_{17}NO_2$ 207.27

2(1*H*)-Pyridinone, 6-cyclohexyl-1-hydroxy-4-methyl-.

6-Cyclohexyl-1-hydroxy-4-methyl-2(1*H*)-pyridone [29342-05-0].

» Ciclopirox contains not less than 98.0 percent and not more than 101.0 percent of $C_{12}H_{17}NO_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, protected from light. Store at a temperature between 15° and 30°.

USP Reference standards ⟨11⟩—

USP Ciclopirox RS

USP Ciclopirox Related Compound A RS
3-Cyclohexyl-4,5-dihydro-5-methyl-5-isoxazolyl acetic acid.

USP Ciclopirox Related Compound B RS
6-Cyclohexyl-4-methyl-2-pyrone.

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy:* ***197K***▲ (CN 1-May-2020)

Loss on drying ⟨731⟩—Dry it in vacuum to constant weight: it loses not more than 1.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds— [NOTE—Carry out the operations avoiding exposure to actinic light. All materials in direct connection with Ciclopirox, like column materials, reagents, solvents, and others should contain only very low amounts of extractable metal cations.]

Mobile phase—Prepare a filtered and degassed mixture of an edetate disodium solution (0.96 in 1000), acetonitrile, and glacial acetic acid (770: 230: 0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Rinsing solution—Prepare a mixture of water, acetonitrile, glacial acetic acid, and acetylacetone (500:500:1:1).

Standard stock solution—Dissolve USP Ciclopirox Related Compound A RS and USP Ciclopirox Related Compound B RS, accurately weighed, in an appropriate volume of acetonitrile and *Mobile phase* solution (approximate ratio, 1:7). Further dilute with *Mobile phase* to obtain a solution having a known final concentration of about 1.5 mg each per mL.

Standard solution A—Dilute 1.0 mL of *Standard stock solution* to 200.0 mL with a mixture of *Mobile phase* and acetonitrile (9:1).

Standard solution B—Dilute 2.0 mL of *Standard solution A* to 10.0 mL with a mixture of *Mobile phase* and acetonitrile (9:1).

Test solution—Dissolve 30 mg of Ciclopirox, accurately weighed, in a mixture of 2 mL of acetonitrile and 15 mL of *Mobile phase*. If necessary, use an ultrasonic bath. Dilute with *Mobile phase* to 20.0 mL.

Resolution solution—Mix 5 mL of *Standard stock solution* with 5 mL of the *Test solution*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a detector capable of recording at both 220 nm and 298 nm and a 4.0-mm × 8-cm column that contains packing L10. [NOTE—Ciclopirox related compound A has an intense absorbance at 220 nm, and 6-cyclohexyl-4-methyl-2(1*H*)-pyridone, ciclopirox related compound B, and ciclopirox have intense absorbances at 298 nm.] The flow rate is about 0.7 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure* at 298 nm: the resolution, *R*, between the ciclopirox related compound B peak and ciclopirox peak is not less than 2.0. Chromatograph the *Standard solution B*, and record the peak responses as directed for *Procedure* at 298 nm: the chromatogram obtained shows at 298 nm a peak corresponding to ciclopirox related compound B with a signal-to-noise ratio of not less than 3. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure* at 298 nm: the tailing factor for the ciclopirox peak is less than 2.0.

Procedure—Separately inject equal volumes (about 10 µL) of *Standard solution A*, *Standard solution B*, and the *Test solution* into the chromatograph, and record the chromatograms. [NOTE—In order to ensure desorption of disruptive metal ions, every new column must be rinsed with the *Rinsing solution* over a period of not less than 15 hours and then with the *Mobile phase* for not less than 5 hours with a flow rate of 0.2 mL per minute. The chromatographic run time is not less than 2.5 times the retention time of the ciclopirox peak.] The relative retention times are about 0.5 for ciclopirox related compound A, 0.9 for 6-cyclohexyl-4-methyl-2(1*H*)-pyridone, 1.0 for ciclopirox, and 1.3 for ciclopirox

www.webofpharma.com

related compound B. The peak response at 220 nm of the ciclopirox related compound A peak in the chromatogram obtained from the *Test solution* is not more than the peak response at 220 nm of the corresponding peak in the chromatogram obtained from *Standard solution A* (0.5%). The sum of responses at 298 nm of the peaks in the chromatogram obtained from the *Test solution* is not more than the peak response at 298 nm of the ciclopirox related compound B peak in the chromatogram obtained from *Standard solution A* (0.5%). At 298 nm disregard any peak due to the solvent and any peak with a response less than the response of the ciclopirox related compound B peak in the chromatogram obtained from *Standard solution B* at 298 nm (0.1%).

Assay—Dissolve 150 mg of Ciclopirox, accurately weighed, in 20 mL of methanol. Add 20 mL of water, mix, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Carry out a blank test. Determine the factor of the 0.1 N sodium hydroxide VS using 100 mg of benzoic acid, accurately weighed, and titrate under the conditions prescribed above. Each mL of 0.1 N sodium hydroxide is equivalent to 20.73 mg of $C_{12}H_{17}NO_2$.

Ciclopirox Topical Solution

DEFINITION

Ciclopirox Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of ciclopirox ($C_{12}H_{17}NO_2$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

[NOTE—Protect the *Standard solution* and *Sample solution* from light.]

Buffer: Transfer 5.25 g of citric acid and 25 mL of 0.1 M edetate disodium to a 1-L volumetric flask, and dilute with water to volume. Adjust with 8.5% diluted sodium hydroxide solution to a pH of 6.5.

Mobile phase: Acetonitrile and *Buffer* (35:65)

Standard solution: 0.2 mg/mL of USP Ciclopirox RS and 1 µg/mL each of USP Ciclopirox Related Compound B RS and USP Ciclopirox Related Compound C RS in methanol

Sample solution: Equivalent to 0.2 mg/mL of ciclopirox in methanol from Topical Solution. Pass through a filter of 0.45-µm pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 303 nm

Column: 4-mm × 12.5-cm; 5-µm packing L1

Column temperature: 30 ± 5°

Flow rate: 0.9 mL/min

Run time: 5 times the retention time of the major peak

Injection size: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—For information only, see *Table 1* for relative retention times of impurities.]

Suitability requirements

Resolution: NLT 3.0 between ciclopirox and ciclopirox related compound B; and NLT 3.0 between ciclopirox related compound C and ciclopirox

Tailing factor: NMT 2.0 for the ciclopirox peak

Relative standard deviation: NMT 2.0% for the ciclopirox peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ciclopirox ($C_{12}H_{17}NO_2$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ciclopirox RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ciclopirox in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩: Meets the requirement

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_T = sum of responses of all the peaks in the *Sample solution*

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Compound	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ciclopirox related compound C	0.54	1.3	0.5
Ciclopirox	1.0	—	—
Ciclopirox related compound B[a]	1.87	—	—
Any unspecified individual impurity	—	1.0	0.2
Total impurities	—	—	1.2

[a] Process impurity already monitored in the drug substance.

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/g, and the total combined molds and yeasts count does not exceed 10^1 cfu/g.

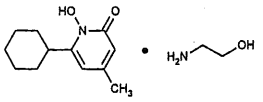
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ciclopirox RS
USP Ciclopirox Related Compound B RS
6-Cyclohexyl-4-methyl-2-pyrone.
$C_{12}H_{16}O_2$ 192.25
USP Ciclopirox Related Compound C RS
6-Cyclohexyl-4-methylpyridin-2(1*H*)-one.
$C_{12}H_{17}NO$ 191.27

Ciclopirox Olamine



$C_{12}H_{17}NO_2 \cdot C_2H_7NO$ 268.35
2(1*H*)-Pyridinone, 6-cyclohexyl-1-hydroxy-4-methyl-, compound with 2-aminoethanol (1:1).
6-Cyclohexyl-1-hydroxy-4-methyl-2(1*H*)-pyridone compound with 2-aminoethanol (1:1) [41621-49-2].

» Ciclopirox Olamine contains not less than 97.5 percent and not more than 101.5 percent of ciclopirox olamine ($C_{12}H_{17}NO_2 \cdot C_2H_7NO$).

Packaging and storage—Preserve in tight containers, protected from light. Store between 5° and 25°.
USP Reference standards ⟨11⟩—
USP Ciclopirox Olamine RS
USP Ciclopirox Related Compound A RS
3-Cyclohexyl-4,5-dihydro-5-methyl-5-isoxazolyl acetic acid.
USP Ciclopirox Related Compound B RS
6-Cyclohexyl-4-methyl-2-pyrone.

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy:* ***197K***▲ (CN 1-May-2020)
pH ⟨791⟩**:** between 8.0 and 9.0, in a mixture with water (1:100).
Residue on ignition ⟨281⟩**:** not more than 0.1%.
Monoethanolamine content—Dissolve about 300 mg, accurately weighed, in 25 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 6.108 mg of C_2H_7NO. The content of monoethanolamine (C_2H_7NO) is not less than 223 mg and not more than 230 mg per g of ciclopirox olamine ($C_{12}H_{17}NO_2 \cdot C_2H_7NO$) found in the *Assay*.
Related compounds— [NOTE—Carry out the operations avoiding exposure to actinic light. All materials that are in direct contact with Ciclopirox Olamine (e.g., column materials, reagents, solvents, etc.) should contain only very low amounts of extractable metal cations.]
Mobile phase—Prepare a filtered and degassed mixture of an edetate disodium solution (0.96 in 1000), acetonitrile, and glacial acetic acid (770: 230: 0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Rinsing solution—Prepare a mixture of water, acetonitrile, glacial acetic acid, and acetylacetone (500:500:1:1).
Standard stock solution—Dissolve USP Ciclopirox Related Compound A RS and USP Ciclopirox Related Compound B RS, accurately weighed, in an appropriate volume of acetonitrile and *Mobile phase* solution (approximate ratio, 1:7). Further dilute with *Mobile phase* to obtain a solution having a known final concentration of about 1.5 mg of each per mL.
Standard solution A—Dilute 1.0 mL of *Standard stock solution* to 200.0 mL with a mixture of *Mobile phase* and acetonitrile (9:1).
Standard solution B—Dilute 2.0 mL of *Standard solution A* to 10.0 mL with a mixture of *Mobile phase* and acetonitrile (9:1).
Test solution—Dissolve 40 mg of Ciclopirox Olamine, accurately weighed, in a mixture of 2 mL of acetonitrile, 20 µL of glacial acetic acid, and 15 mL of *Mobile phase.* If necessary, use an ultrasonic bath to dissolve. Dilute with *Mobile phase* to 20.0 mL, and mix.
Resolution solution—Mix 5 mL of *Standard stock solution* with 5 mL of the *Test solution.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a detector capable of recording at both 220 nm and 298 nm and a 4.0-mm × 8-cm column that contains packing L10. [NOTE—Ciclopirox related compound A has an intense absorbance at 220 nm, and 6-cyclohexyl-4-methyl-2(1*H*)-pyridone, ciclopirox related compound B, and ciclopirox have intense absorbances at 298 nm.] The flow rate is about 0.7 mL per minute. Chromatograph the *Resolution solution* at 298 nm, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the ciclopirox related compound B peak and the ciclopirox peak is not less than 2.0. Chromatograph *Standard solution B* at 298 nm, and record the peak responses as directed for *Procedure:* the chromatogram obtained shows at 298 nm a peak corresponding to ciclopirox related compound B with a signal-to-noise ratio of not less than 3. Chromatograph the *Test solution* at 298 nm, and record the peak responses as directed for *Procedure:* the tailing factor for the ciclopirox peak is less than 2.0.
Procedure—Separately inject equal volumes (about 10 µL) of *Standard solution A, Standard solution B,* and the *Test solution* into the chromatograph, and record the chromatograms. [NOTE—In order to ensure desorption of disruptive metal ions, every new column must be rinsed with the *Rinsing solution* over a period of not less than 15 hours and then with *Mobile phase* for not less than 5 hours with a flow rate of 0.2 mL per minute. The chromatographic run time is not less than 2.5 times the retention time of the ciclopirox peak.] The relative retention times are about 0.5 for ciclopirox related compound A, 0.9 for 6-cyclohexyl-4-methyl-2(1*H*)-pyridone, 1.0 for ciclopirox, and 1.3 for ciclopirox related compound B. The peak response at 220 nm of the ciclopirox related compound A peak in the chromatogram obtained from the *Test solution* is not more than the peak response at 220 nm of the corresponding peak in the chromatogram obtained from *Standard solution A* (0.5% with reference to ciclopirox). The sum of responses at 298 nm of the impurity peaks in the chromatogram obtained from the *Test solution* is not more than the peak response at 298 nm of the ciclopirox related compound B peak in the chromatogram obtained from *Standard solution A* (0.5% with reference to ciclopirox). At 298 nm disregard any peak due to the solvent and any peak with a response less than the response of the ciclopirox related compound B peak in the chromatogram obtained from *Standard solution B* at 298 nm (0.1% with reference to ciclopirox).
Assay—Dissolve 200 mg of Ciclopirox Olamine, accurately weighed, in 2 mL of methanol. Add 38 mL of water, mix, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination,

www.webofpharma.com

and make any necessary corrections. Determine the factor of the 0.1 N sodium hydroxide VS using 100 mg of benzoic acid, accurately weighed, and titrate under the conditions prescribed above. Each mL of 0.1 N sodium hydroxide is equivalent to 26.84 mg of ciclopirox olamine ($C_{12}H_{17}NO_2 \cdot C_2H_7NO$).

Ciclopirox Olamine Cream

» Ciclopirox Olamine Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ciclopirox olamine ($C_{12}H_{17}NO_2 \cdot C_2H_7NO$).

Packaging and storage—Preserve in collapsible tubes, and store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Benzyl Alcohol RS
USP Ciclopirox Olamine RS
Identification— Dilute 4 mL of the *Assay preparation* obtained as directed in the *Assay* with a mixture of methanol and 6.25 N sodium hydroxide (123:2) to make 100 mL: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a similar solution prepared from the *Standard preparation* obtained as directed in the *Assay,* concomitantly measured.
Minimum fill ⟨755⟩: meets the requirements.
pH ⟨791⟩—Add 15 mL of boiling water, previously adjusted with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 6 to 7, to 3.5 g of Cream in a 50-mL centrifuge tube. Place a cap on the tube, and shake vigorously until an emulsion is formed. Loosen the cap, and heat the tube on a steam bath for 10 minutes. Allow to cool, centrifuge, and determine the pH of the aqueous phase: the pH is between 5.0 and 8.0.

Content of benzyl alcohol *(if present)*—
Solvent mixture—Mix chloroform and methanol (4:1).
Internal standard solution—Prepare a solution of 1-nonyl alcohol in *Solvent mixture* containing about 1.75 mg per mL.
Standard preparation—Dilute an accurately weighed quantity of USP Benzyl Alcohol RS, quantitatively and stepwise, with *Solvent mixture* to obtain a solution having a known concentration of about 2 mg per mL. Transfer 5.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.
Test preparation—Transfer 1.0 g of Cream to a 50-mL volumetric flask, add about 30 mL of *Solvent mixture,* and mix. Add 5.0 mL of *Internal standard solution,* dilute with *Solvent mixture* to volume, and mix to obtain a clear solution.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and contains a 4-mm × 2-m glass column packed with 3% phase G3 on 100- to 120-mesh support S1AB. The column is maintained at a temperature of about 100°, the injection port and detector temperatures are maintained at about 315°, and nitrogen is used as the carrier gas at a flow rate of about 45 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the peaks is not less than 1.6; the tailing factor for the benzyl alcohol peak and the internal standard peak is not greater than 3.5; and the relative standard deviation for replicate injections is not more than 3%.
Procedure—Separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [NOTE—After six injections, raise the column temperature to about 300° for about 5 minutes, then cool to 100°.] Calculate the percentage of benzyl alcohol in the Cream taken by the formula:

$$C(R_U/R_S),$$

in which C is the concentration, in mg per mL, of benzyl alcohol in the *Standard preparation;* and R_U and R_S are the peak response ratios of the benzyl alcohol peak to the internal standard peak obtained from the *Test preparation* and the *Standard preparation,* respectively: between 90.0% and 110.0% of the claimed amount is present.

Assay—
Ferrous sulfate solution—Transfer 600 mg of ferrous sulfate to a 25-mL volumetric flask. Add 0.6 mL of glacial acetic acid, dilute with water to volume, and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Ciclopirox Olamine RS in methanol to obtain a solution having a known concentration of about 0.2 mg per mL.
Assay preparation—Transfer an accurately weighed quantity of Cream, equivalent to about 10 mg of ciclopirox olamine, to a 50-mL volumetric flask, add 25 mL of methanol, and shake by mechanical means for about 10 minutes. Dilute with methanol to volume, mix, centrifuge, and use the supernatant.
Procedure—Transfer 4.0 mL of the *Standard preparation,* 4.0 mL of the *Assay preparation,* and 4.0 mL of methanol to provide a blank, to separate 25-mL volumetric flasks. Add 15 mL of methanol to each flask, and mix. Then to each flask add 1.0 mL of *Ferrous sulfate solution,* mix, dilute with methanol to volume, and mix. Store the flasks in the dark for 1 hour. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* against the blank in 1-cm cells at the wavelength of maximum absorbance at about 440 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of ciclopirox olamine ($C_{12}H_{17}NO_2 \cdot C_2H_7NO$) in each g of the Cream taken by the formula:

$$50(C/W)(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Ciclopirox Olamine RS in the *Standard preparation; W* is the weight, in g, of Cream taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Ciclopirox Olamine Topical Suspension

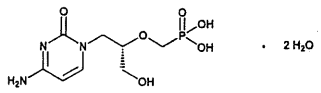
» Ciclopirox Olamine Topical Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ciclopirox olamine ($C_{12}H_{17}NO_2 \cdot C_2H_7NO$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Benzyl Alcohol RS
USP Ciclopirox Olamine RS
Identification—It responds to the *Identification* test under *Ciclopirox Olamine Cream.*
Minimum fill ⟨755⟩: meets the requirements.

www.webofpharma.com

pH ⟨791⟩—Proceed as directed for *pH* under *Ciclopirox Olamine Cream*except to read "Topical Suspension" in place of "Cream" throughout.
Content of benzyl alcohol—Proceed as directed for *Content of benzyl alcohol* under *Ciclopirox Olamine Cream*except to read "Topical Suspension" in place of "Cream" throughout.
Assay—Proceed as directed in the *Assay* under *Ciclopirox Olamine Cream*except to read "Topical Suspension" in place of "Cream" throughout.

Cidofovir



$C_8H_{14}N_3O_6P \cdot 2H_2O$ 315.22
Phosphonic acid, [[2-(4-amino-2-oxo-1(2*H*)-pyrimidyl)-1-(hydroxymethyl)ethoxy]methyl]-, dihydrate, (*S*)-;
1-[(*S*)-3-Hydroxy-2-(phosphonylmethoxy)propyl]cytosine dihydrate [149394-66-1].
Anhydrous
$C_8H_{14}N_3O_6P$ 279.19
[113852-37-2].

DEFINITION
Cidofovir contains NLT 98.0% and NMT 102.0% of cidofovir ($C_8H_{14}N_3O_6P$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
[NOTE—Solutions containing cidofovir are stable at room temperature for 8 h.]
Solution A: Acetonitrile and water (40:60)
Buffer: Dissolve 1.2 g of dibasic ammonium phosphate and 2.0 g of tetrabutylammonium phosphate in 1 L of water. Adjust with ammonium hydroxide to a pH of 9.2.
Mobile phase: *Solution A* and *Buffer* (22:78)
Standard solution: 0.1 mg/mL of USP Cidofovir RS in water
Sample solution: 0.1 mg/mL of Cidofovir in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 274 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cidofovir ($C_8H_{14}N_3O_6P$) in the portion of Cidofovir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cidofovir RS in the *Standard solution* (mg/mL)
C_U = concentration of Cidofovir in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Delete the following:

- ▲**RESIDUE ON IGNITION** ⟨281⟩
Analysis: Perform the ignition at 850 ± 50°; silica crucibles are suitable.
Acceptance criteria: NMT 0.5%▲ (RB 1-Jun-2019)
- **ORGANIC IMPURITIES**
[NOTE—Solutions containing cidofovir are stable at room temperature for 8 h.]
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay* with a run time four times the retention time of cidofovir.
System suitability solution: 1.5 µg/mL each of USP Cidofovir Related Compound A RS and USP Cidofovir Related Compound B RS in water
Standard solution: 0.001 mg/mL of USP Cidofovir RS in water
Sample solution: 1 mg/mL of Cidofovir in water
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between cidofovir related compound A and cidofovir related compound B, *System suitability solution*
Relative standard deviation: NMT 5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Cidofovir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of cidofovir from the *Standard solution*
C_S = concentration of USP Cidofovir RS in the *Standard solution* (mg/mL)
C_U = concentration of Cidofovir in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria
Individual impurities: See *Table 1*. Disregard any impurity peaks less than 0.02%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cidofovir diol analog[a]	0.30	1.3	0.15
Cidofovir related compound A	0.54	0.74	0.15

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cidofovir related compound B	0.63	0.69	0.15
Cidofovir	1.0	—	—
Cidofovir uracil analog[b]	1.4	0.56	0.15
Bromocidofovir[c]	2.0	0.62	0.15
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 1-[(*S*)-2,3-Dihydroxypropyl]cytosine.
[b] 1-[(*S*)-3-Hydroxy-2-(phosphonomethoxy)propyl]uracil.
[c] 1-[(*S*)-3-Bromo-2-(phosphonomethoxy)propyl]cytosine.

• **Enantiomeric Purity**
Mobile phase: Dissolve 1.0 g of cupric sulfate in 1 L of water. Add 1.32 g of L-phenylalanine and sonicate to dissolve.
System suitability solution: 1 mg/mL of USP Cidofovir RS and 0.01 mg/mL of USP Cidofovir Enantiomer RS in water
Standard solution: 0.01 mg/mL of USP Cidofovir Enantiomer RS in water
Sample solution: 1 mg/mL of Cidofovir in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 15°
Column: 15°
Flow rate: 0.5 mL/min
Injection volume: 10 µL
Run time: Two times the retention time of cidofovir
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—Typical relative retention times for cidofovir and cidofovir enantiomer are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 2.0 between cidofovir and cidofovir enantiomer, *System suitability solution*
Relative standard deviation: NMT 5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cidofovir enantiomer in the portion of Cidofovir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cidofovir enantiomer from the *Sample solution*
r_S = peak response of cidofovir enantiomer from the *Standard solution*
C_S = concentration of USP Cidofovir Enantiomer RS in the *Standard solution* (mg/mL)
C_U = concentration of Cidofovir in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS
• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic bacterial count is NMT 10^2 cfu/g. The total combined molds and yeasts count is NMT 10^1 cfu/g.
• **Water Determination** ⟨921⟩, *Method I, Method Ia*
Sample: 0.2 g
Acceptance criteria: 10.5%–12.5%
• **pH** ⟨791⟩
Sample solution: 1 g in 100 mL of water
Acceptance criteria: 2.5–4.5

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Cidofovir RS
USP Cidofovir Enantiomer RS
1-[(*R*)-3-Hydroxy-2-(phosphonomethoxy)propyl] cytosine dihydrate.
$C_8H_{14}N_3O_6P \cdot 2H_2O$ 315.22
USP Cidofovir Related Compound A RS
1-[(*S*)-3-Hydroxy-2-(*O*-ethylphosphonomethoxy)propyl] cytosine.
$C_{10}H_{18}N_3O_6P$ 307.24
USP Cidofovir Related Compound B RS
1-[(*S*)-3-Hydroxy-2-(*O,O*-diethylphosphonomethoxy) propyl]cytosine hydrochloride.
$C_{12}H_{22}N_3O_6P \cdot HCl$ 371.76

Cidofovir Injection

DEFINITION
Cidofovir Injection is a sterile aqueous solution. It contains an amount of cidofovir equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of anhydrous cidofovir ($C_8H_{14}N_3O_6P$).

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Standard solution: 7.5 µg/mL of USP Cidofovir RS in water. Adjust with 0.1 N sodium hydroxide to a pH of 7.5.
Sample solution: Nominally 7.5 µg/mL of cidofovir from Injection in water
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Solution A: Acetonitrile and water (40:60)
Buffer: Dissolve 1.2 g of dibasic ammonium phosphate and 2.0 g of tetrabutylammonium phosphate in 1 L of water. Adjust with ammonium hydroxide to a pH of 9.2.
Mobile phase: *Solution A* and *Buffer* (20:80)
Standard solution: 0.17 mg/mL of USP Cidofovir RS in water. [Note—0.17 mg/mL of USP Cidofovir RS is equivalent to 0.15 mg/mL of cidofovir on the anhydrous basis.]
Sample solution: Nominally 0.15 mg/mL of cidofovir from Injection in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 274 nm

www.webofpharma.com

Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anhydrous cidofovir ($C_8H_{14}N_3O_6P$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cidofovir from the *Sample solution*
r_S = peak response of cidofovir from the *Standard solution*
C_S = concentration of USP Cidofovir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of anhydrous cidofovir in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the anhydrous basis

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay* with a run time NLT 4.5 times the retention time of cidofovir.
Standard solution: 0.0015 mg/mL of USP Cidofovir RS in water
Sample solution: Nominally 1.5 mg/mL of anhydrous cidofovir from Injection in water
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of cidofovir from the *Standard solution*
C_S = concentration of USP Cidofovir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of anhydrous cidofovir in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cidofovir diol analog[a, b]	0.30	—	—
Cidofovir related compound A[b]	0.54	—	—
Cidofovir related compound B[b]	0.63	—	—
Cidofovir	1.0	—	—
Cidofovir uracil analog[c]	1.4	0.56	6.0
Bromocidofovir[b, d]	2.0	—	—
Any individual unspecified impurity	—	—	0.2
Total impurities	—	—	6.0

[a] 1-[(*S*)-2,3-Dihydroxypropyl]cytosine.
[b] These are included in the table for identification only. These are process impurities controlled in the drug substance. They are not to be included in the total impurities.
[c] 1-[(*S*)-3-Hydroxy-2-(phosphonomethoxy)propyl]uracil.
[d] 1-[(*S*)-3-Bromo-2-(phosphonomethoxy)propyl]cytosine.

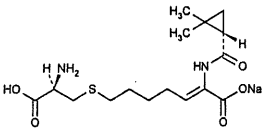
SPECIFIC TESTS

• **pH** ⟨791⟩: 7.1–7.7
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 1 USP Endotoxin Unit/mg of anhydrous cidofovir
• **STERILITY TESTS** ⟨71⟩: Meets the requirements
• **OSMOLALITY AND OSMOLARITY** ⟨785⟩, *Osmolarity*: 550–650 mOsmol/L
• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cidofovir RS
USP Endotoxin RS

Cilastatin Sodium



$C_{16}H_{25}N_2NaO_5S$ 380.43
2-Heptenoic acid, 7-[(2-amino-2-carboxyethyl)thio]-2-[[(2,2-dimethylcyclopropyl)carbonyl]amino]-, monosodium salt, [*R*-[*R**,*S**-(*Z*)]]-.
Sodium (*Z*)-7-[[(*R*)-2-amino-2-carboxyethyl]thio]-2-[(*S*)-2,2-dimethylcyclopropanecarboxamido]-2-heptenoate [81129-83-1].

» Cilastatin Sodium contains not less than 98.0 percent and not more than 101.5 percent of $C_{16}H_{25}N_2NaO_5S$, calculated on the anhydrous and solvent-free basis.

www.webofpharma.com

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution,* and store in a cold place.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile.
USP Reference standards ⟨11⟩—
USP Cilastatin Ammonium Salt RS

Identification—
A: The retention time of the major peak for cilastatin in the chromatogram of the *Test solution,* as obtained in the test for *Chromatographic purity,* corresponds to that in the chromatogram of a similar preparation of USP Cilastatin Ammonium Salt RS.
B: Ignite a small portion of it on a platinum wire in a nonluminous flame: an intense yellow color is imparted to the flame.
Specific rotation ⟨781S⟩: between +41.5° and +44.5°, on the anhydrous and solvent-free basis.
Test solution: 10 mg per mL, in a mixture of methanol and hydrochloric acid (120:1).
Bacterial Endotoxins Test ⟨85⟩ —Where the label states that Cilastatin Sodium is sterile, it contains not more than 0.17 USP Endotoxin Unit per mg of cilastatin.
Sterility Tests ⟨71⟩ —Where the label states that Cilastatin Sodium is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined,* 6 g of specimen dissolved in 200 mL of *Fluid A* being used.
pH ⟨791⟩: between 6.5 and 7.5, in a solution (1 in 100).
Water Determination, *Method I* ⟨921⟩: not more than 2.0%.

Limit of solvents—
Internal standard solution—Transfer 0.5 mL of *n*-propyl alcohol to a 1000-mL volumetric flask, dilute with water to volume, and mix.
Standard solution—Transfer 2.0 mL of acetone, 0.50 mL of methanol, and 0.50 mL of mesityl oxide to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution and 2.0 mL of *Internal standard solution* to a 10-mL volumetric flask, dilute with water to volume, and mix. This solution contains 316 µg of acetone, 79 µg of methanol, and 86 µg of mesityl oxide per mL.
Test solution—Transfer about 200 mg of Cilastatin Sodium, accurately weighed, to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution* and about 5 mL of water, and dissolve by shaking. Dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m capillary column, the internal wall of which is coated with a 1.0-µm film of liquid phase G16. The column temperature is maintained at 50° for 2.5 minutes, then increased at a rate of 8° per minute to 70°, and maintained at 70° for 0.5 minute; the injection port temperature is maintained at 160°; the detector temperature is maintained at 250°; and helium is used as the carrier gas at a flow rate of about 9 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.26 for acetone, 0.35 for methanol, 0.67 for *n*-propyl alcohol, and 1.0 for mesityl oxide; and the relative standard deviation for replicate injections, determined from peak area ratios of each analyte to *n*-propyl alcohol, is not more than 5.0%.
Procedure—Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, using the solvent (water) flush technique; record the chromatograms; and measure the areas for the acetone, methanol, *n*-propyl alcohol, and mesityl oxide peaks. Calculate the percentages of acetone, methanol, and mesityl oxide in the portion of Cilastatin Sodium taken by the formula:

$$(C/W)(R_U/R_S)$$

in which C is the concentration, in µg per mL, of the appropriate analyte in the *Standard solution; W* is the quantity, in mg, of Cilastatin Sodium taken to prepare the *Test solution;* and R_U and R_S are the peak area ratios of the corresponding analyte to *n*-propyl alcohol obtained from the *Test solution* and the *Standard solution,* respectively. Not more than 1.0% of acetone is found; not more than 0.5% of methanol is found; and not more than 0.4% of mesityl oxide is found.

Chromatographic purity—
Solvent—Use water.
Solution A—Prepare a mixture of dilute phosphoric acid (1 in 1000) and acetonitrile (700:300), pass through a filter having a 0.5-µm or finer porosity, and degas.
Solution B—Use dilute phosphoric acid (1 in 1000). Pass through a filter having a 0.5-µm or finer porosity, and degas.
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Test solution—Prepare a solution of Cilastatin Sodium in *Solvent* having a concentration of about 1.6 mg per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.5-mm × 25-cm column containing packing L1. The column is maintained at a constant temperature of about 50°. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	15	85	equilibration
0–30	15→100	85→0	linear gradient

Chromatograph the *Test solution,* and measure the peak responses as directed for *Procedure:* the capacity factor, *k′,* is not less than 10; the column efficiency determined from the cilastatin peak is not less than 3000 theoretical plates; and the tailing factor is not more than 4.5.
Procedure—Separately inject equal volumes (about 20 µL) of the *Test solution* and the *Solvent* into the chromatograph, record the chromatograms, and measure the areas of the peaks. Calculate the chromatographic purity, in percentage, of the portion of Cilastatin Sodium taken by the formula:

$$100r_C/(r_T - r_B - r_A)$$

in which r_C is the area of the cilastatin peak obtained from the *Test solution;* r_T is the sum of the areas of all the peaks obtained from the *Test solution;* r_B is the sum of the areas of all the peaks obtained from the *Solvent;* and r_A is the response of the peak, if any, of nonretained substances, such as acetone, at the solvent front obtained from the *Test solution:* not less than 98.5% is found. Calculate the percentage of each impurity in the portion of Cilastatin Sodium taken by the formula:

$$100r_i/(r_T - r_B - r_A)$$

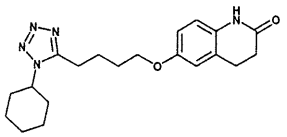
in which r_i is the peak area for each impurity in the chromatogram obtained from the *Test solution* and the other terms are as defined above: not more than 0.5% of any individual impurity is found.
Assay—Transfer about 300 mg of Cilastatin Sodium, accurately weighed, to a suitable beaker, add 30 mL of methanol, and dissolve by swirling. Add 5 mL of water, and titrate potentiometrically with 0.1 N hydrochloric acid to a pH

USP Monographs

www.webofpharma.com

of about 3. Then titrate with 0.1 N sodium hydroxide until three inflection points have been observed. Calculate the titer difference, in mL, between the first and third inflection points. Each mL of 0.1 N sodium hydroxide is equivalent to 19.022 mg of $C_{16}H_{25}N_2NaO_5S$.

Cilostazol



$C_{20}H_{27}N_5O_2$ 369.46

2(1*H*)-Quinolinone, 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl) butoxy]-3,4-dihydro-.

6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydrocarbostyril [73963-72-1].

» Cilostazol contains not less than 98.0 percent and not more than 102.0 percent of $C_{20}H_{27}N_5O_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers, and store at room temperature.

USP Reference standards ⟨11⟩—

USP Cilostazol RS

USP Cilostazol Related Compound A RS
6-Hydroxy-3,4-dihydro-1*H*-quinolin-2-one.
$C_9H_9NO_2$ 163.17

USP Cilostazol Related Compound B RS
6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)-butoxy]-1*H*-quinolin-2-one.
$C_{20}H_{25}N_5O_2$ 367.45

USP Cilostazol Related Compound C RS
1-(4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butyl)-6-(4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy)-3,4-dihydroquinolin-2(1*H*)-one.
$C_{31}H_{45}N_9O_2$ 575.75

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Loss on drying ⟨**731**⟩—Dry it at 110° for 3 hours: it loses not more than 0.3% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chloride ⟨221⟩—

Test solution—Dissolve 0.5 g of Cilostazol in 40 mL of dimethylformamide, add 6 mL of diluted nitric acid and dimethylformamide to make 50 mL.

Control solution—To 0.25 mL of 0.01 M hydrochloric acid add 6 mL of diluted nitric acid and dimethylformamide to make 50 mL.

Procedure—Add 1 mL of silver nitrate TS to the *Test solution* and to the *Control solution*, mix well, and allow to stand for 5 minutes, protecting from direct sunlight. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely. The opalescence developed in the *Test solution* is not more than that of the *Control solution* (0.018%).

Related compounds—

Diluent, Solution A, Solution B, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay*.

Standard solution—Dissolve accurately weighed quantities of USP Cilostazol RS and USP Cilostazol Related Compound C RS in acetonitrile, with sonication if necessary, to obtain a solution having known concentrations of about 0.5 mg per mL of each component. Transfer 4 mL of this solution to a 10-mL volumetric flask, and dilute with water to volume. Further dilute this solution, stepwise if necessary, with *Diluent* to obtain a solution having known concentrations of about 0.4 µg per mL of each component.

Test solution—Transfer about 20 mg of Cilostazol, accurately weighed, to a 50-mL volumetric flask, dissolve in 20 mL of acetonitrile, with sonication if necessary. Dilute with water to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of cilostazol related compound C by the formula:

$$0.1(C_S/C_T)(r_U/r_S)$$

in which C_S is the concentration, in µg per mL, of cilostazol related compound C in the *Standard solution;* C_T is the concentration, in mg per mL, of Cilostazol in the *Test solution;* r_U is the peak response for cilostazol related compound C obtained from the *Test solution;* and r_S is the peak response for cilostazol related compound C obtained from the *Standard solution*. Calculate the percentage of other impurities by the formula:

$$0.1(1/F)(C_S/C_T)(r_U/r_S)$$

in which *F* is the relative response factor from *Table 1* C_S is the concentration, in µg per mL, of cilostazol in the *Standard solution;* C_T is the concentration, in mg per mL, of cilostazol in the *Test solution;* r_U is the peak response for any other impurity obtained from the *Test solution;* and r_S is the peak response for cilostazol obtained from the *Standard solution*.

Table 1

Name	Relative Retention Time	Relative Response Factor (*F*)	Limit (%)
Cilostazol related compound A[1]	0.2	1.7	0.1
Cilostazol related compound B[2]	0.9	0.58	0.1
Cilostazol	1.0	1.0	—
Cilostazol related compound C[3]	1.9	—	0.1
Any other individual impurity	—	1.0	0.1

[1] 6-Hydroxy-3,4-dihydro-1*H*-quinolin-2-one

[2] 6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)-butoxy]-1*H*-quinolin-2-one

[3] 1-(4-(5-Cyclohexyl-1*H*-tetrazol-1-yl)butyl)-6-(4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy)-3,4-dihydroquinolin-2(1*H*)-one

In addition to not exceeding the limits for impurities in *Table 1* not more than 0.4% of total impurities is found.

USP Monographs

www.webofpharma.com

Assay—

Diluent—Use a mixture of water and acetonitrile (60:40).

Solution A—Use a mixture of water and acetonitrile (70:30).

Solution B—Use a mixture of water and acetonitrile (50:50).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare a solution in *Diluent* having known concentrations of about 0.05 mg per mL each of USP Cilostazol RS, USP Cilostazol Related Compound A RS, and USP Cilostazol Related Compound B RS.

Standard preparation—Dissolve an accurately weighed quantity of USP Cilostazol RS in acetonitrile, with sonication if necessary, to obtain a solution having a known concentration of about 1.0 mg per mL. Transfer 4 mL of this solution to a 10-mL volumetric flask, and dilute with water to volume. Further dilute this solution with *Diluent* to obtain a solution having a known concentration of about 0.04 mg per mL.

Assay preparation—Transfer about 20 mg of Cilostazol, accurately weighed, to a 50-mL volumetric flask, dissolve in 20 mL of acetonitrile, sonicate if necessary, dilute with water to volume, and mix. Transfer 1 mL of this solution to a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 10-cm column that contains 3.5-µm packing L7. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–6.5	100→50	0→50	linear gradient
6.5–10	50→0	50→100	linear gradient
10–20	0	100	isocratic
20–20.1	0→100	100→0	linear gradient
20.1–28	100	0	re-equilibration

Chromatograph the *System suitability solution,* identify the components using *Table 1* and record the peak responses as directed for *Procedure:* the resolution, *R,* between cilostazol related compound B and cilostazol is not less than 3.0; the tailing factor for the cilostazol peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{20}H_{27}N_5O_2$ in the portion of Cilostazol taken by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of cilostazol in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cilostazol Tablets

DEFINITION

Cilostazol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cilostazol ($C_{20}H_{27}N_5O_2$).

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S▲** (CN 1-May-2020)

Standard solution: 100 mg/mL of USP Cilostazol RS in chloroform

Sample solution: Transfer the equivalent of 100 mg of cilostazol from finely powdered Tablets into a glass container. Add 1 mL of chloroform, shake for 1 min, and pass through a suitable filter of 0.5-µm or finer pore size.

- **B.** The retention time of the cilostazol peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (7:3:10)

Internal standard solution: 4 mg/mL of benzophenone in methanol

Standard solution: 0.1 mg/mL of USP Cilostazol RS and 0.04 mg/mL of *Internal standard solution* in methanol

Sample solution: Transfer the equivalent of 50 mg of cilostazol from powdered Tablets (NLT 20) into a suitable volumetric flask and add an appropriate quantity of *Internal standard solution.* Dilute with methanol to obtain a solution of 0.1 mg/mL of USP Cilostazol RS and 0.04 mg/mL of the internal standard. Pass a portion of this solution through a membrane filter of 0.5-µm or finer pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 9.0 between the cilostazol and benzophenone peaks, eluted in this order

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cilostazol ($C_{20}H_{27}N_5O_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of cilostazol to the internal standard from the *Sample solution*

R_S = peak response ratio of cilostazol to the internal standard from the *Standard solution*

C_S = concentration of USP Cilostazol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cilostazol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.30% sodium lauryl sulfate in water; 900 mL

Apparatus 2: 75 rpm

Time: 60 min

www.webofpharma.com

Standard solution: 0.28 mg/mL of USP Cilostazol RS in methanol. Dilute this solution with *Medium* to obtain a solution with a final concentration of about 5.6 µg/mL.
Sample solution: Pass NLT 20 mL of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 10 mL. Dilute with *Medium* to obtain a final theoretical concentration of about 5.6 µg/mL, considering complete dissolution of the label claim.
Wavelength: UV 257 nm
Path length: 1 cm
Blank: *Medium*
Calculate the percentage of cilostazol dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = Tablet label claim
V = volume of *Medium*, 900 mL

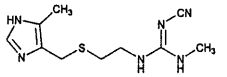
Tolerances: NLT 80% (Q) of the labeled amount of cilostazol is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.3% sodium lauryl sulfate in water; 900 mL, deaerated
Apparatus 2: 75 rpm
Time: 30 min
Standard solution: Prepare a solution containing 1.1 mg/mL of USP Cilostazol RS in methanol. Dilute this solution with 0.5% sodium lauryl sulfate in water to obtain a final concentration of (L/900) mg/mL, where L is the Tablet label claim in mg.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Wavelength: UV 258 nm
Path length: 0.2 cm
Blank: *Medium*
Tolerances: NLT 75% (Q) of the labeled amount of cilostazol is dissolved.
Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.3% sodium lauryl sulfate in water; 900 mL
Apparatus 2: 75 rpm
Time: 60 min
Standard solution, Sample solution, Wavelength, Path length, and **Blank:** Proceed as directed for *Test 1*.
Tolerances: NLT 70% (Q) of the labeled amount of cilostazol is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight and light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cilostazol RS

Cimetidine



$C_{10}H_{16}N_6S$ 252.34
Guanidine, *N*″-cyano-*N*-methyl-*N*′-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]-;
2-Cyano-1-methyl-3-[2-[[(5-methylimidazol-4-yl)methyl]thio]ethyl]guanidine [51481-61-9].

DEFINITION

Cimetidine contains NLT 98.0% and NMT 102.0% of cimetidine ($C_{10}H_{16}N_6S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The UV absorption spectrum of a solution (1 in 80,000) in 0.1 N sulfuric acid exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Cimetidine RS, concomitantly measured.

ASSAY

- **PROCEDURE**

Mobile phase: Transfer 200 mL of methanol and 0.3 mL of phosphoric acid to a 1000-mL volumetric flask, dilute with water to volume, and filter.
Standard stock solution: 0.4 mg/mL of USP Cimetidine RS in methanol and water (1:4), prepared by initially dissolving the USP Cimetidine RS in methanol using 20% of the final volume and diluting that solution with water to volume
Standard solution: 0.01 mg/mL of USP Cimetidine RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: 0.4 mg/mL of Cimetidine in methanol and water (1:4), prepared by initially dissolving the sample in methanol using 20% of the final volume and diluting that solution with water to volume
Sample solution: 0.01 mg/mL of Cimetidine in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor, *k*′: NLT 0.6
Column efficiency: NLT 1000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cimetidine ($C_{10}H_{16}N_6S$) in the portion of Cimetidine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cimetidine RS in the *Standard solution* (mg/mL)

www.webofpharma.com

C_U = concentration of Cimetidine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Mobile phase: Mix 240 mL of methanol, 0.3 mL of 85% phosphoric acid, 940 mg of sodium 1-hexanesulfonate, and sufficient water to make 1 L, and filter.
Standard solution: 0.80 µg/mL of USP Cimetidine RS in *Mobile phase*
Sample solution: 0.4 mg/mL of Cimetidine in *Mobile phase*. Mix, sonicate for 15 min, and then mix again.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 2.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor, *k'*: NLT 3.0
Column efficiency: NLT 2000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Cimetidine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of cimetidine from the *Standard solution*
C_S = concentration of USP Cimetidine RS in the *Standard solution* (µg/mL)
C_U = concentration of Cimetidine in the *Sample solution* (µg/mL)

Acceptance criteria
Any individual impurity: NMT 0.2%
Total impurities: NMT 1.0%

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 139°–144°
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 110° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Cimetidine RS

Cimetidine Injection

DEFINITION

Cimetidine Injection is a sterile solution of Cimetidine Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cimetidine ($C_{10}H_{16}N_6S$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Transfer 200 mL of methanol and 0.3 mL of phosphoric acid to a 1000-mL volumetric flask, dilute with water to volume, and filter.
Standard stock solution: 0.5 mg/mL of USP Cimetidine Hydrochloride RS in a mixture of methanol and water (1:4)
Standard solution: 12.5 µg/mL of USP Cimetidine Hydrochloride RS in *Mobile phase* from *Standard stock solution*
Sample solution: Nominally 10.0 µg/mL of cimetidine, prepared as follows. Transfer an accurately measured volume of Injection, equivalent to about 2 mg of cimetidine, to a 200-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor, *k'*: NLT 0.6
Column efficiency: NLT 1000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cimetidine ($C_{10}H_{16}N_6S$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cimetidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cimetidine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of cimetidine, 252.34
M_{r2} = molecular weight of cimetidine hydrochloride, 288.81

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Unit/mg of cimetidine hydrochloride
- **PH** ⟨791⟩: 3.8–6.0
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Cimetidine Hydrochloride RS

USP Monographs

www.webofpharma.com

Cimetidine Tablets

DEFINITION
Cimetidine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cimetidine ($C_{10}H_{16}N_6S$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Transfer 200 mL of methanol and 0.3 mL of phosphoric acid to a 1000-mL volumetric flask, dilute with water to volume, and filter.
Standard stock solution: 0.4 mg/mL of USP Cimetidine RS in methanol and water (1:4), prepared by initially dissolving the USP Cimetidine RS in methanol using 20% of the final volume and diluting that solution with water to volume
Standard solution: 0.01 mg/mL of USP Cimetidine RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: Weigh, and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to about 100 mg of cimetidine, to a 250-mL volumetric flask. Add 50 mL of methanol, shake for 2 min, add 40 mL of water, sonicate for 15 min, and dilute with water to volume.
Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 200-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor, *k′*: NLT 0.6
Column efficiency: NLT 1000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis: Calculate the percentage of the labeled amount of cimetidine ($C_{10}H_{16}N_6S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cimetidine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cimetidine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm. A 20-mesh basket may be used for 800-mg strength Tablets.
Time: 15 min
Standard solution: USP Cimetidine RS in *Medium*
Sample solution: Filtered solution under test, diluted with *Medium* to a concentration that is similar to that of the *Standard solution*
Detector: UV, the wavelength of maximum absorbance at about 218 nm
Tolerances: NLT 80% (*Q*) of the labeled amount of cimetidine ($C_{10}H_{16}N_6S$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Cimetidine RS

Cimetidine in Sodium Chloride Injection

DEFINITION
Cimetidine in Sodium Chloride Injection is a sterile solution of Cimetidine Hydrochloride and Sodium Chloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cimetidine ($C_{10}H_{16}N_6S$) and NLT 95.0% and NMT 110.0% of the labeled amount of sodium chloride (NaCl).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩ and *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY
- **CIMETIDINE**

Mobile phase: Transfer 200 mL of methanol and 0.3 mL of phosphoric acid to a 1000-mL volumetric flask, dilute with water to volume, and filter.
Standard stock solution: 0.5 mg/mL of USP Cimetidine Hydrochloride RS in a mixture of methanol and water (1:4)
Standard solution: 12.5 µg/mL of USP Cimetidine Hydrochloride RS in *Mobile phase* from *Standard stock solution*
Sample solution: Nominally 10.0 µg/mL of cimetidine, prepared as follows. Transfer an accurately measured volume of Injection, equivalent to about 2 mg of cimetidine, to a 200-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor, *k′*: NLT 0.6
Column efficiency: NLT 1000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cimetidine ($C_{10}H_{16}N_6S$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Cimetidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cimetidine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of cimetidine, 252.34
M_{r2} = molecular weight of cimetidine hydrochloride, 288.81

Acceptance criteria: 90.0%–110.0%

• **SODIUM CHLORIDE**

Sample solution: Dilute a volume of Injection with water to obtain a solution containing 0.5 mg/mL of sodium chloride.

Analysis: Determine the total amount of chloride, *A*, in the *Sample solution*, in mg, by titrating the *Sample solution* with 0.1 N silver nitrate VS, using a silver–silver chloride electrode. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.

To correct for the chloride present as cimetidine hydrochloride, calculate the concentration of chloride, *C*, due to sodium chloride, in mg/mL, in the *Sample solution*:

$$\text{Result} = (A/V) - [W \times (M_{Cl}/M_{r1})]$$

A = total amount of chloride in the *Sample solution* (mg)
V = volume of the Injection taken to prepare the *Sample solution* (mL)
W = quantity of cimetidine in the Injection, as determined in the *Assay* for *Cimetidine* (mg/mL)
M_{Cl} = atomic weight of chloride, 35.453
M_{r1} = molecular weight of cimetidine, 252.34

Calculate the percentage of the labeled amount of sodium chloride (NaCl) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times (M_{NaCl}/M_{Cl}) \times 100$$

C = concentration of chloride due to sodium chloride in the *Sample solution* (mg/mL)
C_U = nominal concentration of sodium chloride in the *Sample solution* (mg/mL)
M_{NaCl} = molecular weight of sodium chloride, 58.443
M_{Cl} = atomic weight of chloride, 35.453

Acceptance criteria: 95.0%–110.0%

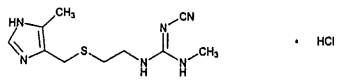
SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.5 USP Endotoxin Unit/mg of cimetidine hydrochloride
• **pH** ⟨791⟩**:** 5.0–7.0
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cimetidine Hydrochloride RS

Cimetidine Hydrochloride



$C_{10}H_{16}N_6S \cdot HCl$ 288.80

Guanidine, *N*″-cyano-*N*-methyl-*N*′-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]-, monohydrochloride; 2-Cyano-1-methyl-3-[2-[[(5-methylimidazol-4-yl)methyl]thio]ethyl]guanidine monohydrochloride [70059-30-2].

DEFINITION

Cimetidine Hydrochloride contains NLT 98.0% and NMT 102.0% of cimetidine hydrochloride ($C_{10}H_{16}N_6S \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 14 µg/mL
Medium: 0.1 N sulfuric acid
Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Transfer 200 mL of methanol and 0.3 mL of phosphoric acid to a 1000-mL volumetric flask, dilute with water to volume, and filter.
Standard stock solution: 0.5 mg/mL of USP Cimetidine Hydrochloride RS in a mixture of methanol and water (1:4)
Standard solution: 12.5 µg/mL of USP Cimetidine Hydrochloride RS in *Mobile phase* from *Standard stock solution*
Sample stock solution: 0.5 mg/mL of Cimetidine Hydrochloride in a mixture of methanol and water, prepared by initially dissolving the sample in water using 20% of the final volume, adding methanol using 20% of the final volume, and diluting that solution with water to volume
Sample solution: 12.5 µg/mL of Cimetidine Hydrochloride in *Mobile phase* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor, *k*′: NLT 0.6
Column efficiency: NLT 1000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cimetidine hydrochloride ($C_{10}H_{16}N_6S \cdot HCl$) in the portion of Cimetidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cimetidine Hydrochloride in the *Standard solution* (mg/mL)

USP Monographs

www.webofpharma.com

C_U = concentration of Cimetidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

• **ORGANIC IMPURITIES**

Mobile phase: Transfer 940 mg of sodium 1-hexanesulfonate to a 1000-mL volumetric flask, add 240 mL of methanol followed by 0.3 mL of phosphoric acid, and dilute with water to volume. Filter before use.

Sample solution: 0.4 mg/mL of Cimetidine Hydrochloride in *Mobile phase*

Diluted sample solution: 0.8 µg/mL of Cimetidine Hydrochloride in *Mobile phase* from the the *Sample solution*

System suitability solution: Dissolve 50 mg of Cimetidine Hydrochloride in 10 mL of 1 N hydrochloric acid, heat on a steam bath for about 10 min (or boil on a hot plate for about 2 min), and allow to cool. Dilute a suitable volume of this solution with *Mobile phase* to obtain a solution containing 2 µg/mL. Use this solution within 24 h of its preparation. Adjusment of the heating step may be necessary to achieve a satisfactory amide analog peak response for the measurement of the resolution between the cimetidine and the amide analog peaks.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Diluted sample solution*

Suitability requirements

Resolution: NLT 4.0 between the cimetidine and the amide analog peaks, *System suitability solution*

Capacity factor, *k'*: NLT 3.0, *Diluted sample solution*

Column efficiency: NLT 2000 theoretical plates, *Diluted sample solution*

Relative standard deviation: NMT 7.0%, *Diluted sample solution*

Analysis

Samples: *Sample solution* and *Diluted sample solution*

Calculate the percentage of each impurity in the portion of Cimetidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response of cimetidine from the *Diluted sample solution*

D = dilution factor to prepare the *Diluted sample solution* from the *Sample solution*, 0.002

Acceptance criteria

Any individual impurity: NMT 0.2%

Total impurities: NMT 1.0%

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 105° for 2 h.

Acceptance criteria: NMT 0.5%

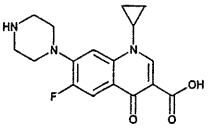
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cimetidine Hydrochloride RS

Ciprofloxacin



$C_{17}H_{18}FN_3O_3$ 331.34

3-Quinolinecarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-;

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid [85721-33-1].

DEFINITION

Ciprofloxacin contains NLT 98.0% and NMT 102.0% of ciprofloxacin ($C_{17}H_{18}FN_3O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1.

Mobile phase: Acetonitrile and *Buffer* (13:87)

Standard solution: 0.5 mg/mL of USP Ciprofloxacin RS prepared as follows. Transfer 12.5 mg of USP Ciprofloxacin RS to a 25-mL volumetric flask. Add 0.1 mL of 7% phosphoric acid, and dilute with *Mobile phase* to volume.

System suitability stock solution: 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*

System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.

Sample solution: Transfer 25 mg of Ciprofloxacin to a 50-mL volumetric flask. Add 0.2 mL of 7% phosphoric acid, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 30 ± 1°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6 between ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*

Tailing factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) in the portion of Ciprofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Ciprofloxacin RS in the *Standard solution* (mg/mL)
C_U = concentration of Ciprofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%, except that where it is intended for use in preparing Ciprofloxacin for Oral Suspension, it is NMT 0.2%.
- **ORGANIC IMPURITIES**

Buffer: Dilute 3.4 mL of phosphoric acid with water to 2000 mL. Adjust with triethylamine to a pH of 3.0 ± 0.1.
Solution A: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Solution A (%)
0	87	13
10	87	13
11	50	50
16	50	50
16.1	87	13
20	87	13

Diluent: *Solution A* and *Buffer* (13:87)
System suitability solution: 7.5 µg/mL each of USP Ciprofloxacin Ethylenediamine Analog RS and USP Ciprofloxacin RS in *Diluent*
Standard stock solution: 0.1 mg/mL each of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin RS prepared as follows. Add suitable amounts of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin RS to a suitable volumetric flask. Add 0.1% of the flask volume of 6 M ammonium hydroxide and dilute with water to volume.
Standard solution: 0.7 µg/mL each of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin RS from *Standard stock solution* in *Diluent*
Sample solution: 0.35 mg/mL of Ciprofloxacin prepared as follows. Transfer 35 mg of Ciprofloxacin to a 100-mL volumetric flask, add 0.2 mL of 7% phosphoric acid, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 263 and 278 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 30 µL
System suitability
Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6.0 between ciprofloxacin ethylenediamine analog and ciprofloxacin at 278 nm, *System suitability solution*
Tailing factor: NMT 2.0 for the ciprofloxacin peak at 278 nm, *Standard solution*
Relative standard deviation: NMT 5.0% for ciprofloxacin at 278 nm; NMT 5.0% for fluoroquinolonic acid at 263 nm, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluoroquinolonic acid in the portion of Ciprofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluoroquinolonic acid at 263 nm from the *Sample solution*
r_S = peak response of fluoroquinolonic acid at 263 nm from the *Standard solution*
C_S = concentration of USP Fluoroquinolonic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of Ciprofloxacin in the *Sample solution* (mg/mL)

Calculate the percentage of the ciprofloxacin ethylenediamine analog and any individual unspecified impurity in the portion of Ciprofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity at 278 nm from the *Sample solution*
r_S = peak response of ciprofloxacin at 278 nm from the *Standard solution*
C_S = concentration of USP Ciprofloxacin RS in the *Standard solution* (mg/mL)
C_U = concentration of Ciprofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Wavelength (nm)	Acceptance Criteria, NMT (%)
Ciprofloxacin ethylenediamine analog	0.70	278	0.2
Ciprofloxacin	1.00	278	—
Fluoroquinolonic acid	1.89	263	0.2
Any individual unspecified impurity	—	278	0.2
Total impurities[a]	—	—	0.5

[a] Total impurities does not include the fluoroquinolonic acid impurity.

SPECIFIC TESTS

- **CLARITY OF SOLUTION**

Sample solution: Dissolve 0.25 g in 10 mL of 0.1 N hydrochloric acid.
Acceptance criteria: A clear to slightly opalescent solution is obtained.

USP Monographs

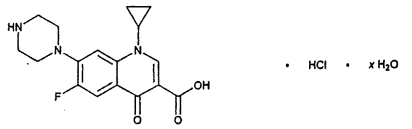
www.webofpharma.com

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: Where it is intended for use in preparing Ciprofloxacin for Oral Suspension, the total microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g. It also meets the requirements for absence of *Salmonella* species and *Escherichia coli*.
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry under vacuum at 120° for 6 h.
  Acceptance criteria: NMT 1.0%, except that where it is labeled as intended for use in preparing Ciprofloxacin for Oral Suspension, 10%–20%
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Where the label states that it is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that it is sterile or where the label states that Ciprofloxacin must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.50 USP Endotoxin Units/mg of ciprofloxacin.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°, and avoid excessive heat.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Where it is intended for use in preparing Ciprofloxacin for Oral Suspension, it is so labeled.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ciprofloxacin RS
  USP Ciprofloxacin Ethylenediamine Analog RS
  1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.
  $C_{15}H_{16}FN_3O_3 \cdot HCl$ 341.77
  USP Fluoroquinolonic Acid RS
  7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.
  $C_{13}H_9ClFNO_3$ 281.67

Ciprofloxacin Hydrochloride



$C_{17}H_{18}FN_3O_3 \cdot HCl \cdot xH_2O$
Sesquihydrate 394.83
Monohydrate 385.82
Anhydrous 367.81
3-Quinolinecarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-, monohydrochloride; 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, monohydrochloride Monohydrate [86393-32-0].

DEFINITION

Ciprofloxacin Hydrochloride contains NLT 98.0% and NMT 102.0% of ciprofloxacin hydrochloride ($C_{17}H_{18}FN_3O_3 \cdot HCl$), calculated on the anhydrous basis. It contains a variable quantity of water.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*

ASSAY

- **PROCEDURE**
  Buffer: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1.
  Mobile phase: Acetonitrile and *Buffer* (13:87)
  Standard solution: 0.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*
  System suitability stock solution: 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*
  System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.
  Sample solution: 0.5 mg/mL of Ciprofloxacin Hydrochloride in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 278 nm
  Column: 4.6-mm × 25-cm; packing L1
  Column temperature: 30 ± 1°
  Flow rate: 1.5 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  [NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 6 between ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*
  Tailing factor: NMT 2.5, *Standard solution*
  Relative standard deviation: NMT 1.5%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of ciprofloxacin hydrochloride ($C_{17}H_{18}FN_3O_3 \cdot HCl$) in the portion of Ciprofloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Ciprofloxacin Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Buffer: Dilute 3.4 mL of phosphoric acid with water to 2000 mL. Adjust with triethylamine to a pH of 3.0 ± 0.1.
  Solution A: Acetonitrile

www.webofpharma.com

Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Solution A (%)
0	87	13
10	87	13
11	50	50
16	50	50
16.1	87	13
20	87	13

Diluent: *Solution A* and *Buffer* (13:87)
System suitability solution: 7.5 µg/mL each of USP Ciprofloxacin Ethylenediamine Analog RS and USP Ciprofloxacin Hydrochloride RS in *Diluent*
Standard stock solution: 0.1 mg/mL each of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin Hydrochloride RS prepared as follows. Add suitable amounts of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin Hydrochloride RS to a suitable volumetric flask. Add 0.1% of the flask volume of 6 M ammonium hydroxide and dilute with water to volume.
Standard solution: 0.7 µg/mL each of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin Hydrochloride RS from *Standard stock solution* in *Diluent*
Sample solution: 0.35 mg/mL of Ciprofloxacin Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 263 and 278 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 30 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6.0 between ciprofloxacin ethylenediamine analog and ciprofloxacin at 278 nm, *System suitability solution*
Tailing factor: NMT 2.0 for the ciprofloxacin peak at 278 nm, *Standard solution*
Relative standard deviation: NMT 5.0% for ciprofloxacin at 278 nm; NMT 5.0% for fluoroquinolonic acid at 263 nm, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluoroquinolonic acid in the portion of Ciprofloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluoroquinolonic acid at 263 nm from the *Sample solution*
r_S = peak response of fluoroquinolonic acid at 263 nm from the *Standard solution*
C_S = concentration of USP Fluoroquinolonic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of Ciprofloxacin Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of ciprofloxacin ethylenediamine analog and any individual unspecified impurity in the portion of Ciprofloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity at 278 nm from the *Sample solution*
r_S = peak response of ciprofloxacin at 278 nm from the *Standard solution*
C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Ciprofloxacin Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Wavelength (nm)	Acceptance Criteria, NMT (%)
Ciprofloxacin ethylenediamine analog	0.70	278	0.2
Ciprofloxacin	1.00	278	—
Fluoroquinolonic acid	1.89	263	0.2
Any individual unspecified impurity	—	278	0.2
Total impurities[a]	—	—	0.5

[a] Total impurities does not include fluoroquinolonic acid impurity.

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 25-mg/mL solution in water
Acceptance criteria: 3.0–4.5
• **WATER DETERMINATION** ⟨921⟩, *Method I*: 4.7%–6.7%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ciprofloxacin Ethylenediamine Analog RS
1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.
$C_{15}H_{16}FN_3O_3 \cdot HCl$ 341.77
USP Ciprofloxacin Hydrochloride RS
USP Fluoroquinolonic Acid RS
7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.
$C_{13}H_9ClFNO_3$ 281.67

Ciprofloxacin Injection

DEFINITION
Ciprofloxacin Injection is a sterile solution of Ciprofloxacin or Ciprofloxacin Hydrochloride in Water for Injection, in 5% Dextrose Injection, or in 0.9% Sodium Chloride Injection prepared with the aid of Lactic Acid. It contains NLT 90.0%

USP Monographs

www.webofpharma.com

and NMT 110.0% of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

IDENTIFICATION

• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1.
Mobile phase: Acetonitrile and *Solution A* (13:87)
Standard solution: 0.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*
System suitability solution: 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.
Sample solution: Equivalent to 0.5 mg/mL of Ciprofloxacin from Injection diluted with *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 25-cm; packing L1
Temperature: 30 ± 1°
Flow rate: 1.5 mL/min
Injection size: 10 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6 between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak
Column efficiency: NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*
Tailing factor: NMT 2.5 for the ciprofloxacin peak, *Standard solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of $C_{17}H_{18}FN_3O_3$ from the portion of Ciprofloxacin Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of ciprofloxacin, 331.34
M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **LACTIC ACID CONTENT**

Mobile phase: Acetonitrile and 0.005 N sulfuric acid (3:17)
Standard solution: 0.8 mg/mL of USP Sodium Lactate RS in water or 4 mg/mL where the Injection is labeled as being a concentrated form
Sample solution: Use the undiluted Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 208 nm
Column: 7.8-mm × 30-cm; packing L17
Temperature: 40 ± 1°
Flow rate: 0.6 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the analyte peak
Relative standard deviation: NMT 2.0%
[NOTE—After each analysis, rinse the column with a mixture of 0.01 N sulfuric acid and acetonitrile to elute the ciprofloxacin from the column. Promptly regenerate the column with 0.01 N sulfuric acid, and the column may be reused or stored.]
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration of lactic acid ($C_3H_6O_3$) in mg/mg of ciprofloxacin:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2})$$

r_U = peak response of lactic acid from the *Sample solution*
r_S = peak response of lactic acid from the *Standard solution*
C_S = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of lactic acid, 90.08
M_{r2} = molecular weight of sodium lactate, 112.07

Acceptance criteria: 0.288–0.352 mg of lactic acid for each mg of ciprofloxacin claimed on the label, except that where the Injection is labeled as being a concentrated form, it contains 0.335–0.409 mg of lactic acid for each mg of ciprofloxacin claimed on the label

• **DEXTROSE CONTENT** (if present)

Sample solution: Undiluted Injection
Analysis: Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).
Calculate the percentage (g/100 mL) of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = A \times R \times (M_{r1}/M_{r2}) \times (100/F)$$

A = 100 mm divided by the length of the polarimeter tube (mm)
R = observed rotation (degrees)
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16
F = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

Acceptance criteria: 4.75–5.25 g/100 mL

• **SODIUM CHLORIDE CONTENT** (if present)

Sample solution: Injection
Analysis: Transfer 10.0 mL of *Sample solution* to a suitable container, dilute with water to 150 mL, add 1.5 mL of potassium chromate TS, and titrate with 0.1 N silver nitrate

www.webofpharma.com

TS. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride (NaCl).
Acceptance criteria: 85.5–94.5 mg

IMPURITIES
ORGANIC IMPURITIES
• **Procedure: Limit of Ciprofloxacin Ethylenediamine Analog**
Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of ciprofloxacin ethylenediamine analog from the portion of Ciprofloxacin Injection taken:

$$\text{Result} = [F \times r_A/(F \times r_A + r_C)] \times 100$$

F = correction factor for ciprofloxacin ethylenediamine analog, 0.7
r_A = ciprofloxacin ethylenediamine analog peak response
r_C = peak response of ciprofloxacin

Acceptance criteria: NMT 0.5%

SPECIFIC TESTS
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements
• **pH** ⟨791⟩: 3.5–4.6, except that where the Injection is labeled as being a concentrated form, its pH is 3.3–3.9
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.50 USP Endotoxin Unit/mg of ciprofloxacin.
• **STERILITY TESTS** ⟨71⟩: It meets the requirements for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
• **COLOR AND ACHROMICITY** ⟨631⟩ (where it is labeled as being a concentrated form): It has no more color than a solution prepared by diluting 5.0 mL of *Matching Fluid O* with 95.0 mL of 0.12 N hydrochloric acid.
• **OTHER REQUIREMENTS:** It meets the requirements for *Container Content for Injections* ⟨697⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, and store in a cool place or at controlled room temperature. Avoid freezing and exposure to light.
• **LABELING:** The label indicates whether the vehicle is Sterile Water for Injection, 5% Dextrose Injection, or 0.9% Sodium Chloride Injection. Label the Injection that has Sterile Water for Injection as the vehicle to indicate that it is a concentrated form that must be diluted to appropriate strength (1–2 mg/mL) with 5% Dextrose Injection or 0.9% Sodium Chloride Injection before administration, and that the resulting solution is stable for up to 14 days when stored in a cool place or at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ciprofloxacin Ethylenediamine Analog RS
1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.
$C_{15}H_{16}FN_3O_3 \cdot HCl$ 341.77
USP Ciprofloxacin Hydrochloride RS
USP Sodium Lactate RS

Ciprofloxacin Ophthalmic Ointment

DEFINITION
Ciprofloxacin Ophthalmic Ointment contains an amount of Ciprofloxacin Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 1.7 g/L of tetrabutylammonium phosphate in water. Adjust with phosphoric acid to a pH of 2.0.
Mobile phase: Methanol and *Buffer* (250:750)
Standard solution: 0.033 mg/mL of USP Ciprofloxacin Hydrochloride RS in 0.1 N hydrochloric acid
System suitability solution: 5 µg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Standard solution*
Sample solution: Transfer an amount nominally equivalent to 750 µg of ciprofloxacin from Ophthalmic Ointment to a screw-capped tube. Add 15 mL of solvent hexane, and shake vigorously until the Ophthalmic Ointment is dispersed. Loosen the cap, and heat in a water bath at 60° for 30 min, with occasional swirling. Remove from the bath, tighten the cap, and shake for 1.5 min while still hot. Add 25.0 mL of 0.1 N hydrochloric acid, and shake vigorously for 1.5 min. Allow the layers to separate, and use the lower, aqueous layer.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for the ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*
Tailing factor: 0.9–2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of ciprofloxacin, 331.34
M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

USP Monographs

www.webofpharma.com

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: It meets the requirements.
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at a temperature between 2° and 25°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ciprofloxacin Ethylenediamine Analog RS
  1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.
  $C_{15}H_{16}FN_3O_3 \cdot HCl$ 341.77
  USP Ciprofloxacin Hydrochloride RS

Ciprofloxacin Ophthalmic Solution

DEFINITION

Ciprofloxacin Ophthalmic Solution is a sterile, aqueous solution of Ciprofloxacin Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.005 M tetrabutylammonium phosphate solution. Adjust with phosphoric acid to a pH of 2.0.
Mobile phase: Methanol and *Solution A* (1:3)
Standard solution: 0.14 mg/mL of USP Ciprofloxacin Hydrochloride RS in water
System suitability solution: 0.01 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Standard solution*
Sample solution: Equivalent to 0.12 mg/mL of ciprofloxacin from Ophthalmic Solution, in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for the ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between the ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*
Capacity factor: 1.5–6 for the ciprofloxacin peak, *Standard solution*
Column efficiency: NLT 500 theoretical plates, *Standard solution*
Tailing factor: 0.9–2.0, *Standard solution*
Relative standard deviation: NMT 2%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of $C_{17}H_{18}FN_3O_3$ in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of ciprofloxacin, 331.34
M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 3.5–5.5
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed under *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ciprofloxacin Ethylenediamine Analog RS
  1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.
  $C_{15}H_{16}FN_3O_3 \cdot HCl$ 341.77
  USP Ciprofloxacin Hydrochloride RS

Ciprofloxacin Tablets

DEFINITION

Ciprofloxacin Tablets contain Ciprofloxacin Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 2.0 ± 0.1.
Solution B: Acetonitrile and *Solution A* (13:87)
Solution C: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1.
Mobile phase: Acetonitrile and *Solution C* (13:87)
Standard solution: 0.2 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Solution B*

www.webofpharma.com

System suitability solution: 0.05 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in the *Standard solution*

Sample solution: Transfer 5 Tablets to a 500-mL volumetric flask, add 400 mL of *Solution B*, and sonicate for about 20 min. Dilute with *Solution B* to volume and pass through a membrane filter of 0.45-µm pore size. Prepare the equivalent of 0.20 mg/mL of ciprofloxacin from the filtrate with *Solution B*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 278 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The retention time for ciprofloxacin is 6.4–10.8 min. The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6 between the ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of ciprofloxacin, 331.34

M_{r2} = molecular weight of ciprofloxacin hydrochloride, 367.81

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Ciprofloxacin Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 276 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: An amount of ciprofloxacin hydrochloride ($C_{17}H_{18}FN_3O_3 \cdot HCl$) equivalent to NLT 80% (*Q*) of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution C, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 0.5 mg/mL each of USP Ciprofloxacin Hydrochloride RS and USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*

Standard solution: 1.0 µg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*

Sample solution: Nominally 0.45 mg/mL of ciprofloxacin prepared as follows. Finely powder NLT 20 Tablets and transfer a portion of the powder to an appropriate volumetric flask. Add *Mobile phase* to about 50% of the flask volume and sonicate as necessary to dissolve. Dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 6.0 between the ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each specified or unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ciprofloxacin ethylenediamine analog	0.68	0.5
Ciprofloxacin	1.0	—
7-Chloro-6-piperazinyl analog[a]	1.2	0.3
Chlorociprofloxacin[b]	2.0	0.3
Any individual unspecified impurity	—	0.2
Total impurities	—	1.0

[a] 7-Chloro-1-cyclopropyl-4-oxo-6-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

[b] 6-Chloro-1-cyclopropyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ciprofloxacin Ethylenediamine Analog RS
1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.
$C_{15}H_{16}FN_3O_3 \cdot HCl$ 341.77
USP Ciprofloxacin Hydrochloride RS

Ciprofloxacin Extended-Release Tablets

DEFINITION
Ciprofloxacin Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: Dilute 2.9 mL of phosphoric acid in water to 1000 mL. Adjust with triethylamine to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (135:865)
System suitability solution: 0.58 mg/mL of USP Ciprofloxacin Hydrochloride RS and 0.5 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*
Standard stock solution: 1.16 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*
Standard solution: 0.058 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase* from *Standard stock solution*
Sample stock solution: Nominally 0.5 mg/mL in *Mobile phase* prepared as follows. Transfer an equivalent to 250 mg of ciprofloxacin from finely powdered Tablets (NLT 20) to a 500-mL volumetric flask. Add 400 mL of *Mobile phase*, place on a rotary shaker for 15 min, and sonicate for 25 min. Allow the solution to cool to room temperature, and dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Sample solution: Nominally 0.05 mg/mL of ciprofloxacin in water from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 6 between the ciprofloxacin and ciprofloxacin ethylenediamine analog peaks, *System suitability solution*
Tailing factor: NMT 4.0 for the ciprofloxacin peak, *System suitability solution*
Relative standard deviation: NMT 2.0% for the ciprofloxacin peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ciprofloxacin from the *Sample solution*
r_S = peak response of ciprofloxacin from the *Standard solution*
C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of ciprofloxacin, 331.34
M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Test 1
Medium: pH 4.5 acetate buffer (transfer 3 g of sodium acetate and 14 mL of 2 N acetic acid to a 1-L volumetric flask, and dilute with water to volume); 900 mL, deaerated
Apparatus 2: 50 rpm
Times: 30, 60, and 120 min
Standard solution: 6.5 µg/mL of USP Ciprofloxacin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. For 500-mg Tablets, transfer 2 mL of the filtrate to a 200-mL volumetric flask, and dilute with *Medium* to volume. For 1000-mg Tablets, transfer 1 mL of the filtrate to a 200-mL volumetric flask, and dilute with *Medium* to volume. Replace the aliquots withdrawn for analysis with fresh portions of *Medium*.
Instrumental conditions
Mode: UV
Analytical wavelength: 277 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) dissolved at each time interval (D_i):

$$D_i = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of ciprofloxacin hydrochloride in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of ciprofloxacin, 331.34
M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*

Percentage of ciprofloxacin dissolved at the first time interval = D_1
Percentage of ciprofloxacin dissolved at the second time interval = $D_2 + [D_1 \times (v/V)]$
Percentage of ciprofloxacin dissolved at the third time interval = $D_3 + [(D_2 + D_1) \times (v/V)]$
v = volume of solution under test removed at each time interval (mL)
Tolerances
For Tablets labeled to contain 500 mg, see *Table 1*.

www.webofpharma.com

Table 1

Time (min)	Amount Dissolved
30	42%–62%
60	62%–87%
120	NLT 80%

For Tablets labeled to contain 1000 mg, see *Table 2*.

Table 2

Time (min)	Amount Dissolved
30	30%–50%
60	50%–70%
120	NLT 80%

The percentages of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Times: 30, 60, and 120 min
Standard solution: 0.62 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* to a concentration similar to the *Standard solution*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: 276 nm
Cell length: 0.1 mm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) dissolved at each time interval (D_i):

$$D_i = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of ciprofloxacin hydrochloride in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of ciprofloxacin, 331.34
M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*

Percentage of ciprofloxacin dissolved at the first time interval = D_1
Percentage of ciprofloxacin dissolved at the second time interval = $D_2 + [D_1 \times (v/V)]$
Percentage of ciprofloxacin dissolved at the third time interval = $D_3 + [(D_2 + D_1) \times (v/V)]$

v = volume of solution under test removed at each time interval (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (i)	Time (min)	Amount Dissolved
1	30	40%–65%
2	60	NLT 60%
3	120	NLT 80%

The percentages of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Times: 30, 60, and 120 min
Standard solution: 0.65 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* to a concentration similar to the *Standard solution*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: 350 nm
Cell length
For 500-mg Tablet strength: 2 mm
For 1000-mg Tablet strength: 1 mm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) dissolved at each time interval (D_i):

$$D_i = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of ciprofloxacin, 331.34
M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*

Percentage of ciprofloxacin dissolved at the first time interval = D_1
Percentage of ciprofloxacin dissolved at the second time interval = $D_2 + [D_1 \times (v/V)]$
Percentage of ciprofloxacin dissolved at the third time interval = $D_3 + [(D_2 + D_1) \times (v/V)]$

v = volume of solution under test removed at each time interval (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (i)	Time (min)	Amount Dissolved
1	30	37%–57%
2	60	55%–75%
3	120	NLT 80%

www.webofpharma.com

The percentages of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, and **System suitability solution:** Prepare as directed in the *Assay*.

Standard stock solution: 0.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*

Standard solution: 1.25 µg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase* from *Standard stock solution*

Sample solution: Nominally 0.5 mg/mL of ciprofloxacin in *Mobile phase* prepared as follows. Transfer an equivalent to 250 mg of ciprofloxacin from finely powdered Tablets (NLT 20) to a 500-mL volumetric flask. Add 400 mL of *Mobile phase*, place on a rotary shaker for 15 min, and sonicate for 25 min with intermittent shaking. Allow the solution to cool to room temperature, and dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 263 and 278 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 6 between the ciprofloxacin and ciprofloxacin ethylenediamine analog peaks at 278 nm, *System suitability solution*

Tailing factor: NMT 2.0 for the ciprofloxacin peak at 278 nm, *Standard solution*

Relative standard deviation: NMT 10.0% for the ciprofloxacin peak at 278 nm, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of decarboxyciprofloxacin in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of decarboxyciprofloxacin at 263 nm from the *Sample solution*

r_S = peak response of ciprofloxacin at 263 nm from the *Standard solution*

C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of ciprofloxacin, 331.34

M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

F = relative response factor of decarboxyciprofloxacin (see *Table 5*)

Calculate the percentage of the other impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity at 278 nm from the *Sample solution*

r_S = peak response of ciprofloxacin at 278 nm from the *Standard solution*

C_S = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of ciprofloxacin, 331.34

M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

F = relative response factor (see *Table 5*)

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Decarboxyciprofloxacin[a]	0.36	1.6	0.2
Desfluorociprofloxacin[b]	0.59	1.0	0.2
Ciprofloxacin ethylenediamine analog[c]	0.68	1.2	0.2
Ciprofloxacin	1.00	—	—
7-Chloro-6-piperazinyl analog[d]	1.20	0.45	0.2
Chlorociprofloxacin[e]	2.10	0.75	0.2
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	0.6

[a] 1-Cyclopropyl-6-fluoro-7-(piperazin-1-yl)quinolin-4(1*H*)-one.
[b] 1-Cyclopropyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.
[c] 7-(2-Aminoethylamino)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.
[d] 7-Chloro-1-cyclopropyl-4-oxo-6-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.
[e] 6-Chloro-1-cyclopropyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ciprofloxacin Ethylenediamine Analog RS
7-(2-Aminoethylamino)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.
$C_{15}H_{16}FN_3O_3$ 305.30

USP Ciprofloxacin Hydrochloride RS

Ciprofloxacin for Oral Suspension

DEFINITION

Ciprofloxacin for Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

www.webofpharma.com

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 13.6 g of sodium acetate in 1000 mL of water and add 1 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.0.
Mobile phase: Methanol and *Solution A* (20:80)
Diluent: 0.1 N hydrochloric acid
Standard stock solution: 1.0 mg/mL of USP Ciprofloxacin RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Ciprofloxacin RS from the *Standard stock solution* in *Mobile phase*
Sample solution: Nominally 0.1 mg/mL of ciprofloxacin prepared as follows. Constitute Ciprofloxacin for Oral Suspension as directed in the labeling. Transfer the entire contents to a tube, and centrifuge. Remove the oil from the centrifuge tube and transfer the contents to a 1000-mL volumetric flask. Add 500 mL of *Diluent*, and sonicate for 15 min with constant shaking. Cool to room temperature, add 50 mL of methanol to dissolve the foam, and dilute with *Diluent* to volume. Centrifuge and discard the oil. Transfer an appropriate quantity of the supernatant to a suitable volumetric flask and dilute with *Mobile phase* to volume. Pass through a suitable filter of 0.2-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm. For *Identification B*, use a diode-array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 40°
Flow rate: 1.2 mL/min
Injection volume: 5 µL
Run time: 1.6 times the retention time of ciprofloxacin
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) in the portion of Ciprofloxacin for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ciprofloxacin from the *Sample solution*
r_S = peak response of ciprofloxacin from the *Standard solution*
C_S = concentration of USP Ciprofloxacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1
Medium: 6.8 g/L of sodium acetate in water. Adjust with glacial acetic acid to a pH of 4.5. Add 0.25 g/L of polyoxyethylene (23) lauryl ether and shake well to dissolve; 900 mL.
Apparatus 2: 100 rpm
Time: 30 min
Buffer: Dissolve 13.6 g of sodium acetate in 1000 mL of water and add 1 mL of triethylamine. Adjust with phosphoric acid to a pH of 4.0.
Mobile phase: Methanol and *Buffer* (30:70)
Standard solution: 0.055 mg/mL of USP Ciprofloxacin RS in *Medium*. Sonication may be used to promote dissolution.
Sample solution: Nominally 0.055 mg/mL of ciprofloxacin prepared as follows. Constitute Ciprofloxacin for Oral Suspension as directed in the labeling. Determine the density, *d* (g/mL), of Ciprofloxacin for Oral Suspension using appropriate means. Using a 5-mL syringe, collect approximately 5 mL of constituted Ciprofloxacin for Oral Suspension, and record the weight. With the paddles lowered, gently empty the contents of each syringe into the bottom of each vessel containing *Medium*. Start rotating the paddles. Reweigh each syringe, and determine the weight (g) of Ciprofloxacin for Oral Suspension delivered into each vessel. At the end of 30 min, remove 10 mL of the solution under test from each vessel, and pass through a suitable filter of 0.2-µm pore size. Transfer the appropriate quantity of filtrate to a suitable volumetric flask, and dilute with *Medium* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 278 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 30°
Flow rate: 1.5 mL/min
Injection volume: 5 µL
Run time: 1.7 times the retention time of ciprofloxacin
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (d/W_U) \times D \times (1/L) \times 100$$

r_U = peak response of ciprofloxacin from the *Sample solution*
r_S = peak response of ciprofloxacin from the *Standard solution*
C_S = concentration of USP Ciprofloxacin RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
d = density of constituted Ciprofloxacin for Oral Suspension (g/mL)
W_U = weight of the portion of constituted Ciprofloxacin for Oral Suspension added to the *Medium* (g)
D = dilution factor for the *Sample solution*, if needed
L = label claim for ciprofloxacin (mg/mL)

www.webofpharma.com

Tolerances: NLT 85% (*Q*) of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Apparatus 2, Time, Buffer, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **Analysis:** Proceed as directed in *Test 1*.

Tolerances: NLT 80% (*Q*) of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) is dissolved.

• **DELIVERABLE VOLUME** ⟨698⟩

For multiple-unit containers

Acceptance criteria: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A and **Mobile phase:** Prepare as directed in the *Assay*.

Diluent: Methanol and 0.1 N hydrochloric acid (70:30)

Standard solution: 0.005 mg/mL of USP Ciprofloxacin RS in *Diluent*

Sample solution: Nominally 0.5 mg/mL of ciprofloxacin prepared as follows. Constitute Ciprofloxacin for Oral Suspension as directed in the labeling. Transfer a sufficient quantity of constituted Ciprofloxacin for Oral Suspension to an appropriate volumetric flask. Add *Diluent* to fill about 60% of the flask volume, sonicate for 10 min, and dilute with *Diluent* to volume. Centrifuge and pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Autosampler: 10°

Column: 40°

Flow rate: 1.2 mL/min

Injection volume: 10 µL

Run time: 70 min

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ciprofloxacin for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of ciprofloxacin from the *Standard solution*

C_S = concentration of USP Ciprofloxacin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ciprofloxacin ethylenediamine analog[a]	0.75	1.3	0.3
Ciprofloxacin	1.0	—	—
7-Chloro-6-piperazinyl analog[b]	1.15	0.47	0.20
Chlorociprofloxacin[c]	2.20	0.61	0.20
Individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	1.0

[a] 7-(2-Aminoethylamino)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

[b] 7-Chloro-1-cyclopropyl-4-oxo-6-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

[c] 6-Chloro-1-cyclopropyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^3 cfu/mL. The total yeasts and molds count does not exceed 10^2 cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ciprofloxacin RS

Ciprofloxacin and Dexamethasone Otic Suspension

DEFINITION

Ciprofloxacin and Dexamethasone Otic Suspension is a sterile, aqueous suspension containing ciprofloxacin hydrochloride and dexamethasone. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) and NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$).

IDENTIFICATION

• **A.** The *Sample solution*, obtained as directed in the *Assay* for *Ciprofloxacin*, exhibits a major peak for ciprofloxacin, the retention time of which corresponds to that of the *Standard solution*, obtained as directed in the *Assay* for *Ciprofloxacin*.

• **B.** The *Sample solution*, obtained as directed in the *Assay* for *Dexamethasone*, exhibits a major peak for dexamethasone, the retention time of which corresponds to that of the *Standard solution*, obtained as directed in the *Assay* for *Dexamethasone*.

www.webofpharma.com

ASSAY

• **CIPROFLOXACIN**

Buffer: Add 6.0 mL of phosphoric acid and 8 g of diethylamine phosphate to 2.0 L of water. Adjust with 50% sodium hydroxide to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (11:89)

System suitability solution: 1.6 µg/mL each of USP Ciprofloxacin Hydrochloride RS and USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*

Standard solution A: 1.48 mg/mL of USP Ciprofloxacin Hydrochloride RS in 0.1 N hydrochloric acid. Dilute with *Mobile phase* to 0.13 mg/mL of ciprofloxacin.

Standard solution B: 0.0025 mg/mL of ciprofloxacin from *Standard solution A* in *Mobile phase*

Sample solution: Nominally 0.12 mg/mL in *Mobile phase* prepared as follows. Transfer the equivalent to 3 mg of ciprofloxacin from freshly mixed Otic Suspension to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 15-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution, Standard solution A,* and *Standard solution B*

Suitability requirements

Resolution: NLT 3.0 between ciprofloxacin and the ciprofloxacin ethylenediamine analog, *System suitability solution*

Column efficiency: NLT 2500 theoretical plates for ciprofloxacin, *System suitability solution*

Tailing factor: NMT 2.0 for ciprofloxacin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution A* and *Standard solution B*

Analysis

Samples: *Standard solution A* and *Sample solution*

Calculate the percentage of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) in the portion of Otic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of ciprofloxacin from the *Sample solution*

r_S = peak response of ciprofloxacin from *Standard solution A*

C_S = concentration of USP Ciprofloxacin Hydrochloride RS in *Standard solution A* (mg/mL)

C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of ciprofloxacin, 331.34

M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

Acceptance criteria: 90.0%–110.0% of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$)

• **DEXAMETHASONE**

Buffer and **Mobile phase:** Prepare as directed in the test for *Limit of Ciprofloxacin Formamide*.

Standard stock solution: 2 mg/mL of USP Dexamethasone RS in acetonitrile

System suitability solution: 0.2 mg/mL of USP Dexamethasone RS and 0.2 mg/mL of USP Dexamethasone Acetate RS in *Mobile phase*

Standard solution A: 0.2 mg/mL of USP Dexamethasone RS from *Standard stock solution* in *Mobile phase*

Standard solution B: 0.004 mg/mL of USP Dexamethasone RS from *Standard solution A* in *Mobile phase*

Sample solution: Nominally 0.2 mg/mL in *Mobile phase* prepared as follows. Transfer freshly mixed Otic Suspension equivalent to 2 mg of dexamethasone to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 15-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution, Standard solution A,* and *Standard solution B*

Suitability requirements

Resolution: NLT 12 between dexamethasone and dexamethasone acetate, *System suitability solution*

Column efficiency: NLT 2000 theoretical plates for dexamethasone, *System suitability solution*

Tailing factor: NMT 2.0 for dexamethasone, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution A* and *Standard solution B*

Analysis

Samples: *Standard solution A* and *Sample solution*

Calculate the percentage of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$) in the portion of Otic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dexamethasone from the *Sample solution*

r_S = peak response of dexamethasone from *Standard solution A*

C_S = concentration of USP Dexamethasone RS in *Standard solution A* (mg/mL)

C_U = nominal concentration of dexamethasone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$)

IMPURITIES

• **LIMIT OF CIPROFLOXACIN FORMAMIDE**

Buffer: Phosphoric acid and water (3:997). Adjust with 50% sodium hydroxide to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (27:73)

Standard stock solution: 0.25 mg/mL of USP Ciprofloxacin Formamide RS in methanol

System suitability solution: 0.025 mg/mL of USP Dexamethasone RS and 0.025 mg/mL of USP Ciprofloxacin Formamide RS prepared as follows. Transfer 2.5 mg of USP Dexamethasone RS and 2.5 mg of USP Ciprofloxacin Formamide RS in a 100-mL volumetric flask, and dissolve in 15 mL of methanol before diluting with *Mobile phase* to volume.

Standard solution: 0.015 mg/mL from *Standard stock solution* in *Mobile phase*

Sample solution: Nominally 0.6 mg/mL in *Mobile phase* prepared as follows. Transfer an amount of freshly mixed Otic Suspension, equivalent to 6 mg, to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 15-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 8 between ciprofloxacin formamide and dexamethasone, *System suitability solution*
Column efficiency: NLT 2000 theoretical plates for ciprofloxacin formamide, *Standard solution*
Tailing factor: NMT 2.0 for ciprofloxacin formamide, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each related compound in the portion of Otic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ciprofloxacin formamide from the *Sample solution*
r_S = peak response of ciprofloxacin formamide from the *Standard solution*
C_S = concentration of USP Ciprofloxacin Formamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5% of the labeled amount of ciprofloxacin

Change to read:

• **CIPROFLOXACIN RELATED COMPOUNDS**
Analysis: From the chromatogram of the *Sample solution*, obtained as directed in the *Assay* for *Ciprofloxacin*, measure the responses for the ciprofloxacin ethylenediamine analog and the other minor peaks. Calculate the percentage of each related compound in the portion of Otic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (100/F)$$

r_U = peak response of each related compound from the *Sample solution*
r_S = peak response of ciprofloxacin from *Standard solution B*
C_S = concentration of USP Ciprofloxacin Hydrochloride RS in *Standard solution B*, calculated on the anhydrous basis (mg/mL)
C_U = nominal concentration of ciprofloxacin in the ▲*Sample solution*▲ (ERR 1-Dec-2018) (mg/mL)
M_{r1} = molecular weight of ciprofloxacin, 331.34
M_{r2} = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81
F = relative response factor (1.3 for ciprofloxacin ethylenediamine analog and 1.0 assumed for all other degradation products)

Acceptance criteria
Ciprofloxacin ethylenediamine analog: NMT 0.4% of the labeled amount of ciprofloxacin
Other single related compound: NMT 0.2%
Sum of all related compounds: NMT 0.8%

Change to read:

• **DEXAMETHASONE RELATED COMPOUNDS**
Analysis: From the chromatogram of the *Sample solution*, obtained as directed in the *Assay* for *Dexamethasone*, measure the responses for the dexamethasone glyoxal analog, the 17-carboxy-17-deoxy analog, and other minor peaks. Calculate the percentage of each related compound in the portion of Otic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each related compound from the *Sample solution*
r_S = peak response of dexamethasone from *Standard solution B*
C_S = concentration of USP Dexamethasone RS in *Standard solution B* (mg/mL)
C_U = nominal concentration of dexamethasone in the ▲*Sample solution*▲ (ERR 1-Dec-2018) (mg/mL)

Acceptance criteria
Dexamethasone glyoxal analog: NMT 1.0%
17-Carboxy-17-deoxy analog: NMT 2.6%
Other single related compound: NMT 0.3%
Sum of all related compounds: NMT 3.5%
[NOTE—The relative retention times for the dexamethasone glyoxal analog (9-Fluoro-11β-hydroxy-16α-methylandrosta-1,4-diene-3-one-17-ylglyoxal) and the 17-carboxy-17-deoxy analog (9-fluoro-11β-hydroxy-16α-methylandrosta-1,4-diene-3-one-17β-carboxylic acid) are about 1.4–1.6 and about 2.8–3.2, respectively.]

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 3.8–4.8
• **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed under *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
• **PARTICLE SIZE**
Carrier fluid: Heat Purified Water to a temperature of 40°–50°, add 100 mg/L of dexamethasone while stirring, cool to room temperature while stirring, pass through a 0.2-µm filter, and store in a clean, covered container.
Sample solution: Dilute a volume of 10 µL of Otic Suspension with *Carrier fluid* to 25 mL.
Analysis
(See *Particulate Matter in Injections* ⟨788⟩, *Light Obscuration Particle Count Test*.)
Analyze the *Sample solution* using an electronic, liquid-borne particle counting system that employs a light obscuration sensor with a suitable sample feeding device.
Acceptance criteria: NLT 99.5% of the particles are ≤25 µm, NLT 99.95% are ≤50 µm, and NLT 99.995% are ≤100 µm.
• **OSMOLALITY AND OSMOLARITY** ⟨785⟩, *Osmolality*: 270–330 mOsmol/kg

ADDITIONAL REQUIREMENTS

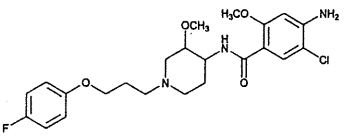
• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Avoid freezing.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ciprofloxacin Ethylenediamine Analog RS
1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.
$C_{15}H_{16}FN_3O_3 \cdot HCl$ 341.77

USP Monographs

www.webofpharma.com

USP Ciprofloxacin Formamide RS
1-Cyclopropyl-6-fluoro-7-(4-formyl-1-piperazinyl)-1,4-dihydro-4-oxo-3-quinolone-carboxylic acid.
$C_{18}H_{18}FN_3O_4$ 359.35
USP Ciprofloxacin Hydrochloride RS
USP Dexamethasone RS
USP Dexamethasone Acetate RS

Cisapride



$C_{23}H_{29}ClFN_3O_4$ 465.95
Benzamide, 4-amino-5-chloro-*N*-[1-[3-(4-fluorophen oxy) propyl]-3-methoxy-4-piperidinyl]-2-methoxy-, *cis*-.
cis-4-Amino-5-chloro-*N*-[1-[3-(*p*-fluorophenoxy)propyl]-3-methoxy-4-piperidyl]-*o*-anisamide [81098-60-4].
Monohydrate 484.0 [260779-88-2].

» Cisapride contains not less than 99.0 percent and not more than 101.0 percent of $C_{23}H_{29}ClFN_3O_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed, light-resistant containers, and store at room temperature.
USP Reference standards ⟨11⟩—
USP Cisapride RS
USP Haloperidol RS
Completeness of solution ⟨641⟩—A solution, 10 mg per mL in methylene chloride, meets the requirements.

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197K***▲ (CN 1-May-2020)
Specific rotation ⟨781S⟩: between −10° and +10°, measured at 20°.
Test solution: 10 mg per mL, in methylene chloride.
Water Determination, *Method I* ⟨921⟩ : between 3.4% and 4.0%.
Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Solution A—Prepare a 20 g per L solution of tetrabutylammonium hydrogen sulfate in water.
Solution B—Use methanol.
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Blank solution—Use methanol.
System suitability solution—Prepare a solution of USP Cisapride RS and USP Haloperidol RS in methanol containing about 0.05 mg per mL and 0.4 mg per mL, respectively.
Test solution 1—Dissolve an accurately weighed quantity of Cisapride, in methanol to obtain a solution having a known concentration of about 10 mg per mL.
Test solution 2—Dilute quantitatively and stepwise *Test solution 1* in methanol to obtain a solution having a known concentration of about 0.05 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 275-nm detector and a 4.0-mm × 10-cm column that contains 3-µm base-deactivated packing L1. The flow rate is about 1.2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–20	80→55	20→45	linear gradient
20–21	55→5	45→95	linear gradient
21–25	5	95	isocratic
25–26	5→80	95→20	return to initial conditions
26–30	80	20	re-equilibration

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the order of elution is cisapride followed by haloperidol, the resolution, *R,* between these two peaks is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0% for the cisapride peak.
Procedure—Inject a volume (about 10 µL) of the *Blank solution, Test solution 1,* and *Test solution 2* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of cisapride impurities in the portion of Cisapride taken by the formula:

$$100(C_S/C_i)(r_i/r_S)$$

in which C_S and C_i are the concentration of cisapride, in mg per mL, of *Test solution 2* and *Test solution 1,* respectively; r_i is the individual peak response of cisapride inpurities in *Test solution 1;* and r_S is cisapride peak area in *Test solution 2:* not more than 0.5% of any cisapride impurity is found, and not more than 1.0% of total impurities is found. Disregard any peak also found in the *Blank solution* and any peak with an area less than 0.1 times the area of the principal peak in the *Test solution 2* chromatogram.
Assay—Dissolve about 0.350 g of Cisapride, accurately weighed, in 70 mL of a mixture of methyl ethyl ketone and acetic acid (7:1). Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 46.60 mg of $C_{23}H_{29}ClFN_3O_4$.

Cisapride Compounded Injection, Veterinary

DEFINITION
Cisapride Compounded Injection, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of cisapride ($C_{23}H_{29}ClFN_3O_4$), calculated on the anhydrous basis.
Prepare Cisapride Compounded Injection, Veterinary 3 mg/mL as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Cisapride (as cisapride monohydrate) powder	30 mg (31.2 mg)
Tartaric acid 10% solution	0.75 mL
Sterile Water for Injection, a sufficient amount to make	10 mL

www.webofpharma.com

Dissolve the *Cisapride monohydrate powder* in 9 mL of *Sterile Water for Injection*. Add the *Tartaric acid 10% solution*. Add sufficient *Sterile Water for Injection* to bring to final volume, and mix well. Pass through a sterile filter of 0.22-µm pore size into a sterile single-dose container. [NOTE—Tartaric acid is added to aid in solubility.]

ASSAY

• **PROCEDURE**

Solution A: Add 0.2 mL of triethylamine to 1000 mL of water.

Mobile phase: Acetonitrile and *Solution A* (65:35)

Standard solution: 0.2 mg/mL of cisapride prepared from USP Cisapride RS in methanol

Sample solution: Transfer 0.67 mL of Injection, Veterinary to a 10-mL volumetric flask, dilute with methanol to volume, and sonicate to mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV-Vis 308 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for cisapride is about 2.0 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cisapride ($C_{23}H_{29}ClFN_3O_4$) in the portion of Injection, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cisapride from the *Sample solution*

r_S = peak response of cisapride from the *Standard solution*

C_S = concentration of USP Cisapride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cisapride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% on the anhydrous basis

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 2.1–3.1

• **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 5.0 USP Endotoxin Units/mg

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** It meets the requirements.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in single-dose sterile glass containers and store in a refrigerator (2°–8°).

• **LABELING:** Label it to indicate that it is for veterinary use only and to state the *Beyond-Use Date*.

Change to read:

BEYOND-USE DATE:

▲In the absence of passing a sterility test and endotoxins test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test and endotoxins test is passed, NMT 90 days after the date on which it was compounded when stored in a refrigerator (2°–8°). ▲ (CN 1-May-2020)

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cisapride RS

Cisapride Compounded Oral Suspension, Veterinary

DEFINITION

Cisapride Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of cisapride ($C_{23}H_{29}ClFN_3O_4$), calculated on the anhydrous basis.

Prepare Cisapride Compounded Oral Suspension, Veterinary 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Cisapride (as cisapride monohydrate) powder	1 g (1.04 g)
Vehicle: A 1:1 mixture of Ora-Plus[a] and Ora-Sweet,[a] a sufficient quantity to make	100 mL

[a] Perrigo Laboratories, Allegan, MI.

Pour the *Cisapride monohydrate powder* into a suitable container. Wet the powder with a small amount of *Vehicle* and triturate to make a smooth paste. Add the *Vehicle* to make the mortar contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: Add 0.2 mL of triethylamine to 1000 mL of water.

Mobile phase: Acetonitrile and *Solution A* (65:35)

Standard solution: 0.2 mg/mL of cisapride prepared from USP Cisapride RS in methanol

Sample solution: Transfer 1.0 mL of Oral Suspension, Veterinary to a 50-mL volumetric flask, dilute with methanol to volume, and sonicate to mix. Filter into an HPLC vial.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV-Vis 308 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for cisapride is about 2.0 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cisapride ($C_{23}H_{29}ClFN_3O_4$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

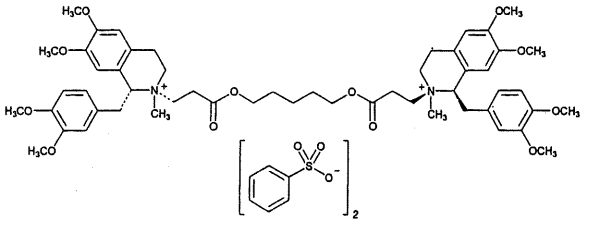
r_U = peak response of cisapride from the *Sample solution*
r_S = peak response of cisapride from the *Standard solution*
C_S = concentration of USP Cisapride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cisapride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% on the anhydrous basis

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.0–5.0

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant plastic containers. Store in a refrigerator (2°–8°) or at controlled room temperature.
- **Labeling:** Label it to indicate that it is for veterinary use only. Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored in a refrigerator (2°–8°) or at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
USP Cisapride RS

Cisatracurium Besylate



$C_{65}H_{82}N_2O_{18}S_2$ 1243.48
Isoquinolinium, 2,2′-[1,5-pentanediylbis[oxy(3-oxo-3,1-propanediyl)]]bis[1-[(3,4-dimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-, dibenzenesulfonate, [1*R*-[1α,2α(1′*R**,2′*R**)]]-;
(1*R*,2*R*)-2-(2-Carboxyethyl)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium benzenesulfonate, pentamethylene ester [96946-42-8].

DEFINITION
Cisatracurium Besylate contains NLT 97.0% and NMT 102.0% of cisatracurium besylate ($C_{65}H_{82}N_2O_{18}S_2$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**

Buffer: 33.3 g/L of ammonium formate prepared as follows. Dissolve 32.8 g of ammonium formate in 984 mL of water, and add 16 mL of anhydrous formic acid.
Mobile phase: Acetonitrile, methanol, and *Buffer* (20:20:60)
Diluent: Acetonitrile, methanol, and water (20:20:60). Add 0.4 mL of anhydrous formic acid per 1 L.
System suitability solution: 0.7 mg/mL of USP Cisatracurium Besylate System Suitability Mixture RS in *Diluent*
Standard solution: 0.7 mg/mL of USP Cisatracurium Besylate RS in *Diluent*
Sample solution: 0.7 mg/mL of Cisatracurium Besylate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25.0-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: NLT 2.5 times the retention time of cisatracurium
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between the peaks for *R-cis-R′-trans*-atracurium and cisatracurium, *System suitability solution*
Tailing factor: NMT 1.7 for cisatracurium, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cisatracurium besylate ($C_{65}H_{82}N_2O_{18}S_2$) in the portion of Cisatracurium Besylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cisatracurium Besylate RS in the *Standard solution* (mg/mL)
C_U = concentration of Cisatracurium Besylate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.1%
- **Organic Impurities**

Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Cisatracurium Besylate taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*. Disregard any peak representing less than 0.09% of the area of the major peak.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzenesulfonic acid[a]	0.10	—
cis-Quaternary acid[b]	0.14	0.2
(*R*)-*N*-Methyllaudanosine[c]	0.16	0.2
(*R*)-Laudanosine[d]	0.20	0.6
cis-Quaternary methyl ester[e]	0.23	0.4
cis-Quaternary alcohol[f]	0.29	0.5
R-trans-R'-trans-Atracurium[g]	0.74	0.2
R-cis-R'-trans-Atracurium[h]	0.87	0.8
Cisatracurium	1.0	—
trans-Monoquaternary compound[i]	1.17	0.5
trans-Monoacrylate[j]	1.28	0.5
cis-Monoquaternary compound[k]	1.39	0.7
cis-cis-Triester analog[l]	1.46	0.4
cis-Monoacrylate[m]	1.56	1.0
Any individual unspecified impurity	—	0.1
Total impurities	—	3.0

[a] This peak is due to the counterion and is not to be reported or included in total impurities.
[b] (1*R*,2*R*)-2-(2-Carboxyethyl)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium.
[c] (*R*)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2,2-dimethyl-1-veratrylisoquinolinium.
[d] (*R*)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinoline.
[e] (1*R*,2*R*)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2-[2-(methoxycarbonyl)ethyl]-2-methyl-1-veratrylisoquinolinium.
[f] (1*R*,2*R*)-1,2,3,4-Tetrahydro-2-(9-hydroxy-3-oxo-4-oxanonyl)-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium.
[g] (1*R*,1′*R*,2*S*,2′*S*)-2,2′-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium).
[h] (1*R*,1′*R*,2*R*,2′*S*)-2,2′-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium).
[i] (1*R*,1′*R*,2*S*)-2-Methyl-2,2′-(3,11-dioxo-4,10-dioxatridecamethylene) bis(1,2,3,4-tetrahydro-6,7-dimethoxy-1-veratrylisoquinolinium).
[j] (1*R*,2*S*)-2-(3,11-Dioxo-4,10-dioxa-12-tridecenyl)-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium).
[k] (1*R*,1′*R*,2*R*)-2-Methyl-2,2′-(3,11-dioxo-4,10-dioxatridecamethylene) bis(1,2,3,4-tetrahydro-6,7-dimethoxy-1-veratrylisoquinolinium).
[l] (1*R*,1′*R*,2*R*,2′*R*)-2,2′-(3,7,15-Trioxo-4,8,14-trioxa-heptadecamethylene) bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium).
[m] (1*R*,2*R*)-2-(3,11-Dioxo-4,10-dioxa-12-tridecenyl)-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium).

- **Limit of Methyl Benzenesulfonate**

[Note—Prepare the solutions immediately before use. Methyl benzenesulfonate is slowly hydrolyzed in aqueous solutions.]

Mobile phase: Acetonitrile and water (45:55)
Internal standard solution: 1.2 mg/mL of methyl *p*-toluenesulfonate in acetonitrile
Standard stock solution A: 1.2 mg/mL of methyl benzenesulfonate in acetonitrile
Standard stock solution B: 48 µg/mL of methyl benzenesulfonate from *Standard stock solution A* in *Mobile phase* prepared as follows. Transfer 2.0 mL of *Standard stock solution A* and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Standard solution: 2.4 µg/mL of methyl benzenesulfonate from *Standard stock solution B* in *Mobile phase*
Sample solution: Nominally 0.2 g/mL of Cisatracurium Besylate in *Mobile phase* prepared as follows. Transfer 1.0 g of Cisatracurium Besylate into a suitable separator. Immediately add 25 µL of *Internal standard solution*, and dissolve the contents in 25 mL of water using vigorous shaking. Add 25 mL of ethyl acetate, and shake vigorously for 2 min. Allow the phases to separate until the aqueous layer is clear and for NMT 2 h. Evaporate the organic layer to dryness in a current of air. Dissolve the residue in 5.0 mL of *Mobile phase* by sonication and gentle swirling.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25.0-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: 5 times the retention time of methyl benzenesulfonate
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between methyl benzenesulfonate and methyl *p*-toluenesulfonate
Relative standard deviation: NMT 2.0% for methyl benzenesulfonate and methyl *p*-toluenesulfonate
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration of methyl benzenesulfonate in the portion of Cisatracurium Besylate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak height ratio of methyl benzenesulfonate to the internal standard from the *Sample solution*

R_S = peak height ratio of methyl benzenesulfonate to the internal standard from the *Standard solution*

C_S = concentration of methyl benzenesulfonate in the *Standard solution* (µg/mL)

C_U = nominal concentration of Cisatracurium Besylate in the *Sample solution* (g/mL)

Acceptance criteria: NMT 10 ppm

SPECIFIC TESTS

- **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10.0 mg/mL in acetonitrile
Acceptance criteria: −60.0° to −54.0° at 20°, calculated on the anhydrous and solvent-free basis
- **Water Determination** ⟨921⟩: NMT 5.0%.
[Note—*Method Ia* or *Method Ic* may be used.]

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store cold, desiccated.
- **USP Reference Standards** ⟨11⟩
USP Cisatracurium Besylate RS

USP Monographs

www.webofpharma.com

USP Cisatracurium Besylate System Suitability Mixture RS
Cisatracurium besylate.
R-trans-R′-trans-Atracurium besylate:
(1*R*,1′*R*,2*S*,2′*S*)-2,2′-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) dibenzenesulfonate.
$C_{65}H_{82}N_2O_{18}S_2$ 1243.48
R-cis-R′-trans-Atracurium besylate:
(1*R*,1′*R*,2*R*,2′*S*)-2,2′-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) dibenzenesulfonate.
$C_{65}H_{82}N_2O_{18}S_2$ 1243.48
Other related compounds.

Cisatracurium Besylate Injection

DEFINITION

Cisatracurium Besylate Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of cisatracurium ($C_{53}H_{72}N_2O_{12}$). Multiple-dose containers may contain benzyl alcohol.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197F▲** (CN 1-May-2020)

Standard solution: 3 mg/mL of USP Cisatracurium Besylate RS in methylene chloride

Sample solution

[NOTE—If an emulsion forms during the extraction with methylene chloride, use a centrifuge to separate the layers.]

For Injections that do not contain benzyl alcohol: Nominally 2 mg/mL of cisatracurium from the Injection in methylene chloride prepared as follows. Transfer an amount of Injection equivalent to 10 mg of cisatracurium into a suitable separator, and extract with 5 mL of methylene chloride. Use the lower methylene chloride layer.

For Injections containing benzyl alcohol: Nominally 2 mg/mL of cisatracurium from the Injection in methylene chloride prepared as follows. Transfer an amount of Injection equivalent to 10 mg of cisatracurium into a suitable separator, and extract with 20.0 mL of ethyl acetate. Transfer the lower aqueous layer into another separator, and extract with 5 mL of methylene chloride. Use the lower methylene chloride layer.

Analysis

Samples: *Standard solution* and *Sample solution*

Add several drops of the *Standard solution* and *Sample solution* to separate potassium bromide plates. Allot the solvent to evaporate, and analyze the resulting residue.

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Acetonitrile, methanol, water, and anhydrous formic acid (21:21:60:1)

Mobile phase: 19.4 g/L of ammonium formate in *Solution A*. Filter under vacuum using a suitable filter.

Diluent: Acetonitrile, methanol, and water (20:20:60). Add 0.4 mL of anhydrous formic acid per 1 L.

System suitability solution: 0.7 mg/mL of USP Cisatracurium Besylate System Suitability Mixture RS in *Diluent*

Standard solution: 0.7 mg/mL of USP Cisatracurium Besylate RS in *Diluent*

Sample solution: Nominally 0.7 mg/mL of cisatracurium besylate from the Injection in *Diluent*, equivalent to 0.5 mg/mL of cisatracurium, prepared as follows. Using a "to contain" pipet, transfer a suitable volume of Injection to an appropriate volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25.0-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between *R-cis-R′-trans*-atracurium and cisatracurium, *System suitability solution*

Tailing factor: NMT 1.7 for cisatracurium, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cisatracurium ($C_{53}H_{72}N_2O_{12}$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of cisatracurium from the *Sample solution*

r_S = peak response of cisatracurium from the *Standard solution*

C_S = concentration of USP Cisatracurium Besylate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cisatracurium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of cisatracurium, 929.14

M_{r2} = molecular weight of cisatracurium besylate, 1243.48

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

- **BENZYL ALCOHOL CONTENT (IF PRESENT)**

Internal standard solution: 4.5 mg/mL of decanol in ethyl acetate

Standard stock solution: 9.0 mg/mL of USP Benzyl Alcohol RS in water

Standard solution: 0.45 mg/mL of benzyl alcohol from the *Standard stock solution* prepared as follows. Transfer 5.0 mL of *Standard stock solution* and 20.0 mL of ethyl acetate into a 50-mL centrifuge tube. Stopper, shake well for 30 s, and allow the phases to separate. Transfer 10.0 mL of the top (organic) layer and 5.0 mL of the *Internal standard solution* into a 50-mL volumetric flask. Dilute with ethyl acetate to volume.

Sample solution: Transfer 5.0 mL of the Injection and 20.0 mL of ethyl acetate into a 50-mL centrifuge tube. Stopper, shake well for 30 s, and allow the phases to separate. Transfer 10.0 mL of the top (organic) layer and 5.0 mL of

USP Monographs

www.webofpharma.com

the *Internal standard solution* into a 50-mL volumetric flask. Dilute with ethyl acetate to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 15-m fused-silica capillary column coated with a 1.0-µm layer of liquid phase G16
Temperatures
Detector: 250°
Injection port: 220°
Column: 140°
Carrier gas: Helium
Flow rate: 5.0 mL/min
Injection volume: 1 µL
Injection type: Split ratio, 2.5:1
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for decanol and benzyl alcohol are 0.62 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between decanol and benzyl alcohol
Relative standard deviation: NMT 2.0% for peak area ratios of benzyl alcohol to decanol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of benzyl alcohol in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/L) \times 100$$

R_U = peak area ratio of benzyl alcohol to the internal standard from the *Sample solution*
R_S = peak area ratio of benzyl alcohol to the internal standard from the *Standard solution*
C_S = concentration of USP Benzyl Alcohol RS in the *Standard stock solution* (mg/mL)
L = label claim of benzyl alcohol (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of benzyl alcohol

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.4 µg/mL of USP Cisatracurium Besylate RS in *Diluent*
Analysis
Samples: *Standard solution, Sample solution,* and *Sensitivity solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of cisatracurium from the *Standard solution*
C_S = concentration of USP Cisatracurium Besylate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cisatracurium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of cisatracurium, 929.14
M_{r2} = molecular weight of cisatracurium besylate, 1243.48

Acceptance criteria: See *Table 1*. Disregard any peaks with responses less than the peak from the *Sensitivity solution*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Besylate[a]	0.10	—
cis-Quaternary acid[b]	0.13	4.3
(*R*)-*N*-Methyllaudanosine[c, d]	0.15	—
(*R*)-Laudanosine[e]	0.19	4.0
cis-Quaternary methyl ester[f, d] and benzyl alcohol[g]	0.22	—
cis-Quaternary alcohol[h]	0.27	5.0
Benzaldehyde[i]	0.40	—
R-trans-R′-trans-Atracurium[j, d]	0.72	—
R-cis-R′-trans-Atracurium[k, d]	0.88	—
Cisatracurium	1.0	—
trans-Monoquaternary compound[l, d]	1.19	—
trans-Monoacrylate[m, d]	1.30	—
cis-Monoquaternary compound[n, d]	1.42	—
cis-cis-Triester analog[o, d]	1.47	—
cis-Monoacrylate[p]	1.58	2.5
Any individual unspecified degradation product	—	0.2
Total degradation products	—	14.4

[a] This peak is due to the counterion and is not to be reported or included in *Total degradation products*.
[b] (1*R*,2*R*)-2-(2-Carboxyethyl)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium benzenesulfonate.
[c] (*R*)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2,2-dimethyl-1-veratrylisoquinolinium benzenesulfonate.
[d] This is a process impurity that is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and is not to be included in the total degradation products.
[e] (*R*)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinoline.
[f] (1*R*,2*R*)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2-[2-(methoxycarbonyl)ethyl]-2-methyl-1-veratrylisoquinolinium benzenesulfonate.
[g] Benzyl alcohol co-elutes with *cis*-quaternary methyl ester. It is monitored using the test for *Benzyl Alcohol Content*.
[h] (1*R*,2*R*)-1,2,3,4-Tetrahydro-2-(9-hydroxy-3-oxo-4-oxanonyl)-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium benzenesulfonate.
[i] Benzaldehyde is a degradant of benzyl alcohol and is not included in the total impurities.
[j] (1*R*,1′*R*,2*S*,2′*S*)-2,2′-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) dibenzenesulfonate.
[k] (1*R*,1′*R*,2*R*,2′*S*)-2,2′-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) dibenzenesulfonate.
[l] (1*R*,1′*R*,2*S*)-2-Methyl-2,2′-(3,11-dioxo-4,10-dioxatridecamethylene) bis(1,2,3,4-tetrahydro-6,7-dimethoxy-1-veratrylisoquinolinium) dibenzenesulfonate.
[m] (1*R*,2*S*)-2-(3,11-Dioxo-4,10-dioxa-12-tridecenyl)-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) benzenesulfonate.
[n] (1*R*,1′*R*,2*R*)-2-Methyl-2,2′-(3,11-dioxo-4,10-dioxatridecamethylene) bis(1,2,3,4-tetrahydro-6,7-dimethoxy-1-veratrylisoquinolinium) dibenzenesulfonate.
[o] (1*R*,1′*R*,2*R*,2′*R*)-2,2′-(3,7,15-Trioxo-4,8,14-trioxa-heptadecamethylene) bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) dibenzenesulfonate.
[p] (1*R*,2*R*)-2-(3,11-Dioxo-4,10-dioxa-12-tridecenyl)-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) benzenesulfonate.

www.webofpharma.com

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.0–3.8
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 8.17 USP Endotoxin Units/mg of cisatracurium besylate
- **Sterility Tests** ⟨71⟩: Meets the requirements for the test for *Sterility of the Product to Be Examined, Membrane Filtration*
- **Other Requirements:** Meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store in a cool place, protected from freezing and light.
- **USP Reference Standards** ⟨11⟩

USP Benzyl Alcohol RS
USP Cisatracurium Besylate RS
USP Cisatracurium Besylate System Suitability Mixture RS
Cisatracurium besylate.
R-trans-R′-trans-Atracurium:
1*R*,1′*R*,2*S*,2′*S*)-2,2′-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) dibenzenesulfonate.
$C_{65}H_{82}N_2O_{18}S_2$ 1243.48
R-cis-R′-trans-Atracurium:
(1*R*,1′*R*,2*R*,2′*S*)-2,2′-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) dibenzenesulfonate.
$C_{65}H_{82}N_2O_{18}S_2$ 1243.48
Other related compounds.

Cisplatin



$Cl_2H_6N_2Pt$ 300.05
Platinum, diamminedichloro-, (*SP*-4-2)-;
cis-Diamminedichloroplatinum [15663-27-1].

DEFINITION
Cisplatin contains NLT 98.0% and NMT 102.0% of cisplatin ($Cl_2H_6N_2Pt$), calculated on the anhydrous basis.
[**Caution**—Cisplatin is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

ASSAY
- **Procedure**

Mobile phase: Ethyl acetate, methanol, dimethylformamide, and degassed water (25:16:5:5)
Standard solution: 1 mg/mL of USP Cisplatin RS in dimethylformamide. Use within 1 h.
Sample solution: 1 mg/mL of Cisplatin in dimethylformamide. Use within 1 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 310 nm
Column: 4.0-mm × 30-cm; packing L8
Flow rate: 2.0 mL/min
Injection volume: 40 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cisplatin ($Cl_2H_6N_2Pt$) in the portion of Cisplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cisplatin RS in the *Standard solution* (mg/mL)
C_U = concentration of Cisplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **Limit of Trichloroammineplatinate**

Mobile phase: 0.4 g/L of ammonium sulfate in water. Adjust with 6 N ammonium hydroxide to a pH of 5.9.
Standard solution: 6 µg/mL of USP Potassium Trichloroammineplatinate RS in saline TS. Protect the solution from light. Use the solution within 4 h.
Sample solution: 0.5 mg/mL of Cisplatin in saline TS. Completely dissolve by stirring by mechanical means for 30 min. Protect the solution from light. Use the solution within 4 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 209 nm
Column: 4.6-mm × 25-cm; packing L14
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[Note—The relative retention times for saline and trichloroammineplatinate are about 0.4 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the saline and trichloroammineplatinate peaks
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of trichloroammineplatinate in the portion of Cisplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of trichloroammineplatinate from the *Sample solution*
r_S = peak area of trichloroammineplatinate from the *Standard solution*
C_S = concentration of USP Potassium Trichloroammineplatinate RS in the *Standard solution* (mg/mL)
C_U = concentration of Cisplatin in the *Sample solution* (mg/mL)

USP Monographs

www.webofpharma.com

M_{r1} = molecular weight of trichloroammineplatinate, 318.48
M_{r2} = molecular weight of potassium trichloroammineplatinate, 357.58

Acceptance criteria: NMT 1.0%

• **LIMIT OF TRANSPLATIN**

Mobile phase: 0.18 M monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.2.

Standard stock solution A: 0.05 mg/mL of USP Transplatin RS in saline TS. Dissolve by stirring by mechanical means for 30 min.

Standard stock solution B: Transfer 5 mL of *Standard stock solution A* to a 25-mL volumetric flask containing 12 mg of USP Cisplatin RS. Dilute with saline TS to volume, and stir by mechanical means for 30 min to dissolve.

System suitability stock solution: 0.05 mg/mL of USP Cisplatin RS in saline TS. Dissolve by stirring by mechanical means for 30 min.

System suitability solution: Transfer 10 mL each of *System suitability stock solution* and *Standard stock solution A* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of thiourea (prepared fresh daily) and 5.0 mL of 1 N hydrochloric acid, and dilute with saline TS to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at 60 ± 0.5° for 60 min. Remove, and cool to room temperature.

Standard solution: Transfer 10 mL of *Standard stock solution B* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of thiourea (prepared fresh daily) and 5.0 mL of 1 N hydrochloric acid, and dilute with saline TS to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at 60 ± 0.5° for 60 min. Remove, and cool to room temperature.

Sample stock solution: 0.5 mg/mL of Cisplatin in saline TS. Dissolve by stirring by mechanical means for 30 min.

Sample solution: Transfer 10 mL of *Sample stock solution* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of thiourea (prepared fresh daily) and 5.0 mL of 1 N hydrochloric acid, and dilute with saline TS to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at 60 ± 0.5° for 60 min. Remove, and cool to room temperature.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L9

Column temperature: 45°

Flow rate: 2.0 mL/min

Injection volume: 20 µL

[NOTE—Condition the *Column* by pumping *Mobile phase* at a flow rate of 2.0 mL/min for 30 min, then at 0.5 mL/min for 30 min, and then again at 2.0 mL/min for 30 min.]

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The retention time for derivatized transplatin is between 5.0 and 9.0 min; or, if it is not, modify the *Mobile phase* as necessary, and recondition the *Column*. The relative retention times for derivatized cisplatin and derivatized transplatin are about 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 1.7 between the derivatized cisplatin and derivatized transplatin peaks, *System suitability solution*

Relative standard deviation: NMT 4.0% for the derivatized transplatin peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of transplatin in the portion of Cisplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of derivatized transplatin from the *Sample solution*
r_S = peak area of derivatized transplatin from the *Standard solution*
C_S = concentration of USP Transplatin RS in the *Standard solution* (mg/mL)
C_U = concentration of Cisplatin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.0%

• **UV PURITY RATIO**

Cleanse all glassware with a mixture of hydrochloric acid and nitric acid (3:1), rinse thoroughly with water, and dry before use. Do not use dichromate for cleaning. Do not use acetone or pressurized air for drying.

Sample: 98.5 ± 0.5 mg of ground Cisplatin

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: Maxima near 301 nm and minima near 246 nm

Cell: 2 cm

Blank: 0.1 N hydrochloric acid

Analysis: Transfer the *Sample* to a 100-mL volumetric flask, and add 0.1 N hydrochloric acid to volume. Using a clean magnetic stir bar, alternately stir at a high speed for 5 min and sonicate for 10 s until complete solution is effected, inverting the flask frequently to remove particles that may cling to the neck. Protect this solution from light, and use within 1 h of preparation. Obtain the absorption spectrum. Calculate the absorbance ratio as follows:

$$\text{Result} = A_{301}/A_{246}$$

A_{301} = absorbance at near 301 nm
A_{246} = absorbance at near 246 nm

Acceptance criteria: NLT 4.5

SPECIFIC TESTS

• **PLATINUM CONTENT**

Sample: 0.5 g of Cisplatin

Analysis: Ignite the *Sample* to constant weight at 800 ± 50°, and weigh the residue. The residue is platinum.

Calculate the platinum content in the portion of Cisplatin taken:

$$\text{Result} = (W_U/W_S) \times 100$$

W_U = weight of platinum
W_S = weight of the *Sample*

Acceptance criteria: 64.42%–65.22% on the anhydrous basis

• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from light. Store at room temperature.

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cisplatin RS
USP Potassium Trichloroammineplatinate RS
Cl_3H_3KNPt 357.58
USP Transplatin RS

Cisplatin for Injection

DEFINITION
Cisplatin for Injection is a sterile, lyophilized mixture of Cisplatin, Mannitol, and Sodium Chloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cisplatin ($Cl_2H_6N_2Pt$).
[CAUTION—Cisplatin is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.]

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHY**
Solution A: 5.6 g of stannous chloride in 10 mL of hydrochloric acid. Stir for 5 min. [NOTE—It is not necessary that all of the solids dissolve.]
Solution B: 0.2 g of potassium iodide in 90 mL of water
Standard solution: 1.0 mg/mL of USP Cisplatin RS, 9 mg/mL of sodium chloride, and 10 mg/mL of D-mannitol in water
Sample solution: Nominally equivalent to 1.0 mg/mL of cisplatin by dissolving the contents of 1 container of Cisplatin for Injection in water, based on the label claim
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Acetone and 1 N nitric acid (9:1)
Spray reagent A: Mix *Solution A* and *Solution B* together. Disregard any precipitate that is formed. Store in the dark. [NOTE—The solution is usable for at least 1 week.]
Spray reagent B: 20 mg/mL of potassium iodide in water
Analysis
Samples: *Standard solution* and *Sample solution*
Develop with *Developing solvent system*, in a suitable chromatographic chamber containing a filter paper lining and equilibrated for 30 min with the *Developing solvent system*, for a distance of about 8 cm from the origin, followed by air drying. Complete the drying by heating in a forced-air oven at about 100° for 1 min. Spray with *Spray reagent A*, heat in an oven at about 100° for 5 min, cool, and spray with *Spray reagent B* to bring out the full color of the spots.
Acceptance criteria: The principal spot from the *Sample solution* corresponds in appearance and R_F value to that produced by the *Standard solution*.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Ethyl acetate, methanol, dimethylformamide, and degassed water (25:16:5:5)
Standard solution: 1 mg/mL of USP Cisplatin RS in dimethylformamide. Use within 1 h.
Sample solution: Nominally equivalent to 1.0 mg/mL of cisplatin in dimethylformamide prepared as follows. Quantitatively dissolve 1 container of Cisplatin for Injection with dimethylformamide by sonicating for 5 min. Pass 5 mL of this solution through a suitable filter and collect the filtrate after discarding the first mL of the filtrate. Use within 1 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 310 nm
Column: 4.0-mm × 30-cm; packing L8
Flow rate: 2.0 mL/min
Injection volume: 40 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cisplatin ($Cl_2H_6N_2Pt$) in the portion of Cisplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cisplatin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cisplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES
• **LIMIT OF TRICHLOROAMMINEPLATINATE**
Mobile phase: 0.4 g/L of ammonium sulfate in water. Adjust with 6 N ammonium hydroxide to a pH of 5.9.
Standard solution: 6 µg/mL of USP Potassium Trichloroammineplatinate RS in saline TS. Protect the solution from light. Use the solution within 4 h.
Sample solution: Nominally equivalent to 0.5 mg/mL of cisplatin by quantitatively dissolving the contents of 1 container of Cisplatin for Injection with water. Protect the solution from light. Use the solution within 4 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 209 nm
Column: 4.6-mm × 25-cm; packing L14
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for saline and trichloroammineplatinate are about 0.4 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the saline and trichloroammineplatinate peaks
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of trichloroammineplatinate in the portion of Cisplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

USP Monographs

www.webofpharma.com

r_U = peak area of trichloroammineplatinate from the *Sample solution*
r_S = peak area of trichloroammineplatinate from the *Standard solution*
C_S = concentration of USP Potassium Trichloroammineplatinate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cisplatin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of trichloroammineplatinate, 318.48
M_{r2} = molecular weight of potassium trichloroammineplatinate, 357.58

Acceptance criteria: NMT 1.0%

• **LIMIT OF TRANSPLATIN**

Mobile phase: 0.18 M monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.2.

Standard stock solution A: 0.05 mg/mL of USP Transplatin RS in saline TS. Dissolve by stirring by mechanical means for 30 min.

Standard stock solution B: Transfer 5 mL of *Standard stock solution A* to a 25-mL volumetric flask containing 12 mg of USP Cisplatin RS. Dilute with saline TS to volume, and stir by mechanical means for 30 min to dissolve.

System suitability stock solution: 0.05 mg/mL of USP Cisplatin RS in saline TS. Dissolve by stirring by mechanical means for 30 min.

System suitability solution: Transfer 10 mL each of *System suitability stock solution* and *Standard stock solution A* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of thiourea (prepared fresh daily) and 5.0 mL of 1 N hydrochloric acid, and dilute with saline TS to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at 60 ± 0.5° for 60 min. Remove, and cool to room temperature.

Standard solution: Transfer 10 mL of *Standard stock solution B* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of thiourea (prepared fresh daily) and 5.0 mL of 1 N hydrochloric acid, and dilute with saline TS to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at 60 ± 0.5° for 60 min. Remove, and cool to room temperature.

Sample stock solution: Nominally equivalent to 0.5 mg/mL of cisplatin by dissolving the contents of 1 container of Cisplatin for Injection with water

Sample solution: Transfer 10 mL of *Sample stock solution* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of thiourea (prepared fresh daily) and 5.0 mL of 1 N hydrochloric acid, and dilute with saline TS to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytef-lined closure, and heat in a heating block at 60 ± 0.5° for 60 min. Remove, and cool to room temperature.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L9
Column temperature: 45°
Flow rate: 2.0 mL/min
Injection volume: 20 µL

[NOTE—Condition the *Column* by pumping the *Mobile phase* at a flow rate of 2.0 mL/min for 30 min, then at 0.5 mL/min for 30 min, and then again at 2.0 mL/min for 30 min.]

System suitability
Samples: *System suitability solution* and *Standard solution*

[NOTE—The retention time for derivatized transplatin is between 5.0 and 9.0 min; or, if it is not, modify the *Mobile phase* as necessary, and recondition the *Column*. The relative retention times for derivatized cisplatin and derivatized transplatin are about 1.0 and 1.3, respectively.]

Suitability requirements
Resolution: NLT 1.7 between the derivatized cisplatin and derivatized transplatin peaks, *System suitability solution*
Relative standard deviation: NMT 4.0% for the derivatized transplatin peak, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of transplatin in the portion of Cisplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of derivatized transplatin from the *Sample solution*
r_S = peak area of derivatized transplatin from the *Standard solution*
C_S = concentration of USP Transplatin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cisplatin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.0%

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample: Constituted as directed in the labeling, using *Sterile Water for Injection*
Acceptance criteria: 3.5–6.2

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 2.0 USP Endotoxin Units/mg of cisplatin.

• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements

• **WATER DETERMINATION** ⟨921⟩, *Method I*
Sample: One container of Cisplatin for Injection
Analysis: Use anhydrous formamide as the extraction solvent, and use the following procedure. Introduce 50 mL of anhydrous formamide into the titration vessel, and titrate with the *Reagent* to the electrometric endpoint. Use the formamide thus dried to rinse a suitable glass syringe equipped with a 22-gauge needle, about 8 cm long. Add the rinse back to the titration vessel, and again titrate the vessel contents, if necessary. Via the syringe, withdraw 5 mL of the formamide thus titrated, and, through the closure of the container, expel the contents into the container. Shake the container to obtain a solution. With the same syringe, withdraw all of the contents of the container, and transfer to the titration vessel. Titrate to the endpoint, adjusting the feeding speed control to the lowest setting, to avoid over-titration.
Acceptance criteria: NMT 2.0%

• **CONSTITUTED SOLUTIONS:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

• **OTHER REQUIREMENTS:** It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*. Protect from light.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cisplatin RS

USP Monographs

www.webofpharma.com

USP Potassium Trichloroammineplatinate RS
Cl_3H_3KNPt 357.58
USP Transplatin RS

Citalopram Oral Solution

DEFINITION
Citalopram Oral Solution contains an amount of citalopram hydrobromide equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of citalopram free base ($C_{20}H_{21}FN_2O$). It may contain a suitable preservative.

IDENTIFICATION
• **A.** The retention time of the citalopram peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

• **PROCEDURE**
Solution A: Methanol and acetonitrile▲(10:90)▲ 2S (USP41)
Buffer: 6.1 g/L of monobasic potassium phosphate in water. Add 1.5 mL of triethylamine per liter of the solution. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: *Solution A* and *Buffer* ▲(28:72)▲ 2S (USP41)
Diluent: Acetonitrile and *Buffer* ▲(25:75)▲ 2S (USP41)
Standard solution: 0.25 mg/mL of USP Citalopram Hydrobromide RS, ▲equivalent to 0.2 mg/mL of citalopram free base in *Diluent*▲ 2S (USP41)
Sample solution: ▲Nominally equivalent to 0.2 mg/mL of citalopram free base, prepared as follows. Transfer a suitable volume of Oral Solution to a suitable volumetric flask.▲ 2S (USP41) Add 50% of the flask volume of *Diluent*, and sonicate at room temperature for 3 min with intermittent shaking. Allow the solution to cool, and dilute with *Diluent* to volume. [NOTE—The *Sample solution* may be passed through either a PVDF or nylon membrane filter of suitable pore size.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. ▲For *Identification B*, use a diode array detector in the range of 210–400 nm.▲ 2S (USP41)
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: ▲NLT▲ 2S (USP41) 2 times the retention time of citalopram
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: ▲NMT 0.73%▲ 2S (USP41)
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of citalopram ($C_{20}H_{21}FN_2O$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of citalopram from the *Sample solution*
r_S = peak response of citalopram from the *Standard solution*
C_S = concentration of USP Citalopram Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of citalopram in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of citalopram free base, 324.39
M_{r2} = molecular weight of citalopram hydrobromide, 405.30

Acceptance criteria: 90.0%–110.0% of citalopram free base ($C_{20}H_{21}FN_2O$)

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
Solution A: Acetonitrile, *methanol*, and tetrahydrofuran▲(85:5:10)▲ 2S (USP41)
Buffer: Dissolve 3.0 g of ▲octanesulfonic acid sodium salt▲ 2S (USP41) in 1 L of water. Add 2 mL of triethylamine and 5 mL of ▲tetrabutylammonium hydroxide, 40 percent in water.▲ 2S (USP41)Mix, and adjust with phosphoric acid to a pH of 3.0.
Mobile phase: *Solution A* and *Buffer* ▲(25:75)▲ 2S (USP41)
Diluent: Acetonitrile and water▲(25:75)▲ 2S (USP41)
System suitability solution: 6 µg/mL of USP Citalopram Related Compound D RS and 1.3 mg/mL of USP Citalopram Hydrobromide RS in *Diluent*
Standard solution: ▲0.0063 mg/mL▲ 2S (USP41) of USP Citalopram Hydrobromide RS in *Diluent*
Sample solution: ▲Nominally equivalent to 1 mg/mL of citalopram free base, prepared as follows. Transfer a suitable volume of Oral Solution to a suitable volumetric flask.▲ 2S (USP41) Add 60% of the flask volume of *Diluent*, and sonicate at room temperature for 3 min with intermittent shaking. Allow the solution to cool, and dilute with *Diluent* to volume. [NOTE—The *Sample solution* may be passed through either a PVDF or nylon membrane filter of suitable pore size.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: ▲NLT▲ 2S (USP41) 2.6 times the retention time of citalopram for the *System suitability solution* and the *Standard solution*; ▲NLT▲ 2S (USP41) 5.7 times the retention time of citalopram for the *Sample solution*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.8 between citalopram and citalopram related compound D, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

USP Monographs

www.webofpharma.com

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ▲each degradation product▲ 2S (USP41) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each ▲degradation product▲ 2S (USP41) from the *Sample solution*

r_S = peak response of citalopram from the *Standard solution*

C_S = concentration of USP Citalopram Hydrobromide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of citalopram in the *Sample solution* (mg/mL)

F = relative response factor for each ▲degradation product▲ 2S (USP41) (see *Table 1*)

M_{r1} = molecular weight of citalopram free base, 324.39

M_{r2} = molecular weight of citalopram hydrobromide, 405.30

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citalopram related compound A[a]	0.29	0.86	0.20
Carboxy citalopram[b]	0.47	0.72	0.20
Desfluorocitalopram[c, d]	0.71	—	—
Citalopram related compound C[e]	0.83	1.8	0.20
Citalopram	1.0	—	—
Citalopram related compound D	1.11	0.95	0.20
Citalopram related compound G[d, f]	3.13	—	—
Citalopram related compound H[d, g]	3.75	—	—
Any individual, unspecified degradation product	—	1.0	0.15
Total ▲degradation products▲ 2S (USP41)	—	—	0.50

[a] 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.

[b] 1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxylic acid.

[c] 1-[3-(Dimethylamino)propyl]-1-phenyl-1,3-dihydroisobenzofuran-5-carbonitrile.

[d] This process impurity is included in the table for identification only. It is controlled in the drug substance, and is not to be reported or included in the total ▲degradation products▲ 2S (USP41) for the drug product.

[e] 3-(3-Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3*H*)-isobenzofuranone.

[f] 3-[5-Chloro-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-*N*,*N*-dimethylpropan-1-amine.

[g] 3-[5-Bromo-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-*N*,*N*-dimethylpropan-1-amine.

SPECIFIC TESTS

- **DELIVERABLE VOLUME** ⟨698⟩: Meets the requirements
- **pH** ⟨791⟩: 3.5–7.0

Change to read:

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed ▲10^2 cfu/mL.▲ 2S (USP41) The total yeasts and molds count does not exceed 5×10^1 cfu/mL. It meets the requirements of the test for the absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant containers at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Citalopram Hydrobromide RS

USP Citalopram Related Compound D RS

[NOTE—May be available as a hydrochloride or hydrobromide salt.]

1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrochloride.

$C_{19}H_{19}FN_2O \cdot HCl$ 346.83

1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrobromide.

$C_{19}H_{19}FN_2O \cdot HBr$ 391.28

Citalopram Tablets

DEFINITION

Citalopram Tablets contain an amount of citalopram hydrobromide equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of citalopram free base ($C_{20}H_{21}FN_2O$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Extract finely ground Tablet powder containing 200 mg of citalopram with 30 mL of water, and filter. Add 1 mL of 1 N sodium hydroxide to the filtrate, and extract with 50 mL of cyclohexane by shaking for 10 min. Pass the cyclohexane layer through a silicone-treated filter paper into a beaker. Reduce the filtrate down to 3 mL, using gentle heat as necessary. Transfer the hot solution to a small centrifuge tube. Induce crystallization while cooling by scratching the side of the test tube with a spatula. Centrifuge the mixture, and decant off the cyclohexane. Dry the residue under vacuum in a desiccator. [NOTE—If crystallization fails to occur in the above procedure, use the following alternative procedure. Extract finely ground Tablet powder containing about 50 mg of citalopram with 10 mL of chloroform in a test tube, and sonicate for 1 min. Centrifuge for 10 min, and filter into a beaker. Evaporate to dryness with nitrogen and, if necessary, induce crystallization by etching the beaker.]

Mix approximately 2 mg of the residue with approximately 300 mg of potassium bromide, and record the IR spectrum.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 1.42 g/L of anhydrous dibasic sodium phosphate in water

USP Monographs

www.webofpharma.com

Diluent: Methanol and *Buffer* (80:20)
Mobile phase: 0.77 mg/mL of dodecyltrimethylammonium bromide in *Diluent*
Internal standard solution: 0.25 mg/mL of USP Citalopram Related Compound F RS in *Diluent*
Standard stock solution: 1.25 mg/mL of USP Citalopram Hydrobromide RS in *Diluent*
Standard solution: 0.025 mg/mL of USP Citalopram Related Compound F RS and 0.125 mg/mL of USP Citalopram Hydrobromide RS from the *Internal standard solution* and the *Standard stock solution*, respectively, in *Diluent*
Sample solution: Transfer 10 Tablets to a 200-mL volumetric flask, add 25 mL of *Buffer*, and shake by mechanical means until disintegrated. Add 100 mL of methanol, and sonicate for about 5 min. Allow to cool to room temperature, and then dilute with *Diluent* to volume. Before taking an aliquot for dilution, allow to stand until the residue settles. Transfer a volume of the clear supernatant to a 50-mL volumetric flask to obtain a final nominal concentration between 0.090 and 0.10 mg/mL of citalopram. Add 5.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume. Pass a portion through a filter (PTFE) having a 0.45-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for citalopram related compound F and citalopram are about 1.36 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between citalopram and citalopram related compound F
Column efficiency: NLT 2000 theoretical plates, calculated from the citalopram peak
Relative standard deviation: NMT 1.5% for the peak response ratio of citalopram to the internal standard
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of citalopram ($C_{20}H_{21}FN_2O$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of citalopram to the internal standard from the *Sample solution*
R_S = peak response ratio of citalopram to the internal standard from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of citalopram, 324.39
M_{r2} = molecular weight of citalopram hydrobromide, 405.30

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Buffer: pH 1.5 buffer (prepared by transferring 118 mL of 1 N hydrochloric acid and 82 mL of 1 N sodium hydroxide to a 1000-mL volumetric flask, diluting with water to volume, and adjusting with 1 N sodium hydroxide to a pH of 1.5)
Medium: *Buffer*, 800 mL, deaerated
Apparatus 1: 100 rpm
Time: 30 min
Standard solution: 12 µg/mL of USP Citalopram Hydrobromide RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Pass through a PVDF filter having a 0.45-µm pore size, and dilute with *Medium* as needed.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 239 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of citalopram ($C_{20}H_{21}FN_2O$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 800 mL
D = dilution factor of the *Sample solution*
M_{r1} = molecular weight of citalopram, 324.39
M_{r2} = molecular weight of citalopram hydrobromide, 405.30
L = label claim of citalopram (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of citalopram ($C_{20}H_{21}FN_2O$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer: 3.15 g/L of monobasic potassium phosphate and 3.60 g/L of dibasic sodium phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) in water
Mobile phase: Methanol, acetonitrile, and *Buffer* (38:7:55). Adjust with phosphoric acid to a pH of 6.5.
Standard stock solution: 0.25 mg/mL of USP Citalopram Hydrobromide RS in *Mobile phase*
System suitability solution: 1 µg/mL each of USP Citalopram Related Compound A RS, USP Citalopram Related Compound B RS, USP Citalopram Related Compound C RS, and USP Citalopram Related Compound E RS in the *Standard stock solution*
Standard solution: 0.625 µg/mL of citalopram hydrobromide from the *Standard stock solution* in *Mobile phase*
Sensitivity solution: 0.05 µg/mL of citalopram hydrobromide from the *Standard solution* in *Mobile phase*
Sample solution: Transfer 10 Tablets to a 200-mL volumetric flask, add 25 mL of *Buffer*, and shake by mechanical means until disintegrated. Add 100 mL of a mixture of methanol and water (1:1), mix, and sonicate for about 5 min. Allow to cool, dilute with a mixture of methanol and water (1:1) to volume, and mix thoroughly. Allow the excipients to settle. Dilute with *Mobile phase*, as necessary, to a final concentration of 0.5 mg/mL of citalopram. Pass a portion of this solution through a polytetrafluoroethylene (PTFE) membrane filter having a 0.45-µm or finer pore size, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1

www.webofpharma.com

Column temperature: 45°
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 3 between citalopram related compound C and citalopram, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 3, *Sensitivity solution*
Analysis
Samples: *System suitability solution*, *Standard solution*, and *Sample solution*
Chromatograph the *System suitability solution*, and identify the components on the basis of their relative retention times given in *Table 1*.
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of the corresponding peak from the *Standard solution*
C_S = concentration of citalopram hydrobromide in the *Standard solution* (mg/mL)
C_U = nominal concentration of citalopram in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of citalopram, 324.39
M_{r2} = molecular weight of citalopram hydrobromide, 405.30
F = relative response factor (see *Table 1*)

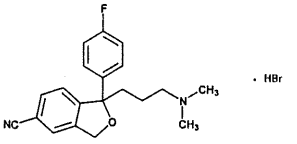
Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citalopram related compound A	0.43	0.77	0.2
Citalopram related compound B	0.60	0.98	0.25
Citalopram related compound C	0.83	0.69	0.25
Citalopram	1.0	—	—
Citalopram related compound E	1.32	0.91	0.1
Any other individual, unidentified impurity	—	1.0	0.2
Total impurities	—	—	0.8

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Citalopram Hydrobromide RS
USP Citalopram Related Compound A RS
1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.
$C_{20}H_{23}FN_2O_2$ 342.22
USP Citalopram Related Compound B RS
1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydroisobenzofuran-5-carbonitrile oxalate.
$C_{20}H_{21}FN_2O_2 \cdot C_2H_2O_4$ 430.43
USP Citalopram Related Compound C RS
3-(3-Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3*H*)-isobenzofuranone oxalate.
$C_{20}H_{19}FN_2O_2 \cdot C_2H_2O_4$ 428.42
USP Citalopram Related Compound E RS
1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydrobenzofuran-5-carbonitrile-*N*-oxide hydrochloride.
$C_{20}H_{21}FN_2O_2 \cdot HCl$ 376.85
USP Citalopram Related Compound F RS
Dimethyl-(1-methyl-3,3-diphenylallyl)amine hydrochloride.
$C_{18}H_{21}N \cdot HCl$ 287.83

Citalopram Hydrobromide



$C_{20}H_{21}FN_2O \cdot HBr$ 405.30
5-Isobenzofurancarbonitrile, 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-, monohydrobromide; 1-[3-(Dimethylamino)propyl]-1-(*p*-fluorophenyl)-5-phthalancarbonitrile monohydrobromide [59729-32-7].

DEFINITION
Citalopram Hydrobromide contains NLT 98.0% and NMT 102.0% of citalopram hydrobromide ($C_{20}H_{21}FN_2O \cdot HBr$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL 191,** *Bromide*
Sample solution: 10 mg/mL of Citalopram Hydrobromide in water
Acceptance criteria: Meets the requirements of test *B*

ASSAY
- **PROCEDURE**
Buffer: Dissolve 1 g of sodium acetate in 800 mL of water, and add 6 mL of triethylamine. Adjust with acetic acid to a pH of 4.6, and dilute with water to 1 L.
Mobile phase: Acetonitrile and *Buffer* (20:80). The apparent pH is 5.0 ± 0.1. Make adjustments, if necessary.
Diluent: Methanol and water (50:50)
System suitability solution: 1 µg/mL each of USP Citalopram Hydrobromide RS and USP Citalopram Related Compound D RS in *Diluent*
Standard solution: 0.625 mg/mL of USP Citalopram Hydrobromide RS in *Diluent*
Sample solution: 0.625 mg/mL of Citalopram Hydrobromide in *Diluent*

USP Monographs

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.3 times the retention time of citalopram
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.8 between citalopram related compound D and citalopram, *System suitability solution*
Column efficiency: NLT 3000 theoretical plates, *Standard solution*
Tailing factor: NMT 3.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Diluent*, *Standard solution*, and *Sample solution*
Verify that there are no interfering peaks, using the *Diluent*.
Calculate the percentage of citalopram hydrobromide ($C_{20}H_{21}FN_2O \cdot HBr$) in the portion of Citalopram Hydrobromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Citalopram Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Citalopram Hydrobromide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Analysis: Moisten the sample with 2 mL of nitric acid and 5 drops of sulfuric acid.
Acceptance criteria: NMT 0.1%

• **ORGANIC IMPURITIES, PROCEDURE 1**
[NOTE—On the basis of the synthetic route used, perform either *Procedure 1* or *Procedure 2*. However, if the chloro and bromo analogs are potential related compounds in the synthetic route used, *Procedure 2* is recommended.]
Buffer, Mobile phase, Diluent, System suitability solution, and Sample solution: Proceed as directed in the *Assay*.
Standard solution: 0.625 µg/mL of USP Citalopram Hydrobromide RS in *Diluent*
Sensitivity solution: 0.0625 µg/mL of USP Citalopram Hydrobromide RS in *Diluent*
Chromatographic system: Proceed as directed in the *Assay*, except use a *Run time* of 1.7 times the retention time of citalopram.
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.8 between citalopram related compound D and citalopram, *System suitability solution*
Tailing factor: 0.8–1.5 for citalopram, *System suitability solution*
Relative standard deviation: NMT 5% for citalopram, *System suitability solution*
Signal-to-noise ratio: NLT 3, *Sensitivity solution*
Analysis
Samples: *Diluent*, *Standard solution*, and *Sample solution*
Verify that there are no interfering peaks, using the *Diluent*.
Calculate the percentage of each impurity in the portion of Citalopram Hydrobromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of citalopram from the *Standard solution*
C_S = concentration of USP Citalopram Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Citalopram Hydrobromide in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of citalopram, 324.39
M_{r2} = molecular weight of citalopram hydrobromide, 405.30
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor[a]	Acceptance Criteria, NMT (%)
Citalopram ketone[b]	0.13	0.34	0.1
Citalopram related compound A	0.18	0.77	0.1
Citalopram open ring[c]	0.26	0.99	0.1
Citalopram related compound B[d]	0.40	0.98	0.1
Citalopram related compound C	0.67	0.69	0.1
Citalopram related compound D	0.90	1.04	0.1
Citalopram	1.0	—	—
Citalopram related compound E[e]	1.29	0.91	0.1
Individual unknown impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] The relative response factors provided are for each impurity relative to citalopram (free base).
[b] 4-(Dimethylamino)-1-(1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl}butan-1-one.
[c] 4-[4-(Dimethylamino)-1-(4-fluorophenyl)-1-hydroxybutyl]-3-(hydroxymethyl)benzonitrile.
[d] 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydroisobenzofuran-5-carbonitrile.
[e] 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile-*N*-oxide.

• **ORGANIC IMPURITIES, PROCEDURE 2**
Buffer: To each L of 2.7 g/L of monobasic potassium phosphate in water prepared, add 1 mL of *N,N*-dimethyloctylamine, and adjust with phosphoric acid to a pH of 3.0.
Solution A: Methanol, tetrahydrofuran, and *Buffer* (24:6:70)
Mobile phase: See *Table 2*.

USP Monographs

www.webofpharma.com

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
18	100	0
40	10	90
45	10	90
46	100	0
55	100	0

Diluent: Acetonitrile and *Buffer* (30:70)
System suitability solution: 1.5 µg/mL each of USP Citalopram Hydrobromide RS, USP Citalopram Related Compound A RS, USP Citalopram Related Compound C RS, USP Citalopram Related Compound D RS, USP Citalopram Related Compound G RS, and USP Citalopram Related Compound H RS in *Diluent*
Standard solution: 1.5 µg/mL of USP Citalopram Hydrobromide RS in *Diluent*
Sample solution: 1.5 mg/mL of Citalopram Hydrobromide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 224 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between citalopram and citalopram related compound D; NLT 4.0 between citalopram related compound G and citalopram related compound H
Tailing factor: NMT 1.5 for citalopram
Relative standard deviation: NMT 2.0% for citalopram
Analysis
Samples: *System suitability solution*, *Standard solution*, and *Sample solution*
Chromatograph the *System suitability solution*, and identify the components on the basis of their relative retention times, as shown in *Table 3*.
Calculate the percentage of each impurity in the portion of Citalopram Hydrobromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of citalopram from the *Standard solution*
C_S = concentration of USP Citalopram Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Citalopram Hydrobromide in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor[a]	Acceptance Criteria, NMT (%)
Bromide[b]	0.24	—	—
Citalopram related compound A	0.40	0.73	0.15
Citalopram related compound C	0.88	1.7	0.15
Citalopram	1.0	—	—
Citalopram related compound D	1.09	0.93	0.15
Citalopram related compound G	2.20	1.2	0.15
Citalopram related compound H	2.30	1.1	0.15
Individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.75

[a] The relative response factors provided are for each impurity relative to citalopram hydrobromide.
[b] This peak is due to the counterion. It is not an impurity and should not be included in the *Total impurities*.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 25 mg/mL of Citalopram Hydrobromide in methanol
Acceptance criteria: –0.2° to +0.2° at 20°
- **pH** ⟨791⟩
Sample solution: 5 mg/mL of Citalopram Hydrobromide in water
Acceptance criteria: 5.5–6.5
- **WATER DETERMINATION,** *Method I* ⟨921⟩
Sample: 250 mg of Citalopram Hydrobromide
Acceptance criteria: NMT 0.5%
- **COMPLETENESS OF SOLUTION**
Blank: 96% alcohol
Sample solution: 25 mg/mL of Citalopram Hydrobromide in 96% alcohol
Analytical wavelength: 410 nm
Acceptance criteria: Absorbance is NMT 0.040 in a 1-cm quartz cell

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** If a procedure for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* procedure the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Citalopram Hydrobromide RS
USP Citalopram Related Compound A RS
1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.
$C_{20}H_{23}FN_2O_2$ 342.41
USP Citalopram Related Compound C RS
3-(3-Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3*H*)-isobenzofuranone oxalate.
$C_{20}H_{19}FN_2O_2 \cdot C_2H_2O_4$ 428.42
USP Citalopram Related Compound D RS
[NOTE—May be available as a hydrochloride or a hydrobromide salt.]
1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrochloride.
$C_{19}H_{19}FN_2O \cdot HCl$ 346.83

www.webofpharma.com

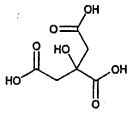
1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrobromide.
$C_{19}H_{19}FN_2O \cdot HBr$ 391.28

USP Citalopram Related Compound G RS
3-[5-Chloro-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-*N*,*N*-dimethylpropan-1-amine hydrobromide.
$C_{19}H_{21}ClFNO \cdot HBr$ 414.74

USP Citalopram Related Compound H RS
3-[5-Bromo-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-*N*,*N*-dimethylpropan-1-amine hydrobromide.
$C_{19}H_{21}BrFNO \cdot HBr$ 459.19

Anhydrous Citric Acid

Portions of the monograph text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (‣ ◂) to specify this fact.



$C_6H_8O_7$ 192.1
1,2,3-Propanetricarboxylic acid, 2-hydroxy-;
Citric acid [77-92-9].

DEFINITION
Anhydrous Citric Acid contains NLT 99.5% and NMT 100.5% of $C_6H_8O_7$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K.**▲ (CN 1-May-2020) Dry the substance to be examined at 105° for 2 h.

ASSAY
• **PROCEDURE**
Sample: 0.550 g of Anhydrous Citric Acid; record weight accurately.
Analysis: Dissolve the *Sample* in 50 mL of water. Add 0.5 mL of phenolphthalein TS. Titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 64.03 mg of $C_6H_8O_7$.
Acceptance criteria: 99.5%–100.5% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%, determined on 1.0 g
• **SULFATE**
Standard sulfate solution A: 1.81 mg/mL of potassium sulfate in 30% alcohol. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with 30% alcohol to volume, and mix. This solution contains 10 µg/mL of sulfate.
Standard sulfate solution B: 1.81 mg/mL of potassium sulfate in water. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 10 µg/mL of sulfate.
Sample stock solution: 66.7 mg/mL of Anhydrous Citric Acid
Sample solution: To 4.5 mL of *Standard sulfate solution A*, add 3 mL of a barium chloride solution (1 in 4), shake, and allow to stand for 1 min. To 2.5 mL of the resulting suspension, add 15 mL of the *Sample stock solution* and 0.5 mL of 5 N acetic acid, and mix.
Standard solution: Prepare as directed for the *Sample solution*, except use 15 mL of *Standard sulfate solution B* instead of the *Sample stock solution*.
Analysis
Samples: *Sample solution* and *Standard solution*
Acceptance criteria: Any turbidity produced in the *Sample solution* after 5 min standing is not greater than that produced in the *Standard solution* (0.015%).
• **LIMIT OF ALUMINUM** (where it is labeled as intended for use in dialysis)
Standard aluminum solution: To 352 mg of aluminum potassium sulfate in a 100-mL volumetric flask, add a few mL of water, swirl to dissolve, add 10 mL of diluted sulfuric acid, dilute with water to volume, and mix. Immediately before use, dilute 1.0 mL of this solution with water to 100.0 mL.
pH 6.0 acetate buffer: Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, dilute with water to 250 mL, and mix.
Sample solution: Dissolve 20.0 g of Anhydrous Citric Acid in 100 mL of water, and add 10 mL of *pH 6.0 acetate buffer*. Extract this solution with successive portions of 20, 20, and 10 mL of a 0.5% solution of 8-hydroxyquinoline in chloroform, combining the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume, and mix.
Standard solution: Prepare a mixture of 2.0 mL of *Standard aluminum solution*, 10 mL of *pH 6.0 acetate buffer*, and 98 mL of water. Extract this mixture as described for the *Sample solution*, dilute the combined extracts with chloroform to volume, and mix.
Blank solution: Prepare a mixture of 10 mL of *pH 6.0 acetate buffer* and 100 mL of water. Extract this mixture as described for *Sample solution*, dilute the combined extracts with chloroform to volume, and mix.
Fluorometric conditions
Excitation wavelength: 392 nm
Emission wavelength: 518 nm
Analysis
Samples: *Sample solution* and *Standard solution*
Determine the fluorescence intensities of the *Samples* in a fluorometer set as directed under *Fluorometric conditions*, using the *Blank solution* to set the instrument to zero.
Acceptance criteria: The fluorescence of the *Sample solution* does not exceed that of the *Standard solution* (0.2 ppm).
• **LIMIT OF OXALIC ACID**
Sample stock solution: 0.80 g of Anhydrous Citric Acid in 4 mL of water
Sample solution: To the *Sample stock solution* add 3 mL of hydrochloric acid and 1 g of granular zinc, boil for 1 min, and allow to stand for 2 min. Transfer the supernatant to a test tube containing 0.25 mL of a phenylhydrazine hydrochloride solution (1 in 100), and heat to boiling. Cool rapidly, transfer to a graduated cylinder, and add an equal volume of hydrochloric acid and 0.25 mL of a potassium ferricyanide solution (1 in 20). Shake, and allow to stand for 30 min.
Standard solution: Prepare as directed for the *Sample solution*, except use 4 mL of 0.10 mg/mL oxalic acid solution, equivalent to 0.0714 mg/mL of anhydrous oxalic acid, instead of the *Sample stock solution*. [NOTE—Prepare concomitantly with the *Sample solution*.]

USP Monographs

www.webofpharma.com

Analysis
Samples: *Sample solution* and *Standard solution*
Acceptance criteria: Any pink color produced in the *Sample solution* is not more intense than that produced in the *Standard solution* (0.036%).

SPECIFIC TESTS

• **• BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** The level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Anhydrous Citric Acid is used can be met. Where the label states that Anhydrous Citric Acid must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Anhydrous Citric Acid is used can be met.•

Change to read:

• **CLARITY OF SOLUTION**
[NOTE—The *Sample solution* is to be compared to *Standard suspension A* in diffused daylight 5 min after preparation of *Standard suspension A*.]
Hydrazine sulfate solution: 10 mg/mL of hydrazine sulfate in water. Allow to stand for 4–6 h before use.
Methenamine solution: Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.
Primary opalescent suspension: Transfer 25.0 mL of *Hydrazine sulfate solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. [NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.]
Opalescence standard: Dilute 15.0 mL of *Primary opalescent suspension* with water to 1000 mL. [NOTE—This suspension should not be used beyond 24 h after preparation.]
Standard suspension A: Dilute 5.0 mL of *Opalescence standard* with 95 mL of water.
Standard suspension B: Dilute 10.0 mL of *Opalescence standard* with 90 mL of water.
Sample solution: 200 mg/mL of Anhydrous Citric Acid in water
Analysis
Samples: *Standard suspension A*, *Standard suspension B*, water, and *Sample solution*
Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension A*, *Standard suspension B*, and water to separate matching test tubes. Compare the *Sample solution*, *Standard suspension A*, *Standard suspension B*, and water in diffused daylight, viewing vertically against a black background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)). [NOTE—The diffusion of light must be such that *Standard suspension A* can readily be distinguished from water, and that *Standard suspension B* can readily be distinguished from *Standard suspension A*.]
Acceptance criteria: The *Sample solution* shows the same clarity as that of water or its opalescence is not more pronounced than *Standard suspension A*.

Change to read:

• **COLOR OF SOLUTION**
Standard stock solution A: Ferric chloride CS, cobaltous chloride CS, and dilute hydrochloric acid (10 g/L) (2.4: 0.6: 7.0)
Standard stock solution B: Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and dilute hydrochloric acid (10 g/L) (2.4: 1.0: 0.4: 6.2)
Standard stock solution C: Ferric chloride CS, cobaltous chloride CS, and cupric sulfate CS (9.6: 0.2: 0.2)
[NOTE—Prepare the *Standard solutions* immediately before use.]
Standard solution A: Dilute 2.5 mL of *Standard stock solution A* with dilute hydrochloric acid (10 g/L) to 100 mL.
Standard solution B: Dilute 2.5 mL of *Standard stock solution B* with dilute hydrochloric acid (10 g/L) to 100 mL.
Standard solution C: Dilute 0.75 mL of *Standard stock solution C* with dilute hydrochloric acid (10 g/L) to 100 mL.
Sample solution: Prepare as directed in the test for *Clarity of Solution*.
Analysis 1
Samples: Water and *Sample solution*
Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer water to a separate matching test tube. Compare the *Sample solution* and water in diffused daylight, viewing vertically against a white background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)).
Acceptance criteria 1: The *Sample solution* is not more intensely colored than water. If more intensely colored, follow *Analysis 2*.
Analysis 2
Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*
Transfer a sufficient portion of *Standard solution A*, *Standard solution B*, and *Standard solution C* to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Compare the *Sample solution* from *Analysis 1* to *Standard solution A*, *Standard solution B*, and *Standard solution C* in diffused daylight, viewing vertically against a white background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)).
Acceptance criteria 2: The *Sample solution* is not more intensely colored than *Standard solutions A, B,* and *C*.

• **READILY CARBONIZABLE SUBSTANCES**
Sample: 1.0 g of powdered Anhydrous Citric Acid
Analysis: Transfer the *Sample* to a 22-mm × 175-mm test tube previously rinsed with 10 mL of sulfuric acid and allowed to drain for 10 min. Add 10 mL of sulfuric acid, agitate until solution is complete, and immerse in a water bath at 90 ± 1° for 60 ± 0.5 min, keeping the level of the acid below the level of the water during the entire period. Cool the tube in running water, and transfer the acid to a color-comparison tube.
Acceptance criteria: The color of the acid is not darker than that of a similar volume of *Matching Fluid K* (see *Color and Achromicity* ⟨631⟩) in a matching tube, the tubes being observed vertically against a white background.

• **• STERILITY TESTS** ⟨71⟩**:** Where the label states that Anhydrous Citric Acid is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ in the relevant dosage form monograph(s) in which Anhydrous Citric Acid is used.•

• **WATER DETERMINATION,** *Method I* ⟨921⟩
Sample: 2.0 g of Anhydrous Citric Acid
Acceptance criteria: NMT 1.0%

USP Monographs

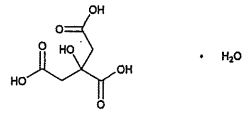
www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **•PACKAGING AND STORAGE:** Preserve in tight containers. No storage requirements specified.
- **LABELING:** Where it is intended for use in dialysis solutions, it is so labeled. Where Anhydrous Citric Acid must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled. Where Anhydrous Citric Acid is sterile, it is so labeled.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Citric Acid RS

Citric Acid Monohydrate

Portions of the monograph text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (• •) to specify this fact.



$C_6H_8O_7 \cdot H_2O$ 210.14

1,2,3-Propanetricarboxylic acid, 2-hydroxy-, monohydrate [5949-29-1].

DEFINITION

Citric Acid Monohydrate contains one molecule of water of hydration. It contains NLT 99.5% and NMT 100.5% of $C_6H_8O_7$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)**:** Dry the substance to be examined at 105° for 2 h.

ASSAY

- **PROCEDURE**

  Sample: 0.550 g of Citric Acid Monohydrate. Record the weight accurately.

  Analysis: Dissolve the *Sample* in 50 mL of water, and add 0.5 mL of phenolphthalein TS. Titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 64.03 mg of $C_6H_8O_7$.

  Acceptance criteria: 99.5%–100.5% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%, determined on 1.0 g
- **SULFATE**

  Standard sulfate solution A: 1.81 mg/mL of potassium sulfate in 30% alcohol. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with 30% alcohol to volume, and mix. This solution contains 10 µg/mL of sulfate.

  Standard sulfate solution B: 1.81 mg/mL of potassium sulfate. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 10 µg/mL of sulfate.

  Sample stock solution: 66.7 mg/mL of Citric Acid Monohydrate

  Sample solution: To 4.5 mL of *Standard sulfate solution A*, add 3 mL of a barium chloride solution (1 in 4), shake, and allow to stand for 1 min. To 2.5 mL of the resulting suspension add 15 mL of the *Sample stock solution* and 0.5 mL of 5 N acetic acid, and mix.

  Standard solution: Prepare as directed in the *Sample solution*, except use 15 mL of *Standard sulfate solution B* instead of the *Sample stock solution*.

  Analysis

  Samples: *Sample solution* and *Standard solution*

  Acceptance criteria: Any turbidity produced in the *Sample solution* after 5 min standing is not greater than that produced in the *Standard solution* (0.015%).

- **LIMIT OF ALUMINUM** (where it is labeled as intended for use in dialysis)

  Standard aluminum solution: To 352 mg of aluminum potassium sulfate in a 100-mL volumetric flask, add a few mL of water, swirl to dissolve, add 10 mL of diluted sulfuric acid, and dilute with water to volume. Immediately before use, dilute 1.0 mL of this solution with water to 100.0 mL.

  pH 6.0 acetate buffer: Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, dilute with water to 250 mL, and mix.

  Sample solution: Dissolve 20.0 g of Citric Acid Monohydrate in 100 mL of water, and add 10 mL of *pH 6.0 acetate buffer*. Extract this solution with successive portions of 20, 20, and 10 mL of a 0.5% solution of 8-hydroxyquinoline in chloroform, combining the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume, and mix.

  Standard solution: Prepare a mixture of 2.0 mL of *Standard aluminum solution*, 10 mL of *pH 6.0 acetate buffer*, and 98 mL of water. Extract this mixture as described for the *Sample solution*, dilute the combined extracts with chloroform to volume, and mix.

  Blank solution: Prepare a mixture of 10 mL of *pH 6.0 acetate buffer* and 100 mL of water. Extract this mixture as described for the *Sample solution*, dilute the combined extracts with chloroform to volume, and mix.

  Fluorometric conditions

  Excitation wavelength: 392 nm

  Emission wavelength: 518 nm

  Analysis

  Samples: *Sample solution* and *Standard solution*

  Determine the fluorescence intensities of the *Samples* in a fluorometer set as directed under *Fluorometric conditions*, using the *Blank solution* to set the instrument to zero.

  Acceptance criteria: The fluorescence of the *Sample solution* does not exceed that of the *Standard solution* (0.2 ppm).

- **LIMIT OF OXALIC ACID**

  Sample stock solution: 0.80 g of Citric Acid Monohydrate in 4 mL of water

  Sample solution: To the *Sample stock solution* add 3 mL of hydrochloric acid and 1 g of granular zinc, boil for 1 min, and allow to stand for 2 min. Transfer the supernatant to a test tube containing 0.25 mL of a phenylhydrazine hydrochloride solution (1 in 100), and heat to boiling. Cool rapidly, transfer to a graduated cylinder, and add an equal volume of hydrochloric acid and 0.25 mL of a potassium ferricyanide solution (1 in 20). Shake and allow to stand for 30 min.

  Standard solution: Prepare as directed for the *Sample solution*, except use 4 mL of 0.10 mg/mL oxalic acid solution, equivalent to 0.0714 mg/mL of anhydrous oxalic acid, instead of the *Sample stock solution*. [NOTE—Prepare concomitantly with the *Sample solution*.]

  Analysis

  Samples: *Sample solution* and *Standard solution*

www.webofpharma.com

Acceptance criteria: Any pink color produced in the *Sample solution* is not more intense than that produced in the *Standard solution* (0.036%).

SPECIFIC TESTS

• **‣ BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** The level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Citric Acid Monohydrate is used can be met. Where the label states that Citric Acid Monohydrate must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Citric Acid Monohydrate is used can be met.◂

Change to read:

• **CLARITY OF SOLUTION**

[NOTE—The *Sample solution* is to be compared to *Standard suspension A* in diffused daylight 5 min after preparation of *Standard suspension A*.]

Hydrazine sulfate solution: 10 mg/mL of hydrazine sulfate in water. Allow to stand for 4–6 h before use.

Methenamine solution: Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

Primary opalescent suspension: Transfer 25.0 mL of *Hydrazine sulfate solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. [NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.]

Opalescence standard: Dilute 15.0 mL of *Primary opalescent suspension* with water to 1000 mL. [NOTE—This suspension should not be used beyond 24 h after preparation.]

Standard suspension A: Dilute 5.0 mL of *Opalescence standard* with 95 mL of water.

Standard suspension B: Dilute 10.0 mL of *Opalescence standard* with 90 mL of water.

Sample solution: 200 mg/mL of Citric Acid Monohydrate in water

Analysis

Samples: *Standard suspension A*, *Standard suspension B*, water, and *Sample solution*

Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension A*, *Standard suspension B*, and water to separate matching test tubes. Compare the *Sample solution*, *Standard suspension A*, *Standard suspension B*, and water in diffused daylight, viewing vertically against a black background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)). [NOTE—The diffusion of light must be such that *Standard suspension A* can readily be distinguished from water, and that *Standard suspension B* can readily be distinguished from *Standard suspension A*.]

Acceptance criteria: The *Sample solution* shows the same clarity as that of water or its opalescence is not more pronounced than *Standard suspension A*.

Change to read:

• **COLOR OF SOLUTION**

Standard stock solution A: Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and dilute hydrochloric acid (10 g/L) (2.4: 0.6: 0: 7.0)

Standard stock solution B: Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and dilute hydrochloric acid (10 g/L) (2.4: 1.0: 0.4: 6.2)

Standard stock solution C: Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and dilute hydrochloric acid (10 g/L) (9.6: 0.2: 0.2: 0)

[NOTE—Prepare the *Standard solutions* immediately before use.]

Standard solution A: Transfer 2.5 mL of *Standard stock solution A*, and dilute with dilute hydrochloric acid (10 g/L) to 100 mL.

Standard solution B: Transfer 2.5 mL of *Standard stock solution B*, and dilute with dilute hydrochloric acid (10 g/L) to 100 mL.

Standard solution C: Transfer 0.75 mL of *Standard stock solution C*, and dilute with dilute hydrochloric acid (10 g/L) to 100 mL.

Sample solution: Prepare as directed in the test for *Clarity of Solution*.

Analysis 1

Samples: Water and *Sample solution*

Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer water to a separate matching test tube. Compare the *Sample solution* and water in diffused daylight, viewing vertically against a white background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)).

Acceptance criteria 1: The *Sample solution* is not more intensely colored than water. If more intensely colored, follow *Analysis 2*.

Analysis 2

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Transfer a sufficient portion of *Standard solution A*, *Standard solution B*, and *Standard solution C* to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Compare the *Sample solution* from *Analysis 1* to *Standard solution A*, *Standard solution B*, and *Standard solution C* in diffused daylight, viewing vertically against a white background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)).

Acceptance criteria 2: The *Sample solution* is not more intensely colored than *Standard solutions A*, *B*, and *C*.

• **READILY CARBONIZABLE SUBSTANCES**

Sample: 1.0 g powdered Citric Acid Monohydrate

Analysis: Transfer the *Sample* to a 22-mm × 175-mm test tube previously rinsed with 10 mL of sulfuric acid and allowed to drain for 10 min. Add 10 mL of sulfuric acid, agitate until solution is complete, and immerse in a water bath at 90 ± 1° for 60 ± 0.5 min, keeping the level of the acid below the level of the water during the entire period. Cool the tube in running water, and transfer the acid to a color-comparison tube.

Acceptance criteria: The color of the acid is not darker than that of a similar volume of *Matching Fluid K* (see *Color and Achromicity* ⟨631⟩) in a matching tube, the tubes being observed vertically against a white background.

• **‣ STERILITY TESTS** ⟨71⟩**:** Where the label states that Citric Acid Monohydrate is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ in the relevant dosage form monograph(s) in which Citric Acid Monohydrate is used.◂

• **WATER DETERMINATION,** *Method I* ⟨921⟩

Sample: 0.5 g of Citric Acid Monohydrate

Acceptance criteria: 7.5%–9.0%

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- ***PACKAGING AND STORAGE:** Preserve in tight containers. No storage requirements specified.
- **LABELING:** Where it is intended for use in dialysis solutions, it is so labeled. Where Citric Acid Monohydrate must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled. Where Citric Acid Monohydrate is sterile, it is so labeled.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Citric Acid RS

Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation

DEFINITION

Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation is a sterile solution of Citric Acid, Magnesium Oxide, and Sodium Carbonate in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amounts of citric acid ($C_6H_8O_7 \cdot H_2O$), magnesium oxide (MgO), and sodium carbonate (Na_2CO_3).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩ and *Magnesium* ⟨191⟩
- **B.**

Sample solution: 10 mL of Irrigation

Analysis: Add 1 mL of mercuric sulfate TS to the *Sample solution*, heat to boiling, and add a few drops of potassium permanganate TS.

Acceptance criteria: A white precipitate is formed.

ASSAY

- **CITRIC ACID**

Mobile phase, Standard preparation 1, and Chromatographic system: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Assay preparation for citric acid/citrate assay: Nominally 20 µg/mL of citrate from Irrigation in 1 mM of sodium hydroxide prepared as follows. Transfer a suitable volume of Irrigation to an appropriately sized volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Sample solution (for the assay of citric acid/citrate)*.

Analysis

Samples: *Standard preparation 1* and *Assay preparation for citric acid/citrate assay*

Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩, *Procedure*.

Calculate the percentage of the labeled amount of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) in the portion of Irrigation taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of citrate from the *Assay preparation for citric acid/citrate assay*

r_S = peak response of citrate from *Standard preparation 1*

C_S = concentration of *Standard preparation 1* (µg/mL)

C_U = nominal concentration of citric acid monohydrate in the *Assay preparation for citric acid/citrate assay* (µg/mL)

M_{r1} = molecular weight of citric acid monohydrate, 210.14

M_{r2} = molecular weight of citrate, 189.10

Acceptance criteria: 95.0%–105.0%

- **MAGNESIUM OXIDE**

Sample solution: A volume of Irrigation, nominally equivalent to 40 mg of magnesium oxide

Analysis: Transfer the *Sample solution* to a beaker containing 130 mL of water heated to 75° ± 5°, and add 4 mL of ammonium chloride TS and then 5 mL of ammonium hydroxide. Mix, and add slowly, with stirring, 8 mL of 8-hydroxyquinoline TS. After allowing to stand for 30 min at 75°, filter through a sintered-glass crucible, previously dried and weighed. Wash the precipitate with 50 mL of a warm mixture of water and 6 N ammonium hydroxide (45:5), followed by 50 mL of cool water. Dry the crucible and contents at 105° for 3 h, cool, and weigh.

Determine the equivalent of magnesium oxide (MgO) in the portion of Irrigation taken by multiplying the weight of the $C_{18}H_{12}MgN_2O_2 \cdot 2H_2O$ so obtained by 0.1156 (mg of MgO).

Calculate the percentage of the labeled amount of magnesium oxide (MgO) in the portion of Irrigation taken.

Acceptance criteria: 95.0%–105.0%

- **SODIUM CARBONATE**

Sodium chloride stock solution: 4.75 mg/mL of sodium chloride, previously dried at 105° for 2 h, in water

Internal standard solution: 0.636 mg/mL of lithium chloride in water

Standard solution: 0.0475 mg/mL of sodium chloride and 0.6296 mg/mL of lithium chloride prepared from an appropriate mixture of *Sodium chloride stock solution* and *Internal standard solution*

Sample stock solution: Nominally equivalent to 4.4 mg/mL of sodium carbonate from Irrigation diluted with water

Sample solution: 0.044 mg/mL of sodium carbonate and 0.6296 mg/mL of lithium chloride prepared from an appropriate mixture of *Sample stock solution* and *Internal standard solution*

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths: 591 and 671 nm

Analysis

Samples: *Internal standard solution*, *Standard solution*, and *Sample solution*

Concomitantly determine the emittances of the *Standard solution* and the *Sample solution*, adjusting the instrument with *Internal standard solution* to zero emittance.

Calculate the percentage of the labeled amount of sodium carbonate (Na_2CO_3) in the portion of Irrigation taken:

$$\text{Result} = (r_{U,591}/r_{U,671}) \times (r_{S,671}/r_{S,591}) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_{U,591}$ = emittance reading from the *Sample solution* at 591 nm

$r_{U,671}$ = emittance reading from the *Sample solution* at 671 nm

$r_{S,671}$ = emittance reading from the *Standard solution* at 671 nm

$r_{S,591}$ = emittance reading from the *Standard solution* at 591 nm

C_S = concentration of sodium chloride in the *Standard solution* (mg/mL)

C_U = nominal concentration of sodium carbonate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of sodium carbonate, 105.99

www.webofpharma.com

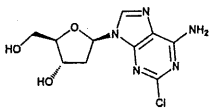
M_{r2} = two times the molecular weight of sodium chloride, 116.88

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 3.8–4.2
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains not more than 2.80 USP Endotoxin Units per mL.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, except that the container may be designed to empty rapidly and may exceed 1000 mL in capacity.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I or Type II glass.

Cladribine



$C_{10}H_{12}ClN_5O_3$ 285.69
Adenosine, 2-chloro-2′-deoxy-;
2-Chloro-2′-deoxyadenosine [4291-63-8].

DEFINITION
Cladribine contains NLT 98.0% and NMT 102.0% of cladribine ($C_{10}H_{12}ClN_5O_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: Dissolve 9.96 g of triethylamine phosphate in 500 mL of water. Add another 500 mL of water, and adjust with potassium hydroxide to a pH of 6.1.
Diluent: Methanol and water (10:90)
Mobile phase: Methanol and *Buffer* (22:78)
System suitability solution: 0.02 mg/mL each of USP Cladribine RS and USP Cladribine Related Compound A RS in *Diluent*
Standard solution: 0.5 mg/mL of USP Cladribine RS in *Diluent*
Sample solution: 0.5 mg/mL of Cladribine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between the cladribine and cladribine related compound A peaks, *System suitability solution*
Tailing factor: NMT 2.0 for the cladribine peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cladribine ($C_{10}H_{12}ClN_5O_3$) in the portion of Cladribine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cladribine RS in the *Standard solution* (mg/mL)
C_U = concentration of Cladribine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer, Diluent, Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between cladribine and cladribine related compound A
Tailing factor: NMT 2.0 for the cladribine peak
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Cladribine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity
r_T = sum of the responses of all the peaks

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2,6-Diaminopurine-2′-deoxyriboside	0.41	0.20
2′-Deoxyadenosine	0.47	0.20
2-Chloroadenine	0.60	0.20
Cladribine related compound A	0.91	0.20
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL in dimethylformamide
Acceptance criteria: −17.0° to −21.0°

USP Monographs

www.webofpharma.com

• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 4.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and protect from light. Store between 2° and 8°.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cladribine RS

USP Cladribine Related Compound A RS

2-Methoxy-2′-deoxyadenosine.

$C_{11}H_{15}N_5O_4$ 281.27

Cladribine Injection

DEFINITION

Cladribine Injection is a clear, colorless, sterile, preservative-free, isotonic solution. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cladribine ($C_{10}H_{12}ClN_5O_3$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 0.05 mg/mL of cladribine in water

Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 9.96 g of triethylamine phosphate, accurately weighed, in 500 mL of water, and add another 500 mL of water. Adjust with potassium hydroxide to a pH of 6.1. [NOTE—Alternatively, dissolve 13.5 mL of triethylamine in 1 L of water, and adjust with phosphoric acid to a pH of 6.1.]

Mobile phase: Methanol and *Buffer* (22:78)

Diluent: Methanol and water (10:90)

System suitability solution: 0.02 mg/mL each of USP Cladribine RS and USP Cladribine Related Compound A RS in *Diluent*

Standard solution: 0.5 mg/mL of USP Cladribine RS in *Diluent*

Sample solution: Nominally, equivalent to 0.5 mg/mL of cladribine in *Diluent* from Injection

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between cladribine and cladribine related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for the cladribine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cladribine ($C_{10}H_{12}ClN_5O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Cladribine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cladribine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, Diluent, System suitability solution, and **Sample solution:** Proceed as directed in the *Assay*.

Standard solution: 0.01 mg/mL of USP Cladribine RS in *Diluent*

Chromatographic system: Proceed as directed in the *Assay*. In addition, the run time is NLT 2.5 times of the retention time of the cladribine peak for the *Sample solution*.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between cladribine and cladribine related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for the cladribine peak, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of cladribine from the *Standard solution*

C_S = concentration of USP Cladribine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cladribine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2,6-Diaminopurine-2′-de-oxyriboside	0.41	0.2
2′-Deoxyadenosine	0.47	0.2
2-Chloroadenine	0.60	0.5
Cladribine related compound A[a]	0.91	0.2
Cladribine	1.0	—
Any individual, unspecified impurity	—	0.2

www.webofpharma.com

Table 1 *(continued)*

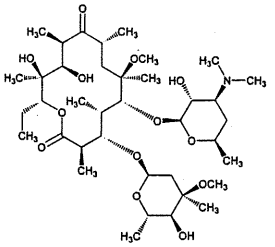
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	2.0

[a] 2-Methoxy-2′-deoxyadenosine.

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 55 USP Endotoxin Units/mg of cladribine
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **pH** ⟨791⟩: 5.5–8.0
- **OSMOLALITY AND OSMOLARITY,** *Osmolality* ⟨785⟩: 250–370 mOsmol/kg
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements for small-volume injections.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-use clear flint glass vials. Store refrigerated at 2°–8°C. Protect from light.
- **LABELING:** Label it to indicate that it is to be diluted with 0.9% Sodium Chloride Injection USP for the single daily dose and to be diluted with bacteriostatic 0.9% Sodium Chloride Injection USP (0.9% benzyl alcohol preserved) to prepare the 7-day infusion solution.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cladribine RS
  USP Cladribine Related Compound A RS
  2-Methoxy-2′-deoxyadenosine.
  $C_{11}H_{15}N_5O_4$ 281.27

Clarithromycin



$C_{38}H_{69}NO_{13}$ 747.95
Erythromycin, 6-*O*-methyl-;
6-*O*-Methylerythromycin [81103-11-9].

DEFINITION
Clarithromycin contains NLT 96.0% and NMT 102.0% of clarithromycin ($C_{38}H_{69}NO_{13}$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of *Standard solution 1*, as obtained in the *Assay.*

ASSAY
- **PROCEDURE**

Solution A, Solution B, Mobile phase, Diluent, Standard solution 1, Standard solution 2, Standard solution 4, Sample solution, and **Chromatographic system:** Proceed as directed in *Organic Impurities.*

System suitability

Samples: *Standard solution 1*, *Standard solution 2*, and *Standard solution 4*

[NOTE—See the relative retention times in *Table 2*. The typical retention time for clarithromycin is about 11 min.]

Suitability requirements

Peak-to-valley ratio: NLT 3.0, *Standard solution 4*
The *Peak-to-valley ratio* is calculated as follows:

$$\text{Result} = H_P/H_V$$

H_P = height above the baseline of the clarithromycin impurity D peak, *Standard solution 4*
H_V = height above the baseline of the lowest point of the curve separating the clarithromycin impurity D peak from the clarithromycin peak, *Standard solution 4*

Tailing factor: NMT 1.7, *Standard solution 2*
Relative standard deviation: NMT 1.5%, *Standard solution 1*

Analysis

Samples: *Standard solution 1* and *Sample solution*
Calculate the percentage of clarithromycin ($C_{38}H_{69}NO_{13}$) in the portion of Clarithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from *Standard solution 1*
C_S = concentration of USP Clarithromycin RS in *Standard solution 1* (mg/mL)
C_U = concentration of Clarithromycin in the *Sample solution* (mg/mL)

Acceptance criteria: 96.0%–102.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩

Sample: 0.5 g
Acceptance criteria: NMT 0.2%
- **ORGANIC IMPURITIES**

Solution A: 4.76 g/L of monobasic potassium phosphate. Adjust with dilute phosphoric acid (1 in 10) or potassium hydroxide (45% w/v) to a pH of 4.4. Pass this solution through a C18 filtration kit.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
32	40	60
34	40	60
36	75	25
42	75	25

Diluent: Acetonitrile and *water* (1:1)

USP Monographs

www.webofpharma.com

Standard solution 1: 1.5 mg/mL of USP Clarithromycin RS in acetonitrile and *water* (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with *water* to volume.
Standard solution 2: 75 µg/mL of USP Clarithromycin RS from *Standard solution 1* in *Diluent*
Standard solution 3: 7.5 µg/mL of USP Clarithromycin RS from *Standard solution 2* in *Diluent*
Standard solution 4: 1.5 mg/mL of USP Clarithromycin Identity RS in acetonitrile and *water* (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with *water* to volume.
Sample solution: 1.5 mg/mL of Clarithromycin in acetonitrile and *water* (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 10-cm; packing L1
Column temperature: 40°
Flow rate: 1.1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution 2* and *Standard solution 4*
[NOTE—See the relative retention times in *Table 2*. The typical retention time for clarithromycin is about 11 min.]
Suitability requirements
Peak-to-valley ratio: NLT 3.0, *Standard solution 4*
The *Peak-to-valley ratio* is calculated as follows:

$$\text{Result} = H_P / H_V$$

H_P = height above the baseline of the clarithromycin impurity D peak, *Standard solution 4*
H_V = height above the baseline of the lowest point of the curve separating the clarithromycin impurity D peak from the clarithromycin peak, *Standard solution 4*

Tailing factor: NMT 1.7, *Standard solution 2*
Analysis
Samples: *Diluent*, *Standard solution 2*, *Standard solution 3*, *Standard solution 4*, and *Sample solution*
Calculate the percentage of each impurity in the portion of Clarithromycin taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times (1/F) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of clarithromycin from *Standard solution 3*
C_S = concentration of USP Clarithromycin RS in *Standard solution 3* (mg/mL)
C_U = concentration of Clarithromycin in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: The reporting threshold is 0.1%. Disregard the peaks eluting before impurity I and after impurity H.
Any individual impurity: NMT 1.0%. NMT four impurities exceed 0.4%.
Total impurities: NMT 3.5%

Table 2

Name	Relative Retention Time	Relative Response Factor
Clarithromycin impurity I[a]	0.38	1.0
Clarithromycin impurity A[b] (clarithromycin F)	0.42	1.0
Clarithromycin impurity J[c]	0.63	1.0
Clarithromycin impurity L[d]	0.74	1.0
Clarithromycin impurity B[e]	0.79	1.0
Clarithromycin impurity M[f]	0.81	1.0
Clarithromycin impurity C[g]	0.89	1.0
Clarithromycin impurity D[h]	0.96	1.0
Clarithromycin	1.0	—
Clarithromycin impurity N[i]	1.15	1.0
Clarithromycin related compound A[j]	1.27	1.0
Clarithromycin impurity F[k]	1.33	1.0
Clarithromycin impurity P[l]	1.35	1.0
Clarithromycin impurity O[m]	1.38	1.0
Clarithromycin impurity K[n]	1.59	1.0
Clarithromycin impurity G[o]	1.72	3.7
Clarithromycin impurity H[p]	1.82	6.7

[a] 3-*O*-Decladinosyl-6-*O*-methylerythromycin A.
[b] 2-Demethyl-2-(hydroxymethyl)-6-*O*-methylerythromycin A.
[c] Erythromycin A (*E*)-9-oxime.
[d] 6-*O*-Methylerythromycin (*Z*)-9-oxime.
[e] 6-*O*-Methyl-15-norerythromycin A.
[f] 3″-*N*-Demethyl-6-*O*-methylerythromycin A (*E*)-9-oxime.
[g] 6-*O*-Methylerythromycin A (*E*)-9-oxime.
[h] 3″-*N*-Demethyl-6-*O*-methylerythromycin A.
[i] (10*E*)-10,11-Didehydro-11-deoxy-6-*O*-methylerythromycin A.
[j] 6,11-Di-*O*-methylerythromycin A.
[k] 6,12-Di-*O*-methylerythromycin A.
[l] 4′,6-Di-*O*-methylerythromycin A.
[m] 6-*O*-Methylerythromycin A (*Z*)-9-(*O*-methyloxime).
[n] (1*S*,2*R*,5*R*,6*S*,7*S*,8*R*,9*R*,11*Z*)-2-Ethyl-6-hydroxy-9-methoxy-1,5,7,9,11,13-hexamethyl-8-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexapyranosyl]oxy]-3,15-dioxabicyclo[10.2.1]pentadeca-11,13-dien-4-one (3-*O*-decladinosyl-8,9:10,11-dianhydro-6-*O*-methylerythromycin A)-9,12-hemiketal.
[o] 6-*O*-Methylerythromycin A (*E*)-9-(*O*-methyloxime).
[p] 3″-*N*-Demethyl-3′-*N*-formyl-6-*O*-methylerythromycin A.

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 10 mg/mL in methylene chloride
  Acceptance criteria: −94° to −102° (at 20°)
- **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
- **pH** ⟨791⟩
  Sample: 2-mg/mL suspension in methanol and *water* (1:19)
  Acceptance criteria: 8.0–10.0
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clarithromycin RS
  USP Clarithromycin Identity RS
  This is a mixture of clarithromycin, clarithromycin impurity D (3″-*N*-demethyl-6-*O*-methylerythromycin A; $C_{37}H_{67}NO_{13}$; 733.9), and other impurities.

USP 43 Monographs

www.webofpharma.com

Clarithromycin for Oral Suspension

DEFINITION

Clarithromycin for Oral Suspension is a dry mixture of Clarithromycin, dispersing agents, diluents, preservatives, and flavorings. It contains NLT 90.0% and NMT 115.0% of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$), the labeled amount being 25 mg or 50 mg/mL when constituted as directed in the labeling.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer A: 0.067 M monobasic potassium phosphate
Buffer B: 0.067 M dibasic potassium phosphate
Mobile phase: Methanol and *Buffer A* (60:40), adjusted with phosphoric acid to a pH of 3.5. Pass through a suitable filter.
Standard stock solution: Equivalent to 2.1 mg/mL of clarithromycin from USP Clarithromycin RS in methanol
Standard solution: 0.415 mg/mL of clarithromycin from *Standard stock solution* in *Mobile phase*
Sample stock solution: Constitute the Clarithromycin for Oral Suspension as directed in the labeling. Transfer an aliquot of the suspension, equivalent to 1–2 g of clarithromycin, with the aid of 330 mL of *Buffer B*, to a 1000-mL volumetric flask containing 50 mL of *Buffer B*. Shake by mechanical means for 30 min, and dilute with methanol to volume. Sonicate for about 30 min, and allow to cool. Dilute with methanol to volume, add a magnetic stirring bar, and stir for 60 min. Allow to settle, and use the clear supernatant.
Sample solution: Transfer an aliquot of the *Sample stock solution*, nominally equivalent to 20 mg of clarithromycin, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and pass through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Columns
Guard (optional): Packing L1
Analytical: 4.6-mm × 15-cm; packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 1.0–1.7
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) in the portion of the constituted Clarithromycin for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area response from the *Sample solution*
r_S = peak area response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of clarithromycin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩ (for powder packaged in single-unit containers): Meets the requirements
- **DELIVERABLE VOLUME** ⟨698⟩ (for powder packaged in multiple-unit containers): Meets the requirements

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample: Use the suspension constituted as directed in the labeling.
Acceptance criteria: 4.0–5.4

- **LOSS ON DRYING** ⟨731⟩

Sample: 1 g
Analysis: Dry under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Clarithromycin RS

Clarithromycin Tablets

DEFINITION

Clarithromycin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol and 0.067 M monobasic potassium phosphate (13:7). Adjust with phosphoric acid to a pH of 4.0, and pass through a suitable filter.
System suitability stock solution: 625 µg/mL of USP Clarithromycin Related Compound A RS in methanol
System suitability solution: 125 µg/mL of USP Clarithromycin RS from the *Standard stock solution* and 125 µg/mL of USP Clarithromycin Related Compound A RS from the *System suitability stock solution* in *Mobile phase*
Standard stock solution: 625 µg/mL of clarithromycin from USP Clarithromycin RS dissolved in methanol. Shake, and sonicate to facilitate dissolution.
Standard solution: 125 µg/mL of clarithromycin from *Standard stock solution* in *Mobile phase*. Pass through a suitable filter.
Sample stock solution: Nominally 4 mg/mL of clarithromycin from finely powdered Tablets in methanol. Shake by mechanical means for 30 min to disperse, and allow any insoluble matter to settle.
Sample solution: 120 µg/mL of clarithromycin from the *Sample stock solution* in *Mobile phase*. Pass through a filter of 0.5-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
[NOTE—A guard column containing packing L1 may be added.]
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20–50 µL

www.webofpharma.com

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for clarithromycin and clarithromycin related compound A are 0.75 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between clarithromycin and clarithromycin related compound A, *System suitability solution*
Tailing factor: 0.9–1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of clarithromycin in the *Standard solution* (µg/mL)
C_U = nominal concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Buffer: Prepare a solution containing 13.61 mg/mL of sodium acetate trihydrate in water. Prepare another solution by diluting 5.7 mL of glacial acetic acid with water to 1 L. Combine portions of the two solutions to obtain a pH of 5.0.
Medium: *Buffer*, 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Mobile phase, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Standard stock solution: 625 µg/mL of clarithromycin from USP Clarithromycin RS dissolved in *Buffer*. Shake, and sonicate to facilitate dissolution.
Standard solution: 125 µg/mL of clarithromycin from the *Standard stock solution* in *Mobile phase*. Pass through a suitable filter.
Sample solution: Dilute with *Mobile phase* to yield a solution containing nominally 125 µg/mL of clarithromycin.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = nominal concentration of the *Sample solution* (µg/mL)

Tolerances: NLT 80% (*Q*) of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: 4.76 g/L of monobasic potassium phosphate adjusted with dilute phosphoric acid (1 in 10) or 4.5% (w/v) of potassium hydroxide to a pH of 4.4
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
32	40	60
34	40	60
36	75	25
42	75	25

Diluent: Acetonitrile and water (1:1)
System suitability solution: 1.5 mg/mL of USP Clarithromycin Identity RS in acetonitrile and water (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with water to volume.
Standard stock solution: 1.5 mg/mL of USP Clarithromycin RS in acetonitrile and water (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with water to volume.
Standard solution 1: 0.075 mg/mL of USP Clarithromycin RS from *Standard stock solution* in *Diluent*
Standard solution 2: 0.0075 mg/mL of USP Clarithromycin RS from *Standard solution 1* in *Diluent*
Sample solution: Nominally 1.5 mg/mL of clarithromycin from finely powdered Tablets in acetonitrile and water (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with water to volume. Sonicate, and pass through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1.1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution 1*
[NOTE—See *Table 2* for relative retention times. The typical retention time for clarithromycin is about 11 min.]
Suitability requirements
Peak-to-valley ratio: NLT 3.0 between clarithromycin and clarithromycin impurity D, *System suitability solution*. Calculate as follows:

$$\text{Result} = H_P/H_V$$

H_P = height above the baseline of the clarithromycin impurity D peak
H_V = height above the baseline of the lowest point of the curve separating the clarithromycin impurity D peak from the clarithromycin peak

Tailing factor: NMT 1.7, *Standard solution 1*
Analysis
Samples: *Standard solution 2* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from *Standard solution 2*
C_S = concentration of clarithromycin in *Standard solution 2* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: The reporting threshold is 0.1%.Disregard the peaks eluting before impurity I and after impurity H.
Any individual impurity: NMT 1.0%; NMT four impurities exceed 0.4%.
Total impurities: NMT 3.5%

Table 2

Name	Relative Retention Time	Relative Response Factor
Clarithromycin impurity I[a]	0.38	1.0
Clarithromycin impurity A[b] (clarithromycin F)	0.42	1.0
Clarithromycin impurity J[c]	0.63	1.0
Clarithromycin impurity L[d]	0.74	1.0
Clarithromycin impurity B[e]	0.79	1.0
Clarithromycin impurity M[f]	0.81	1.0
Clarithromycin impurity C[g]	0.89	1.0
Clarithromycin impurity D[h]	0.96	1.0
Clarithromycin	1.0	—
Clarithromycin impurity N[i]	1.15	1.0
Clarithromycin related compound A[j]	1.27	1.0
Clarithromycin impurity F[k]	1.33	1.0
Clarithromycin impurity P[l]	1.35	1.0
Clarithromycin impurity O[m]	1.38	1.0
Clarithromycin impurity K[n]	1.59	1.0
Clarithromycin impurity G[o]	1.72	3.7
Clarithromycin impurity H[p]	1.82	6.7

[a] 3-*O*-Decladinosyl-6-*O*-methylerythromycin A.
[b] 2-Demethyl-2-(hydroxymethyl)-6-*O*-methylerythromycin A.
[c] Erythromycin A (*E*)-9-oxime.
[d] 6-*O*-Methylerythromycin (*Z*)-9-oxime.
[e] 6-*O*-Methyl-15-norerythromycin A.
[f] 3″-*N*-Demethyl-6-*O*-methylerythromycin A (*E*)-9-oxime.
[g] 6-*O*-Methylerythromycin A (*E*)-9-oxime.
[h] 3″-*N*-Demethyl-6-*O*-methylerythromycin A.
[i] (10*E*)-10,11-Didehydro-11-deoxy-6-*O*-methylerythromycin A.
[j] 6,11-Di-*O*-methylerythromycin A.
[k] 6,12-Di-*O*-methylerythromycin A.
[l] 4′,6-Di-*O*-methylerythromycin A.
[m] 6-*O*-Methylerythromycin A (*Z*)-9-(*O*-methyloxime).
[n] (1*S*,2*R*,5*R*,6*S*,7*S*,8*R*,9*R*,11*Z*)-2-Ethyl-6-hydroxy-9-methoxy-1,5,7,9,11,13-hexamethyl-8-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexapyranosyl]oxy]-3,15-dioxabicyclo[10.2.1]pentadeca-11,13-dien-4-one (3-*O*-decladinosyl-8,9:10,11-dianhydro-6-*O*-methylerythromycin A)-9,12-hemiketal.
[o] 6-*O*-Methylerythromycin A (*E*)-9-(*O*-methyloxime).
[p] 3″-*N*-Demethyl-3″-*N*-formyl-6-*O*-methylerythromycin A.

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry a portion of powdered Tablets under vacuum at a pressure not exceeding 5 mm of mercury at 110° for 3 h.
Acceptance criteria: NMT 6.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Clarithromycin RS
USP Clarithromycin Identity RS
This is a mixture of clarithromycin, clarithromycin impurity D (3″-*N*-demethyl-6-*O*-methylerythromycin A; $C_{37}H_{67}NO_{13}$ 733.9), and other impurities.
USP Clarithromycin Related Compound A RS
6,11-Di-*O*-methylerythromycin A.
$C_{39}H_{71}NO_{13}$ 761.98

Clarithromycin Extended-Release Tablets

DEFINITION
Clarithromycin Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Buffer A: 0.067 M monobasic potassium phosphate
Mobile phase: Methanol and *Buffer A* (13:7). Adjust with phosphoric acid to a pH of 4.0. Pass through a suitable filter.
Standard stock solution: 625 µg/mL of clarithromycin from USP Clarithromycin RS in methanol. Shake and sonicate, if necessary, to facilitate dissolution.
Standard solution: 125 µg/mL of clarithromycin in *Mobile phase* from *Standard stock solution*. Pass through a suitable filter.
System suitability stock solution: 625 µg/mL of USP Clarithromycin Related Compound A RS in methanol
System suitability solution: 125 µg/mL of USP Clarithromycin Related Compound A RS from *System suitability stock solution* and 125 µg/mL of clarithromycin from *Standard stock solution* in *Mobile phase*
Sample stock solution: Transfer nominally 2000 mg of clarithromycin from finely powdered Tablets to a 500-mL volumetric flask with the aid of methanol. Add about 350 mL of methanol, and shake by mechanical means for 30 min. Dilute with methanol to volume, and sonicate for 30 min. Cool to room temperature, and allow to stand for at least 16 h. Mix, allow any insoluble matter to settle, and use the supernatant.
Sample solution: Transfer 3.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Columns
Guard (optional): Packing L1
Analytical: 4.6-mm × 15-cm; packing L1

www.webofpharma.com

Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20–50 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for clarithromycin and clarithromycin related compound A are about 0.75 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between clarithromycin and clarithromycin related compound A, *System suitability solution*
Tailing factor: 0.9–1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of clarithromycin in the *Standard solution* (µg/mL)
C_U = nominal concentration of clarithromycin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1
Buffer B: Dissolve 816.5 g of monobasic potassium phosphate and 48 g of sodium hydroxide in about 4 L of water, mix, and dilute with water to 20 L. Adjust with either concentrated phosphoric acid or 1 N sodium hydroxide to a pH of 6.0 ± 0.05.
Medium: *Buffer B*; 900 mL
Apparatus 2: 75 rpm
Times: 30, 45, 60, and 120 min
Standard solutions: Prepare five solutions of USP Clarithromycin RS dissolved in acetonitrile and diluted with *Medium*, with known concentrations over a range of about 60–600 µg/mL.
Sample solution: Use portions of the solution under test passed through a polyethylene filter of 35-µm pore size.
Chromatographic system: Proceed as directed in the *Assay*, except the *Injection volume* is 50 µL.
Analysis
Samples: *Standard solutions* and *Sample solution*
Perform a linear regression analysis to generate a standard curve using the peak area of each *Standard solution* versus its concentration. Determine the percentage of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) dissolved at each specified time interval, using the peak area of each *Sample solution* and the linear regression statistics for the *Standard solutions*.
Tolerances: The percentages of the labeled amounts of clarithromycin ($C_{38}H_{69}NO_{13}$) dissolved at the times specified conform to *Table 1*.

Table 1

Level	Time (min)	Amount Dissolved, Individual Limits (%)	Amount Dissolved, Average Limits (%)
L1	30	NMT 65	—
	45	55–85	—
	60	NLT 75	—
	120	NLT 85	—
L2	30	NMT 75	NMT 65
	45	45–95	55–85
	60	NLT 65	NLT 75
	120	NLT 75	NLT 85
L3	30	NMT 2 Tablets release more than 75%, and no individual Tablet releases more than 85%	NMT 65
	45	NMT 2 Tablets are outside the range of 45%–95%, and no individual Tablet is outside the range of 35%–105%	55–85
	60	NMT 2 Tablets release less than 65%, and no individual Tablet releases less than 55%	NLT 75
	120	NMT 2 Tablets release less than 75%, and no individual Tablet releases less than 65%	NLT 85

Test 2
If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Buffer C: 0.05 M phosphate buffer with a pH of 6.8, containing 0.5% of sodium lauryl sulfate
Medium: *Buffer C*; 900 mL, degassed by sonication and vacuum
Apparatus 1: 100 rpm
Times: 2, 12, and 24 h
Buffer D: 9.2 g/L of monobasic sodium phosphate monohydrate in water, adjusted with phosphoric acid to a pH of 2.5 prior to final dilution
Mobile phase: Methanol and *Buffer D* (65:35)
Standard solution: 0.56 mg/mL of USP Clarithromycin RS in a solution of methanol and *Medium* (1 in 10). Dissolve first in methanol using 10% of the final volume, and dilute with *Medium* to volume.
Sample solution: Centrifuge the solution under test at 2500 rpm for 10 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) dissolved at each time point (Q_T):

USP Monographs

www.webofpharma.com

$$Q_2 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_{12} = [Q_2 \times (V_S/V)] + [(r_U/r_S) \times (C_S/L) \times (V - V_S) \times 100]$$

$$Q_{24} = [Q_2 \times (V_S/V)] + [Q_{12} \times V_S/(V - 2V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 2V_S) \times 100]$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of clarithromycin in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of the sample withdrawn at each time point (mL)
L = label claim (mg/Tablet)

Tolerances: The percentages of the labeled amounts of clarithromycin ($C_{38}H_{69}NO_{13}$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
2	NMT 20
12	45–70
24	NLT 80

Test 3

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Buffer E: Dissolve 3.59 g of sodium acetate trihydrate and 11.0 mL of 2 N acetic acid in 1000 mL of water. Adjust with 2 N acetic acid to a pH of 4.75.

Medium: *Buffer E*; 1000 mL

Apparatus 1: 10 mesh; 50 rpm

Times: 1, 2, 4, 8, and 12 h

Buffer F: 9.12 g/L of monobasic potassium phosphate in water

Mobile phase: Methanol and *Buffer F* (65:35). Adjust with phosphoric acid to a pH of 4.0.

Standard stock solution: 625 µg/mL of clarithromycin from USP Clarithromycin RS in methanol. Shake and sonicate, if necessary, to dissolve.

Standard solution: 125 µg/mL of clarithromycin from *Standard stock solution* in *Mobile phase*

System suitability stock solution: 625 µg/mL of USP Clarithromycin Related Compound A RS in methanol

System suitability solution: 125 µg/mL of clarithromycin related compound A from *System suitability stock solution* and 125 µg/mL of clarithromycin from *Standard stock solution* in *Mobile phase*

Sample solution: Withdraw 10 mL of the solution under test from each vessel and replace with 10 mL of *Medium*. Transfer 3 mL of the withdrawn solution to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass through a filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clarithromycin and clarithromycin related compound A are about 0.75 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between clarithromycin and clarithromycin related compound A, *System suitability solution*

Tailing factor: 0.9–2, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) dissolved at each time point (Q_T):

$$Q_1 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_2 = [Q_1 \times (V_S/V)] + [(r_U/r_S) \times (C_S/L) \times (V - V_S) \times 100]$$

$$Q_4 = [Q_1 \times (V_S/V)] + [Q_2 \times V_S/(V - 2V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 2V_S) \times 100]$$

$$Q_8 = [Q_1 \times (V_S/V)] + [Q_2 \times V_S/(V - 2V_S)] + [Q_4 \times V_S/(V - 3V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 3V_S) \times 100]$$

$$Q_{12} = [Q_1 \times (V_S/V)] + [Q_2 \times V_S/(V - 2V_S)] + [Q_4 \times V_S/(V - 3V_S)] + [Q_8 \times V_S/(V - 4V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 4V_S) \times 100]$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of clarithromycin in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
V_S = volume of the sample withdrawn at each time point (mL)
L = label claim (mg/Tablet)

Tolerances: The percentages of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Table 3

Time (h)	Amount Dissolved (%)
1	NMT 15
2	10–30
4	35–55
8	NLT 80
12	NLT 90

Test 4

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Buffer G: 6.8 g/L of potassium dihydrogen phosphate and 0.18 g/L of sodium hydroxide in water. Adjust with dilute sodium hydroxide or phosphoric acid to a pH of 6.0 ± 0.1.

Medium: *Buffer G*; 900 mL

Apparatus 2: 50 rpm

Times: 2, 4, 8, and 12 h

Buffer H: 6.8 g/L of potassium dihydrogen phosphate in water. Adjust with dilute sodium hydroxide or phosphoric acid to a pH of 4.5 ± 0.1.

Mobile phase: Methanol and *Buffer H* (64:36)

www.webofpharma.com

Standard solution: 0.4 mg/mL of USP Clarithromycin RS in methanol and *Medium* (4:96). Dissolve first in *Medium*, using 60% of the final volume. Sonicate about 10 min until dissolved. Add methanol, using 4% of the final volume. Dilute with *Medium* to volume.
Sample solution: Use the solution under test, passed through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 203 nm
Column: 4.0-mm × 12.5-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the concentration, in mg/mL, of clarithromycin ($C_{38}H_{69}NO_{13}$) in the *Sample solution* at each time point:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) dissolved at each time point (Q_T):

$$Q_2 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_4 = [Q_2 \times (V_S/V)] + [(r_U/r_S) \times (C_S/L) \times (V - V_S) \times 100]$$

$$Q_8 = [Q_2 \times (V_S/V)] + [Q_4 \times V_S/(V - 2V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 2V_S) \times 100]$$

$$Q_{12} = [Q_2 \times (V_S/V)] + [Q_4 \times V_S/(V - 2V_S)] + [Q_8 \times V_S/(V - 3V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 3V_S) \times 100]$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of clarithromycin in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
V_S = volume of the sample withdrawn at each time point (mL)
L = label claim (mg/Tablet)

Tolerances: The percentages of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Table 4

Time (h)	Amount Dissolved (%)
2	NMT 25
4	20–40
8	45–75
12	NLT 80

Test 5
If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: Phosphate buffer, pH 6.0 (6.8 g/L of monobasic potassium phosphate and 0.18 g/L of sodium hydroxide in water. Adjust with dilute sodium hydroxide or phosphoric acid to a pH of 6.0 ± 0.1); 900 mL
Apparatus 2: 50 rpm, with sinker (see *Dissolution* ⟨711⟩, *Figure 2a*)
Times: 2, 4, 8, and 14 h
Buffer: 9.11 g/L of monobasic potassium phosphate in water. Adjust with dilute sodium hydroxide or phosphoric acid to a pH of 2.5 ± 0.05.
Mobile phase: Acetonitrile and *Buffer* (55:45)
Standard solution: 0.55 mg/mL of USP Clarithromycin RS in *Medium*. Sonicate to dissolve prior to dilution to final volume.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.22-µm pore size. Replace the portion of solution withdrawn with an equal volume of *Medium*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 50°
Flow rate: 0.25 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of clarithromycin ($C_{38}H_{69}NO_{13}$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of clarithromycin in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 5*.

www.webofpharma.com

Table 5

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	NMT 25
2	4	20–40
3	8	45–75
4	14	NLT 80

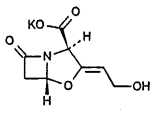
The percentages of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clarithromycin RS
  USP Clarithromycin Related Compound A RS
  6,11-Di-*O*-methylerythromycin A.
  $C_{39}H_{71}NO_{13}$ 762.00

Clavulanate Potassium



$C_8H_8KNO_5$ 237.25

4-Oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3-(2-hydroxyethylidene)-7-oxo-, monopotassium salt, 2*R*-(2α, 3*Z*,5α)-;
Potassium (*Z*)-(2*R*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate [61177-45-5].

DEFINITION

Clavulanate Potassium contains the equivalent of NLT 75.5% and NMT 92.0% of clavulanic acid ($C_8H_9NO_5$), calculated on the anhydrous basis.

IDENTIFICATION

- **A.** The retention time of the major peak for clavulanic acid of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Potassium*: Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: 7.8 mg/mL of monobasic sodium phosphate in water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 4.4 ± 0.1 before final dilution.
Mobile phase: Methanol and *Solution A* (1:19)
Standard solution: 0.25 mg/mL of USP Clavulanate Lithium RS in water
System suitability solution: 0.5 mg/mL of amoxicillin dissolved in *Standard solution*
Sample solution: 0.25 mg/mL of Clavulanate Potassium in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4-mm × 30-cm; 3- to 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for clavulanic acid and amoxicillin are about 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.5 between the amoxicillin and clavulanic acid peaks, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clavulanic acid ($C_8H_9NO_5$) in each mg of Clavulanate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clavulanate Lithium RS in the *Standard solution* (mg/mL)
C_U = concentration of Clavulanate Potassium in the *Sample solution* (mg/mL)
P = designated potency of USP Clavulanate Lithium RS, in µg/mg of clavulanic acid
F = unit conversion factor, 0.001 mg/µg

Acceptance criteria: 75.5%–92.0% on the anhydrous basis

IMPURITIES

- **PROCEDURE 1**

Solution A: 0.05 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 4.0 ± 0.1.
Solution B: Methanol and *Solution A* (1:1)
System suitability solution: 0.1 mg/mL each of USP Clavulanate Lithium RS and amoxicillin in *Solution A*
Mobile phase: See *Table 1*.
[NOTE—The system is equilibrated for 15 min with 100% *Solution A*.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
15	50	50
18	50	50
24	100	0

Standard solution: 0.1 mg/mL of USP Clavulanate Lithium RS in *Solution A*
Sample solution: 10.0 mg/mL of Clavulanate Potassium in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1

www.webofpharma.com

Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for clavulanic acid and amoxicillin are about 1.0 and 2.5, respectively.]
Suitability requirements
Resolution: NLT 13 between the clavulanic acid and amoxicillin peaks, *System suitability solution*
Tailing factor: NMT 2.0 for the clavulanic acid peak, *System suitability solution*
Relative standard deviation: NMT 2%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the Clavulanate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of an individual impurity from the *Sample solution*
r_S = peak response of clavulanic acid from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of Clavulanate Potassium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of clavulanate potassium, 237.3
M_{r2} = molecular weight of clavulanate lithium, 205.1

Acceptance criteria
Total impurities: NMT 2%

• **PROCEDURE 2: LIMIT OF CLAVAM-2-CARBOXYLATE POTASSIUM**

Mobile phase: 0.1 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 4.0 ± 0.1.
Standard solution: 5 µg/mL of USP Clavam-2-carboxylate Potassium RS in water
Sample solution: 10 mg/mL of Clavulanate Potassium in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4-mm × 30-cm; 3- to 10-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for clavam-2-carboxylic acid and clavulanic acid are about 0.7 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clavam-2-carboxylate potassium in the portion of Clavulanate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clavam-2-carboxylate Potassium RS in the *Standard solution* (µg/mL)
C_U = concentration of Clavulanate Potassium in the *Sample solution* (mg/mL)
F = unit conversion factor, 0.001 mg/µg

Acceptance criteria: NMT 0.01%

• **PROCEDURE 3: LIMIT OF ALIPHATIC AMINES**

Internal standard solution: 50 µL of 3-methyl-2-pentanone in water to 100 mL
Standard solution: Dissolve 80.0 mg of each of the following amines in 2 N hydrochloric acid: 1,1-dimethylethylamine, diethylamine, tetramethylethylenediamine, 1,1,3,3-tetramethylbutylamine, and *N,N'*-diisopropylethylenediamine. Dilute with 2 N hydrochloric acid to 200.0 mL. Transfer 5.0 mL of this solution to a centrifuge tube. Add 5.0 mL of *Internal standard solution*, 10.0 mL of 2 N sodium hydroxide, 5.0 mL of isobutyl alcohol, and 5 g of sodium chloride. Shake for 1 min, and centrifuge to separate the layers. Use the upper layer.
Sample solution: Transfer 1.0 g of Clavulanate Potassium to a centrifuge tube, add 5.0 mL of *Internal standard solution*, 5.0 mL of 2 N sodium hydroxide, 10.0 mL of water, 5.0 mL of isobutyl alcohol, and 5 g of sodium chloride. Shake for 1 min, and centrifuge to separate the layers. Use the upper layer.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 50-m capillary fused silica; 5-µm film coating of stationary phase G41
Temperatures
Injection port: 200°
Detector: 250°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
35	—	35	7
35	30	150	15

Carrier gas: Helium
Flow rate: 8 mL/min
Injection type: Split ratio, 1:10
Injection volume: 1 µL
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—See *Table 3* for relative retention times.]

Table 3

Name	Relative Retention Time
1,1-Dimethylethylamine	0.55
Diethylamine	0.76
3-Methyl-2-pentanone (internal standard)	1.0
Tetramethylethylenediamine	1.07
1,1,3,3-Tetramethylbutylamine	1.13
N,N'-Diisopropylethylenediamine	1.33

USP Monographs

www.webofpharma.com

Table 3 *(continued)*

Name	Relative Retention Time
Bis(2-methylamino)ethyl ether[a]	1.57

[a] The relative retention time for this compound is provided for information only; bis(2-methylamino)ethyl ether is not a component of the *Standard solution*.

Calculate the percentage of each individual impurity in the portion of Clavulanate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each individual impurity from the *Sample solution*
r_S = peak response for the relevant analyte from the *Standard solution*
C_S = concentration of the relevant analyte in the *Standard solution* (mg/mL)
C_U = concentration of Clavulanate Potassium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual impurity for which no relevant reference compound is provided in the *Standard solution* by the same formula, except for r_S, using the peak response corresponding to the 1,1-dimethylethylamine peak.

Acceptance criteria

Total of all aliphatic amines: NMT 0.2%

- **PROCEDURE 4: LIMIT OF 2-ETHYLHEXANOIC ACID**

Internal standard solution: 1 mg/mL of 3-cyclohexylpropionic acid in cyclohexane

Standard solution: 1.5 mg/mL of 2-ethylhexanoic acid in *Internal standard solution*. Transfer 1.0 mL of this solution to a centrifuge tube, and add 4.0 mL of 4 N hydrochloric acid. Shake for 1 min, and allow the phases to separate, centrifuging if necessary. Withdraw the lower phase, and reserve the upper phase. To the lower phase add 1.0 mL of *Internal standard solution*, and shake for 1 min. Allow the phases to separate, centrifuging if necessary. Withdraw the upper phase, and combine with the reserved upper layer.

Sample solution: Transfer 300 mg of Clavulanate Potassium to a centrifuge tube. Add 4.0 mL of 4 N hydrochloric acid, and shake with two successive 1.0-mL portions of the *Internal standard solution*. Allow the phases to separate, centrifuging if necessary. Use the combined upper phases.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 25-m capillary fused silica; 1-µm film coating of stationary phase G35

Temperatures

Injection port: 200°

Detector: 300°

Column: See *Table 4*.

Table 4

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	2
40	30	200	3

Carrier gas: Hydrogen

Flow rate: 100 cm/s

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the 2-ethylhexanoic acid and 3-cyclohexylpropionic acid peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 2-ethylhexanoic acid in the portion of Clavulanate Potassium taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of 2-ethylhexanoic acid to 3-cyclohexylpropionic acid from the *Sample solution*
R_S = peak response ratio of 2-ethylhexanoic acid to 3-cyclohexylpropionic acid from the *Standard solution*
C_S = concentration of 2-ethylhexanoic acid in the *Standard solution* (mg/mL)
C_U = concentration of Clavulanate Potassium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.8%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Clavulanate Potassium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.03 USP Endotoxin Units/mg.
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined,Membrane Filtration***:** Where the label states that Clavulanate Potassium is sterile, it meets the requirements when tested as directed.
- **pH** ⟨791⟩

Sample: 10 mg/mL

Acceptance criteria: 5.5–8.0

- **WATER DETERMINATION** ⟨921⟩, *Method I***:** NMT 1.5%

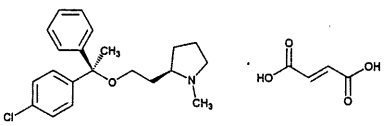
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Clavam-2-carboxylate Potassium RS

USP Clavulanate Lithium RS

Clemastine Fumarate



$C_{21}H_{26}ClNO \cdot C_4H_4O_4$ 459.96

Pyrrolidine, 2-[2-[1-(4-chlorophenyl)-1-phenylethoxy]ethyl]-1-methyl-, [*R*-(*R**,*R**)]-, (*E*)-2-butenedioate (1:1); (+)-(2*R*)-2-[2-[[[(*R*)-*p*-Chloro-α-methyl-α-phenylbenzyl]-oxy] ethyl]-1-methylpyrrolidine fumarate (1:1) [14976-57-9].

DEFINITION

Clemastine Fumarate contains NLT 98.0% and NMT 102.0% of clemastine fumarate ($C_{21}H_{26}ClNO \cdot C_4H_4O_4$), calculated on the dried basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197M▲** (CN 1-May-2020)
- **B.** The retention times of the fumarate and clemastine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 4.1 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0.
Mobile phase: Methanol, acetonitrile, and *Buffer* (35:35:30)
Standard solution: 0.28 mg/mL of USP Clemastine Fumarate RS in *Mobile phase*
Sample solution: 0.28 mg/mL of Clemastine Fumarate in *Mobile phase*
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 5-cm; 1.8-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 5 µL
Run time: 3 min
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8 for clemastine
Relative standard deviation: NMT 0.73% for clemastine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clemastine fumarate ($C_{21}H_{26}ClNO \cdot C_4H_4O_4$) in the portion of Clemastine Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clemastine from the *Sample solution*
r_S = peak response of clemastine from the *Standard solution*
C_S = concentration of USP Clemastine Fumarate RS in the *Standard solution* (mg/mL)
C_U = concentration of Clemastine Fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer: 4.1 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0.
Solution A: Methanol, acetonitrile, and *Buffer* (35:35:30)
Solution B: Methanol, acetonitrile, and *Buffer* (40: 37.5: 22.5)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
3.1	0	100
18	0	100
18.1	100	0
25	100	0

Standard stock solution 1: 0.14 mg/mL of USP Clemastine Fumarate RS in *Solution A*. Sonication may be needed to aid dissolution.
Standard stock solution 2: 0.14 mg/mL of USP 4-Chlorobenzophenone RS in methanol. Sonication may be needed to aid dissolution.
Sensitivity solution: 0.14 µg/mL of USP Clemastine Fumarate RS in *Solution A* from *Standard stock solution 1*
Standard solution: 0.28 µg/mL of USP Clemastine Fumarate RS and 0.36 µg/mL of USP 4-Chlorobenzophenone RS in *Solution A* from *Standard stock solution 1* and *Standard stock solution 2*, respectively
Sample solution: 0.28 mg/mL of Clemastine Fumarate in *Solution A*
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 5-cm; 1.8-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 50 µL
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between clemastine and 4-chlorobenzophenone, *Standard solution*
Relative standard deviation: NMT 2.0% for clemastine, *Standard solution*
Signal-to-noise ratio: NLT 50 for clemastine, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 4-chlorobenzophenone in the portion of Clemastine Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 4-chlorobenzophenone from the *Sample solution*
r_S = peak response of 4-chlorobenzophenone from the *Standard solution*
C_S = concentration of USP 4-Chlorobenzophenone RS in the *Standard solution* (µg/mL)
C_U = concentration of Clemastine Fumarate in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Clemastine Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual unspecified impurity from the *Sample solution*
r_S = peak response of clemastine from the *Standard solution*
C_S = concentration of USP Clemastine Fumarate RS in the *Standard solution* (mg/mL)
C_U = concentration of Clemastine Fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks having areas less than 0.05% of clemastine.

www.webofpharma.com

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fumaric acid[a]	0.5	—
Clemastine	1.0	—
4-Chlorobenzophenone	1.7	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

[a] Salt counter ion is included in this table for identification purposes only.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL in methanol
  Acceptance criteria: +15.0° to +18.0° (T = 20°)
- **pH** ⟨791⟩
  Sample solution: 100 mg/mL suspension
  Acceptance criteria: 3.2–4.2
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° to constant weight.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature not exceeding 25°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP 4-Chlorobenzophenone RS
  4-Chlorobenzophenone.
  $C_{13}H_9ClO$ 216.66
  USP Clemastine Fumarate RS

Clemastine Fumarate Tablets

DEFINITION

Clemastine Fumarate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clemastine fumarate ($C_{21}H_{26}ClNO \cdot C_4H_4O_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the clemastine peak of the *Diluted sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY

- **PROCEDURE**
  Solution A: 9.47 g/L of anhydrous dibasic sodium phosphate in water
  Solution B: 9.08 g/L of monobasic potassium phosphate in water
  Solution C: *Solution A* and *Solution B* (612:388)
  Buffer: *Solution C* and water (25:75)
  Mobile phase: Methanol and *Buffer* (83:17)
  Diluent: Methanol and water (50:50)
  Standard solution: 0.14 mg/mL of USP Clemastine Fumarate RS in *Diluent*
  Sample solution: Transfer a quantity of NLT 20 finely powdered Tablets, equivalent to 14 mg of clemastine fumarate, to a 200-mL conical flask. Pipet 100 mL of *Diluent* into the flask, shake for 30 min, centrifuge, and filter the supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 10-µm packing L7
Flow rate: 4 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clemastine fumarate ($C_{21}H_{26}ClNO \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clemastine Fumarate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clemastine fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Buffer: Dissolve 20.0 g of citric acid monohydrate in 1000 mL of water, add 22.0 mL of sodium hydroxide solution (3 in 10) and 8.8 mL of hydrochloric acid, and dilute with water to 2000 mL. Adjust, if necessary, with sodium hydroxide solution (1 in 2) to a pH of 4.0.
  Medium: *Buffer*; 500 mL
  Apparatus 2: 50 rpm
  Time: 30 min
  Standard solution: USP Clemastine Fumarate RS in *Medium* with a similar concentration to the *Sample solution*
  Sample solution: Centrifuge 60 mL of the solution under test for 20 min at 4000 rpm.
  Instrumental conditions
  Mode: UV
  Analytical wavelength: About 420 nm
  Blank: *Medium*
  Analysis
  Samples: *Standard solution*, *Sample solution*, and *Blank*
  Transfer 50.0 mL of the *Samples* to individual 125-mL separatory funnels, and treat each of the solutions as follows. Add 10 mL of methyl orange solution (2 in 10,000), mix, add 20.0 mL of chloroform, shake simultaneously by mechanical means for 10 min, remove the chloroform layer, and centrifuge the chloroform layer for 10 min at 4000 rpm. Use the *Blank* to set the instrument.
  Calculate the percentage of the labeled amount of clemastine fumarate ($C_{21}H_{26}ClNO \cdot C_4H_4O_4$) dissolved.
  Tolerances: NLT 75% (Q) of the labeled amount of clemastine fumarate ($C_{21}H_{26}ClNO \cdot C_4H_4O_4$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**
  Buffer: 4.1 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0.
  Solution A: Methanol, acetonitrile, and *Buffer* (35:35:30)
  Solution B: Methanol, acetonitrile, and *Buffer* (40: 37.5: 22.5)

www.webofpharma.com

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
3.1	0	100
18	0	100
18.1	100	0
25	100	0

Standard stock solution 1: 0.14 mg/mL of USP Clemastine Fumarate RS in *Solution A*. Sonicate for NLT 5 min or until dissolved.
Standard stock solution 2: 0.14 mg/mL of USP 4-Chlorobenzophenone RS in methanol. Sonicate for NLT 5 min or until dissolved.
System suitability solution: 2.8 µg/mL each of USP Clemastine Fumarate RS and USP 4-Chlorobenzophenone RS in *Solution A* from *Standard stock solution 1* and *Standard stock solution 2*
Sensitivity solution: 0.14 µg/mL of USP Clemastine Fumarate RS in *Solution A* from *Standard stock solution 1*
Standard solution: 2.8 µg/mL of USP Clemastine Fumarate RS in *Solution A* from *Standard stock solution 1*
Sample solution: Nominally 0.28 mg/mL of clemastine fumarate from Tablets in *Solution A* prepared as follows. Transfer a quantity of NLT 20 finely powdered Tablets, equivalent to 14 mg of clemastine fumarate, to a 50-mL volumetric flask. Add 25 mL of *Solution A*, shake the flask for NLT 30 min, and sonicate for NLT 15 min. Dilute with *Solution A* to volume. Pass an aliquot through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate.
Diluted sample solution: Nominally 2.8 µg/mL of clemastine fumarate in *Solution A* from *Sample solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm. For *Identification* test *B*, use a diode array detector in the range of 200–300 nm.
Column: 4.6-mm × 5-cm; 1.8-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[NOTE—The relative retention times for clemastine and 4-chlorobenzophenone are 1.0 and 1.7, respectively.]
Suitability requirements
Resolution: NLT 1.5 between clemastine and 4-chlorobenzophenone, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution*, *Diluted sample solution*, and *Sample solution*
[NOTE—The *Diluted sample solution* is used for *Identification* test *B*.]
Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

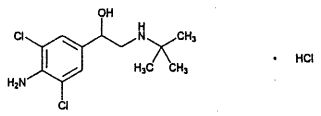
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of clemastine from the *Standard solution*
C_S = concentration of USP Clemastine Fumarate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clemastine fumarate in the *Sample solution* (mg/mL)

Acceptance criteria
Any unspecified degradation product: NMT 0.5%
Total impurities: NMT 2.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP 4-Chlorobenzophenone RS
4-Chlorobenzophenone.
$C_{13}H_9ClO$ 216.66
USP Clemastine Fumarate RS

Clenbuterol Hydrochloride



$C_{12}H_{18}Cl_2N_2O \cdot HCl$ 313.65
Ethanol, 1-(4-amino-3,5-dichlorophenyl)-2-(*tert*-butylamino), hydrochloride;
4-Amino-α-[(*tert*-butylamino)methyl]-3,5-dichlorobenzyl alcohol, hydrochloride [21898-19-1].

DEFINITION
Clenbuterol Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{12}H_{18}Cl_2N_2O \cdot HCl$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY
- **PROCEDURE**
Sample solution: Dissolve 0.25 g in 50 mL of alcohol, and add 5.0 mL of 0.01 N hydrochloric acid.
Analysis: Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Read the volume added between the two points of inflection. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N sodium hydroxide is equivalent to 31.37 mg of $C_{12}H_{18}Cl_2N_2O \cdot HCl$.
Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
INORGANIC IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.1%, from 1–2 g

USP Monographs

www.webofpharma.com

ORGANIC IMPURITIES
• **Procedure**
Buffer: Dissolve 3.0 g of sodium 1-decanesulfonate and 5.0 g of monobasic potassium phosphate in 900 mL of water, adjust with dilute phosphoric acid (1 in 10) to a pH of 3.0, and dilute with water to 1000 mL.
Mobile phase: Acetonitrile, methanol, and *Buffer* (2:2:6)
System suitability solution: 0.2 mg/mL each of USP Clenbuterol Related Compound B RS and Clenbuterol Hydrochloride in *Mobile phase*
Sample solution 1: 2.0 mg/mL of Clenbuterol Hydrochloride in *Mobile phase*
Sample solution 2: 2.0 µg/mL of Clenbuterol Hydrochloride in *Mobile phase*, from *Sample solution 1*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.0-mm × 12.5-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 0.5 mL/min
Injection size: 5 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 4.0 between clenbuterol related compound B and clenbuterol
Relative standard deviation: NMT 2.0% for the clenbuterol peak
Analysis
Samples: *Sample solution 1* and *Sample solution 2*
Calculate the percentage of impurities in the portion of $C_{12}H_{18}Cl_2N_2O \cdot HCl$ taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

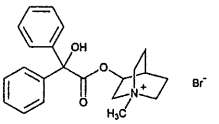
r_U = peak response of each impurity from *Sample solution 1*
r_S = peak response of clenbuterol from *Sample solution 2*
C_S = concentration of Clenbuterol Hydrochloride in *Sample solution 2* (mg/mL)
C_U = concentration of Clenbuterol Hydrochloride in *Sample solution 1* (mg/mL)

Acceptance criteria
Individual impurities: 0.1%
Total impurities: NMT 0.2%
[NOTE—The reporting level for impurities is 0.05%.]

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample: 30 mg/mL in water, filter as necessary
Acceptance criteria: −10° to +10° at 20°
• **pH** ⟨791⟩**:** 5.0–7.0
Sample: 50 mg/mL in carbon dioxide-free water
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.
• **LABELING:** Label it to indicate that it is for veterinary use only.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clenbuterol Hydrochloride RS
USP Clenbuterol Related Compound B RS
1-(4-Amino-3,5-dichlorophenyl)-2-*tert*-butylaminoethanone hydrochloride.
$C_{12}H_{16}Cl_2N_2O \cdot HCl$ 311.64

Clidinium Bromide



$C_{22}H_{26}BrNO_3$ 432.35
1-Azoniabicyclo[2.2.2]octane, 3-[(hydroxydiphenylacetyl)oxy]-1-methyl-, bromide, (±)-;
(±)-3-Hydroxy-1-methylquinuclidinium bromide benzilate [3485-62-9].

DEFINITION
Clidinium Bromide contains NLT 99.0% and NMT 100.5% of $C_{22}H_{26}BrNO_3$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.
• **C. BROMIDE**
Sample solution: 50 mg/mL
Analysis: To 2 mL of the *Sample solution* add a few drops of 2 N nitric acid and 1 mL of silver nitrate TS.
Acceptance criteria: A yellowish white precipitate is formed.

ASSAY
• **PROCEDURE**
Sample: 1.2 g
Analysis: Dissolve the *Sample* in 80 mL of glacial acetic acid, warming if necessary to effect solution. Cool, and add 15 mL of mercuric acetate TS. Titrate with 0.1 N perchloric acid in dioxane VS, determining the endpoint potentiometrically. Perform a blank determination (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 43.24 mg of $C_{22}H_{26}BrNO_3$.
Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **ORGANIC IMPURITIES**
Standard solution: 100 mg/mL of USP Clidinium Bromide RS in 0.1 N methanolic hydrochloric acid
Sample solution: 100 mg/mL of Clidinium Bromide in 0.1 N methanolic hydrochloric acid
Reference solution: Dissolve 100 mg of USP Clidinium Bromide RS in 1.0 mL of 0.1 N methanolic hydrochloric acid, and add 20 µL of a solution of 25.0 mg of USP Clidinium Bromide Related Compound A RS in 1.0 mL of 0.1 N methanolic hydrochloric acid.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: Acetone, methanol, hydrochloric acid, and water (70:20:5:5)
Spray reagent: Dissolve 850 mg of bismuth subnitrate in a mixture of 10 mL of glacial acetic acid and 40 mL of water. In a separate container, dissolve 20 g of potassium iodide in 50 mL of water. Mix the two solutions, and dilute

USP Monographs

www.webofpharma.com

with dilute sulfuric acid (1 in 10) to 500 mL. Add 7.5 g ± 2.5 g of iodine, and mix until the solution is complete.

Chromatographic plates: Predevelop suitable thin-layer chromatographic plates by placing in a chromatographic chamber saturated with the *Developing solvent system*, and allow the *Developing solvent system* to move about 15 cm. Remove the plates from the chamber, dry at 105° for 15 min, and cool.

Analysis 1 (3-quinuclidinyl benzilate): Apply the *Standard solution* and the *Sample solution* to a *Chromatographic plate.* Place the plate in an unsaturated chromatographic chamber containing freshly prepared *Developing solvent system*, and allow the solvent front to move 10 cm. Remove the plate, dry at 105° for 10 min, cool, and spray with potassium iodoplatinate TS.

Acceptance criteria 1: The *Sample solution* shows no spot at an R_F value (about 0.8) corresponding to that of 3-quinuclidinyl benzilate.

Analysis 2 (limit of clidinium bromide related compound A): Apply the *Sample solution* and *Reference solution* to a second *Chromatographic plate.* Place the plate in an unsaturated chromatographic chamber containing freshly prepared *Developing solvent system*, and allow the solvent front to move 15 cm. Remove the plate, dry at 105° for 10 min, cool, and spray with the *Spray reagent.*

Acceptance criteria 2: Any spot from the *Sample solution* at an R_F value of about 0.4 is not greater in size or intensity than the minor spot of the *Reference solution:* NMT 0.5% of clidinium bromide related compound A is found.

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩**:** Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩
  USP Clidinium Bromide RS
  USP Clidinium Bromide Related Compound A RS
  3-Hydroxy-1-methylquinuclidinium bromide.
  $C_8H_{16}BrNO$ 222.13

Clindamycin Injection

DEFINITION

Clindamycin Injection contains an amount of Clindamycin Phosphate in Water for Injection equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$). It may be frozen.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

- **Procedure**

Mobile phase: Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, mix, and filter. Ensure that the concentration of acetonitrile in the *Mobile phase* is NLT 22% and NMT 25% to retain the correct elution order.

System suitability stock solution: 0.1 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*

System suitability solution: 25 µg/mL of USP Benzyl Alcohol RS from *System suitability stock solution* and 0.25 mg/mL of USP Clindamycin Phosphate RS, in *Mobile phase*

Standard solution: 0.24 mg/mL of USP Clindamycin Phosphate RS in *Mobile phase*

Sample stock solution: Nominally 3 mg/mL of clindamycin from Injection in *Mobile phase*

Sample solution: 0.21 mg/mL of clindamycin from *Sample stock solution* in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for clindamycin phosphate and benzyl alcohol are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between clindamycin phosphate and benzyl alcohol, *System suitability solution*
Relative standard deviation: NMT 2.5, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$) in the portion of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of clindamycin phosphate from the *Sample solution*
r_S = peak response of clindamycin phosphate from the *Standard solution*
C_S = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clindamycin in the *Sample solution* (mg/mL)
P = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 5.5–7.0
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 0.58 USP Endotoxin Unit/mg of clindamycin
- **Particulate Matter in Injections** ⟨788⟩**:** Meets the requirements for small-volume injections
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, or in suitable plastic containers.
- **Labeling:** Meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.* Where it is maintained in the frozen state, the label states that it is to be thawed just before use, describes the conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
- **USP Reference Standards** ⟨11⟩
  USP Benzyl Alcohol RS
  USP Clindamycin Phosphate RS

USP Monographs

www.webofpharma.com

Clindamycin for Injection

DEFINITION

Clindamycin for Injection contains an amount of Clindamycin Phosphate equivalent to NLT 758 µg/mg of clindamycin ($C_{18}H_{33}ClN_2O_5S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197M▲** (CN 1-May-2020)

Sample: Undried specimen

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: Add 14 mL of phosphoric acid to 4000 mL of HPLC grade water. Add 10 mL of ammonium hydroxide, and adjust with ammonium hydroxide to a pH of 3.90 ± 0.05.

Solution A: Acetonitrile and methanol (9:1)

Diluent: *Solution A* and *Buffer* (1:4)

Solution B: *Solution A* and *Buffer* (2:23)

Solution C: *Solution A* and *Buffer* (12:13)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	95	5
40	5	95
41	95	5
46	95	5

Standard solution: 2.2 mg/mL of USP Clindamycin Phosphate RS in *Diluent*, prepared as follows. Shake briefly, and sonicate for 5 min to dissolve. Allow to cool to ambient temperature.

Sample solution: Nominally 2.2 mg/mL of clindamycin phosphate in *Diluent* from Clindamycin for Injection, prepared as follows. Shake briefly, and sonicate for 5 min to dissolve. Allow to cool to ambient temperature.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between clindamycin phosphate and its nearest related compound. Calculate the resolution using peak widths at half height.

Peak-to-valley ratio: NLT 2.5

Calculate the peak-to-valley ratio:

$$\text{Result} = H_p / H_v$$

H_p = height above the baseline of the nearest related compound peak

H_v = height above the baseline of the valley between clindamycin phosphate and its nearest related compound

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity of clindamycin ($C_{18}H_{33}ClN_2O_5S$), in µg/mg, in the portion of Clindamycin for Injection taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times P$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clindamycin phosphate in the *Sample solution* (mg/mL)

P = potency of clindamycin in the USP Clindamycin Phosphate RS (µg/mg)

Acceptance criteria: NLT 758 µg/mg on the anhydrous basis

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** Contains NMT 0.58 USP Endotoxin Unit/mg of clindamycin
- **STERILITY TESTS ⟨71⟩**

Sample solution: 6 g of Clindamycin for Injection aseptically dissolved in 200 mL of *Fluid A*

Acceptance criteria: Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*

- **pH ⟨791⟩**

Sample solution: Nominally 10 mg/mL of clindamycin in water from Clindamycin for Injection

Acceptance criteria: 3.5–4.5

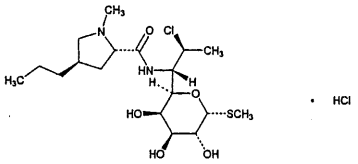
- **WATER DETERMINATION, *Method I* ⟨921⟩:** NMT 6.0%
- **CRYSTALLINITY ⟨695⟩:** Meets the requirements
- **Other Requirements:** It responds to the *Identification* test in *Clindamycin Phosphate*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Clindamycin Phosphate RS

Clindamycin Hydrochloride



$C_{18}H_{33}ClN_2O_5S \cdot HCl$ 461.44

$C_{18}H_{33}ClN_2O_5S \cdot HCl \cdot H_2O$ 479.47

L-*threo*-α-D-*galacto*-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[(1-methyl-4-propyl-2-pyrrolidinyl)- carbonyl] amino]-1-thio-, (2*S*-*trans*)-, monohydrochloride;

Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-*threo*-α-D-*galacto*-octopyranoside monohydrochloride [21462-39-5].

Monohydrate [58207-19-5].

USP Monographs

www.webofpharma.com

DEFINITION

Clindamycin Hydrochloride is the hydrated hydrochloride salt of clindamycin, a substance produced by the chlorination of lincomycin. It has a potency equivalent to NLT 800 µg/mg of $C_{18}H_{33}ClN_2O_5S$.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197M▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 8 N potassium hydroxide to a pH of 7.5.
Mobile phase: Acetonitrile and *Buffer* (9:11)
Standard solution: 1 mg/mL of USP Clindamycin Hydrochloride RS in *Mobile phase*
Sample solution: 1 mg/mL of Clindamycin Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
[NOTE—USP Clindamycin Hydrochloride RS contains clindamycin B and 7-epiclindamycin as minor components.]
Resolution: NLT 2.4 between clindamycin B and 7-epiclindamycin and NLT 3.0 between 7-epiclindamycin and clindamycin
Tailing factor: NMT 1.2 for the clindamycin peak
Relative standard deviation: NMT 1.0% for the clindamycin peak
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms for a period of time that is twice the retention time of the clindamycin peak.
Calculate the potency of $C_{18}H_{33}ClN_2O_5S$, in µg/mg, in the portion of Clindamycin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Clindamycin Hydrochloride in the *Sample solution* (mg/mL)
P = potency of clindamycin in USP Clindamycin Hydrochloride RS (µg/mg)

Acceptance criteria: NLT 800 µg/mg

IMPURITIES

ORGANIC IMPURITIES

- **Procedure**

Buffer and **Mobile phase:** Prepare as directed in the *Assay*.
Standard stock solution: 0.5 mg/mL of USP Lincomycin Hydrochloride RS and 1 mg/mL of USP Clindamycin Hydrochloride RS in *Mobile phase*
Standard solution: 50 µg/mL of USP Lincomycin Hydrochloride RS and 100 µg/mL of USP Clindamycin Hydrochloride RS from *Standard stock solution* in *Mobile phase*
Sample solution: 5 mg/mL of Clindamycin Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms for a period of time that is six times the retention time of clindamycin.
Calculate the percentage of lincomycin in the portion of Clindamycin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of lincomycin from the *Sample solution*
r_S = peak response of lincomycin from the *Standard solution*
C_S = concentration of USP Lincomycin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Clindamycin Hydrochloride in the *Sample solution* (mg/mL)
P = potency of USP Lincomycin Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Calculate the percentage of all other related compounds in the portion of Clindamycin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each individual related compound, other than lincomycin, from the *Sample solution*
r_S = peak response of clindamycin from the *Standard solution*
C_S = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of USP Clindamycin Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lincomycin[a]	0.4	—
Clindamycin B	0.65	2.0
7-Epiclindamycin	0.8	4.0
Clindamycin	1.0	—
Any other individual related compound	—	1.0

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total related compounds[b]	—	6.0

[a] Lincomycin is controlled in the total of all related compounds. There is no individual acceptance criterion for this compound.
[b] Total of all related compounds including lincomycin.

SPECIFIC TESTS
- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **pH** ⟨791⟩: 3.0–5.5, in a 100-mg/mL solution
- **WATER DETERMINATION,** *Method I* ⟨921⟩: 3.0%–6.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Clindamycin Hydrochloride RS
L-*threo*-α-D-*galacto*-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[(1-methyl-4-propyl-2-pyrrolidinyl)-carbonyl]amino]-1-thio-, (2*S*-*trans*)-, monohydrochloride.
$C_{18}H_{33}ClN_2O_5S \cdot HCl$ 461.45

USP Lincomycin Hydrochloride RS
D-*erythro*-α-D-*galacto*-Octopyranoside, methyl 6,8-dideoxy-6-[[(1-methyl-4-propyl-2-pyrrolidinyl) carbonyl]amino]-1-thio-, monohydrochloride, monohydrate, (2*S*-*trans*)-.
$C_{18}H_{34}N_2O_6S \cdot HCl \cdot H_2O$ 461.02

Clindamycin Hydrochloride Capsules

DEFINITION
Clindamycin Hydrochloride Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, relative to the internal standard, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Add 2 g of *dl*-10-camphorsulfonic acid, 1 g of ammonium acetate, and 1 mL of glacial acetic acid to 200 mL of water in a 500-mL volumetric flask, and mix to dissolve. Dilute with methanol to volume, and mix. Adjust, if necessary, with hydrochloric acid or a sodium hydroxide solution (1 in 2) to a pH of 6.0 ± 0.1.
Internal standard solution: Add 0.5 mL of phenylethyl alcohol to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Standard solution: 18 mg/mL of USP Clindamycin Hydrochloride RS in *Internal standard solution*
Sample solution: Equivalent to 15 mg/mL of clindamycin, from the contents of NLT 20 Capsules, in *Internal standard solution*; shake for 30 min; centrifuge or filter, if necessary, to obtain a clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4-mm × 30-cm stainless steel; packing L1
Flow rate: 1 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for the internal standard and clindamycin are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 5.0 between the analyte and internal standard
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clindamycin ($C_{18}H_{33}ClN_2O_5S$) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P \times F \times 100$$

R_U = peak response ratio of clindamycin to the internal standard from the *Sample solution*
R_S = peak response ratio of clindamycin to the internal standard from the *Standard solution*
C_S = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clindamycin in the *Sample solution* (mg/mL)
P = potency of clindamycin in USP Clindamycin Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Mobile phase: Dissolve 16 g of *dl*-10-camphorsulfonic acid, 8 g of ammonium acetate, and 8 mL of glacial acetic acid in 1600 mL of water. Add 2400 mL of methanol, and adjust with hydrochloric acid or 5 N sodium hydroxide to a pH of 6.0 ± 0.05.
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Standard solution: Prepare a solution of USP Clindamycin Hydrochloride RS in *Medium* having a known concentration similar to that expected in the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 3-µm packing L1
Flow rate: 2 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 7.0%

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clindamycin Hydrochloride RS

Add the following:

▲Clindamycin Hydrochloride Compounded Oral Solution

DEFINITION
Clindamycin Hydrochloride Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$).
Prepare Clindamycin Hydrochloride Compounded Oral Solution 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Clindamycin (as Clindamycin Hydrochloride)	1 g (calculate)[a]
Suspendlt,[b] a sufficient quantity to make	100 mL

[a] Calculate the amount of clindamycin hydrochloride powder based on the salt conversion and potency obtained from the Certificate of Analysis.
[b] PCCA, Houston, TX.

Place the *Clindamycin Hydrochloride* in a suitable container and triturate to a fine powder. Add a small amount of *Suspendlt* and mix well to form a smooth paste. Add a sufficient amount of *Suspendlt* to make a liquid that is pourable. Transfer contents stepwise and quantitatively to a calibrated container using the remainder of the *Suspendlt*. Add sufficient *Suspendlt* to bring to final volume, and mix well.

ASSAY
- **PROCEDURE**

Solution A: 50 mM monobasic sodium phosphate adjusted with phosphoric acid to a pH of 2.9
Mobile phase: Acetonitrile and *Solution A* (15:85)
Standard solution: 0.01 mg/mL of clindamycin prepared from USP Clindamycin Hydrochloride RS in *Solution A*
Sample solution: Transfer 0.01 mL of Oral Solution to a 10-mL volumetric flask, and dilute with *Solution A* to volume. Mix on a vortex mixer for 30 s.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 2.1-mm × 10-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for clindamycin is about 3.5 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clindamycin from the *Sample solution*
r_S = peak response of clindamycin from the *Standard solution*
C_S = concentration of clindamycin in the *Standard solution* (mg/mL)
C_U = nominal concentration of clindamycin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.7–5.7

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clindamycin Hydrochloride RS▲ (USP 1-Aug-2019)

Clindamycin Hydrochloride Oral Solution

DEFINITION
Clindamycin Hydrochloride Oral Solution contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$).

IDENTIFICATION
- **A.** The retention time of the clindamycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 8 N potassium hydroxide to a pH of 7.5.
Mobile phase: Acetonitrile and *Buffer* (450:550). Increasing the proportion of acetonitrile in the *Mobile phase* decreases the retention time, and decreasing it increases the resolution between 7-epiclindamycin and clindamycin.
▲**Peroxide solution:** Transfer 7 mL of hydrogen peroxide to a 100-mL volumetric flask and dilute with water to volume.
System suitability solution: 2 mg/mL of USP Clindamycin Hydrochloride RS prepared as follows. Transfer a weighed amount of USP Clindamycin Hydrochloride RS to a suitable volumetric flask and add *Peroxide solution* to fill 10% of the final volume. Dilute with *Mobile phase* to volume. [NOTE—Clindamycin when treated with *Peroxide solution* generates two degradation products. Peroxide degradation product 1 has a relative retention time of 0.36 and peroxide degradation product 2 has a relative retention time of 0.41.]▲ (USP 1-Aug-2019)
Standard solution: 1 mg/mL of USP Clindamycin Hydrochloride RS in *Mobile phase*
Sample solution: Nominally equivalent to 0.85 mg/mL of clindamycin from Oral Solution in *Mobile phase*

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
▲Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between the peroxide degradation product 1 and peroxide degradation product 2 peaks, *System suitability solution*
Tailing factor: NMT 1.2, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*▲ (USP 1-Aug-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲the labeled amount of▲ (USP 1-Aug-2019) clindamycin ($C_{18}H_{33}ClN_2O_5S$) in ▲the portion▲ (USP 1-Aug-2019) of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area response from the *Sample solution*
r_S = peak area response from the *Standard solution*
C_S = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clindamycin in the *Sample solution* (mg/mL)
P = potency of clindamycin in USP Clindamycin Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Uniformity of Dosage Units** ⟨905⟩
For Oral Solution packaged in single-unit containers: Meets the requirements
• **Deliverable Volume** ⟨698⟩**:** Meets the requirements

IMPURITIES

Add the following:

▲• Organic Impurities
Buffer, Mobile phase, Peroxide solution, System suitability solution, and Chromatographic system: ᵎ Proceed as directed in the *Assay*.
Standard solution: 0.05 mg/mL of USP Clindamycin Hydrochloride RS in *Mobile phase*
Sensitivity solution: 0.005 mg/mL of USP Clindamycin Hydrochloride RS in *Mobile phase* from *Standard solution*
Sample solution: Nominally 5 mg/mL of clindamycin in *Mobile phase* prepared as follows. Transfer an appropriate volume of Oral Solution to a suitable volumetric flask. Dilute with *Mobile phase* to volume.
System suitability
Samples: *System suitability solution, Standard solution,* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between the peroxide degradation product 1 and peroxide degradation product 2 peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of clindamycin from the *Standard solution*
C_S = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clindamycin in the *Sample solution* (mg/mL)
P = potency of clindamycin in USP Clindamycin Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lincomycin[a]	0.32	—
Clindamycin B[b]	0.65	2.0
7-Epiclindamycin[c]	0.80	4.0
Clindamycin	1.0	—
Any other individual impurity	—	1.0
Total impurities[d]	—	6.0

[a] Lincomycin is controlled in the total of all related compounds. There is no individual acceptance criterion for this compound.
[b] Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-ethylpyrrolidine-2-carboxamido]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside.
[c] Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside.
[d] Total of all related compounds including lincomycin.▲ (USP 1-Aug-2019)

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 2.5–6.0

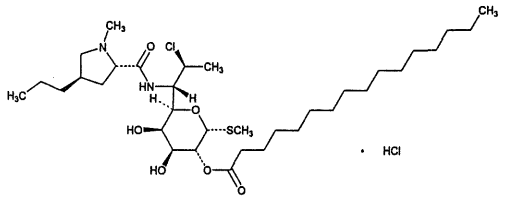
ADDITIONAL REQUIREMENTS

Change to read:

• **Packaging and Storage:** Preserve in tight containers. ▲Store at controlled room temperature.▲ (USP 1-Aug-2019)
• **Labeling:** Label Oral Solution to indicate that it is intended for veterinary use only.
• **USP Reference Standards** ⟨11⟩
USP Clindamycin Hydrochloride RS

www.webofpharma.com

Clindamycin Palmitate Hydrochloride



$C_{34}H_{63}ClN_2O_6S \cdot HCl$ 699.85

L-*threo*-α-D-*galacto*-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[(1-methyl-4-propyl-2-pyrrolidinyl) carbonyl] amino]-1-thio-2-hexadecanoate, monohydrochloride, (2*S*-*trans*)-;

Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-*threo*-α-D-*galacto*-octopyranoside 2-palmitate monohydrochloride [25507-04-4].

DEFINITION

Clindamycin Palmitate Hydrochloride has a potency equivalent to NLT 540 µg of clindamycin ($C_{18}H_{33}ClN_2O_5S$)/mg.

IDENTIFICATION

Change to read:

▲• **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Mobile phase: Dissolve 2 g of docusate sodium and 1.54 g of ammonium acetate in a mixture of 2 mL of glacial acetic acid and 75 mL of water. Dilute with methanol to 1 L. Pass through a suitable filter, and degas.

Standard solution: 14 mg/mL of USP Clindamycin Palmitate Hydrochloride RS in *Mobile phase*

Sample solution: 14 mg/mL of Clindamycin Palmitate Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1.2 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the potency in µg/mg of $C_{18}H_{33}ClN_2O_5S$ in the portion of Clindamycin Palmitate Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response of clindamycin palmitate from the *Sample solution*

r_S = peak response of clindamycin palmitate from the *Standard solution*

C_S = concentration of USP Clindamycin Palmitate Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of Clindamycin Palmitate Hydrochloride in the *Sample solution* (mg/mL)

P = potency of clindamycin in USP Clindamycin Palmitate Hydrochloride RS (µg/mg)

Acceptance criteria: NLT 540 µg/mg

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.5%

SPECIFIC TESTS

• **pH** ⟨791⟩: 2.8–3.8, in a 10 mg/mL solution

• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 3.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Clindamycin Palmitate Hydrochloride RS

Clindamycin Palmitate Hydrochloride for Oral Solution

DEFINITION

Clindamycin Palmitate Hydrochloride for Oral Solution is a dry mixture of Clindamycin Palmitate Hydrochloride and one or more suitable buffers, colors, diluents, flavors, and preservatives. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$), the labeled amount being 15 mg/mL when constituted as directed in the labeling.

ASSAY

• **PROCEDURE**

Solution A: 300 mg/mL of sodium carbonate

Internal standard solution: 5 mg/mL of cholesteryl benzoate in chloroform

Standard solution: Transfer 150 mg of USP Clindamycin Palmitate Hydrochloride RS to a glass-stoppered, 15-mL conical centrifuge tube. Add 5 mL of water, 5.0 mL of *Internal standard solution*, and 1 mL of *Solution A*. Insert the stopper, shake vigorously for NLT 10 min, and centrifuge. Remove the upper aqueous layer, and transfer 1.0 mL of the lower chloroform layer to a 15-mL centrifuge tube. Add 1.0 mL of pyridine and 1.0 mL of acetic anhydride. Agitate the tube to ensure complete mixing, cover the top of the centrifuge tube with a plastic cap through which a small hole has been punched, heat at 100° for 2.5 h, and allow to cool. Mix, and centrifuge if necessary. Use the clear solution.

Sample solution: Constitute the Clindamycin Palmitate Hydrochloride for Oral Solution as directed in the labeling, and transfer 5.0 mL of the constituted solution to a glass-stoppered, 15-mL conical centrifuge tube. Add 5.0 mL of *Internal standard solution* and 1 mL of *Solution A*. Insert the stopper, shake vigorously for NLT 10 min, and centrifuge. Remove the upper aqueous layer, and transfer 1.0 mL of the lower chloroform layer to a 15-mL centrifuge tube. Add 1.0 mL of pyridine and 1.0 mL of acetic anhydride. Agitate the tube to ensure complete mixing, cover the top of the centrifuge tube with a plastic cap through which a small hole has been punched, heat at 100° for 2.5 h, and allow to cool. Mix, and centrifuge if necessary. Use the clear solution.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.6-m × 3-mm glass; packing 1% phase G36 on support S1AB

www.webofpharma.com

Temperature
Column: 290°
Detector: 320°
Carrier gas: Dry helium
Flow rate: 60 mL/min
Injection size: 1.0 µL
System suitability
Sample: *Standard solution*
The elution order is: cholesteryl benzoate, clindamycin palmitate.
Suitability requirements: In a suitable chromatogram, the peaks are completely resolved.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$) in each mL of the solution constituted from Clindamycin Palmitate Hydrochloride for Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P \times 100$$

R_U = internal standard ratio (peak response of clindamycin palmitate/peak response of cholesteryl benzoate) from the *Sample solution*
R_S = internal standard ratio (peak response of clindamycin palmitate/peak response of cholesteryl benzoate) from the *Standard solution*
C_S = concentration of USP Clindamycin Palmitate Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clindamycin palmitate hydrochloride in the *Sample solution* (mg/mL)
P = potency of clindamycin in USP Clindamycin Palmitate Hydrochloride RS (µg/mg)

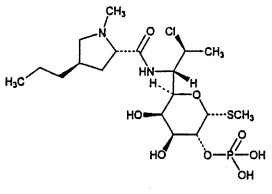
Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
For solids packaged in single-unit containers: Meets the requirements
• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

SPECIFIC TESTS
• **PH** ⟨791⟩**:** 2.5–5.0, in the solution constituted as directed in the labeling
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 3.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clindamycin Palmitate Hydrochloride RS

Clindamycin Phosphate



$C_{18}H_{34}ClN_2O_8PS$ 504.96
L-*threo*-α-D-*galacto*-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-, 2-(dihydrogen phosphate), (2*S*-*trans*)-;
Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-*threo*-α-D-*galacto*-octopyranoside 2-(dihydrogen phosphate) [24729-96-2].

DEFINITION
Clindamycin Phosphate has a potency equivalent to NLT 758 µg/mg of clindamycin ($C_{18}H_{33}ClN_2O_5S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Standard: Add 0.2 mL of water to 50 mg of USP Clindamycin Phosphate RS, and heat to dissolve. Evaporate to dryness under vacuum, and dry the residue at 100°–105° for 2 h.
Sample: Add 0.2 mL of water to 50 mg of Clindamycin Phosphate, and heat to dissolve. Evaporate to dryness under vacuum, and dry the residue at 100°–105° for 2 h.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: Add 14 mL of phosphoric acid to 4000 mL of water. Add 10 mL of ammonium hydroxide, and adjust with ammonium hydroxide to a pH of 5.6 ± 0.1.
Solution B: Acetonitrile and methanol (900:100)
Solution C: *Solution B* and *Solution A* (80:920)
Solution D: *Solution B* and *Solution A* (480:520)
Diluent: *Solution B* and *Solution A* (20:80)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution C (%)	Solution D (%)
0	95	5
40	5	95
41	95	5
46	95	5

System suitability solution: 2.2 mg/mL of USP Clindamycin Phosphate System Suitability RS in *Diluent*. Shake, and sonicate to dissolve.
Standard solution: 2.2 mg/mL of USP Clindamycin Phosphate RS in *Diluent*. Shake, and sonicate to dissolve.
Sample solution: 2.2 mg/mL of Clindamycin Phosphate in *Diluent*. Shake, and sonicate to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between clindamycin phosphate and 7-epiclindamycin phosphate, *System suitability solution*

USP Monographs

www.webofpharma.com

Tailing factor: NMT 2.0 for clindamycin phosphate, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity of clindamycin ($C_{18}H_{33}ClN_2O_5S$), in µg/mg, in the portion of Clindamycin Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)
C_U = concentration of Clindamycin Phosphate in the *Sample solution* (mg/mL)
P = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg)

Acceptance criteria: NLT 758 µg/mg on the anhydrous basis

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Solution C, Solution D, Diluent, Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 14 µg/mL of USP Clindamycin Phosphate RS in *Diluent*. Shake, and sonicate to dissolve.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between 7-epiclindamycin phosphate and clindamycin phosphate, *System suitability solution*
Tailing factor: NMT 2.0 for clindamycin phosphate, *Standard solution*
Relative standard deviation: NMT 5.0% for clindamycin phosphate, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Clindamycin Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of clindamycin phosphate from the *Standard solution*
C_S = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)
C_U = concentration of Clindamycin Phosphate, corrected for water content, in the *Sample solution* (mg/mL)
P = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg)
F_1 = conversion factor, 0.001 mg/µg
F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting level is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lincomycin phosphate[a]	0.36	1.0	1.0
Lincomycin[b]	0.50	2.0	0.5
Clindamycin B phosphate[c]	0.77	1.0	1.5
7-Epiclindamycin phosphate[d]	0.89	1.0	0.8
Clindamycin 3-phosphate[e]	0.93	1.0	0.3
Clindamycin phosphate	1.0	—	—
Clindamycin[f]	1.4	1.0	0.5
Any individual, unspecified impurity	—	1.0	1.0
Total impurities	—	—	4.0

[a] Methyl 6,8-dideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside 2-phosphate.
[b] Methyl 6,8-dideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside.
[c] Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-ethylpyrrolidine-2-carboxamido]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside 2-phosphate.
[d] Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside 2-phosphate.
[e] Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside 3-phosphate.
[f] Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside.

SPECIFIC TESTS

• **CRYSTALLINITY** ⟨695⟩: Meets the requirements
• **PH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 3.5–4.5
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 6.0%
• **STERILITY TESTS** ⟨71⟩
Sample solution: 6 g of specimen aseptically dissolved in 200 mL of *Fluid A*
Analysis: Test as directed in the *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
Acceptance criteria: It meets the requirements where the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.58 USP Endotoxin Units/mg of clindamycin, where the label states that Clindamycin Phosphate is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store below 30°.
• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clindamycin Phosphate RS
USP Clindamycin Phosphate System Suitability RS
Contains clindamycin phosphate and the following impurity:
7-Epiclindamycin phosphate;

USP Monographs

www.webofpharma.com

Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside 2-phosphate.
$C_{18}H_{34}ClN_2O_8PS$
504.96

Clindamycin Phosphate Vaginal Cream

DEFINITION
Clindamycin Phosphate Vaginal Cream contains an amount of clindamycin phosphate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$).

IDENTIFICATION
- **A.** The relative retention time of the major peak for clindamycin phosphate of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, and mix.
System suitability solution: 0.6 mg/mL each of USP Clindamycin Phosphate RS and USP Clindamycin Hydrochloride RS in *Mobile phase*
Standard solution: 0.25 mg/mL of USP Clindamycin Phosphate RS in *Mobile phase*
Sample solution: Nominally 0.2 mg/mL of clindamycin in *Mobile phase* from Cream, prepared as follows. Transfer a suitable portion of Cream to a stoppered conical flask, and add *Mobile phase*. Add about 10 glass beads (about 10 mm in diameter). Insert the stopper securely in the flask, and shake by mechanical means at 50° for 1 h. Cool in an ice bath for 20 min, and centrifuge. Pass a portion of the cloudy lower layer through a filter of 2-µm or finer pore size, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for clindamycin phosphate and clindamycin are about 1.0 and 1.5, respectively.]
Suitability requirements
Resolution: NLT 6.0 between clindamycin phosphate and clindamycin, *System suitability solution*
Column efficiency: NLT 1700 theoretical plates, *System suitability solution*
Tailing factor: NMT 1.3, *System suitability solution*
Relative standard deviation: NMT 2.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*.
Calculate the percentage of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clindamycin in the *Sample solution* (mg/mL)
P = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.0–6.0, determined on the undiluted Cream

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Clindamycin Hydrochloride RS
USP Clindamycin Phosphate RS

Clindamycin Phosphate Gel

DEFINITION
Clindamycin Phosphate Gel contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$).

IDENTIFICATION
- **A.** The retention time of the clindamycin phosphate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, and mix.
System suitability solution: 0.6 mg/mL each of USP Clindamycin Phosphate RS and USP Clindamycin Hydrochloride RS in *Mobile phase*
Standard solution: 0.25 mg/mL of USP Clindamycin Phosphate RS in *Mobile phase*
Sample solution: Nominally 0.2 mg/mL of clindamycin in *Mobile phase* from Gel. Shake by mechanical means for 30 min. Centrifuge a portion of the solution, and if necessary, filter a portion of the supernatant. Use the clear filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for clindamycin phosphate and clindamycin are 1.0 and 1.5, respectively.]
Suitability requirements
Resolution: NLT 6.0 between the clindamycin phosphate and clindamycin peaks, *System suitability solution*
Column efficiency: NLT 1700 theoretical plates, *System suitability solution*
Calculate as follows:

$$\text{Result} = (t_r/W_{h/2})^2 \times 5.545$$

www.webofpharma.com

t_r = retention time
$W_{h/2}$ = peak width at half height

Tailing factor: NMT 1.3, *System suitability solution*
Relative standard deviation: NMT 2.5%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clindamycin in the *Sample solution* (mg/mL)
P = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Minimum Fill** ⟨755⟩: Meets the requirements

SPECIFIC TESTS
• **pH** ⟨791⟩: 4.5–6.5

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Clindamycin Hydrochloride RS
USP Clindamycin Phosphate RS

Clindamycin Phosphate Topical Solution

DEFINITION
Clindamycin Phosphate Topical Solution contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, mix, and filter. Ensure that the concentration of acetonitrile in the *Mobile phase* is NLT 22% and NMT 25% to retain the correct elution order.
System suitability stock solution 1: 4 mg/mL of 4′-hydroxyacetophenone in acetonitrile
System suitability stock solution 2: 0.04 mg/mL of 4′-hydroxyacetophenone from *System suitability stock solution 1* in *Mobile phase*
Standard solution: 0.24 mg/mL of USP Clindamycin Phosphate RS in *Mobile phase*
System suitability solution: Mix 1 part of *System suitability stock solution 2* with 3 parts of *Standard solution*.
Sample solution: Equivalent to 0.2 mg/mL of clindamycin from Topical Solution in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[Note—The relative retention times for clindamycin phosphate and 4′-hydroxyacetophenone are about 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between clindamycin phosphate and 4′-hydroxyacetophenone, *System suitability solution*
Relative standard deviation: NMT 2.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$) in the portion of the Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clindamycin in the *Sample solution* (mg/mL)
P = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 4.0–7.0

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Clindamycin Phosphate RS

Clindamycin Phosphate Topical Suspension

DEFINITION
Clindamycin Phosphate Topical Suspension contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, mix, and filter.

USP Monographs

www.webofpharma.com

System suitability solution: 0.6 mg/mL each of USP Clindamycin Phosphate RS and USP Clindamycin Hydrochloride RS, in the *Mobile phase*
Standard solution: 0.25 mg/mL of USP Clindamycin Phosphate RS in the *Mobile phase*
Sample solution: Equivalent to 0.2 mg/mL of clindamycin from Topical Suspension in *Mobile phase*. Prepare as follows. Using a suitable hypodermic needle and syringe, transfer a suitable aliquot of Topical Suspension to a suitable volumetric flask, dilute with *Mobile phase* to volume, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for clindamycin phosphate and clindamycin are about 1.0 and 1.5, respectively.]
Suitability requirements
Resolution: NLT 6.0 between the clindamycin phosphate and clindamycin peaks, *System suitability solution*
Column efficiency: NLT 1700 theoretical plates, *System suitability solution*, calculated from the peak width at half height
Tailing factor: NMT 1.3, *System suitability solution*
Relative standard deviation: NMT 2.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$) in the portion of the Topical Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clindamycin in the *Sample solution* (mg/mL)
P = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS
• **pH** ⟨791⟩: 4.5–6.5

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clindamycin Hydrochloride RS
USP Clindamycin Phosphate RS

Clindamycin Phosphate Vaginal Inserts

DEFINITION
Clindamycin Phosphate Vaginal Inserts contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
Sample: Transfer a Vaginal Insert into a suitable container, add 120 mL of methylene chloride, insert a stopper, and shake until the Vaginal Insert is completely dissolved. Using a vacuum, pass through a methylene chloride-compatible filter having a 0.45-µm pore size. Rinse the filter with several portions of methylene chloride, and allow the filter to air-dry. Use the white residue to prepare the mineral oil dispersion for the test.
Acceptance criteria: The IR absorption of the *Sample* exhibits maxima at the same wavelengths as that of a similar preparation of USP Clindamycin Phosphate RS.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 10.54 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, and mix.
System suitability solution: 0.24 mg/mL of USP Clindamycin Phosphate RS and 6 µg/mL of USP Clindamycin Hydrochloride RS, in *Buffer*
Standard solution: 0.24 mg/mL of USP Clindamycin Phosphate RS in *Buffer*
Sample solution: Transfer 1 Vaginal Insert to a suitable 100-mL container. Add 40 mL of isooctane, and seal the container tightly with a teflon-lined septum and crimp cap. Shake vigorously for about 15 min until all of the Vaginal Insert is dissolved. Add 40.0 mL of *Buffer*. Recap the container tightly, and shake vigorously for NLT 30 min, taking care to avoid leakage. Allow the layers to separate, and remove a volume of the lower aqueous layer sufficient to perform the following steps. Pass the aqueous solution through a filter having a 5-µm or finer pore size, discarding the first 2 mL of the filtrate. Collect the remaining filtrate, and prepare a solution equivalent to 0.2 mg/mL of clindamycin with *Buffer*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 35 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between clindamycin phosphate and clindamycin hydrochloride, *System suitability solution*
Calculate as follows.

$$\text{Result} = [(t_2 - t_1)/(w_{h1} + w_{h2})] \times 1.177$$

www.webofpharma.com

t_2 = retention time of the second peak
t_1 = retention time of the first peak
w_{h1} = height at half width of the first peak
w_{h2} = height at half width of the second peak

Relative standard deviation: NMT 2.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$) equivalent in the Vaginal Insert taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clindamycin in the *Sample solution* (mg/mL)
P = potency of clindamycin in the USP Clindamycin Phosphate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%. Use as the *Assay* value the average of the determinations obtained in the test for *Uniformity of Dosage Units* ⟨905⟩, *Content Uniformity*.

PERFORMANCE TESTS

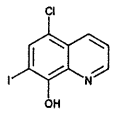
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, at controlled room temperature, or in a cool place.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clindamycin Hydrochloride RS
USP Clindamycin Phosphate RS

Clioquinol



C_9H_5ClINO 305.50
8-Quinolinol, 5-chloro-7-iodo-;
5-Chloro-7-iodo-8-quinolinol [130-26-7].

DEFINITION

Clioquinol, dried over phosphorus pentoxide for 5 h, contains NLT 93.0% and NMT 100.5% of clioquinol (C_9H_5ClINO).

IDENTIFICATION

• **A.**

Standard solution: Prepare as directed for the *Standard solution* in the *Assay*, except use 1.0 mL of pyridine instead of the *Internal standard solution*.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Analytical wavelength: 267 nm
Medium: 3 N hydrochloric acid
Sample solution: 5 µg/mL
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

• **C.**

Sample: 100 mg
Analysis: Heat the *Sample* with 5 mL of sulfuric acid.
Acceptance criteria: Copious violet vapors of iodine are evolved.

ASSAY

• **PROCEDURE**

Internal standard solution: 2 mg/mL of pyrene in pyridine
Standard stock solution: 3 mg/mL of USP Clioquinol RS in a mixture of pyridine and *n*-hexane (4:1)
Standard solution: Transfer 1.0 mL of the *Standard stock solution* to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, attach the cap, and mix. Heat in a water bath at 50° for 15 min, and then cool to ambient temperature.
Sample stock solution: 3 mg/mL of Clioquinol, previously dried, in a mixture of pyridine and *n*-hexane (4:1)
Sample solution: Transfer 1.0 mL of the *Sample stock solution* to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, and attach the cap. Heat in a water bath at 50° for 15 min, and then cool to ambient temperature.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 1.83-m × 2-mm glass; packed with 3% liquid phase G3 on 80- to 100-mesh support S1AB
Temperatures
Column: The initial temperature is 200° for a conditioning period of NLT 16 h (not connected to the detector) and is then reduced to 165°.
Injection port: 170°
Detector: 250°
Carrier gas: Helium
Flow rate: 30 mL/min for helium. Hydrogen and air are introduced into the detector at rates of 25 and 500 mL/min, respectively.
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for clioquinol and pyrene are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3 between the clioquinol and the internal standard peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clioquinol (C_9H_5ClINO) in the portion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of clioquinol to the internal standard from the *Sample solution*
R_S = peak response ratio of clioquinol to the internal standard from the *Standard solution*

USP Monographs

www.webofpharma.com

C_S = concentration of USP Clioquinol RS in the *Standard solution* (mg/mL)
C_U = concentration of Clioquinol in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–100.5%

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.5%

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample over phosphorus pentoxide for 5 h.
Acceptance criteria: NMT 0.5%

• **FREE IODINE AND IODIDE**
Control solution: Dilute 2.0 mL of potassium iodide solution (1 in 6000) with water to 10 mL, add 6 mL of 2 N sulfuric acid, 1 mL of potassium dichromate TS, and 2 mL of chloroform, and shake for 15 s (0.05% of iodide).
Sample: 1.0 g
Analysis:
Shake the *Sample* with 20 mL of water for 30 s, allow to stand for 5 min, and filter. To 10 mL of the filtrate add 1 mL of 2 N sulfuric acid, then add 2 mL of chloroform, and shake: no violet color appears in the chloroform (free iodine). To the mixture add 5 mL of 2 N sulfuric acid and 1 mL of potassium dichromate TS, and shake for 15 s.
Acceptance criteria: The color of the chloroform layer is no deeper than that produced in the *Control solution.*

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clioquinol RS

Clioquinol Cream

DEFINITION

Clioquinol Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of clioquinol (C_9H_5ClINO) in a suitable cream base.

IDENTIFICATION

• **A.**
Standard solution: Prepare as directed for the *Standard solution* in the *Assay,* except use 1.0 mL of pyridine instead of the *Internal standard solution.*
Acceptance criteria: The retention time of the major peak of the *Sample solution,* as obtained in the *Assay,* corresponds to that of the *Standard solution.*

• **B.**
Sample solution: Place nominally 25 mg of clioquinol in a 100-mL volumetric flask, add 75 mL of dilute hydrochloric acid (1 in 4), and heat on a steam bath to melt the Cream, shaking vigorously to extract the clioquinol. Cool under running water, and add dilute hydrochloric acid (1 in 4) to volume. Filter through paper, and dilute 3 mL of the filtrate with dilute hydrochloric acid (1 in 4) to 100 mL.
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Clioquinol RS, concomitantly measured.

ASSAY

• **PROCEDURE**
Internal standard solution: 2 mg/mL of pyrene in pyridine
Standard stock solution: 3 mg/mL of USP Clioquinol RS in a mixture of pyridine and *n*-hexane (4:1)
Standard solution: Transfer 1.0 mL of the *Standard stock solution* to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution,* attach the cap, and mix. Heat in a water bath at 50° for 15 min, and then cool to ambient temperature.
Sample stock solution: Transfer nominally 150 mg of clioquinol from Cream to a 60-mL separator. Place the separator on its side in a vacuum oven at a pressure of 10 mm of mercury at 45° for 4 h. Remove the separator from the oven, allow to cool, add 15 mL of a mixture of pyridine and *n*-hexane (4:1), and insert a polytef stopper. Transfer the mixture to a 50-mL volumetric flask, and rinse the separator with two 15-mL portions of the same solvent, shaking each time for 30 s. Transfer both rinsings to the volumetric flask, and dilute with the same solvent to volume.
Sample solution: Transfer 1.0 mL of the *Sample stock solution* to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution,* and attach the cap. Heat in a water bath at 50° for 15 min, and then cool to ambient temperature.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability.*)
Mode: GC
Detector: Flame ionization
Column: 1.83-m × 2-mm glass; packed with 3% liquid phase G3 on 80- to 100-mesh support S1AB
Temperatures
Column: The initial temperature is 200° for a conditioning period of NLT 16 h (not connected to the detector) and is then reduced to 165°.
Injection port: 170°
Detector: 250°
Carrier gas: Helium
Flow rate: 30 mL/min for helium. Hydrogen and air are introduced into the detector at rates of 25 and 500 mL/min, respectively.
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for clioquinol and pyrene are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3 between the clioquinol and the internal standard peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clioquinol (C_9H_5ClINO) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of clioquinol to the internal standard from the *Sample solution*
R_S = peak response ratio of clioquinol to the internal standard from the *Standard solution*
C_S = concentration of USP Clioquinol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clioquinol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clioquinol RS

www.webofpharma.com

Clioquinol Ointment

DEFINITION

Clioquinol Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of clioquinol (C_9H_5ClINO) in a suitable ointment base.

IDENTIFICATION

• **A.**

Standard solution: Prepare as directed for the *Standard solution* in the *Assay*, except use 1.0 mL of pyridine instead of the *Internal standard solution*.

Acceptance criteria: The retention time of the major peak of the *Sample solution*, as obtained in the *Assay*, corresponds to that of the *Standard solution*.

• **B.**

Sample solution: Place nominally 25 mg of clioquinol from Ointment in a 100-mL volumetric flask, add 75 mL of dilute hydrochloric acid (1 in 4), and heat on a steam bath to melt the Ointment, shaking vigorously to extract the clioquinol. Cool under running water, and add dilute hydrochloric acid (1 in 4) to volume. Filter through paper, and dilute 3 mL of the filtrate with dilute hydrochloric acid (1 in 4) to 100 mL.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Clioquinol RS, concomitantly measured.

ASSAY

• **PROCEDURE**

Internal standard solution: 2 mg/mL of pyrene in pyridine

Standard stock solution: 3 mg/mL of USP Clioquinol RS in a mixture of pyridine and *n*-hexane (4:1)

Standard solution: Transfer 1.0 mL of the *Standard stock solution* to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, and attach the cap. Heat in a water bath at 50° for 15 min, and then cool to ambient temperature.

Sample solution: Transfer nominally 150 mg of clioquinol from Ointment to a 125-mL separator. Add 75 mL of *n*-hexane, then add 15 mL of dimethylformamide, and mix for 1 min. Allow the layers to separate, and transfer the lower layer to a 50-mL volumetric flask. Repeat the extraction with separate 15- and 10-mL portions of dimethylformamide, and transfer the lower layers to the 50-mL volumetric flask. Dilute with dimethylformamide to volume. Transfer 1.0 mL of this solution to a screw-capped glass vial fitted with a septum, and evaporate at 60° under a stream of nitrogen to dryness. Add 1.0 mL of a mixture of pyridine and *n*-hexane (4:1) to the residue, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, and attach the cap. Heat in a water bath at 50° for 15 min, then cool to ambient temperature.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 1.83-m × 2-mm glass; packed with 3% liquid phase G3 on 80- to 100-mesh support S1AB

Temperatures

Column: The initial temperature is 200° for a conditioning period of NLT 16 h (not connected to the detector) and is then reduced to 165°.

Injection port: 170°

Detector: 250°

Carrier gas: Helium

Flow rate: 30 mL/min for helium. Hydrogen and air are introduced into the detector at rates of 25 and 500 mL/min, respectively.

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for clioquinol and pyrene are 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3 between the clioquinol and the internal standard peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clioquinol (C_9H_5ClINO) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of clioquinol to the internal standard from the *Sample solution*

R_S = peak response ratio of clioquinol to the internal standard from the *Standard solution*

C_S = concentration of USP Clioquinol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clioquinol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Clioquinol RS

Compound Clioquinol Topical Powder

DEFINITION

Compound Clioquinol Topical Powder contains NLT 22.5% and NMT 27.5% of clioquinol (C_9H_5ClINO).

Clioquinol	250 g
Lactic Acid	25 g
Zinc Stearate	200 g
Lactose	525 g
To make	1000 g

Mix the *Lactic Acid* with *Lactose*. Then add *Clioquinol* and *Zinc Stearate* to the mixture, and mix.

IDENTIFICATION

• **A.**

Sample: A quantity of Topical Powder equivalent to 30 mg of clioquinol

Analysis: Place the *Sample* in a glass-stoppered, 50-mL conical flask, add 20 mL of 1 N sulfuric acid, and shake for 5 min. Filter, and transfer 5 mL of the filtrate to a glass-stoppered test tube. Add 5 drops of potassium dichromate TS and 2 mL of chloroform, and shake well.

Acceptance criteria: A red-violet color develops in the chloroform layer.

ASSAY

• **PROCEDURE**

Standard solution: 5 µg/mL of USP Clioquinol RS in 3 N hydrochloric acid

Sample solution: Transfer a quantity of Topical Powder equivalent to 50 mg of clioquinol to a 200-mL volumetric

USP Monographs

www.webofpharma.com

flask. Add 100 mL of 3 N hydrochloric acid, and shake by mechanical means for 15 min. Dilute with 3 N hydrochloric acid to volume. Filter a portion of the solution. Dilute 4.0 mL of the filtrate with 3 N hydrochloric acid to 200.0 mL, and mix.

Blank: 3 N hydrochloric acid

Instrumental conditions

Mode: UV

Analytical wavelength: 267 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the absorbances of the *Standard solution* and the *Sample solution* at the wavelength of maximum absorbance.

Calculate the percentage of clioquinol (C_9H_5ClINO) in the portion of Topical Powder taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of clioquinol in the *Standard solution* (µg/mL)

C_U = nominal concentration of clioquinol in the *Sample solution* (µg/mL)

Acceptance criteria: 22.5%–27.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clioquinol RS

Clioquinol and Hydrocortisone Cream

DEFINITION

Clioquinol and Hydrocortisone Cream contains NLT 90.0% and NMT 110.0% of the labeled amounts of clioquinol (C_9H_5ClINO) and hydrocortisone ($C_{21}H_{30}O_5$) in a suitable cream base.

IDENTIFICATION

- **A.** The retention time of the clioquinol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Clioquinol*.
- **B.** The retention time of the hydrocortisone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Hydrocortisone*.

ASSAY

- **CLIOQUINOL**

Internal standard solution: 2 mg/mL of pyrene in pyridine

Standard stock solution: 3 mg/mL of USP Clioquinol RS in a mixture of pyridine and *n*-hexane (4:1)

Standard solution: Transfer 1.0 mL of the *Standard stock solution*, 1.0 mL of *N,O*-bis(trimethylsilyl)acetamide, and 1.0 mL of *Internal standard solution* to a suitable screw-capped glass vial fitted with a polytef-lined septum, and mix. Heat on a water bath at 50° for 15 min, and cool to room temperature.

Sample solution: Transfer nominally 150 mg of clioquinol from Cream to a 60-mL separator. Place the separator on its side in a vacuum oven at 45° for 4 h. Remove the separator, cool to room temperature, and add 15.0 mL of a mixture of pyridine and hexane (4:1). Insert the stopper in the separator, and mix until the specimen is completely dispersed. Transfer the contents of the separator to a 50-mL volumetric flask, rinse the separator with two 15-mL portions of a mixture of pyridine and hexane (4:1), collecting the rinsings in the volumetric flask, and dilute with the same solvent mixture to volume. Immediately transfer 1 mL of this solution to a dry, screw-capped glass vial, and evaporate with the aid of gentle heat and a stream of nitrogen to dryness. Dissolve the residue in 1.0 mL of a mixture of pyridine and hexane (4:1), add 1 mL each of *N,O*-bis(trimethylsilyl)acetamide and *Internal standard solution* to the screw-capped glass vial fitted with a polytef-lined septum, and mix. Heat on a water bath at 50° for 15 min, and cool to room temperature.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m; packed with 3% liquid phase G3 on 80- to 100-mesh support S1AB

Temperatures

Column: 165°

Injection port: 170°

Detector: 250°

Carrier gas: Dry helium

Flow rate: 30 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for clioquinol and pyrene are 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the analyte and internal standard peaks

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms to obtain NLT 40% of maximum recorder response, and measure the peak response of each component.

Calculate the percentage of the labeled amount of clioquinol (C_9H_5ClINO) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of clioquinol to the internal standard from the *Sample solution*

R_S = peak response ratio of clioquinol to the internal standard from the *Standard solution*

C_S = concentration of USP Clioquinol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clioquinol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

- **HYDROCORTISONE**

Mobile phase: Acetonitrile, methanol, and water (1:1:2.75)

Standard stock solution: 1 mg/mL of USP Hydrocortisone RS in alcohol

Standard solution: 100 µg/mL of USP Hydrocortisone RS in alcohol from the *Standard stock solution*

Sample solution: Transfer nominally 10 mg of hydrocortisone from Cream to a 50-mL centrifuge tube. Add 30 mL of alcohol, and heat on a steam bath just to boiling. Shake for 15 min, and centrifuge. Transfer the supernatant extract to a 100-mL volumetric flask. Repeat the extraction with two 20-mL portions of alcohol, combining the extracts in the 100-mL volumetric flask. Add alcohol to volume, mix, and filter.

www.webofpharma.com

System suitability stock solution: 0.5 mg/mL of methylparaben in alcohol
System suitability solution: Transfer 2 mL of *System suitability stock solution* and 20 mL of *Standard stock solution* into a 200-mL volumetric flask, and dilute with alcohol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Columns
Guard: Packing L2
Analytical: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for methylparaben and hydrocortisone are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the hydrocortisone and methylparaben peaks
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clioquinol RS
USP Hydrocortisone RS

Clioquinol and Hydrocortisone Ointment

DEFINITION
Clioquinol and Hydrocortisone Ointment contains NLT 90.0% and NMT 110.0% of the labeled amounts of clioquinol (C_9H_5ClINO) and hydrocortisone ($C_{21}H_{30}O_5$) in a suitable ointment base.

IDENTIFICATION
• **A.** The retention time of the clioquinol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Clioquinol*.
• **B.** The retention time of the hydrocortisone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Hydrocortisone*.

ASSAY
• **CLIOQUINOL**
Internal standard solution: 2 mg/mL of pyrene in pyridine
Standard stock solution: 3 mg/mL of USP Clioquinol RS in a mixture of pyridine and *n*-hexane (4:1)
Standard solution: Transfer 1.0 mL of the *Standard stock solution*, 1.0 mL of *N,O*-bis(trimethylsilyl)acetamide, and 1.0 mL of *Internal standard solution* to a suitable screw-capped glass vial fitted with a polytef-lined septum, and mix. Heat on a water bath at 50° for 15 min, and cool to room temperature.
Sample solution: Transfer nominally 150 mg of clioquinol from Ointment to a 125-mL separator. Add 75 mL of *n*-hexane, insert the stopper in the separator, and mix until the specimen is completely dispersed. Extract with 25 mL of dimethylformamide, collecting the extract in a 50-mL volumetric flask. Repeat the extraction with two 10-mL portions of dimethylformamide, collecting the extracts in the 50-mL volumetric flask, and dilute with dimethylformamide to volume. Transfer 1.0 mL of this solution to a suitable size screw-capped vial, and evaporate the solution with the aid of nitrogen at 60° to dryness. Dissolve the residue in 1.0 mL of a mixture of pyridine and hexane (4:1), and pipet 1.0 mL of *N,O*-bis(trimethylsilyl) acetamide and 1.0 mL of *Internal standard solution* into the glass vial, fitted with a polytef-lined septum, and securely close. Heat the vial on a water bath at 50° for 15 min, and cool to room temperature.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 2-mm × 1.8-m; packed with 3% liquid phase G3 on 80- to 100-mesh support SIAB
Temperatures
Column: 165°
Injection port: 170°
Detector: 250°
Carrier gas: Dry helium
Flow rate: 30 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for clioquinol and pyrene are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the analyte and internal standard peaks
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms to obtain NLT 40% of maximum recorder response, and measure the peak response of each component.
Calculate the percentage of the labeled amount of clioquinol (C_9H_5ClINO) taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of clioquinol to the internal standard from the *Sample solution*
R_S = peak response ratio of clioquinol to the internal standard from the *Standard solution*
C_S = concentration of USP Clioquinol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clioquinol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

USP Monographs

www.webofpharma.com

• **HYDROCORTISONE**
Mobile phase: Acetonitrile, methanol, and water (1:1:2.75)
Standard stock solution: 1 mg/mL of USP Hydrocortisone RS in alcohol
Standard solution: *Standard stock solution* and alcohol (1:9)
Sample solution: Transfer nominally 10 mg of hydrocortisone from Ointment to a 50-mL centrifuge tube. Add 30 mL of alcohol, and heat on a steam bath just to boiling. Shake for 15 min, and centrifuge. Transfer the supernatant extract to a 100-mL volumetric flask. Repeat the extraction with two 20-mL portions of alcohol, combining the extracts in the 100-mL volumetric flask. Add alcohol to volume, mix, and filter.
System suitability stock solution: 0.5 mg/mL of methylparaben in alcohol
System suitability solution: Transfer 2 mL of *System suitability stock solution* and 20 mL of *Standard stock solution* into a 200-mL volumetric flask, and dilute with alcohol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Columns
Guard: Packing L2
Analytical: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for methylparaben and hydrocortisone are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the hydrocortisone and methylparaben peaks
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

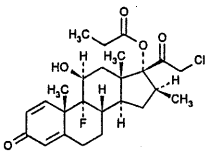
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clioquinol RS
USP Hydrocortisone RS

Clobetasol Propionate



$C_{25}H_{32}ClFO_5$ 466.97
Pregna-1,4-diene-3,20-dione, 21-chloro-9-fluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)-, (11β,16β)-;
21-Chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate [25122-46-7; 25122-41-2].

DEFINITION
Clobetasol Propionate contains NLT 97.0% and NMT 102.0% of $C_{25}H_{32}ClFO_5$, calculated on the dried basis.

IDENTIFICATION

Change to read:

▲• **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Solution A: 0.05 M monobasic sodium phosphate. Adjust with 85% phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile, methanol, and *Solution A* (19:4:17)
Internal standard solution: 0.2 mg/mL of beclomethasone dipropionate in methanol
Standard solution: Dissolve a quantity of USP Clobetasol Propionate RS in methanol and *Internal standard solution* to obtain a final solution of 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate.
System suitability solution: 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*
Sample solution: Transfer 4 mg of Clobetasol Propionate to a 100-mL volumetric flask, add 40.0 mL of *Internal standard solution*, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A
Column efficiency: NLT 5000 theoretical plates for the clobetasol propionate peak
Tailing factor: NMT 2.0 for the clobetasol propionate peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]

Calculate the percentage of $C_{25}H_{32}ClFO_5$ in the portion of Clobetasol Propionate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*

R_S = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*

C_S = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%, using a platinum crucible

ORGANIC IMPURITIES

• **Procedure**

Solution A, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Sample solution: 0.1 mg/mL of Clobetasol Propionate in *Mobile phase*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Clobetasol Propionate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area for each impurity

r_T = sum of the areas of all of the peaks

Acceptance criteria

Any individual impurity: NMT 1.0%

Total impurities: NMT 2.5%

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** ⟨741⟩: Approximately 196°

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩: +98° to +104° at 20°

Sample solution: 10 mg/mL in dioxane

• **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 3 h: it loses NMT 2.0% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Clobetasol Propionate RS

USP Clobetasol Propionate Related Compound A RS

9α-Fluoro-11β-hydroxy-16β-methyl 3-oxo-androsta-1,4-diene-17(*R*)-spiro-2′-[4′-chloro-5′-ethylfuran-3′(2′*H*)-one].

$C_{25}H_{30}ClFO_4$ 448.96

Clobetasol Propionate Cream

DEFINITION

Clobetasol Propionate Cream is Clobetasol Propionate in a suitable cream base. It contains NLT 90.0% and NMT 115.0% of the labeled amount of clobetasol propionate ($C_{25}H_{32}ClFO_5$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 0.6 mg/mL of USP Clobetasol Propionate RS in chloroform

Test solution: Transfer a portion of Cream equivalent to 0.75 mg of clobetasol propionate to a 25-mL, plastic-stoppered centrifuge tube. Add 10 mL of methanol, and cap. Heat in a 60° water bath for 4 min, remove the tube from the bath, and shake vigorously. Repeat the heating and shaking. Cool to room temperature, add 3.5 mL of water, and mix. Centrifuge at 3500 rpm for 10 min. Transfer 5 mL of the supernatant to a 100-mL separator, add 1 g of sodium chloride and 10 mL of water, and mix. Extract with 5 mL of chloroform by shaking for 1 min, collect the lower layer, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 0.5 mL of chloroform.

Developing solvent system: Chloroform, acetone, and alcohol (100:10:5)

Acceptance criteria: The R_F value of the principal spot obtained from the *Test solution* corresponds to that from the *Standard solution*.

ASSAY

• **PROCEDURE**

Buffer: 0.05 M monobasic sodium phosphate. Adjust with 50% sodium hydroxide solution to a pH of 5.5.

Mobile phase: Acetonitrile, methanol, and *Buffer* (95:20:85)

Internal standard solution: 0.2 mg/mL of beclomethasone dipropionate in methanol

System suitability solution: 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*

Standard solution: 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate prepared as follows. Transfer 1.0 mg of USP Clobetasol Propionate RS to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with methanol to volume.

Sample solution: Nominally 0.04 mg/mL of clobetasol propionate. In a suitable flask, dissolve a portion of Cream equivalent to 1.0 mg of clobetasol propionate in 10.0 mL of the *Internal standard solution* and 15.0 mL of methanol, and shake vigorously to disperse the Cream. Centrifuge at about 3500 rpm for 10 min, and pass a portion of the supernatant through a filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A
Column efficiency: NLT 5000 theoretical plates for the clobetasol propionate peak
Tailing factor: NMT 2.0 for the clobetasol propionate peak
Relative standard deviation: NMT 2.0% for the clobetasol propionate peak

Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]
Calculate the percentage of clobetasol propionate ($C_{25}H_{32}ClFO_5$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*
R_S = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*
C_S = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.
• **pH** ⟨791⟩**:** 4.5–7.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers. Store at controlled room temperature. Do not refrigerate.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clobetasol Propionate RS
USP Clobetasol Propionate Related Compound A RS
9α-Fluoro-11β-hydroxy-16β-methyl 3-oxo-androsta-1,4-diene-17(*R*)-spiro-2′-[4′-chloro-5′-ethylfuran-3′(2′*H*)-one].
$C_{25}H_{30}ClFO_4$ 448.96

Clobetasol Propionate Ointment

DEFINITION
Clobetasol Propionate Ointment is Clobetasol Propionate in a suitable ointment base. It contains NLT 90.0% and NMT 115.0% of the labeled amount of clobetasol propionate ($C_{25}H_{32}ClFO_5$).

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 0.5 mg/mL of of USP Clobetasol Propionate RS in chloroform
Test solution: Nominally 0.5 mg/mL of clobetasol propionate. Transfer a portion of Ointment equivalent to 1.0 mg of clobetasol propionate to a 25-mL, plastic-stoppered centrifuge tube. Add 10 mL of methanol, and cap. Heat in a 70° water bath for 4 min, remove the tube from the bath, and shake vigorously. Repeat the heating and shaking. Freeze the mixture in an ice bath for 5 min, and centrifuge at about 3500 rpm for 10 min. Transfer 5 mL of the supernatant to a suitable vial. Evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 1.0 mL of chloroform.
Developing solvent system: Chloroform, acetone, and alcohol (100:10:5)
Acceptance criteria: The R_F value of the principal spot obtained from the *Test solution* corresponds to that from the *Standard solution*.

ASSAY
• **PROCEDURE**
Buffer: 0.05 M monobasic sodium phosphate. Adjust with 85% phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile, methanol, and *Buffer* (95:20:85)
Internal standard solution: 0.2 mg/mL of beclomethasone dipropionate in methanol
System suitability solution: 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*
Standard solution: Dissolve a quantity of USP Clobetasol Propionate RS in methanol and *Internal standard solution* to obtain a final solution of 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate
Sample solution: Nominally 0.04 mg/mL of clobetasol propionate. Transfer a portion of Ointment equivalent to 1.0 mg of clobetasol propionate into a 125-mL separatory funnel. Add 30 mL of hexane, 10.0 mL of the *Internal standard solution*, and shake. Collect the lower layer in a 25-mL volumetric flask. Extract the hexane remaining in the separatory funnel with two 5-mL portions of *Mobile phase*, and combine all of the extracts in the 25-mL volumetric flask. Dilute with *Mobile phase* to volume, and pass a portion through a filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A
Column efficiency: NLT 5000 theoretical plates for the clobetasol propionate peak
Tailing factor: NMT 2.0 for the clobetasol propionate peak
Relative standard deviation: NMT 2.0% for the clobetasol propionate peak

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]
Calculate the percentage of clobetasol propionate ($C_{25}H_{32}ClFO_5$) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*
R_S = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*
C_S = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.
- **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers. Store at controlled room temperature. Do not refrigerate.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Clobetasol Propionate RS
USP Clobetasol Propionate Related Compound A RS
9α-Fluoro-11β-hydroxy-16β-methyl 3-oxo-androsta-1,4-diene-17(*R*)-spiro-2′-[4′-chloro-5′-ethylfuran-3′(2′*H*)-one].
$C_{25}H_{30}ClFO_4$ 448.96

Clobetasol Propionate Topical Solution

DEFINITION
Clobetasol Propionate Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of clobetasol propionate ($C_{25}H_{32}ClFO_5$).

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 0.75 mg/mL of USP Clobetasol Propionate RS in chloroform
Test solution: Nominally 0.75 mg/mL of clobetasol propionate. Transfer a portion of Topical Solution equivalent to 1.5 mg of clobetasol to a 50-mL separatory funnel. Add 5 mL of water, and extract with 5 mL of chloroform. Collect the lower layer through a cotton wool plug, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 2 mL of chloroform.
Developing solvent system: Chloroform, acetone, and alcohol (100:10:5)
Acceptance criteria: The R_F value of the principal spot obtained from the *Test solution* corresponds to that from the *Standard solution*.

ASSAY
- **PROCEDURE**
Buffer: 0.05 M monobasic sodium phosphate. Adjust with 85% phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile, methanol, and *Buffer* (95:20:85)
Internal standard solution: 0.2 mg/mL of beclomethasone dipropionate in methanol
System suitability solution: 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*
Standard solution: 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate prepared as follows. Transfer 1.0 mg of USP Clobetasol Propionate RS to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with methanol to volume.
Sample solution: Nominally 0.04 mg/mL of clobetasol propionate. Transfer a portion of Topical Solution equivalent to 1.0 mg of clobetasol propionate to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A
Column efficiency: NLT 5000 theoretical plates for the clobetasol propionate peak
Tailing factor: NMT 2.0 for the clobetasol propionate peak
Relative standard deviation: NMT 2.0% for the clobetasol propionate peak
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]
Calculate the percentage of clobetasol propionate ($C_{25}H_{32}ClFO_5$) in the portion of Topical Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*
R_S = ratio of the clobetasol propionate peak area to the Internal standard peak area from the *Standard solution*
C_S = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

www.webofpharma.com

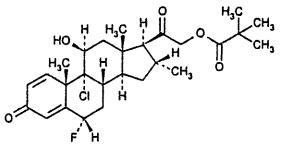
SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.
- **pH** ⟨791⟩: 4.5–6.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature. Do not refrigerate.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clobetasol Propionate RS
  USP Clobetasol Propionate Related Compound A RS
  9α-Fluoro-11β-hydroxy-16β-methyl 3-oxo-androsta-1,4-diene-17(*R*)-spiro-2′-[4′-chloro-5′-ethylfuran-3′(2′*H*)-one].
  $C_{25}H_{30}ClFO_4$ 448.96

Clocortolone Pivalate



$C_{27}H_{36}ClFO_5$ 495.02

Pregna-1,4-diene-3,20-dione, 9-chloro-21-(2,2-dimethyl-1-oxopropoxy)-6-fluoro-11-hydroxy-16-methyl-, (6α,11β,16α)-.

9-Chloro-6α-fluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-pivalate [34097-16-0].

» Clocortolone Pivalate contains not less than 97.0 percent and not more than 103.0 percent of $C_{27}H_{36}ClFO_5$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Clocortolone Pivalate RS

Color and clarity of solution—A 1 in 100 solution in chloroform is clear and practically colorless.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020)

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 15 µg per mL.
Medium: methanol.
Absorptivities at 238 nm, calculated on the dried basis, do not differ by more than 3.0%.

Specific rotation ⟨781S⟩: between +125° and +135°.
Test solution: 40 mg per mL, in chloroform.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.2%, a 100-mg test specimen being used.

Chromatographic purity—
Test solution—Accurately weigh about 100 mg of Clocortolone Pivalate, and transfer to a 25-mL volumetric flask. Dissolve in a mixture of chloroform and methanol (1:1), and dilute with the same solvent to volume.
Standard solution—Using an accurately weighed quantity of USP Clocortolone Pivalate RS, prepare a solution in a mixture of chloroform and methanol (1:1) having a known concentration of about 4 mg per mL.
Procedure—Score a 20- × 20-cm thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture into three equal sections to be used for the *Test solution*, the blank, and the *Standard solution*, respectively. Activate the plate at 105° for 30 minutes before use. Apply 100 µL each of the *Test solution* and the *Standard solution* as streaks 2.5 cm from the bottom of the appropriate section of the plate, and dry the streaks with a gentle current of air. Using a solvent system consisting of a mixture of cyclohexane and ethyl acetate (2:1), develop the chromatogram in a suitable chromatographic chamber lined with absorbent paper and previously equilibrated, until the solvent front has moved 15 cm above the line of application. Air-dry the plate, and develop the chromatogram a second time using the same chromatographic system. Air-dry the plate, and locate the principal band occupied by the *Standard solution* by viewing under UV light. Mark this band as well as corresponding bands in the blank and *Test solution* sections of the plate. Quantitatively remove the silica gel from each band, and transfer to separate glass-stoppered, 50-mL centrifuge tubes. Add 25.0 mL of methanol to each tube, shake for not less than 20 minutes, and centrifuge. Concomitantly determine the absorbances of the supernatants from the *Test solution* and the *Standard solution* against the blank at the wavelength of maximum absorbance at about 238 nm, with a suitable spectrophotometer. Calculate the percentage of chromatographic impurities in the *Test solution* taken by the formula:

$$100 - [100(C_S/C_U)(A_U/A_S)]$$

in which C_S is the concentration, in mg per mL, of USP Clocortolone Pivalate RS in the *Standard solution*; C_U is the concentration, in mg per mL, of the *Test solution*; and A_U and A_S are the absorbances of the solutions from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% of total impurities is found.

Assay—
Standard preparation—Dissolve an accurately weighed quantity of USP Clocortolone Pivalate RS in chloroform to obtain a solution having a known concentration of about 0.75 mg per mL. Dilute an accurately measured volume of this solution with methanol, and mix to obtain a *Standard preparation* having a known concentration of about 30 µg per mL.
Assay preparation—Accurately weigh about 75 mg of Clocortolone Pivalate, and transfer to a 100-mL volumetric flask. Dissolve in chloroform, dilute with chloroform to volume, and mix. Transfer 4.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix.
Procedure—Transfer 10.0-mL portions of the *Standard preparation* and the *Assay preparation* to separate glass-stoppered, 50-mL conical flasks, and evaporate on a steam bath to dryness. To each flask, and to a third flask to provide the blank, add 15.0 mL of a solution containing 250 mg of isoniazid and 0.3 mL of hydrochloric acid in 500 mL of

www.webofpharma.com

methanol. Swirl the contents of the flasks to dissolve the residues. Insert the stoppers securely in the flasks, and place in a water bath at 60° for 2.5 hours. Cool to room temperature. Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells against the blank at the wavelength of maximum absorbance at about 405 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of $C_{27}H_{36}ClFO_5$ in the portion of Clocortolone Pivalate taken by the formula:

$$2.5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Clocortolone Pivalate RS in the *Standard preparation*, and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Clocortolone Pivalate Cream

» Clocortolone Pivalate Cream contains not less than 90.0 percent and not more than 110.0 percent of $C_{27}H_{36}ClFO_5$ in a suitable cream base. It may contain suitable preservatives.

Packaging and storage—Preserve in collapsible tubes or in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Clocortolone Pivalate RS
Identification—Place a portion of Cream, equivalent to about 1 mg of clocortolone pivalate, in a suitable separator. Add 5 mL of water, and extract with 10 mL of chloroform. Evaporate the chloroform layer to dryness, and dissolve the residue in 2 mL of methanol. Apply 20 µL of this test solution and 20 µL of a Standard solution of USP Clocortolone Pivalate RS in chloroform containing about 0.5 mg per mL about 1.5 cm from the bottom of a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of cyclohexane and ethyl acetate (2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.
Minimum fill ⟨755⟩: meets the requirements.
pH ⟨791⟩: between 5.0 and 7.0, in a 1 in 10 aqueous dispersion.
Particle size determination—Place a small portion of Cream on a microscope slide, apply a cover slide, press slightly, and examine under 40× objective magnification using a suitable microscope equipped with polarized light. Scan the complete slide preparation, and record the size of the largest crystal found in reference to a calibrated grid: no particle in the Cream is greater than 50 microns when measured in the longitudinal axis.

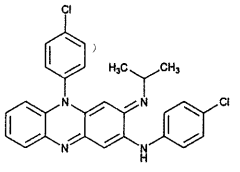
Assay—
Standard preparation—Dissolve an accurately weighed quantity of USP Clocortolone Pivalate RS in methanol to obtain a solution having a known concentration of about 0.06 mg per mL.

Assay preparation—Using a plastic syringe equipped with a suitable cannula, transfer an accurately weighed quantity of Cream, equivalent to about 3 mg of clocortolone pivalate, to a 50-mL volumetric flask. Add about 25 mL of methanol, and warm the flask in a 60° water bath for about 10 minutes, with occasional swirling, to disperse the Cream. Cool to room temperature, dilute with methanol to volume, and mix. Allow any insoluble material to settle.
Procedure—Transfer 10.0 mL of the *Standard preparation* to a 25-mL volumetric flask. Transfer 10.0-mL portions of the *Assay preparation* into two separate 25-mL volumetric flasks labeled *Assay preparation* and *Assay blank*, respectively. Evaporate the contents of the three flasks with the aid of a stream of air or nitrogen to dryness. Transfer 20.0 mL of a solution containing 250 mg of isoniazid and 0.3 mL of hydrochloric acid in 500 mL of methanol to the flasks containing the *Standard preparation*, the *Assay preparation*, and a fourth 25-mL volumetric flask labeled *Reagent blank*. Pipet 20 mL of acidified methanol solution (0.3 mL of hydrochloric acid diluted with methanol to 500 mL) into the flask labeled *Assay blank*. Insert the stoppers securely in the flasks, and place in a water bath at 60° for 2.5 hours, occasionally swirling the contents of each flask. Cool the flasks to room temperature, dilute with the acidified methanol solution to volume, and mix. Centrifuge the *Assay preparation* at high speed for 10 minutes. Concomitantly determine the absorbances of the *Standard preparation*, the *Assay preparation*, the *Assay blank*, and the *Reagent blank* against acidified methanol solution as the solvent blank in 1-cm cells at the wavelength of maximum absorbance at about 390 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of $C_{27}H_{36}ClFO_5$ in the portion of Cream taken by formula:

$$50C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Clocortolone Pivalate RS in the *Standard preparation*; A_U is the absorbance of the *Assay preparation*, corrected for the *Assay blank* and the *Reagent blank*; and A_S is the absorbance of the *Standard preparation* corrected for the *Reagent blank*.

Clofazimine



$C_{27}H_{22}Cl_2N_4$ 473.40
2-Phenazinamine, *N*,5-bis(4-chlorophenyl)-3,5-dihydro-3-[(1-methylethyl)imino]-;
3-(*p*-Chloroanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-(isopropylimino)phenazine [2030-63-9].

DEFINITION
Clofazimine contains NLT 98.0% and NMT 102.0% of clofazimine ($C_{27}H_{22}Cl_2N_4$), calculated on the dried basis.

Change to read:

IDENTIFICATION
• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

www.webofpharma.com

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 4.5 mg/mL of sodium dodecyl sulfate, 1.7 mg/mL of tetrabutylammonium hydrogen sulfate, and 1.8 mg/mL of disodium hydrogen phosphate in water. Adjust with dilute phosphoric acid (about 8.5%) to a pH of 3.0 in 90% of the volume before diluting with water to volume.

Mobile phase: Acetonitrile and *Buffer* (65:35)

Standard solution: 0.05 mg/mL of USP Clofazimine RS in *Mobile phase*

Sample solution: 0.05 mg/mL of Clofazimine in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for the clofazimine peak, *Standard solution*

Relative standard deviation: NMT 0.73% for the clofazimine peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of clofazimine ($C_{27}H_{22}Cl_2N_4$) in the portion of Clofazimine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Clofazimine RS in the *Standard solution* (mg/mL)

C_U = concentration of Clofazimine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 0.5 mg/mL of USP Clofazimine RS and 1.5 µg/mL of USP Clofazimine Related Compound B RS in *Mobile phase*

Standard solution: 0.5 µg/mL of USP Clofazimine RS and 5.0 µg/mL of USP Clofazimine Related Compound B RS in *Mobile phase*

Sample solution: 0.5 mg/mL of Clofazimine in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the clofazimine and clofazimine related compound B peaks, *System suitability solution*

Relative standard deviation: NMT 2.8% for the clofazimine peak and NMT 2.0% for the clofazimine related compound B peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of clofazimine related compound B in the portion of Clofazimine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clofazimine related compound B from the *Sample solution*

r_S = peak response of clofazimine related compound B from the *Standard solution*

C_S = concentration of USP Clofazimine Related Compound B RS in the *Standard solution* (mg/mL)

C_U = concentration of Clofazimine in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Clofazimine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*

r_S = peak response of clofazimine from the *Standard solution*

C_S = concentration of USP Clofazimine RS in the *Standard solution* (mg/mL)

C_U = concentration of Clofazimine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Clofazimine related compound B	0.81	1.0
Clofazimine	1.00	—
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 105° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Clofazimine RS

USP Clofazimine Related Compound B RS

5-(4-Chlorophenyl)-3-(isopropylimino)-*N*-phenyl-3,5-dihydrophenazin-2-amine.

$C_{27}H_{23}ClN_4$ 438.95

Clofazimine Capsules

DEFINITION

Clofazimine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of clofazimine ($C_{27}H_{22}Cl_2N_4$).

www.webofpharma.com

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 4.5 mg/mL of sodium dodecyl sulfate, 1.7 mg/mL of tetrabutylammonium hydrogen sulfate, and 1.8 mg/mL of disodium hydrogen phosphate in water. Adjust with dilute phosphoric acid (about 8.5%) to a pH of 3.0 in 90% of the volume before diluting with water to volume.

Mobile phase: Acetonitrile and *Buffer* (65:35)

System suitability solution: 0.5 mg/mL of USP Clofazimine RS and 1.5 µg/mL of USP Clofazimine Related Compound B RS in *Mobile phase*

Standard solution: 0.05 mg/mL of USP Clofazimine RS in *Mobile phase*

Sample stock solution: Nominally 0.5 mg/mL of clofazimine in *Mobile phase* prepared as follows. Remove as completely as possible the contents of NLT 20 Capsules, and mix. Transfer the weighed portion of the combined contents of the Capsules, equivalent to about 500 mg of clofazimine, into a 250-mL conical flask. Add 50 mL of *Mobile phase* in increments, shake well, and quantitatively transfer into a 1000-mL volumetric flask. Repeat this process until transfer of all the Capsule contents is complete and make up the volume of the flask with *Mobile phase*. Stir at a high speed to make the solution homogenous.

Sample solution: Nominally 0.05 mg/mL of clofazimine from the *Sample stock solution* in *Mobile phase* prepared as follows. Filter 20 mL of the *Sample stock solution* into a beaker. Transfer 1.0 mL of the filtered solution into a 10-mL volumetric flask, and dilute to volume with *Mobile phase*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm. For *Identification* test *B*, use a diode array detector in the range of 190 nm–400 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between clofazimine and clofazimine related compound B peaks, *System suitability solution*

Tailing factor: NMT 1.5 for the clofazimine peak, *Standard solution*

Relative standard deviation: NMT 1.0% for the clofazimine peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clofazimine ($C_{27}H_{22}Cl_2N_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clofazimine from the *Sample solution*

r_S = peak response of clofazimine from the *Standard solution*

C_S = concentration of USP Clofazimine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clofazimine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 15 min

Analysis: Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting the rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

Tolerances: The requirements are met if all of the Capsules tested rupture in NMT 15 min. If 1 or 2 of the Capsules rupture in more than 15 but NMT 30 min, repeat the test on 12 additional Capsules. NMT 2 of the total of 18 Capsules tested rupture in more than 15 min but NMT 30 min.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.5 µg/mL of USP Clofazimine RS and 5 µg/mL of USP Clofazimine Related Compound B RS in *Mobile phase*

Sample solution: Nominally 0.5 mg/mL of clofazimine in *Mobile phase* prepared as follows. Remove as completely as possible the contents of NLT 20 Capsules, and mix. Transfer the weighed portion of the combined contents of the Capsules, equivalent to about 500 mg of clofazimine, into a 250-mL conical flask. Add 50 mL of *Mobile phase* in increments, shake well, and quantitatively transfer into a 1000-mL volumetric flask. Repeat this process until transfer of all the Capsule contents is complete and make up the flask volume with *Mobile phase*. Stir at a high speed to make the solution homogenous and filter.

Suitability requirements

Resolution: NLT 2.0 between the clofazimine and clofazimine related compound B peaks, *System suitability solution*

Relative standard deviation: NMT 2.8% for the clofazimine peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of clofazimine related compound B in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clofazimine related compound B from the *Sample solution*

r_S = peak response of clofazimine related compound B from the *Standard solution*

C_S = concentration of USP Clofazimine Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clofazimine in the *Sample solution* (mg/mL)

Calculate the percentage of unspecified impurities in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of impurities from the *Sample solution*

www.webofpharma.com

r_S = peak response of clofazimine from the *Standard solution*
C_S = concentration of USP Clofazimine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clofazimine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

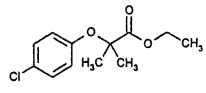
Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Clofazimine related compound B	0.81	1.0
Clofazimine	1.00	—
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clofazimine RS
  USP Clofazimine Related Compound B RS
  5-(4-Chlorophenyl)-3-(isopropylimino)-*N*-phenyl-3,5-dihydrophenazin-2-amine.
  $C_{27}H_{23}ClN_4$ 438.95

Clofibrate



$C_{12}H_{15}ClO_3$ 242.70
Propanoic acid, 2-(4-chlorophenoxy)-2-methyl-, ethyl ester.
Ethyl 2-(*p*-chlorophenoxy)-2-methylpropionate [637-07-0].

» Clofibrate contains not less than 97.0 percent and not more than 103.0 percent of $C_{12}H_{15}ClO_3$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Clofibrate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197F*▲ (CN 1-May-2020)

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: methanol.
Refractive index ⟨831⟩: between 1.500 and 1.505, at 20°.

Acidity—Mix 10.0 g with 100 mL of neutralized alcohol, add 3 drops of phenolphthalein TS, and titrate with 0.10 N sodium hydroxide: not more than 0.90 mL is required for neutralization.
Water Determination, *Method I* ⟨921⟩: not more than 0.2%.

Chromatographic purity—
Standard preparation—Prepare a solution in chloroform having known concentrations of about 0.1 mg of USP Clofibrate RS and 0.03 mg of *p*-chlorophenol per mL. To 10.0 mL of this solution add 5.0 µL of tributyrin, and mix.
Test preparation—To 10.0 mL of Clofibrate add 5.0 µL of tributyrin, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a split injector with a 20:1 split ratio, a flame-ionization detector, and a 0.53-mm × 15-m column to the internal walls of which is bonded a 1.5-µm film of phase G1. The chromatograph is programmed to maintain the column temperature at 120° for 1 minute, then to increase the temperature at a rate of 5° per minute for 12 minutes, and finally to maintain a temperature of 180° for 9 minutes. Maintain the injection port at 210° and the detector block at 220°. Helium is used as the carrier gas flowing at a rate of about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*:the relative retention times are about 0.2 for *p*-chlorophenol, 0.55 for clofibrate, and 1.0 for tributyrin.
Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 1 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity, other than *p*-chlorophenol, in the Clofibrate taken by the formula:

$$0.1C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Clofibrate RS in the *Standard preparation*, R_U is the ratio of the response of each individual impurity peak (other than the *p*-chlorophenol peak) to that of the tributyrin peak obtained from the *Test preparation*, and R_S is the ratio of the response of the clofibrate peak to that of the tributyrin peak obtained from the *Standard preparation*:the percentage of any impurity, other than *p*-chlorophenol, does not exceed 0.01%, and the total percentage of all such impurities does not exceed 0.12%.
Limit of *p*-chlorophenol—Use the chromatograms obtained in the test for *Chromatographic purity*. Calculate the percentage of *p*-chlorophenol in the portion of Clofibrate taken by the formula:

$$0.1C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of *p*-chlorophenol in the *Standard preparation*, and R_U and R_S are the ratios of the responses of the *p*-chlorophenol and tributyrin peaks obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 0.003% of *p*-chlorophenol is found.

Assay—
Ion-exchange resin—To a beaker containing 75 mL of 1 N sodium hydroxide add about 3 g of a 50- to 100-mesh strongly basic styrene-divinylbenzene anion-exchange resin, and allow the mixture to stand for about 15 minutes, with occasional stirring. Wash the resin with water until the last washing is neutral to litmus paper, then wash with three 50-mL portions of methanol.
Ion-exchange column—Place a plug of glass wool in the base of a 1- × 15-cm ion-exchange tube, and transfer to the tube a

www.webofpharma.com

sufficient amount of *Ion-exchange resin*, slurried in methanol, to produce a column bed height of from 6 cm to 8 cm.

Standard preparation—Dissolve an accurately weighed quantity of USP Clofibrate RS in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution having a known concentration of about 20 µg per mL.

Assay preparation—Transfer about 200 mg of Clofibrate, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 10.0 mL of this solution to the *Ion-exchange column*, and collect the eluate in a 100-mL volumetric flask. Rinse the column with 25 mL of methanol, collect the rinsing in the volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 226 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_{12}H_{15}ClO_3$ in the portion of Clofibrate taken by the formula:

$$10C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Clofibrate RS in the *Standard preparation*; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Clofibrate Capsules

» Clofibrate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{12}H_{15}ClO_3$.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Clofibrate RS

Identification—Capsules respond to *Identification* test *A* under *Clofibrate*.

Dissolution ⟨711⟩—
Medium: sodium lauryl sulfate solution (5 in 100); 1000 mL.
Apparatus 2: 100 rpm.
Time: 180 minutes.

Determine the amount of $C_{12}H_{15}ClO_3$ dissolved by employing the following method.

Mobile phase—Prepare a degassed and filtered mixture of methanol and water (80:20).

Standard solution—Transfer about 20 mg of USP Clofibrate RS, accurately weighed, to a 50-mL volumetric flask. Add 20 mL of methanol, mix to dissolve the clofibrate, dilute with water to volume, and mix. Dilute an aliquot quantitatively with methanol to obtain a final solution having a known concentration of about 80 µg per mL.

Test solution—Dilute a 5.0-mL portion of the solution under test to 25.0 mL, using methanol. Allow to stand for 5 minutes, and filter.

Chromatographic system—The liquid chromatograph is equipped with a 226-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal portions (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of $C_{12}H_{15}ClO_3$ dissolved.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{12}H_{15}ClO_3$ is dissolved in 180 minutes.

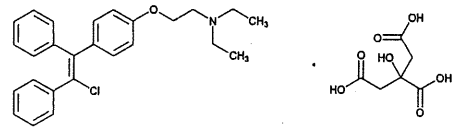
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—Proceed with Capsules as directed in the *Assay*—under *Clofibrate*, using the following as the *Assay preparation:* Weigh accurately not less than 20 Capsules in a tared weighing bottle. With a sharp blade, carefully open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any liquid from the emptied capsules by washing with several small portions of ether. Discard the washings, and allow the capsules to dry in a jet of dry air until the odor of ether no longer is perceptible. Weigh the empty capsules in the tared weighing bottle, and calculate the average net weight per capsule. Transfer an accurately weighed amount of capsule contents, equivalent to about 200 mg of clofibrate, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 10.0 mL of this solution to the *Ion-exchange column*, and collect the eluate in a 100-mL volumetric flask. Rinse the column with 25 mL of methanol, collect the rinsings in the volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Calculate the quantity, in mg, of $C_{12}H_{15}ClO_3$ in the portion of Capsules taken by the formula:

$$10C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Clofibrate RS in the *Standard preparation;* and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation,* respectively.

Clomiphene Citrate



$C_{26}H_{28}ClNO \cdot C_6H_8O_7$ 598.08

Ethanamine, 2-[4-(2-chloro-1,2-diphenylethenyl)phenoxy]-*N*,*N*-diethyl-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1); 2-[*p*-(2-Chloro-1,2-diphenylvinyl)phenoxy]triethylamine citrate (1:1) [50-41-9].

DEFINITION

Clomiphene Citrate contains NLT 98.0% and NMT 102.0% of a mixture of the (*E*)- and (*Z*)-geometric isomers of clomiphene citrate ($C_{26}H_{28}ClNO \cdot C_6H_8O_7$), calculated on the anhydrous basis. It contains NLT 30.0% and NMT 50.0% of the *Z*-isomer, [(*Z*)-2-[4-(2-chloro-1,2-diphenylethenyl) phenoxy]-*N*,*N*-diethylethanamine 2-hydroxy-1,2,3-propanetricarboxylate (1:1).

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet Spectroscopy*: **197U▲** (CN 1-May-2020)

Medium: 0.1 N hydrochloric acid
Sample solution: 20 µg/mL
Acceptance criteria: Meets the requirements

- **C. IDENTIFICATION TESTS—GENERAL,** *Citrate* ⟨191⟩

ASSAY

- **PROCEDURE**

Use low-actinic glassware for all solutions.
Mobile phase: Methanol, water, and triethylamine (55: 45: 0.3). Adjust with phosphoric acid to a pH of 2.5.
System suitability solution: 2 µg/mL of USP Clomiphene Related Compound A RS and 50 µg/mL of USP Clomiphene Citrate RS, in *Mobile phase*
Standard solution: 50 µg/mL of USP Clomiphene Citrate RS, in *Mobile phase*
Sample stock solution: 0.5 mg/mL of Clomiphene Citrate, in *Mobile phase*, filtered
Sample solution: 50 µg/mL of Clomiphene Citrate, in *Mobile phase*, from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 233 nm
Column: 4.6-mm × 25-cm; packing L26
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for clomiphene related compound A, (*Z*)-isomer, and (*E*)-isomer are about 0.9, 1.0, and 1.2, respectively.]
Suitability requirements
Resolution: NLT 1.0 between clomiphene related compound A and (*Z*)-isomer; NLT 1.5 between (*Z*)-isomer and (*E*)-isomer, *System suitability solution*
Column efficiency: NLT 2000 theoretical plates for the (*E*)-isomer, *Standard solution*
Tailing factor: NMT 3.0 for the (*E*)-isomer, *Standard solution*
Relative standard deviation: NMT 2.0% for both (*E*)- and (*Z*)-isomers, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clomiphene citrate ($C_{26}H_{28}ClNO \cdot C_6H_8O_7$) in the portion of Clomiphene Citrate taken:

$$\text{Result} = [(r_{UE} + r_{UZ})/(r_{SE} + r_{SZ})] \times (C_S/C_U) \times 100$$

r_{UE} = peak response of the (*E*)-isomer from the *Sample solution*
r_{UZ} = peak response of the (*Z*)-isomer from the *Sample solution*
r_{SE} = peak response of the (*E*)-isomer from the *Standard solution*
r_{SZ} = peak response of the (*Z*)-isomer from the *Standard solution*
C_S = concentration of USP Clomiphene Citrate RS in the *Standard solution* (µg/mL)
C_U = concentration of Clomiphene Citrate in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

OTHER COMPONENTS

- **CONTENT OF (*Z*)-ISOMER**

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of (*Z*)-isomer in the portion of Clomiphene Citrate taken:

$$\text{Result} = (r_Z/r_T) \times 100$$

r_Z = peak response of the (*Z*)-isomer from the *Sample solution*
r_T = sum of all the peak responses of the *Sample solution*

Acceptance criteria: 30.0%–50.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Use low-actinic glassware for all solutions.
Buffer: Acetonitrile, diethylamine, and water (40: 0.8: 60). Adjust with phosphoric acid to a pH of 6.2.
Solution A: *Buffer* and water (90:10)
Solution B: *Buffer*
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3.0	100	0
23.0	0	100
33.0	0	100
33.5	100	0
40.0	100	0

System suitability stock solution: 0.28 mg/mL of USP Clomiphene Related Compound A RS in *Buffer*
System suitability solution: 1.25 mg/mL of USP Clomiphene Citrate RS and 0.028 mg/mL of USP Clomiphene Related Compound A RS, prepared as follows. Transfer 12.5 mg of USP Clomiphene Citrate RS into a 10-mL volumetric flask, add 1.0 mL of *System suitability stock solution*, and dilute with *Buffer* to volume.
Standard solution: 0.025 mg/mL of USP Clomiphene Citrate RS in *Buffer*
Sample solution: 1.25 mg/mL of Clomiphene Citrate in *Buffer*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 233 nm
Column: 4.6-mm × 10-cm; 2.6-µm packing L7
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]

USP Monographs

www.webofpharma.com

Suitability requirements
Relative standard deviation: NMT 5.0% from the sum of the peak areas of the (*E*)- and (*Z*)-isomers, *Standard solution*
Peak-to-valley ratio: The ratio of the height of the clomiphene related compound A peak to the height of the valley between clomiphene related compound A and clomiphene peaks is NLT 15, *System suitability solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Clomiphene Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = sum of the peak responses of (*E*)- and (*Z*)-isomers of clomiphene from the *Standard solution*
C_S = concentration of USP Clomiphene Citrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Clomiphene Citrate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Clomiphene benzophenone analog[a]	0.10	0.51	1.0
Clomiphene tritylphenone analog[b]	0.13	1.0	1.0
Clomiphene keto analog[c]	0.33	1.0	1.0
Clomiphene related compound A	0.92	1.0	2.0
Clomiphene *Z*-isomer	0.98	—	—
Clomiphene *E*-isomer	1.00	—	—
2-Chloroclomiphene[d]	1.57	1.0	
2-Chloroclomiphene[d]	1.63	1.0	1.0[e]
4-Chloroclomiphene[f]	1.70	1.0	
4-Chloroclomiphene[f]	1.77	1.0	1.0[e]
Deschloroclomiphene chlorophenyl analog[g]	2.36	0.77	
Deschloroclomiphene chlorophenyl analog[g]	2.48	0.77	1.0[e]
Benzyl clomiphene[h]	2.67	0.74	0.15
Benzyl clomiphene[h]	2.76	0.81	0.15
Any other unspecified impurity	—	1.0	0.10
Total impurities	—	—	2.5

[a] {4-[2-(Diethylamino)ethoxy]phenyl}(phenyl)methanone.
[b] 2,2-Bis{4-[2-(diethylamino)ethoxy]phenyl}-1,2-diphenylethanone.
[c] 2-{4-[2-(Diethylamino)ethoxy]phenyl}-1,2-diphenylethan-1-one.
[d] (*E,Z*)-2-[2-Chloro-4-(2-chloro-1,2-diphenylvinyl)phenoxy]-*N,N*-diethylethan-1-amine.
[e] The sum of these geometric isomers is NMT 1.0%.
[f] (*E,Z*)-2-{4-[2-Chloro-2-(4-chlorophenyl)-1-phenylvinyl]phenoxy}-*N,N*-diethylethan-1-amine.
[g] (*E,Z*)-2-{4-[1,2-Bis(4-chlorophenyl)vinyl]phenoxy}-*N,N*-diethylethan-1-amine.
[h] (*E,Z*)-2-{4-[1-(4-Benzylphenyl)-2-chloro-2-phenylvinyl]phenoxy}-*N,N*-diethylethan-1-amine.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clomiphene Citrate RS
USP Clomiphene Related Compound A RS
(*E,Z*)-2-[4-(1,2-▲Diphenylvinyl)▲ (ERR 1-Jun-2019)phenoxy]-*N,N*-diethylethanamine hydrochloride.
▲Also known as (*E,Z*)-2-[4-(1,2-Diphenylethenyl) phenoxy]-*N,N*-diethylethanamine hydrochloride.▲ (ERR 1-Jun-2019)
$C_{26}H_{29}NO \cdot HCl$ 407.98

Clomiphene Citrate Tablets

DEFINITION
Clomiphene Citrate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of clomiphene citrate ($C_{26}H_{28}ClNO \cdot C_6H_8O_7$).

IDENTIFICATION
• **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩
Sample solution: Place a portion of finely powdered Tablets equivalent to about 30 mg of clomiphene citrate in a centrifuge tube containing a 30-mL solution of methanol and 0.1 N hydrochloric acid (1:2). Insert the stopper, and place the tube in a water bath at about 37° for 15 min. Shake occasionally. Centrifuge, and place the clear supernatant in a separator. Extract with one 40-mL and two 25-mL portions of hexanes, and discard the extract. Render the aqueous solution alkaline with 1 N sodium hydroxide, and extract the precipitated base with one 50-mL and two 25-mL portions of hexanes. Wash the combined extracts with two portions of water. Dry the extract with anhydrous sodium sulfate, and remove the hexanes by evaporation under reduced pressure. Add about 1.0 mL of carbon disulfide to the residue, and dissolve.
Standard solution: Prepare as directed in the *Sample solution*, but use USP Clomiphene Citrate RS.
Analysis
Samples: *Sample solution* and *Standard solution*
Determine the absorption spectra.
Acceptance criteria: Meet the requirements

www.webofpharma.com

ASSAY

• **ANALYSIS**

Mobile phase: Methanol, water, and triethylamine (55: 45: 0.3). Adjust with phosphoric acid to a pH of 2.5.

System suitability solution: 0.002 mg/mL of USP Clomiphene Related Compound A RS and 0.05 mg/mL of USP Clomiphene Citrate RS in *Mobile phase*. Use actinic glassware for the *Standard solution* and *Sample solution*.

Standard solution: 0.05 mg/mL of USP Clomiphene Citrate RS, in *Mobile phase*

Sample stock solution: Nominally 0.5 mg/mL of clomiphene citrate prepared in *Mobile phase* as follows. Transfer an equivalent of 50 mg of clomiphene citrate from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add about 50 mL of *Mobile phase*, and stir using a magnetic bar for 30 min. Remove the magnetic bar from the flask, dilute with *Mobile phase* to volume, and filter.

Sample solution: Nominally 0.05 mg/mL of clomiphene citrate prepared from the *Sample stock solution* in *Mobile phase*. Filter, and discard the first 10 mL. [NOTE—This solution is stable for NLT 24 h.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 233 nm

Column: 4.6-mm × 25-cm; packing L26

Flow rate: 1.0 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution* [NOTE—The relative retention times for clomiphene related compound A, (*Z*)-isomer, and (*E*)-isomer are about 0.9, 1.0, and 1.2, respectively.]

Suitability requirements

Resolution: NLT 1.0 between clomiphene related compound A and (*Z*)-isomer, and NLT 1.5 between (*Z*)-isomer and (*E*)-isomer, *System suitability solution*

Column efficiency: NLT 2000 theoretical plates for the (*E*)-isomer, *Standard solution*

Tailing factor: NMT 3.0 for the (*E*)-isomer, *Standard solution*

Relative standard deviation: NMT 2.0% for both (*E*)- and (*Z*)-isomers, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clomiphene citrate ($C_{26}H_{28}ClNO \cdot C_6H_8O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Clomiphene Citrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clomiphene citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: USP Clomiphene Citrate RS in 0.1 N hydrochloric acid

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with 0.1 N hydrochloric acid to a concentration similar to the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 232 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clomiphene citrate ($C_{26}H_{28}ClNO \cdot C_6H_8O_7$) dissolved.

Tolerances: NLT 75% (*Q*) of the labeled amount of clomiphene citrate ($C_{26}H_{28}ClNO \cdot C_6H_8O_7$) dissolved

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Clomiphene Citrate RS

USP Clomiphene Related Compound A RS

(*E,Z*)-2-[4-(1,2-diphenylethenyl)phenoxy]-*N,N*-diethylethanamine hydrochloride.

$C_{26}H_{29}NO \cdot HCl$ 407.98

Clomipramine Hydrochloride Compounded Oral Suspension, Veterinary

DEFINITION

Clomipramine Hydrochloride Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of clomipramine hydrochloride ($C_{19}H_{23}ClN_2 \cdot HCl$). Prepare Clomipramine Hydrochloride Compounded Oral Suspension, Veterinary 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Clomipramine Hydrochloride tablets[a] equivalent to	100 mg of clomipramine hydrochloride
Vehicle: a 1:1 mixture of Ora-Plus[b] and Ora-Sweet SF[b], a sufficient quantity to make	100 mL

[a] Clomicalm 20-mg tablets, Novartis Animal Health, Greensboro, NC.

[b] Perrigo, Allegan, MI.

Place the *Clomipramine Hydrochloride tablets* in a suitable container and comminute to a fine powder. Wet the powder with a small amount of *Vehicle* and triturate to make a smooth paste. Add the *Vehicle* to make the mortar contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: Methanol and acetonitrile (50:50)

Solution B: 25 mM monobasic potassium phosphate adjusted with phosphoric acid to a pH of 3.2

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	40	60
4	50	50

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
10	50	50
10.5	40	60
20	40	60

Standard solution: 0.1 mg/mL of clomipramine hydrochloride prepared from USP Clomipramine Hydrochloride RS and methanol. Vortex for about 30 s until dissolved.
Sample solution: Transfer 1.0 mL of Oral Suspension, Veterinary to a 10-mL volumetric flask, and rinse the pipette with about 2 mL of methanol. Vortex for 30 s and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 2.0-mm × 10-cm; 2.5-µm packing L1
Column temperature: 50°
Flow rate: 0.275 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for clomipramine hydrochloride is about 8.4 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clomipramine hydrochloride ($C_{19}H_{23}ClN_2 \cdot HCl$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

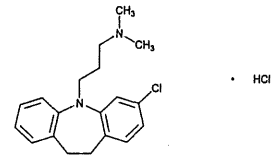
r_U = peak response of clomipramine hydrochloride from the *Sample solution*
r_S = peak response of clomipramine hydrochloride from the *Standard solution*
C_S = concentration of USP Clomipramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clomipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 3.8–4.8

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
• **BEYOND-USE DATE:** NMT 90 days after the day on which it was compounded when stored in a refrigerator or at controlled room temperature
• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*. Label it to state that it is for veterinary use.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clomipramine Hydrochloride RS

Clomipramine Hydrochloride



$C_{19}H_{23}ClN_2 \cdot HCl$ 351.31
5H-Dibenz[*b,f*]azepine-5-propanamine, 3-chloro-10,11-dihydro-*N,N*-dimethyl-, monohydrochloride;
3-Chloro-5-[3-(dimethylamino)propyl]-10,11-dihydro-5*H*-dibenz[*b,f*]azepine monohydrochloride [17321-77-6].

DEFINITION
Clomipramine Hydrochloride contains NLT 98.0% and NMT 102.0% of clomipramine hydrochloride ($C_{19}H_{23}ClN_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Medium: 0.1 N hydrochloric acid
Solution: 100 µg/mL
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 1.0% at the wavelength of maximum absorbance.

ASSAY
• **PROCEDURE**
Solution A: 55 g/L of sodium 1-heptanesulfonate prepared as follows. Transfer a suitable quantity of sodium 1-heptanesulfonate to an appropriate volumetric flask. Dissolve in 50% of the flask volume of water, and dilute with glacial acetic acid to volume.
Mobile phase: Transfer 20.0 mL of *Solution A* and 2.0 mL of triethylamine to a 500-mL volumetric flask, and dilute with water to volume. Transfer this solution to a 1-L volumetric flask, adjust with phosphoric acid to a pH of 3.2 ± 0.1, dilute with acetonitrile to volume, filter, and degas.
System suitability solution: 0.07 mg/mL of USP Desipramine Hydrochloride RS and 0.10 mg/mL of USP Imipramine Hydrochloride RS in methanol
Standard solution: 0.32 mg/mL of USP Clomipramine Hydrochloride RS in methanol
Sample solution: 0.32 mg/mL of Clomipramine Hydrochloride in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for desipramine and imipramine are 0.85 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 0.5 between desipramine and imipramine

www.webofpharma.com

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of clomipramine hydrochloride ($C_{19}H_{23}ClN_2 \cdot HCl$) in the portion of Clomipramine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clomipramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Clomipramine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩**:** NMT 0.1%
- **Organic Impurities, Procedure 1**

Solution A and **System suitability solution:** Proceed as directed in the *Assay*.

Mobile phase: Transfer 20.0 mL of *Solution A*, 2.0 mL of triethylamine, and 500 mL of water to a suitable container. Adjust with phosphoric acid to a pH of 3.2 ± 0.1, and dilute with water to 625 mL. Transfer to a 1-L volumetric flask, and dilute with acetonitrile to volume.

Sample solution: 2 mg/mL of Clomipramine Hydrochloride in methanol

Chromatographic system and **System suitability:** Proceed as directed in the *Assay*, except use an *Injection volume* of 5 µL.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Clomipramine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity
r_T = sum of all the peak responses

Acceptance criteria: See *Table 1*.

- **Organic Impurities, Procedure 2**

Solution A, Mobile phase, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Sample solution: 2 mg/mL of Clomipramine Hydrochloride in methanol

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Clomipramine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity
r_T = sum of the responses of all the peaks

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual, unspecified impurity	—	0.5
Total impurities[a]	—	2.0

[a] Sum of all impurities from *Organic Impurities, Procedure 1* and *Organic Impurities, Procedure 2*.

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: 100 mg/mL of Clomipramine Hydrochloride in water

Acceptance criteria: 3.5–5.0

- **Loss on Drying** ⟨731⟩

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨11⟩

USP Clomipramine Hydrochloride RS
USP Desipramine Hydrochloride RS
USP Imipramine Hydrochloride RS

Clomipramine Hydrochloride Capsules

DEFINITION

Clomipramine Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of clomipramine hydrochloride ($C_{19}H_{23}ClN_2 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

- **Procedure**

▲**Solution A:** Add 2.5 mL of acetic acid to 1 L of water. Adjust with stronger ammonia water to a pH of 7.5.

Solution B: Acetonitrile, methanol, and stabilizer-free tetrahydrofuran (80:15:5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	65	35
2.0	65	35
5.0	50	50
11.0	20	80

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
14.0	20	80
14.1	65	35
18.0	65	35

Diluent: *Solution A* and acetonitrile (50:50)
System suitability solution: 0.2 mg/mL of USP Clomipramine Hydrochloride RS and 0.005 mg/mL of USP Clomipramine Related Compound A RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Clomipramine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.2 mg/mL of clomipramine hydrochloride from Capsules in *Diluent* prepared as follows. Transfer a sufficient portion of the contents of Capsules (NLT 20) to a suitable volumetric flask. Add 60% of the final flask volume of *Diluent*. Shake by mechanical means for about 30 min. Dilute with *Diluent* to volume. Centrifuge to obtain a clear supernatant and use the clear supernatant. [NOTE—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 30°
Flow rate: 0.3 mL/min
Injection volume: 2 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between clomipramine and clomipramine related compound A, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clomipramine hydrochloride ($C_{19}H_{23}ClN_2 \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clomipramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clomipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% ▲(USP 1-May-2019)

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.1 N hydrochloric acid VS; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: USP Clomipramine Hydrochloride RS in *Medium*
Sample solution: ▲Pass the solution under test through a suitable filter and use the filtrate.▲ (USP 1-May-2019) Dilute with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.
Instrumental conditions
▲(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)▲ (USP 1-May-2019)
Mode: UV
Analytical wavelength: ▲▲ (USP 1-May-2019) 252 nm
Analysis
Samples: *Standard solution* and *Sample solution*
▲Calculate the percentage of the labeled amount of clomipramine hydrochloride ($C_{19}H_{23}ClN_2 \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Clomipramine Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 500 mL
L = label claim (mg/Capsule)▲ (USP 1-May-2019)

Tolerances: NLT 80% (Q) of the labeled amount of clomipramine hydrochloride ($C_{19}H_{23}ClN_2 \cdot HCl$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.01 N hydrochloric acid TS; 500 mL
Apparatus 2: 75 rpm with sinkers
Time: 15 min
Solution A: 55 g/L of sodium 1-heptanesulfonate in solution prepared as follows. Transfer a suitable amount of sodium 1-heptanesulfonate to an appropriate volumetric flask. Add 50% of the flask volume of water and dilute with glacial acetic acid to volume.
Solution B: To an appropriate volumetric flask, add 4% of the flask volume of *Solution A* and 0.4% of the flask volume of triethylamine. Dilute with water to volume.
Mobile phase: To an appropriate volumetric flask, add 50% of the flask volume of *Solution B*. Adjust the resulting solution with phosphoric acid to a pH of 3.2. Dilute with acetonitrile to volume.
Standard stock solution: 0.5 mg/mL of USP Clomipramine Hydrochloride RS in *Medium*. Sonication may be used to promote dissolution.
Standard solution: (L/500) mg/mL of USP Clomipramine Hydrochloride RS from the *Standard stock solution* in *Medium*, where L is the label claim in mg/Capsule
Sample solution: Pass a portion of the solution under test through a suitable filter and discard NLT the first 5 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 1.4 times the retention time of clomipramine

www.webofpharma.com

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clomipramine hydrochloride ($C_{19}H_{23}ClN_2 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clomipramine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of the *Medium*, 500 mL
L = label claim of clomipramine hydrochloride (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of clomipramine hydrochloride ($C_{19}H_{23}ClN_2 \cdot HCl$) is dissolved.

Change to read:

• **Uniformity of Dosage Units** ⟨905⟩
Procedure for content uniformity
Standard solution: 30 µg/mL of USP Clomipramine Hydrochloride RS in methanol
Sample stock solution: Transfer the contents of 1 Capsule to a 100-mL volumetric flask with the aid of methanol. Add about 75 mL of methanol, shake by mechanical means for 1 h, and dilute with methanol to volume.
Sample solution: Nominally 30 µg/mL of clomipramine hydrochloride from the *Sample stock solution* in methanol
Instrumental conditions
▲(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)▲ (USP 1-May-2019)
Mode: UV
Analytical wavelength: ▲▲ (USP 1-May-2019) 252 nm
Cell: 1 cm
Blank: Methanol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clomipramine hydrochloride ($C_{19}H_{23}ClN_2 \cdot HCl$) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Clomipramine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of clomipramine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

Add the following:

▲• **Organic Impurities**
Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability solution: 0.5 mg/mL of USP Clomipramine Hydrochloride RS and 0.0025 mg/mL of USP Clomipramine Related Compound A RS in *Diluent*
Standard solution: 0.0025 mg/mL each of USP Clomipramine Hydrochloride RS, USP Clomipramine Related Compound C RS, and USP Imipramine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of clomipramine hydrochloride from Capsules in *Diluent* prepared as follows. Transfer a sufficient portion of the contents of Capsules (NLT 20) to a suitable volumetric flask. Add 60% of the final flask volume of *Diluent*. Shake by mechanical means for about 30 min. Dilute with *Diluent* to volume. Centrifuge to obtain a clear supernatant and use the clear supernatant. [Note—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between clomipramine and clomipramine related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0% for all standard peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified impurity from the *Sample solution*
r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of clomipramine hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of clomipramine from the *Standard solution*
C_S = concentration of USP Clomipramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clomipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.03%.

USP Monographs

www.webofpharma.com

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Imipramine	0.77	1.0
Clomipramine related compound C	0.87	0.5
Clomipramine	1.00	—
Clomipramine related compound A[a]	1.1	—
Clomipramine related compound D[a,b]	1.2	—
Clomipramine related compound F[a,c]	1.5	—
Any individual unspecified impurity	—	0.5
Total impurities	—	2.0

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.
[b] 3-(3,7-Dichloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine hydrochloride.
[c] 3-Chloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine. ▲ (USP 1-May-2019)

ADDITIONAL REQUIREMENTS

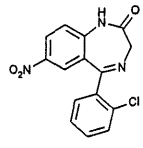
Change to read:

- **PACKAGING AND STORAGE:** ▲Preserve in tight, light-resistant containers. Protect from moisture. Store at controlled room temperature.▲ (USP 1-May-2019)
- **LABELING:** The labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clomipramine Hydrochloride RS
  ▲USP Clomipramine Related Compound A RS
  *N*¹-[3-(3-Chloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)propyl]-*N*¹,*N*³,*N*³-trimethylpropane-1,3-diamine dihydrochloride.
  $C_{23}H_{32}ClN_3 \cdot 2HCl$ 458.89
  USP Clomipramine Related Compound C RS
  3-(3-Chloro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine hydrochloride, monohydrate.
  $C_{19}H_{21}ClN_2 \cdot HCl \cdot H_2O$ 367.31▲ (USP 1-May-2019)
  USP Imipramine Hydrochloride RS

Clonazepam



$C_{15}H_{10}ClN_3O_3$ 315.71
2*H*-1,4-Benzodiazepin-2-one, 5-(2-chlorophenyl)-1,3-dihydro-7-nitro-.
5-(*o*-Chlorophenyl)-1,3-dihydro-7-nitro-2*H*-1,4-benzodiazepin-2-one [1622-61-3].

» Clonazepam contains not less than 98.0 percent and not more than 102.0 percent of $C_{15}H_{10}ClN_3O_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, at room temperature.
USP Reference standards ⟨11⟩—
USP Clonazepam RS
USP Clonazepam Related Compound A RS
3-Amino-4-(2-chlorophenyl)-6-nitrocarbostyril.
$C_{15}H_{10}ClN_3O_3$ 315.72
USP Clonazepam Related Compound B RS
2-Amino-2′-chloro-5-nitrobenzophenone.
$C_{13}H_9ClN_2O_3$ 276.68
USP Clonazepam Related Compound C RS
2-Bromo-2′-(2-chlorobenzoyl)-4′-nitroacetanilide.

Change to read:

▲ ***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: 197K▲ (CN 1-May-2020)
Melting range ⟨741⟩: between 237° and 240°.
Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Limit of clonazepam related compound C—
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.
Test solution—Dissolve an accurately weighed quantity of Clonazepam in acetone to obtain a solution having a concentration of 25 mg per mL.
Standard solution—Dissolve an accurately weighed quantity of USP Clonazepam Related Compound C RS in acetone to obtain a solution having a known concentration of 50 µg per mL.
Application volume: 20 µL.
Developing solvent system: a mixture of acetone and *n*-heptane (3:2).
Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. After air-drying the plate, heavily spray the plate with 2 M sulfuric acid, and dry at 105° for 15 minutes. Successively spray the plate with 0.01 M sodium nitrite, 9 mM ammonium sulfamate, and *N*-(1-naphthyl)ethylenediamine dihydrochloride TS, and dry the plate with a current of air. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with that of the principal spot in the chromatogram of the *Standard solution:* no secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot obtained from the *Standard solution* (0.2%).

Related compounds—
Buffer solution, Mobile phase, Diluent, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay.*
Test preparation—Use the *Assay preparation.*
Procedure—Inject a volume (about 50 µL) of the *Test preparation* into the chromatograph, record the chromatogram, and measure the responses for all of the peaks. Calculate the percentage of each impurity in the portion of Clonazepam taken by the formula:

$$100 Pr_i/(r_C + \Sigma Pr_i)$$

in which P is the relative response factor, which is 1.84 for clonazepam related compound A, 0.94 for clonazepam related compound B, and 1 for all other impurities; r_i is the peak

www.webofpharma.com

response for each impurity obtained from the *Test preparation;* and r_C is the peak response for clonazepam in the *Test preparation:* not more than 0.1% of clonazepam related compound A or of clonazepam related compound B is found, not more than 0.2% of any other impurity is found, and the sum of all other impurities is not more than 0.3%.

Assay—

Buffer solution—Transfer about 6.6 g of anhydrous dibasic ammonium phosphate to a 1-L volumetric flask, dissolve in 950 mL of water, adjust with 1 N phosphoric acid or 1 N sodium hydroxide to a pH of 8.0, dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution*, methanol, and tetrahydrofuran (60:52:13). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of water, methanol, and tetrahydrofuran (60:52:13).

Standard preparation—Dissolve an accurately weighed quantity of USP Clonazepam RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.1 mg per mL.

System suitability solution—Dissolve suitable quantities of USP Clonazepam Related Compound A RS, USP Clonazepam Related Compound B RS, and USP Clonazepam RS in *Diluent* to obtain a solution containing about 0.04 mg per mL of each Reference Standard.

Assay preparation—Transfer about 10 mg of Clonazepam, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 2.2 for clonazepam related compound A, 2.5 for clonazepam related compound B, and 1.0 for clonazepam; and the resolution, *R*, between clonazepam related compound A and clonazepam related compound B is not less than 2.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{15}H_{10}ClN_3O_3$ in the portion of Clonazepam taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Clonazepam RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Clonazepam Compounded Oral Suspension

DEFINITION

Clonazepam Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$).

Prepare Clonazepam Compounded Oral Suspension 0.1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Clonazepam	10 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

If using tablets, comminute the tablets into a fine powder in a suitable mortar, or add *Clonazepam* powder to the mortar. Add approximately 10 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol, acetonitrile, and water (30:30:40). Filter and degas.

Standard solution: 25 µg/mL of USP Clonazepam RS in acetonitrile

Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 2.5 mL of the sample into a 10-mL volumetric flask, and dilute with acetonitrile to volume to obtain a solution having a nominal concentration of 25 µg/mL of clonazepam.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 10-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for clonazepam is about 7 min.]

Suitability requirements

Relative standard deviation: NMT 1.8% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Clonazepam RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of clonazepam in the *Sample solution* (µg/mL)

USP Monographs

www.webofpharma.com

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.6–4.6

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- **Beyond-Use Date:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator
- **Labeling:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
  USP Clonazepam RS

Clonazepam Tablets

» Clonazepam Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$).

Packaging and storage—Preserve in tight, light-resistant containers, at room temperature.

USP Reference standards ⟨11⟩—
USP Clonazepam RS
USP Clonazepam Related Compound A RS
3-Amino-4-(2-chlorophenyl)-6-nitrocarbostyril.
$C_{15}H_{10}ClN_3O_3$ 315.72
USP Clonazepam Related Compound B RS
2-Amino-2′-chloro-5-nitrobenzophenone.
$C_{13}H_9ClN_2O_3$ 276.68

Identification—
A: Place an amount of finely powdered Tablets, equivalent to about 10 mg of clonazepam, in a 125-mL separator. Add 25 mL of water, shake for 2 minutes, and extract with two 40-mL portions of chloroform. Pass the extracts through anhydrous sodium sulfate, combine them, and evaporate at room temperature with the aid of a stream of nitrogen to dryness. Wash the residue with three 10-mL portions of solvent hexane: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Clonazepam RS.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: degassed water; 900 mL.
Apparatus 2: 75 rpm.
Time: 45 minutes.
Determine the amount of Clonazepam dissolved, using the following method.

Mobile phase—Prepare a filtered and degassed mixture of water, methanol, and acetonitrile (40:30:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Prepare a solution of USP Clonazepam RS in methanol having a known concentration of about 0.05 mg per mL. Quantitatively dilute a portion of this solution with *Dissolution Medium* to obtain a *Standard solution* having a known concentration similar to the expected concentration in the solution under test.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph replicate injections of the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%; and the tailing factor is not more than 2.0.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of $C_{15}H_{10}ClN_3O_3$ dissolved by comparison of the peak responses obtained from the *Standard solution* and the test solution.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{15}H_{10}ClN_3O_3$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Related compounds—
Buffer solution, Mobile phase, Diluent, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Clonazepam.*

Test preparation—Use the *Assay preparation.*

Procedure—Inject a volume (about 50 µL) of the *Test preparation* into the chromatograph, record the chromatogram, and measure the responses for all of the peaks. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100Pr_i/(r_C + \Sigma Pr_i)$$

in which P is the relative response factor, which is 2.45 for the peak with a relative retention time of 0.7 (if present), 1.84 for clonazepam related compound A, 0.94 for clonazepam related compound B, and 1 for all other impurities; r_i is the peak response for each impurity obtained from the *Test preparation;* and r_C is the peak response for clonazepam in the *Test preparation:* not more than 0.8% for the peak at relative retention time 0.7, not more than 0.4% of clonazepam related compound A, and not more than 1.0% of clonazepam related compound B are found; not more than 0.2% of any other impurity is found; and the sum of all other impurities is not more than 0.5%.

Assay—
Buffer solution, Mobile phase, Diluent, Standard preparation, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Clonazepam.*

Assay preparation—Weigh and finely powder not fewer than 10 Tablets. Transfer an accurately weighed amount of powder, equivalent to about 10 mg of clonazepam, to a 100-mL volumetric flask; dissolve, with sonication, in 75 mL of *Diluent;* cool to room temperature, dilute with *Diluent* to volume, mix, and filter, discarding the first few mL of the filtrate.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of clonazepam ($C_{15}H_{10}ClN_3O_3$) in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Clonazepam RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

www.webofpharma.com

Clonazepam Orally Disintegrating Tablets

DEFINITION
Clonazepam Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (25:25:50)

Standard solution: 0.01 mg/mL of USP Clonazepam RS in *Mobile phase*

Sample solution: Nominally 0.01 mg/mL of clonazepam in *Mobile phase* prepared as follows. Transfer an equivalent to about 2 mg of clonazepam, from finely powdered Tablets (NLT 20), to a 200-mL volumetric flask. Add 120 mL of *Mobile phase* and sonicate for about 15 min with intermittent shaking. Shake the flask on a mechanical shaker for about 30 min. Dilute with *Mobile phase* to volume. Pass a portion of this solution through a nylon membrane filter of 0.45-µm or finer pore size. Use the filtrate after discarding the first 4 mL. This solution is stable for 48 h at room temperature.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.2 mL/min
Injection volume: 60 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clonazepam from the *Sample solution*
r_S = peak response of clonazepam from the *Standard solution*
C_S = concentration of USP Clonazepam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clonazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISINTEGRATION** ⟨701⟩: NMT 60 s
- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 60 min
Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay* except for *Injection volume*.
Injection volume: 100 µL
Standard stock solution: 0.01 mg/mL of USP Clonazepam RS in *Mobile phase*
Standard solution: Dilute the *Standard stock solution* with *Medium* according to the Tablet strength. See *Table 1* for the concentration of the *Standard solution* corresponding to each Tablet strength.

Table 1

Tablet Strength (mg/Tablet)	Standard Solution (µg/mL of clonazepam)
0.125	0.125
0.25	0.25
0.5	0.50
1.0	1.0
2.0	2.0

Sample solution: Pass a portion of the solution under test through a nylon membrane filter of 0.45-µm pore size, discarding the first few milliliters.

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of clonazepam from the *Sample solution*
r_S = peak response of clonazepam from the *Standard solution*
C_S = concentration of USP Clonazepam RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
- **ORGANIC IMPURITIES**

Mobile phase: Proceed as directed in the *Assay*.
System suitability solution: 0.2 µg/mL each of USP Clonazepam Related Compound A RS and USP Clonazepam Related Compound B RS in *Mobile phase*
Standard stock solution: Use the *Standard solution* from the *Assay*.
Standard solution: 0.2 µg/mL of USP Clonazepam RS from the *Standard stock solution* in *Mobile phase*
Sample solution: Nominally 0.1 mg/mL of clonazepam from Tablets prepared as follows. Transfer an equivalent to about 2 mg of clonazepam, from finely powdered Tablets (NLT 20), to a 50-mL volumetric flask. Pipet 20.0 mL of *Mobile phase* into the flask, and sonicate for about 2 min with intermittent shaking. Do not dilute to volume. Shake the flask for 30 min on a mechanical shaker. Pass a portion of this solution through a nylon membrane filter of 0.45-µm

www.webofpharma.com

or finer pore size, and use the filtrate after discarding the first 4 mL of the filtrate.
Chromatographic system: Proceed as directed in the *Assay* except for *Injection volume.*
Injection volume: 100 µL
Run time: NLT 4 times the retention time of clonazepam
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between clonazepam related compound A and clonazepam related compound B, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 6.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of clonazepam from the *Standard solution*
C_S = concentration of USP Clonazepam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clonazepam in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peaks with a relative retention time less than 0.63.

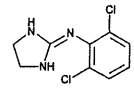
Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Clonazepam	1.0	—	—
Clonazepam related compound A	1.71	0.67	0.4
Clonazepam related compound B	2.25	0.79	1.0
Any other unspecified degradation product	—	1.0	0.2
Total impurities	—	—	2.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Clonazepam RS
USP Clonazepam Related Compound A RS
3-Amino-4-(2-chlorophenyl)-6-nitrocarbostyril.
$C_{15}H_{10}ClN_3O_3$ 315.72
USP Clonazepam Related Compound B RS
2-Amino-2′-chloro-5-nitrobenzophenone.
$C_{13}H_9ClN_2O_3$ 276.68

Clonidine



$C_9H_9Cl_2N_3$ 230.09
Benzenamine, 2,6-dichloro-*N*-2-imidazolidinylidene-;
2-[(2,6-Dichlorophenyl)imino]imidazolidine [4205-90-7].

DEFINITION

Clonidine contains NLT 99.0% and NMT 101.0% of clonidine ($C_9H_9Cl_2N_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Medium: 0.01 N hydrochloric acid
Sample solution: 0.3 mg/mL of Clonidine in *Medium*
Acceptance criteria: Absorptivities are about 2.1 and 1.8 for the maxima at 271 nm and 279 nm, respectively, calculated on the dried basis.

ASSAY

- **PROCEDURE**

Sample solution: 190 mg of Clonidine in 100 mL of glacial acetic acid
Analysis: Titrate with 0.1 N perchloric acid VS, using a silver–silver chloride glass combination electrode with liquid junction. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 23.01 mg of clonidine ($C_9H_9Cl_2N_3$).
Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩

Analysis: Ignite at 500 ± 25° to constant weight.
Acceptance criteria: NMT 0.1%

- **ORGANIC IMPURITIES**

1 M phosphoric acid: 115 g/L of phosphoric acid in water
Buffer: 4 g/L of monobasic potassium phosphate in water. Adjust with *1 M phosphoric acid* to a pH of 4.0.
Solution A: Acetonitrile and *Buffer* (75:25)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Solution A (%)
0	90	10
15	30	70
15.1	90	10
20	90	10

Acetylclonidine solution: 0.05 mg/mL of USP Clonidine Related Compound A RS, prepared as follows. Initially dissolve USP Clonidine Related Compound A RS in acetonitrile (1 mg/mL), and dilute with *Buffer* to volume.

www.webofpharma.com

System suitability solution: 0.86 mg/mL of USP Clonidine RS and 0.86 µg/mL of clonidine related compound A from *Acetylclonidine solution* in *Buffer*
Standard solution: 8.6 µg/mL of USP Clonidine RS in *Buffer*
Sample solution: 0.86 mg/mL of Clonidine in *Buffer*
Blank: *Buffer*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 3.0-mm × 15-cm; 5-µm packing L56
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5 between clonidine and acetylclonidine, *System suitability solution*
Tailing factor: NMT 2.5 for clonidine, *System suitability solution*
Relative standard deviation: NMT 5%, *Standard solution*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of each impurity in the portion of Clonidine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of clonidine from the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria
Individual impurity: NMT 0.1%
Total impurities: NMT 0.2%

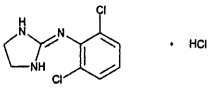
SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample at 60° under vacuum to constant weight.
Acceptance criteria: NMT 0.5%
• **Appearance of Solution**
Color of solution
Standard solution: 4.8 µg/mL of potassium chromate in water
Sample solution: 100 mg/mL in methanol
Acceptance criteria: The color of 10 mL of *Sample solution* is not more intense than that of 10 mL of *Standard solution* when compared in matched color-comparison tubes (see *Visual Comparison* ⟨630⟩).
Turbidity
Sample solution: Prepare as directed in *Color of solution*.
Acceptance criteria: 10 mL of *Sample solution* has no more turbidity than 10 mL of methanol when compared in matched color-comparison tubes (see *Visual Comparison* ⟨630⟩).

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at room temperature.
• **USP Reference Standards** ⟨11⟩
USP Clonidine RS
USP Clonidine Related Compound A RS
1-Acetyl-2-(2,6-dichlorophenylamino)-2-(4,5-dihydroimidazol).
$C_{11}H_{11}Cl_2N_3O$ 272.13

Clonidine Hydrochloride



$C_9H_9Cl_2N_3 \cdot HCl$ 266.55
Benzenamine, 2,6-dichloro-*N*-2-imidazolidinylidene-, monohydrochloride;
2-[(2,6-Dichlorophenyl)imino]imidazolidine monohydrochloride [4205-91-8].

DEFINITION

Change to read:

Clonidine Hydrochloride contains NLT ▲98.0%▲ (USP 1-May-2020) and NMT ▲102.0%▲ (USP 1-May-2020) of clonidine hydrochloride ($C_9H_9Cl_2N_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

Change to read:

• **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)
• **C. Identification Tests—General** ⟨191⟩, *Chemical Identification Tests, Chloride*: Meets the requirements

ASSAY

Change to read:

• **Procedure**
Solution A: 1.0 mL/L of triethylamine in water
Mobile phase: Acetonitrile and *Solution A* (32:68). Adjust with phosphoric acid to a pH of 6.9.
▲▲ (USP 1-May-2020)
Standard solution: 0.05 mg/mL of USP Clonidine Hydrochloride RS in *Mobile phase*▲▲ (USP 1-May-2020)
Sample solution: 0.05 mg/mL of Clonidine Hydrochloride in *Mobile phase*▲▲ (USP 1-May-2020)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
▲**Run time:** NLT 3 times the retention time of clonidine▲ (USP 1-May-2020)
System suitability
Sample: ▲▲ (USP 1-May-2020)*Standard solution*
Suitability requirements
▲▲ (USP 1-May-2020)

www.webofpharma.com

Tailing factor: NMT 2.0
Relative standard deviation: NMT ▲0.73%▲ (USP 1-May-2020)

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clonidine hydrochloride ($C_9H_9Cl_2N_3 \cdot HCl$) in the portion of Clonidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clonidine from the *Sample solution*
r_S = peak response of clonidine from the *Standard solution*
C_S = concentration of USP Clonidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Clonidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: ▲98.0%–102.0%▲ (USP 1-May-2020) on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

• **ORGANIC IMPURITIES**

Solution A and **Mobile phase:** Prepare as directed in the *Assay*.

Standard stock solution: 0.06 mg/mL each of USP Clonidine Hydrochloride RS, USP Clonidine Related Compound A RS, and 2,6-dichloroaniline in acetonitrile ▲▲ (USP 1-May-2020)

Standard solution: 0.6 µg/mL each of USP Clonidine Hydrochloride RS, ▲USP Clonidine Related Compound A RS, and 2,6-dichloroaniline▲ (USP 1-May-2020) in *Mobile phase* from the *Standard stock solution*

▲**Sensitivity solution:** 0.06 µg/mL each of USP Clonidine Hydrochloride RS, USP Clonidine Related Compound A RS, and 2,6-dichloroaniline in *Mobile phase* from the *Standard solution*

Sample stock solution: 1.5 mg/mL of Clonidine Hydrochloride in *Mobile phase* prepared as follows. Transfer 150 mg of Clonidine Hydrochloride to a 100-mL volumetric flask. Add *Mobile phase* to about 60% of the volume of the flask, sonicate for 5 min, and then dilute with *Mobile phase* to volume.▲ (USP 1-May-2020)

Sample solution: 150 µg/mL of Clonidine Hydrochloride in *Mobile phase* ▲from the *Sample stock solution*▲ (USP 1-May-2020)

Chromatographic system: ▲Proceed as directed in the *Assay*, except for the *Run time*.▲ (USP 1-May-2020)
Run time: ▲NLT 6▲ (USP 1-May-2020) times the retention time of clonidine

System suitability
Samples: *Standard solution* ▲and *Sensitivity solution*
[NOTE—See *Table 1* for the relative retention times.▲ (USP 1-May-2020)]
Suitability requirements
Resolution: NLT 2.0 between clonidine related compound A and 2,6-dichloroaniline, *Standard solution* ▲▲ (USP 1-May-2020)
Relative standard deviation: NMT 5% for ▲clonidine, clonidine related compound A, and 2,6-dichloroaniline,▲ (USP 1-May-2020) *Standard solution*
▲**Signal-to-noise ratio:** NLT 10 for clonidine, clonidine related compound A, and 2,6-dichloroaniline, *Sensitivity solution*▲ (USP 1-May-2020)

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clonidine related compound A and 2,6-dichloroaniline in the portion of Clonidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clonidine related compound A or 2,6-dichloroaniline from the *Sample solution*
r_S = peak response of clonidine related compound A or 2,6-dichloroaniline from the *Standard solution*
C_S = concentration of USP Clonidine Related Compound A RS or 2,6-dichloroaniline in the *Standard solution* ▲(µg/mL)▲ (USP 1-May-2020)
C_U = concentration of Clonidine Hydrochloride in the *Sample solution* ▲(µg/mL)▲ (USP 1-May-2020)

Calculate the percentage of any ▲▲ (USP 1-May-2020) unspecified impurity in the portion of Clonidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any ▲▲ (USP 1-May-2020) unspecified impurity from the *Sample solution*
r_S = peak response of clonidine from the *Standard solution*
C_S = concentration of USP Clonidine Hydrochloride RS in the *Standard solution* ▲(µg/mL)▲ (USP 1-May-2020)
C_U = concentration of Clonidine Hydrochloride in the *Sample solution* ▲(µg/mL)▲ (USP 1-May-2020)

Acceptance criteria: See *Table 1*. ▲The reporting threshold is▲ (USP 1-May-2020) 0.04%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Clonidine ▲▲ (USP 1-May-2020)	1.0	—
Clonidine related compound A▲▲ (USP 1-May-2020)	2.5	0.1
2,6-Dichloroaniline	3.3	0.1
Any ▲▲ (USP 1-May-2020) unspecified impurity	—	0.1
Total impurities	—	0.2

▲▲ (USP 1-May-2020)

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: 50 mg/mL
Acceptance criteria: 3.5–5.5

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clonidine Hydrochloride RS

www.webofpharma.com

USP Clonidine Related Compound A RS
1-Acetyl-2-(2,6-dichlorophenylimino)-imidazolidine).
$C_{11}H_{11}Cl_2N_3O$ 272.13

Add the following:

▲Clonidine Hydrochloride Compounded Oral Suspension

DEFINITION

Clonidine Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of clonidine hydrochloride ($C_9H_9Cl_2N_3 \cdot HCl$).

Prepare Clonidine Hydrochloride Compounded Oral Suspension 0.01 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Clonidine Hydrochloride tablets,[a] equivalent to	1 mg of clonidine hydrochloride
Ora-Blend,[b] a sufficient quantity to make	100 mL

[a] Clonidine Hydrochloride 0.2-mg tablets, Qualitest, Huntsville, AL.
[b] Perrigo, Allegan, MI.

Place the *Clonidine Hydrochloride Tablets* in a suitable container and triturate to a fine powder. Add a small amount of *Ora-Blend* and mix well to form a smooth paste. Add a sufficient amount of *Ora-Blend* to make the mortar contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the remainder of the *Ora-Blend*. Add sufficient *Ora-Blend* to bring to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: 10 mM of monobasic potassium phosphate adjusted with 6 N potassium hydroxide to a pH of 8.0

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Acetonitrile (%)
0	98	2
60	98	2
60.1	70	30
75	70	30
75.1	98	2
85	98	2

Standard stock solution: 0.1 mg/mL of USP Clonidine Hydrochloride RS in water

Standard solution: Transfer 0.5 mL of the *Standard stock solution* to a 50-mL volumetric flask and dilute with water to volume.

Sample solution: Transfer 1.0 mL of Oral Suspension into a 10-mL volumetric flask and dilute with water to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L96
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 100 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for clonidine hydrochloride is about 71.3 min.]
Suitability requirements
Tailing factor: NMT 3.0
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clonidine hydrochloride ($C_9H_9Cl_2N_3 \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clonidine hydrochloride from the *Sample solution*
r_S = peak response of clonidine hydrochloride from the *Standard solution*
C_S = concentration of USP Clonidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clonidine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.0–5.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.

• **BEYOND-USE DATE:** NMT 14 days after the date on which it was compounded when stored at controlled room temperature. NMT 90 days after the date on which it was compounded when stored in a refrigerator

• **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clonidine Hydrochloride RS▲ (USP 1-May-2020)

Clonidine Hydrochloride Tablets

DEFINITION

Clonidine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clonidine hydrochloride ($C_9H_9Cl_2N_3 \cdot HCl$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 10 mg/mL of USP Clonidine Hydrochloride RS in methanol

Sample solution: Transfer an equivalent to 1 mg of clonidine hydrochloride, from a quantity of finely powdered Tablets, to a separator containing 30 mL of water and 5 mL of 1 N sodium hydroxide. Swirl gently to dissolve the sample specimen, and extract with 20 mL of

www.webofpharma.com

chloroform. Allow the layers to separate, and filter the chloroform extract. Evaporate the filtrate to dryness, and dissolve the residue in 0.1 mL of methanol.

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 2 µL
Developing solvent system: Methanol and ammonium hydroxide (200:3)

Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in the chapter. Position the plate in a chromatographic chamber, and develop in *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Solution A: 2.2 mg/mL of sodium 1-octanesulfonate in water
Mobile phase: Methanol, *Solution A*, and phosphoric acid (500:500:1). Adjust with 1 N sodium hydroxide to a pH of 3.0, and pass through a suitable filter of 0.45-µm pore size.
2,6-Dichloroaniline stock solution: 12 µg/mL of 2,6-dichloroaniline in *Mobile phase*
Standard stock solution: 100 µg/mL of USP Clonidine Hydrochloride RS in *Mobile phase*
Standard solution: 1 µg/mL of clonidine hydrochloride from the *Standard stock solution* in *Mobile phase*
System suitability solution: 2 µg/mL of USP Clonidine Hydrochloride RS and 2.4 µg/mL of 2,6-dichloroaniline in *Mobile phase*, from the *Standard stock solution* and *2,6-Dichloroaniline stock solution*, respectively
Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer the equivalent to 0.1 mg of clonidine hydrochloride from powder to a 100-mL volumetric flask. Add about 60 mL of *Mobile phase*, shake by mechanical means for 15–30 min, dilute with *Mobile phase* to volume, and mix. Centrifuge a portion of this solution to obtain a clear solution.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; packing L7, deactivated for basic compounds
Flow rate: 1.5 mL/min
Injection volume: 50 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for clonidine and 2,6-dichloroaniline are about 0.5 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 1.5 for the clonidine peak, *System suitability solution*
Column efficiency: NLT 3500 theoretical plates for the clonidine peak, *System suitability solution*
Relative standard deviation: NMT 2.0% for the clonidine peak, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clonidine hydrochloride ($C_9H_9Cl_2N_3 \cdot HCl$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = nominal concentration of clonidine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: Proceed as directed in the *Assay*, except use *Medium* instead of *Mobile phase*.
Analysis: Proceed as directed in the *Assay*.
Tolerances: NLT 75% (*Q*) of the labeled amount of clonidine hydrochloride ($C_9H_9Cl_2N_3 \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clonidine Hydrochloride RS

Clonidine Hydrochloride and Chlorthalidone Tablets

DEFINITION

Clonidine Hydrochloride and Chlorthalidone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of chlorthalidone ($C_{14}H_{11}ClN_2O_4S$) and NLT 90.0% and NMT 110.0% of the labeled amount of clonidine hydrochloride ($C_9H_9Cl_2N_3 \cdot HCl$).

IDENTIFICATION

• **A.**
Sample solution: Transfer an amount of powdered Tablets, equivalent to 3 mg of clonidine hydrochloride, to a beaker. Add 30 mL of water, stir for 5 min, and pass through a filter of medium pore size into a sintered-glass funnel. Transfer the filtrate to a separator, add 5 mL of 0.1 N sodium hydroxide, and extract with 20 mL of chloroform, collecting the chloroform extract in a separator. Extract the chloroform phase with 15 mL of 0.01 N hydrochloric acid, collecting the acid extract in a beaker. Remove any residual chloroform from the acid extract by heating on a steam bath.
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Clonidine Hydrochloride RS, concomitantly measured.

• **B. INFRARED ABSORPTION**
Sample: Transfer 10 powdered Tablets to a 50-mL beaker. Add 10 mL of methanol, boil on a steam bath for 5 min, and filter. Add 20 mL of water to the filtrate, and boil on a steam bath for 5 min under a current of air. Cool, with stirring, in ice until crystals form. Filter the crystals, and dry at 105° for 1 h.

www.webofpharma.com

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Chlorthalidone RS.

• **C.** The retention times of the chlorthalidone and clonidine hydrochloride peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 1 g/L of monobasic ammonium phosphate in water

Mobile phase: Methanol, acetonitrile, and *Buffer* (100:100:800)

Clonidine hydrochloride standard stock solution: 1500/ µg/mL of USP Clonidine Hydrochloride RS in *Buffer*; *I* is the ratio of the labeled amount, in mg, of clonidine hydrochloride to the labeled amount, in mg, of chlorthalidone per Tablet.

Standard solution: 150/ µg/mL of USP Clonidine Hydrochloride RS and 150 µg/mL of USP Chlorthalidone RS prepared as follows. Transfer 15 mg of USP Chlorthalidone RS to a 100-mL volumetric flask, dissolve in 10 mL of methanol, and add 25 mL of *Buffer* and 10.0 mL of *Clonidine hydrochloride standard stock solution*. Dilute with *Buffer* to volume.

Sample solution: Transfer an amount equivalent to 15 mg of chlorthalidone from powdered Tablets (NLT 20). Add 10 mL of methanol, and sonicate for 5 min. Add 40 mL of *Buffer*, and sonicate until the solution is free from agglomerates. Allow to cool to ambient temperature, dilute with *Buffer* to volume, and centrifuge.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; packing L7

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for clonidine hydrochloride and chlorthalidone are about 0.2 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3 between the clonidine hydrochloride and chlorthalidone peaks

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of clonidine hydrochloride ($C_9H_9Cl_2N_3 \cdot HCl$) and chlorthalidone ($C_{14}H_{11}ClN_2O_4S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clonidine hydrochloride or chlorthalidone from the *Sample solution*

r_S = peak response of clonidine hydrochloride or chlorthalidone from the *Standard solution*

C_S = concentration of USP Clonidine Hydrochloride RS or USP Chlorthalidone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of clonidine hydrochloride or chlorthalidone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 100 rpm

Time: 60 min

Sample solution: Pipet 20 mL of a centrifuged portion of the solution under test into a 25-mL volumetric flask, and dilute with 0.5% monobasic ammonium phosphate solution to volume. Use the resulting solution as the *Sample solution*.

Analysis: Proceed as directed in the *Assay*, making any necessary volumetric adjustments.

Tolerances: NLT 50% (*Q*) of the labeled amount of chlorthalidone ($C_{14}H_{11}ClN_2O_4S$) and NLT 80% (*Q*) of the labeled amount of clonidine hydrochloride ($C_9H_9Cl_2N_3 \cdot HCl$) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements for *Content Uniformity* with respect to both clonidine hydrochloride and chlorthalidone

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Chlorthalidone RS

USP Clonidine Hydrochloride RS

Clonidine Transdermal System

DEFINITION

Clonidine Transdermal System contains NLT 80.0% and NMT 120.0% of the labeled amount of clonidine ($C_9H_9Cl_2N_3$).

[NOTE—Throughout the following procedures, avoid the use of tetrahydrofuran stabilized with butylated hydroxytoluene (BHT). In the presence of peroxides, BHT may react with clonidine, producing impurity peaks.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Buffer solution: 242.28 g/L of tris(hydroxymethyl) aminomethane in water. Adjust with dilute hydrochloric acid to a pH of 9.2.

Sample: Carefully peel the release liner from each Transdermal System, and place a number of Transdermal Systems equivalent to 25 mg of clonidine into a 50-mL screw-capped centrifuge tube. Add 5 mL of chloroform, and mix on a vortex mixer for 5 min. Allow to stand for 30 min, and mix intermittently on a vortex mixer. Transfer the chloroform solution to another 50-mL centrifuge tube, and wash the residue with an additional 3 mL of chloroform, combining the extracts. Add 2 mL of 0.5 N hydrochloric acid to the extract, mix on a vortex mixer for 1 min, and centrifuge at about 1000 rpm for 4 min. Remove and discard the bottom chloroform layer. Extract the aqueous layer with 4 mL of chloroform. Centrifuge at 1000 rpm for an additional 5 min, and again discard the bottom chloroform layer. Add 5 mL of *Buffer solution* and 3 mL of methylene chloride. Mix on a vortex mixer for 1 min. Centrifuge at 1000 rpm for 4 min. Transfer the bottom methylene chloride layer into a 100-mL beaker, and dry the methylene chloride with anhydrous sodium sulfate (about 1/4 liquid height). Decant, and evaporate to dryness with

USP Monographs

www.webofpharma.com

a stream of nitrogen. Dry at 105° for 30 min, and allow to cool in a desiccator.

Analysis: Determine the IR spectrum of the *Sample solution* and USP Clonidine RS in the wavelength region of 3500–600 cm^{-1}.

Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer solution: 2.5 mL of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer solution* (60:40). [NOTE—Stir the solution for 30 min.]

Diluent: Tetrahydrofuran and methanol (1:1)

System suitability solution: 250 µg/mL of USP Clonidine RS and 10 µg/mL of USP Clonidine Related Compound B RS in *Diluent*

Standard stock solution: 1 mg/mL of USP Clonidine RS in tetrahydrofuran

Standard solutions: Prepare a minimum of four *Standard solutions* from the *Standard stock solution* in *Diluent* that bracket the expected clonidine concentration in the sample. The standard concentrations should be within the range of 50–300 µg/mL. [NOTE—The *Standard solutions* are stable for up to 2 days if stored at 4°.]

Sample solution: 357 µg/mL of clonidine prepared as follows. Remove each Transdermal System from its package, discard the release liner from each system, and transfer into a 50-mL centrifuge tube with a Teflon-lined screw cap. Add the appropriate volume of tetrahydrofuran as listed in *Table 1*.

Table 1

For systems containing about 2.5 mg of clonidine	7.0 mL
For systems containing about 5.0 mg of clonidine	14.0 mL
For systems containing about 7.5 mg of clonidine	21.0 mL

Mix vigorously on a vortex mixer until the systems are washed down and fully submerged in the tetrahydrofuran. Let the systems soak in tetrahydrofuran for about 5 min, and mix on a vortex mixer until the systems are completely delaminated. Allow the systems to remain submerged for an additional 60 min, mixing on a vortex mixer every 30 min. Add methanol in a volume equal to the volume of tetrahydrofuran, and mix vigorously on a vortex mixer. The solution turns milky. Centrifuge for 10 min at 2000 rpm. Use the supernatant as the *Sample solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 and 242 nm

[NOTE—The detector is programmed initially to 242 nm and switched to 210 nm after the elution of the clonidine peak but before the elution of the clonidine related compound B peak.]

Column: 4.6-mm × 15-cm; packing L10

Flow rate: 1 mL/min

Injection size: 25 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for clonidine and clonidine related compound B are 1.0 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 2.0 between clonidine and clonidine related compound B

Capacity factor (*k'*): NLT 0.6 for clonidine

Tailing factor: NMT 3.0 for both clonidine and clonidine related compound B

Relative standard deviation: NMT 2.0% for the clonidine peak area

Analysis

Samples: At least three *Standard solutions* that will bracket the expected sample concentration range and the *Sample solution*

Calculate the peak response ratios of the analyte, and plot the results. Determine the linear regression equation of the standards by the mean-square method, and record the linear regression equation and the correlation coefficient: it should be NLT 0.995.

Calculate the percentage of the labeled amount of clonidine ($C_9H_9Cl_2N_3$) in the Transdermal System taken:

$$\text{Result} = (C_S/C_U) \times 100$$

C_S = concentration of clonidine from the linear regression analysis (µg/mL)

C_U = nominal concentration of clonidine in the *Sample solution* (µg/mL)

Acceptance criteria: 80.0%–120.0%

PERFORMANCE TESTS

Change to read:

• **DRUG RELEASE** ⟨724⟩

Test 1

Medium: 0.001 M phosphoric acid; 80 mL for systems containing 5 mg or less of clonidine; 200 mL for systems containing more than 5 mg of clonidine

Times: 8, 24, 96, and 168 h

Apparatus 7: Proceed as directed in the chapter, using the transdermal system holder-angled disk (see *Drug Release* ⟨724⟩, *Figure 5a*). The appropriate size of the holder, 1.42 or 1.98 inches, should be chosen based on the size of the system to prevent overhang. Use 100-mL beakers for *Medium* volumes of 80 mL and 300-mL beakers for *Medium* volumes of 200 mL. Gently press the Transdermal System to a dry, smooth, square piece of cellulose membrane, or equivalent, with the adhesive side against the membrane. Attach the membrane/system to a suitable inert sample holder with a Viton O-ring, or equivalent, so that the backing of the system is adjacent to and centered on the bottom of the sample holder. Trim the excess cellulose membrane with scissors. Suspend each sample holder from the arm of a reciprocating shaker so that each system is continuously immersed in a beaker containing the specified volume of *Medium*. The filled beakers are weighed and pre-equilibrated to 32.0 ± 0.3° before immersing the test sample. Agitate the sample in an up-down motion at a frequency of 30 cycles/min with an amplitude of 2.0 ± 0.1 cm. The *Medium* must be added daily to the beakers during each interval to maintain sample immersion. At the end of each time interval, transfer the test sample to a fresh beaker containing the appropriate volume of *Medium*, weighed and pre-equilibrated to 32.0 ± 0.3°.

Mobile phase: 0.1% solution of triethylamine in a mixture of methanol and water (30:70). Adjust with phosphoric acid to a pH of 6.0 ± 0.2.

System suitability solution: 10 µg/mL of USP Clonidine RS in 0.001 M phosphoric acid

USP Monographs

www.webofpharma.com

Standard solutions: Prepare a minimum of four *Standard solutions* of USP Clonidine RS in 0.001 M phosphoric acid having known concentrations of clonidine similar to those of the *Sample solutions*.
Sample solutions: At the end of each release interval, allow the beakers to cool to room temperature, and make up for evaporative *Medium* losses by adding *Medium* to obtain the original weight, then mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 25 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Capacity factor (k'): NLT 0.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solutions* and *Sample solutions*
Construct a standard curve of concentration (µg/mL) of clonidine in the *Standard solutions* versus peak area by linear regression analysis. The correlation coefficient is NLT 0.995.
Calculate the release rate of clonidine:

$$\text{Result} = CV/TA$$

C = concentration of clonidine in the sample of the standard curve (µg/mL)
V = volume of the *Medium* (mL)
T = time (h)
A = area of the Transdermal System (cm²)

Tolerances: See *Table 2*.

Table 2

Time (h)	Time for Sampling (h)	Release Rate (µg/h/cm²)
0–8	8	7.5–16.0
8–24	24	1.5–4.6
24–96	96	1.5–4.6
96–168	168	1.5–3.3

The release rate of clonidine ($C_9H_9Cl_2N_3$) from the Transdermal System, expressed as µg/h/cm² at the times specified, conforms to *Drug Release* ⟨724⟩, *Acceptance Table 1*.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 2*.
Medium: 0.01 N hydrochloric acid; 500 mL for systems labeled as 0.1 mg/day, 900 mL for systems labeled as 0.2 or 0.3 mg/day
Apparatus 6: 100 rpm. Apply double-sided tape around the lower-most circumference of the cylinder, overlapping the ends to prevent peeling of the tape end from the cylinder. Remove the outer layer of the tape. Attach the Transdermal System to the cylinder with the backing side against the double-sided tape and the longitudinal axis parallel to the bottom of the cylinder. Carefully smooth the system to remove any air bubbles, and remove the release liner from the system. For systems requiring 500 mL of *Medium*, apply the double-sided tape to the system such that the bottom edge of each is NMT 2 mm from the bottom of the cylinder to prevent evaporation during the test from exposure to air. After setting the cylinder in the vessel, cover the vessel to minimize evaporation.
Times: 6, 48, 96, and 168 h
Buffer: 0.3% triethylamine in 0.025 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 6.20 ± 0.10.
Mobile phase: *Buffer* and tetrahydrofuran (94:6)
Standard solutions: Solutions containing 0.7, 3.0, 5.3, 7.5, and 9.8 µg/mL of USP Clonidine RS in *Medium*. A small amount of methanol (not exceeding 10% of the final volume) can be used to solubilize clonidine.
Sample solution: 1.5 mL aliquots of the solution under test. After sampling the last time point, measure the volume of *Medium* remaining in the vessel.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Columns
Guard: 3.0-mm × 4-mm; packing L1
Analytical: 4.6-mm × 15-cm; packing L1
Flow rate: 1.0 mL/min
Injection size: 50 µL
System suitability
Sample: 5.3 µg/mL of the *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solutions* and *Sample solution*
Construct a standard curve of concentration (µg/mL) of clonidine in the *Standard solutions* versus peak area by linear regression analysis. The correlation coefficient is NLT 0.997. Calculate the release rate of clonidine.
Calculate the volume loss rate in mL/h (L):

$$L = [V - F + (N \times 1.5)]/T$$

V = initial volume of *Medium* (mL)
F = final volume of *Medium* (mL)
N = number of sampling time points
T = total elapsed time between start of run and final volume measurement (h)

Calculate the volume (mL) at each sampling time adjusted for evaporation (V_{adj}):

$$V_{adj} = V - (L \times t_C) - [(n - 1) \times 1.5]$$

t_C = cumulative time for the sample withdrawal (6, 48, 96, or 168 h)
n = sampling number (1, 2, 3, or 4 for the 6-, 48-, 96-, and 168-h sampling times, respectively)

Calculate the release rate of clonidine (µg/h/cm²):

$$\text{Result} = [(r_U - b) \times V_{adj}]/(m \times A \times t_i)$$

r_U = peak response from the *Sample solution*
b = y-intercept of the standard curve
m = slope of the standard curve
A = area of the system (cm²)
t_i = interval time (h)

Tolerances: See *Table 3*.

USP Monographs

www.webofpharma.com

Table 3

Time (h)	Time for Sampling (h)	Interval Time (h)	Release Rate (µg/h/cm²)
0–6	6	6	7.6–12.0
6–48	48	42	1.7–2.5
48–96	96	48	2.0–2.9
96–168	168	72	1.7–2.6

The release rate of clonidine ($C_9H_9Cl_2N_3$) from the Transdermal System, expressed as µg/h/cm² at the times specified, conforms to *Drug Release* ⟨724⟩, *Acceptance Table 1*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 3*.

Medium: 100 mM acetate buffer, pH 5.0, with 0.01% of cetyltrimethylammonium bromide (13.6 g/L of sodium acetate monohydrate in water, adjust with glacial acetic acid to a pH of 5.0, and add 0.1 g/L of cetyltrimethylammonium bromide); 900 mL

Apparatus 5: 100 rpm, with the 76-mm disk

Times: 8, 24, 96, and 168 h

Solution A: 2.4 g/L of octanesulfonic acid sodium salt and 2 mL/L of phosphoric acid in water

Mobile phase: Methanol and *Solution A* (45:55). Adjust with 10 N sodium hydroxide to a pH of 3.0.

Standard stock solution: 1 mg/mL of USP Clonidine RS in methanol

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration similar to the expected concentration in the *Sample solution*, considering complete drug release.

Sample solution: Apply double-sided adhesive tape to the stainless steel disk to cover enough of the disk area so that the entire patch is secured by the tape. Apply a Transdermal System with the release liner intact to the adhesive layer on the stainless steel disk. Press the backing film of the patch to the adhesive tape with the clear release liner film of the system facing up. Peel the release liner from the affixed system on the disk assembly, and place the disk assembly flat on the bottom of the vessel with the exposed transdermal adhesive side up and parallel to the bottom edge of the paddle blade. Lower the paddle, and start the equipment. At each sampling time withdraw an appropriate volume of the solution under test.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; packing L7

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection size: 30 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.8

Relative standard deviation: NMT 2.0%

Analysis:

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of clonidine ($C_9H_9Cl_2N_3$) in the *Medium* (mg/mL) at each time point:

$$C_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

i = interval, where $i = 1$ at 8 h, $i = 2$ at 24 h, $i = 3$ at 96 h, $i = 4$ at 168 h

Calculate the rate of clonidine ($C_9H_9Cl_2N_3$) released in µg/h/cm² at each time point:

$$\text{Result} = [(C_i - C_{i-1}) \times V_i \times 1000]/[S \times (T_i - T_{i-1})]$$

$$V_i = V_0 - [(i - 1) \times V_A]$$

V_i = volume of *Medium* at a given time point

V_0 = initial volume of *Medium*, 900 mL

V_A = volume of *Medium* withdrawn at each time point

1000 = conversion factor from mg to µg

S = system size in cm²

T_i = current time point

T_{i-1} = previous time point

Tolerances: See *Table 4*.

Table 4

Time (h)	Release Rate (µg/h/cm²)
8	▲5.5 ▲ (RB 1-Jun-2019)–11.0
24	2.5–5.5
96	2.5–5.0
168	2.0–3.8

The release rate of clonidine ($C_9H_9Cl_2N_3$) from the Transdermal System, expressed as µg/h/cm² at the times specified, conforms to *Drug Release* ⟨724⟩, *Acceptance Table 1*.

• **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements

IMPURITIES

• **Organic Impurities**

Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard stock solution: 1 mg/mL of USP Clonidine Related Compound B RS in tetrahydrofuran

Standard solutions: Prepare a minimum of four *Standard solutions* in *Diluent* that bracket the expected clonidine related compound B concentration in the sample. The standard concentrations should be within the range of 0.2–10.0 µg/mL.

[Note—The *Standard solutions* are stable for up to 2 days if stored at 4°.]

Analysis

Samples: At least three *Standard solutions* that will bracket the expected sample concentration range and the *Sample solution*

Measure the responses for clonidine related compound B. Calculate the peak response ratios of the analyte, and plot the results. Determine the linear regression equation of the standards by the mean-square method, and record the linear regression equation and the correlation coefficient: it should be NLT 0.995. Determine the concentration of clonidine related compound B.

Calculate the amount, in µg/cm², of clonidine related compound B in the portion of the Transdermal System taken:

$$\text{Result} = CV/A$$

USP Monographs

www.webofpharma.com

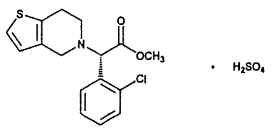
C = concentration of clonidine related compound B from the linear regression analysis (µg/mL)
V = volume of the *Sample solution* (mL)
A = area of the sample system (cm²)

Acceptance criteria: NMT 10.0 µg/cm²

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in sealed, single-dose containers at a temperature not exceeding 30°.
- **LABELING:** The label states the total amount of clonidine in the Transdermal System and the release rate, in mg/day, for the duration of the application of one system. When more than one *Drug Release* test is given, the labeling states the *Drug Release* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clonidine RS
  USP Clonidine Related Compound B RS
  2-[(*E*)-2,6-Dichlorophenylimino]-1-(1-{2-[(*E*)-2,6-dichlorophenylimino]-imidazolidin-1-yl}-ethyl) imidazolidine.
  $C_{20}H_{20}Cl_4N_6$ 486.23

Clopidogrel Bisulfate



$C_{16}H_{16}ClNO_2S \cdot H_2SO_4$ 419.90
Thieno[3,2-*c*]pyridine-5(4*H*)-acetic acid, α-(2-chlorophenyl)-6,7-dihydro-, methyl ester, (*S*)-, sulfate (1:1);
Methyl (+)-(*S*)-α-(*o*-chlorophenyl)-6,7-dihydrothieno[3,2-*c*] pyridine-5(4*H*)-acetate, sulfate (1:1) [120202-66-6].

DEFINITION

Clopidogrel Bisulfate contains NLT 97.0% and NMT 101.5% of clopidogrel bisulfate ($C_{16}H_{16}ClNO_2S \cdot H_2SO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**
  [NOTE—For all clopidogrel related compounds, the concentrations are expressed as bisulfate salts. Use bisulfate salt equivalents stated on USP Reference Standards labels to calculate the concentrations as appropriate.]
  Buffer: 1.36 g/L of monobasic potassium phosphate in water
  Mobile phase: Acetonitrile and *Buffer* (25:75)
  System suitability stock solution: 100 µg/mL of USP Clopidogrel Bisulfate RS and 200 µg/mL of USP Clopidogrel Related Compound B RS in methanol
  System suitability solution: 2.5 µg/mL of USP Clopidogrel Bisulfate RS and 5.0 µg/mL of USP Clopidogrel Related Compound B RS in *Mobile phase* from *System suitability stock solution*
  Standard stock solution: 1.0 mg/mL of USP Clopidogrel Bisulfate RS in methanol
  Standard solution: 0.1 mg/mL in *Mobile phase* from the *Standard stock solution*
  Sample stock solution: 1 mg/mL of Clopidogrel Bisulfate in methanol
  Sample solution: 0.1 mg/mL in *Mobile phase*, from *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 220 nm
  Column: 4.6-mm × 15-cm; packing L57
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for the two enantiomers of clopidogrel related compound B and for clopidogrel are 0.8, 1.2, and 1.0, respectively.]
  Suitability requirements
  Resolution: Greater than 2.5 between clopidogrel and the first enantiomer of clopidogrel related compound B, *System suitability solution*
  Relative standard deviation: NMT 1.0% from clopidogrel bisulfate, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of clopidogrel bisulfate ($C_{16}H_{16}ClNO_2S \cdot H_2SO_4$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **ORGANIC IMPURITIES**
  [NOTE—For all clopidogrel related compounds, the concentrations are expressed as bisulfate salts. Use bisulfate salt equivalents stated on USP Reference Standards labels to calculate the concentrations as appropriate.]
  Buffer: 0.96 g/L sodium 1-pentanesulfonate. Adjust with phosphoric acid to a pH of 2.5.
  Solution A: Acetonitrile
  Solution B: Methanol
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Solution A (%)	Solution B (%)
0	85	10	5
3	85	10	5
48	30	65	5
68	30	65	5

www.webofpharma.com

Diluent: Acetonitrile and *Buffer* (60:40)
System suitability solution: 6.5 mg/mL of USP Clopidogrel Bisulfate RS and 0.01 mg/mL each of USP Clopidogrel Related Compound A RS and USP Clopidogrel Related Compound B RS in *Diluent*
Standard solution: 6.5 µg/mL of USP Clopidogrel Bisulfate RS in *Diluent*
Sample solution: 6.5 mg/mL of Clopidogrel Bisulfate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for clopidogrel related compound A, clopidogrel, and clopidogrel related compound B are given in *Table 2*.]
Suitability requirements
Peak-to-valley ratio (H_P/H_V): NLT 10 where H_P is the height above the baseline of the peak due to clopidogrel related compound B and H_V is the height above the baseline of the lowest point of the curve separating clopidogrel related compound B and clopidogrel, *System suitability solution*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clopidogrel related compound A, clopidogrel related compound B, and any other individual impurity in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clopidogrel related compound A, clopidogrel related compound B, or any other impurity from the *Sample solution*
r_S = peak response of clopidogrel from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Clopidogrel related compound A[a]	0.4	0.2
Clopidogrel	1.0	—
Clopidogrel related compound B[b]	1.1	0.3
Any other impurity[c]	—	0.10
Total impurities	—	0.5

[a] (+)-(*S*)-(*o*-Chlorophenyl)-6,7-dihydrothieno[3,2-*c*]pyridine-5(4*H*)-acetic acid.
[b] Methyl (+/−)-(*o*-chlorophenyl)-4,5-dihydrothieno[2,3-*c*]pyridine-6(7*H*)-acetate.
[c] Disregard any peak less than 0.05%.

• **LIMIT OF CLOPIDOGREL RELATED COMPOUND C**
Mobile phase: Heptane and dehydrated alcohol (85:15)
Standard solution: 0.02 mg/mL each of USP Clopidogrel Bisulfate RS, USP Clopidogrel Related Compound B RS, and USP Clopidogrel Related Compound C RS prepared as follows. Dissolve a quantity of USP Clopidogrel Bisulfate RS, USP Clopidogrel Related Compound B RS, and USP Clopidogrel Related Compound C RS in dehydrated alcohol (about 50% of the volume of the flask), and dilute with heptane to volume.
Sample solution: 2 mg/mL of Clopidogrel Bisulfate prepared as follows. Transfer 100 mg of Clopidogrel Bisulfate to a 50-mL volumetric flask, dissolve in 25 mL of dehydrated alcohol, and dilute with heptane to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 10-µm packing L80
Flow rate: 0.8 mL/min
Injection volume: 10 µL
Run time: 1.25 times the retention time of clopidogrel
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for clopidogrel related compound B, clopidogrel, and clopidogrel related compound C are 0.7, 1.0, and 0.6, respectively.]
Suitability requirements
Resolution: NLT 2.0 between clopidogrel related compound C and clopidogrel related compound B
Signal-to-noise ratio: NLT 20 for clopidogrel related compound C peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clopidogrel related compound C in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clopidogrel related compound C from *Sample solution*
r_S = peak response of clopidogrel related compound C from *Standard solution*
C_S = concentration of the clopidogrel related compound C in *Standard solution* (mg/mL)
C_U = concentration of Clopidogrel Bisulfate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clopidogrel Bisulfate RS
USP Clopidogrel Related Compound A RS
(+)-(*S*)-(*o*-Chlorophenyl)-6,7-dihydrothieno[3,2-*c*]pyridine-5(4*H*)-acetic acid, hydrochloride.
$C_{15}H_{14}ClNO_2S \cdot HCl$ 344.26
USP Clopidogrel Related Compound B RS
Methyl (+/−)-(*o*-chlorophenyl)-4,5-dihydrothieno[2,3-*c*]pyridine-6(7*H*)-acetate, hydrochloride.
$C_{16}H_{17}Cl_2NO_2S$ 358.28
USP Clopidogrel Related Compound C RS
Methyl (−)-(*R*)-(*o*-chlorophenyl)-6,7-dihydrothieno[3,2-*c*]pyridine-5(4*H*)-acetate, hydrogen sulfate.
$C_{16}H_{16}ClNO_2S \cdot H_2SO_4$ 419.90

www.webofpharma.com

Clopidogrel Compounded Oral Suspension

DEFINITION

Clopidogrel Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of clopidogrel ($C_{16}H_{16}ClNO_2S$).

Prepare Clopidogrel Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Clopidogrel tablet(s)[a] equivalent to	525 mg
Vehicle: A 1:1 mixture of Ora-Plus[b] and Ora-Sweet[b], a sufficient quantity to make	105 mL

[a] Clopidogrel 75-mg tablets, Dr. Reddy's Laboratory Limited, Bridgewater, NJ.
[b] Perrigo Pharmaceuticals, Allegan, MI.

Crush the *Clopidogrel tablet(s)* to a fine powder using a mortar and pestle or by other mechanical means. Wet the powder with a small amount of *Vehicle*, and triturate to make a smooth paste. Add the *Vehicle* to make the contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: 10 mM sodium phosphate adjusted with phosphoric acid to a pH of 3.0. Pass through a nylon filter of 0.45-µm pore size, and degas.

Mobile phase: Acetonitrile and *Solution A* (65:35)

Diluent: Water adjusted with phosphoric acid to a pH of 3.0

Standard stock solution: 5 mg/mL of clopidogrel prepared from USP Clopidogrel Bisulfate RS and *Diluent*. Mix well, and sonicate for 3 min. Store at 2°–8°.

Standard solution: Transfer 2.0 mL of the *Standard stock solution* to a 1-L volumetric flask, and dilute with *Diluent* to volume. Mix well, centrifuge a portion of the solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

Sample solution: Shake each bottle of Oral Suspension thoroughly. Transfer 2.0 mL of the Oral Suspension to a 1-L volumetric flask, and dilute with *Diluent* to volume. Mix well, centrifuge a portion of the solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 2.1-mm × 25-cm; 5-µm packing L7

Temperatures

Column: 35°

Autosampler: 5°

Flow rate: 0.3 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for clopidogrel is about 7.4 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clopidogrel ($C_{16}H_{16}ClNO_2S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clopidogrel from the *Sample solution*

r_S = peak response of clopidogrel from the *Standard solution*

C_S = concentration of clopidogrel in the *Standard solution* (mg/mL)

C_U = nominal concentration of clopidogrel in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 2.1–3.1

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored at 2°–8° or at controlled room temperature

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Clopidogrel Bisulfate RS

Clopidogrel Tablets

» Clopidogrel Tablets contain Clopidogrel Bisulfate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of clopidogrel ($C_{16}H_{16}ClNO_2S$).

Packaging and storage—Preserve in well-closed containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—

USP Clopidogrel Bisulfate RS

USP Clopidogrel Related Compound A RS
(+)-(*S*)-(*o*-Chlorophenyl)-6,7-dihydrothieno[3,2-*c*] pyridine-5(4*H*)-acetic acid.

USP Clopidogrel Related Compound B RS
Methyl (±)-(*o*-chlorophenyl)-4,5-dihydrothieno[2,3-*c*] pyridine-6(7*H*)-acetate, hydrochloride.

USP Clopidogrel Related Compound C RS
Methyl (–)-(*R*)-(*o*-chlorophenyl)-6,7-dihydrothieno[3,2-*c*]pyridine-5(4*H*)-acetate, hydrogen sulfate.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—*Spectral range:* 250 to 300 nm.

Solution—Use the test solution prepared as directed in the test for *Uniformity of dosage units*.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

www.webofpharma.com

Dissolution ⟨711⟩—

Medium: pH 2.0 hydrochloric acid buffer (see *Buffer Solutions* under *Reagents, Indicators, and Solutions*); 1000 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Standard solution—Dissolve an accurately weighed quantity of USP Clopidogrel Bisulfate RS in 20.0 mL of methanol, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration corresponding to that of the solution under test.

Procedure—Determine the amount of $C_{16}H_{16}ClNO_2S$ dissolved by employing UV absorption at a wavelength of about 240 nm on filtered portions of the solution under test in comparison with the *Standard solution*.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{16}H_{16}ClNO_2S$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—Using a suitable volumetric flask, place 1 Tablet in 50.0 mL of 0.1 N hydrochloric acid. Sonicate for 5 minutes, and cool. Quantitatively transfer 5.0 mL of this solution to the flask, and dilute with 0.1 N hydrochloric acid to 50.0 mL. Pass a portion of the solution through a suitable filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate. Determine the amount of clopidogrel by employing UV absorption at the wavelength of maximum absorbance at about 270 nm, in comparison with a Standard solution having a known concentration of USP Clopidogrel Bisulfate RS in 0.1 N hydrochloric acid.

Related compounds— [NOTE—For all clopidogrel related compounds, the concentrations are expressed as bisulfate salts. Use bisulfate salt equivalents stated on USP Reference Standards labels to calculate the concentrations as appropriate.]

Phosphate buffer and *Mobile phase*—Prepare as directed in the *Assay* under *Clopidogrel Bisulfate*.

System suitability solution—Dissolve accurately weighed quantities of USP Clopidogrel Bisulfate RS and USP Clopidogrel Related Compound B RS in methanol, and dilute with methanol to obtain a solution having concentrations of about 100 µg per mL and 200 µg per mL, respectively. Transfer 5 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Standard solution—Dissolve accurately weighed quantities of USP Clopidogrel Bisulfate RS, USP Clopidogrel Related Compound A RS, and USP Clopidogrel Related Compound C RS in methanol to obtain a solution having known concentrations of about 40 µg per mL, 250 µg per mL, and 300 µg per mL, respectively. Transfer 5 mL of this solution to a 200-mL volumetric flask, and dilute with *Mobile phase* to volume. This solution contains about 1 µg of clopidogrel bisulfate per mL, 6 µg of clopidogrel related compound A per mL, and 7.5 µg of clopidogrel related compound C per mL.

Test solution—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of clopidogrel (free base), to a 200-mL volumetric flask, add 5 mL of methanol, dilute with *Mobile phase* to volume, and mix. Allow to stand for 10 minutes, and mix. Pass a portion of this solution through a filter having a 0.45-µm or finer porosity, and use the filtrate after discarding the first 5 mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and 4.6-mm × 15-cm column that contains packing L57. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 and 1.2 for the two enantiomers of clopidogrel related compound B and 1.0 for clopidogrel; and the resolution, *R,* between clopidogrel and the first enantiomer of clopidogrel related compound B is greater than 2.5. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.5 for clopidogrel related compound A, 1.0 for clopidogrel and 2.0 for clopidogrel related compound C; and the relative standard deviation for replicate injections is not more than 15% for each peak.

Procedure—Inject equal volumes (about 10 µL) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of clopidogrel related compounds A and C in the portion of Tablets taken by the formula:

$$20(321.82/419.90)(C/W)(r_U/r_S)$$

in which 321.82 is the molecular weight of clopidogrel; 419.90 is the molecular weight of clopidogrel bisulfate; *C* is the concentration, in µg per mL, of the relevant clopidogrel related compound in the *Standard solution; W* is the weight, in mg, of clopidogrel in the portion of Tablets used to prepare the *Test solution* based on the labeled quantity of clopidogrel per Tablet, Tablet weight, and the weight of the portion of Tablets used; and r_U and r_S are the peak responses of the corresponding related compounds obtained from the *Test solution* and the *Standard solution,* respectively.

Calculate the percentage of any other impurity (excluding clopidogrel related compound B) in the portion of Tablets taken by the formula:

$$20(321.82/419.90)(C_C/W)(r_U/r_S)$$

in which C_C is the concentration of clopidogrel bisulfate, in µg per mL, in the *Standard solution;* r_U is the peak response of any other impurity obtained from the *Test solution;* r_S is the peak response of clopidogrel peak obtained from the *Standard solution;* and the other terms are as defined above: not more than 1.2% of clopidogrel related compound A is found, not more than 1.5% of clopidogrel related compound C is found, not more than 0.2% of any other single impurity (excluding clopidogrel related compound B) is found, and not more than 2.5% of total impurities (excluding clopidogrel related compound B) is found.

Assay— [NOTE—For all clopidogrel related compounds, the concentrations are expressed as bisulfate salts. Use bisulfate salt equivalents stated on USP Reference Standards labels to calculate the concentrations as appropriate.]

Phosphate buffer, Mobile phase, and *Chromatographic system*—Proceed as directed in the *Assay* under *Clopidogrel Bisulfate.*

System suitability preparation—Dissolve accurately weighed quantities of USP Clopidogrel Bisulfate RS and USP Clopidogrel Related Compound B RS in methanol, and quantitatively dilute with methanol to obtain a solution having concentrations of about 100 µg per mL and 200 µg per mL, respectively. Transfer 5 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Clopidogrel Bisulfate RS in methanol to obtain a solution having a known concentration of about 0.1 mg of clopidogrel bisulfate per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of clopidogrel (base), to a 100-mL volumetric flask, and add 50 mL of methanol. Sonicate for 5 minutes, and stir for 30 minutes. Dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to the flask, dilute with methanol to 50.0 mL, and mix. Pass a portion of this solution through a filter having a 0.45-µm or

www.webofpharma.com

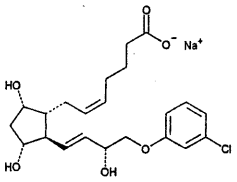
finer porosity, and use the filtrate after discarding the first 5 mL.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the analyte peaks. Calculate the quantity, in mg, of clopidogrel ($C_{16}H_{16}ClNO_2S$) in the portion of Tablets taken by the formula:

$$1000(321.82/419.90)C(r_U/r_S)$$

in which 321.82 is the molecular weight of clopidogrel; 419.90 is the molecular weight of clopidogrel bisulfate; C is the concentration, in mg per mL, of USP Clopidogrel Bisulfate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cloprostenol Sodium



$C_{22}H_{28}ClNaO_6$ 446.90

5-Heptenoic acid, 7-[2-[4-(3-chlorophenoxy)-3-hydroxy-1-butenyl]-3,5-dihydroxycyclopentyl]-, [1α(*Z*),2β(1*E*,3*R**),3α,5α]-, sodium salt, (±)-;
(±)-Sodium (*Z*)-7-[(1*R**,2*R**,3*R**,5*S**)-2-[(*E*)-(3*R**)-4-(*m*-chlorophenoxy)-3-hydroxy-1-butenyl]-3,5-dihydroxycyclopentyl]-5-heptenoate [55028-72-3].

DEFINITION

Cloprostenol Sodium contains NLT 97.5% and NMT 102.5% of cloprostenol sodium ($C_{22}H_{28}ClNaO_6$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩

ASSAY

- **PROCEDURE**

Mobile phase: Chromatographic hexane, dehydrated alcohol, and glacial acetic acid (900:100:1)
Standard solution: 0.8 mg/mL of USP Cloprostenol Sodium RS in dehydrated alcohol
Sample solution: 0.8 mg/mL of Cloprostenol Sodium in dehydrated alcohol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Flow rate: 1.8 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for the cloprostenol peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cloprostenol sodium ($C_{22}H_{28}ClNaO_6$) in the portion of Cloprostenol Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cloprostenol Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Cloprostenol Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.5% on the anhydrous basis

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase: Chromatographic hexane, dehydrated alcohol, and glacial acetic acid (930:70:1)
Sample solution: 20 mg/mL of Cloprostenol Sodium in dehydrated alcohol
Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay,* except use a run time of NLT 2 times the retention time of cloprostenol.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Cloprostenol Sodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of the responses all the peaks from the *Sample solution*

Acceptance criteria: Disregard any peak below 0.05%.
Individual impurities: NMT 1.0%
Total impurities: NMT 2.5%

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩

Sample solution: 50 mg dissolved in 1 mL of dehydrated alcohol
Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS

- **LABELING:** Label it to indicate that it is for veterinary use only.
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Cloprostenol Sodium RS

Cloprostenol Injection

» Cloprostenol Injection is a sterile solution of Cloprostenol Sodium in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cloprostenol ($C_{22}H_{29}ClO_6$).

www.webofpharma.com

Packaging and storage—Preserve in single-dose or multiple-dose containers protected from light. Store at controlled room temperature.
Labeling—Label it to indicate that it is for veterinary use only, and to indicate the strength as the equivalent amount of cloprostenol per dose.
USP Reference standards ⟨11⟩—
USP Cloprostenol Sodium RS
USP Hydrocortisone Acetate RS

Identification—
A: The retention time of the cloprostenol peak in the chromatogram of the *Assay preparation* corresponds to that of the cloprostenol peak in the chromatogram of the *Standard preparation,* obtained as directed in the *Assay.*
B: It meets the requirements of the test for *Sodium* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 2500 USP Endotoxin Units per mg of cloprostenol.
Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

Related compounds—
Mobile phase and *System suitability solution*—Prepare as directed in the *Assay.*
Standard solution—Prepare as directed for *Standard preparation* under *Assay.*
Test solution—Prepare as directed for *Assay preparation.*
Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay.*
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Injection taken by the formula:

$$100(C_S/C_T)(r_i/r_S)$$

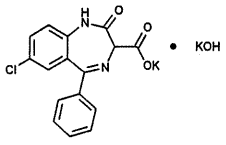
in which C_S is the concentration, in mg per mL, of USP Cloprostenol Sodium RS in the *Standard solution*; C_T is the concentration, in mg per mL, of cloprostenol in the *Test solution*; r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response of cloprostenol obtained from the *Standard solution*: not more than 1.0% of any individual impurity is found, and not more than 2.5% of total impurities is found. Disregard any peak below 0.05%.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
pH 2.5 Monobasic sodium phosphate solution—Prepare an aqueous solution containing 2.4 mg of monobasic sodium phosphate dihydrate per mL of solution. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase—Prepare a filtered and degassed mixture of *pH 2.5 Monobasic sodium phosphate solution* and acetonitrile (73:27). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve an accurately weighed quantity of USP Cloprostenol Sodium RS and USP Hydrocortisone Acetate RS in dehydrated alcohol, and dilute with *Mobile phase* to obtain a solution containing about 0.25 mg of cloprostenol sodium and 0.5 mg of hydrocortisone acetate per mL of solution.
Standard preparation—Dissolve an accurately weighed quantity of USP Cloprostenol Sodium RS in dehydrated alcohol, and dilute quantitatively, and stepwise if necessary, with dehydrated alcohol to obtain a solution having a known concentration of about 0.1 mg per mL.
Assay preparation—Dilute a volume of Injection in dehydrated alcohol and dilute quantitatively, and stepwise if necessary, with dehydrated alcohol to obtain a solution containing 0.1 mg of cloprostenol per mL of solution, based on the label claim.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 5-mm × 25-cm column that contains packing L1. The flow rate is about 1.8 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure*: the resolution, *R,* between the hydrocortisone acetate peak and the cloprostenol peak is not less than 6. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the cloprostenol peak. Calculate the percentage label claim of cloprostenol ($C_{22}H_{29}ClO_6$) in the portion of Injection taken by the formula:

$$100(C_S/C_U)(r_U/r_S)(M_1/M_2)$$

in which C_S is the concentration, in mg per mL, of USP Cloprostenol Sodium RS in the *Standard preparation;* C_U is the concentration, in mg per mL, of cloprostenol in the *Assay preparation;* r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively, M_1 is the molecular weight of cloprostenol (424.92), and M_2 is the molecular weight of cloprostenol sodium (446.90).

Clorazepate Dipotassium



$C_{16}H_{11}ClK_2N_2O_4$ 408.92
1*H*-1,4-Benzodiazepine-3-carboxylic acid, 7-chloro-2,3-dihydro-2-oxo-5-phenyl-, potassium salt compound with potassium hydroxide (1:1).
Potassium 7-chloro-2,3-dihydro-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-carboxylate compound with potassium hydroxide (1:1) [57109-90-7].

» Clorazepate Dipotassium contains not less than 98.5 percent and not more than 101.5 percent of $C_{16}H_{11}ClK_2N_2O_4$, calculated on the dried basis.

Packaging and storage—Preserve under nitrogen in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP 2-Amino-5-chlorobenzophenone RS
$C_{13}H_{10}ClNO$ 231.68
USP Nordazepam RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.
$C_{15}H_{11}ClN_2O$ 270.72
USP Clorazepate Dipotassium RS

www.webofpharma.com

Identification—

Change to read:

A: ▲Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197M▲ (CN 1-May-2020)

Change to read:

B: ▲Spectroscopic Identification Tests ⟨197⟩, Ultraviolet-Visible Spectroscopy: 197U▲ (CN 1-May-2020)—
Solution: 7 µg per mL.
Medium: sodium hydroxide solution (1 in 2500).

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 1 hour: it loses not more than 0.5% of its weight.

Related compounds—
TEST 1—
Phosphate buffer solution—Dissolve about 13.8 g of monobasic sodium phosphate in 500 mL of water, adjust with 2.5 N sodium hydroxide to a pH of 8.0, and mix.
Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and *Phosphate buffer solution* (5:4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Dissolve about 5 mL of 2,6-dimethylaniline in 50 mL of hexane, and carefully add dropwise hydrochloric acid to precipitate the amine hydrochloride. Filter through a sintered-glass funnel, wash the solid precipitate with hexane, and allow the precipitate to dry. Transfer about 50 mg of the dried precipitate of 2,6-dimethylaniline hydrochloride to a 100-mL volumetric flask, add 10.0 mL of *Phosphate buffer solution* and 40 mL of water, and dilute with acetonitrile to volume.
Standard solution—Dissolve an accurately weighed quantity of USP Nordazepam RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 75 µg per mL. Transfer 4.0 mL of this solution to a 50-mL conical flask, add 4.0 mL of 0.7 M potassium carbonate, 2.0 mL of *Internal standard solution,* and 15.0 mL of water. Insert a stopper, and mix.
Test solution—Transfer an accurately weighed quantity of about 50 mg of Clorazepate Dipotassium to a 50-mL conical flask. Add 4.0 mL of 0.7 M potassium carbonate, and start stirring the solution. Add 2 mL of *Internal standard solution* and 19.0 mL of water. Stop stirring about 5 minutes after the addition of the 0.7 M potassium carbonate solution. [NOTE—Prepare fresh immediately before each injection.]
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 232-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention time for 2,6-dimethylaniline is about 0.8 and 1.0 for nordazepam; the relative standard deviation of the peak area ratio of nordazepam to 2,6-dimethylaniline for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of nordazepam in the portion of Clorazepate Dipotassium taken by the formula:

$$2500(C/W)\,(R_i/R_S)$$

in which C is the concentration, in mg per mL, of USP Nordazepam RS in the *Standard solution; W* is the weight, in mg, of Clorazepate Dipotassium taken to prepare the *Test solution; R_i* is the peak area ratio of any impurity to 2,6-dimethylaniline obtained from the *Test solution;* and R_S is the peak area ratio of nordazepam to 2,6-dimethylaniline obtained from the *Standard solution:* not more than 0.5% of nordazepam is found and not more than 0.1% of any individual impurity is found.
TEST 2—
Diluent—Prepare a mixture of 0.001 N sodium hydroxide and acetonitrile (1:1).
Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and a 1 M solution of tetrabutylammonium hydroxide in methanol (110:90:1), adjust with phosphoric acid to a pH of 7.7, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution—Dissolve an accurately weighed quantity of USP 2-Amino-5-chlorobenzophenone RS in *Diluent,* and dilute quantitatively, and stepwise if necessary, with *Diluent,* to obtain a solution having a known concentration of about 0.0026 mg per mL.
Test solution—Transfer about 300 mg of Clorazepate Dipotassium, accurately weighed, to a glass test tube. Add 10.0 mL of *Diluent,* and vigorously mix on a vortex mixer for about 90 seconds. [NOTE—Prepare fresh immediately before each injection.]
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 238-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation of the peak height for replicate injections is not more than 3.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Clorazepate Dipotassium taken by the formula:

$$1000(C/W)(r_i/r_S)$$

in which C is the concentration, in mg per mL, of USP 2-Amino-5-chlorobenzophenone RS in the *Standard solution; W* is the weight, in mg, of sample taken; r_i is the peak height of each impurity obtained from the *Test solution;* and r_S is the peak height of 2-amino-5-chlorobenzophenone obtained from the *Standard solution:* not more than 0.1% of 2-amino-5-chlorobenzophenone is found, not more than 0.1% of any other individual impurity is found, and not more than 1.0% of total impurities in *Test 1* and *Test 2* is found.

Assay—Transfer about 150 mg of Clorazepate Dipotassium, accurately weighed, to a 250-mL beaker, add 100 mL of glacial acetic acid, and stir until dissolved. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass electrode and a calomel electrode containing a 1 in 100 solution of lithium perchlorate in glacial acetic acid. Perform a blank determination (see *Titrimetry* ⟨541⟩), and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 13.63 mg of $C_{16}H_{11}ClK_2N_2O_4$.

Clorazepate Dipotassium Tablets

» Clorazepate Dipotassium Tablets contain not less than 90.0 percent and not more than 110.0 percent of clorazepate dipotassium ($C_{16}H_{11}ClK_2N_2O_4$).

USP Monographs

www.webofpharma.com

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP 2-Amino-5-chlorobenzophenone RS
$C_{13}H_{10}ClNO$ 231.68
USP Nordazepam RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.
$C_{15}H_{11}ClN_2O$ 270.72
USP Clorazepate Dipotassium RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{16}H_{11}ClK_2N_2O_4$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 240 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Clorazepate Dipotassium RS in the same *Medium.*
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{16}H_{11}ClK_2N_2O_4$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.
PROCEDURE FOR CONTENT UNIFORMITY—
Standard solution—Dissolve an accurately weighed quantity of USP Clorazepate Dipotassium RS in 0.01 M sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with 0.01 M sodium hydroxide to obtain a solution having a known concentration of about 7.6 µg per mL.
Test solution—Transfer 1 Tablet to a suitable container, add 200 mL of 0.01 M sodium hydroxide, and homogenize for not less than 3 minutes. Centrifuge a portion of this solution for 15 minutes, and filter the supernatant, discarding the first 20 mL. Dilute an accurately measured portion of the filtrate with 0.01 M sodium hydroxide to obtain a solution having a known concentration of about 7.6 µg per mL.
Procedure—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* in 1-cm cells at the wavelength of maximum absorbance at about 231 nm, with a suitable spectrophotometer, using 0.01 M sodium hydroxide as the blank.

Related compounds—
METHOD I—
Phosphate buffer solution—Dissolve about 13.8 g of monobasic sodium phosphate in 500 mL of water, adjust with 1 N sodium hydroxide to a pH of 8.0, and mix.
Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and *Phosphate buffer solution* (5:4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution—Dissolve an accurately weighed quantity of USP Nordazepam RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 66 µg per mL. Transfer 4.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of 0.7 M potassium carbonate and 3.0 mL of acetonitrile, dilute with water to volume, mix, and filter.
Test solution—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 15 mg of clorazepate dipotassium, to a suitable container. Add 5 mL of acetonitrile, 5 mL of 0.7 M potassium carbonate, and 15 mL of water, stir for 10 minutes, and filter. [NOTE—Prepare fresh before each injection, and use within 3 minutes.]
Chromatographic system—The liquid chromatograph is equipped with a 232-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for not less than twice the retention time of nordazepam, and measure the peak responses. Calculate the quantity, in mg, of each impurity in the portion of Tablets taken by the formula:

$$25C(r_i/r_S)$$

in which C is the concentration, in mg per mL, of USP Nordazepam RS in the *Standard solution;* r_i is the peak response of each impurity obtained from the *Test solution;* and r_S is the peak response for nordazepam obtained from the *Standard solution:* not more than 2.0% of nordazepam is found.

METHOD II—
Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and a 1 M solution of tetrabutylammonium hydroxide in methanol (110:90:1), adjust with phosphoric acid to a pH of 7.7, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution—Dissolve an accurately weighed quantity of USP 2-Amino-5-chlorobenzophenone RS in acetonitrile to obtain a solution having a known concentration of about 0.50 mg per mL. Dilute with water to obtain a solution having a known concentration of about 0.25 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with a mixture of 0.1 mM sodium hydroxide and acetonitrile (7:3) to volume, and mix. Transfer 15 mL of this solution to a 50-mL volumetric flask, dilute with a mixture of 0.1 mM sodium hydroxide and acetonitrile (7:3) to volume, and mix.
Test solution—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 15 mg of clorazepate dipotassium, to a suitable container, add 10 mL of a mixture of 0.1 mM sodium hydroxide and acetonitrile (7:3), mix, shake by mechanical means for 10 minutes, and filter.
Chromatographic system—The liquid chromatograph is equipped with a 238-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of each impurity in the portion of Tablets taken by the formula:

$$10C(r_i/r_S)$$

in which C is the concentration, in mg per mL, of USP 2-Amino-5-chlorobenzophenone RS in the *Standard solution;* r_i is the peak response of each impurity obtained from the *Test solution;* and r_S is the response of the 2-amino-5-chlorobenzophenone peak obtained from the *Standard solution:* the sum of all impurities, other than nordazepam, found in *Method I* and *Method II* is not more than 0.5%.

USP Monographs

www.webofpharma.com

Assay—

Buffer solution—Transfer 5.0 mL of 1 M tetrabutylammonium hydroxide in methanol to a 1-L volumetric flask, dilute with water to volume, adjust with phosphoric acid to a pH of 7.5, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Clorazepate Dipotassium RS in 0.01 M sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with 0.01 M sodium hydroxide to obtain a solution having a known concentration of about 60 µg per mL. Shake by mechanical means for 15 minutes, and filter.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of clorazepate dipotassium, to a suitable container, add 200 mL of 0.01 M sodium hydroxide, and homogenize for not less than 3 minutes. Transfer 15 mL of this solution to a 100-mL volumetric flask, dilute with 0.01 M sodium hydroxide to volume, mix, and filter.

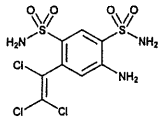
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses of the major peaks. Calculate the quantity, in mg, of clorazepate dipotassium ($C_{16}H_{11}ClK_2N_2O_4$) in the portion of Tablets taken by the formula:

$$1333C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Clorazepate Dipotassium RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Clorsulon



$C_8H_8Cl_3N_3O_4S_2$ 380.66

1,3-Benzenedisulfonamide, 4-amino-6-(trichloroethenyl)-; 4-Amino-6-(trichlorovinyl)-*m*-benzenedisulfonamide [60200-06-8].

DEFINITION

Clorsulon contains NLT 98.0% and NMT 101.0% of clorsulon ($C_8H_8Cl_3N_3O_4S_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

[NOTE—Store the *Standard solution* and the *Sample solution* in low-actinic glassware.]

Mobile phase: Acetonitrile, glacial acetic acid, and water (30: 0.1: 70)

Standard solution: 0.1 mg/mL of USP Clorsulon RS in *Mobile phase*

Sample stock solution: 1 mg/mL of Clorsulon in *Mobile phase*

Sample solution: 0.1 mg/mL of Clorsulon in *Mobile phase,* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 30 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 7400 theoretical plates

Tailing factor: NMT 1.4

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of clorsulon ($C_8H_8Cl_3N_3O_4S_2$) in the portion of Clorsulon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Clorsulon RS in the *Standard solution* (mg/mL)

C_U = concentration of Clorsulon in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

[NOTE—Store the *Standard solutions* and the *Sample solution* in low-actinic glassware.]

Standard solution A: 10 mg/mL of USP Clorsulon RS in methanol

Standard solution B: 0.1 mg/mL of USP Clorsulon RS in methanol, from *Standard solution A*

Sample solution: 10 mg/mL of Clorsulon in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Developing solvent system: Chloroform and methanol (4:1)

Analysis 1

Samples: *Standard solution A, Standard solution B,* and *Sample solution*

Apply 10 µL each of the *Sample solution* and *Standard solution A,* and 5 and 10 µL of *Standard solution B.* Allow the spots to dry. Develop in *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate, mark the solvent front, allow

USP Monographs

www.webofpharma.com

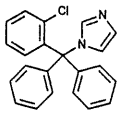
the solvent to evaporate, and examine the plate under short-wavelength UV light.
Acceptance criteria 1: The chromatograms show principal spots at the same R_F value.
Analysis 2: Estimate the amounts of any additional spots observed in the chromatograms of the *Sample solution* in *Analysis 1* by comparing them with the spots in the two chromatograms of *Standard solution B*, corresponding to 0.5% and 1.0% of impurities.
Acceptance criteria 2
Any individual impurities: 0.5%; no spot other than the principal spot of the *Sample solution* is larger or more intense than that of the principal spot of the 5-µL portion of *Standard solution B*.
Total impurities: NMT 2.0%

SPECIFIC TESTS
- **MELTING RANGE** ⟨741⟩: 197°–203°
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample under vacuum at 100° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clorsulon RS

Clotrimazole



$C_{22}H_{17}ClN_2$ 344.84
1*H*-Imidazole, 1-[(2-chlorophenyl)diphenylmethyl]-;
1-(*o*-Chloro-α,α-diphenylbenzyl)imidazole [23593-75-1].

DEFINITION
Clotrimazole contains NLT 98.0% and NMT 102.0% of $C_{22}H_{17}ClN_2$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: 4.35 mg/mL of dibasic potassium phosphate
Mobile phase: Acetonitrile and *Buffer* (3:1). Pass through a membrane filter having a 0.2-µm or finer pore size. The ratio of volumes may be changed to obtain the required resolution.
Standard solution: 0.5 mg/mL of USP Clotrimazole RS in methanol
System suitability solution: 0.1 mg/mL each of USP Clotrimazole RS and USP Clotrimazole Related Compound A RS in methanol
Sample solution: 0.5 mg/mL of Clotrimazole in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 25 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for clotrimazole and clotrimazole related compound A are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between clotrimazole and clotrimazole related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{22}H_{17}ClN_2$ in the portion of Clotrimazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clotrimazole from the *Sample solution*
r_S = peak response of clotrimazole from the *Standard solution*
C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
INORGANIC IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES
- **Procedure 1: Limit of Imidazole**

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Standard solution: 500 µg/mL of USP Imidazole RS in chloroform
Sample solution: 100 mg/mL of Clotrimazole in chloroform
Application volume: 5 µL
Developing solvent system: Methanol and chloroform (3:2)
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed for *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*. After air-drying the plate for 5 min, place it in a closed container with a dish containing 100 g of iodine in a shallow layer, and allow to remain for 60 min. Remove the plate from the container, and observe the chromatogram.
Acceptance criteria: Any brown spot from the *Sample solution* at an R_F value corresponding to the principal spot from the *Standard solution* is not greater in size or intensity than the principal spot from the *Standard solution:* NMT 0.5% of imidazole.

- **Procedure 2: Limit of Clotrimazole Related Compound A**

Buffer, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 50 µg/mL of USP Clotrimazole Related Compound A RS prepared by dissolving in methanol using about 75% of the final flask volume. Dilute with *Buffer* to volume.

USP Monographs

www.webofpharma.com

Sample solution: Transfer 100 mg of Clotrimazole to a 10-mL volumetric flask, add 5 mL of methanol to dissolve, add 2.5 mL of *Buffer*, dilute with methanol to volume, and mix.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of clotrimazole related compound A in the portion of Clotrimazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clotrimazole related compound A from the *Sample solution*

r_S = peak response of clotrimazole related compound A from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩**:** Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** ⟨11⟩

USP Clotrimazole RS

USP Clotrimazole Related Compound A RS

(*o*-Chlorophenyl)diphenylmethanol.

$C_{19}H_{15}ClO$ 294.78

USP Imidazole RS

Clotrimazole Cream

DEFINITION

Clotrimazole Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: 4.35 mg/mL of dibasic potassium phosphate

Mobile phase: Acetonitrile and *Buffer* (3:1)

[Note—The ratio of volumes may be changed to obtain the required resolution.]

Standard solution: 0.5 mg/mL of USP Clotrimazole RS in methanol

System suitability solution: 0.1 mg/mL each of USP Clotrimazole RS and USP Clotrimazole Related Compound A RS in methanol

Sample solution: Transfer the equivalent of 25 mg of clotrimazole from the Cream to a 50-mL screw-capped centrifuge tube. Add 25.0 mL of methanol, and heat at 50° in a water bath for 5 min, with occasional shaking. Remove the tube from the bath, and shake vigorously for 5 min. Cool in a methanol–ice bath for 15 min, and promptly centrifuge. Transfer the supernatant to a 50-mL volumetric flask. Add 20.0 mL of methanol to the residue in the centrifuge tube, and repeat the extraction starting with "heat at 50° in a water bath". Transfer the supernatant to the volumetric flask containing the supernatant from the first extraction, dilute with methanol to volume, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 25 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for clotrimazole and clotrimazole related compound A are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 2.0 between clotrimazole and clotrimazole related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{22}H_{17}ClN_2$ in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clotrimazole from the *Sample solution*

r_S = peak response of clotrimazole from the *Standard solution*

C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in collapsible tubes or tight containers, at a temperature between 2° and 30°.
- **Labeling:** Cream that is packaged and labeled for use as a vaginal preparation shall be labeled Clotrimazole Vaginal Cream.
- **USP Reference Standards** ⟨11⟩

USP Clotrimazole RS

USP Clotrimazole Related Compound A RS

(*o*-Chlorophenyl)diphenylmethanol.

$C_{19}H_{15}ClO$ 294.78

Clotrimazole Lotion

DEFINITION

Clotrimazole Lotion contains NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$).

IDENTIFICATION

- **A.** The retention time of the major peak for clotrimazole of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: 4.35 g/L of dibasic potassium phosphate in water

Mobile phase: Methanol and *Buffer* (3:1). Pass through a filter of 0.5-µm or finer pore size.

Internal standard solution: 0.07 mg/mL of testosterone propionate in dehydrated alcohol

Standard stock solution A: 2 mg/mL of USP Clotrimazole RS in dehydrated alcohol

Standard stock solution B: 0.1 mg/mL of USP Clotrimazole Related Compound A RS in dehydrated alcohol

www.webofpharma.com

Standard solution: *Standard stock solution A*, *Standard stock solution B*, and *Internal standard solution* (5.0: 5.0: 10.0)
Sample solution: Nominally 1 mg/mL, prepared as follows. Transfer the equivalent of 10 mg of clotrimazole from freshly mixed Lotion to a screw-capped, 50-mL centrifuge tube. Add 10.0 mL of *Internal standard solution*, place the cap on the tube, and heat at 50° in a water bath for 5 min, with occasional shaking. Remove the tube from the bath, and shake vigorously for 5 min. Cool in a methanol–ice bath for 15 min, and promptly centrifuge. Transfer the supernatant to a test tube. Add 10.0 mL of dehydrated alcohol to the residue in the centrifuge tube, and repeat the extraction as directed above, beginning with "place the cap on the tube". Transfer the supernatant to the test tube containing the supernatant from the first extraction.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Columns
Guard: 2.1-mm × 6-cm, 10-µm packing L2
Analytical: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for clotrimazole related compound A, clotrimazole, and testosterone propionate are 0.9, 1.0, and 1.5, respectively.]
Suitability requirements
Resolution: NLT 1.2 between clotrimazole related compound A and clotrimazole, and NLT 1.9 between clotrimazole and testosterone propionate
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount clotrimazole ($C_{22}H_{17}ClN_2$) in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of clotrimazole to testosterone propionate from the *Sample solution*
R_S = peak response ratio of clotrimazole to testosterone propionate from the *Standard solution*
C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **ORGANIC IMPURITIES: LIMIT OF CLOTRIMAZOLE RELATED COMPOUND A**
Analysis: Using the chromatograms of the *Standard solution* and *Sample solution* as obtained in the *Assay*, calculate the percentage of clotrimazole related compound A in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of clotrimazole related compound A to testosterone propionate from the *Sample solution*
R_S = peak response ratio of clotrimazole related compound A to testosterone propionate from the *Standard solution*
C_S = concentration of USP Clotrimazole Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole related compound A in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 5%

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
• **pH** ⟨791⟩**:** 5.0–7.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature between 2° and 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clotrimazole RS
USP Clotrimazole Related Compound A RS
(*o*-Chlorophenyl)diphenylmethanol.
$C_{19}H_{15}ClO$ 294.78

Clotrimazole Lozenges

DEFINITION
Clotrimazole Lozenges contain NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$) in a suitable molded base.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 0.3 g/L of anhydrous monobasic sodium phosphate and 0.35 g/L of anhydrous dibasic sodium phosphate in water. The resulting solution has a pH of 6.6–7.0.
Mobile phase: Acetonitrile and *Buffer* (1:1)
Diluent: Acetonitrile and water (1:1)
Standard solution: 0.2 mg/mL of USP Clotrimazole RS in *Diluent*
Sample solution: Nominally 0.2 mg/mL of clotrimazole in *Diluent* prepared as follows. Transfer a portion of powdered Lozenges (from NLT 20 Lozenges) equivalent to 5 mg of clotrimazole to a 25-mL volumetric flask. Dilute with *Diluent* to volume. Sonicate for about 10 min, and centrifuge at 3500 rpm for about 15 min at ambient temperature to obtain a clear supernatant. Use the clear supernatant for injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 206 nm. For *Identification* test *B* use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L85
Flow rate: 1 mL/min
Injection volume: 8 µL
Run time: 1.25 times the retention time of clotrimazole
System suitability
Sample: *Standard solution*

USP Monographs

www.webofpharma.com

Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$) in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clotrimazole from the *Sample solution*
r_S = peak response of clotrimazole from the *Standard solution*
C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 500 mL, deaerated
Apparatus 2: 50 rpm
Time: 45 min
Determine the amount of clotrimazole ($C_{22}H_{17}ClN_2$) dissolved by using the following method.
Buffer A: 4.4 mg/mL of dibasic potassium phosphate in water
Buffer B: 17.4 mg/mL of dibasic potassium phosphate in water
Mobile phase: Methanol and *Buffer A* (4:1)
Diluent: Methanol and *Buffer B* (60:40)
Standard stock solution: 0.02 mg/mL of USP Clotrimazole RS in *Medium*
Standard solution: 4 µg/mL from the *Standard stock solution* in *Diluent*
Sample solution: Withdraw 25 mL of the solution under test from the vessel. Pass through a polyvinylidene difluoride filter of 0.45-µm pore size, and discard the first 10 mL of the filtrate. Transfer 5.0 mL of filtrate to a 25-mL volumetric flask, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 3.9-mm × 7.5-cm; packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim of clotrimazole (mg/Lozenge)
D = dilution factor for the *Sample solution*, 5
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer, Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1 µg/mL each of USP Clotrimazole RS, USP Clotrimazole Related Compound A RS, and USP Imidazole RS in *Diluent*
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between clotrimazole related compound A and imidazole peaks; NLT 4.0 between clotrimazole and clotrimazole related compound A peaks
Relative standard deviation: NMT 2.0% for clotrimazole, clotrimazole related compound A, and imidazole
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified impurity in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding specified impurity from the *Sample solution*
r_S = peak response of the corresponding specified impurity from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of clotrimazole from the *Standard solution*
C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Imidazole	0.5	0.5
Clotrimazole related compound A	0.7	0.5
Clotrimazole	1.0	—
Any unspecified impurity	—	0.2
Total impurities	—	2.0

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clotrimazole RS
  USP Clotrimazole Related Compound A RS
  (*o*-Chlorophenyl)diphenylmethanol.
  $C_{19}H_{15}ClO$ 294.78
  USP Imidazole RS

Clotrimazole Topical Solution

DEFINITION
Clotrimazole Topical Solution is a solution of Clotrimazole in a suitable nonaqueous, hydrophilic solvent. It contains NLT 90.0% and NMT 115.0% of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the clotrimazole peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY
- **PROCEDURE**

Buffer: 0.3 g/L of monobasic sodium phosphate, anhydrous and 0.35 g/L of dibasic sodium phosphate, anhydrous in water. The resulting solution has a pH of 6.6–7.0.
Mobile phase: Acetonitrile and *Buffer* (50:50)
Diluent: Acetonitrile and water (50:50)
Standard solution: 0.2 mg/mL of USP Clotrimazole RS in *Diluent*
Sample solution: Nominally equivalent to 0.2 mg/mL of clotrimazole from Topical Solution in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 206 nm. For *Identification* test *B*, use a diode array detector in the range of 200–300 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L85
Flow rate: 1 mL/min
Injection volume: 8 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clotrimazole from the *Sample solution*
r_S = peak response of clotrimazole from the *Standard solution*
C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

IMPURITIES
- **ORGANIC IMPURITIES**

Buffer, Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.001 mg/mL each of USP Clotrimazole RS, USP Imidazole RS, and USP Clotrimazole Related Compound A RS in *Diluent*
System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 4 between imidazole and clotrimazole related compound A, and between clotrimazole and clotrimazole related compound A
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of the labeled amount of clotrimazole related compound A and imidazole in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clotrimazole related compound A or imidazole from the *Sample solution*
r_S = peak response of clotrimazole related compound A or imidazole from the *Standard solution*
C_S = concentration of USP Clotrimazole Related Compound A RS or USP Imidazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of the clotrimazole from the *Standard solution*
C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Imidazole	0.5	0.5
Clotrimazole related compound A	0.7	0.5
Clotrimazole	1	—
Any unspecified impurity	—	0.2
Total impurities	—	2.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers at a temperature between 2° and 30°.

USP Monographs

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Clotrimazole RS
USP Clotrimazole Related Compound A RS
(*o*-Chlorophenyl)diphenylmethanol.
$C_{19}H_{15}ClO$ 294.78
USP Imidazole RS
$C_3H_4N_2$ 68.08

Clotrimazole Vaginal Inserts

DEFINITION
Clotrimazole Vaginal Inserts contain NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 0.3 g/L of anhydrous monobasic sodium phosphate and 0.35 g/L of anhydrous dibasic sodium phosphate in water. The resulting solution has a pH of 6.6–7.0.
Mobile phase: Acetonitrile and *Buffer* (1:1)
Diluent: Acetonitrile and water (1:1)
Standard solution: 0.2 mg/mL of USP Clotrimazole RS in *Diluent*
Sample solution: Nominally 0.2 mg/mL of clotrimazole in *Diluent* prepared as follows. Transfer a portion of powdered Vaginal Inserts (from NLT 20 Vaginal Inserts) equivalent to 5 mg of clotrimazole to a 25-mL volumetric flask. Dilute with *Diluent* to volume. Sonicate for about 10 min, and centrifuge at 3500 rpm for about 15 min at ambient temperature to obtain a clear supernatant. Use the clear supernatant for injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 206 nm. For *Identification* test *B* use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L85
Flow rate: 1 mL/min
Injection volume: 8 µL
Run time: 1.25 times the retention time of clotrimazole
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$) in the portion of Vaginal Inserts taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clotrimazole from the *Sample solution*
r_S = peak response of clotrimazole from the *Standard solution*
C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISINTEGRATION** ⟨701⟩
Time: 20 min
Acceptance criteria: Meet the requirements
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES
• **ORGANIC IMPURITIES**
Buffer, Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1 µg/mL each of USP Clotrimazole RS, USP Clotrimazole Related Compound A RS, and USP Imidazole RS in *Diluent*
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between clotrimazole related compound A and imidazole peaks; NLT 4.0 between clotrimazole and clotrimazole related compound A peaks
Relative standard deviation: NMT 2.0% for clotrimazole, clotrimazole related compound A, and imidazole
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified impurity in the portion of Vaginal Inserts taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding specified impurity from the *Sample solution*
r_S = peak response of the corresponding specified impurity from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Vaginal Inserts taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of clotrimazole from the *Standard solution*
C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Imidazole	0.5	0.5

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Clotrimazole related compound A	0.7	0.5
Clotrimazole	1.0	—
Any unspecified impurity	—	0.2
Total impurities	—	2.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clotrimazole RS
  USP Clotrimazole Related Compound A RS
  (*o*-Chlorophenyl)diphenylmethanol.
  $C_{19}H_{15}ClO$ 294.78
  USP Imidazole RS

Clotrimazole and Betamethasone Dipropionate Cream

DEFINITION

Clotrimazole and Betamethasone Dipropionate Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$) and an amount of betamethasone dipropionate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$), in a suitable cream base.

IDENTIFICATION

- **A.** The retention times of the major peaks for clotrimazole and betamethasone dipropionate of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay* for clotrimazole and betamethasone.

ASSAY

- **PROCEDURE**

Buffer: 6.6 g/L of dibasic ammonium phosphate in water

Mobile phase: Prepare a mixture of methanol and *Buffer* (7:3), and adjust with phosphoric acid to a pH of 7.0 ± 0.2. Pass through a membrane filter having a 0.45-µm or finer pore size, and degas.

Internal standard solution: 0.15 mg/mL of progesterone in alcohol

Clotrimazole stock solution: 5 mg/mL of USP Clotrimazole RS in alcohol

Betamethasone dipropionate stock solution: 6.4*f* mg/mL of USP Betamethasone Dipropionate RS in alcohol, *f* being the ratio of the labeled amount of betamethasone (in mg/g) to the labeled amount of clotrimazole (in mg/g) in the Cream

Clotrimazole related compound A stock solution: 0.5 mg/mL of USP Clotrimazole Related Compound A RS in methanol

Standard solution: Transfer 1.0 mL of *Clotrimazole related compound A stock solution* to a suitable container, and evaporate to dryness in a water bath at room temperature under a stream of nitrogen. To the residue add 2.0 mL each of *Clotrimazole stock solution*, *Betamethasone dipropionate stock solution*, and *Internal standard solution*.

Sample solution: Weigh a portion of Cream equivalent to 10 mg of clotrimazole, and transfer to a screw-capped, 50-mL centrifuge tube. Add 2.0 mL of *Internal standard solution* and 4.0 mL of alcohol, place the cap on the tube, and heat at 60° in a water bath for 10 min, with occasional shaking. Remove the tube from the bath, cool in an ice bath for 20 min, and promptly centrifuge. Transfer a portion of the supernatant to a test tube, and use as the *Sample solution*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 10-µm packing L1
Flow rate: 1.7 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for betamethasone dipropionate, clotrimazole related compound A, progesterone, and clotrimazole are about 1.0, 1.2, 1.4, and 1.7, respectively.]
Suitability requirements
Resolution: NLT 1.0 between betamethasone dipropionate and clotrimazole related compound A, NLT 1.5 between clotrimazole related compound A and progesterone, and NLT 1.8 between progesterone and clotrimazole
Relative standard deviation: NMT 2.0% determined from clotrimazole and betamethasone dipropionate and NMT 4.0% determined from clotrimazole related compound A

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of clotrimazole to progesterone from the *Sample solution*
R_S = peak response ratio of clotrimazole to progesterone from the *Standard solution*
C_S = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of betamethasone dipropionate to progesterone from the *Sample solution*
R_S = peak response ratio of betamethasone dipropionate to progesterone from the *Standard solution*
C_S = concentration of USP Betamethasone Dipropionate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of betamethasone in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of betamethasone, 392.46
M_{r2} = molecular weight of betamethasone dipropionate, 504.60

Acceptance criteria: 90.0%–110.0% of the labeled amount of clotrimazole ($C_{22}H_{17}ClN_2$); 90.0%–110.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$)

USP Monographs

www.webofpharma.com

IMPURITIES

• **ORGANIC IMPURITIES: LIMIT OF CLOTRIMAZOLE RELATED COMPOUND A**

Buffer, Mobile phase, Internal standard solution, Clotrimazole stock solution, Betamethasone dipropionate stock solution, Clotrimazole related compound A stock solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Use as directed in the *Assay*.

Analysis: Using the chromatograms of the *Standard solution* and *Sample solution* as obtained in the *Assay*, calculate the percentage of clotrimazole related compound A in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of clotrimazole related compound A to progesterone from the *Sample solution*

R_S = peak response ratio of clotrimazole related compound A to progesterone from the *Standard solution*

C_S = concentration of USP Clotrimazole Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 5.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Betamethasone Dipropionate RS

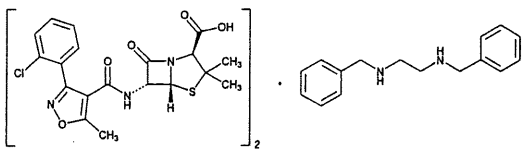
USP Clotrimazole RS

USP Clotrimazole Related Compound A RS

(*o*-Chlorophenyl)diphenylmethanol.

$C_{19}H_{15}ClO$ 294.78

Cloxacillin Benzathine



$(C_{19}H_{18}ClN_3O_5S)_2 \cdot C_{16}H_{20}N_2$ 1112.11

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6- [[[3-(2-chlorophenyl)-5-methyl-4-isoxazolyl] carbonyl]amino]-3,3-dimethyl-7-oxo-, [2*S*-(2α,5α,6β)]-, compd. with *N*,*N*′-bis(phenylmethyl)-1,2-ethanediamine (2:1); (2*S*,5*R*,6*R*)-6-[3-(*o*-Chlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with *N*,*N*′-dibenzylethylenediamine (2:1) [23736-58-5].

DEFINITION

Cloxacillin Benzathine has a potency equivalent to NLT 704 µg and NMT 821 µg of cloxacillin ($C_{19}H_{18}ClN_3O_5S$) per mg, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: To 20 mg of Cloxacillin Benzathine add 5 mL of 5 N sodium hydroxide. Heat the solution on a steam bath for 20 min, and cool. Add 1 mL of this solution to 10 mL of 1.2 N sulfuric acid in a separator, and extract with 50 mL of ether. Wash the ether extract with 30 mL of water, and extract the ether layer with 50 mL of 0.1 N sodium hydroxide.

Standard solution: Use 15 mg of USP Cloxacillin Sodium RS, and follow the same procedures as described in the *Sample solution*.

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Buffer: 0.1 M monobasic sodium phosphate in water, prepared by dissolving 55.2 g of monobasic sodium phosphate in water, and diluting with water to 4 L

Mobile phase: Acetonitrile and *Buffer* (1:3). Adjust with phosphoric acid or 1 N sodium hydroxide to a pH of 4.6 ± 0.2. Pass through a 0.45-µm nylon filter, and degas. [NOTE—The retention time of cloxacillin is very sensitive to the acetonitrile content of the *Mobile phase*.]

Diluent: 0.05 M monobasic sodium phosphate in water. Mix acetonitrile and the resulting solution (2:3). Adjust with phosphoric acid or 1 N sodium hydroxide to a pH of 6.4.

Standard solutions: In duplicate, 112 µg/mL of USP Cloxacillin Sodium RS in *Diluent*

Sample solutions: In duplicate, 128 µg/mL of Cloxacillin Benzathine in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 10-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solutions*

Suitability requirements: Peak areas of the two *Standard solutions* agree within 98%–102%.

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solutions* and *Sample solutions*

Calculate the quantity, in µg, of cloxacillin ($C_{19}H_{18}ClN_3O_5S$) in each mg of Cloxacillin Benzathine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = average peak areas of cloxacillin from the *Sample solutions*

r_S = average peak areas of cloxacillin from the *Standard solutions*

USP Monographs

www.webofpharma.com

C_S = concentration of USP Cloxacillin Sodium RS in the *Standard solutions* (µg/mL)
C_U = concentration of Cloxacillin Benzathine in the *Sample solutions* (µg/mL)
P = assigned potency of USP Cloxacillin Sodium RS (µg of cloxacillin per mg)

Acceptance criteria: 704–821 µg/mg on the anhydrous basis

SPECIFIC TESTS
- **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
- **pH** ⟨791⟩
  Sample solution: 10 mg/mL of suspension
  Acceptance criteria: 3.0–6.5
- **STERILITY TESTS** ⟨71⟩**:** Where the label states that Cloxacillin Benzathine is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*, except use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, and shake the tubes once daily.
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 5.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is for veterinary use only. Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cloxacillin Benzathine RS
  USP Cloxacillin Sodium RS

Cloxacillin Benzathine Intramammary Infusion

DEFINITION
Cloxacillin Benzathine Intramammary Infusion is a suspension of Cloxacillin Benzathine in a suitable oil vehicle. It has a potency equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of cloxacillin ($C_{19}H_{18}ClN_3O_5S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Transfer a quantity of Intramammary Infusion, equivalent to 500 mg of cloxacillin, to a 50-mL centrifuge tube. Add 25 mL of toluene, mix, and centrifuge. Decant and discard the toluene. Wash the residue with four 25-mL portions of toluene, sonicating for 30 s after each addition of toluene. Dry the residue under vacuum over silica gel.
  Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**
  Buffer: 0.1 M monobasic sodium phosphate in water prepared by dissolving 55.2 g of monobasic sodium phosphate in water, and diluting with water to 4 L
  Mobile phase: Acetonitrile and *Buffer* (1:3). Adjust with phosphoric acid or 1 N sodium hydroxide to a pH of 4.6 ± 0.2. Pass through a 0.45-µm nylon filter, and degas. [NOTE—The retention time of cloxacillin is very sensitive to the acetonitrile content of the *Mobile phase*.]
  Diluent: 0.05 M monobasic sodium phosphate in water. Mix acetonitrile and the resulting solution (2:3). Adjust with phosphoric acid or 1 N sodium hydroxide to a pH of 6.4.
  Standard solutions: In duplicate, 112 µg/mL of USP Cloxacillin Sodium RS in *Diluent*
  Sample solutions: Nominally 100 µg/mL of cloxacillin prepared as follows. In duplicate, quantitatively express the entire contents of a syringe of Intramammary Infusion into a 500-mL volumetric flask. Add 300 mL of methanol, and stir for 45 ± 1 min. Dilute with methanol to volume, and stir for an additional 10 ± 1 min. Immediately transfer 45 mL of the resulting solution to a 50-mL polypropylene centrifuge tube, and centrifuge for 10 min. From the supernatant remove an aliquot, and dilute with a sufficient volume of *Diluent* to prepare a solution containing nominally 100 µg/mL of cloxacillin.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 220 nm
  Column: 4.6-mm × 25-cm; 10-µm packing L1
  Column temperature: 40°
  Flow rate: 1.5 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *Standard solutions*
  Suitability requirements: Peak areas of the two *Standard solutions* agree within 98%–102%.
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2%
  Analysis
  Samples: *Standard solutions* and *Sample solutions*
  Calculate the percentage of the labeled amount of cloxacillin ($C_{19}H_{18}ClN_3O_5S$) in each syringe of Intramammary Infusion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = average peak areas of cloxacillin from the *Sample solutions*
r_S = average peak areas of cloxacillin from the *Standard solutions*
C_S = concentration of cloxacillin in the *Standard solutions* (µg/mL)
C_U = nominal concentration of cloxacillin in the *Sample solutions* (µg/mL)

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** Where the label states that it is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*, except use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, and shake the tubes once daily.
- **WATER DETERMINATION,** *Method I* ⟨921⟩
  Analysis: Use 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.
  Acceptance criteria: NMT 1.0%

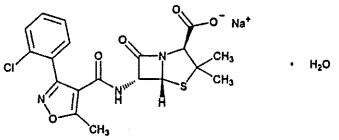
USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in disposable syringes that are well-closed containers, except that where the Intramammary Infusion is labeled as sterile, the individual syringes or cartons are sealed and tamper-proof so that sterility is assured at time of use.
- **LABELING:** Label it to indicate that it is for veterinary use only. Intramammary Infusion that is sterile may be so labeled.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cloxacillin Benzathine RS
  USP Cloxacillin Sodium RS

Cloxacillin Sodium



$C_{19}H_{17}ClN_3NaO_5S \cdot H_2O$ 475.88

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[3-(2-chlorophenyl)-5-methyl-4-isoxazolyl]carbonyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, monohydrate, [2*S*-(2α,5α,6β)]-;
Monosodium (2*S*,5*R*,6*R*)-6-[3-(*o*-chlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate [7081-44-9].

Anhydrous

$C_{19}H_{17}ClN_3NaO_5S$ 457.87
[642-78-4].

DEFINITION

Cloxacillin Sodium contains the equivalent of NLT 825 µg/mg of cloxacillin ($C_{19}H_{18}ClN_3O_5S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Protect solutions containing cloxacillin from light.

Solution A: 1.18 g/L of sodium 1-hexanesulfonate monohydrate and 0.8 mL/L of ammonium hydroxide in water, adjusted with phosphoric acid to a pH of 2.9–3.1

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	45	55
2	45	55
2.5	35	65
5	35	65

Return to the original conditions and re-equilibrate the system.

Diluent: Acetonitrile and water (50:50)

System suitability stock solution: 0.1 mg/mL of USP Cloxacillin Related Compound D RS in *Diluent*. Sonicate as needed to dissolve.

System suitability solution: 0.001 mg/mL of USP Cloxacillin Related Compound D RS from *System suitability stock solution* and 0.1 mg/mL of USP Cloxacillin Sodium RS in *Diluent*. Store this solution at 4°.

Standard solution: 0.1 mg/mL of USP Cloxacillin Sodium RS in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

Sample solution: 0.1 mg/mL of Cloxacillin Sodium in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Column: 40°
Autosampler: 4°
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for cloxacillin and cloxacillin related compound D are about 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between cloxacillin and cloxacillin related compound D, *System suitability solution*
Tailing factor: 0.8–1.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of cloxacillin ($C_{19}H_{18}ClN_3O_5S$) in the portion of Cloxacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cloxacillin Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Cloxacillin Sodium in the *Sample solution* (mg/mL)
P = potency of cloxacillin in USP Cloxacillin Sodium RS (µg/mg)

Acceptance criteria: NLT 825 µg/mg

IMPURITIES

- **ORGANIC IMPURITIES**

Protect solutions containing cloxacillin from light.

Solution A, Solution B, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Mobile phase: See *Table 2*.

www.webofpharma.com

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
30	35	65

Return to the original conditions and re-equilibrate the system.

System suitability stock solution: 0.1 mg/mL of USP Cloxacillin Related Compound D RS in *Diluent*

System suitability solution: 0.01 mg/mL of USP Cloxacillin Related Compound D RS from *System suitability stock solution* and 1 mg/mL of USP Cloxacillin Sodium RS in *Diluent*. Store this solution at 4°.

Standard solution: 0.01 mg/mL of USP Cloxacillin Sodium RS in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

Sample solution: 1 mg/mL of Cloxacillin Sodium in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between cloxacillin related compound D and cloxacillin, *System suitability solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 2.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cloxacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Cloxacillin Sodium RS in the *Standard solution* (mg/mL)

C_U = concentration of Cloxacillin Sodium in the *Sample solution* (mg/mL)

P = potency of cloxacillin in USP Cloxacillin Sodium RS (µg/mg)

F_1 = conversion factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. The reporting threshold is 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound A[a]	0.12	0.24	1.0
Cloxacillin penicilloic acid[b]	0.49	0.65	1.0
Cloxacillin penilloic acid[c, d]	0.70 0.72	1.0	1.0
Cloxacillin related compound D[e]	0.89	1.0	1.0
Cloxacillin	1.0	—	—
Tiocloxacillin[f]	1.18	1.0	1.0
Cloxacillin penicillamide[g]	1.25	1.0	1.0
Cloxacillin penicilloic penicillamide[h]	1.54	1.0	1.0
Any individual unspecified impurity	—	1.0	1.0
Total impurities	—	—	5.0

[a] 6-Aminopenicillanic acid; (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[b] (4*S*)-2-{Carboxy[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[c] (4*S*)-2-{[3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[d] The system resolves two isomers. The limit is for the sum of the isomers.

[e] 3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxylic acid.

[f] (2*R*,5*R*,6*R*)-6-[3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbothioic *S*-acid.

[g] (2*S*,5*R*,6*R*)-6-{(2*S*,5*R*,6*R*)-6-[3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[h] (2*S*,5*R*,6*R*)-6-{(*R*)-2-[(2*R*,4*S*)-4-Carboxy-5,5-dimethylthiazolidin-2-yl]-2-[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido]acetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

• **DIMETHYLANILINE** ⟨223⟩: Meets the requirements

SPECIFIC TESTS

• **CRYSTALLINITY** ⟨695⟩: Meets the requirements

• **pH** ⟨791⟩

Sample solution: 10 mg/mL in water

Acceptance criteria: 4.5–7.5

• **STERILITY TESTS** ⟨71⟩: Meets the requirements where the label states that Cloxacillin Sodium is sterile. If the test for *Direct Inoculation of the Culture Medium* is used, perform the procedure as directed in the chapter with the following exceptions. Use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube. Use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube. Shake the tubes once daily.

• **WATER DETERMINATION,** *Method I* ⟨921⟩: 3.0%–5.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature not exceeding 25°.

• **LABELING:** Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cloxacillin Related Compound D RS

3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxylic acid.

$C_{11}H_8ClNO_3$ 237.64

USP Cloxacillin Sodium RS

www.webofpharma.com

Cloxacillin Sodium Capsules

DEFINITION
Cloxacillin Sodium Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cloxacillin ($C_{19}H_{18}ClN_3O_5S$).

ASSAY
• **PROCEDURE**
Buffer: 0.02 M monobasic potassium phosphate in water, adjusted with 2 N sodium hydroxide to a pH of 6.8
Mobile phase: Acetonitrile and *Buffer* (20:80)
Standard solution: 0.55 mg/mL of USP Cloxacillin Sodium RS in *Buffer*
Sample solution: Nominally 0.5 mg/mL of cloxacillin in *Buffer*, prepared as follows. Mix the contents of NLT 10 Capsules. Transfer a suitable portion of the powder to a volumetric flask, dilute with *Buffer* to volume, and stir for 10 min. Pass a portion of the solution through a suitable filter, discarding the first 5 mL of the filtrate. Use the clear filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cloxacillin ($C_{19}H_{18}ClN_3O_5S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cloxacillin Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cloxacillin in the *Sample solution* (mg/mL)
P = potency of cloxacillin in USP Cloxacillin Sodium RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.05 M pH 6.8 potassium phosphate buffer; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Buffer, Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Standard solution: USP Cloxacillin Sodium RS in *Medium*
Sample solution: Sample per the chapter.
Tolerances: NLT 80% (*Q*) of the labeled amount of cloxacillin ($C_{19}H_{18}ClN_3O_5S$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 5.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cloxacillin Sodium RS

Cloxacillin Sodium Intramammary Infusion

DEFINITION
Cloxacillin Sodium Intramammary Infusion is a suspension of Cloxacillin Sodium in a suitable natural or chemically modified vegetable oil vehicle with a suitable dispersing agent. It has a potency equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of cloxacillin ($C_{19}H_{18}ClN_3O_5S$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Transfer a quantity of Intramammary Infusion, equivalent to 500 mg of cloxacillin, to a 50-mL centrifuge tube. Add 15 mL of isooctane, mix, and centrifuge. Decant and discard the isooctane. Wash the residue with two 15-mL portions of isooctane and two 15-mL portions of ethyl ether, and discard the washings. Dry the residue in a current of air.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Sample solution: Expel the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of *Buffer B.1* and 1.0 mL of polysorbate 80, and blend for 3–5 min. Allow to stand for 10 min, and dilute a measured volume of the aqueous phase with *Buffer B.1* to obtain a test dilution having a concentration assumed to be equal to the median dose level of the Standard.
Analysis: Proceed as directed in the chapter for Cloxacillin.
Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS
• **STERILITY TESTS** ⟨71⟩**:** Where the label states that it is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*, except use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, and shake the tubes once daily.
• **WATER DETERMINATION,** *Method I* ⟨921⟩
Analysis: Use 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in disposable syringes that are well-closed containers, except that where the Intramammary Infusion is labeled as sterile, the individual syringes or cartons are sealed and tamper-proof so that sterility is assured at time of use.

www.webofpharma.com

- **LABELING:** Label it to indicate that it is for veterinary use only. Intramammary Infusion that is sterile may be so labeled.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cloxacillin Sodium RS

Cloxacillin Sodium for Oral Solution

DEFINITION

Cloxacillin Sodium for Oral Solution is a dry mixture of Cloxacillin Sodium and one or more suitable buffers, colors, flavors, and preservatives. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cloxacillin ($C_{19}H_{18}ClN_3O_5S$).

ASSAY

- **PROCEDURE**

Buffer: 0.02 M of monobasic potassium phosphate in water, adjusted with 2 N sodium hydroxide to a pH of 6.8
Mobile phase: Acetonitrile and *Buffer* (20:80)
Standard solution: 0.55 mg/mL of USP Cloxacillin Sodium RS in *Buffer*
Sample solution: Nominally 0.5 mg/mL of cloxacillin in *Buffer*, prepared as follows. Constitute Cloxacillin Sodium for Oral Solution as directed in the labeling. Transfer a suitable portion of the resulting solution to a volumetric flask, dilute with *Buffer* to volume, mix, and stir for 15 min. Pass a portion of the solution through a suitable filter, discarding the first 5 mL of the filtrate. Use the clear filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cloxacillin ($C_{19}H_{18}ClN_3O_5S$) in the portion of Cloxacillin Sodium for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cloxacillin Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cloxacillin in the *Sample solution* (mg/mL)
P = potency of cloxacillin in USP Cloxacillin Sodium RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  For solids packaged in single-unit containers
  Acceptance criteria: Meets the requirements

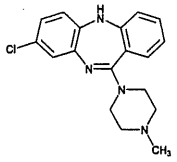
SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample solution: Constitute as directed in the labeling.
  Acceptance criteria: 5.0–7.5
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cloxacillin Sodium RS

Clozapine



$C_{18}H_{19}ClN_4$ 326.82
5*H*-Dibenzo[*b*,*e*][1,4]diazepine, 8-chloro-11-(4-methyl-1-piperazinyl)-;
8-Chloro-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine [5786-21-0].

DEFINITION

Clozapine contains NLT 98.0% and NMT 102.0% of clozapine ($C_{18}H_{19}ClN_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, triethylamine, and water (800: 0.75: 200)
Diluent: Methanol and water (80:20)
Standard solution: 0.1 mg/mL of USP Clozapine RS in *Diluent*
System suitability stock solution: Transfer 10 mg of USP Clozapine RS to a suitable container, add 5 mL of 0.1 N hydrochloric acid, and heat for 2 h at 90°. Transfer this solution to a 100-mL volumetric flask, add 15 mL of water, and dilute with methanol to volume.
System suitability solution: *Standard solution* and *System suitability stock solution* (1:1)
Sample solution: 0.1 mg/mL of Clozapine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection size: 10 µL
Run time: 3 times the retention time of clozapine
System suitability
Samples: *Standard solution* and *System Suitability solution*
Suitability requirements
Resolution: NLT 1.5 between clozapine and any other peak, *System suitability solution*

USP Monographs

www.webofpharma.com

Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clozapine ($C_{18}H_{19}ClN_4$) in the portion of Clozapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of clozapine from the *Sample solution*
r_S = peak response of clozapine from the *Standard solution*
C_S = concentration of USP Clozapine RS in the *Standard solution* (mg/mL)
C_U = concentration of Clozapine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Diluent: Methanol and water (80:20)
Buffer: 2.0 g/L of monobasic potassium phosphate. Adjust with phosphoric acid (85%) to a pH of 2.4. [NOTE—The pH of this solution must not be below 2.4.]
Solution A: Filtered and degassed mixture of acetonitrile, methanol, and *Buffer* (1:1:8)
Solution B: Filtered and degassed mixture of acetonitrile, methanol, and *Buffer* (4:4:2)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
24	0	100
29	0	100
40	100	0

System suitability solution: Dissolve 4 mg of USP Clozapine Resolution Mixture RS in 4 mL of methanol, add 1 mL of water, and dilute with *Diluent* to 10 mL.
Standard solution: 0.75 µg/mL of USP Clozapine RS in *Diluent*
Sample solution: 0.75 mg/mL prepared as follows. Transfer a suitable quantity of Clozapine to a suitable volumetric flask. Dissolve in 80% of the flask volume of methanol, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection size: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times are provided in *Table 2*.]
Suitability requirements
Resolution: NLT 2.5 between *Impurity C* and clozapine, *System suitability solution*
Relative standard deviation: NMT 5.0% for clozapine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Disregard any peak with an area less than 0.5 times the area of the clozapine peak from the *Standard solution*.]
Calculate the percentage of each related compound and any unknown impurity in the portion of Clozapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of clozapine from the *Standard solution*
C_S = concentration of USP Clozapine RS from the *Standard solution* (mg/mL)
C_U = concentration of Clozapine from the *Sample solution* (mg/mL)
F = relative response factor of the impurity (see *Table 2*)

Acceptance criteria: See *Table 2*

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity C[a]	0.9	1.0	0.3
Clozapine	1.0	—	—
Impurity D[b]	1.1	0.35	0.2
Impurity A[c]	1.6	1.2	0.1
Impurity B[d]	1.7	1.0	0.2
Individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.6

[a] 8-Chloro-11-(piperazin-1-yl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine.
[b] 4-Chloro-*N* ^{1}-(2-[(4-methylpiperazin-1-yl)carbonyl]phenyl)benzene-1,2-diamine.
[c] 8-Chloro-5,10-dihydro-11*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one.
[d] 11,11′-(Piperazine-1,4-diyl)bis(8-chloro-5*H*-dibenzo[*b*,*e*][1,4]diazepine).

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Clozapine RS
USP Clozapine Resolution Mixture RS
Contains the following components:
Clozapine.
Impurity A: 8-chloro-5,10-dihydro-11*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one.
Impurity B: 11,11′-(piperazine-1,4-diyl)bis(8-chloro-5*H*-dibenzo[*b*,*e*][1,4]dizepine).
Impurity C: 8-chloro-11-(piperazin-1-yl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine.
Impurity D: 4-chloro-*N*^{1}-(2-[(4-methylpiperazin-1-yl)carbonyl]phenyl)benzene-1,2-diamine.

USP Monographs

www.webofpharma.com

Clozapine Tablets

DEFINITION
Clozapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clozapine ($C_{18}H_{19}ClN_4$).

IDENTIFICATION
- **A.** The R_F value of the principal spot of the *Sample solution* corresponds to those of the principal spots of the *Standard solutions*, as obtained in the test for *Organic Impurities*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Methanol, triethylamine, and water (800: 0.75: 200)

Standard solution: 0.125 mg/mL of USP Clozapine RS prepared as follows. Transfer the required amount of USP Clozapine RS to a suitable volumetric flask. Dissolve in 80% of the flask volume of methanol. [NOTE—Dissolve the Reference Standard in methanol, and dilute with water to obtain the final concentration. The final solvent composition of methanol and water is about 8:2.]

System suitability stock solution: Transfer 10 mg of clozapine to a suitable container, add 5 mL of 0.1 N hydrochloric acid, and heat for 2 h at 90°. Transfer this solution to a 100-mL volumetric flask, add 15 mL of water, and dilute with methanol to volume.

System suitability solution: *Standard solution* and *System suitability stock solution* (1:1)

Sample solution: Transfer 125 mg of clozapine from a quantity of finely powdered Tablets (NLT 20) to a 1-L volumetric flask. Dissolve in 640 mL of methanol, sonicate for 10 min, dilute with water to volume, mix, and filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm
Column: 4.0-mm × 25-cm; packing L7
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between the clozapine peak and any other peak, *System suitability solution*
Column efficiency: NLT 1500 theoretical plates, *Standard solution*
Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clozapine ($C_{18}H_{19}ClN_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Clozapine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clozapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: pH 4.0 acetate buffer prepared as follows. Dissolve 2 g of sodium hydroxide in 450 mL of water. Adjust with glacial acetic acid to a pH of 4.0. Dilute to 1 L; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: USP Clozapine RS in *Medium* in a concentration similar to the one expected in the *Sample solution*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: 290 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of clozapine ($C_{18}H_{19}ClN_4$) dissolved.
Tolerances: NLT 85% (*Q*) of the labeled amount of clozapine ($C_{18}H_{19}ClN_4$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES
- **ORGANIC IMPURITIES**

Diluent: Chloroform and methanol (4:1)
Standard stock solution: 5.0 mg/mL of USP Clozapine RS in *Diluent*
Standard solutions: Dilute portions of the *Standard stock solution* with *Diluent* to obtain the following solutions.

Standard solution	Dilution	Concentration (µg/mL of RS)	Percentage (for comparison with Sample)
A	1 in 200	25	0.5
B	1 in 250	20	0.4
C	1 in 333	15	0.3
D	1 in 500	10	0.2
E	1 in 1000	5	0.1

Sample solution: Transfer an equivalent to 125 mg of clozapine from a portion of finely powdered Tablets (NLT 20) to a 25-mL volumetric flask. Dissolve in 20 mL of *Diluent*, shake by mechanical means for 15 min, dilute with *Diluent* to volume, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: *n*-Heptane, chloroform, dehydrated alcohol, and ammonium hydroxide (30:30:30:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots of the *Sample solution* with those of the principal spots of the *Standard solutions*.
Acceptance criteria: No secondary spot of the *Sample solution* is larger or more intense than the principal spot of *Standard solution A* (NMT 0.5%), and the sum of the intensities of the secondary spots from the *Sample solution* corresponds to NMT 2.0%.

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Clozapine RS

Coal Tar

DEFINITION
Coal Tar is the tar obtained as a by-product during the destructive distillation of bituminous coal at temperatures in the range of 900°–1100°. It may be processed further either by extraction with alcohol and suitable dispersing agents and maceration times or by fractional distillation with or without the use of suitable organic solvents.

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩
  Sample: 100 mg
  Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.

Coal Tar Ointment

» Prepare Coal Tar Ointment as follows.

Coal Tar	10 g
Polysorbate 80	5 g
Zinc Oxide Paste	985 g
to make	1000 g

Blend the Coal Tar with the Polysorbate 80, and incorporate the mixture with the Zinc Oxide Paste.

Packaging and storage—Preserve in tight containers.

Coal Tar Topical Solution

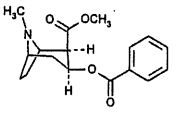
» Prepare Coal Tar Topical Solution as follows.

Coal Tar	200 g
Polysorbate 80	50 g
Alcohol, a sufficient quantity, to make	1000 mL

Mix the Coal Tar with 500 g of washed sand (see under *Reagents* in the section *Reagents, Indicators, and Solutions*), and add the Polysorbate 80 and 700 mL of Alcohol. Macerate the mixture for 7 days in a closed vessel with frequent agitation. Filter, and rinse the vessel and the filter with sufficient Alcohol to make the product measure 1000 mL.

Packaging and storage—Preserve in tight containers.
Alcohol Determination ⟨611⟩: between 81.0% and 86.0% of C_2H_5OH.

Cocaine



$C_{17}H_{21}NO_4$ 303.35
8-Azabicyclo[3.2.1]octane-2-carboxylic acid, 3-(benzoyloxy)-8-methyl-, methyl ester, [1*R*-(*exo*,*exo*)]-.
Methyl 3β-hydroxy-1αH,5αH-tropane-2β-carboxylate benzoate (ester) [50-36-2].

» Cocaine, dried over phosphorus pentoxide for 3 hours, contains not less than 99.0 percent and not more than 101.0 percent of $C_{17}H_{21}NO_4$.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Cocaine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 15 µg per mL.
Medium: dilute hydrochloric acid (1 in 120).
Absorptivities at 233 nm, calculated on the dried basis, do not differ by more than 3.0%.

B: It meets the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩, USP Cocaine Hydrochloride RS being used, and sodium carbonate TS being used in place of sodium hydroxide TS.

C: Dissolve about 100 mg in a mixture of 0.4 mL of dilute hydrochloric acid (1 in 12) and water to make 5 mL, and add 5 drops of chromium trioxide solution (1 in 20): a yellow precipitate is formed, and it quickly redissolves when the mixture is shaken. Add 1 mL of hydrochloric acid: a permanent, orange-colored, crystalline precipitate is formed.

D: Dissolve about 10 mg in 1 mL of dilute hydrochloric acid (1 in 600), and evaporate on a steam bath just to dryness. Dissolve the residue in 2 drops of water, and add 1 mL of potassium permanganate solution (1 in 300): a violet, crystalline precipitate is formed, and it appears brownish violet when collected on a filter, and shows characteristic violet-red crystalline aggregates under the low power of a microscope, similar to those obtained from USP Cocaine Hydrochloride RS.
Melting range, *Class I* ⟨741⟩: between 96° and 98°.
Loss on drying ⟨731⟩—Dry it over phosphorus pentoxide for 3 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

www.webofpharma.com

Readily carbonizable substances ⟨271⟩—Dissolve about 500 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid A*.
Limit of cinnamyl-cocaine and other reducing substances—Dissolve about 300 mg of finely powdered Cocaine in 1 mL of dilute hydrochloric acid (1 in 12) with the aid of heat, if necessary, and dilute with water to 15 mL. Mix 5 mL of this solution with 0.3 mL of dilute sulfuric acid (1 in 35) and 0.1 mL of potassium permanganate solution (1 in 300): the violet color does not disappear entirely within 30 minutes.
Limit of isoatropyl-cocaine—Dilute in a beaker 5 mL of the solution of Cocaine prepared in the test for *Cinnamyl-cocaine and other reducing substances* with 80 mL of water, add 0.2 mL of 6 N ammonium hydroxide, and stir the solution vigorously for 5 minutes, occasionally rubbing the inner wall of the beaker with a stirring rod: a crystalline precipitate of cocaine is formed, and the supernatant is clear.
Assay—Dissolve about 600 mg of Cocaine, previously dried and accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 30.34 mg of $C_{17}H_{21}NO_4$.

Cocaine Hydrochloride

$C_{17}H_{21}NO_4 \cdot HCl$ 339.81
8-Azabicyclo[3.2.1]octane-2-carboxylic acid, 3-(benzoyloxy)-8-methyl-, methyl ester, hydrochloride, 1*R*-(*exo*,*exo*)-.
Methyl 3β-hydroxy-1αH,5αH-tropan-2β-carboxylate, benzoate (ester) hydrochloride [53-21-4].

» Cocaine Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of $C_{17}H_{21}NO_4 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Cocaine Hydrochloride RS

Identification—
A: It meets the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩, sodium carbonate TS being used in place of 1 N sodium hydroxide.
B: To 5 mL of a solution (1 in 50) add 5 drops of chromium trioxide solution (1 in 20): a yellow precipitate is formed, and it quickly redissolves when the mixture is shaken gently. Add 1 mL of hydrochloric acid: a permanent, orange-colored crystalline precipitate is formed.
C: To a solution of about 10 mg in 2 drops of water add 1 mL of 0.1 N potassium permanganate: a violet, crystalline precipitate is formed, and it appears brownish violet when collected on a filter, and shows characteristic, violet-red crystalline aggregates under the low power of a microscope.
D: It responds to the tests for *Chloride* ⟨191⟩.
Specific rotation ⟨781S⟩: between −71° and −73°.
Test solution: 20 mg, previously dried, per mL, in water.
Acidity—Dissolve 500 mg in 10 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 0.50 mL is required to produce a yellow color.
Loss on drying ⟨731⟩—Dry it over silica gel for 3 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Readily carbonizable substances ⟨271⟩—Dissolve 500 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid F*.
Limit of cinnamyl-cocaine and other reducing substances—To 5 mL of a solution (1 in 50) add 0.3 mL of 1 N sulfuric acid and 0.10 mL of 0.10 N potassium permanganate: the violet color does not disappear entirely within 30 minutes.
Limit of isoatropyl-cocaine—Dilute 5 mL of a solution (1 in 50) in a beaker with 80 mL of water, add 0.2 mL of 6 N ammonium hydroxide, stir the solution vigorously during 5 minutes, occasionally rubbing the inner wall of the beaker with a stirring rod: a crystalline precipitate of cocaine is formed, and the supernatant is clear.
Assay—Dissolve about 500 mg of Cocaine Hydrochloride, accurately weighed, in a mixture of 40 mL of glacial acetic acid and 10 mL of mercuric acetate TS. Add 2 drops of quinaldine red TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 33.98 mg of $C_{17}H_{21}NO_4 \cdot HCl$.

Cocaine Hydrochloride Tablets for Topical Solution

» Cocaine Hydrochloride Tablets for Topical Solution contain not less than 91.0 percent and not more than 109.0 percent of the labeled amount of $C_{17}H_{21}NO_4 \cdot HCl$.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Cocaine Hydrochloride RS

Identification—
A: Add 5 drops of chromium trioxide solution (1 in 20) to 5 mL of a filtered solution of Tablets, equivalent to cocaine hydrochloride solution (1 in 50): a yellow precipitate is formed and it redissolves when the mixture is shaken. On the addition of 1 mL of hydrochloric acid, a permanent, yellowish orange, crystalline precipitate is formed.
B: Dissolve a portion of powdered Tablets, equivalent to about 10 mg of cocaine hydrochloride, in 1 mL of water, filter, and add 2 mL of 0.1 N potassium permanganate: a red-purple, crystalline precipitate, which appears brown when collected on a filter, is formed, and it shows characteristic, crystalline aggregates under the low power of a microscope.
C: Add silver nitrate TS, dropwise, to a filtered solution of Tablets, equivalent to cocaine hydrochloride solution (1 in 20): a white precipitate is formed, and it is insoluble in nitric acid.
Disintegration ⟨701⟩: 15 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Place 1 Tablet in a 100-mL volumetric flask, add 50 mL of water, and shake the flask until the tablet is dissolved. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate, if necessary, with water to provide a solution containing approximately 80 µg of cocaine hydrochloride per mL. Concomitantly determine the absorbances of this test solution and a Standard solution of USP Cocaine Hydrochloride RS in the same medium having a known concentration of about 80 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 275 nm, with a suitable spectrophotometer, using water as the blank.

USP Monographs

www.webofpharma.com

Calculate the quantity, in mg, of $C_{17}H_{21}NO_4 \cdot HCl$ in the Tablet by the formula:

$$(T/D)C(A_U/A_S)$$

in which T is the labeled quantity, in mg, of cocaine hydrochloride in the Tablet; D is the concentration, in µg per mL, of cocaine hydrochloride in the test solution, based upon the labeled quantity per Tablet and the extent of dilution; C is the concentration, in µg per mL, of USP Cocaine Hydrochloride RS in the Standard solution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—Weigh and finely powder not fewer than 20 Tablets. Dissolve an accurately weighed portion of the powder, equivalent to about 60 mg of cocaine hydrochloride, in 10 mL of water, render the solution slightly alkaline with 6 N ammonium hydroxide, and completely extract the cocaine with small successive portions of ether. Evaporate the combined ether extracts on a steam bath to one-half their volume, transfer the remaining liquid to a separator, and wash with three 5-mL portions of water. Shake the water washings with a small portion of ether, and add the ether washing to the combined ether extracts. Add 10.0 mL of 0.05 N sulfuric acid VS to the ether solution, agitate the mixture thoroughly, and draw off the acidified water layer into a beaker. Wash the ether with two small portions of water, add the washings to the acid liquid, and titrate the excess acid with 0.02 N sodium hydroxide VS, using methyl red TS as the indicator. Each mL of 0.05 N sulfuric acid is equivalent to 16.99 mg of $C_{17}H_{21}NO_4 \cdot HCl$.

Cod Liver Oil

Change to read:

DEFINITION

Cod Liver Oil is the partially destearinated fixed oil obtained from fresh livers of *Gadus morrhua* L. and other species of Fam. Gadidae. Cod Liver Oil contains, in each gram, NLT 180 µg ▲▲ 2S (USP41) and NMT 750 µg ▲▲ 2S (USP41) of vitamin A and NLT 1.5 µg ▲▲ 2S (USP41) and NMT 6.25 µg ▲▲ 2S (USP41) of vitamin D.

Cod Liver Oil may be flavored by the addition of NMT 1% of a suitable flavor or a mixture of flavors. A suitable antioxidant may be added.

IDENTIFICATION

• **A. Presence of Vitamin A**

Sample solution: 25 mg/mL of Cod Liver Oil in chloroform

Analysis: To 1 mL of the *Sample solution* add 10 mL of antimony trichloride TS.

Acceptance criteria: A blue color results immediately.

Change to read:

• **B. Fatty Acid Profile**

Antioxidant solution: 0.05 mg/mL of butylated hydroxytoluene in hexanes

System suitability solution: Prepare a mixture containing equal amounts of methyl palmitate, methyl stearate, methyl arachidate, and methyl behenate in *Antioxidant solution*.

Standard stock solution: 45 mg/mL of USP Cod Liver Oil RS in *Antioxidant solution*

Standard solution: Transfer 2.0 mL of the *Standard stock solution* into a ▲glass[1]▲ 2S (USP41) tube, and evaporate with a gentle stream of nitrogen. Add 1.5 mL of a 2% solution of sodium hydroxide in methanol, cap tightly with a polytetrafluoroethylene-lined cap, mix, and heat in a water bath for 7 min. Cool, add 2 mL of a 120-mg/mL solution of boron trichloride in methanol, cover with nitrogen, cap tightly, mix, and heat in a water bath for 30 min. Cool to 40°–50°, add 1 mL of isooctane, cap, and mix in a vortex mixer or shake vigorously for at least 30 s. Immediately add 5 mL of saturated sodium chloride solution, cover with nitrogen, cap, and mix in a vortex mixer or shake thoroughly for at least 15 s. Allow the upper layer to become clear, and transfer to a separate tube. Shake the methanol layer once more with 1 mL of isooctane, and combine the isooctane extracts. Wash the combined extracts twice with 1 mL of water, and dry over anhydrous sodium sulfate.

Sample solution: Prepare as directed for the *Standard solution*, except replace USP Cod Liver Oil RS with Cod Liver Oil.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m fused silica capillary; bonded with a 0.25-µm film of phase G16

Temperatures

Injector: 250°

Detector: 280°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	1	225	20

Carrier gas: Helium

Injection volume: 1 µL

Injection type: Split ratio, 200:1

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Chromatogram similarity: The chromatogram from the *Standard solution* is similar to the reference chromatogram supplied with USP Cod Liver Oil RS. Identify the retention times of the relevant fatty acid methyl esters by comparing the chromatogram of the *Standard solution* with the reference chromatogram supplied with USP Cod Liver Oil RS.

Resolution: NLT 1.3 between methyl oleate and methyl *cis*-vaccinate, and that between methyl gadoleate and methyl gondoate is sufficient for purposes of identification and area measurement, *Standard solution*

Theoretical area percentages: 24.4 ± 1 for methyl palmitate, 24.8 ± 1 for methyl stearate, 25.2 ± 1 for methyl arachidate, and 25.6 ± 1 for methyl behenate, *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the retention times of the relevant fatty acid methyl esters in the *Sample solution* by comparing the chromatogram of the *Sample solution* with that of the *Standard solution*.

[1] Suitable glass tube may be obtained from VWR cat. no. 391-0171.

www.webofpharma.com

Determine the number of fatty acid methyl esters in the *Sample solution*. The number of fatty acid methyl ester peaks exceeding 0.05% of the total area of fatty acid methyl esters is at least 24, and the 24 largest peaks of the methyl esters account for more than 90% of the total area. (These correspond to the following, in common elution order: 14:0, 15:0, 16:0, 16:1 *n*–7, 16:4 *n*–1, 18:0, 18:1 *n*–9, 18:1 *n*–7, 18:2 *n*–6, 18:3 *n*–3, 18:4 *n*–3, 20:1 *n*–11, 20:1 *n*–9, 20:1 *n*–7, 20:2 *n*–6, 20:4 *n*–6, 20:3 *n*–3, 20:4 *n*–3, 20:5 *n*–3, 22:1 *n*–11, 22:1 *n*–9, 21:5 *n*–3, 22:5 *n*–3, and 22:6 *n*–3.)

Calculate the area percentage for each fatty acid methyl ester in the portion of Cod Liver Oil taken:

$$\text{Result} = (r_A/r_B) \times 100$$

r_A = peak area of each individual fatty acid methyl ester in the *Sample solution*

r_B = total area from all peaks, except the solvent peak and butylated hydroxytoluene, in the *Sample solution*

Acceptance criteria: The *Sample solution* meets the limits described in *Table 2*.

Table 2

Fatty Acid	Shorthand Notation	Lower Limit (Area %)	Upper Limit (Area %)
Saturated fatty acids			
Myristic acid	14:0	2.0	6.0
Palmitic acid	16:0	7.0	14.0
Stearic acid	18:0	1.0	4.0
Monounsaturated fatty acids			
Palmitoleic acid	16:1 *n*–7	4.5	11.5
cis-Vaccenic acid	18:1 *n*–7	2.0	7.0
Oleic acid	18:1 *n*–9	12.0	21.0
Gadoleic acid	20:1 *n*–11	1.0	5.5
Gondoic acid	20:1 *n*–9	5.0	17.0
Erucic acid	22:1 *n*–9	0	1.5
Cetoleic acid	22:1 *n*–11	5.0	12.0
Polyunsaturated fatty acids			
Linoleic acid	18:2 *n*–6	0.5	3.0
α-Linolenic acid	18:3 *n*–3	0	2.0
Moroctic acid	18:4 *n*–3	0.5	4.5
Eicosapentaenoic acid	20:5 *n*–3	7.0	16.0
Docosahexaenoic acid	22:6 *n*–3	6.0	18.0

ASSAY

Change to read:

- **VITAMIN A**

Sample: 500 mg to 1 g of Cod Liver Oil

Analysis: Proceed as directed in *Vitamin A Assay* ⟨571⟩, ▲*Assay, Chemical Methods, Procedure 1.*▲ 2S (USP41)

Acceptance criteria: 180 ▲▲ 2S (USP41) to 750 µg ▲▲ 2S (USP41) of vitamin A per gram of Cod Liver Oil

Change to read:

- **VITAMIN D:** ▲Proceed as directed in *Vitamin D Assay* ⟨581⟩, *Assay, Chromatographic Methods, Procedure 8.*▲ 2S (USP41)

Acceptance criteria: 1.5 ▲▲ 2S (USP41) to 6.25 µg ▲▲ 2S (USP41) of vitamin D per gram of Cod Liver Oil

SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩: 0.918–0.927
- **COLOR:** When viewed transversely in a tall, cylindrical, standard oil-specimen bottle of colorless glass of about 120-mL capacity, the color of the Cod Liver Oil is not more intense than that of a mixture of cobaltous chloride CS, ferric chloride CS, and water (11:76:33), in a similar bottle of the same internal diameter.
- **NONDESTEARINATED COD LIVER OIL**

Sample: Cod Liver Oil

Analysis: Fill a tall, cylindrical, standard oil-specimen bottle of 120-mL capacity with the *Sample* at a temperature between 23° and 28°, insert the stopper, and immerse the bottle in a mixture of ice and water for 3 h.

Acceptance criteria: The oil remains clear and does not deposit stearin.

- **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Unsaponifiable Matter*: NMT 1.30%
- **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Acid Value*

Sample solution: Mix 15 mL of alcohol with 15 mL of ether, add 5 drops of phenolphthalein TS, and neutralize with 0.1 N sodium hydroxide. Dissolve 2.0 g of Cod Liver Oil in the mixture, and boil the oil solution gently under a reflux condenser for 10 min.

Analysis: Cool, and titrate the mixture with 0.1 N sodium hydroxide VS to the production of a pink color that persists after shaking for 30 s.

Acceptance criteria: NMT 1.0 mL of 0.1 N sodium hydroxide is required.

- **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Iodine Value*: 145–180
- **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Saponification Value*: 180–192

[NOTE—If carbon dioxide has been used as a preservative, expose the Cod Liver Oil in a shallow dish in a vacuum desiccator for 24 h before weighing the specimen for determination of the saponification value.]

- **FATS AND FIXED OILS** ⟨401⟩, *Procedures, Anisidine Value*: NMT 30

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. It may be bottled or otherwise packaged in containers from which air has been expelled by the production of a vacuum or by an inert gas.
- **LABELING:** The vitamin A potency and vitamin D potency, when designated on the label, are expressed in USP Units/g of oil. The potencies may be expressed also in metric units, on the basis that 1 USP Vitamin A Unit equals 0.3 µg and 40 USP Vitamin D Units equals 1 µg. Where the content of docosahexaenoic acid or eicosapentaenoic acid is claimed, state the concentration in mg/g.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

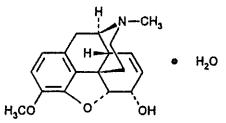
▲▲ (CN 1-May-2018)

USP Cod Liver Oil RS

▲▲ (CN 1-May-2018)

www.webofpharma.com

Codeine



$C_{18}H_{21}NO_3 \cdot H_2O$ 317.38
Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-, monohydrate, (5α,6α)-.
7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol monohydrate [6059-47-8].
Anhydrous 299.37
[76-57-3].

» Codeine, dried at 80° for 4 hours, contains not less than 98.5 percent and not more than 100.5 percent of $C_{18}H_{21}NO_3$.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Codeine Sulfate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—Proceed as directed with the codeine test specimen and the codeine standard specimen obtained from 50 mg of USP Codeine Sulfate RS dissolved in 15 mL of water, then rendered alkaline with 6 N ammonium hydroxide and extracted with several 10-mL portions of chloroform, followed by evaporation of the combined chloroform extracts on a steam bath to dryness, and drying at 80° for 4 hours.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 100 µg per mL.
Medium: 0.1 N sulfuric acid.
Absorptivity at 284 nm, calculated on the dried basis, is between 112.9% and 119.9% of that of USP Codeine Sulfate RS.
Melting range ⟨741⟩—When previously dried, it melts between 154° and 158°, but the range between beginning and end of melting does not exceed 2°.
Loss on drying ⟨731⟩—Dry it at 80° for 4 hours: it loses not more than 6.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Readily carbonizable substances ⟨271⟩—Dissolve 10 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid S*.
Chromatographic purity—Prepare a solution of it in dehydrated alcohol containing 40 mg per mL (*Solution A*). Dilute 2.0 mL of *Solution A* with dehydrated alcohol to 100 mL (*Solution B*). Dilute 1.0 mL of *Solution A* with dehydrated alcohol to 100 mL (*Solution C*). Apply separate 10-µL volumes of *Solution A*, *Solution B*, and *Solution C* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of dehydrated alcohol, cyclohexane, and ammonium hydroxide (72:30:6) until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, and allow the solvent to evaporate. Spray the plate with a reagent prepared by mixing 3 mL of chloroplatinic acid solution (1 in 10) with 97 mL of water, followed by the addition of 100 mL of potassium iodide solution (6 in 100), and examine the chromatogram: no spot obtained from *Solution A*, other than the principal spot and any spot observed at the origin, is more intense than the principal spot obtained from *Solution B* (2%); and not more than one such spot having an R_F greater than that of the principal spot is more intense than the principal spot obtained from *Solution C* (1%).
Limit of morphine—Dissolve about 50 mg of potassium ferricyanide in 10 mL of water, and add 1 drop of ferric chloride TS and 1 mL of a neutral or slightly acid solution of Codeine (1 in 100) prepared with the aid of sulfuric acid: no blue color is produced immediately.
Assay—Dissolve about 400 mg of Codeine, previously dried and accurately weighed, by warming it in 30.0 mL of 0.1 N sulfuric acid VS. Cool, and add 10 mL of water. Add methyl red TS, and titrate the excess acid with 0.1 N sodium hydroxide VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 0.1 N sulfuric acid is equivalent to 29.94 mg of $C_{18}H_{21}NO_3$.

Codeine Phosphate

$C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$ 406.37
Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-, (5α,6α)-, phosphate (1:1) (salt), hemihydrate.
7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol phosphate (1:1) (salt) hemihydrate [41444-62-6].
Anhydrous 397.37
[52-28-8].

» Codeine Phosphate contains not less than 99.0 percent and not more than 101.5 percent of $C_{18}H_{21}NO_3 \cdot H_3PO_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store up to 40° as permitted by the manufacturer.
USP Reference standards ⟨11⟩—
USP Codeine Phosphate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: Neutralize a solution (1 in 50) with 6 N ammonium hydroxide, and add silver nitrate TS: a yellow precipitate of silver phosphate is formed, and it is soluble in 2 N nitric acid and in 6 N ammonium hydroxide.
Acidity—Dissolve 100 mg in 20 mL of water, and titrate with 0.010 N sodium hydroxide to a pH of 5.4, using a pH meter: not more than 1.0 mL of 0.010 N sodium hydroxide is required.
Water Determination, *Method I* ⟨921⟩: not more than 3.0%.
Chloride—To 10 mL of a solution (1 in 100), acidified with nitric acid, add a few drops of silver nitrate TS: no opalescence is produced immediately.
Sulfate—To 10 mL of a solution (1 in 100) add a few drops of barium chloride TS: no turbidity is produced immediately.

www.webofpharma.com

Limit of morphine—Dissolve about 50 mg of potassium ferricyanide in 10 mL of water, and add 1 drop of ferric chloride TS and 1 mL of a solution of Codeine Phosphate (1 in 100): no blue color is produced immediately.
Chromatographic purity—Using Codeine Phosphate, proceed as directed in the test for *Chromatographic purity* under *Codeine* except to use a mixture of 0.01 N hydrochloric acid and dehydrated alcohol (4:1), instead of dehydrated alcohol, to prepare *Solution A*, *Solution B*, and *Solution C*.
Assay—Dissolve about 1 g of Codeine Phosphate, accurately weighed, in 50 mL of glacial acetic acid, warming slightly if necessary to effect solution, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 39.74 mg of $C_{18}H_{21}NO_3 \cdot H_3PO_4$.

Codeine Phosphate Injection

» Codeine Phosphate Injection is a sterile solution of Codeine Phosphate in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$.
[NOTE—Do not use the Injection if it is more than slightly discolored or contains a precipitate.]

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Codeine Phosphate RS

Identification—
A: Dilute a volume of Injection, equivalent to about 90 mg of codeine phosphate, with water to about 10 mL, add 1 drop of hydrochloric acid, and extract with three 10-mL portions of chloroform, discarding the chloroform extracts. Add 6 N ammonium hydroxide until the solution is alkaline, and extract with several 10-mL portions of chloroform. Evaporate the combined chloroform extracts on a steam bath to dryness, and dry at 80° for 4 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima at the same wavelengths as that of the codeine obtained by similarly treating 1 mL of a solution of USP Codeine Phosphate RS (1 in 100).
B: A volume of Injection, equivalent to about 60 mg of codeine phosphate, responds to *B:* test *B:* under *Codeine Phosphate*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 5.8 USP Endotoxin Units per mg of codeine phosphate.
pH ⟨791⟩: between 3.0 and 6.0.
Limit of morphine—Diluted with water to a concentration of 5 mg of codeine phosphate per mL, it meets the requirements of the test for *Limit of morphine*— under *Codeine Phosphate*.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer an accurately measured volume of Injection, equivalent to about 75 mg of codeine phosphate, to a small separator, and add about 15 mL of water. Add 2 drops of phosphoric acid, and extract with four 10-mL portions of chloroform, collecting the chloroform extracts in a separator. Wash the combined chloroform extracts with 10 mL of water, and add the water wash to the first separator containing the sample. Discard the chloroform extracts. Proceed as directed in the *Assay*— under *Codeine Phosphate Tablets*, beginning with "render the solution alkaline with 6 N ammonium hydroxide." Each mL of 0.02 N sulfuric acid is equivalent to 8.128 mg of $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$.

Codeine Phosphate Compounded Oral Solution

DEFINITION
Codeine Phosphate Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$).
Prepare Codeine Phosphate Compounded Oral Solution 3 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Codeine Phosphate	300 mg
Purified Water	1.3 mL
Ora-Sweet,[a] a sufficient quantity to make	100 mL

[a] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place *Codeine Phosphate* powder in a suitable calibrated container. Add *Purified Water* and mix to dissolve. Add the *Ora-Sweet* to bring to final volume, and mix well.

ASSAY
• **PROCEDURE**
Buffer: 25 mM monobasic potassium phosphate adjusted with phosphoric acid to a pH of 2.5
Mobile phase: Methanol and *Buffer* (30:70). Filter and degas.
Internal standard solution: 0.5 mg/mL of theophylline
Standard stock solution: 1.0 mg/mL of USP Codeine Phosphate RS
Standard solution: Pipet 0.5 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 0.6 mL of the *Internal standard solution*, dilute with water to volume, and pass through a membrane filter of 0.2-µm pore size to obtain a solution with nominal concentrations of 5 µg/mL of codeine phosphate and 3 µg/mL of theophylline.
Sample solution: Shake the Oral Solution thoroughly by hand. Transfer 2 mL of Oral Solution from each bottle into a 50-mL volumetric flask, and dilute with water to volume. Transfer 4.17 mL of the diluted sample to a 100-mL volumetric flask, add 0.6 mL of *Internal standard solution*, dilute with water to volume, and filter to obtain a solution with nominal concentrations of 5 µg/mL of codeine phosphate and 3 µg/mL of theophylline.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention times for codeine phosphate and theophylline are about 1.9 and 3.8 min, respectively.]

USP Monographs

www.webofpharma.com

Suitability requirements
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of codeine phosphate to the internal standard from the *Sample solution*
R_S = peak response ratio of codeine phosphate to the internal standard from the *Standard solution*
C_S = concentration of USP Codeine Phosphate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of codeine phosphate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of codeine phosphate hemihydrate, 406.37
M_{r2} = molecular weight of anhydrous codeine phosphate, 397.37

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.7–4.7

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature.
- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded, when stored at controlled room temperature
- **Labeling:** Label it to indicate the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
  USP Codeine Phosphate RS

Codeine Phosphate Tablets

» Codeine Phosphate Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Codeine Phosphate RS

Identification—
A: Digest a quantity of finely powdered Tablets, equivalent to about 100 mg of codeine phosphate, with 15 mL of water and 5 mL of 2 N sulfuric acid for 1 hour. Filter, if necessary, and wash any undissolved residue with a few mL of water. Render the filtrate alkaline with 6 N ammonium hydroxide, extract with several small portions of chloroform, and proceed as directed in *Identification*— test *Identification*— under *Codeine Phosphate Injection*, beginning with "Evaporate the combined chloroform extracts." The specified results are observed.
B: To a quantity of finely powdered Tablets, equivalent to about 100 mg of codeine phosphate, add 10 mL of water and 2 drops of 2 N sulfuric acid. Digest, with frequent shaking, for 15 minutes, and filter. Neutralize 5 mL of the filtrate with 6 N ammonium hydroxide, and add silver nitrate TS: a yellow precipitate of silver phosphate is formed, and it is soluble in diluted nitric acid and in 6 N ammonium hydroxide.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 284 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Codeine Phosphate RS in the same *Medium*.
Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Transfer 1 Tablet, previously crushed or finely powdered, to a 50-mL volumetric flask, add 25 mL of water, and shake to dissolve. Dilute with water to volume, and filter, if necessary, discarding the first 20 mL of the filtrate. Transfer an aliquot of the filtrate, equivalent to about 6 mg of codeine phosphate, to a 50-mL volumetric flask containing 2 mL of 3 N hydrochloric acid, and dilute with water to volume. Dissolve an accurately weighed quantity of USP Codeine Phosphate RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 120 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 284 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ in the Tablet taken by the formula:

$$2.5(C/V)(A_U/A_S)(406.37/397.37)$$

in which C is the concentration, in µg per mL, of USP Codeine Phosphate RS in the Standard solution; V is the volume, in mL, of the aliquot taken of the solution of the Tablet; A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively; and 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively.
Limit of morphine—A 1-mL portion of the filtrate from *Identification* test *B* meets the requirements of the test for *Limit of morphine*— under *Codeine Phosphate.*
Assay—Weigh and finely powder not fewer than 20 Tablets. Accurately weigh a portion of the powder, equivalent to about 150 mg of codeine phosphate, and transfer to a 100-mL volumetric flask. Add 20 mL of 0.5 N sulfuric acid, and shake the mixture occasionally during 2 hours. Add water to volume, mix, and filter through a filtering crucible. Transfer to a separator an accurately measured portion of the filtrate, equivalent to not less than 75 mg of codeine phosphate, render the solution alkaline with 6 N ammonium hydroxide, and completely extract the alkaloid with successive 15-mL portions of chloroform. Evaporate the combined chloroform solution on a steam bath nearly to dryness. Dissolve the residue in about 2 mL of methanol, heating, if necessary, add methyl red TS, and titrate with 0.02 N sulfuric acid VS to a faint pink color. Add about 40 mL of freshly boiled, cooled water, and complete the titration with 0.02 N sulfuric acid VS. Each mL of 0.02 N sulfuric acid is equivalent to 8.128 mg of $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$.

www.webofpharma.com

Codeine Sulfate

$(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O$ 750.85

$(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4$ 696.82

Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-, (5α,6α)-, sulfate (2:1) (salt), trihydrate; 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol sulfate (2:1) (salt) trihydrate [6854-40-6].
Anhydrous [1420-53-7].

DEFINITION
Codeine Sulfate, dried at 105° for 3 h, contains NLT 98.0% and NMT 102.0% of $(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4$.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 100 µg/mL in water
Acceptance criteria: Absorptivities at 284 nm do not differ by more than 3.0%, calculated on the dried basis.

- **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩**:** Meets the requirements

ASSAY
- **PROCEDURE**

Buffer: Dissolve 4.0 g of potassium phosphate monobasic in 2000 mL of water, and adjust with phosphoric acid to a pH of 3.0 ± 0.1.
Solution A: 5 mM sodium heptane sulfonate in methanol and *Buffer* (3:7). [NOTE—Dissolve 1.0 g sodium heptane sulfonate for each L of *Mobile phase* produced, and filter.]
Solution B: 5 mM sodium heptane sulfonate in methanol and *Buffer* (11:9). [NOTE—Dissolve 1.0 g sodium heptane sulfonate for each L of *Mobile phase* produced, and filter.]
Diluent: *Solution A*
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
16	0	100
16.5	100	0
24	100	0

Standard solution: 1 mg/mL of USP Codeine Sulfate RS in *Diluent*
Sample solution: 1 mg/mL of Codeine Sulfate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of codeine sulfate $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4]$ in the portion of Codeine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of codeine sulfate from the *Sample solution*
r_S = peak area of codeine sulfate from the *Standard solution*
C_S = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Codeine Sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES
INORGANIC IMPURITIES
- **Residue on Ignition** ⟨281⟩**:** NMT 0.1%

ORGANIC IMPURITIES
- **Procedure 1**

Buffer, Solution A, Solution B, Diluent, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 1 mg/mL of USP Codeine Sulfate RS in *Diluent*
Standard solution: 0.01 mg/mL of USP Codeine Sulfate RS in *Diluent* from *Standard stock solution*
Sensitivity solution: 0.5 µg/mL of USP Codeine Sulfate RS in *Diluent* from *Standard stock solution*
Sample solution: 1 mg/mL of Codeine Sulfate in *Diluent*
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NLT 0.5 and NMT 1.5, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Codeine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of codeine sulfate from the *Standard solution*
C_S = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Codeine Sulfate in the *Sample solution* (mg/mL)
F = relative response factor of the related compounds (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.5%.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
10-Hydroxy-codeine[a]	0.81	1.35	0.15

www.webofpharma.com

Impurity Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
Codeine-*N*-oxide[b]	0.90	1.0	0.15
Codeine sulfate	1.00	—	—
Norcodeine[c]	1.09	1.0	0.15
Codeinone[d]	1.16	1.0	0.15
Codeine methyl ether[e]	1.34	1.0	1.0
Individual unspecified impurities	—	—	0.10

[a] 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,10-diol.
[b] 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol *N*-oxide.
[c] 7,8-Didehydro-4,5α-epoxy-3-methoxymorphinan-6α-ol.
[d] 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-one.
[e] 7,8-Didehydro-4,5α-epoxy-3,6α-dimethoxy-17-methylmorphinan.

• **PROCEDURE 2: LIMIT OF MORPHINE**
Analysis: Dissolve 50 mg of potassium ferricyanide in 10 mL of water, and add 1 drop of ferric chloride TS and 1 mL of a 10 mg/mL solution of Codeine Sulfate.
Acceptance criteria: No blue color is produced immediately.

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 20 mg/mL, in water
Acceptance criteria: −112.5° to −115.0°
• **ACIDITY**
Analysis: Dissolve 500 mg in 15 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide.
Acceptance criteria: NMT 0.30 mL is required for neutralization.
• **WATER DETERMINATION,** *Method III* ⟨921⟩**:** Dry 500 mg at 105° for 3 h: it loses between 6.0% and 7.5% of its weight.
• **READILY CARBONIZABLE SUBSTANCES TEST** ⟨271⟩
Sample solution: Dissolve 10 mg in 5 mL of sulfuric acid.
Acceptance criteria: The solution has no more color than *Matching Fluid S*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Codeine Sulfate RS

Codeine Sulfate Oral Solution

DEFINITION

Codeine Sulfate Oral Solution contains NLT 93.0% and NMT 105.0% of the labeled amount of codeine sulfate $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4]$.

IDENTIFICATION

• **A.** The retention time of the codeine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B. THIN-LAYER CHROMATOGRAPHY**
Diluent: Methanol and water (25:75)
Standard solution: 1 mg/mL of USP Codeine Sulfate RS in *Diluent*
Sample solution: 1 mg/mL of codeine sulfate in *Diluent*
Chromatographic system
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Methanol, ethyl acetate, water, and ammonium hydroxide (135: 85: 1: 0.5)
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the plate until the solvent front has moved about three-fourths of the length of the plate, remove it, mark the solvent front, and allow the solvent to evaporate. Place the plate in an iodine chamber, and visualize it for NLT 5 min.
Acceptance criteria: The principal spot from the *Sample solution* corresponds in R_F value to that from the *Standard solution*.

ASSAY

• **PROCEDURE**
[NOTE—Solutions are stable for 4 days at room temperature when stored in amber glassware.]
Buffer: 0.02 M ammonium acetate. Dissolve 1.54 g of ammonium acetate in 1 L of water, and adjust with glacial acetic acid to a pH of 4.2 ± 0.1.
Solution A: Dissolve 1.71 g of sodium decanesulfonic acid in 1 L of methanol and *Buffer* mixture (33:67). Pass through a filter of 0.45-µm pore size.
Solution B: Dissolve 1.71 g of sodium decanesulfonic acid in 1 L of methanol and *Buffer* mixture (63:37). Pass through a filter of 0.45-µm pore size.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3.3	60	40
20	0	100
21	0	100
22	100	0
28	100	0

Diluent: Methanol and water (25:75)
Standard solution: 1.2 mg/mL of USP Codeine Sulfate RS in *Diluent*. Prepare by adding 70% of the flask volume of *Diluent*, and sonicate to dissolve. Dilute with *Diluent* to volume.
Sample solution: 1.2 mg/mL of codeine sulfate in *Diluent*, prepared by adding 70% of the flask volume of *Diluent*, then swirling, and letting it sit for 10 min. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 40 ± 2°
Flow rate: 1.2 mL/min
Injection volume: 30 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of codeine sulfate $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4]$ in each mL of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of codeine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–105.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer, Solution A, Solution B, Mobile phase, Diluent, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.6 µg/mL of USP Codeine Sulfate RS in *Diluent* from the *Standard solution*
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Relative standard deviation: NMT 2.0%, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Oral Solution taken:

$$(r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response of each individual impurity from the *Sample solution*
r_S = response of codeine sulfate in the *Standard solution*
C_S = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of codeine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Related Compound	Relative Retention Time	Acceptance Criteria, NMT (%)
Codeine-*N*-oxide[a]	0.65	0.15
Codeine	1.00	—
Codeinone[b]	1.16	0.15
Individual unspecified degradant	—	0.15
Total impurities	—	0.5

[a] 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol *N*-oxide.
[b] 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-one.

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/mL. The total yeasts and molds count does not exceed 20 cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.
• **PH** ⟨791⟩**:** 2.8–3.8

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store at controlled room temperature in a well-sealed container.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Codeine Sulfate RS

Codeine Sulfate Tablets

DEFINITION

Codeine Sulfate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of codeine sulfate trihydrate $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O]$.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.**
Standard solution and **Sample solution:** Proceed as directed in the *Assay*.
Analysis: Inject 2 µL each of the *Standard solution* and the *Sample solution* using the *Chromatographic system* in the *Assay*.
Acceptance criteria: The spectrum of the codeine peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the corresponding peaks of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Solution A: Acetonitrile and 0.1% ammonium hydroxide (1.0 mL of concentrated ammonium hydroxide and 1000 mL of water) (1:19)
Solution B: Acetonitrile and 0.1% ammonium hydroxide (9:11)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	0	100
41	100	0
50	100	0

[NOTE—The *Standard solution* and *Sample solution*, for the degradation products, are stable for 4 days when stored at room temperature in amber vials.]
Diluent: 0.5% phosphoric acid (5 mL of concentrated phosphoric acid and 1000 mL of water)
Standard solution: 1.2 mg/mL of USP Codeine Sulfate RS in *Diluent*
Sample solution: Nominally 1.2 mg/mL of codeine sulfate trihydrate in *Diluent*. Dissolve 20 Tablets in 80% of the flask volume of *Diluent* and sonicate for 15–30 min with occasional swirling before diluting with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

USP Monographs

www.webofpharma.com

Detector: UV 282 nm. For *Identification* test *B*, use a diode-array detector in the range of 210–400 nm.
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 40 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of codeine sulfate trihydrate $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O]$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of codeine sulfate from the *Sample solution*
r_S = peak area of codeine sulfate from the *Standard solution*
C_S = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of codeine sulfate trihydrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of codeine sulfate trihydrate, 750.85
M_{r2} = molecular weight of codeine sulfate, anhydrous, 696.81

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: Water; 500 mL
Apparatus 2: 25 rpm
Time: 45 min
Detector: UV maxima at about 284 nm
Cell: 1 cm
Blank: *Medium*
Standard solution: USP Codeine Sulfate RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.8-µm pore size.
Tolerances: NLT 75% (*Q*) of the labeled amount of codeine sulfate trihydrate $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O]$ is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Solution A, Solution B, Mobile phase, Diluent, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Sensitivity solution: 0.6 µg/mL of USP Codeine Sulfate RS from the *Standard solution* in *Diluent*
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of codeine sulfate from the *Standard solution*
C_S = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of codeine sulfate trihydrate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

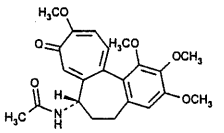
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Codeine-*N*-oxide[a]	0.39	1.25	0.2
Codeine sulfate	1.00	—	—
Codeinone[b]	1.10	1.0	0.3
Individual unspecified degradant	—	1.0	0.2
Total impurities	—	—	0.5

[a] 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol *N*-oxide.
[b] 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-one.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature. Protect from moisture and light.
• **USP Reference Standards** ⟨11⟩
USP Codeine Sulfate RS

Colchicine



$C_{22}H_{25}NO_6$ 399.44
Acetamide, *N*-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[*a*]heptalen-7-yl)-, (*S*)-;
Colchicine [64-86-8].

DEFINITION

Colchicine is an alkaloid contained in various species of *Colchicum* and in other genera. It contains NLT 94.0% and NMT 101.0% of colchicine ($C_{22}H_{25}NO_6$), calculated on the anhydrous, solvent-free basis.
[**Caution**—Colchicine is extremely poisonous.]

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
[Note—Disregard any peak occurring at 1735 cm^{-1}.]

ASSAY

• **Procedure**
[Note—Perform all dilutions in low-actinic glassware.]

www.webofpharma.com

Mobile phase: Dilute 45 mL of 0.5 M *monobasic potassium phosphate* with water to 450 mL. Add 530 mL of methanol, cool to room temperature, and add *methanol* to bring the volume to 1000 mL. Adjust with 0.5 M *phosphoric acid* to a pH of 5.5 ± 0.05, and pass through a 0.45-µm membrane filter.
Standard solution: 6 µg/mL of USP Colchicine RSin a mixture of *methanol* and *water* (1:1). This solution is stable for 4 months when stored tightly stoppered and in the dark.
Sample solution: 6 µg/mL of Colchicine in a mixture of *methanol* and *water* (1:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing *L7*
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 4500 theoretical plates
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the responses for all peaks recorded during 1.5 times the retention time for colchicine.
Calculate the percentage of colchicine ($C_{22}H_{25}NO_6$) in the portion of Colchicine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Colchicine RS in the *Standard solution* (µg/mL)
C_U = concentration of Colchicine in the *Sample solution* (µg/mL)

Acceptance criteria: 94.0%–101.0% on the anhydrous, solvent-free basis

IMPURITIES

• **LIMIT OF COLCHICEINE**
Analysis: To 5 mL of a 10-mg/mL solution of colchicine, add 2 drops of *ferric chloride TS*.
Acceptance criteria: No definite green color is produced.
• **LIMIT OF ETHYL ACETATE**
Internal standard solution: Dilute 0.5 mL of *n*-propyl alcohol with *water* to 100 mL.
Standard solution: Transfer 1.0 mL of ethyl acetate, 0.5 mL of diacetone alcohol, and 0.5 mL of *n*-propyl alcohol to a 1000-mL volumetric flask and dilute with *water* to volume. Mix well. This solution contains 0.9 mg/mL of ethyl acetate.
Sample solution: Add 250 mg of Colchicine, accurately weighed, to a 10-mL volumetric flask, and dissolve in about 8.0 mL of *water*. Add 1.0 mL of *Internal standard solution*, dilute with *water* to volume, and mix well.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m; coated with a 1-µm film of phase G16
Temperatures
Injection port: 180°
Detector: 220°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	20
40	20	200	10

Carrier gas: Helium
Flow rate: 5.72 mL/min
Injection volume: 2 µL
Injection type: Split ratio, 15:1
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 15.0% for the peak response ratios of ethyl acetate to the *n*-propyl alcohol peak, and of diacetone alcohol to the *n*-propyl alcohol peak, in replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ethyl acetate in the portion of Colchicine taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of ethyl acetate to *n*-propyl alcohol from the *Sample solution*
R_S = peak response ratio of ethyl acetate to *n*-propyl alcohol from the *Standard solution*
C_S = concentration of ethyl acetate in the *Standard solution* (mg/mL)
C_U = concentration of Colchicine in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 8.0%
• **CHROMATOGRAPHIC PURITY**
Procedure: Proceed as directed in the *Assay*.
Acceptance criteria: NMT 5.0% other than that due to colchicine, eluting within 1.5 times the retention time for colchicine
• **RESIDUAL SOLVENTS** ⟨467⟩**:** Meets the requirements, except that the limit of chloroform is 100 ppm

SPECIFIC TESTS

• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL, in alcohol
Acceptance criteria: −240° to −250°, calculated on the anhydrous, solvent-free basis
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Colchicine RS

Colchicine Injection

» Colchicine Injection is a sterile solution of $C_{22}H_{25}NO_6$ in Water for Injection, prepared from Colchicine with the aid of Sodium Hydroxide. It contains not less than 90.0 percent and not more

www.webofpharma.com

than 110.0 percent of the labeled amount of $C_{22}H_{25}NO_6$.
[**CAUTION**—Colchicine is extremely poisonous.]

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Colchicine RS

Identification—
A: Transfer a volume of Injection, equivalent to about 2 mg of colchicine, to a separator. Add 5 mL of water, and extract with 15 mL of chloroform. Evaporate the chloroform extract, using mild heat, to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Colchicine RS.
B: The UV absorption spectrum of the Injection, diluted with water to a concentration of about 10 µg of colchicine per mL, exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Colchicine RS, concomitantly measured.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 166.7 USP Endotoxin Units per mg of colchicine.
pH ⟨791⟩: between 6.0 and 7.2, in a solution of Injection containing 1.0 mg of potassium chloride in each mL.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay— [NOTE—Perform all dilutions in low-actinic glassware.]
Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *ASSAY* under *Colchicine.*
Assay preparation— [NOTE—Prepare immediately before use.] Transfer an accurately measured volume, *V* mL, of Injection, equivalent to about 1 mg of colchicine, to a 50-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. Pipet 30 mL of this solution into a 100-mL volumetric flask, dilute with the same mixture to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *ASSAY* under *Colchicine,* and measure the responses for the colchicine peaks. Calculate the quantity, in mg, of $C_{22}H_{25}NO_6$ in each mL of the Injection taken by the formula:

$$(C/6V)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Colchicine Tablets

» Colchicine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{22}H_{25}NO_6$.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Colchicine RS
Identification—Weigh a portion of ground Tablets, equivalent to about 20 mg of colchicine, triturate with 20 mL of water, allow the solids to settle, and filter the supernatant into a separator. Extract with 30 mL of chloroform. Evaporate the chloroform extract, using mild heat, to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Colchicine RS.
Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
[NOTE—Conduct this procedure without delay, under subdued light, and using low-actinic glassware.]
Medium: water; 500 mL.
Apparatus 1: 100 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{22}H_{25}NO_6$ dissolved, employing the procedure set forth in the *Assay* under *Colchicine,* making any necessary modifications.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{22}H_{25}NO_6$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay— [NOTE—Perform all dilutions in low-actinic glassware.]
Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Colchicine.*
Assay preparation— [NOTE—Prepare immediately before use.] Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 0.6 mg of colchicine, to a 100-mL volumetric flask, add about 50 mL of a mixture of methanol and water (1:1), and shake by mechanical means for 15 minutes, rinsing down the walls of the flask at about 8 minutes. Dilute with the same mixture to volume, and pass through a 0.45-µm membrane filter.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Colchicine,* and measure the responses for the colchicine peaks. Calculate the quantity, in mg, of $C_{22}H_{25}NO_6$ in the portion of Tablets taken by the formula:

$$0.1C(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Colestipol Hydrochloride

» Colestipol Hydrochloride is an insoluble, high molecular weight basic anion-exchange copolymer of diethylenetriamine and 1-chloro-2,3-epoxypropane with approximately one out of five amino nitrogens protonated. Each g binds not less than 1.1 mEq and not more than 1.6 mEq of sodium cholate, calculated as cholate binding capacity.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Colestipol Hydrochloride RS

Change to read:

Identification—▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—Prepare the test specimen and the Standard specimen by mixing about 3 to 4 mg of Colestipol Hydrochloride and of USP Colestipol Hydrochloride RS, respectively, with about 150 mg of potassium bromide.

USP Monographs

www.webofpharma.com

pH ⟨791⟩—Prepare a 10% (w/w) suspension of it in deionized water in a clean vial. Insert the stopper, shake at approximately 10-minute intervals for 1 hour, and centrifuge. Transfer a portion of the clear supernatant to a suitable container, and record the pH as soon as the reading has stabilized: the pH is between 6.0 and 7.5.

Loss on drying ⟨731⟩—Dry it in vacuum at a pressure of about 5 mm of mercury at 75° for 16 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.3%.

Content of chloride—

Test preparation—Using about 20 mg of Colestipol Hydrochloride, accurately weighed, proceed as directed under *Oxygen Flask Combustion* ⟨471⟩, 10 mL of 0.05 N sodium hydroxide being used as the absorbing liquid. Do not allow the paper specimen wrapper to come in contact with the liquid, and ignite the paper with an IR igniter. After combustion is complete, shake the flask vigorously, and allow to stand, with frequent shaking, for about 40 minutes or until no cloudiness is present. Transfer the solution to a 50-mL beaker. Wash the flask with two 5-mL portions of water and two 10-mL portions of alcohol, adding each washing to the beaker, and add 0.2 mL of nitric acid.

Reagent blank preparation—Using a paper specimen wrapper, complete the combustion, and allow the mixture to stand for about 40 minutes or until no cloudiness is present, as directed under *Test preparation*. Transfer the solution so obtained to a 50-mL beaker. Wash the combustion flask with two 5-mL portions of water and two 10-mL portions of alcohol, adding the washings to the beaker, and add 0.2 mL of nitric acid.

Procedure—Titrate the *Test preparation* and the *Reagent blank preparation* with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-silver chloride electrode and a glass reference electrode (see *Titrimetry* ⟨541⟩). Determine the volume of 0.05 N silver nitrate VS consumed by the test specimen taken by the formula:

$$V - V_B$$

in which V and V_B are the volumes, in mL, of titrant used for the *Test preparation* and the *Reagent blank preparation*, respectively. Each mL of 0.05 N silver nitrate is equivalent to 1.773 mg of Cl: the chloride content is between 6.5% and 9.0%, calculated on the dried basis.

Water absorption—Transfer about 5 g of Colestipol Hydrochloride, accurately weighed, to a dry, 100-mL plastic container, and add about 80 g of water, accurately weighed. Cover the container, and allow the suspension to equilibrate for 72 hours. With the aid of vacuum, filter the slurry transferred to a medium-porosity, fritted-glass funnel, and collect the filtrate in a tared plastic container. Disconnect the vacuum 2 minutes after the collection of the last portion of the filtrate. Weigh the container and the filtrate, and determine the weight, in g, of the filtrate. Determine the amount of water absorbed by subtracting the weight of the filtrate from the weight of water taken for the test, and divide the weight, in g, of the absorbed water by the weight, in g, of Colestipol Hydrochloride taken: each g absorbs between 3.3 g and 5.3 g of water.

Cholate binding capacity—

Cholate solution—Dissolve accurately weighed quantities of sodium cholate and sodium chloride in water, and quantitatively dilute with water to obtain a solution having known concentrations of 10.0 mg of sodium cholate per mL and 9.0 mg of sodium chloride per mL. Adjust the solution by the dropwise addition of hydrochloric acid to a pH of 6.4 ± 0.1.

Test preparation—Transfer 1.0 ± 0.01 g of Colestipol Hydrochloride to a glass-stoppered, 125-mL conical flask. Add 100.0 mL of *Cholate solution*, insert the stopper securely in the flask, shake vigorously for 90 minutes with the flask positioned horizontally on a platform shaker, remove the flask from the shaker, and allow the solids to settle for 5 minutes.

Procedure—Transfer 20.0 mL of supernatant from the *Test preparation* to a 40-mL beaker, transfer 20.0 mL of *Cholate solution* to a second 40-mL beaker, and adjust both solutions by the dropwise addition of 1 N sodium hydroxide to a pH of 10.5 ± 0.5. Titrate both solutions with 0.1 N hydrochloric acid VS, determining the endpoints potentiometrically, and measure the titrant volume corresponding to the difference between the midpoints of the two inflections in the titration curves obtained for each solution (see *Titrimetry* ⟨541⟩). Determine the volume of titrant equivalent to the bound cholate by subtracting the volume of 0.1 N hydrochloric acid VS used in titrating the *Test preparation* from that used in titrating the *Cholate solution*. Calculate the *Cholate binding capacity*, in mEq per g, taken by the formula:

$$5VN/W$$

in which V is the volume, in mL, of titrant equivalent to the bound cholate; N is the normality of the 0.1 N hydrochloric acid VS; and W is the weight, in g, of Colestipol Hydrochloride taken for the *Test preparation*. The *Cholate binding capacity* is between 1.1 mEq per g and 1.6 mEq per g.

Water-soluble substances—Transfer 5.0 g of Colestipol Hydrochloride, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 80.0 mL of water, insert the stopper in the flask, and mount the flask in a water-bath shaker maintained at 37 ± 1°. Operate the shaker for 72 hours, remove the flask from the shaker, and filter the contents twice —first through a premoistened 0.45-µm nylon membrane filter and then through a 0.45-µm PVDF filter, collecting the filtrate in a tared 125-mL conical flask. Rinse any residual test material in the flask with two 5-mL portions of water, pass the washings through the filters, and combine the filtrates from the washings with the filtrate from the test mixture. Evaporate the filtrate to dryness, filtered air or nitrogen being used, if necessary, to aid in the evaporation. Dry the residue in a vacuum oven maintained at 75° for 1 hour, allow to cool in a desiccator, and weigh: not more than 0.5% of water-soluble substances is found in the portion of Colestipol Hydrochloride taken.

Colestipol exchange capacity—

Resin base preparation—Combine not less than 2 g of Colestipol Hydrochloride and 100 mL of 1 N sodium hydroxide in a 125-mL conical flask, insert a stopper in the flask, secure the flask on a platform shaker, and shake the mixture for 3 to 4 hours. Filter the suspension through a coarse-porosity, fritted-glass funnel, and wash the resin with 500 mL of water. Transfer the resin to a 1000-mL beaker, add 200 mL of water, allow to stand for 10 minutes, filter the suspension, and check the pH of the filtrate. Repeat the washing procedure with 200-mL portions of water until the pH of the filtrate is below 8 (as much as 5000 mL of water may be required). Dry the colestipol base resin so obtained and the funnel at a pressure of about 5 mm of mercury at 60° for 16 hours. Break up any aggregates, and store the *Resin base preparation* in a desiccator.

Procedure—Transfer about 1.0 g of the *Resin base preparation* to a 125-mL conical flask, add 100.0 mL of 0.20 N hydrochloric acid, insert a stopper in the flask, and shake the mixture by mechanical means for 2.5 hours. Filter a portion of the suspension through a pledget of glass wool, and transfer 8.0 mL of the filtrate (test preparation) to a 25-mL beaker. Transfer 5.0 mL of the same 0.20 N hydrochloric acid that was

USP Monographs

www.webofpharma.com

used to equilibrate the resin to a second 25-mL beaker, and add 5.0 mL of water. Titrate both solutions with 0.2 N sodium hydroxide VS, determining the endpoints potentiometrically (see *Titrimetry* ⟨541⟩), and calculate the *Colestipol exchange capacity*, in mEq per g, taken by the formula:

$$(100N/W)[(V_b/5) - (V_a/8)]$$

in which N is the normality of the sodium hydroxide VS; W is the weight, in g, of the *Resin base preparation* taken; V_b is the volume, in mL, of titrant used to neutralize the 5.0-mL aliquot of 0.20 N hydrochloric acid; and V_a is the volume, in mL, of titrant used to neutralize the residual acid in the test preparation. Each g exchanges not less than 9.0 mEq and not more than 11.0 mEq of sodium hydroxide, calculated as colestipol exchange capacity.

Colestipol Hydrochloride for Oral Suspension

» Colestipol Hydrochloride for Oral Suspension is a mixture of Colestipol Hydrochloride with a suitable flow-promoting agent. Each g binds not less than 1.1 mEq and not more than 1.6 mEq of sodium cholate, calculated as the cholate binding capacity.

Packaging and storage—Preserve in tight, single-dose or multiple-dose containers.
USP Reference standards ⟨11⟩—
USP Colestipol Hydrochloride RS

Change to read:

Identification, ▲ ***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy* **: *197K*** ▲(CN 1-May-2020)—Prepare the test specimen and the Standard specimen by mixing about 5 mg of Colestipol Hydrochloride for Oral Suspension and 5 mg of USP Colestipol Hydrochloride RS, respectively, with about 100 to 125 mg of potassium bromide.
Minimum fill ⟨755⟩: meets the requirements for powders.
Water-soluble substances—Transfer 5.0 g of Colestipol Hydrochloride for Oral Suspension, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 80.0 mL of water, insert the stopper in the flask, and mount the flask in a water-bath shaker maintained at 37 ± 1°. Operate the shaker for 72 hours, remove the flask from the shaker, and filter the contents twice—first through a premoistened 0.45-µm nylon membrane filter and then through a 0.45-µm PVDF filter, collecting the filtrate in a tared 100-mL fused quartz crucible. Rinse any residual test material in the flask with two 5-mL portions of water, pass the washings through the filters, and combine the filtrates from the washings with the filtrate from the test mixture. Evaporate the filtrate to dryness, filtered air or nitrogen being used, if necessary, to aid in the evaporation. Dry the residue in a vacuum oven maintained at 75° for 1 hour, allow to cool in a desiccator, and weigh. Calculate the initial percentage of water-soluble substances in the portion of Colestipol Hydrochloride for Oral Suspension taken. Again heat the residue in a muffle furnace maintained at 800 ± 25° for 4 hours, allow to cool in a desiccator, and weigh. Calculate the percentage of inert ingredients present. Calculate the actual percentage of water-soluble substances in the portion of Colestipol Hydrochloride for Oral Suspension taken by subtracting the percentage of inert ingredients from the initial percentage of water-soluble substances found. Not more than 0.5% of water-soluble substances is found.
Other requirements: meets the requirements of the tests for *Cholate binding capacity* and *pH* under *Colestipol Hydrochloride*.

Colestipol Hydrochloride Tablets

» Colestipol Hydrochloride Tablets contain Colestipol Hydrochloride. Each Tablet binds not less than 1.1 mEq and not more than 1.6 mEq of sodium cholate per g of the labeled amount of colestipol hydrochloride, calculated as cholate binding capacity.

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Colestipol Hydrochloride RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197K***▲(CN 1-May-2020)—
Test specimen—Completely remove the coating film from a Tablet with a suitable implement, and grind the contents into fine powder. To about 30 to 40 mg of the powder, add about 15 mL of methanol, shake vigorously for 3 minutes, then sonicate for 10 minutes, and shake for another 3 minutes. Pass through a suitable paper filter, wash the residue 3 times, each time with 10 mL of methanol. [NOTE—A qualitative paper filter, with a coarse porosity and a particle retention 20-25 µm, is suitable.] Dry the residue at 60° in vacuum for 2 hours. Mix about 4 mg of the dried sample with about 150 mg of potassium bromide.
Standard specimen—Mix about 3 to 4 mg of USP Colestipol Hydrochloride RS with about 150 mg of potassium bromide.

Uniformity of dosage units—
Sodium chloride solution, Cholate solution, 0.09 M Buffer solution pH 2.5, Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the test for *Cholate binding capacity*.
Test solution—Transfer 1 Tablet to a 100-mL volumetric flask, dilute with *Cholate solution* to volume, and stir for 120 minutes. Let the sample settle down for at least 10 minutes, and filter a portion using a 0.45-µm PVDF filter, discarding the first 5 mL of the filtrate.
Procedure—Proceed as directed in the test for *Cholate binding capacity,* except to inject the *Test solution* instead of the *Test preparation*.
Select not fewer than 30 Tablets. Test 10 Tablets individually as directed above. The requirements are met if the cholate binding capacity in each of the 10 Tablets lies within the range of 1.15 to 1.55 mEq per g of the labeled amount of colestipol hydrochloride, and the relative standard deviation is not more than 6.0%.
If 1 Tablet is outside the range of 1.15 to 1.55 mEq per g and no Tablet is outside the range of 1.01 to 1.69 mEq per g, or if the relative standard deviation is greater than 6.0%, or if both conditions prevail, test 20 additional Tablets. The requirements are met if not more than 1 Tablet of the 30 is outside the range of 1.15 to 1.55 mEq per g and no Tablet is outside the range of 1.01 to 1.69 mEq per g of the labeled

www.webofpharma.com

amount of colestipol hydrochloride, and the relative standard deviation for 30 Tablets does not exceed 7.8%.

pH ⟨791⟩—Transfer 5 g of ground Tablets to a suitable flask, add 50 mL of deionized water, close the flask with a stopper, and stir for about 30 minutes or until the Tablets completely disintegrate. Centrifuge to obtain a clear supernatant: the pH is between 5.5 and 7.5.

Cholate binding capacity—

0.09 M Buffer solution pH 2.5—Dissolve 12.5 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 2.5 ± 0.05.

Mobile phase—Prepare a mixture of *0.09 M Buffer solution pH 2.5,* acetonitrile, and methanol (50:36:14), mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Sodium chloride solution—Prepare a solution in water containing 9.0 mg of sodium chloride per mL.

Cholate solution—Transfer an accurately weighed quantity of sodium cholate to a suitable volumetric flask, add *Sodium chloride solution* to about 80% of the final volume, sonicate to dissolve, and dilute with *Sodium chloride solution* to volume to obtain a solution having a known concentration of 10.0 mg of sodium cholate per mL on the anhydrous basis. [NOTE—Determine the water content of sodium cholate titrimetrically at the time of use.] Adjust the solution by the dropwise addition of 0.5 N hydrochloric acid to a pH of 6.45 ± 0.05. [NOTE—Do not allow the pH to go below 6.40 at any time.] Use this solution as soon as possible after preparation.

Test preparation—Transfer 10 Tablets to a glass-stoppered 1.5-L flask. Add 1000.0 mL of *Cholate solution,* insert the stopper securely in the flask, and stir for 120 minutes. Filter a portion using a 0.45-µm PVDF filter, discarding the first 5 mL of the filtrate.

Standard preparation—Dilute a portion of the *Cholate solution* with *Sodium chloride solution,* to obtain a solution having a known concentration of about 4.0 mg of sodium cholate per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L7. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 2000 theoretical plates; the tailing is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the concentration of the unbound cholate, C_T, in the *Test preparation* by the formula:

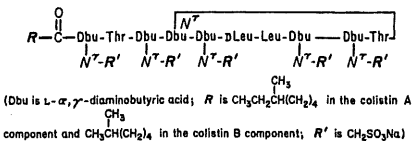
$$C_S \times (r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of sodium cholate in the *Standard preparation;* and r_U and r_S are the cholate peak areas obtained from the *Test preparation* and the *Standard preparation,* respectively. Calculate the cholate binding capacity, in mEq per g of the labeled amount of colestipol hydrochloride, by the formula:

$$(1000/430.6)(C_{CH} - C_T)/NL$$

in which 1000 is a conversion coefficient to g; 430.6 is the molecular weight of sodium cholate; C_{CH} is the concentration, in mg per mL, of sodium cholate in the *Cholate solution; N* is the number of Tablets taken to prepare the *Test preparation;* and *L* is the labeled amount of colestipol hydrochloride, in g per Tablet.

Colistimethate Sodium



$C_{58}H_{105}N_{16}Na_5O_{28}S_5$ (colistin A component) 1749.82

$C_{57}H_{103}N_{16}Na_5O_{28}S_5$ (colistin B component) 1735.79

Colistimethate sodium.

Pentasodium colistinmethanesulfonate [8068-28-8; 21362-08-3].

» Colistimethate Sodium has a potency equivalent to not less than 390 µg of colistin per mg.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩—
USP Colistimethate Sodium RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197K***▲ (CN 1-May-2020)

pH ⟨791⟩: between 6.5 and 8.5, in a solution containing 10 mg per mL.

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 7.0% of its weight.

Free colistin—Dissolve 80 mg in 3 mL of water, and add 0.05 mL of silicotungstic acid solution (1 in 10): no immediate precipitate is formed.

Other requirements—Where the label states that Colistimethate Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial Endotoxins Test 85*— under *Colistimethate for Injection.* Where the label states that Colistimethate Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial Endotoxins Test 85*— under *Colistimethate for Injection.*

Assay—

Assay preparation—Dissolve a suitable quantity of Colistimethate Sodium, accurately weighed, in 2.0 mL of water, add a sufficient accurately measured volume of *Buffer B.6* to obtain a solution having a convenient concentration.

Procedure—Proceed as directed for Colistimethate Sodium under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively

USP Monographs

www.webofpharma.com

with *Buffer B.6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Colistimethate for Injection

» Colistimethate for Injection contains an amount of Colistimethate Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of colistin.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Colistimethate Sodium RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 2.0 USP Endotoxin Units per mg of colistin.
Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
Other requirements—It responds to the *Identification* test and meets the requirements for *pH*, *Loss on drying*, and *Free colistin*— under *Colistimethate Sodium.* It meets also the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions* and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

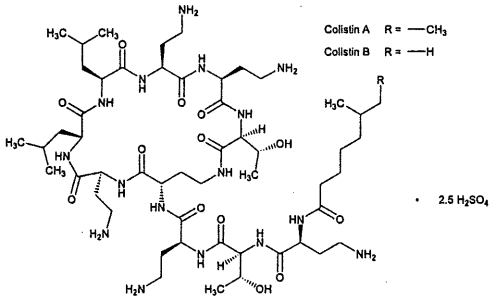
Assay—

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Colistimethate for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Buffer B.6* to obtain a solution having a convenient concentration.

Assay preparation 2 (where the label states the quantity of colistin equivalent in a given volume of constituted solution) —Constitute Colistimethate for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Buffer B.6* to obtain a solution having a convenient concentration.

Procedure—Proceed as directed for Colistimethate Sodium under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer B.6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Colistin Sulfate



Colistin, sulfate.
Colistins sulfate [1264-72-8].

» Colistin Sulfate is the sulfate salt of an antibacterial substance produced by the growth of *Bacillus polymyxa* var. *colistinus.* It has a potency equivalent to not less than 500 µg of colistin per mg.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Colistin Sulfate RS

Identification—

A: Dissolve about 20 mg in 2 mL of buffer solution, prepared by adjusting 50 mL of 1 M monobasic potassium phosphate with 1 N sodium hydroxide to a pH of 7.0, diluting with water to 100 mL, and mixing. Add 0.2 mL of ninhydrin solution (1 in 200) and boil: a purple color is produced.

B: It meets the requirements of the tests for *Sulfate* ⟨191⟩.

C: Liquid Chromatographic Identification Test—

Mobile phase—Prepare a mixture of 0.1 M tribasic sodium phosphate and acetonitrile (77:23), and adjust with phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Prepare a solution of USP Colistin Sulfate RS in *Mobile phase* having a concentration of about 6 mg per mL. Protect this solution from light.

Test solution—Prepare a solution of Colistin Sulfate in *Mobile phase* having a concentration of about 6 mg per mL. Protect this solution from light.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 212-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and record the chromatograms. The chromatogram obtained from the *Test solution* corresponds qualitatively with that obtained from the *Standard solution,* exhibiting a major peak corresponding to colistin A and minor peaks at relative retention times of about 0.55 (colistin B) and 0.8.

pH ⟨791⟩: between 4.0 and 7.0, in a solution containing 10 mg per mL.

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 7.0% of its weight.

Assay—Proceed as directed for Colistin under *Antibiotics—Microbial Assays* ⟨81⟩.

www.webofpharma.com

Colistin Sulfate for Oral Suspension

» Colistin Sulfate for Oral Suspension is a dry mixture of Colistin Sulfate with or without one or more suitable buffers, colors, diluents, dispersants, and flavors. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of colistin.

Packaging and storage—Preserve in tight containers, protected from light.
USP Reference standards ⟨11⟩—
USP Colistin Sulfate RS

Uniformity of dosage units ⟨905⟩—
FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 5.0 and 6.0, in the suspension constituted as directed in the labeling.
Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.0% of its weight.
Assay—Constitute Colistin Sulfate for Oral Suspension as directed in the labeling. Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩ using an accurately measured volume of this suspension, freshly mixed and free from air bubbles, diluted quantitatively with *Buffer B.6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Colistin and Neomycin Sulfates and Hydrocortisone Acetate Otic Suspension

» Colistin and Neomycin Sulfates and Hydrocortisone Acetate Otic Suspension is a sterile suspension containing the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of colistin, not less than 90.0 percent and not more than 125.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$). It contains one or more suitable buffers, detergents, dispersants, and preservatives.
[NOTE—Where Colistin and Neomycin Sulfates and Hydrocortisone Acetate Otic Suspension is prescribed, without reference to the quantity of colistin, neomycin, or hydrocortisone acetate contained therein, a product containing 3.0 mg of colistin, 3.3 mg of neomycin, and 10 mg of hydrocortisone acetate per mL shall be dispensed.]

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Colistin Sulfate RS
USP Neomycin Sulfate RS
USP Hydrocortisone Acetate RS

Sterility Tests ⟨71⟩: meets the requirements, 0.25 mL from each container being transferred directly to 90 mL of each medium.
pH ⟨791⟩: between 4.8 and 5.2.
Assay for colistin—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using a freshly mixed, accurately measured volume of Otic Suspension diluted quantitatively and stepwise with *Buffer B.6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.
Assay for neomycin—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using a freshly mixed, accurately measured volume of Otic Suspension diluted quantitatively and stepwise with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for hydrocortisone acetate—
Reagent blank—Dilute 200 mL of 22 N sulfuric acid with 100 mL of dehydrated alcohol.
Phenylhydrazine reagent—Dissolve 43.33 mg of phenylhydrazine hydrochloride in 100 mL of *Reagent blank*.
Standard preparation—Dissolve a suitable quantity of USP Hydrocortisone Acetate RS, accurately weighed, in chloroform, and dilute quantitatively and stepwise with chloroform to obtain a solution having a known concentration of about 10 µg per mL.
Assay preparation—Transfer 5.0 mL of freshly mixed Otic Suspension to a 125-mL separator. Extract with three 20-mL portions of chloroform, filtering each chloroform extract through a pledget of cotton previously saturated with chloroform, collect the filtrates in a 100-mL volumetric flask, dilute with chloroform to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute with chloroform to volume, and mix. Pipet 20 mL of this solution into a 100-mL volumetric flask, dilute with chloroform to volume, and mix.
Procedure—Pipet 50 mL each of the *Standard* and the *Assay preparation* into separate 125-mL separators, add 2 mL of 0.1 N sodium hydroxide to each separator, shake, and allow the layers to separate. Filter both chloroform layers through glass wool, and collect the filtrates in separate beakers. Pipet two 20-mL portions of each chloroform filtrate into separate 125-mL separators. Add 25.0 mL of *Phenylhydrazine reagent* to one separator each of the filtrates from the *Standard preparation* and the *Assay preparation*, respectively, and add 25.0 mL of *Reagent blank* to the remaining two separators. Shake all four separators well, allow the layers to separate, and discard the chloroform layers. Drain the aqueous layers into separate centrifuge tubes, and centrifuge for 2 minutes. Pipet 10 mL of each solution into separate glass-stoppered test tubes. Place the tubes in a water bath maintained at a temperature of 60° for 30 minutes, then cool the solution to room temperature. Concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 410 nm, with a suitable spectrophotometer, using water to set the instrument. Calculate the quantity, in mg, of hydrocortisone acetate ($C_{23}H_{32}O_6$) in each mL of the Otic Suspension taken by the formula:

$$C(A_U - A_{UB}/A_S - A_{SB})$$

in which C is the concentration, in µg per mL, of USP Hydrocortisone Acetate RS in the *Standard preparation*; A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* treated with *Phenylhydrazine reagent*, respectively; and A_{UB} and A_{SB} are the absorbances of the solution from the *Assay preparation* and the

www.webofpharma.com

Standard preparation treated with the *Reagent blank*, respectively.

Collodion

DEFINITION
Collodion contains NLT 5.0%, by weight, of pyroxylin.

Pyroxylin	40 g
Ether	750 mL
Alcohol	250 mL
To make about	1000 mL

To the *Pyroxylin* in a suitable container add the *Alcohol* and *Ether*, and insert the stopper into the container. Shake the mixture occasionally until the *Pyroxylin* is dissolved. [**CAUTION**—Collodion is highly flammable.]

IDENTIFICATION
- **A.**
  Analysis: Expose a thin layer to air, leaving a transparent, tenacious film.
  Acceptance criteria: The film of pyroxylin so obtained burns rapidly with a yellow flame.
- **B.**
  Analysis: Mix with an equal volume of water.
  Acceptance criteria: A viscid, stringy mass of pyroxylin is produced.

ASSAY
- **PROCEDURE**
  Sample: 10 mL
  Analysis: Quickly pour the *Sample* into a tared flask, insert the stopper, and weigh the *Assay* charge accurately. Remove the stopper, warm on a steam bath, and add 10 mL of water dropwise, with constant stirring. Evaporate the mixture on a steam bath, and dry the residue at 105° for 4 h.
  Acceptance criteria: NLT 5.0%, by weight

OTHER COMPONENTS
- **ALCOHOL DETERMINATION** ⟨611⟩
  Internal standard solution: Acetone and 1,2-dichloroethane (20:80) in a glass-stoppered graduated cylinder
  Standard stock solutions: Transfer 10-, 20-, and 30-mL portions of dehydrated alcohol into separate 100-mL volumetric flasks, dilute with 1,2-dichloroethane to volume, and mix.
  Standard solutions: Mix 10 mL of each *Standard stock solution* with 15 mL of 1,2-dichloroethane, 10 mL of hexane, and 10.0 mL of *Internal standard solution* in separate glass-stoppered, 50-mL graduated cylinders.
  Sample solution: To 10 mL of Collodion in a glass-stoppered, 50-mL graduated cylinder add 15 mL of 1,2-dichloroethane, 10 mL of hexane, and 10.0 mL of *Internal standard solution*. Mix, and allow the precipitate to settle.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: GC
  Detector: Thermal conductivity
  Column: 1.8-m × 3.5-mm glass; packing S3
  Temperatures
  Column: 150°
  Injection port: 200°
  Detector: 250°
  Carrier gas: Helium
  Flow rate: 50 mL/min
  Injection volume: 4 µL
  Analysis
  Samples: *Standard solutions* and *Sample solution*
  Calculate the relative response factor, *F*, for each *Standard solution* taken:

$$F = C_S / R_S$$

C_S = concentration of alcohol in the *Standard solution*, as a percentage (v/v)
R_S = peak response ratio of alcohol to acetone from the respective *Standard solution*

Calculate the percentage of alcohol (C_2H_5OH) in the portion of Collodion taken:

$$\text{Result} = R_U \times F_a$$

R_U = peak response ratio of alcohol to acetone from the *Sample solution*
F_a = average of the individual *F* values

Acceptance criteria: 22.0%–26.0%

SPECIFIC TESTS
- **SPECIFIC GRAVITY** ⟨841⟩: 0.765–0.775
- **ACIDITY**
  Sample: 5 mL
  Analysis: Add the *Sample* to 5 mL of water.
  Acceptance criteria: The liquid separated from the pyroxylin is not acid to litmus.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight containers, at a temperature not exceeding 30°, remote from fire.
- **LABELING:** The label bears a caution statement to the effect that Collodion is highly flammable.

Flexible Collodion

DEFINITION
Prepare Flexible Collodion as follows.

Camphor	20 g
Castor Oil	30 g
Collodion, a sufficient quantity to make	1000 g

Weigh the ingredients, successively, into a dry, tared bottle. Insert the stopper in the bottle, and shake the mixture until the *Camphor* is dissolved.

IDENTIFICATION
- **A.**
  Analysis: Expose a thin layer to air, leaving a transparent, tenacious film.
  Acceptance criteria: The film exhibits a distinct odor of camphor. The film of pyroxylin so obtained burns rapidly with a yellow flame.
- **B.**
  Analysis: Mix with an equal volume of water.
  Acceptance criteria: A viscid, stringy mass of pyroxylin is produced.

USP Monographs

www.webofpharma.com

OTHER COMPONENTS

- **ALCOHOL DETERMINATION** ⟨611⟩

Internal standard solution: Acetone and 1,2-dichloro ethane (20:80) in a glass-stoppered, graduated cylinder

Standard stock solutions: Transfer 10-, 20-, and 30-mL portions of dehydrated alcohol into separate 100-mL volumetric flasks, dilute with 1,2-dichloroethane to volume, and mix.

Standard solutions: Mix 10 mL of each *Standard stock solution* with 15 mL of 1,2-dichloroethane, 10 mL of hexane, and 10.0 mL of *Internal standard solution* in separate, glass-stoppered, 50-mL graduated cylinders.

Sample solution: To 10 mL of Flexible Collodion in a glass-stoppered, 50-mL graduated cylinder add 15 mL of 1,2-dichloroethane, 10 mL of hexane, and 10.0 mL of *Internal standard solution*. Mix, and allow the precipitate to settle.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Thermal conductivity

Column: 1.8-m × 3.5-mm glass; support S3

Temperatures

Column: 150°

Injection port: 200°

Detector: 250°

Carrier gas: Helium

Flow rate: 50 mL/min

Injection volume: 4 µL

Analysis

Samples: *Standard solutions* and *Sample solution*

Calculate the relative response factor, *F*, for each *Standard solution* taken:

$$F = C_S/R_S$$

C_S = concentration of alcohol in the *Standard solution*, as a percentage (v/v)

R_S = peak response ratio of alcohol to acetone from the respective *Standard solution*

Calculate the percentage of alcohol (C_2H_5OH) in the portion of Flexible Collodion taken:

$$\text{Result} = R_U \times F_A$$

R_U = peak response ratio of alcohol to acetone from the *Sample solution*

F_A = average of the individual *F* values

Acceptance criteria: 21.0%–25.0%

SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩: 0.770–0.790

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight containers, at a temperature not exceeding 30°, remote from fire.
- **LABELING:** The label bears a caution statement to the effect that Flexible Collodion is highly flammable.

Colloidal Oatmeal

DEFINITION

Colloidal Oatmeal is the powder resulting from the grinding and further processing of whole oat grain meeting U.S. Standards for Number 1 or Number 2 oats (7 CFR 810.1001).

IDENTIFICATION

- **A.**

Analysis: Prepare a smooth mixture of 10 g of Colloidal Oatmeal and 100 mL of warm water. Stir for 10 min.

Acceptance criteria: The resulting slurry has a characteristic slippery feel and shows the development of slimy, viscous strands.

- **B.** A water slurry is colored reddish violet to deep blue by iodine TS.

IMPURITIES

- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨561⟩: NMT 2.5% on the dried basis

SPECIFIC TESTS

- **NITROGEN DETERMINATION,** *Method I* ⟨461⟩: NLT 2.0% on the dried basis
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^4 cfu/g; the total combined molds and yeasts count does not exceed 150 cfu/g.
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 120° for 4 h.

Acceptance criteria: NMT 10%

- **PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING** ⟨786⟩: NMT 3% of the total particles exceed 150 µm in size and NMT 20% of the total particles exceed 75 µm in size.
- **FAT CONTENT**

Sample: 4 g from material previously dried under vacuum at 100° for about 5 h

Analysis: Extract the fat from the *Sample* with anhydrous ethyl ether, using a continuous extraction apparatus, the extraction period being 4 h at a condensation rate of 5–6 drops/s. Evaporate the ether from the extract, transfer it to a tared beaker, and dry at 100° to constant weight. Perform a blank determination. Calculate the percentage of fat found, corrected for the blank.

Acceptance criteria: NLT 0.2% on the previously dried basis

- **VISCOSITY—ROTATIONAL METHODS** ⟨912⟩

Sample solution: Transfer 25 g of Colloidal Oatmeal in small portions, with stirring at 1000 rpm over a 1-min period, to 500 mL of water contained in a beaker, maintained at 45° and equipped with a variable speed mixer. Stir for 5 min after the addition of the last portion of oatmeal. Allow the suspension to stand for 90 min, and equilibrate to ambient temperature. Stir the suspension at 800 rpm for 1 min.

Apparatus: Equip a suitable rotational viscometer with a spindle having a cylinder 1.88 cm in diameter and 6.25 cm high attached to a shaft 0.32 cm in diameter, the distance from the top of the cylinder to the lower tip of the shaft being 0.75 cm, and the immersion depth being 8.15 cm (No. 1 spindle).

Analysis: Determine and record the viscosity of the suspension, with the spindle rotating at 60 rpm. Convert to centipoise by multiplying the reading by the constant for the viscometer spindle and speed employed.

Acceptance criteria: The average of three viscosities obtained is greater than 1 and less than 100 centipoises.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

www.webofpharma.com

Construct Human Fibroblasts in Bilayer Synthetic Scaffold

DEFINITION

Construct Human Fibroblasts in Bilayer Synthetic Scaffold is a nonliving monolayer skin substitute derived from neonatal foreskins. It is composed of fibroblasts, an extracellular matrix, and a nylon mesh bonded to a transparent, semi-permeable silicone membrane. Human fibroblasts are seeded onto the nylon mesh. The fibroblasts proliferate within the nylon mesh and secrete human matrix proteins. Following freezing, no cellular metabolic activity remains.

The fibroblast-cell banks, from which Construct Human Fibroblasts in Bilayer Synthetic Scaffold is derived, test negative for human and animal viruses and retroviruses, and are also tested for normal cell morphology, human karyology, and isoenzymes. Maternal blood sera are tested for evidence of infection with human immunodeficiency virus types 1 and 2, hepatitis B and C viruses, syphilis, and human T-lymphotropic virus type 1, and are found negative for the purpose of donor selection.

Construct Human Fibroblasts in Bilayer Synthetic Scaffold is manufactured with sterile components under aseptic conditions within the final package. All materials derived from bovine sources originate from countries free of bovine spongiform encephalopathy. During subsequent screening of the fibroblast cell strain at various stages in the manufacturing process, testing for these same viruses, as well as Epstein-Barr virus and human T-lymphotropic virus type 2, is carried out and found to be negative. The final product is inspected and tested to ensure that the product meets specifications.

SPECIFIC TESTS

- **HISTOLOGICAL CHARACTERIZATION**

Buffered formalin: Prepare a solution containing 10% (w/v) formaldehyde solution and 1.0%–1.5% methanol in a suitable buffer, adjusted to a pH of 6.8–7.2.[1]

Preparation of tissue for staining: Cut Construct Human Fibroblasts in Bilayer Synthetic Scaffold into 3-mm × 3-mm sections. Place three sections into suitable histological tissue cassettes,[2] and insert the cassettes into suitable histological cassette basket(s).[3]

Embedding and sectioning: At a temperature of 40°, sequentially immerse the histological cassette basket(s) in separate solutions of *Buffered formalin* (2 h), two changes of 80% alcohol (30 min/step), alcohol (30 min), three changes of dehydrated alcohol (30 min/step), suitable histological xylene substitute (30 min),[4] and two changes of suitable xylene substitute (30 min/step). Immerse the histological cassette basket(s) into molten paraffin[5] that is at a temperature of 60° for 30 min. Remove the cassette basket(s), and immerse in a fresh container of molten paraffin at 60° for 60 min. Remove the histological tissue cassette from the container and basket, and disassemble. Fill preheated embedding molds with molten paraffin heated to 56°–60°, and place on top of a preheated warming platform that is designed for histology work. Transfer Construct Human Fibroblasts in Bilayer Synthetic Scaffold specimens from the cassettes using forceps, and place specimens into individual molds. Orient the specimens in molds so as to cut cross-sections. Cool the paraffin by sliding the mold down the platform to its cool side until the paraffin has solidified. Maintain specimen orientation with forceps during cooling, removing the forceps when the paraffin becomes translucent. Slide the paraffin block onto a histological cold plate to rapidly cool the block. Trim the paraffin block with a new single-edged razor blade to form a rectangle or slight trapezoid to within 5 mm of the tissue mass, if necessary. Cool the block at 4° for 15–30 min. Clamp the tissue block into the block holder of the microtome. Fill a histological tissue flotation water bath with fresh water, add an appropriate amount of a suitable histological adhesive,[6] and heat to 5° less than the melting point of the paraffin. Properly mount and adjust the tissue and paraffin block into a microtome. Set the microtome to make cuts 5 µm thick with a blade angle of 5 ± 2°. Insert a sharp stainless steel microtome knife that has been properly honed, or a new disposable microtome knife, into the knife holder. Cut a ribbon that contains 6–10 sections of Construct Human Fibroblasts in Bilayer Synthetic Scaffold. Pick up the ribbon with forceps, and stretch it across the tissue flotation water bath. Separate 2–3 adjacent sections from the ribbon on the water bath. The selected sections should not be compressed, wrinkled, or scratched. Pick up the selected sections by dipping a microscope slide into the water bath under the floating sections, and gently lift the slide out of the water. For each staining procedure, prepare three slides from each of the three starting Construct Human Fibroblasts in Bilayer Synthetic Scaffold 3-mm × 3-mm sections. Allow the mounted sections to air-dry completely, or dry the slide in a 60° oven for 1 h.

Hematoxylin–eosin staining

Hematoxylin–alcohol solution: Dissolve 2.5 g of hematoxylin in 25.0 mL of dehydrated alcohol with heating.

Potassium alum solution: Dissolve 50.0 g of potassium alum in 500 mL of water with heating.

Hematoxylin staining solution: Mix *Hematoxylin–alcohol solution* and *Potassium alum solution*. Bring to a boil as rapidly as possible with constant stirring. Do not heat for more than 1 min. Slowly add 0.185 g of sodium iodate. Reheat to a simmer until the solution becomes a deep purple. Remove from heat, and quickly cool. Filter daily before use.

10% acid alcohol: Add 5.0 mL of hydrochloric acid to 495 mL of 70% alcohol.

Eosin solution: Dissolve 1 g of eosin Y in 100 mL of alcohol. Filter daily before use.

Analysis: Sequentially immerse the microscope slide with affixed tissue, as prepared in *Preparation of tissue for staining*, in three changes of a suitable histological, aliphatic xylene substitute (2 min/step), three changes of dehydrated alcohol (1 min/step), alcohol (20 s), running tap water rinse (1 min), *Hematoxylin staining solution* (4–5 min), running tap water rinse (1 min), *10% acid alcohol* (15 s), running tap water rinse (1 min), a suitable bluing reagent[7] (20–30 s), running tap water rinse (1 min), alcohol (20 s), *Eosin solution* (10–20 s, until a reddish-brown color is obtained in the tissue), three changes of dehydrated alcohol (10 s/step), and three changes of a suitable histological xylene substitute (10 s/

[1] A suitable *Buffered formalin* can be obtained from VWR International, 1310 Goshen Pkwy., West Chester, PA 19380.

[2] A suitable histological tissue cassette can be obtained from Sakura Finetek U.S.A., Inc., 1750 West 214th St., Torrance, CA 90501.

[3] A suitable histological tissue cassette basket can be obtained from Sakura Finetek U.S.A., Inc., 1750 West 214th St., Torrance, CA 90501.

[4] A suitable histological xylene substitute is Citrosolve® Clearing Agent, available from Fisher Scientific, 200 Park Ln., Pittsburgh, PA 15275.

[5] A suitable paraffin for use is Tissue Prep* 2 Embedding Media, available from Fisher Scientific, 200 Park Ln., Pittsburgh, PA 15275.

[6] A suitable histological adhesive for use is Histoslide® Adhesive, which can be obtained from Poly Scientific Research Corp., 70 Cleveland Ave., Bay Shore, NY 11706-1282.

[7] A suitable bluing reagent can be obtained from Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178.

USP Monographs

www.webofpharma.com

step). Adjust the above immersion times as needed to suitably stain the tissue. Remove the slide from the last histological xylene substitute wash, and blot dry the back of the slide. Do not allow the tissue to dry. Affix a coverslip over the tissue using a coverslip mountant.

Acceptance criteria: Using USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 1 (hematoxylin–eosin stained)[8] for comparison, the nylon-scaffold mesh, silicone membrane, and secreted collagen-based matrix are present, and the tissue contains normal human fibroblast distributed throughout the secreted matrix, and resembles normal human papillary dermis. The fibroblasts appear elongated and spindle shaped. The tissue contains about 10^6 cells/cm^2 and about 500 cells/mm along the section.

Collagen staining

Bouins' solution: Mix 75.0 mL of 1.22% picric acid solution, 25.0 L of dimethoxymethane, and 5.0 L of acetic acid.

Weigert's iron hematoxylin solution A: Dissolve 1.0 g of hematoxylin in 100 mL of alcohol.

Weigert's iron hematoxylin solution B: Mix 4.0 mL of 29% ferric chloride, 95.0 mL of water, and 1.0 mL of hydrochloric acid.

Weigert's iron hematoxylin working solution: Combine *Weigert's iron hematoxylin solution A* and *Weigert's iron hematoxylin solution B* (1:1). Pass the solution through a suitable filter of 0.45-µm pore size. Prepare fresh as needed.

Gomori's trichrome solution: Mix 1.0 mL of acetic acid and 100 mL of water. Dissolve 0.6 g of chromotrope 2R, 0.3 g of Fast Green FCF, and 0.6 g of phosphotungstic acid.

1% acetic acid: Dilute 1 mL of glacial acetic acid with water to make 100 mL of solution.

Analysis: Sequentially immerse the microscope slide with affixed tissue, as prepared in *Preparation of tissue for staining*, in three changes of a suitable histological, aliphatic xylene substitute (2 min/step), three changes of dehydrated alcohol (1 min/step), alcohol (20 s), and running tap water rinse (1 min). Immerse the slide in *Bouins' solution*, and place in a 42° water bath for 1 h. Rinse the slide in water for 10 s. Sequentially immerse the slide in *Weigert's iron hematoxylin working solution* (10 min) and running tap water rinse (10 min). Rinse the slide in water for 10 s, and immerse in *Gomori's trichrome solution* (3–5 min). Rinse the slide in *1% acetic acid* for 20 s. Sequentially immerse the slide in three changes of alcohol (10 s/step) and three changes of a suitable histological, aliphatic xylene substitute (10 s/step). Affix a coverslip over the tissue using a suitable coverslip mountant. Nuclei will stain black; cytoplasm, keratin, and muscle fibers will stain red; and collagen and mucin will stain blue.

Acceptance criteria: Using USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 2 for comparison, collagen is found throughout the extracellular matrix. The tissue contains normal human fibroblast distributed throughout the secreted matrix and resembles normal human papillary; dermis, muscle fibers, and keratin are absent.

Distribution of fibronectin

Tris-saline buffer: Combine 0.1 M tris–(hydroxymethyl) aminomethane hydrochloride and 0.15 M sodium chloride, and adjust to a pH of 7.8.

3% Hydrogen peroxide: Dilute 30 mL of hydrogen peroxide with water or methanol.

Diaminobenzidine solution: Use a suitable solution.[9]

Hematoxylin staining solution: Prepare as directed for *Hematoxylin–eosin staining*.

Analysis: The microscope slide with affixed tissue as prepared in *Preparation of tissue for staining* is dried either overnight at 37° or for 1 h at 60°. The microscope slide with affixed tissue as prepared in *Preparation of tissue for staining* is sequentially immersed in three changes of a suitable histological, aliphatic xylene substitute (2 min/step), three changes of dehydrated alcohol (1 min/step), alcohol (20 s), and running tap water rinse (1 min). Sequentially immerse the slide in *Tris-saline buffer* (10 min), *3% hydrogen peroxide* (30 min), three changes of *Tris-saline buffer* (1 min/step), a suitable normal rabbit serum[10] (30 min), water (5 min), and three changes of *Tris-saline buffer* (1 min/step). Incubate the slide with a suitable solution of rabbit anti-human fibronectin antibody,[11] diluted using a suitable antibody diluent[12] to an antibody concentration of 21.0 ± 2.1 mg/L for 1 h. Sequentially immerse the slide in water (5 min) and three changes of *Tris-saline buffer* (1 min/step). Place enough drops of a biotinylated goat anti-rabbit antibody solution[13] to cover the tissue section, and incubate for 30 min. Sequentially immerse the slide in water (5 min) and three changes of *Tris-saline buffer* (1 min/step). Place enough drops of a streptavidin conjugated horseradish peroxidase solution[14] to cover the tissue section, and incubate for 30 min. Sequentially immerse the slide in water (5 min) and three changes of *Tris-saline buffer* (1 min/step). Incubate the slide with *Diaminobenzidine solution* for 1–5 min, until a suitable difference in staining is seen by comparison with a control in which the fibronectin (primary) antibody is omitted. Sequentially immerse the slide in water (1 min), *Hematoxylin staining solution* (4–5 min), and water (1 min). Do not allow the tissue to dry. Affix a coverslip over the tissue using a low-viscosity, aqueous, synthetic-resin coverslip mountant.

Acceptance criteria: Using USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 3 (diaminobenzidine–hematoxylin stained) for comparison, fibronectin binds to collagen and is found throughout the extracellular matrix. Fibronectin is found colocalizing with the collagen fibers. The intensity of staining may vary from region to region of the slide.

• **METABOLIC ACTIVITY ASSESSMENT**

DPBS solution A: Dissolve 1.32 g of calcium chloride and 1.21 g of magnesium sulfate heptahydrate in 2 L of water.

DPBS solution B: Dissolve 80.0 g of sodium chloride, 2.0 g of potassium chloride, 11.5 g of dibasic sodium phosphate,

[8] These photomicrographs are available as a CD from the USP Reference Standards collection, available to the user through USP Customer Services. To order these and other Reference Standards, call 1-800-227-8772 (U.S. and Canada), +1-301-881-0666 or 00-800-4875-5555 (select Europe); or go online to www.usp.org. Order item number 1535857.

[9] A suitable Diaminobenzidine solution can be obtained from Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178; catalog number D-6815.

[10] A suitable normal rabbit serum can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

[11] Suitable rabbit anti-human fibronectin antibodies can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

[12] Suitable antibody diluent can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

[13] Suitable biotinylated goat anti-rabbit antibody solution can be obtained from BioGenex, 4600 Norris Canyon Rd., San Ramon, CA 94583.

[14] A suitable streptavidin conjugated horseradish peroxidase solution can be obtained from BioGenex, 4600 Norris Canyon Rd., San Ramon, CA 94583.

www.webofpharma.com

2.0 g of monobasic potassium phosphate, 10.0 g of glucose, 0.36 g of sodium phosphate, 0.5 g of streptomycin sulfate, and 1,000,000 USP Units of penicillin G sodium in 8 L of water.

DPBS working solution: Mix *DPBS solution B* and *DPBS solution A* (8:2). Pass the solution through a filter of 0.22-µm pore size.

Dulbecco's modified Eagle's tissue culture medium: Prepare a solution that contains the components listed in *Table 1*.

Table 1

Component	mg/L
Calcium chloride	264.9
Ferric nitrate nonahydrate	0.10
Potassium chloride	400.0
Magnesium sulfate heptahydrate	200.0
Sodium chloride	6,400.0
Sodium bicarbonate	3,700.0
Sodium phosphate, monobasic (monohydrate)	125.0
Dextrose	4,500.0
Phenol red	15.0
Sodium pyruvate	110.0
L-Arginine hydrochloride	84.0
L-Cystine	48.0
Aminoacetic acid	30.0
L-Histidine hydrochloride monohydrate	42.0
L-Isoleucine	104.8
L-Leucine	104.8
L-Lysine hydrochloride	146.2
L-Methionine	30.0
L-Phenylalanine	66.0
L-Serine	42.0
L-Threonine	95.2
L-Tryptophan	16.0
L-Tyrosine	72.0
L-Valine	93.6
D-Calcium pantothenate	4.0
Choline chloride	4.0
Folic acid	4.0
Inositol	7.0
Nicotinamide	4.0
Pyridoxine hydrochloride	4.0
Riboflavin	0.40
Thiamine hydrochloride	4.0

L-Glutamine solution: Prepare 100 mL of a solution containing 2.92 g of L-glutamine.

Sodium pyruvate solution: Prepare 100 mL of a solution containing 1.10 g of sodium pyruvate.

Antibiotic-antimycotic solution: Prepare 100 mL of a solution containing 0.85 g of sodium chloride, 10,000 USP Units of penicillin G sodium, 10,000 µg of streptomycin (base), and 25 µg of amphotericin B in water.

Assay stock medium: Mix 1000 mL of *Dulbecco's modified Eagle's tissue culture medium*, 10 mL of *L-Glutamine solution*, 10 mL of *Sodium pyruvate solution*, 10 mL of *Antibiotic-antimycotic solution*, and 20 mL of fetal bovine serum.[15]

MTT–assay solution: Dissolve 0.50 g of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide in 1 L of *Assay stock medium*, using constant stirring.

Sterilize the solution by passing it through a filter of 0.2-µm pore size.

MTT formazan stock solution: 100 µg/mL of 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan in isopropyl alcohol

MTT formazan calibration solutions: 15, 30, 45, 60, and 75 µg/mL of MTT formazan, using *MTT formazan stock solution* and diluting with isopropyl alcohol

Analysis: Thaw Construct Human Fibroblasts in Bilayer Synthetic Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Bilayer Synthetic Scaffold unit. Cut three 11-mm × 11-mm sections of Construct Human Fibroblasts in Bilayer Synthetic Scaffold, and immerse the sections into separate, 3.0-mL portions of *MTT–assay solution*. Incubate for 2 h at 37 ± 2° in a 3%–7% CO_2–air environment with shaking on an orbital shaker at 150–200 rpm. After incubation, remove from the 37°, 3%–7% CO_2–air environment. Remove the *MTT–assay solution*, and rinse twice with *DPBS working solution*. Immerse the Construct Human Fibroblasts in Bilayer Synthetic Scaffold in 2 mL of isopropyl alcohol, and incubate at ambient temperature for 1 h with shaking on an orbital shaker at approximately 125 rpm. Transfer 200-µL aliquots of the five *MTT formazan calibration solutions*, in triplicate, and 200-µL aliquots of the three isopropyl alcohol extracts of Construct Human Fibroblasts in Bilayer Synthetic Scaffold to a suitable 96-well, flat-bottom plate. Read the absorbance of each aliquot at 540 nm, using 200 µL of isopropyl alcohol as the blank.

Plot the responses of the *MTT formazan calibration solutions* versus concentration, in µg of MTT formazan/mL, and calculate the regression line using the least-squares method.

System suitability requirements: The test is considered valid if the regression line has a square of the correlation coefficient NLT 0.95.

Acceptance criteria: The absorbance value of individual Construct Human Fibroblasts in Bilayer Synthetic Scaffold sections at 540 nm is less than 0.1.

- **DNA CONTENT**

Cell culture water: Sterile water containing NMT 0.005 USP Endotoxin Unit/mL

DNA extraction buffer: Transfer 850 mL of *Cell culture water* to a sterile, 1-L graduated container. Dissolve 12.110 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 3.802 g of edetate disodium, 23.380 g of sodium chloride, and 0.080 g of sodium dodecyl sulfate, with stirring. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 7.0. Dilute with *Cell culture water* to 1 L.

Proteinase K solution: Prepare a solution of Tritirachium album proteinase K in 10 mM of 2-amino-2-hydroxymethyl-1,3-propanediol, adjusted to a pH of 7.5, having an activity of 600 units/mL.[16]

[15] A suitable fetal bovine serum can be obtained from HyClone, 925 West 1800 South, Logan, UT 84321; catalog number SH30070.03.

[16] A suitable Proteinase K solution can be obtained from Roche Diagnostics Corp., Roche Applied Sciences, P.O. Box 50414, 9115 Hague Rd., Indianapolis, IN 46250-0414.

www.webofpharma.com

Working DNA extraction buffer: Add 1.22 mL of *Proteinase K solution* to 38.78 mL of *DNA extraction buffer*, and mix.

Dilution buffer: Transfer 850 mL of *Cell culture water* to a sterile, 1-L graduated container. Add 1.211 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 3.802 g of edetate disodium, and 5.844 g of sodium chloride, with stirring. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 7.0. Dilute with *Cell culture water* to 1 L.

DPBS without Ca^{++}, Mg^{++} solution: Prepare a solution containing 8.00 g/L of sodium chloride, 1.15 g/L of dibasic sodium phosphate (anhydrous), 0.20 g/L of potassium chloride, and 0.20 g/L of monobasic potassium phosphate in water.

Calf thymus DNA solution: Prepare a solution containing 1 mg/L of calf thymus DNA in *DPBS without Ca^{++}, Mg^{++} solution*, mixing thoroughly for 12–24 h at ambient temperature. Dilute the resulting solution with *DPBS without Ca^{++}, Mg^{++} solution* to prepare a solution containing 50 µg/mL of calf thymus DNA, mixing thoroughly on a vortex mixer for 10 min.

Calf thymus DNA calibration solutions: Prepare four calibration solutions containing 5, 10, 15, and 20 µg/mL of calf thymus DNA, using *Calf thymus DNA solution*, and diluting with *DPBS without Ca^{++}, Mg^{++} solution*.

DNA staining solution: Prepare a solution containing 0.5 µg of 2′-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi(1H-benzimidazole) trihydrochloride pentahydrate/mL of *Dilution buffer*. Store in low-actinic glassware.

Analysis: Thaw Construct Human Fibroblasts in Bilayer Synthetic Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Bilayer Synthetic Scaffold unit. Cut three 11-mm × 11-mm sections of Construct Human Fibroblasts in Bilayer Synthetic Scaffold. To each of three microcentrifuge tubes add 1 mL of *DPBS without Ca^{++}, Mg^{++} solution*. Immerse a single Construct Human Fibroblasts in Bilayer Synthetic Scaffold 11-mm × 11-mm section into each microcentrifuge tube to remove the cryopreservative. Aspirate the *DPBS without Ca^{++}, Mg^{++} solution* from each tube, and replace with 1 mL of *Working DNA extraction buffer*, making sure that each Construct Human Fibroblasts in Bilayer Synthetic Scaffold is completely submerged in the extraction buffer. Incubate the samples in a 56°–60° water bath for 4–18 h. Sonicate for 10–15 s using an ultrasonic cell disrupter to achieve complete cellular disruption of the tissue and to mix the contents of the tube. Centrifuge the microcentrifuge tubes at 12,000–15,000 × g to pellet non-DNA material. Transfer three 50-µL aliquots of each sample supernatant to individual wells of a 96-well black plate suitable for performing fluorescent analysis. Transfer triplicate 50-µL aliquots of each of the *Calf thymus DNA calibration solutions* to the 96-well plate, as well as a 50-µL aliquot of *DPBS working solution* for the blank. Add 150 µL of *DNA staining solution* to all wells containing the tissue samples, *Calf thymus DNA calibration solutions*, and the blank. Cover with aluminum foil, and place in a dark cabinet for 30–45 min at 15°–30°. Read the fluorescence of each well, using an excitation wavelength of 355 nm and an emission wavelength of 460 nm, blanking against the *DPBS without Ca^{++}, Mg^{++} solution* well. Plot the responses of the *Calf thymus DNA calibration solutions* versus concentration, in µg/mL of calf thymus DNA, and calculate the regression line using the least-squares method. The test is considered valid if the %CV of the replicate values is less than 15%, the slope is 4.48–6.27, the *y*-intercept is between −2.04 and 3.65, and the square of the correlation coefficient is NLT 0.990. From the regression line so obtained, determine the amount of DNA, in µg/11-mm × 11-mm sample.

Acceptance criteria: The amount of DNA of an individual Construct Human Fibroblasts in Bilayer Synthetic Scaffold 11-mm × 11-mm section is between 6 and 14 µg.

• **TOTAL COLLAGEN CONTENT**

DPBS without Ca^{++}, Mg^{++} solution: Proceed as directed for *DNA Content*.

DPBS with Ca^{++}, Mg^{++} solution: 8.00 mg/mL of sodium chloride, 1.15 mg/mL of dibasic sodium phosphate (anhydrous), 0.20 mg/mL of potassium chloride, 0.20 mg/mL of monobasic potassium phosphate, 0.10 mg/mL of magnesium chloride hexahydrate, and 0.10 mg/mL of calcium chloride (anhydrous) in water

Collagenase extraction solution: At least 250 Units/mL of *Clostridium histolyticum* collagenase, type 2, in *DPBS with Ca^{++}, Mg^{++} solution*

2% Acetic acid solution: Mix 10 mL of acetic acid with 490 mL of water.

Collagen standard stock solution: 2 mg/mL of collagen, type I, in *2% Acetic acid solution*

Collagen calibration standards: Cut polyglactin mesh[17] into seventeen 11-mm × 11-mm squares, and place one square into 17 individual wells of a 24-well plate. Each well of the 24-well plate has a surface area of 220 mm^2 and a volume of 3.5 mL. In quadruplicate, prepare wells containing 0.050, 0.100, 0.200, and 0.400 mg of collagen by adding 25, 50, 100, and 200 µL, respectively, of the *Collagen standard stock solution*. The remaining well to which no *Collagen standard stock solution* has been added is used as the blank. Allow the wells to air dry.

Sirius red solution: 1 mg/mL of Direct Red 80 in saturated picric acid

1% (*p-tert*-Octylphenoxy) polyethoxyethanol solution: Mix 10 mL of (*p-tert*-Octylphenoxy) polyethoxyethanol in 990 mL of *DPBS with Ca^{++}, Mg^{++} solution*.

Analysis: Thaw Construct Human Fibroblasts in Bilayer Synthetic Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Bilayer Synthetic Scaffold unit. Cut three 11-mm × 11-mm sections of Construct Human Fibroblasts in Bilayer Synthetic Scaffold. Place each test section into separate wells of a 24-well plate. Add 200 µL of *1% (p-tert-Octylphenoxy) polyethoxyethanol solution* to each sample. Shake on a rotating platform shaker at 100–150 rpm for 60–70 min at room temperature. Aspirate off the *1% (p-tert-Octylphenoxy) polyethoxyethanol solution*, and rinse three times with 2 mL of *DPBS without Ca^{++}, Mg^{++} solution*. Transfer each of the collagen standards to individual wells of the 24-well plate. Add 0.5 mL of *Sirius red solution* to each test sample and collagen standards. Shake on a rotating platform shaker at 100–150 rpm for 60 min at room temperature. Aspirate off the *Sirius red solution* from each well. Rinse each well twice with 2 mL of *DPBS without Ca^{++}, Mg^{++} solution*. Add an additional 2 mL of *DPBS without Ca^{++}, Mg^{++} solution* to each well, and allow to stand for 2 min. Aspirate off the *DPBS without Ca^{++}, Mg^{++} solution*, and rinse twice more with 2 mL of *DPBS without Ca^{++}, Mg^{++} solution*. Aspirate off all traces of *DPBS without Ca^{++}, Mg^{++} solution*. Add 0.5 mL of *Collagenase extraction solution* to each well containing the *Collagen calibration standards*. Add 2.0 mL of *Collagenase extraction solution* to each well containing test samples. Rotate the plate on an orbital rotator at 150 rpm for 90 min at 37°. Transfer 200 µL from each well, to a

[17] A suitable polyglactin mesh can be obtained from Ethicon Co., Johnson & Johnson Corp., 425 Hoes Ln., P.O. Box 6800, Piscataway, NJ 08855.

USP Monographs

www.webofpharma.com

suitable 96-well, flat-bottom plate. Read the absorbance of each aliquot at 540 nm. Dilute the Construct Human Fibroblasts in Bilayer Synthetic Scaffold sample preparation further with *DPBS without Ca ++, Mg ++ solution* if the absorbance is greater than the absorbance of the highest of the *Collagen calibration standards*.

Plot the responses of the *Collagen calibration standards* versus the amount, in mg of collagen, and calculate the regression line using the least-squares method.

System suitability requirements: The test is considered valid if the slope is 3.00–5.00 and the square of the correlation coefficient is ≥ 0.950.

Determine the collagen content, in mg, of an 11- × 11-mm section of Construct Human Fibroblasts in Bilayer Synthetic Scaffold from the regression line, and by using the following equation:

$$\text{Result} = D \times A \times SC_{SR}$$

D = dilution factor (normally 4, unless the sample had to be further diluted)
A = absorbance at 540 nm
SC_{SR} = slope of the regression line of the standards calculated above

Acceptance criteria: The amount of collagen in individual Construct Human Fibroblasts in Bilayer Synthetic Scaffold 11- × 11-mm samples is 0.50–4.0 mg.

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩

Sample solution: Thaw Construct Human Fibroblasts in Bilayer Synthetic Scaffold by placing the tissue, still in its polycarbonate cassette contained in a plastic covering bag, in a water bath heated to a maximum of 37° for 15–20 min until no visible ice remains in the cassette. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Bilayer Synthetic Scaffold unit.

Analysis: Remove the unit from the polycarbonate cassette, and immerse in 25 mL of LAL Reagent Water. Extract for 60 min at 37° with shaking on an orbital shaker set at 125 revolutions/min. Remove a 4-mL aliquot of the extract for testing.

Acceptance criteria: NMT 0.5 USP Endotoxin Unit/mL

• **STERILITY TESTS** ⟨71⟩

Sample solution: Thaw Construct Human Fibroblasts in Bilayer Synthetic Scaffold by placing the tissue, still in its polycarbonate cassette contained in a plastic covering bag, in a water bath heated to a maximum of 37° for 15–20 min until no visible ice remains in the cassette. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Bilayer Synthetic Scaffold unit.

Analysis: Perform the test on 20 mL of the cryopreservative.

Acceptance criteria: Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Construct Human Fibroblasts in Bilayer Synthetic Scaffold is aseptically packaged and supplied frozen in a clear plastic cassette containing two, approximately 12.5- × 19-cm units. The solution within the cassette is a phosphate-buffered cryoprotectant solution used to facilitate long-term storage. A clear plastic bag surrounds the cassette for its protection. Construct Human Fibroblasts in Bilayer Synthetic Scaffold should be stored at a temperature of –70° to –20° for no longer than 18 months.

• **LABELING:** The label indicates the dimensions of the Construct Human Fibroblasts in Bilayer Synthetic Scaffold material enclosed. It contains the expiry date, required storage conditions, and the lot number. The label cautions that Construct Human Fibroblasts in Bilayer Synthetic Scaffold is not to be used if the package shows signs of damage. Additional labeling requirements include instructions on the proper thawing and handling of Construct Human Fibroblasts in Bilayer Synthetic Scaffold and the time frame for use after package opening.

• **USP REFERENCE STANDARDS,** *Authentic Visual References* ⟨11⟩**:** USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrographs. These three photomicrographs represent examples of passing units, prepared as directed in *Hematoxylin–eosin staining*, *Collagen staining*, and *Distribution of fibronectin*. They are specified to assist in ascertaining histological quality. The fibroblasts are embedded in an extracellular matrix that they have secreted (USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 1). The collagen (USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 2) and fibronectin (USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 3) are to be found throughout the extracellular matrix. The nylon fibers (yellow in USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 1) and the silicone backing (gray in USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 1) are frequently visible, although easily lost during processing.

Construct Human Fibroblasts in Polyglactin Scaffold

DEFINITION

Construct Human Fibroblasts in Polyglactin Scaffold is a living monolayer skin substitute derived from neonatal foreskins. It is composed of fibroblasts, an extracellular matrix, and a bioabsorbable scaffold. Human fibroblasts are seeded onto a bioabsorbable, nonantigenic and nonpyrogenic mesh scaffold composed of polyglactin, a copolymer of glycolide and lactide. The fibroblast-cell banks, from which Construct Human Fibroblasts in Polyglactin Scaffold are derived have passed applicable donor eligibility requirements for relevant communicable diseases. Maternal blood sera are tested for evidence of infection with human immunodeficiency virus types 1 and 2, hepatitis B and C viruses, syphilis, and human T-lymphotropic virus type 1 and are found negative for the purpose of donor selection. Construct Human Fibroblasts in Polyglactin Scaffold is manufactured with sterile components under aseptic conditions within the final package. Reagents used in the manufacture of Construct Human Fibroblasts in Polyglactin Scaffold are tested and found free of viruses, retroviruses, endotoxins, and mycoplasma before use. All materials derived from bovine sources originate from countries free of bovine spongiform encephalopathy. During subsequent screening of the fibroblast cell strain at various stages in the manufacturing process, testing for these same viruses, as well as Epstein-Barr virus and human T-lymphotropic virus type 2, is carried out and found to be negative. The final product is inspected and tested to ensure that the product meets specifications.

SPECIFIC TESTS

• **HISTOLOGICAL EVALUATION**

Buffered formalin: Prepare a solution containing 10% (w/v) formaldehyde solution and 1.0%–1.5% methanol in a suitable buffer, adjusted to a pH of 6.8–7.2.[1]

[1] A suitable *Buffered formalin* can be obtained from VWR International, 1310 Goshen Pkwy., West Chester, PA 19380.

USP Monographs

www.webofpharma.com

Preparation of tissue for staining: Cut Construct Human Fibroblasts in Polyglactin Scaffold into 3-mm × 3-mm sections. Place three sections into suitable histological tissue cassettes,[2] and insert the cassettes into suitable histological cassette basket(s).[3] At a temperature of 40°, sequentially immerse the histological cassette basket(s) in separate solutions of *Buffered formalin* (2 h), two changes of 80% alcohol (30 min/step), alcohol (30 min), three changes of dehydrated alcohol (30 min/step), suitable histological xylene substitute (30 min),[4] and two changes of suitable xylene substitute (30 min/step). Immerse the histological cassette basket(s) into molten paraffin[5] that is at a temperature of 60° for 30 min. Remove the cassette basket(s), and immerse in a fresh container of molten paraffin at 60° for 60 min. Remove the histological tissue cassette from the container and basket, and disassemble. Fill preheated embedding molds with molten paraffin heated to 56°–60°, and place on top of a preheated warming platform that is designed for histology work. Transfer Construct Human Fibroblasts in Polyglactin Scaffold specimens from the cassettes using forceps, and place specimens into individual molds. Orient the specimens in molds so as to cut cross-sections. Cool the paraffin by sliding the mold down the platform to its cool side until the paraffin has solidified. Maintain specimen orientation with forceps during cooling, removing the forceps when the paraffin becomes translucent. Slide the paraffin block onto a histological cold plate to rapidly cool the block. Trim the paraffin block with a new single-edged razor blade to form a rectangle or slight trapezoid to within 5 mm of the tissue mass, if necessary. Cool the block at 4° for 15–30 min. Clamp the tissue block into the block holder of the microtome. Fill a histological tissue flotation water bath with fresh water, add an appropriate amount of a suitable histological adhesive,[6] and heat to 5° less than the melting point of the paraffin. Properly mount and adjust the tissue and paraffin block into a microtome. Set the microtome to make cuts 5-µm thick with a blade angle of 5 ± 2°. Insert a sharp stainless steel microtome knife that has been properly honed or a new disposable microtome knife into the knife holder. Cut a ribbon that contains 6–10 sections of Construct Human Fibroblasts in Polyglactin Scaffold. Pick up the ribbon with forceps, and stretch it across the tissue flotation water bath. Separate 2–3 adjacent sections from the ribbon on the water bath. The selected sections should not be compressed, wrinkled, or scratched. Pick up the selected sections by dipping a microscope slide into the water bath under the floating sections, and gently lift the slide out of the water. For each staining procedure, prepare three slides from each of the three starting Construct Human Fibroblasts in Polyglactin Scaffold 3-mm × 3-mm sections. Allow the mounted sections to air-dry completely, or dry the slide in a 60° oven for 1 h.

Hematoxylin–eosin staining

Hematoxylin–alcohol solution: Dissolve 2.5 g of hematoxylin in 25.0 mL of dehydrated alcohol with heating.

Potassium alum solution: Dissolve 50.0 g of potassium alum in 500 mL of water with heating.

Hematoxylin staining solution: Mix *Hematoxylin–alcohol solution* and *Potassium alum solution*. Bring to a boil as rapidly as possible with constant stirring. Do not heat for more than 1 min. Slowly add 0.185 g of sodium iodate. Reheat to a simmer until the solution becomes a deep purple. Remove from heat, and quickly cool. Filter daily before use.

10% Acid alcohol: Add 5.0 mL of hydrochloric acid to 495 mL of 70% alcohol.

Eosin solution: Dissolve 1 g of eosin Y in 100 mL of alcohol. Filter daily before use.

Analysis: Sequentially immerse the microscope slide with affixed tissue, as prepared in *Preparation of tissue for staining*, in three changes of a suitable histological, aliphatic xylene substitute (2 min/step), three changes of dehydrated alcohol (1 min/step), alcohol (20 s), running tap water rinse (1 min), *Hematoxylin staining solution* (4–5 min), running tap water rinse (1 min), *10% Acid alcohol* (15 s), running tap water rinse (1 min), a suitable bluing reagent[7] (20–30 s), running tap water rinse (1 min), alcohol (20 s), *Eosin solution* (10–20 s, until a reddish-brown color is obtained in the tissue), three changes of dehydrated alcohol (10 s/step), and three changes of a suitable histological xylene substitute (10 s/step). Adjust the above immersion times as needed to suitably stain the tissue. Remove the slide from the last histological xylene substitute wash, and blot dry the back of the slide. Do not allow the tissue to dry. Affix a coverslip over the tissue using a coverslip mountant.

Acceptance criteria: Using USP Construct Human Fibroblasts in Polyglactin Scaffold Reference Photomicrograph 1[8] for comparison, the test article shows a polyglactin scaffold mesh and a secreted collagen-based matrix; the tissue contains normal human fibroblast distributed throughout the secreted matrix and resembles normal human papillary dermis. Fibroblasts appear elongated and spindle-shaped. The tissue, which is 100–300 µm thick, contains 10^6 cells/cm^2.

Collagen staining

Bouins's solution: Mix 75 mL of 1.22% picric acid solution, 25 L of dimethoxymethane, and 5.0 L of acetic acid.

Weigert's iron hematoxylin solution A: Dissolve 1 g of hematoxylin in 100 mL of alcohol.

Weigert's iron hematoxylin solution B: Mix 4.0 mL of 29% ferric chloride, 95.0 mL of water, and 1.0 mL of hydrochloric acid.

Weigert's iron hematoxylin working solution: Mix equal volumes of *Weigert's iron hematoxylin solution A* and *Weigert's iron hematoxylin solution B*. Pass the solution through a filter of 0.45-µm pore size. Prepare fresh as needed.

Gomori's trichrome solution: Mix 1.0 mL of acetic acid and 100 mL of water. Dissolve 0.6 g of chromotrope 2R, 0.3 g of Fast Green FCF, and 0.6 g of phosphotungstic acid.

1% Acetic acid: Dilute 1 mL of glacial acetic acid with water to make 100 mL of solution.

Analysis: The microscope slide with affixed tissue, as prepared in *Preparation of tissue for staining*, is sequentially

[2] A suitable histological tissue cassette can be obtained from Sakura Finetek U.S.A., Inc., 1750 West 214th St., Torrance, CA 90501.

[3] A suitable histological tissue cassette basket can be obtained from Sakura Finetek U.S.A., Inc., 1750 West 214th St., Torrance, CA 90501.

[4] A suitable histological xylene substitute is Citrosolve® Clearing Agent, available from Fisher Scientific, 200 Park Ln., Pittsburgh, PA 15275.

[5] A suitable paraffin for use is Tissue Prep* 2 Embedding Media, available from Fisher Scientific, 200 Park Ln., Pittsburgh, PA 15275.

[6] A suitable histological adhesive for use is Histoslide® Adhesive, which can be obtained from Poly Scientific Research Corp., 70 Cleveland Ave., Bay Shore, NY 11706-1282.

[7] A suitable bluing reagent can be obtained from Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178.

[8] These photomicrographs are available as a CD from the USP Reference Standards collection, available to the user through USP Customer Services. To order these and other Reference Standards, call 1-800-227-8772 (U.S. and Canada), +1-301-881-0666 or 00-800-4875-5555 (select Europe); or go online to www.usp.org. Order item number 1535868.

www.webofpharma.com

immersed in three changes of a suitable histological, aliphatic xylene substitute (2 min/step), three changes of dehydrated alcohol (1 min/step), alcohol (20 s), and running tap water rinse (1 min). Immerse the slide in *Bouins's solution*, and place in a 42° water bath for 1 h. Rinse the slide in water for 10 s. Sequentially immerse the slide in *Weigert's iron hematoxylin working solution* (10 min) and running tap water rinse (10 min). Rinse the slide in water for 10 s, and immerse in *Gomori's trichrome solution* (3–5 min). Rinse the slide in *1% acetic acid* for 20 s. Sequentially immerse the slide in three changes of alcohol (10 s/step) and three changes of a suitable histological, aliphatic xylene substitute (10 s/step). Affix a coverslip over the tissue using a suitable coverslip mountant. Nuclei will stain black; cytoplasm, keratin, and muscle fibers will stain red; and collagen and mucin will stain blue.

Acceptance criteria: Using USP Construct Human Fibroblasts in Polyglactin Scaffold Reference Photomicrograph 2 for comparison, the tissue contains normal human fibroblast distributed throughout the secreted matrix and resembles normal human papillary; dermis, muscle fibers, and keratin are absent.

Distribution of fibronectin

Tris-saline buffer: Prepare a solution containing 0.1 M tris-(hydroxymethyl)aminomethane hydrochloride and 0.15 M sodium chloride, adjusted to a pH of 7.8.

3% Hydrogen peroxide: Dilute 30 mL of hydrogen peroxide with water or methanol

Diaminobenzidine solution: Use a suitable solution.[9]

Hematoxylin staining solution: Prepare as directed for *Hematoxylin–eosin staining*.

Analysis: The microscope slide with affixed tissue as prepared in *Preparation of tissue for staining* is dried either overnight at 37° or for 1 h at 60°. The microscope slide with affixed tissue as prepared in *Preparation of tissue for staining* is sequentially immersed in three changes of a suitable histological, aliphatic xylene substitute (2 min/step), three changes of dehydrated alcohol (1 min/step), alcohol (20 s), and running tap water rinse (1 min). Sequentially immerse the slide in *Tris-saline buffer* (10 min), *3% hydrogen peroxide* (30 min), three changes of *Tris-saline buffer* (1 min/step), a suitable normal rabbit serum[10] (30 min), water (5 min), and three changes of *Tris-saline buffer* (1 min/step). Incubate the slide with a suitable solution of rabbit anti-human fibronectin antibody,[11] diluted using a suitable antibody diluent[12] to an antibody concentration of 21.0 ± 2.1 mg/L for 1 h. Sequentially immerse the slide in water (5 min) and three changes of *Tris-saline buffer* (1 min/step). Place enough drops of a biotinylated goat anti-rabbit antibody solution[13] to cover the tissue section, and incubate for 30 min. Sequentially immerse the slide in water (5 min) and three changes of *Tris-saline buffer* (1 min/step). Place enough drops of a streptavidin conjugated horseradish peroxidase solution[14] to cover the tissue section, and incubate for 30 min. Sequentially immerse the slide in water (5 min) and three changes of *Tris-saline buffer* (1 min/step). Incubate the slide with *Diaminobenzidine solution* for 1–5 min, until a suitable difference in staining is seen by comparison with a control in which the fibronectin (primary) antibody is omitted. Sequentially immerse the slide in water (1 min), *Hematoxylin staining solution* (4–5 min), and water (1 min). Do not allow the tissue to dry. Affix a coverslip over the tissue using a low-viscosity, aqueous, synthetic-resin coverslip mountant.

Acceptance criteria: Using USP Construct Human Fibroblasts in Polyglactin Scaffold Reference Photomicrograph 3 for comparison, fibronectin is found colocalizing with the collagen fibers. The intensity of staining may vary from region to region of the slide.

• **METABOLIC ACTIVITY ASSESSMENT**

DPBS solution A: Dissolve 1.32 g of calcium chloride and 1.21 g of magnesium sulfate heptahydrate in 2 L of water.

DPBS solution B: Dissolve 80.0 g of sodium chloride, 2.0 g of potassium chloride, 11.5 g of dibasic sodium phosphate, 2.0 g of monobasic potassium phosphate, 10.0 g of glucose, 0.36 g of sodium phosphate, 0.5 g of streptomycin sulfate, and 1,000,000 USP Units of penicillin G sodium in 8 L water.

DPBS working solution: Mix *DPBS solution B* with *DPBS solution A* (8:2). Pass the solution through a filter of 0.22-µm pore size.

Dulbecco's modified Eagle's tissue culture medium: Prepare a solution that contains the components listed in *Table 1*.

Table 1

Component	mg/L
Calcium chloride	264.9
Ferric nitrate nonahydrate	0.10
Potassium chloride	400.0
Magnesium sulfate heptahydrate	200.0
Sodium chloride	6,400.0
Sodium bicarbonate	3,700.0
Sodium phosphate, monobasic (monohydrate)	125.0
Dextrose	4,500.0
Phenol red	15.0
Sodium pyruvate	110.0
L-Arginine hydrochloride	84.0
L-Cystine	48.0
Aminoacetic acid	30.0
L-Histidine hydrochloride monohydrate	42.0
L-Isoleucine	104.8
L-Leucine	104.8
L-Lysine hydrochloride	146.2
L-Methionine	30.0
L-Phenylalanine	66.0
L-Serine	42.0
L-Threonine	95.2
L-Tryptophan	16.0
L-Tyrosine	72.0

[9] A suitable Diaminobenzidine solution can be obtained from Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178; catalog number D-6815.

[10] A suitable normal rabbit serum can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

[11] Suitable rabbit anti-human fibronectin antibodies can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

[12] Suitable antibody diluent can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

[13] Suitable biotinylated goat anti-rabbit antibody solution can be obtained from BioGenex, 4600 Norris Canyon Rd., San Ramon, CA 94583.

[14] A suitable streptavidin conjugated horseradish peroxidase solution can be obtained from BioGenex, 4600 Norris Canyon Rd., San Ramon, CA 94583.

www.webofpharma.com

Table 1 *(continued)*

Component	mg/L
L-Valine	93.6
D-Calcium pantothenate	4.0
Choline chloride	4.0
Folic acid	4.0
Inositol	7.0
Nicotinamide	4.0
Pyridoxine hydrochloride	4.0
Riboflavin	0.40
Thiamine hydrochloride	4.0

L-Glutamine solution: Prepare a 100-mL solution containing 2.92 g of L-glutamine.

Sodium pyruvate solution: Prepare 100 mL of a solution containing 1.10 g of sodium pyruvate.

Antibiotic–antimycotic solution: Prepare 100 mL of a solution containing 0.85 g of sodium chloride, 10,000 USP Units of penicillin G sodium, 10,000 µg of streptomycin (base), and 25 µg of amphotericin B.

Assay stock medium: Mix 1000 mL of *Dulbecco's modified Eagle's tissue culture medium*, 10 mL of *L-Glutamine solution*, 10 mL of *Sodium pyruvate solution*, 10 mL of *Antibiotic–antimycotic solution*, and 20 mL of fetal bovine serum.[15]

MTT-assay solution: Dissolve 0.50 g of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide in 1 L of *Assay stock medium*, using constant stirring. Sterilize the solution by passing it through a filter of 0.2-µm pore size.

MTT formazan stock solution: 100 µg/mL of 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan in isopropyl alcohol

MTT formazan calibration solutions: Prepare calibration solutions of 15, 30, 45, 60, and 75 µg/mL of MTT formazan, using *MTT formazan stock solution* and diluting with isopropyl alcohol.

Analysis: Thaw Construct Human Fibroblasts in Polyglactin Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Polyglactin Scaffold unit. Cut three 11-mm × 11-mm sections of Construct Human Fibroblasts in Polyglactin Scaffold, and immerse the sections into separate, 3.0 mL portions of *MTT-assay solution*. Incubate for 2 h at 37 ± 2° in a 3%–7% CO_2–air environment with shaking on an orbital shaker at 150–200 rpm. After incubation remove from the 37°, 3%–7% CO_2–air environment. Remove the *MTT-assay solution*, and rinse twice with *DPBS working solution*. Immerse the Construct Human Fibroblasts in Polyglactin Scaffold in 2 mL of isopropyl alcohol, and incubate at ambient temperature for 1 h with shaking on an orbital shaker at approximately 125 rpm. Transfer 200-µL aliquots of the five *MTT formazan calibration solutions*, in triplicate, and 200-µL aliquots of the three isopropyl alcohol extracts of Construct Human Fibroblasts in Polyglactin Scaffold to a suitable 96-well flat-bottom plate. Read the absorbance of each aliquot at 540 nm, using 200 µL of isopropyl alcohol as the blank. Plot the responses of the *MTT formazan calibration solutions* versus concentration, in µg/mL of MTT formazan, and calculate the regression line using the least-squares method.

System suitability: The test is considered valid if the regression line has a square of the correlation coefficient NLT 0.95.

Acceptance criteria: The absorbance value of individual, thawed Construct Human Fibroblasts in Polyglactin Scaffold sections at 540 nm is between 0.30 and 0.86.

• **DNA CONTENT**

Cell culture water: Sterile water containing NMT 0.005 USP Endotoxin Unit/mL

DNA extraction buffer: Transfer 850 mL of *Cell culture water* to a sterile, 1-L graduated container. Dissolve 12.110 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 3.802 g of edetate disodium, 23.380 g of sodium chloride, and 0.080 g of sodium dodecyl sulfate, with stirring. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 7.0. Dilute with *Cell culture water* to 1 L.

Proteinase K solution: Prepare a solution of *Tritirachium album* proteinase K in 10 mM of 2-amino-2-hydroxymethyl-1,3-propanediol, adjusted to a pH of 7.5, having an activity of 600 units/mL.[16]

Working DNA extraction buffer: Add 1.22 mL of *Proteinase K solution* to 38.78 mL of *DNA extraction buffer* and mix.

Dilution buffer: Transfer 850 mL of *Cell culture water* to a sterile, 1-L graduated container. Add 1.211 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 3.802 g of edetate disodium, and 5.844 g of sodium chloride, with stirring. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 7.0. Dilute with *Cell culture water* to 1 L.

DPBS without Ca^{++}, Mg^{++} solution: Prepare a solution containing 8.00 g/L of sodium chloride, 1.15 g/L of dibasic sodium phosphate (anhydrous), 0.20 g/L of potassium chloride, and 0.20 g/L of monobasic potassium phosphate in water.

Calf thymus DNA solution: Prepare a solution containing 1 mg/L of calf thymus DNA in *DPBS without Ca^{++}, Mg^{++} solution*, mixing thoroughly for 12–24 h at ambient temperature. Dilute the resulting solution with *DPBS without Ca^{++}, Mg^{++} solution* to prepare a solution containing 50 µg/mL of calf thymus DNA, mixing thoroughly on a vortex mixer for 10 min.

Calf thymus DNA calibration solutions: Prepare four calibration solutions containing 5, 10, 15, and 20 µg/mL of calf thymus DNA, using *Calf thymus DNA solution* and diluting with *DPBS without Ca^{++}, Mg^{++} solution*.

DNA staining solution: Prepare a solution containing 0.5 µg of 2′-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi(1H-benzimidazole) trihydrochloride pentahydrate/mL of *Dilution buffer*. Store in low-actinic glassware.

Analysis: Thaw Construct Human Fibroblasts in Polyglactin Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Polyglactin Scaffold unit. Cut three 11-mm × 11-mm sections of Construct Human Fibroblasts in Polyglactin Scaffold. To each of three microcentrifuge tubes add 1 mL of *DPBS without Ca^{++}, Mg^{++} solution*. Immerse a single Construct Human Fibroblasts in Polyglactin Scaffold 11-mm × 11-mm section into each microcentrifuge tube to remove the cryopreservative. Aspirate the *DPBS without Ca^{++}, Mg^{++} solution* from each tube, and replace with 1 mL of *Working DNA extraction buffer*, making sure that each Construct Human Fibroblasts in Polyglactin Scaffold is completely

[15] A suitable fetal bovine serum can be obtained from HyClone, 925 West 1800 South, Logan, UT 84321; catalog number SH30070.03.

[16] A suitable *Proteinase K solution* can be obtained from Roche Diagnostics Corp., Roche Applied Sciences, P.O. Box 50414, 9115 Hague Rd., Indianapolis, IN 46250-0414.

USP Monographs

www.webofpharma.com

submerged in the extraction buffer. Incubate the samples in a 56°–60° water bath for 4–18 h. Sonicate for 10–15 s using an ultrasonic cell disrupter to achieve complete cellular disruption of the tissue and to mix the contents of the tube. Centrifuge the microcentrifuge tubes at 12,000–15,000 × g to pellet non-DNA material. Transfer three 50-µL aliquots of each sample supernatant to individual wells of a 96-well black plate suitable for performing fluorescent analysis. Transfer triplicate 50-µL aliquots of each of the *Calf thymus DNA calibration solutions* to the 96-well plate, as well as a 50-µL aliquot of *DPBS working solution* for the blank. Add 150 µL of *DNA staining solution* to all wells containing the tissue samples, *Calf thymus DNA calibration solutions*, and the blank. Cover with aluminum foil, and place in a dark cabinet for 30–45 min at 15°–30°. Read the fluorescence of each well, using an excitation wavelength of 355 nm and an emission wavelength of 460 nm, blanking against the *DPBS without Ca ++, Mg ++ solution* well.

Plot the responses of the *Calf thymus DNA calibration solutions* versus concentration, in µg/mL of calf thymus DNA, and calculate the regression line using the least-squares method. From the regression line so obtained, determine the amount of DNA in µg/11-mm × 11-mm sample.

System suitability requirements: The test is considered valid if the %CV of the replicate values is less than 15%, the slope is between 4.48 and 6.27, the *y*-intercept is between –2.04 and 3.65, and the square of the correlation coefficient is NLT 0.990.

Acceptance criteria: The amount of DNA of an individual Construct Human Fibroblasts in Polyglactin Scaffold 11-mm × 11-mm section is between 6 and 15 µg.

• **TOTAL COLLAGEN CONTENT**

DPBS without Ca^{++}, Mg^{++} solution: Prepare as directed for *DNA Content*.

DPBS with Ca^{++}, Mg^{++} solution: 8.00 mg/mL of sodium chloride, 1.15 mg/mL of dibasic sodium phosphate (anhydrous), 0.20 mg/mL of potassium chloride, and 0.20 mg/mL of monobasic potassium phosphate, 0.10 mg/mL of magnesium chloride hexahydrate, and 0.10 mg/mL of calcium chloride (anhydrous) in water

Collagenase extraction solution: At least 250 Units/mL of *Clostridium histolyticum* collagenase, type 2, in *DPBS with Ca ++, Mg ++ solution*

2% Acetic acid solution: Mix 10 mL of acetic acid with 490 mL of water.

Collagen standard stock solution: 2 mg/mL of collagen, type I, in *2% Acetic acid solution*

Collagen calibration standards: Cut polyglactin mesh[17] into 17 11-mm × 11-mm squares, and place one square into 17 individual wells of a 24-well plate. Each well of the 24-well plate has a surface area of 220 mm^2 and a volume of 3.5 mL. In quadruplicate, prepare wells containing 0.050, 0.100, 0.200, and 0.400 mg of collagen by adding 25, 50, 100, and 200 µL, respectively, of the *Collagen standard stock solution*. The remaining well to which no *Collagen standard stock solution* has been added is used as the blank. Allow the wells to air-dry.

Sirius red solution: 1 mg/mL of Direct Red 80 in saturated picric acid

1% (*p-tert*-Octylphenoxy)polyethoxyethanol solution: Mix 10 mL of (*p-tert*-Octylphenoxy)polyethoxyethanol in 990 ml of *DPBS with Ca ++, Mg ++ solution*.

Analysis: Thaw Construct Human Fibroblasts in Polyglactin Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Polyglactin Scaffold unit. Cut three 11-mm × 11-mm sections of Construct Human Fibroblasts in Polyglactin Scaffold. Place each test section into separate wells of a 24-well plate. Add 200 µL of *1% (p-tert-Octylphenoxy)polyethoxyethanol solution* to each sample. Shake on a rotating platform shaker at 100–150 rpm for 60–70 min at room temperature. Aspirate off the *1% (p-tert-Octylphenoxy)polyethoxyethanol solution*, and rinse three times with 2 mL of *DPBS without Ca ++, Mg ++ solution*. Transfer each of the collagen standards to individual wells of the 24-well plate. Add 0.5 mL of *Sirius red solution* to each test sample and collagen standards. Shake on a rotating platform shaker at 100–150 rpm for 60 min at room temperature. Aspirate off the *Sirius red solution* from each well. Rinse each well twice with 2 mL of *DPBS without Ca ++, Mg ++ solution*. Add an additional 2 mL of *DPBS without Ca ++, Mg ++ solution* to each well, and allow to stand for 2 min. Aspirate off the *DPBS without Ca ++, Mg ++ solution*, and rinse twice more with 2 mL of *DPBS without Ca ++, Mg ++ solution*. Aspirate off all traces of *DPBS without Ca ++, Mg ++ solution*. Add 0.5 mL of *Collagenase extraction solution* to each well containing the *Collagen calibration standards*. Add 2.0 mL of *Collagenase extraction solution* to each well containing test samples. Rotate the plate on an orbital rotator at 150 rpm for 90 min at 37°. Transfer 200 µL from each well, to a suitable 96-well, flat-bottom plate. Read the absorbance of each aliquot at 540 nm. Dilute the Construct Human Fibroblasts in Polyglactin Scaffold sample preparation further with *DPBS without Ca ++, Mg ++ solution* if the absorbance is greater than the absorbance of the highest of the *Collagen calibration standards*. Plot the responses of the *Collagen calibration standards* versus the amount, in mg of collagen, and calculate the regression line using the least-squares method. Determine the mg of collagen/Construct Human Fibroblasts in Polyglactin Scaffold section from the regression line and using the following equation:

$$\text{Result} = D \times A \times SC_{SR}$$

D = dilution factor (normally 4, unless the sample had to be further diluted)

A = absorbance at 540 nm

SC_{SR} = slope of the regression line of the standards calculated above

System suitability requirements: The test is considered valid if the slope of the regression line is between 3.00 and 5.00 and the square of the correlation coefficient is greater than or equal to 0.950.

Acceptance criteria: The amount of collagen in individual Construct Human Fibroblasts in Polyglactin Scaffold 11-mm × 11-mm samples is 0.40–2.0 mg.

• **STERILITY TESTS** ⟨71⟩

Sample solution: Thaw Construct Human Fibroblasts in Polyglactin Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to a temperature of 34°–37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Polyglactin Scaffold unit.

Analysis: Perform the test on 20 mL of the cryopreservative.

Acceptance criteria: Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩

Sample: Thaw Construct Human Fibroblasts in Polyglactin Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to a temperature of 34°–37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Polyglactin Scaffold

[17] A suitable polyglactin mesh can be obtained from Ethicon Co., Johnson & Johnson Corp., 425 Hoes Ln., P.O. Box 6800, Piscataway, NJ 08855.

USP Monographs

www.webofpharma.com

unit. Remove the unit from the ethyl vinyl acetate bag, and immerse in 25 mL of LAL Reagent Water.

Analysis: Extract for 60 min at 37° with shaking on an orbital shaker set at 125 revolutions/min. Remove a 4-mL aliquot of the extract for testing.

Acceptance criteria: It contains NMT 0.5 USP Endotoxin Unit/mL.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Construct Human Fibroblasts in Polyglactin Scaffold is aseptically packaged and supplied frozen in a clear ethyl vinyl acetate bag. The solution within the bag is a saline-based cryoprotectant supplemented with 10% dimethyl sulfoxide and bovine serum to facilitate long-term storage. An aluminum–plastic foil envelope surrounds the bag for its protection. Construct Human Fibroblasts in Polyglactin Scaffold should be stored at –75 ± 10° for no longer than 6 months.
- **LABELING:** The label indicates the dimensions of the Construct Human Fibroblasts in Polyglactin Scaffold material enclosed. It contains the expiry date, the required storage conditions, and the lot number. The label cautions that Construct Human Fibroblasts in Polyglactin Scaffold is not to be used if the package shows signs of damage. Additional labeling requirements include instructions on the proper thawing and handling of Construct Human Fibroblasts in Polyglactin Scaffold, the time frame for use after package opening, and a statement that cytotoxic agents are not to be used.
- **USP REFERENCE STANDARDS,** *Authentic Visual References* ⟨11⟩
  USP Construct Human Fibroblasts in Polyglactin Scaffold Reference Photomicrographs
  [NOTE—These three photomicrographs represent examples of passing units. They are specified to assist in ascertaining histological quality.]

Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold

DEFINITION

Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold is a living, bilayered skin substitute derived from neonatal foreskins manufactured under Class 100 sterile conditions. The upper, epidermal layer is formed by human keratinocytes and has a well-differentiated stratum corneum. The inner, dermal layer is composed of human fibroblasts in a bovine Type I collagen lattice. Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold does not contain Langerhans cells, melanocytes, macrophages, lymphocytes, blood vessels, hair follicles, or any other epidermally derived components. The fibroblast and keratinocyte cell banks from which Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold is derived test negative for human and animal viruses, retroviruses, bacteria, fungi, yeast, mycoplasma, and tumorigenicity. The cell banks are also tested for normal human karyology and isoenzymes. The final product is tested for morphology, cell viability, and physical container integrity. Used tissue culture media are tested for mycoplasma and sterility. All materials derived from bovine sources originate from countries free of bovine spongiform encephalopathy.

SPECIFIC TESTS

- **HISTOLOGICAL CHARACTERIZATION**

2.0 M Monobasic potassium phosphate: Dissolve 13.61 g of anhydrous monobasic potassium phosphate in 50 mL of water.

2.0 M Dibasic potassium phosphate: Dissolve 17.42 g of anhydrous dibasic potassium phosphate in 50 mL of water.

Phosphate-buffered saline solution (pH 7.1–7.5): Combine 3.6 mL of *2.0 M Monobasic potassium phosphate*, 16.4 mL of *2.0 M Dibasic potassium phosphate*, 8 g of sodium chloride, and 1 L of water. Mix thoroughly.

0.3% Acid alcohol: To 100 mL of 70% alcohol, add 0.3 mL of hydrochloric acid and mix.

Hematoxylin–alcohol solution: Dissolve 2.5 g of hematoxylin in 25.0 mL of dehydrated alcohol, with heating.

Potassium alum solution: Dissolve 50.0 g of potassium alum in 500 mL of water, with heating.

Hematoxylin staining solution: Mix the *Hematoxylin–alcohol solution* and the *Potassium alum solution*, and heat to boiling as rapidly as possible with constant stirring. Do not heat for more than 1 min. Slowly add 0.185 g of sodium iodate, and reheat to a simmer until the solution becomes a deep purple. Remove from the heat, and quickly cool. Filter daily before use.

Bluing agent: Dissolve 200 mg of sodium bicarbonate and 40 mg of lithium carbonate in 63 mL of water and 37 mL of methanol and mix.

Eosin solution: Dissolve 1 g of eosin Y in 100 mL of alcohol. Filter daily before use.

Analysis: Remove three 2-cm diameter circular tissues from every 30-cm^2 section of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold (NLT 30% of the total unit area), using the appropriate size biopsy punch. Cut with a circular rocking motion to prevent crushing the tissue. Immerse the sections in 3.7% dimethoxymethane for 30 min, using a gentle rocking motion. Remove the sections, and lay on a cutting surface, dermal side (glossy side) down. Cut an approximately 3-mm-wide strip through the center of the specimen, using a new, single-edged razor blade. Place the strips in a histological microwave cassette, using suitable biopsy pads premoistened with *Phosphate-buffered saline solution (pH 7.1–7.5)* to hold the strips in place. Insert the cassette into a histological microwave processing rack, place the rack inside a suitable microwave container, and add sufficient *Phosphate-buffered saline solution (pH 7.1–7.5)* to completely cover the rack. Place the container in a microwave oven suitable for histological work,[1] and heat for 4 min at 55°.

Remove the *Phosphate-buffered saline solution (pH 7.1–7.5)*, and add enough dehydrated alcohol to completely cover the rack. Return the container to the microwave oven, and heat for 4 min at 67°. Remove the alcohol, and add enough dehydrated isopropyl alcohol to completely cover the rack. Return the container to the microwave oven, and heat for 4 min at 74°.

Remove the isopropyl alcohol, and add enough suitable grade paraffin[2] that has been melted and held at 84° prior to use, to completely cover the rack. Return the container to the microwave oven, and heat for 7 min at 84°. Remove the histological microwave cassette from the container and rack while the paraffin is still melted, and disassemble,

[1] A microwave oven suitable for histological preparation can be obtained from Energy Beam Sciences, Inc., 11 Bowles Road, P.O. Box 14508, Agawam, MA or equivalent.

[2] A suitable paraffin for use is Accumate™ Tissue Embedding/Infiltration Medium, which can be obtained from Sigma Diagnostics, P.O. Box 468, St. Louis, MO 63178 or equivalent.

www.webofpharma.com

discarding the biopsy pads. Fill preheated embedding molds with molten paraffin[3] heated to 60°, and place on top of a preheated warming platform that is designed for histological work. Using forceps, remove the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold specimens from the cassette, and place the specimens in individual molds. Orient the specimens in the molds to enable cutting of a cross-or longitudinal section. Cool the paraffin by sliding the mold down the platform to its cool side until the paraffin has solidified. Maintain the specimen orientation with forceps during cooling, removing the forceps when the paraffin becomes translucent. Slide the paraffin block onto a histological cold plate to rapidly cool the block. Trim the paraffin block with a new single-edged razor blade to form a rectangle or slight trapezoid to within 5 mm of the tissue mass, if necessary. Cool the block at 4° for 15 to 30 min, and clamp the paraffin block into the block holder of the microtome. Fill a histological tissue-flotation water bath with fresh water, add an appropriate amount of a suitable histological adhesive,[4] and heat to a temperature 5° lower than the melting point of the paraffin. Properly mount the paraffin block into a microtome, adjusting as necessary. Set the microtome to make 5-µm thick cuts with a blade angle of 5 ± 2°. Insert into the knife holder a sharp stainless steel microtome knife that has been properly honed or a new disposable microtome knife, and cut a ribbon that contains 6–10 sections of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold. Pick up the ribbon with forceps, and stretch it across the tissue-flotation water bath. Separate 2 to 3 adjacent sections from the ribbon on the water bath. The selected sections should not be compressed, wrinkled, or scratched. Pick up the selected sections by dipping a microscope slide into the water bath under the floating sections, and gently lift the slide out of the water. Allow the mounted sections to air-dry completely, or dry the slide in a 60° oven for 1 h. The microscope slide with affixed tissue is sequentially immersed in 3 changes of a suitable histological, aliphatic xylene substitute,[5] 5 min/step, followed by two changes of dehydrated alcohol, 3 min/step. Sequentially immerse the slide in alcohol (for 3 min), running water rinse (3 min), *Hematoxylin staining solution* (6 min), running water rinse (7 min), *0.3% Acid alcohol* (6 s), running water rinse (5 min), *Bluing agent* (1 s), running water rinse (5 min), *Eosin solution* (2 min), two changes of alcohol (3 min each step), four changes of dehydrated alcohol (3 min each step), and four changes of a suitable histological xylene substitute (3 min each step). Adjust the above immersion times as needed to suitably stain the tissue. Remove the slide from the last histological xylene substitute wash, and blot dry the back of the slide. Do not allow the tissue to dry. Affix a coverslip over the tissue, using a suitable coverslip mountant.

Microscopic specifications: A light microscope with 4×, 10×, 20×, and 40× objectives installed in a revolving nosepiece; a 10× widefield ocular with 10 to 19 mm per 100 microdisk reticle installed; and a 10× widefield ocular with grid reticle installed

Microscopic and morphological characteristics: Score the three Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold sections for epidermal and dermal aspects, using the light microscope. Evaluate the slides from each of the sections taken. Average the aspect values for each section (n = 3) to determine the overall aspect score for the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold unit. When examined microscopically, Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold shows a bilayered construct resembling the epidermal and dermal layers of human skin. Using USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrographs of passing and failing articles for comparison, Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold meets the requirements for epidermal aspects, including epidermal coverage, epidermal development, and keratinocyte aspect, and meets the requirements for dermal aspects, including dermal matrix thickness, fibroblast density, and matrix aspect, as described below.

Epidermal aspects

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 1 for an example of a passing unit.]

Acceptance criteria for Epidermal coverage: NLT 95% of the dermal matrix present on the slide is covered with epidermal keratinocytes.

Acceptance criteria for Epidermal development: NLT 70% of the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold epithelium is composed of three distinct cell layers.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 2 for an example of a failing unit.]

The basal cell layer of the epithelium is at least 1 cell thick, consisting of keratinocytes with a cuboidal-columnar shape.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 3 for an example of a failing unit.]

The suprabasal layer is composed of stratified cells and is at least 5 cells thick. Suprabasal cells closest to the basal layer are cuboidal in shape; cells become progressively stratified the closer they are to the uppermost, squamous cell layer. The squamous cell layer on the apical surface is cornified and at least 1 cell thick.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 4 for an example of a failing unit.]

The uppermost cell layer of the epithelium is analogous to the stratum corneum of human skin and is composed of one or more rows of flat, scaly cells that are nonliving and keratinized.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 5 for an example of a failing unit.]

Acceptance criteria for Keratinocyte aspects: NLT 95% of the basal keratinocytes have basophilic cytoplasm that neither has distinct vacuoles nor is necrotic. See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 6 for an example of a failing unit. NLT 80% of suprabasal cells (excluding those in the upper 20% of the cell layer closest to the squamous layer) have basophilic cytoplasm. Furthermore, these basophilic suprabasal cells do not have distinct vacuoles and are neither necrotic nor keratinized.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrographs 7 and 8 for examples of failing units.]

[3] A suitable paraffin for use is Paraplast® X-Tra Tissue Embedding Medium ASTM, melting point 50–54°, which can be obtained from Fisher Scientific, 300 Industry Drive, Pittsburgh, PA 15275 or equivalent.

[4] A suitable histological adhesive for use is Histoslide® Adhesive, which can be obtained from Poly Scientific R & D Corp., 70 Cleveland Ave., Bay Shore, NY 11706-1282 or equivalent.

[5] A suitable histological xylene substitute is Shandon Xylene Substitute from Thermo Scientific (http://www.thermoscientific.com) or equivalent.

www.webofpharma.com

Dermal aspects

Five randomly selected fields/slide will be evaluated for dermal matrix thickness and fibroblast density. The five fields will be averaged to obtain the final value for each section.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 1 for an example of a passing unit.]

Acceptance criteria for Dermal aspects

Dermal matrix thickness: The Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold dermal layer is NLT 40 µm thick and is composed of several rows of flat dermal cells.

Fibroblast density: The dermal matrix contains an average of at least four nonpyknotic nuclei present per microscopic field (field = 20 grid squares of reticle when using the 10× widefield ocular and 40× objective).

Matrix aspect: NLT 95% of the dermal matrix collagen stains uniformly with no large holes or inclusions present.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrographs 9 and 10 for examples of failing units.]

• **GENE EXPRESSION PROFILE**

RNA extraction solution: Use an aqueous phenol and guanidine isothiocyanate solution suitable for RNA extraction.[6]

DEPC-treated water: Add 0.2 mL of diethylpyrocarbonate (DEPC) to 100 mL of sterile Purified Water, shake vigorously, and allow to stand for at least 12 h. Autoclave the resulting solution for 15 min, using the liquid cycle, to inactivate residual DEPC. Prepare fresh as needed.

5X Reaction buffer: Prepare a solution of 375 mM potassium chloride, 15 mM magnesium chloride, and 250 mM tris(hydroxymethyl) aminomethane hydrochloride. Adjust to a pH of 8.3.

10X Reaction buffer: Prepare a solution of 500 mM potassium chloride and 100 mM tris(hydroxymethyl) aminomethane hydrochloride. Adjust to a pH of 8.3.

Oligo-deoxythymidine solution: Prepare a 20-mM oligo-deoxythymidine (primer length: 18) solution, using a suitable buffer.[7]

dNTP solution I: Using a suitable buffer,[7] prepare a solution of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, and deoxythymidine triphosphate in which the concentration of each component is 10 mM.

dNTP solution II: Prepare a solution, in water, of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, and deoxythymidine triphosphate, in which the concentration of each component is 10 mM.

Ribonuclease inhibitor solution: Prepare a solution containing 40 units of ribonuclease inhibitor/mL of a suitable buffer.[7]

Reverse transcriptase buffer: Prepare a solution of 0.1 M sodium chloride, 0.1 mM edetate disodium, 1.0 mM dithiothreitol, 0.01% nonylphenol polyoxyethylene ether, 50% glycerin, and 200 mM tris(hydroxymethyl) aminomethane hydrochloride. Adjust to a pH of 7.5.

Reverse transcriptase solution: Prepare a solution containing 200 units of Moloney-Murine Leukemia Virus reverse transcriptase/µL in *Reverse transcriptase solution*.

DNA primer pairs: Prepare individual 20-µM solutions of the following DNA primer pairs, using deoxyribonuclease- and ribonuclease-free water.

Table 1

Transforming growth factor β 1 (TGFβ 1):	
TGFβ 1-3′	agg ctc caa atg tag ggg cag g
TGFβ 1-5′	gcc ctg gac acc aac tat tgc t
Interleukin-1α (IL1α):	
IL1α-3′	tag tgc cgt gag ttt ccc aga aga aga gga gg
IL1α-5′	caa gga gag cat ggt ggt agt agc aac caa cg
Interleukin-4 (IL4):	
IL4-3′	acg tac tct ggt tgg ctt cct tca cag gac ag
IL4-5′	cgg caa ctt tga cca cgg aca caa gtg cga ta
Platelet-derived growth factor A:	
PDGF-A-3′	ctg ctt cac cga gtg cta caa tac ttg ct
PDGF-A-5′	aga agt cca ggt gag gtt aga gga gcat
Glyceraldehyde-3-phosphate dehydrogenase:	
G3PDH-3′	cat gtg ggc cat gag gtc cac cac
G3PDH-5′	tga agg tcg gag tca acg gat ttg gt

DNA polymerase solution: Prepare a solution containing five units of deoxyribonucleic acid polymerase/mL of a solution of 100 mM potassium chloride, 0.1 mM edetate disodium, 1 mM dithiothreitol, 0.5% polyoxyethylene (20) sorbitan monolaurate, 0.5% nonylphenol polyoxyethylene ether, 50% glycerin, and 20 mM tris(hydroxymethyl) aminomethane hydrochloride. Adjust to a pH of 8.0.

Analysis

RNA extraction: Remove three 2-cm diameter circular sections from every 30 cm² of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold (NLT 30% of the total unit area), using the appropriate size biopsy punch. Transfer each piece of tissue to individual polypropylene microcentrifuge tubes. Add 1.0 mL of *RNA extraction solution* to each tube, homogenize by repetitive pipetting, and incubate the samples for 5 min at room temperature. To each tube, add 0.2 mL of chloroform, mix on a vortex mixer, and centrifuge at 12,000 × *g* for 15 min at 2°–8°. Transfer the upper, aqueous phase to a second tube, add 0.5 mL of isopropanol, and incubate for 30 min to overnight at –20°. Centrifuge at 12,000 × *g* for 15 min, discard the supernatants by aspiration, and add 75% alcohol to each pellet. Mix the sample on a vortex mixer, centrifuge at 12,000 × *g* for 2 min, and discard the supernatants by aspiration without disturbing the RNA pellets. Recentrifuge at 12,000 × *g* for 2 min, and remove the remaining supernatants with a small-volume (20 µL or smaller capacity) micropipet. Air-dry the pellets for 5 min at room temperature by keeping the microcentrifuge cap off, and resuspend each pellet in 50 µL of *DEPC-treated*

[6] A suitable RNA extraction solution is Trizol® reagent, which can be obtained from Life Technologies, (https://www.lifetechnologies.com) or equivalent.

[7] A suitable buffer can be obtained from the RT-for-PCR Kit, Clontech, 1290 Terra Bella Ave., Mountain View, CA 94043 or equivalent.

www.webofpharma.com

water. Bring absorbance into linear range by diluting 5 µL of each suspension with 195 µL of *DEPC-treated water*. Transfer the samples to suitable quartz microplates or cuvettes and determine the absorbance of the RNA solution at wavelengths of 260 and 280 nm, using a spectrophotometer and *DEPC-treated water* as the blank. The ratio of the absorbance at 260 versus 280 nm should be NLT 1.65. If this ratio is less than 1.65, mix the resuspended pellet by repetitive pipetting, and repeat the dilution step and absorbance measurement. If this fails to raise the absorbance ratio, repeat the RNA extraction for that sample by adding 1 mL of *RNA extraction solution*, and proceed as above, beginning with "incubate the sample for 5 min at room temperature".

Determine the concentration of RNA, in µg/mL:

$$\text{Result} = F \times A \times D$$

F = conversion factor, 40
A = absorbance at 260 nm
D = dilution factor

Adjust the volume of the RNA solutions with additional *DEPC-treated water* to bring the concentration of RNA to about 80 µg/mL. If the absorbance at 260 nm is less than 0.05, discard the sample, and repeat the RNA extraction on a fresh sample.

Synthesis of cDNA: To separate, individual thin-walled polymerase chain reaction (PCR) tubes, add 12.5 µL of the RNA solution from samples 1, 2, and 3 (3 reaction tubes total). Add 1 µL of *Oligo-deoxythymidine solution* to each tube, and incubate at 72° for 2 min to anneal the oligo-deoxythymidine to the mRNA. Place the tubes in an ice bath, and to each tube, add 4 µL of *5X Reaction buffer*, 1 µL of *dNTP solution I*, 0.5 µL of *Ribonuclease inhibitor solution*, and 1 µL of *Reverse transcriptase solution*. Incubate at 42° for 1 h to synthesize cDNA, and then incubate at 94° for 5 min to inactivate the reverse transcriptase. To each tube, add 80 µL of *DEPC-treated water* and mix.

cDNA positive control[8]

Polymerase chain reaction amplification of cDNA: For each of the five *DNA primer pairs*, label five individual centrifuge tubes (5 tubes total). Add the following to each centrifuge tube: 135.8 µL of *DEPC-treated water*; 10.5 µL of *dNTP solution II*; 21 µL of *10X Reaction buffer*; 3.5 µL of the appropriate 5′ primer; 3.5 µL of the appropriate 3′ primer; and 12.6 µL of 25 mM magnesium chloride. Close, mix on a vortex mixer, and pulse spin in a microcentrifuge. Add 2.1 µL of *DNA polymerase solution* to each centrifuge tube, and mix by repetitive pipetting. For each primer pair, transfer 27 µL of the resulting solution to five thin-walled PCR tubes. There should be a total of 25 PCR tubes. Add the following to the PCR tubes of each primer set:

Table 2

PCR tube number	Sample
1	3 µL Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold sample 1 cDNA
2	3 µL Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold sample 2 cDNA
3	3 µL Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold sample 3 cDNA
4	3 µL *cDNA positive control*
5 Negative control	3 µL *DEPC-treated water*

Repeat for the remaining primer pairs. The positive control contains authentic cDNA of *Transforming growth factor β*, *Interleukin-1α*, *Interleukin-4*, *Platelet-derived growth factor A*, and *Glyceraldehyde-3-phosphate dehydrogenase*, as appropriate for each primer set. Pulse spin the PCR tubes in a microcentrifuge to mix, and place the tubes in a single PCR thermal cycler. Cycling conditions are as follows.

Table 3

Melting temperature	94°
Melting time	45 s
Anneal temperature	58°
Anneal time	45 s
Elongation temperature	72°
Elongation time	2 min
Number of cycles	30
Final elongation temperature	72°
Final elongation time	2 min

Terminate the PCR amplification by heating each tube to 72° for 7 min.

Electrophoresis identification

Tris-boric acid buffer: 89 mM of tris(hydroxymethyl) aminomethane, 89 mM of boric acid, and 2 mM of edetate disodium

6X Loading buffer: A solution containing 15% of a branched polymeric sucrose (400 kDa), 0.25% bromophenol blue, and 0.25% xylene cyanole FF

Ethidium bromide solution: 10 mg/mL Ethidium bromide in *Tris-boric acid buffer*

Agarose gel: A horizontal 2% agarose[9] gel in *Tris-boric acid buffer*

Once the gel is set, remove the comb, and place the gel into the electrophoresis chamber with the comb end of the gel situated closest to the cathode terminal. Fill the electrophoresis chamber with *Tris-boric acid buffer* until the buffer reaches 3–5 mm over the surface of the gel.

100-bp DNA ladder markers: A solution containing 10 DNA fragments covering the range of 100–1000 base pairs (bp) in 100-bp increments, with a total DNA content of approximately 100 ng/µL (15–20 ng of DNA per band) in an appropriate buffer[10]

Analysis: Dilute the 25 PCR samples prepared in the *Polymerase chain reaction amplification of cDNA* with *6X Loading buffer* so that the final concentration of the buffer is one-sixth of its original concentration. Load 5 µL of the *100-bp DNA ladder markers* in the first lane of the agarose gel. Load 10 µL of each PCR sample into each gel well, and attach the cathode to the terminal close to the loaded wells. Attach the anode to the terminal farthest from the loaded wells, and apply 120 V to the gel. Run the gel until the

[8] A suitable cDNA positive control can be obtained from Clontech, 1290 Terra Bella Ave., Mountain View, CA 94043.

[9] An agarose suitable for electrophoresis analysis of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold cytokine PCR product is SeaKem® GTG agarose and can be obtained from Lonza Inc., 90 Boroline Road, Allendale, NJ 07401 or equivalent.

[10] A suitable buffered solution of *100-bp DNA ladder markers* can be obtained from Lonza Inc., 90 Boroline Road, Allendale, NJ 07401 or equivalent.

USP Monographs

www.webofpharma.com

bromophenol blue is two-thirds the length of the gel. Remove the gel from the electrophoresis apparatus, and place it in a tray containing enough *Ethidium bromide solution* to cover the gel. Slowly agitate the gel on a shaker table for 30 min. Completely remove the *Ethidium bromide solution* from the tray, add an equal amount of *Tris-boric acid buffer*, and slowly agitate the gel on a shaker table for 60 min. Place the gel on a 312-nm UV light source, photograph the gel, and inspect the image for bands that have migrated from each individual well. If a band appears, it is verified for size in base pairs by comparing it to the lane for the *100-bp DNA ladder markers*.

System suitability: If a band appears and it is of the appropriate size, it is considered positive. The analysis is considered valid if the positive controls show the appropriately sized cDNA–PCR products, no PCR product bands appear in the negative controls, and all bands are observed to be visually discrete.

Acceptance criteria: The lanes of the agarose gel that correspond to Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold show cDNA bands for *Interleukin-1α* (expected PCR product band size of 491 base pairs, limit of detection NLT 9.6×10^{-21} moles); *Platelet-derived growth factor A* (expected PCR product band size of 304 base pairs, limit of detection NLT 1.5×10^{-20} moles); *Transforming growth factor β1* (expected PCR product band size of 161 base pairs, limit of detection NLT 1.5×10^{-20}) moles; and *Glyceraldehyde-3-phosphate dehydrogenase* (expected PCR product band size of 983 base pairs); but not *Interleukin-4* (expected PCR product band size of 344 base pairs, limit of detection NLT 1.5×10^{-22} moles). If one of the replicates tested yields results discordant with the other two replicates, repeat the test, and accept only if all three replicates are concordant.

- **BARRIER INTEGRITY ASSESSMENT**

Ham's F-12 tissue culture medium: Prepare a solution that contains the following:

Table 4

Component	mg/mL
L-Alanine	8.91
L-Arginine hydrochloride	210.7
L-Asparagine monohydrate	15.01
L-Aspartic acid	13.30
L-Cysteine hydrochloride monohydrate	35.12
L-Glutamic acid	14.70
L-Glutamine	146.2
Aminoacetic acid	7.51
L-Histidine hydrochloride monohydrate	20.96
L-Isoleucine	3.94
L-Leucine	13.12
L-Lysine hydrochloride	36.54
L-Methionine	4.48
L-Phenylalanine	4.96
L-Proline	34.53
L-Serine	10.51
L-Threonine	11.91
L-Tryptophan	2.04
L-Tyrosine disodium	6.71

Table 4 *(continued)*

Component	mg/mL
L-Valine	11.71
Calcium chloride	44.00
Cupric sulfate, pentahydrate	0.0025
Ferric sulfate, heptahydrate	0.834
Potassium chloride	223.7
Magnesium chloride	57.22
Sodium chloride	7599.0
Sodium phosphate, dibasic	142.0
Zinc sulfate, heptahydrate	0.863
D-Biotin	0.0073
D-Calcium pantothenate	0.238
Choline chloride	13.96
Folic acid	1.30
Hypoxanthine	4.04
Inositol	18.02
Niacinamide	0.0366
Pyridoxine hydrochloride	0.0617
Riboflavin	0.0376
Thiamine hydrochloride	0.337
Thymidine	0.727
Cyanocobalamin	1.36
α-Lipoic acid	0.206
Linoleic acid	0.0841
Dextrose	1801.6
Phenol red, sodium	1.30
Sodium pyruvate	110.0
Putrescine dihydrochloride	0.161
Sodium bicarbonate	1176.0

Tritiated water: 2.0 µCi/mL

Percutaneous absorption apparatus: Prepare the apparatus as described below.[11]

Six-well cell culture plate: The dimensions are inner diameter, 35 mm; depth, 18 mm.

Cell culture well insert: Each well is a plastic cylinder with inner length, 15 mm; inner diameter, 24 mm; outer diameter, 27 mm, with a flanged end extending 4 mm from the outer diameter. The inner diameter opposite the flanged end is covered by a taut polycarbonate membrane having a porosity of 3 µm. The flange should allow the *Cell culture well insert* to be suspended in the well of a *Six-well cell culture plate*, leaving a 3-mm space between the bottom of the *Cell culture well insert* and the inner bottom surface of the *Six-well cell culture plate*.

Percutaneous absorption insert: Use a polytetrafluoroethylene cylinder having the following dimensions: length, 20 mm; inner diameter, 20 mm; outer diameter, 23 mm with a flanged end extending 3

[11] A suitable *Percutaneous absorption apparatus*, not including the *Percutaneous absorption insert*, is a Costar® 6-well culture cluster, flat bottom with lid, and a Costar® Transwell®, 24 mm in a 6-well cluster plate with lid and can be obtained from Corning Life Sciences, 836 North Street, Building 300, Suite 3401, Tewksbury, MA 01876 or equivalent.

www.webofpharma.com

mm from the outer diameter. Ten mm from the flanged end of the cylinder, the inner diameter begins to funnel so that the inner diameter at 10 mm from the flanged end is 20 mm, and the inner diameter at 15 mm from the flanged end is 8 mm. From 15 to 20 mm from the flanged end, the inner diameter remains at 8 mm. The outer diameter of the cylinder remains constant at 23 mm. The flanged end is considered to be the top of the component.

Silicon grease: Use high-vacuum silicon grease suitable for glass.[12]

Analysis: Fill each well of the *Six-well cell culture* plate with 1.5 mL of *Ham's F-12 tissue culture medium*. Remove two 2-cm circular sections from every 30 cm^2 of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold (NLT 20% of the total unit area), using the appropriate size biopsy punch. Transfer each excised section to a separate *Cell culture well insert*, dermal side down on the polycarbonate membrane. Using forceps, gently smooth out the section to remove any wrinkles. Apply a narrow ring of *Silicon grease* to the underside of the *Percutaneous absorption insert*, and place the insert into the *Cell culture well insert*, grease side down, onto the epidermal surface of the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold biopsy, with slight pressure to form a tight seal. Do not allow any grease to enter the 8-mm diameter exposed area of the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold surface. Place the *Cell culture well insert* containing the *Percutaneous absorption insert* into one of the wells of the *Six-well cell culture plate* containing 1.5 mL of *Ham's F-12 tissue culture medium*. Apply 1.0 mL of *Tritiated water* to the exposed surface of the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold unit in the *Percutaneous absorption insert*, and incubate at ambient temperature for 6 h. At the end of each h, transfer the *Cell culture well insert* containing the *Percutaneous absorption insert* to a new well within the *Six-well cell culture plate* containing 1.5 mL of fresh *Ham's F-12 tissue culture medium*. After the 6-h incubation, remove the *Cell culture well insert*. Remove a 0.5-mL aliquot of *Ham's F-12 tissue culture medium* from each well of the *Six-well cell culture plate*, and transfer into individual scintillation vials. Dispense 0.5 mL of *Tritiated water* to a separate scintillation vial as a control. Add 4.5 mL of a suitable scintillation cocktail[13] to each scintillation vial and gently mix. Place the scintillation vials into a liquid scintillation counter, and count the emissions in the tritium spectrum for 60 s. Average the counts for each of the six time points (punch average) and duplicate sections (unit average). Determine the percent penetration per h:

$$\text{Result} = F \times (C_S/C_C)$$

F = conversion factor, 150
C_S = counts/min of the 0.5-mL aliquot of the *Ham's F-12 tissue culture medium* taken at the end of the incubation period
C_C = counts/min in the 0.5-mL aliquot of *Tritiated water*

Acceptance criteria: NMT 1.97% penetration is found.

• **METABOLIC ACTIVITY ASSESSMENT**

Dulbecco's modified Eagle's tissue culture medium: Prepare a solution that contains the following:

Table 5

Component	mg/L
Calcium chloride	264.9
Ferric nitrate, nonahydrate	0.10
Potassium chloride	400.0
Magnesium sulfate, heptahydrate	200.0
Sodium chloride	6,400.0
Sodium bicarbonate	3,700.0
Sodium phosphate, monobasic (monohydrate)	125.0
Dextrose	4,500.0
Phenol red	15.0
Sodium pyruvate	110.0
L-Arginine hydrochloride	84.0
L-Cystine	48.0
Aminoacetic acid	30.0
L-Histidine hydrochloride monohydrate	42.0
L-Isoleucine	104.8
L-Leucine	104.8
L-Lysine hydrochloride	146.2
L-Methionine	30.0
L-Phenylalanine	66.0
L-Serine	42.0
L-Threonine	95.2
L-Tryptophan	16.0
L-Tyrosine	72.0
L-Valine	93.6
D-Calcium pantothenate	4.0
Choline chloride	4.0
Folic acid	4.0
Inositol	7.0
Nicotinamide	4.0
Pyridoxine hydrochloride	4.0
Riboflavin	0.40
Thiamine hydrochloride	4.0

MTT solution: Dissolve 0.33 g of (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide in 1 L of *Dulbecco's modified Eagle's tissue culture medium*, with constant stirring. Pass the solution through a suitable size filter having a 0.2-µm porosity.

0.04 N Acidified isopropyl alcohol: Add 3.45 mL of hydrochloric acid to 1 L of isopropyl alcohol, and mix thoroughly. Store at room temperature no longer than 6 months.

Analysis: Immerse the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold in separate 40.0-mL portions of *MTT solution*, making sure that about 20 mL of *MTT solution* is under the sample article, and 20 mL of *MTT solution* is on the surface. Take care not to produce any bubbles. Incubate for 3 h at 37°, in an environment of air enriched with 10% carbon dioxide. After incubation, remove from the 37°, 10% carbon dioxide-enriched air environment. Transfer the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold to a suitable

[12] A suitable *Silicon grease* is High Vacuum Silicon Lubricant for Glass and can be obtained from Dow Corning Corporation, P.O. Box 0994, Midland, MI 48686-0994.

[13] A suitable scintillation cocktail is OptiPhase Supermix Perkin-Elmer Life Sciences, Inc., 549 Albany St., Boston, MA 02118 or equivalent.

www.webofpharma.com

cutting surface, and, using an appropriate biopsy punch, remove three 8-mm diameter circular sections from every 30 cm^2 of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold (5% of unit area). Transfer each punch to individual snap-top test tubes. Add 0.9 mL of *0.04 N Acidified isopropyl alcohol* to each tube, making sure that the tissue is completely submerged. If not submerged, use forceps to place the sample into the *0.04 N Acidified isopropyl alcohol*. Cap each tube tightly, place on an orbital shaker, and shake for 1 h at a moderate setting. After 1 h, remove the tubes from the orbital shaker, and mix each tube on a vortex mixer. Inspect the tubes to make sure that the tissue samples continue to be submerged. If not, use forceps or another device to resubmerge the tissues. Return the tubes to the orbital shaker, and continue to shake for an additional 1 h. Remove the tubes from the orbital shaker, mix the tubes on a vortex mixer, and transfer a 0.2-mL aliquot to a suitable 96-well flat-bottom plate. Read the absorbance of each sample at 570 nm, using 0.2 mL of *0.04 N Acidified isopropyl alcohol* as the blank.

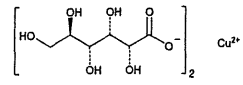
Acceptance criteria: The average absorbance value is NLT 0.237.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold is aseptically packaged in a Class 100 environment in single-use containers that preserve cell viability and product integrity. Store at controlled room temperature for NMT 5 days, and do not subject to freezing temperatures. The atmosphere within the package contains air enriched with 10% carbon dioxide. The device is translucent and off-white in color. The upper, epidermal surface is dull with small irregularities resulting from the cornification of keratinocytes, while the bottom surface is smooth and shiny in appearance. The device is packaged so that the dermal layer (glossy layer) is closest to the agarose-based nutrient medium. The packaging permits easy observation of the medium and provides ready access to the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold when needed. The medium contains all of the required nutrients for the living cell components of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold plus an appropriate, nontoxic, pH-sensitive dye to indicate package breaches or microbial contamination. The medium should appear pink (pH 6.8–7.7) when compared to the enclosed pH color chart.
- **LABELING:** Label it to indicate the dimensions of the enclosed Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold, the expiry date, the required storage conditions, and the lot number. The label indicates that the enclosed Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold and surrounding medium are to be examined for signs of contamination or deterioration. The label also contains a pH color code to be used for determination of the acceptability of the pH of the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold medium. The label cautions that Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold is not to be used if the package shows signs of damage or microbial contamination. Label it to indicate that sterile techniques are to be used in handling Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold and that cytotoxic agents are not to be used. Label it to indicate the time frame for use after package opening.
- **USP REFERENCE STANDARDS,** *Authentic Visual References* ⟨11⟩
  USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrographs

[NOTE—These 10 photomicrographs represent examples of both passing and failing Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold units. They are specified to assist in ascertaining histological quality.]

Copper Gluconate



$C_{12}H_{22}CuO_{14}$ 453.84

Copper, bis(D-gluconato-O^1,O^2)-;
Copper D-gluconate (1:2) [527-09-3].

DEFINITION

Copper Gluconate contains NLT 98.0% and NMT 102.0% of copper gluconate ($C_{12}H_{22}CuO_{14}$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Copper* ⟨191⟩**:** A 50-mg/mL solution meets the requirements.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: 10 mg/mL of Copper Gluconate, heating in a water bath at 60°, if necessary, to dissolve

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the *Spray reagent*. Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.

ASSAY

- **PROCEDURE**

Sample: 1.5 g of Copper Gluconate

Blank: 100 mL of water

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Indirect titration

Titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 100 mL of water. Add 2 mL of glacial acetic acid and 5 g potassium iodide, mix, and titrate with *Titrant* to a light yellow color. Add 2 g of ammonium thiocyanate, and mix. Add 3 mL of starch TS, and continue titrating to a milk-white endpoint. Perform the blank determination.

Calculate the percentage of copper gluconate ($C_{12}H_{22}CuO_{14}$) in the *Sample* taken:

www.webofpharma.com

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 453.8 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Standard solution: 1.0 mL of 0.020 N hydrochloric acid
Sample: 1.0 g
Acceptance criteria: NMT 0.07%
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Standard solution: 1.0 mL of 0.020 N sulfuric acid
Sample: 2.0 g
Acceptance criteria: NMT 0.05%
• **ARSENIC,** *Method I* ⟨211⟩
Test preparation: 1.0 g in 35 mL of water
Acceptance criteria: NMT 3 ppm
• **LIMIT OF LEAD**
[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]
Standard stock solution: Transfer 10.0 mL of lead nitrate stock solution TS to a 100-mL volumetric flask. Add 40 mL of water and 5 mL of nitric acid, and dilute with water to volume.
Standard solution: Transfer 0.40 mL of *Standard stock solution* to a 100-mL volumetric flask. Add 50 mL of water and 1 mL of nitric acid, and dilute with water to volume. This solution contains 0.04 µg/mL of lead.
Sample stock solution: Transfer 4 g of Copper Gluconate to a 100-mL volumetric flask. Add 50 mL of water and 5 mL of nitric acid, and sonicate to dissolve the specimen. Dilute with water to volume. Transfer 4.0 mL of this solution to a second 100-mL volumetric flask. Add 50 mL of water and 1 mL of nitric acid, dilute with water to volume, and mix.
Blank: Transfer 1.2 mL of nitric acid to a 100-mL volumetric flask and dilute with water to volume.
Sample solution A: Mix 10.0 mL of the *Sample stock solution* with 10.0 mL of *Blank*. This solution contains 0.00 µg/mL of added lead from the *Standard solution*.
Sample solution B: Mix 10.0 mL of the *Sample stock solution* with 4.0 mL of the *Standard solution* and 6.0 mL of *Blank*. This solution contains 0.008 µg/mL of added lead from the *Standard solution*.
Sample solution C: Mix 10.0 mL of the *Sample stock solution* with 7.0 mL of the *Standard solution* and 3.0 mL of *Blank*. This solution contains 0.014 µg/mL of added lead from the *Standard solution*.
Sample solution D: Mix 10.0 mL of the *Sample stock solution* with 10.0 mL of the *Standard solution*. This solution contains 0.020 µg/mL of added lead from the *Standard solution*.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Graphite furnace atomic absorption spectrophotometry
Analytical wavelength: 283.3 nm
Lamp: Lead hollow-cathode
Argon flow rate: 3 L/min, or as noted
Graphite tube temperature: See *Table 1*.

Table 1

Temperature (°)	Time (s)
70	10
90	60
120	15
250 (no gas flow)	5
250	10
250 (no gas flow)	2
2000	3.2

Injection volume: 20 µL
Analysis
Samples: *Blank* and *Sample solutions A,B, C,* and *D*
The graphite tube is temperature-programmed to reach 2000° in about 2 min, as shown in *Table 1*. When the temperature reaches 2000°, determine the absorbance at 283.3 nm, corrected for background absorption. Inject the *Sample solutions* and *Blank*, and determine the absorbances. Correct the absorbance values from the *Sample solutions* by subtracting from each the absorbance value from the *Blank*. Plot the corrected absorbances of the *Sample solutions* versus their added lead concentrations, in µg/mL. Draw the straight line best fitting the four points, and extrapolate the line until it intercepts the concentration axis. From the intercept, determine the concentration, *C*, in µg/mL, of lead in *Sample solution A*.
Calculate the content of lead in the portion of Copper Gluconate taken:

$$\text{Result} = (C \times V)/W$$

C = concentration of lead in the *Sample solution A* (µg/mL), determined from the intercept of the linear regression line
V = volume of solvent taken to prepare the *Sample solution A* (mL)
W = weight of Calcium Gluconate taken to prepare the *Sample solution A* (g)

Acceptance criteria: NMT 25 µg/g
• **REDUCING SUBSTANCES**
Sample: 1.0 g of Copper Gluconate
Blank: 10 mL of water
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 0.1 N iodine VS
Back-titrant: 0.1 N sodium thiosulfate VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 250-mL conical flask, add 10 mL of water to dissolve the *Sample*, then add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid, and titrate with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the blank determination.
Calculate the percentage of reducing substances (as dextrose) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F]/W\} \times 100$$

www.webofpharma.com

V_B = *Back-titrant* volume consumed by the *Blank* (mL)
V_S = *Back-titrant* volume consumed by the *Sample* (mL)
N = actual normality of the *Back-titrant* (mEq/mL)
F = equivalency factor, 27 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Potassium Gluconate RS

Corticotropin Injection

DEFINITION

Corticotropin Injection is a sterile solution, in a suitable diluent, of the material containing the polypeptide hormone having the property of increasing the rate of secretion of adrenal corticosteroids, which is obtained from the anterior lobe of the pituitary of mammals used for food by humans. Its potency is NLT 80.0% and NMT 125.0% of the potency stated on the label in USP Corticotropin Units. It may contain a suitable antimicrobial agent.

IDENTIFICATION

- **A. HPLC**

Solution A: 0.1% trifluoroacetic acid
Solution B: 0.1% trifluoroacetic acid in acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
1	75	25
17	70	30
27	70	30
27.5	20	80
32	20	80
32.5	75	25
35	75	25

Standard solution: 18.7 USP Corticotropin Units/mL of USP Corticotropin RS
Sample solution: 22 USP Corticotropin Units/mL of Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorescence; excitation 295 nm, emission 355 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Temperatures
Sample tray: 35°
Column: 35°
Flow rate: 1.0 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation of the retention time: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The retention time of the corticotropin peak of the *Sample solution* corresponds to that of the *Standard solution*.

- **B.** Meets the requirements of the *Assay*

ASSAY

Change to read:

- **PROCEDURE**

Standard solutions: ▲Add▲ (USP 1-Aug-2019) gelatin TS to an opened container of USP Corticotropin RS, and mix to obtain a solution with a concentration of 2.0 USP Corticotropin Units/mL. Using gelatin TS as a diluent, prepare three diluted *Standard solutions* such that the respective concentrations of corticotropin constitute a geometric series such as 1:2:4 or 1:3:9 and such that the quantity of corticotropin in each 0.5 mL lies within the range of 10–300 milliunits.
Sample solutions: In the same manner as the *Standard solutions* preparation, using the same diluent, dilute the Injection to make three *Sample solutions* corresponding in concentration to those of the *Standard solutions*.
The animals: Select healthy rats, of the same but either sex, that have been raised on a diet fully adequate with respect to vitamin and mineral content. Anesthetize the rats and remove the hypophysis from each by application of gentle suction through a fine-tipped tube. Between 16 and 48 h after the operation, select those rats weighing 80–180 g, but restrict the selection so that no rat is more than 30% heavier than the lightest, and the number of rats is an exact multiple of 6. Separate the selected rats into 6 groups, equal in size, of NLT 6 rats each, and assign at random one of the three diluted *Standard solutions* or one of the three *Sample solutions* to each group.
Analysis: Inject all rats of each group subcutaneously with the assigned test doses. Three h after the injection, anesthetize the rats, and remove both adrenal glands from each rat, free them from adhering tissue, and promptly weigh each pair on a suitable balance to the nearest 0.2 mg. Place the weighed glands from each rat in suitable vessels each containing 8.0 mL of metaphosphoric acid solution (1 in 40), and pulverize the glands by grinding with a small quantity of washed sand. Cover each vessel, and proceed similarly until all glands have been extracted.
Ascorbic acid determination: Filter the metaphosphoric acid extracts, and pipet 4 mL of each filtrate into suitable vessels each containing 4.0 mL of indophenol–acetate TS. Mix by shaking, and read the absorbance at 520 nm, with a suitable spectrophotometer. From the observed absorbance and the standard curve prepared as directed below, calculate the amount of ascorbic acid in mg/100 g of adrenal gland tissue. Prepare a standard concentration–absorbance curve, using three ascorbic acid solutions containing, respectively, 6.0, 8.0, and 10.0 µg/mL of in metaphosphoric acid solution (1 in 40). Pipet into each of three suitable vessels, preferably spectrophotometer cells, 4 mL of indophenol–acetate TS. Add 4.0 mL of one of the three standard ascorbic acid solutions to one of the cells, mix, and promptly read the absorbance from the same instrument and under the same conditions as for the adrenal gland extracts. Repeat the process for the other two standard ascorbic acid solutions, plot the concentration–absorbance values, and draw the straight line best fitting the three plotted points.

USP Monographs

www.webofpharma.com

Calculation: If there are no missing data (i.e., all groups of rats are the same size, *f*), then the following may be used. [NOTE—If there are missing values, then suitable software can be used and standard procedures followed for parallel line bioassays, including assessment of parallelism and linearity.] Tabulate the observed concentration of ascorbic acid in the adrenal glands of each rat, designated by the symbol y_{jkl}, where j = *S* (Standard) or *U* ▲(Sample),▲ (USP 1-Aug-2019) k = 1, 2, or 3 for the three doses, and l = 1, . . ., f rats. Total the values of the y_{jkl}'s in each group as:

$$T_{jk} = \sum_{l=1}^{f} y_{jkl}$$

Then determine the following quantities:

$$T_a = \sum_{k=1}^{3} (T_{Uk} - T_{Sk})$$

$$T_b = (T_{U3} - T_{U1}) + (T_{S3} - T_{S1})$$

$$V = (T_{U3} - T_{U1})/(T_{S3} - T_{S1})$$

$$T_q = (T_{U3} - 2T_{U2} + T_{U1}) + (T_{S3} - 2T_{S2} + T_{S1})$$

$$T_{aq} = (T_{U3} - 2T_{U2} + T_{U1}) - (T_{S3} - 2T_{S2} + T_{S1})$$

$$s^2 = \frac{1}{n}\left[\sum_{j,k,l} y_{jkl}^2 - \frac{1}{f}\sum_{j,k} T_{jk}^2\right] \text{ where } n = 6(f-1)$$

or $\frac{1}{6}s_{jk}^2$ where $s_{jk}^2 = \frac{1}{f-1}\sum_{l=1}^{f}\left(y_{jkl} - \frac{T_{jk}}{f}\right)^2$

$$F = \frac{T_q^2 + T_{aq}^2}{24fs^2}$$

If $V \geq 0.75$ and $V \leq 1.33$, then the data satisfy parallelism. If $F \leq F_{.05,2,n}$, where $F_{.05,2,n}$ is the upper 0.05 percentage point of an *F* distribution with 2 and n degrees of freedom, then the data satisfy linearity. If both conditions are satisfied, determine the logarithm of potency of the Injection, *M*, taken as:

$$M = M' + \log R,$$
$$\text{where } M' = 4iT_a/(3T_b)$$

i = interval between successive log doses ▲[log(higher dose) – log(lower dose)]▲ (USP 1-Aug-2019) of both the *Standard solution* and the *Sample solution*

R = v_S/v_U, the ratio of the high dose of the *Standard solution* in USP Corticotropin Units (v_S) to the high dose of the ▲*Sample solution*▲ (USP 1-Aug-2019) in mL (v_U)

Determine the width, *L*, of the log confidence interval as:

$$L = 2\sqrt{(C-1)\left(C(M')^2 + \frac{8}{3}i^2\right)}$$
$$\text{where } C = T_b^2/\left(T_b^2 - 4fs^2t_{.025,n}^2\right)$$

and $t_{.025,n}$ is the upper one-sided 0.025 percentage point (or two-sided 0.05 percentage point) of a *t*-distribution with n degrees of freedom.

Replication: Repeat the entire determination at least once. Test the agreement among the two or more independent determinations, and compute the weight for each (see *Design and Analysis of Biological Assays* ⟨111⟩, *Combination of Independent Assays*). Calculate the weighted mean log-potency $\overline{M}$ and its confidence interval, L_c (see *Design and Analysis of Biological Assays* ⟨111⟩, *The Confidence Interval and Limits of Potency*). The potency, P_*, is satisfactory if P_* = antilog $\overline{M}$ is 80%–125% of the labeled potency and if the confidence interval does not exceed 0.40.

Acceptance criteria: 80.0%–125.0%

IMPURITIES

- **VASOPRESSIN ACTIVITY**

Solution A: Dissolve 6.6 g of dibasic ammonium phosphate in about 950 mL of water, and adjust with concentrated phosphoric acid to a pH of 3.0. Dilute with water to 1 L.

Mobile phase: Acetonitrile and *Solution A* (13:87). Filter and degas.

[NOTE—The retention time of the vasopressin peak is very sensitive to small changes in the acetonitrile concentration.]

Standard solution: Dissolve the entire contents of a vial of USP Vasopressin RS in a known volume of *Solution A*, and dilute with *Solution A* to obtain a final solution containing 0.1 USP Vasopressin Units/mL.

Sample solution: Dissolve the entire contents of a vial of the Injection in a known volume of *Solution A*, and dilute with *Solution A* to obtain a final solution containing 2.0 USP Corticotropin Units/mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: About 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the vasopressin activity in USP Vasopressin Units/USP Corticotropin Unit:

$$\text{Result} = C_S \times [(r_U/r_S)/2]$$

C_S = concentration of the *Standard solution* (USP Vasopressin Units/mL)
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

Acceptance criteria: NMT 0.05 USP Vasopressin Units/USP Corticotropin Unit

SPECIFIC TESTS

- **pH** ⟨791⟩: 3.0–7.0
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 3.1 USP Endotoxin Units/USP Corticotropin Unit
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store in a cold place.
- **LABELING:** If the labeling of Injection recommends intravenous administration, include specific information on dosage.
- **USP REFERENCE STANDARDS** ⟨11⟩

www.webofpharma.com

USP Corticotropin RS
USP Vasopressin RS

Corticotropin for Injection

DEFINITION

Corticotropin for Injection is the sterile, dry material containing the polypeptide hormone having the property of increasing the rate of secretion of adrenal corticosteroids, which is obtained from the anterior lobe of the pituitary of mammals used for food by humans. Its potency is NLT 80.0% and NMT 125.0% of the potency stated on the label in USP Corticotropin Units. It may contain a suitable antimicrobial agent and suitable diluents and buffers.

IDENTIFICATION

• **A. HPLC**

Solution A: 0.1% trifluoroacetic acid
Solution B: 0.1% trifluoroacetic acid in acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
1	75	25
17	70	30
27	70	30
27.5	20	80
32	20	80
32.5	75	25
35	75	25

Standard solution: 18.7 USP Corticotropin Units/mL of USP Corticotropin RS
Sample solution: 22 USP Corticotropin Units/mL of Corticotropin for Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorescence; excitation 295 nm, emission 355 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Temperatures
Sample tray: 35°
Column: 35°
Flow rate: 1.0 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation of the retention time: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The retention time of the corticotropin peak of the *Sample solution* corresponds to that of the *Standard solution*.

• **B.** Meets the requirements of the *Assay*

ASSAY

Change to read:

• **PROCEDURE**

Standard solutions: ▲Add▲ (USP 1-Aug-2019) gelatin TS to an opened container of USP Corticotropin RS, and mix to obtain a solution with a concentration of 2.0 USP Corticotropin Units/mL. Using gelatin TS as a diluent, prepare three diluted *Standard solutions* such that the respective concentrations of corticotropin constitute a geometric series such as 1:2:4 or 1:3:9 and such that the quantity of corticotropin in each 0.5 mL lies within the range of 10–300 milliunits.

Sample solutions: Constitute the Corticotropin for Injection as directed in the labeling. In the same manner as the *Standard solutions* preparation, using the same diluent, dilute the reconstituted Corticotropin for Injection to make three *Sample solutions* corresponding in concentration to those of the *Standard solutions*.

The animals: Select healthy rats, of the same but either sex, that have been raised on a diet fully adequate with respect to vitamin and mineral content. Anesthetize the rats and remove the hypophysis from each by application of gentle suction through a fine-tipped tube. Between 16 and 48 h after the operation, select those rats weighing 80–180 g, but restrict the selection so that no rat is more than 30% heavier than the lightest, and the number of rats is an exact multiple of 6. Separate the selected rats into 6 groups, equal in size, of NLT 6 rats each, and assign at random one of the three diluted *Standard solutions* or one of the three *Sample solutions* to each group.

Analysis: Inject all rats of each group subcutaneously with the assigned test doses. Three h after the injection, anesthetize the rats, and remove both adrenal glands from each rat, free them from adhering tissue, and promptly weigh each pair on a suitable balance to the nearest 0.2 mg. Place the weighed glands from each rat in suitable vessels each containing 8.0 mL of metaphosphoric acid solution (1 in 40), and pulverize the glands by grinding with a small quantity of washed sand. Cover each vessel, and proceed similarly until all glands have been extracted.

Ascorbic acid determination: Filter the metaphosphoric acid extracts, and pipet 4 mL of each filtrate into suitable vessels each containing 4.0 mL of indophenol–acetate TS. Mix by shaking, and read the absorbance at 520 nm, with a suitable spectrophotometer. From the observed absorbance and the standard curve prepared as directed below, calculate the amount of ascorbic acid in mg/100 g of adrenal gland tissue. Prepare a standard concentration–absorbance curve, using three ascorbic acid solutions containing, respectively, 6.0, 8.0, and 10.0 µg/mL of in metaphosphoric acid solution (1 in 40). Pipet into each of three suitable vessels, preferably spectrophotometer cells, 4 mL of indophenol–acetate TS. Add 4.0 mL of one of the three standard ascorbic acid solutions to one of the cells, mix, and promptly read the absorbance from the same instrument and under the same conditions as for the adrenal gland extracts. Repeat the process for the other two standard ascorbic acid solutions, plot the concentration–absorbance values, and draw the straight line best fitting the three plotted points.

Calculation: If there are no missing data (i.e., all groups of rats are the same size, *f*), then the following may be used. [NOTE—If there are missing values, then suitable software can be used and standard procedures followed for parallel line bioassays, including assessment of parallelism and linearity.] Tabulate the observed concentration of ascorbic acid in the adrenal glands of each rat, designated by the

USP Monographs

www.webofpharma.com

symbol y_{jkl}, where $j = S$ (Standard) or U▲(Sample),▲ (USP 1-Aug-2019) $k = 1$, 2, or 3 for the three doses, and $l = 1, \ldots, f$ rats. Total the values of the y_{jkl}'s in each group as:

$$T_{jk} = \sum_{l=1}^{f} y_{jkl}$$

Then determine the following quantities:

$$T_a = \sum_{k=1}^{3} (T_{Uk} - T_{Sk})$$

$$T_b = (T_{U3} - T_{U1}) + (T_{S3} - T_{S1})$$

$$V = (T_{U3} - T_{U1})/(T_{S3} - T_{S1})$$

$$T_q = (T_{U3} - 2T_{U2} + T_{U1}) + (T_{S3} - 2T_{S2} + T_{S1})$$

$$T_{aq} = (T_{U3} - 2T_{U2} + T_{U1}) - (T_{S3} - 2T_{S2} + T_{S1})$$

$$s^2 = \frac{1}{n}\left[\sum_{j,k,l} y_{jkl}^2 - \frac{1}{f}\sum_{j,k} T_{jk}^2\right] \text{ where } n = 6(f-1)$$

$$\text{or } \frac{1}{6}s_{jk}^2 \text{ where } s_{jk}^2 = \frac{1}{f-1}\sum_{l=1}^{f}\left(y_{jkl} - \frac{T_{jk}}{f}\right)^2$$

$$F = \frac{T_q^2 + T_{aq}^2}{24fs^2}$$

If $V \geq 0.75$ and $V \leq 1.33$, then the data satisfy parallelism. If $F \leq F_{.05,2,n}$, where $F_{.05,2,n}$ is the upper 0.05 percentage point of an F distribution with 2 and n degrees of freedom, then the data satisfy linearity. If both conditions are satisfied, determine the logarithm of potency of the Corticotropin for Injection, M, taken as:

$$M = M' + \log R,$$
$$\text{where } M' = 4iT_a/(3T_b)$$

i = interval between successive log doses ▲[log(higher dose) − log(lower dose)]▲ (USP 1-Aug-2019) of both the *Standard solution* and the *Sample solution*

R = v_S/v_U, the ratio of the high dose of the *Standard solution* in USP Corticotropin Units (v_S) to the high dose of the ▲*Sample solution*▲ (USP 1-Aug-2019) in mL (v_U)

Determine the width, L, of the log confidence interval as:

$$L = 2\sqrt{(C-1)\left(C(M')^2 + \frac{8}{3}i^2\right)}$$
$$\text{where } C = T_b^2/\left(T_b^2 - 4fs^2t_{.025,n}^2\right)$$

and $t_{.025,n}$ is the upper one-sided 0.025 percentage point (or two-sided 0.05 percentage point) of a t-distribution with n degrees of freedom.

Replication: Repeat the entire determination at least once. Test the agreement among the two or more independent determinations, and compute the weight for each (see *Design and Analysis of Biological Assays* ⟨111⟩, *Combination of Independent Assays*). Calculate the weighted mean log-potency M and its confidence interval, L_c (see *Design and Analysis of Biological Assays* ⟨111⟩, *The Confidence Interval and Limits of Potency*). The potency, P, is satisfactory if P = antilog M is 80%–125% of the labeled potency and if the confidence interval does not exceed 0.40.

Acceptance criteria: 80.0%–125.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

- **VASOPRESSIN ACTIVITY**

Solution A: Dissolve 6.6 g of dibasic ammonium phosphate in 950 mL of water, and adjust with concentrated phosphoric acid to a pH of 3.0. Dilute with water to 1 L.

Mobile phase: Acetonitrile and *Solution A* (13:87)
[NOTE—The retention time of the vasopressin peak is very sensitive to small changes in the acetonitrile concentration.]

Standard solution: Dissolve the entire contents of a vial of USP Vasopressin RS in a known volume of *Solution A*, and dilute with *Solution A* to obtain a final solution containing 0.1 USP Vasopressin Units/mL.

Sample solution: Dissolve the entire contents of a vial of Corticotropin for Injection in a known volume of *Solution A*, and dilute with *Solution A* to obtain a final solution containing 2.0 USP Corticotropin Units/mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the vasopressin activity in USP Vasopressin Units/USP Corticotropin Unit:

$$\text{Result} = C_S \times [(r_U/r_S)/2]$$

C_S = concentration of the *Standard solution* (USP Vasopressin Units/mL)
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

Acceptance criteria: NMT 0.05 USP Vasopressin Units/USP Corticotropin Unit

SPECIFIC TESTS

- **pH** ⟨791⟩: 2.5–6.0, in a solution constituted as directed in the labeling supplied by the manufacturer
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 3.1 USP Endotoxin Units/USP Corticotropin Unit
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and Clarity of Solutions* and **LABELING** ⟨7⟩, *Labels and Labeling for Injectable Products*: Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for Constitution*.
- **LABELING:** If the labeling of Corticotropin for Injection recommends intravenous administration, include specific information on dosage.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Corticotropin RS
USP Vasopressin RS

www.webofpharma.com

Repository Corticotropin Injection

DEFINITION

Repository Corticotropin Injection is Corticotropin in a solution of partially hydrolyzed gelatin. Its potency is NLT 80.0% and NMT 125.0% of the potency stated on the label in USP Corticotropin Units. It may contain a suitable antimicrobial agent.

IDENTIFICATION

• **A.** Meets the requirements of the *Assay*

ASSAY

Change to read:

• **PROCEDURE**

Standard solutions: ▲Add▲ (USP 1-Aug-2019)gelatin TS to an opened container of USP Corticotropin RS, and mix to obtain a solution having a concentration of 2.0 USP Corticotropin Units/mL. Using gelatin TS as a diluent, prepare three diluted *Standard solutions* such that the respective concentrations of corticotropin constitute a geometric series such as 1:2:4 or 1:3:9 and such that the quantity of corticotropin in each 0.5 mL lies within the range of 10–300 milliunits.

Sample solutions: In the same manner as the *Standard solutions* preparation, using the same diluent, dilute the Injection to make three *Sample solutions* corresponding in concentration to those of the *Standard solutions*.

The animals: Select healthy rats, of the same but either sex, that have been raised on a diet fully adequate with respect to vitamin and mineral content. Anesthetize the rats and remove the hypophysis from each by application of gentle suction through a fine-tipped tube. Between 16 and 48 h after the operation, select those rats weighing 80–180 g, but restrict the selection so that no rat is more than 30% heavier than the lightest, and the number of rats is an exact multiple of 6. Separate the selected rats into 6 groups, equal in size, of NLT 6 rats each, and assign at random one of the three diluted *Standard solutions* or one of the three *Sample solutions* to each group.

Analysis: Inject all rats of each group subcutaneously with the assigned test doses. Three h after the injection, anesthetize the rats, and remove both adrenal glands from each rat, free them from adhering tissue, and promptly weigh each pair on a suitable balance to the nearest 0.2 mg. Place the weighed glands from each rat in suitable vessels each containing 8.0 mL of metaphosphoric acid solution (1 in 40), and pulverize the glands by grinding with a small quantity of washed sand. Cover each vessel, and proceed similarly until all glands have been extracted.

Ascorbic acid determination: Filter the metaphosphoric acid extracts, and pipet 4 mL of each filtrate into suitable vessels each containing 4.0 mL of indophenol–acetate TS. Mix by shaking, and read the absorbance at 520 nm, with a suitable spectrophotometer. From the observed absorbance and the standard curve prepared as directed below, calculate the amount of ascorbic acid in mg/100 g of adrenal gland tissue. Prepare a standard concentration–absorbance curve, using three ascorbic acid solutions containing, respectively, 6.0, 8.0, and 10.0 µg/mL of in metaphosphoric acid solution (1 in 40). Pipet into each of three suitable vessels, preferably spectrophotometer cells, 4 mL of indophenol–acetate TS. Add 4.0 mL of one of the three standard ascorbic acid solutions to one of the cells, mix, and promptly read the absorbance from the same instrument and under the same conditions as for the adrenal gland extracts. Repeat the process for the other two standard ascorbic acid solutions, plot the concentration–absorbance values, and draw the straight line best fitting the three plotted points.

Calculation: If there are no missing data (i.e., all groups of rats are the same size, f), then the following may be used. [NOTE—If there are missing values, then suitable software can be used and standard procedures followed for parallel line bioassays, including assessment of parallelism and linearity.] Tabulate the observed concentration of ascorbic acid in the adrenal glands of each rat, designated by the symbol y_{jkl}, where j = S (Standard) or U ▲(Sample),▲ (USP 1-Aug-2019) k = 1, 2, or 3 for the three doses, and l = 1, . . ., f rats. Total the values of the y_{jkl}'s in each group as:

$$T_{jk} = \sum_{l=1}^{f} y_{jkl}$$

Then determine the following quantities:

$$T_a = \sum_{k=1}^{3} (T_{Uk} - T_{Sk})$$

$$T_b = (T_{U3} - T_{U1}) + (T_{S3} - T_{S1})$$

$$V = (T_{U3} - T_{U1}) \Big/ (T_{S3} - T_{S1})$$

$$T_q = (T_{U3} - 2T_{U2} + T_{U1}) + (T_{S3} - 2T_{S2} + T_{S1})$$

$$T_{aq} = (T_{U3} - 2T_{U2} + T_{U1}) - (T_{S3} - 2T_{S2} + T_{S1})$$

$$s^2 = \tfrac{1}{n}\left[\sum_{j,k,l} y_{jkl}^2 - \tfrac{1}{f}\sum_{j,k} T_{jk}^2\right] \text{ where } n = 6(f-1)$$

$$\text{or } = \tfrac{1}{6} s_{jk}^2 \text{ where } s_{jk}^2 = \tfrac{1}{f-1}\sum_{l=1}^{f} \left(y_{jkl} - \frac{T_{jk}}{f}\right)^2$$

$$F = \frac{T_q^2 + T_{aq}^2}{24 f s^2}$$

If $V \geq 0.75$ and $V \leq 1.33$, then the data satisfy parallelism. If $F \leq F_{.05,2,n}$, where $F_{.05,2,n}$ is the upper 0.05 percentage point of an F distribution with 2 and n degrees of freedom, then the data satisfy linearity. If both conditions are satisfied, determine the logarithm of potency of the Injection, M, taken as:

$$M = M' + \log R,$$

$$\text{where } M' = 4iT_a/(3T_b)$$

i = interval between successive log doses ▲[log(higher dose) – log(lower dose)]▲ (USP 1-Aug-2019) of both the *Standard solution* and the *Sample solution*

R = v_S/v_U, the ratio of the high dose of the *Standard solution* in USP Corticotropin Units (v_S) to the high dose of the ▲*Sample solution*▲ (USP 1-Aug-2019) in mL (v_U)

Determine the width, L, of the log confidence interval as:

$$L = 2\sqrt{(C-1)(C(M')^2 + \tfrac{8}{3}i^2)}$$

$$\text{where } C = T_b^2 \Big/ (T_b^2 - 4fs^2 t_{.025,n}^2)$$

USP Monographs

www.webofpharma.com

and $t_{.025,n}$ is the upper one-sided 0.025 percentage point (or two-sided 0.05 percentage point) of a *t*-distribution with *n* degrees of freedom.

Replication: Repeat the entire determination at least once. Test the agreement among the two or more independent determinations, and compute the weight for each (see *Design and Analysis of Biological Assays* ⟨111⟩, *Combination of Independent Assays*). Calculate the weighted mean log-potency $\overline{M}$ and its confidence interval, L_c (see *Design and Analysis of Biological Assays* ⟨111⟩, *The Confidence Interval and Limits of Potency*). The potency, P_*, is satisfactory if P_* = antilog $\overline{M}$ is 80%–125% of the labeled potency and if the confidence interval does not exceed 0.40.

Acceptance criteria: 80.0%–125.0%

IMPURITIES

• **VASOPRESSIN ACTIVITY**

Solution A: Dissolve 6.6 g of dibasic ammonium phosphate in about 950 mL of water, and adjust with concentrated phosphoric acid to a pH of 3.0. Dilute with water to 1 L.

Mobile phase: Acetonitrile and *Solution A* (13:87). Filter and degas.

[NOTE—The retention time of the vasopressin peak is very sensitive to small changes in the acetonitrile concentration.]

Standard solution: Dissolve the entire contents of a vial of USP Vasopressin RS in a known volume of *Solution A*, and dilute with *Solution A* to obtain a final solution containing 0.1 USP Vasopressin Units/mL.

Sample solution: Dissolve the entire contents of a vial of Injection in a known volume of *Solution A*, and dilute with *Solution A* to obtain a final solution containing 2.0 USP Corticotropin Units/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: About 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the vasopressin activity in USP Vasopressin Units/USP Corticotropin Unit:

$$\text{Result} = C_S \times [(r_U/r_S)/2]$$

C_S = concentration of the *Standard solution* (USP Vasopressin Units/mL)

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

Acceptance criteria: NMT 0.05 USP Vasopressin Units/USP Corticotropin Unit

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 3.0–7.0

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 3.1 USP Endotoxin Units/USP Corticotropin Unit

• **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

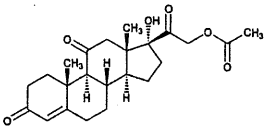
• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Corticotropin RS

USP Vasopressin RS

Cortisone Acetate



$C_{23}H_{30}O_6$ 402.48

Pregn-4-ene-3,11,20-trione, 21-(acetyloxy)-17-hydroxy-; 17,21-Dihydroxypregn-4-ene-3,11,20-trione 21-acetate [50-04-4].

DEFINITION

Cortisone Acetate contains NLT 97.0% and NMT 102.0% of cortisone acetate ($C_{23}H_{30}O_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)**:** [NOTE—Methods described in ⟨197K⟩ or ⟨197A⟩ may be used.]▲ 2S (USP41)

Sample: Dissolve in methanol, evaporate the methanol on a steam bath, and dry at 105° for 30 min.

Acceptance criteria: Meets the requirements

Change to read:

• **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

• **PROCEDURE**

▲**Solution A:** Water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	70	30
20	70	30
27	30	70
27.1	70	30
30	70	30

Diluent: Acetonitrile, glacial acetic acid, and water (70:1:30)

System suitability solution: 1000 µg/mL of USP Cortisone Acetate RS and 150 µg/mL of USP Hydrocortisone Acetate RS in *Diluent*

Standard solution: 1000 µg/mL of USP Cortisone Acetate RS in *Diluent*

www.webofpharma.com

Sample solution: 1000 µg/mL of Cortisone Acetate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 15 µL
System suitability
[NOTE—See *Table 2* for the relative retention times.]
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 4.2 between cortisone acetate and hydrocortisone acetate, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cortisone acetate ($C_{23}H_{30}O_6$) in the portion of Cortisone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cortisone Acetate RS in the *Standard solution* (µg/mL)
C_U = concentration of Cortisone Acetate in the *Sample solution* (µg/mL)▲ 2S (USP41)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

Change to read:

• **RESIDUE ON IGNITION** ⟨281⟩: ▲NMT 0.50%▲ 2S (USP41)

Change to read:

• **ORGANIC IMPURITIES**
▲**Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Sample solution,** and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1 µg/mL of USP Cortisone Acetate RS and 5 µg/mL of USP Hydrocortisone Acetate RS in *Diluent*
System suitability
[NOTE—See *Table 2* for the relative retention times.]
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 4.2 between cortisone acetate and hydrocortisone acetate, *System suitability solution*
Relative standard deviation: NMT 5.0%, hydrocortisone acetate and cortisone acetate, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of hydrocortisone acetate in the portion of Cortisone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydrocortisone acetate from the *Sample solution*
r_S = peak response of hydrocortisone acetate from the *Standard solution*
C_S = concentration of USP Hydrocortisone Acetate RS in the *Standard solution* (µg/mL)
C_U = concentration of Cortisone Acetate in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Cortisone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*
r_S = peak response of cortisone acetate from the *Standard solution*
C_S = concentration of USP Cortisone Acetate RS in the *Standard solution* (µg/mL)
C_U = concentration of Cortisone Acetate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Hydrocortisone acetate	0.8	0.5
Cortisone acetate	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	1.5▲ 2S (USP41)

SPECIFIC TESTS
• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL in dioxane
Acceptance criteria: +208° to +217°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 30 min.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cortisone Acetate RS
▲USP Hydrocortisone Acetate RS▲ 2S (USP41)

Cortisone Acetate Tablets

DEFINITION
Cortisone Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cortisone acetate ($C_{23}H_{30}O_6$).

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Powder a number of Tablets equivalent to 25 mg of cortisone acetate. Add 25 mL of solvent hexane, and extract for 15 min with occasional agitation. Decant and discard the supernatant, then extract the residue with 5 mL of chloroform, with frequent agitation, for 5 min. Filter, add 10 mL of methanol to the filtrate, mix, evaporate the solvent on a steam bath with the aid of a current of air, then dry the residue at 105° for 30 min. Use the residue.
Acceptance criteria: Meet the requirements

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

• **PROCEDURE**
▲Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	70	30
20	70	30
27	30	70
27.1	70	30
30	70	30

Diluent: Acetonitrile, glacial acetic acid, and water (70:1:30)
System suitability solution: 1000 µg/mL of USP Cortisone Acetate RS and 150 µg/mL of USP Hydrocortisone Acetate RS in *Diluent*
Standard solution: 1000 µg/mL of USP Cortisone Acetate RS in *Diluent*
Sample solution: Nominally 1000 µg/mL of cortisone acetate from Tablets in *Diluent*, prepared as follows. Transfer an appropriately weighed portion of finely powdered Tablets (NLT 20) to a suitable volumetric flask. Add 80% of the final flask volume of *Diluent* and sonicate for 10 min. Dilute with *Diluent* to volume and mix well. Pass through a suitable filter of 0.2-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 15 µL
System suitability
[NOTE—See *Table 2* for the relative retention times.]
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 4.2 between cortisone acetate and hydrocortisone acetate, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cortisone acetate ($C_{23}H_{30}O_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cortisone Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cortisone acetate in the *Sample solution* (µg/mL)▲ 2S (USP41)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Medium: 0.5% sodium lauryl sulfate solution; 1000 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: ▲0.00555 mg/mL▲ 2S (USP41) of USP Cortisone Acetate RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 242 nm
Cell: 1 cm
Analysis
Samples: *Standard solution* and *Sample solution*
▲Calculate the percentage of the labeled amount of cortisone acetate ($C_{23}H_{30}O_6$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Cortisone Acetate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
D = dilution factor for the *Sample solution*, if applicable
L = label claim (mg/Tablet)▲ 2S (USP41)

Tolerances: NLT 75% (Q) of the labeled amount of cortisone acetate ($C_{23}H_{30}O_6$) is dissolved.

Change to read:

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: ▲Meet the requirements▲ 2S (USP41)

USP Monographs

www.webofpharma.com

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 5 µg/mL of USP Cortisone Acetate RS in *Diluent*

System suitability

[NOTE—See *Table 2* for the relative retention times.]

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.2 between cortisone acetate and hydrocortisone acetate, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified degradation product from the *Sample solution*

r_S = peak response of cortisone acetate from the *Standard solution*

C_S = concentration of USP Cortisone Acetate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of cortisone acetate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Hydrocortisone acetate[a]	0.8	—
Cortisone acetate	1.0	—
Any individual unspecified degradation product	—	0.5
Total degradation products	—	1.5

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product. ▲2S (USP41)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cortisone Acetate RS

▲USP Hydrocortisone Acetate RS▲ 2S (USP41)

Cosyntropin

SYSMEHFRWG KPVGKKRRPV KVYP

$C_{136}H_{210}N_{40}O_{31}S$ 2933

$\alpha^{1\text{-}24}$-Corticotropin [16960-16-0].

DEFINITION

Cosyntropin is a synthetic peptide whose sequence is identical to the first 24 amino acids of human adrenocorticotropic hormone (ACTH). Cosyntropin contains NLT 90% and NMT 102% of cosyntropin ($C_{136}H_{210}N_{40}O_{31}S$), calculated on the anhydrous, acetic acid-free basis.

IDENTIFICATION

• **A.** The retention time of the cosyntropin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. AMINO ACID ANALYSIS**

Hydrolysis solution: 12 N hydrochloric acid containing a small crystal (0.1%–1.0%) of phenol

Sample hydrolysate preparation: Transfer a portion of cosyntropin into a vial, weigh it, and dissolve in water to a concentration of 3 mg/mL. Transfer 70 mg of this solution to a vacuum hydrolysis tube. Add 70 µL of *Hydrolysis solution*, seal the tube, and heat for 24 h at 110°. Cool the tube, and remove the solvents at reduced pressure. Resuspend the hydrolysate residue in 1 mL of 0.020 M hydrochloric acid, and filter.

Solution A: Prepare a solution having a composition of 140 mM sodium acetate and 17 mM triethylamine, and adjust with phosphoric acid to a pH of 5.02.[1]

Solution B: Acetonitrile and water (60:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
0.5	99	1
18	95	5
19	91	9
29.5	77	23
40	77	23
40.01	40	60
50	40	60

Sample solution: Add 140 µL of 0.2 M borate buffer (pH 8.8) to 20 µL of *Sample hydrolysate preparation*. Add 40 µL of 10 mM 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC). Incubate for 2 min at room temperature. Transfer to an insert in an HPLC vial, seal with a silicone cap, and incubate for 10 min at 55°.

Standard solution: Prepare a solution having known equimolar amounts of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine with half the equimolar amount of L-cystine. [NOTE—Suitable concentrations are 0.1 mM and 0.05 mM, respectively.] Derivatize with AQC as outlined for *Sample solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

[1] A suitable substitute is Eluant A from Waters Corporation, catalog number WAT052890.

www.webofpharma.com

Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Sample solution* and *Standard solution*
Suitability requirements: All 17 amino acid peaks must be visible in the *Standard solution.*
Resolution: NLT 1.5 between each of the 17 amino acid peaks in the *Standard solution.* [NOTE—In those cases where the peak does not recover to the baseline, the following criteria will be applied: $(hp - hv)/hp \times 100 \pm 90\%$, where *hp* is the height of the minor peak, and *hv* is the height of the valley between the peaks.]
Analysis
Samples: *Sample solution* and *Standard solution*
First record and measure the responses for each amino acid peak in the *Standard solution.* Express the content of each amino acid in moles.
Calculate the mean nmol of the amino acids:

Result = (nmol found in the *Analysis* for Glu, Gly, Val, Phe, Lys, Arg, Pro)/17

Divide the nmol of each amino acid by the *Result* to determine the amino acid ratios that must meet the *Acceptance criteria.*
Acceptance criteria: 3.5–4.7 of lysine; 0.9–1.1 of histidine; 2.7–3.3 of arginine; 1.1–2.2 of serine; 0.9–1.1 of glutamic acid; 2.5–3.5 of proline; 1.8–2.2 of glycine; 0.9–1.1 of methionine; 1.7–2.2 of tyrosine; 0.9–1.1 of phenylalanine; 2.7–3.3 of valine

ASSAY

• **PROCEDURE**
Mobile phase: 365 mL of acetonitrile, 10 mL of glacial acetic acid, and 10 g of ammonium sulfate. Dilute with water to 2 L, and a pH of about 3.3.
Standard solution: 1.0 mg/mL of USP Cosyntropin Acetate RS
Sample solution: 1.0 mg/mL of Cosyntropin
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 50 µL
Run time: 50 min
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between the main cosyntropin peak and the reduced Trp-cosyntropin peak in the *Standard solution*
Retention time: 16–20 min for the cosyntropin peak, *Standard solution*
Relative standard deviation: NMT 2.0% for the cosyntropin peak from three replicate injections of the *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cosyntropin ($C_{136}H_{210}N_{40}O_{31}S$) in the portion of Cosyntropin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cosyntropin from the *Sample solution*
r_S = peak response of cosyntropin from the *Standard solution*
C_S = concentration of cosyntropin in each vial of USP Cosyntropin Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Cosyntropin, calculated on the anhydrous, acetic acid-free basis, in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–102.0% of cosyntropin on the anhydrous, acetic acid-free basis

IMPURITIES

• **ORGANIC IMPURITIES, RELATED PEPTIDES**
Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*
Peak identification solution: 1.0 mg/mL of cosyntropin in 1% (v/v) glacial acetic acid. Add 50 µL of 30% hydrogen peroxide:water (1:999). Let stand for 2 h to produce the main impurity, cosyntropin sulfoxide.
System suitability
Samples: *Standard solution* and *Peak identification solution*
Suitability requirements
Resolution: NLT 1.0 between the main cosyntropin peak and the reduced Trp-cosyntropin peak in the *Standard solution*
Retention time: 16–20 min for the cosyntropin peak, *Standard solution*
Relative retention time of main impurity in *Peak identification solution*: About 0.4
Relative standard deviation: NMT 2.0% for the cosyntropin peak from three replicate injections of the *Standard solution*
Analysis
Sample: *Sample solution*
Integrate the chromatogram using the normalization procedure.
Calculate the percentage of cosyntropin-related impurities in the portion of Cosyntropin taken:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = peak response of any individual impurity from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria
Individual impurities: See *Table 2.*

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cosyntropin sulfoxide	0.4	2
Reduced Trp in cosyntropin	0.9	2
Any other individual impurity	—	1
Total impurities	—	5

OTHER COMPONENTS

• **ACETIC ACID IN PEPTIDES** ⟨503⟩**:** 8%–13% using a 1-mg/mL *Test Solution* prepared by dissolving 10 mg of Cosyntropin in 10 mL of *Solution A* and *Solution B* (95:5)

USP Monographs

www.webofpharma.com

- **TRIFLUOROACETIC ACID CONTENT:** Perform the method contained in ⟨503⟩, substituting a suitable quantity of trifluoroacetic acid for the *Standard solution*. The portion of Cosyntropin taken shall contain NMT 0.5% trifluoroacetic acid.

SPECIFIC TESTS

- **UV ABSORPTION SPECTROPHOTOMETRY**
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Wavelength range: 240–280 nm
Sample solution: 0.2 mg/mL in 0.1 M hydrochloric acid
Acceptance criteria: Absorptivity of 0.51–0.61, calculated on the anhydrous and acetic acid-free basis, at the maximum wavelength of 276 nm
Ratio: A276/A248, 2.4–2.9
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 10 USP Endotoxin Units/mg of cosyntropin.
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count is less than 100 cfu/g.
- **WATER DETERMINATION,** *Method 1c* ⟨921⟩: NMT 10.0%

ADDITIONAL REQUIREMENTS

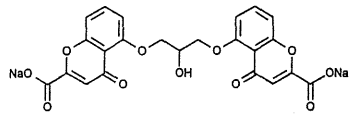
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at 2°–8°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Cosyntropin Acetate RS

Purified Cotton

» Purified Cotton is the hair of the seed of cultivated varieties of *Gossypium hirsutum* Linné, or of other species of *Gossypium* (Fam. Malvaceae), freed from adhering impurities, deprived of fatty matter, bleached, and sterilized in its final container.

Packaging and storage—Package it in rolls of not more than 500 g of a continuous lap, with a light-weight paper running under the entire lap, the paper being of such width that it may be folded over the edges of the lap to a distance of at least 25 mm, the two together being tightly and evenly rolled, and enclosed and sealed in a well-closed container. It may be packaged also in other types of containers if these are so constructed that the sterility of the product is maintained.
Labeling—Its label bears a statement to the effect that the sterility cannot be guaranteed if the package bears evidence of damage or if the package has been opened previously.
Sterility Tests ⟨71⟩: meets the requirements.
Acidity or alkalinity—Thoroughly saturate about 10 g with 100 mL of recently boiled and cooled water, then with the aid of a glass rod press out two 25-mL portions of the water into white porcelain dishes. To one portion add 3 drops of phenolphthalein TS, and to the other portion add 1 drop of methyl orange TS: no pink color develops in either portion.
Residue on ignition ⟨281⟩—Place about 5 g, accurately weighed, in a porcelain or platinum dish, and moisten with 2 N sulfuric acid. Gently heat the cotton until it is charred, then ignite more strongly until the carbon is completely consumed: not more than 0.20% of residue remains.
Water-soluble substances—Place 10 g, accurately weighed, in a beaker containing 1000 mL of water, and boil gently for 30 minutes, adding water as required to maintain the volume. Pour the water through a funnel into another vessel, and press out the excess water from the cotton with a glass rod. Wash the cotton in the funnel with two 250-mL portions of boiling water, pressing the cotton after each washing. Filter the combined extract and washings, and wash the filter thoroughly with hot water. Evaporate the combined extract and washings to a small volume, transfer to a tared porcelain or platinum dish, evaporate to dryness, and dry the residue at 105° to constant weight: the residue weighs not more than 35 mg (0.35%).
Fatty matter—Pack 10 ± 0.01 g in a Soxhlet extractor provided with a tared receiver, and extract with ether for 5 hours at a rate such that the ether siphons over not less than four times per hour. The ether solution in the flask shows no trace of blue, green, or brownish color. Evaporate the extract to dryness, and dry at 105° for 1 hour: the weight of the residue does not exceed 70 mg (0.7%).
Dyes—Pack about 10 g in a narrow percolator, and extract slowly with alcohol until the percolate measures 50 mL: when observed downward through a column 20 cm in depth, the percolate may show a yellowish color, but neither a blue nor a green tint.
Other foreign matter—The pinches of it taken for the determination of *Fiber length* contain no oil stains or metallic particles.
Fiber length and Absorbency—Remove it from its wrappings, and condition it for not less than 4 hours in a standard atmosphere of 65 ± 2% relative humidity at 21 ± 1.1° (70 ± 2°F), before determining the *Fiber length* and *Absorbency*.
Fiber length—Determine the fiber length of Purified Cotton as directed under *Cotton—Fiber Length* ⟨691⟩: not less than 60% of the fibers, by weight, are 12.5 mm or greater in length, and not more than 10% of the fibers, by weight, are 6.25 mm or less in length.
Absorbency—Proceed as directed under *Cotton—Absorbency Test* ⟨691⟩: submersion is complete in 10 seconds at a temperature of 25°, and the cotton retains not less than 24 times its weight of water.

Cromolyn Sodium



$C_{23}H_{14}Na_2O_{11}$ 512.33
4*H*-1-Benzopyran-2-carboxylic acid, 5,5′-[(2-hydroxy-1,3-propanediyl)bis(oxy)bis[4-oxo-, disodium salt];
Disodium 5,5′-[(2-hydroxytrimethylene)dioxy]bis[4-oxo-4*H*-1-benzopyran-2-carboxylate] [15826-37-6].

DEFINITION
Cromolyn Sodium contains NLT 98.0% and NMT 101.0% of cromolyn sodium ($C_{23}H_{14}Na_2O_{11}$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
Analysis: Dry at 105° to constant weight.
Acceptance criteria: The infrared spectrum of the *Sample solution* is consistent with that of a similarly prepared USP Cromolyn Sodium RS.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

• **C. Identification Tests—General** ⟨191⟩, *Chemical Identification Tests, Sodium*: Meets the requirements

ASSAY

• **Procedure**

Buffer: 2.1 g/L of sodium acetate in water. Adjust with glacial acetic acid to a pH of 5.5.
Mobile phase: Methanol and *Buffer* (20:80)
Standard solution: 0.1 mg/mL of USP Cromolyn Sodium RS in water
Sample solution: 0.1 mg/mL of Cromolyn Sodium in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.7 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of cromolyn
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cromolyn sodium ($C_{23}H_{14}Na_2O_{11}$) in the portion of Cromolyn Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cromolyn from the *Sample solution*
r_S = peak response of cromolyn from the *Standard solution*
C_S = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Cromolyn Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the anhydrous basis

IMPURITIES

• **Organic Impurities**

Diluent A: Acetonitrile and water (20:80)
Diluent B: Methanol and water (30:70)
Buffer: 2.1 g/L of sodium acetate in water. Adjust with glacial acetic acid to a pH of 5.5.
Solution A: Methanol and *Buffer* (20:80)
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
20	43	57
30	43	57
32	100	0
35	100	0

[Note—The *Sample solution* and *Standard solutions* are stable for 6 h at 4°.]

Standard stock solution: 0.15 mg/mL of USP Cromolyn Related Compound A RS and 0.06 mg/mL of USP Cromolyn Related Compound B RS, in acetonitrile
Standard solution A: 0.0075 mg/mL of USP Cromolyn Related Compound A RS and 0.003 mg/mL of USP Cromolyn Related Compound B RS from *Standard stock solution* in *Diluent A*
Standard solution B: 0.0015 mg/mL of USP Cromolyn Sodium RS in *Diluent B*
Sensitivity solution: 0.75 µg/mL of USP Cromolyn Sodium RS from *Standard solution B* in *Diluent B*
Sample solution: 1.5 mg/mL of Cromolyn Sodium in *Diluent B*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 4°
Column: 35°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution A*, *Standard solution B*, and *Sensitivity solution*
[Note—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.5 between cromolyn related compound A and cromolyn related compound B, *Standard solution A*
Relative standard deviation: NMT 5.0%, *Standard solution B*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Calculate the percentage of each cromolyn related compound in the portion of Cromolyn Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cromolyn related compound A or cromolyn related compound B from the *Sample solution*
r_S = peak response of cromolyn related compound A or cromolyn related compound B from *Standard solution A*
C_S = concentration of the corresponding cromolyn related compound in *Standard solution A* (mg/mL)
C_U = concentration of Cromolyn Sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any specified unidentified impurity, 2-acetylresorcinol, or each unspecified impurity in the portion of Cromolyn Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the specified unidentified impurity, 2-acetylresorcinol, or each unspecified impurity from the *Sample solution*
r_S = peak response of cromolyn from *Standard solution B*
C_S = concentration of USP Cromolyn Sodium RS in *Standard solution B* (mg/mL)
C_U = concentration of Cromolyn Sodium in the *Sample solution* (mg/mL)

www.webofpharma.com

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cromolyn	1.0	—
Specified unidentified impurity[a]	1.57	0.15
2-Acetylresorcinol[b]	2.5	0.10
Cromolyn related compound A	4.2	0.10
Cromolyn related compound B	4.35	0.10
Any other individual unspecified impurity	—	0.10
Total impurities	—	0.5

[a] Manufacturing process impurity with an indeterminate chemical structure.
[b] 1-(2,6-Dihydroxyphenyl)ethan-1-one; also known as 2,6-dihydroxyacetophenone.

• **LIMIT OF OXALATE**
Standard solution: To 0.35 mg of oxalic acid in 20 mL of water, add 5.0 mL of iron salicylate TS, and dilute with water to 50 mL.
Sample solution: To 100 mg of Cromolyn Sodium in 20 mL of water, add 5.0 mL of iron salicylate TS, and dilute with water to 50 mL.
Instrumental conditions
Mode: Vis
Analytical wavelength: 480 nm
Blank: Water
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the absorbance of the *Standard solution* and the *Sample solution* at 480 nm.
Acceptance criteria: The absorbance of the *Sample solution* is NLT that of the *Standard solution* (0.35%).

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 10.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cromolyn Sodium RS
USP Cromolyn Related Compound A RS
1,3-Bis(2-acetyl-3-hydroxyphenoxy)propan-2-ol.
$C_{19}H_{20}O_7$ 360.36
USP Cromolyn Related Compound B RS
Diethyl 5,5′-[(2-hydroxypropane-1,3-diyl)bis(oxy)]bis(4-oxo-4*H*-chromene-2-carboxylate).
$C_{27}H_{24}O_{11}$ 524.48

Cromolyn Sodium Inhalation Powder

» Cromolyn Sodium Inhalation Powder is a mixture of equal parts of Lactose and Cromolyn Sodium contained in a hard gelatin capsule. It contains not less than 95.0 percent and not more than 125.0 percent of the labeled amount of cromolyn sodium ($C_{23}H_{14}Na_2O_{11}$).

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature. Avoid excessive heat.
USP Reference standards ⟨11⟩—
USP Cromolyn Sodium RS
Identification—It meets the requirements of *Identification* test *B* under *Cromolyn Sodium*.
Uniformity of dosage units ⟨905⟩: meets the requirements.

Assay—
pH 7.4 Sodium phosphate buffer—Prepare as directed in the *Assay* under *Cromolyn Sodium*.
Assay preparation—Remove and accurately weigh the contents of not fewer than 20 capsules of Cromolyn Sodium Inhalation Powder, and transfer the combined contents to a 250-mL volumetric flask. Dissolve in 100 mL of water, dilute with water to volume, and mix. Transfer an aliquot of this solution, equivalent to 8 mg of cromolyn sodium, to a 250-mL volumetric flask, add 1 mL of *pH 7.4 Sodium phosphate buffer*, dilute with water to volume, and mix.
Standard preparation—Dissolve a suitable quantity of USP Cromolyn Sodium RS, previously dried in vacuum at 105° to constant weight and accurately weighed, in water to obtain a solution having a known concentration of about 350 µg per mL. Transfer 10 mL of this solution to a 100-mL volumetric flask, add 1 mL of *pH 7.4 Sodium phosphate buffer*, dilute with water to volume, and mix. The final concentration is about 35 µg per mL.
Procedure—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 326 nm, with a suitable spectrophotometer, using a solution of *pH 7.4 Sodium phosphate buffer* (1 in 250) as the blank. Calculate the quantity, in mg, of cromolyn sodium ($C_{23}H_{14}Na_2O_{11}$) in the aliquot taken by the formula:

$$0.25C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Cromolyn Sodium RS in the *Standard preparation;* and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Cromolyn Sodium Inhalation Solution

DEFINITION
Cromolyn Sodium Inhalation Solution is a sterile, aqueous solution of Cromolyn Sodium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cromolyn sodium ($C_{23}H_{14}Na_2O_{11}$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 5.6 g/L of monobasic potassium phosphate and 22.2 g/L of myristyltrimethylammonium bromide in water. Adjust with sodium hydroxide solution to a pH of 6.5.
Mobile phase: Methanol and *Buffer* (55:45)
Diluent: Acetonitrile and water (30:70)

USP Monographs

www.webofpharma.com

System suitability solution: 0.5 mg/mL of USP Cromolyn Sodium RS and 0.02 mg/mL each of USP Cromolyn Related Compound A RS and USP Cromolyn Related Compound B RS in *Diluent*
Standard solution: 0.5 mg/mL of USP Cromolyn Sodium RS in *Diluent*
Sample solution: Nominally equivalent to 0.5 mg/mL of cromolyn sodium from a volume of Inhalation Solution in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 326 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between cromolyn related compound B and cromolyn related compound A, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cromolyn sodium ($C_{23}H_{14}Na_2O_{11}$) in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cromolyn from the *Sample solution*
r_S = peak response of cromolyn from the *Standard solution*
C_S = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer, Mobile phase, Diluent, and System suitability solution: Proceed as directed in the *Assay*.
Standard solution: 0.002 mg/mL each of USP Cromolyn Related Compound A RS, USP Cromolyn Related Compound B RS, and USP Cromolyn Sodium RS in *Diluent*
Sample solution: Nominally equivalent to 2 mg/mL of cromolyn sodium from a volume of Inhalation Solution in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 326 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L7
Temperatures
Column: 40°
Autosampler: 4°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: 2 times the retention time of cromolyn
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between cromolyn related compound B and cromolyn related compound A; NLT 2.0 between cromolyn related compound A and cromolyn, *System suitability solution*
Relative standard deviation: NMT 3% for six replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cromolyn related compound A or cromolyn related compound B in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cromolyn related compound A or cromolyn related compound B from the *Sample solution*
r_S = peak response of cromolyn related compound A or cromolyn related compound B from the *Standard solution*
C_S = concentration of the corresponding cromolyn related compound in the *Standard solution* (mg/mL)
C_U = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of cromolyn from the *Standard solution*
C_S = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cromolyn related compound B	0.4	1.0
Cromolyn related compound A	0.5	1.0
Cromolyn	1.0	—
Any unspecified individual degradation product	—	1.0
Total impurities	—	2.0

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 4.0–7.0
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-unit double-ended glass ampuls or in low-density polyethylene ampuls. Store at controlled room temperature, protected from light.

www.webofpharma.com

• **LABELING:** The label indicates that the Inhalation Solution is not to be used if it contains a precipitate.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cromolyn Related Compound A RS
1,3-Bis(2-acetyl-3-hydroxyphenoxy) propan-2-ol.
$C_{19}H_{20}O_7$ 360.36
USP Cromolyn Related Compound B RS
Diethyl 5,5′-[(2-hydroxypropane-1,3-diyl)bis(oxy)]bis(4-oxo-4*H*-chromene-2-carboxylate).
$C_{27}H_{24}O_{11}$ 524.48
USP Cromolyn Sodium RS

Cromolyn Sodium Nasal Solution

DEFINITION
Cromolyn Sodium Nasal Solution is an aqueous solution of Cromolyn Sodium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cromolyn sodium ($C_{23}H_{14}Na_2O_{11}$). It may contain suitable stabilizers.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Buffer: 5.6 g/L of monobasic potassium phosphate and 22.2 g/L of myristyltrimethylammonium bromide in water. Adjust with sodium hydroxide solution to a pH of 6.5.
Mobile phase: Methanol and *Buffer* (55:45)
Diluent: Acetonitrile and water (30:70)
System suitability solution: 0.5 mg/mL of USP Cromolyn Sodium RS and 0.02 mg/mL each of USP Cromolyn Related Compound A RS and USP Cromolyn Related Compound B RS in *Diluent*
Standard solution: 0.5 mg/mL of USP Cromolyn Sodium RS in *Diluent.* Sonication may be needed to aid dissolution.
Sample solution: Nominally equivalent to 0.5 mg/mL of cromolyn sodium from a volume of Nasal Solution in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 326 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between cromolyn related compound B and cromolyn related compound A; NLT 2.0 between cromolyn related compound A and cromolyn, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cromolyn sodium ($C_{23}H_{14}Na_2O_{11}$) in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cromolyn from the *Sample solution*
r_S = peak response of cromolyn from the *Standard solution*
C_S = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase, Diluent, and **System suitability solution:** Proceed as directed in the *Assay.*
Standard solution: 0.002 mg/mL each of USP Cromolyn Related Compound A RS, USP Cromolyn Related Compound B RS, and USP Cromolyn Sodium RS in *Diluent*
Sample solution: Nominally equivalent to 2 mg/mL of cromolyn sodium from a volume of Nasal Solution in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 326 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L7
Temperatures
Autosampler: 4°
Column: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: 2 times the retention time of cromolyn
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between cromolyn related compound B and cromolyn related compound A; NLT 2.0 between cromolyn related compound A and cromolyn, *System suitability solution*
Relative standard deviation: NMT 3% for six replicate injections for each peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cromolyn related compound A or cromolyn related compound B in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cromolyn related compound A or cromolyn related compound B from the *Sample solution*
r_S = peak response of cromolyn related compound A or cromolyn related compound B from the *Standard solution*
C_S = concentration of the corresponding cromolyn related compound in the *Standard solution* (mg/mL)
C_U = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

www.webofpharma.com

r_S = peak response of cromolyn from the *Standard solution*
C_S = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cromolyn related compound B	0.4	1.0
Cromolyn related compound A	0.5	1.0
Cromolyn	1.0	—
Any unspecified individual degradation product	—	1.0
Total impurities	—	2.0

SPECIFIC TESTS
• **pH** ⟨791⟩: 4.0–7.0

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Cromolyn Related Compound A RS
1,3-Bis(2-acetyl-3-hydroxyphenoxy) propan-2-ol.
$C_{19}H_{20}O_7$ 360.36
USP Cromolyn Related Compound B RS
Diethyl 5,5′-[(2-hydroxypropane-1,3-diyl)bis(oxy)]bis(4-oxo-4*H*-chromene-2-carboxylate).
$C_{27}H_{24}O_{11}$ 524.48
USP Cromolyn Sodium RS

Cromolyn Sodium Ophthalmic Solution

DEFINITION
Cromolyn Sodium Ophthalmic Solution is a sterile, aqueous solution of Cromolyn Sodium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cromolyn sodium ($C_{23}H_{14}Na_2O_{11}$). It may contain suitable antimicrobial and stabilizing agents.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Buffer: 5.6 g/L of monobasic potassium phosphate and 22.2 g/L of myristyltrimethylammonium bromide in water. Adjust with sodium hydroxide solution to a pH of 6.5.
Mobile phase: Methanol and *Buffer* (55:45)
Diluent: Acetonitrile and water (30:70)
System suitability solution: 0.5 mg/mL of USP Cromolyn Sodium RS and 0.02 mg/mL each of USP Cromolyn Related Compound A RS and USP Cromolyn Related Compound B RS in *Diluent*
Standard solution: 0.5 mg/mL of USP Cromolyn Sodium RS in *Diluent*. Sonication may be needed to aid dissolution.
Sample solution: Nominally equivalent to 0.5 mg/mL of cromolyn sodium from a volume of Ophthalmic Solution in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 326 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between cromolyn related compound B and cromolyn related compound A; NLT 2.0 between cromolyn related compound A and cromolyn sodium, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cromolyn sodium ($C_{23}H_{14}Na_2O_{11}$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cromolyn from the *Sample solution*
r_S = peak response of cromolyn from the *Standard solution*
C_S = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **Organic Impurities**
Mobile phase, Diluent, and **System suitability solution:** Proceed as directed in the *Assay*.
Standard solution: 0.002 mg/mL each of USP Cromolyn Related Compound B RS, USP Cromolyn Related Compound A RS, and USP Cromolyn Sodium RS in *Diluent*
Sample solution: Nominally equivalent to 2 mg/mL of cromolyn sodium from a volume of Ophthalmic Solution in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 326 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L7
Temperatures
Autosampler: 4°
Column: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: 2 times the retention time of cromolyn
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between cromolyn related compound B and cromolyn related compound A; NLT 2.0 between cromolyn related compound A and cromolyn sodium, *System suitability solution*

www.webofpharma.com

Relative standard deviation: NMT 3% for six replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each cromolyn related compound in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cromolyn related compound A or cromolyn related compound B from the *Sample solution*

r_S = peak response of cromolyn related compound A or cromolyn related compound B from the *Standard solution*

C_S = concentration of the corresponding cromolyn related compound in the *Standard solution* (mg/mL)

C_U = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of cromolyn from the *Standard solution*

C_S = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cromolyn related compound B	0.4	0.75
Cromolyn related compound A	0.5	0.75
Cromolyn sodium	1.0	—
Any individual unspecified degradation product	—	0.75
Total impurities	—	2.0

SPECIFIC TESTS

- **pH** ⟨791⟩: 4.0–7.0
- **Sterility Tests** ⟨71⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

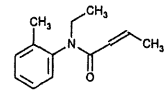
- **Packaging and Storage:** Preserve in tight, light-resistant, single-dose or multiple-dose containers. Ophthalmic Solution that is packaged in multiple-dose containers contains a suitable antimicrobial agent. Store between 15° and 30°.
- **USP Reference Standards** ⟨11⟩

USP Cromolyn Related Compound A RS
1,3-Bis(2-acetyl-3-hydroxyphenoxy) propan-2-ol.
$C_{19}H_{20}O_7$ 360.36

USP Cromolyn Related Compound B RS
Diethyl 5,5′-[(2-hydroxypropane-1,3-diyl)bis(oxy)]bis(4-oxo-4*H*-chromene-2-carboxylate).
$C_{27}H_{24}O_{11}$ 524.48

USP Cromolyn Sodium RS

Crotamiton



$C_{13}H_{17}NO$ 203.28

2-Butenamide, *N*-ethyl-*N*-(2-methylphenyl)-.
N-Ethyl-*o*-crotonotoluidide [483-63-6].

» Crotamiton is a mixture of *cis* and *trans* isomers containing not less than 97.0 percent and not more than 103.0 percent of $C_{13}H_{17}NO$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Crotamiton RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197F*▲ (CN 1-May-2020)

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: cyclohexane.
C: To about 10 mL of a saturated solution in water add a few drops of potassium permanganate TS: a brown color is produced, and a brown precipitate is formed on standing.

Specific gravity ⟨841⟩: between 1.008 and 1.011 at 20°.

Refractive index ⟨831⟩: between 1.540 and 1.543 at 20°.

Residue on ignition ⟨281⟩: not more than 0.1%.

Bound halogen—Place 4 drops in a 3-mm (ID) test tube, and add calcium oxide to a height of 1 cm. Heat the tube in a flame, starting from the top, until the reaction is complete, then ignite for a short time. Transfer the contents to a beaker containing 10 mL of water, acidify with nitric acid, and filter. To the filtrate add 0.2 mL of silver nitrate solution (1 in 60): any opalescence obtained is not more than that obtained from a blank solution treated in the same manner.

Assay—Transfer about 50 mg of Crotamiton, accurately weighed, to a 100-mL volumetric flask, add cyclohexane to volume, and mix. Transfer 10.0 mL of this solution to a 250-mL volumetric flask, dilute with cyclohexane to volume, and mix. Determine the absorbance of this solution and of a solution of USP Crotamiton RS in the same medium having a known concentration of about 20 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 242 nm, with a suitable spectrophotometer, using cyclohexane as the blank. Calculate the quantity, in mg, of $C_{13}H_{17}NO$ in the Crotamiton taken by the formula:

USP Monographs

www.webofpharma.com

$$2.5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Crotamiton RS in the Standard solution; and A_U and A_S are the absorbances of the assay solution and the Standard solution, respectively.

Crotamiton Cream

» Crotamiton Cream contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{13}H_{17}NO$.

Packaging and storage—Preserve in collapsible tubes or tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Crotamiton RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay preparation*.
Minimum fill ⟨755⟩: meets the requirements.

Assay—
Internal standard solution—Dissolve butyl benzoate in methanol to obtain a solution containing about 17.5 mg per mL.
Mobile phase—Prepare a suitable degassed and filtered mixture of acetonitrile and water (3:2).
Standard solution—Dissolve a suitable quantity of USP Crotamiton RS, accurately weighed, in methanol to obtain a solution having a known concentration of about 1 mg per mL.
Standard preparation—Pipet 10 mL of *Standard solution* and 5 mL of *Internal standard solution* into a 50-mL volumetric flask, dilute with methanol to volume, and mix.
Assay preparation—Transfer an accurately weighed portion of Crotamiton Cream, equivalent to about 50 mg of crotamiton, to a tared 50-mL volumetric flask. Add about 25 mL of methanol, and shake and sonicate to disperse the cream. Dilute with methanol to volume, and mix. Filter about 20 mL through moderately retentive filter paper. Pipet 10 mL of the clear filtrate and 5 mL of *Internal standard solution* into a 50-mL volumetric flask, dilute with methanol to volume, and mix.
Procedure—Inject equal volumes of the *Standard preparation* and the *Assay preparation* into a liquid chromatograph (see *Chromatography* ⟨621⟩) equipped with a 254-nm detector and a 4.6-mm × 25-cm stainless steel column that contains packing L1. In a suitable chromatogram, the resolution, R, between peaks for crotamiton and butyl benzoate is not less than 3.0; and the lowest and highest peak response ratios (R_S) of three replicate injections of the *Standard preparation* agree within 2.0%. Calculate the quantity, in mg, of $C_{13}H_{17}NO$ in the portion of Cream taken by the formula:

$$250C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Crotamiton RS in the *Standard preparation;* and R_U and R_S are the peak response ratios of the crotamiton peak and the butyl benzoate peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cupric Chloride

$CuCl_2 \cdot 2H_2O$ 170.48
$CuCl_2$ 134.45
Copper chloride ($CuCl_2$) dihydrate;
Copper(2+) chloride dihydrate [10125-13-0].
Anhydrous [7447-39-4].

DEFINITION
Cupric Chloride contains NLT 99.0% and NMT 100.5% of $CuCl_2$, calculated on the dried basis.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: A solution (1 in 20) meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL,** *Copper* ⟨191⟩: A solution (1 in 20) meets the requirements.

ASSAY

Change to read:

- **PROCEDURE**

Sample solution: 8 mg/mL of Cupric Chloride in water
Analysis: ▲To 50 mL of the *Sample solution*▲ (ERR 1-Dec-2018) add 4 mL of acetic acid and 3 g of potassium iodide. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 2 g of potassium thiocyanate and 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 13.45 mg of Cupric Chloride ($CuCl_2$).
Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES
- **LIMIT OF SODIUM**

Sample stock solution: Transfer 10.0 g of Cupric Chloride to a 100-mL volumetric flask, add water, and swirl to dissolve. Add 5 mL of nitric acid, and dilute with water to volume. This solution contains 0.1 g/mL of cupric chloride.
Sample solutions: To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*. Add 2 mL of potassium chloride solution (1 in 20) to each flask, and dilute with water to volume.
Analysis: Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.
Acceptance criteria: NMT 0.02%

Table 1

Limit Test	Wave-length (nm)	Sample Weight (g)	Reference Ion Added (mg)	Flame Type	Back-ground Correc-tion
Sodium	589.0	0.1	0.01/0.02	Air–acety-lene	No
Potassium	766.5	0.1	0.01/0.02	Air–acety-lene	No
Calcium	422.7	2.0	0.05/0.10	Air–acety-lene	No
Iron	248.3	2.0	0.05/0.10	Air–acety-lene	Yes
Nickel	232.0	2.0	0.10/0.20	Air–acety-lene	No

www.webofpharma.com

• **LIMIT OF POTASSIUM**
Sample stock solution: Prepare as directed in the test for *Limit of Sodium*.
Sample solutions: To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.
Analysis: Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.
Acceptance criteria: NMT 0.01%

• **LIMIT OF CALCIUM**
Sample stock solution: Prepare as directed in the test for *Limit of Sodium*.
Sample solutions: To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.
Analysis: Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.
Acceptance criteria: NMT 0.005%

• **LIMIT OF IRON**
Sample stock solution: Prepare as directed in the test for *Limit of Sodium*.
Sample solutions: To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.
Analysis: Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.
Acceptance criteria: NMT 0.005%

• **LIMIT OF NICKEL**
Sample stock solution: Prepare as directed in the test for *Limit of Sodium*.
Sample solutions: To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.
Analysis: Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.
Acceptance criteria: NMT 0.01%

• **INSOLUBLE MATTER**
Sample: 10 g of Cupric Chloride
Analysis: Transfer the *Sample* to a 250-mL beaker. Add 100 mL of water and 2 mL of hydrochloric acid. Cover the beaker, and heat to boiling. Digest the hot solution on a steam bath for 1 h, and pass through a tared, filtering crucible of fine-pore size. Rinse the beaker with hot water, passing the rinsings through the filter, and finally wash the filter with additional hot water. Retain the combined filtrate and washings for the test for *Limit of Sulfate*. Dry the filter at 105°.
Acceptance criteria: The residue weighs NMT 1.0 mg (0.01%).

• **LIMIT OF SULFATE**
Analysis: Heat to boiling the combined filtrate and washings retained from the test for *Insoluble Matter*. Add 10 mL of barium chloride TS, digest for 2 h on a steam bath, and allow to stand overnight. Pass the solution through a tared, porcelain filtering crucible of medium-pore size, and wash the residue with two 10-mL portions of hot water. Ignite at 800 ± 25° to constant weight.
Acceptance criteria: The weight of the residue, corrected for the weight obtained in a blank test, does not exceed 1.2 mg (0.005%).

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩: Dry a sample at 105° for 16 h: it loses 20.9%-21.4% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

Cupric Chloride Injection

DEFINITION
Cupric Chloride Injection is a sterile solution of Cupric Chloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of copper (Cu).

IDENTIFICATION
• **A.** The *Sample solution* exhibits an absorption maximum at about 325 nm when prepared and tested as directed in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**
Sodium chloride solution: 1.35 g/L of sodium chloride
Standard stock solution: Transfer 1.000 g of copper to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, and dilute with 0.2 N nitric acid to volume. This solution contains 1000 µg/mL of copper. Store in a polyethylene bottle.
Standard solutions: Pipet 15 mL of *Standard stock solution* into a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer 4.0, 5.0, and 6.0 mL of this solution to separate 100-mL volumetric flasks containing 10 mL of *Sodium chloride solution*, dilute the contents of each flask with water to volume, and mix. These *Standard solutions* contain 2.4, 3.0, and 3.6 µg of copper per mL, respectively.
Sample stock solution: Transfer a volume of Injection, equivalent to 2 mg of copper, into 100 mL of water.
Sample solution: Pipet 15 mL of the *Sample stock solution* into a 100-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the Injection, calculate the amount, in mg, of sodium chloride in the initial dilution, and add sufficient *Sodium chloride solution* to bring the total ▲sodium chloride▲ (ERR 1-Dec-2018) content of this flask to 13.5 mg. Dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption
Analytical wavelength: 324.8 nm (copper emission line)
Lamp: Copper hollow-cathode
Flame: Air–acetylene
Blank: *Sodium chloride solution* and water (1 in 10)
Analysis
Samples: *Standard solutions* and *Sample solution*
Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of copper, and draw the straight line best fitting the three plotted points. From

www.webofpharma.com

the graph so obtained, determine the concentration, C, in µg/mL, of copper in the *Sample solution*.

Calculate the percentage of copper in the portion of Injection taken:

$$\text{Result} = [(C/V) \times F \times V_1 \times D] \times (100/L)$$

C = concentration of copper in the *Sample solution* (µg/mL)
V = volume of Injection (mL)
F = conversion factor from µg to mg, 1/1000
V_1 = volume of the *Sample stock solution*, 100 mL
D = dilution factor from the *Sample solution*, 100/15
L = label claim (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 1.5–2.5
- **Bacterial Endotoxins Test** ⟨85⟩: It contains NMT 250.0 USP Endotoxin Units/mg of copper.
- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements for small-volume injections
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.
- **Labeling:** Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid before administration.

Cupric Sulfate

$CuSO_4 \cdot 5H_2O$ 249.69

$CuSO_4$ 159.61

Sulfuric acid, copper(2+) salt (1:1), pentahydrate; Copper(2+) sulfate (1:1) pentahydrate [7758-99-8]. Anhydrous [7758-98-7].

DEFINITION

Cupric Sulfate is anhydrous or contains five molecules of water of hydration. It contains NLT 98.5% and NMT 100.5% of cupric sulfate ($CuSO_4$), calculated on the dried basis.

IDENTIFICATION

- **A. Identification Tests—General,** *Sulfate* ⟨191⟩: A 100-mg/mL solution meets the requirements.
- **B. Identification Tests—General,** *Copper* ⟨191⟩: A 100-mg/mL solution meets the requirements.

ASSAY

- **Procedure**

Sample solution: Place 650 mg of Cupric Sulfate in a weighed container fitted with a ground-glass stopper. Dry, allow to cool in a desiccator, and weigh again to obtain the weight of the sample. Dissolve in 50 mL of water. Add 4 mL of 6 N acetic acid and 3 g of potassium iodide.

Titrimetric system

Mode: Direct titration
Titrant: 0.1 N sodium thiosulfate VS
Endpoint detection: Visual

Analysis: Titrate the liberated iodine in the *Sample solution* with the *Titrant*, adding about 2 g of potassium thiocyanate and 3 mL of starch TS as the endpoint is approached. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 15.96 mg of cupric sulfate ($CuSO_4$).

Acceptance criteria: 98.5%–100.5% on the dried basis

IMPURITIES

- **Limit of Sodium**

Sample stock solution: 0.2 g/mL of cupric sulfate in water, prepared as follows. Transfer 40.0 g of Cupric Sulfate to a 200-mL volumetric flask, add water, and swirl to dissolve. Add 5 mL of nitric acid, and dilute with water to volume.

Sample solutions: To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*. Add 2 mL of potassium chloride solution (1 in 20) to each flask, and dilute with water to volume.

Analysis: Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), analyze the *Sample solutions* by the method of standard addition analysis given in *Table 1*.

Table 1

Limit Test	Wavelength (nm)	Sample Weight (g)	Reference Ion Added (mg)	Flame Type	Background Correction
Sodium	589.0	0.05	0.005/0.01	Air–acetylene	No
Potassium	766.5	0.4	0.02/0.04	Air–acetylene	No
Calcium	422.7	0.8	0.02/0.04	Nitrous oxide–acetylene	No
Iron	248.3	4.0	0.12/0.24	Air–acetylene	Yes
Nickel	232.0	4.0	0.10/0.20	Air–acetylene	No

Acceptance criteria: NMT 0.02%

- **Limit of Potassium**

Sample stock solution: Prepare as directed in the test for *Limit of Sodium*.

Sample solutions: To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.

Analysis: Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), analyze the *Sample solutions* by the method of standard addition analysis given in *Table 1*.

Acceptance criteria: NMT 0.01%

- **Limit of Calcium**

Sample stock solution: Prepare as directed in the test for *Limit of Sodium*.

Sample solutions: To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.

Analysis: Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), analyze the *Sample solutions* by the method of standard addition analysis given in *Table 1*.

Acceptance criteria: NMT 0.005%

- **Limit of Iron**

Sample stock solution: Prepare as directed in the test for *Limit of Sodium*.

USP Monographs

www.webofpharma.com

Sample solutions: To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.
Analysis: Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), analyze the *Sample solutions* by the method of standard addition analysis given in *Table 1*.
Acceptance criteria: NMT 0.003%

• **LIMIT OF NICKEL**
Sample stock solution: Prepare as directed in the test for *Limit of Sodium*.
Sample solutions: To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.
Analysis: Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), analyze the *Sample solutions* by the method of standard addition analysis given in *Table 1*.
Acceptance criteria: NMT 0.005%

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry it at 250° to constant weight.
Acceptance criteria: 33.0%–36.5% for the pentahydrate form; NMT 1.0% for the anhydrous form

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **LABELING:** Label the product to indicate whether it is anhydrous or it is the pentahydrate.

Cupric Sulfate Injection

» Cupric Sulfate Injection is a sterile solution of Cupric Sulfate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of copper (Cu).

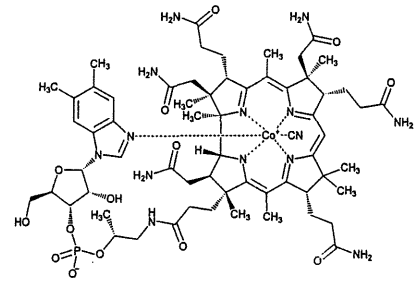
Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.
Labeling—Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.
Identification—The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 325 nm when tested as directed for *Procedure* in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 250.0 USP Endotoxin Units per mg of copper.
pH ⟨791⟩: between 2.0 and 3.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Change to read:

Assay—
Sodium chloride solution, Copper stock solution, and *Standard preparations*—Prepare as directed in the *ASSAY* under *Cupric Chloride Injection*.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 2 mg of copper, to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 15 mL of this solution into a 100-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the Injection, calculate the amount, in mg, of sodium chloride in the initial dilution, and add sufficient *Sodium chloride solution* to bring the total ▲sodium chloride▲ (ERR 1-Dec-2018) content of this flask to 13.5 mg. Dilute with water to volume, and mix.
Procedure—Proceed as directed for *ASSAY* in the *Assay* under *Cupric Chloride Injection*.

Cyanocobalamin



$C_{63}H_{88}CoN_{14}O_{14}P$ 1355.37
Vitamin B_{12} [68-19-9].

DEFINITION
Cyanocobalamin contains NLT 96.0% and NMT 102.0% of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Wavelength range: 200–700 nm
Sample solution: Prepare as directed in the *Assay*.
Acceptance criteria: The absorption spectrum exhibits maxima at 278 ± 1, 361 ± 1, and 550 ± 2 nm. The absorbance ratio A_{361}/A_{278} is 1.70–1.90, and the absorbance ratio A_{361}/A_{550} is 3.15–3.40.

• **B.**
Sample solution: Fuse 1 mg of Cyanocobalamin with 50 mg of potassium pyrosulfate in a porcelain crucible. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling.
Analysis: Add 1 drop of phenolphthalein TS, and add sodium hydroxide solution (100 mg/mL), dropwise, until just pink. Add 500 mg of sodium acetate, 0.5 mL of 1 N acetic acid, and 0.5 mL of nitroso R salt solution (2 mg/mL). Add 0.5 mL of hydrochloric acid, and boil for 1 min.
Acceptance criteria: A red or orange-red color appears immediately after the addition of nitroso R salt. The red color persists after boiling with the addition of hydrochloric acid.

• **C. HPLC**
Mobile phase and **Chromatographic system:** Proceed as directed in the test for *Related Compounds*.
Standard solution: 50 µg/mL of cyanocobalamin from USP Cyanocobalamin (Crystalline) RS in *Mobile phase*. Use within 1 h.

USP Monographs

www.webofpharma.com

Sample solution: 50 µg/mL of Cyanocobalamin in *Mobile phase*. Use within 1 h.
Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**
Sample solution: 30 µg/mL of Cyanocobalamin in water
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 361 nm
Cell: 1 cm
Blank: Water
Analysis
Sample: *Sample solution*
Calculate the percentage of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) in the portion of Cyanocobalamin taken:

$$\text{Result} = A_U/(A_S \times C_U)$$

A_U = absorbance of the *Sample solution*
A_S = specific absorbance ($E^{1\%}$) of cyanocobalamin at 361 nm (100 mL · g^{-1} · cm^{-1}), 207
C_U = concentration of Cyanocobalamin in the *Sample solution* (g/mL)

Acceptance criteria: 96.0%–102.0% on the dried basis

IMPURITIES

• **RELATED COMPOUNDS**
Solution A: 10 g/L of disodium hydrogen phosphate in water
Mobile phase: Mixture of methanol and *Solution A* (26.5:73.5). Adjust with phosphoric acid to a pH of 3.5.
System suitability solution: Dissolve 25 mg of Cyanocobalamin in 10 mL of water, warming if necessary. Allow to cool, add 5 mL of a 1.0-g/L solution of tosylchloramide sodium and 0.5 mL of 0.05 M hydrochloric acid, and then dilute with water to 25 mL. Shake and allow to stand for 5 min. Dilute 1.0 mL of this solution with *Mobile phase* to 10 mL, and inject immediately.
Quantitative limit solution: 1 µg/mL of Cyanocobalamin in *Mobile phase*. Use within 1 h.
Sample solution: 1 mg/mL of Cyanocobalamin in *Mobile phase*. Use within 1 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 361 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 35°
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Quantitative limit solution*
[NOTE—The *System suitability solution* should exhibit two major peaks, cyanocobalamin and 7β, 8β-lactocyanocobalamin. The relative retention times for the two peaks are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.5 between cyanocobalamin and 7β, 8β-lactocyanocobalamin, *System suitability solution*
Signal-to-noise ratio: NLT 5.0 for the major peak, *Quantitative limit solution*
Analysis
Sample: *Sample solution*
[NOTE—The run time should be at least three times the retention time of cyanocobalamin peak.]
Identify the impurities listed in *Table 1*, and measure the peak responses.
Calculate the percentage of individual impurities in the portion of Cyanocobalamin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*. [NOTE—Disregard any peak less than 0.1%.]

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cyanocobalamin	1.0	—
7β,8β-Lactocyanocobalamin	1.2	1.0
50-Carboxycyanocobalamin	1.4	0.5
34-Methylcyanocobalamin	1.5	2.0
32-Carboxycyanocobalamin	1.6	1.0
8-epi-Cyanocobalamin	2.5	1.0
Any other unidentified impurity	—	0.5
Total impurities	—	3.0

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Sample: 25 mg
Analysis: Dry the *Sample* in a suitable vacuum drying apparatus at 105° and at a pressure of NMT 5 mm of mercury for 2 h.
Acceptance criteria: NMT 12.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cyanocobalamin (Crystalline) RS

Cyanocobalamin Injection

» Cyanocobalamin Injection is a sterile solution of Cyanocobalamin in Water for Injection, or in Water for Injection rendered isotonic by the addition of Sodium Chloride. It contains not less than 95.0 percent and not more than 115.0 percent of the labeled amount of anhydrous cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$).

Packaging and storage—Preserve in light-resistant, single-dose or multiple-dose containers, preferably of Type I glass, and store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Cyanocobalamin (Crystalline) RS

www.webofpharma.com

Identification—The absorption spectrum, in the range of 300 nm to 550 nm, of the solution employed for measurement of absorbance in the *Assay* exhibits maxima at the same wavelengths as that of a similar solution of USP Cyanocobalamin (Crystalline) RS, concomitantly measured, and the ratio A_{361}/A_{550} is between 3.15 and 3.40.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.4 USP Endotoxin Unit per µg of cyanocobalamin.
pH ⟨791⟩: between 4.5 and 7.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Dilute, if necessary, an accurately measured volume of Injection, equivalent to not less than 300 µg of cyanocobalamin, quantitatively and stepwise with water to a concentration of about 30 µg per mL. Dissolve an accurately weighed quantity of USP Cyanocobalamin (Crystalline) RS in water, and dilute quantitatively and stepwise with water to obtain a Standard solution having a known concentration of about 30 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 361 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in µg, of $C_{63}H_{88}CoN_{14}O_{14}P$ in each mL of the Injection taken by the formula:

$$10(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Cyanocobalamin (Crystalline) RS in the Standard solution; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Cyanocobalamin Tablets

DEFINITION
Cyanocobalamin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay, Procedure 1* or *Procedure 2*.

ASSAY
[NOTE—Where more than one assay procedure is given in the monograph, the requirements may be met by following any one of the specified procedures. The procedure used is stated in the labeling only if *Procedure 1* is not used.]

• **PROCEDURE 1**
[NOTE—Use low-actinic glassware throughout this procedure.]
Mobile phase: Methanol and water (7:13)
Standard solution: 5 µg/mL of cyanocobalamin from USP Cyanocobalamin (Crystalline) RS in water
Sample solution: Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 500 µg of cyanocobalamin, to a 100-mL volumetric flask, add 60 mL of water, and sonicate for 5 min. Dilute with water to volume, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 361 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of cyanocobalamin from USP Cyanocobalamin (Crystalline) RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cyanocobalamin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

• **PROCEDURE 2**
[NOTE—Use low-actinic glassware throughout this procedure. Inject samples within 30 min.]
Buffer: Dissolve 470.5 mg of low UV hexanesulfonic acid sodium salt in water, add 1 mL of phosphoric acid, dilute with water to 1000 mL, and mix. Adjust with 50% potassium hydroxide to a pH of 3.5.
Mobile phase: Acetonitrile and *Buffer*. See *Table 1* for gradient.

Table 1

Time (min)	Acetonitrile (%)	Buffer (%)
0	1.0	99.0
0.5	1.0	99.0
1.2	2.3	97.7
1.4	5.0	95.0
2.5	7.0	93.0
5.0	18.0	82.0
5.5	25.0	75.0
6.5	25.0	75.0
7.0	1.0	99.0
8.0	1.0	99.0

Standard solution: 1 µg/mL of cyanocobalamin from USP Cyanocobalamin (Crystalline) RS in water
Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 25 µg of cyanocobalamin, to a suitable Erlenmeyer flask with a stopper, pipet 25 mL of water, sonicate for 5 min, and shake vigorously for 2 min. Pass through a membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: UPLC
Detector: UV 361 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 35°
Flow rate: 0.5 mL/min
Injection volume: 15 µL

USP Monographs

www.webofpharma.com

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

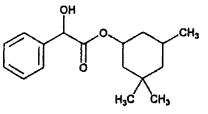
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of cyanocobalamin from USP Cyanocobalamin (Crystalline) RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cyanocobalamin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISINTEGRATION** ⟨701⟩
Medium: Water
Time: 30 min. If the label recommends to disintegrate the Tablets in the mouth before swallowing: NMT 3 min
Acceptance criteria: Meet the requirements
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **LABELING:** Tablets that are intended to be disintegrated in the mouth before swallowing are so labeled. The labeling states with which assay procedure the product complies only if *Procedure 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cyanocobalamin (Crystalline) RS

Cyclandelate



$C_{17}H_{24}O_3$ 276.37
3,3,5-Trimethylcyclohexanol α-phenyl-α-hydroxyacetate; 1,5-*cis*-3,3,5-Trimethylcyclohexyl 2-hydroxy-2-phenyl acetate [456-59-7].

DEFINITION
Cyclandelate contains NLT 98.0% of cyclandelate ($C_{17}H_{24}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩**,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Sample solution: 0.5 mg/mL of cyclandelate
Medium: Alcohol
Acceptance criteria: The solution exhibits absorption maxima between 250 and 254 nm, between 256 and 260 nm, and between 262 and 266 nm.
• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Sample solution: 10 mg/mL of cyclandelate in alcohol
Chromatographic system
Application volume: 5 µL
Developing solvent system: Hexane, ethyl acetate, and glacial acetic acid (80:20:10)
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (800:200)
System suitability solution: 0.2 mg/mL of USP Cyclandelate RS and 0.08 mg/mL of dicyclohexyl phthalate in *Mobile phase*
Standard solution: 0.2 mg/mL of USP Cyclandelate RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Cyclandelate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 228 nm
Column: 4.0-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 7 between cyclandelate and dicyclohexyl phthalate, *System suitability solution*
Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cylcandelate ($C_{17}H_{24}O_3$) in the portion of Cyclandelate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cyclandelate RS in the *Standard solution* (mg/mL)
C_U = concentration of Cyclandelate in the *Sample solution* (mg/mL)

Acceptance criteria: NLT 98.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
• **ORGANIC IMPURITIES**
Mobile phase, System suitability solution, Standard solution, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution 1: 0.03 mg/mL of cyclandelate in *Mobile phase* prepared as follows. Pipet 3.0 mL of the *Sample solution* into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: 1 mg/mL of Cyclandelate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Proceed as directed in the *Assay*, except for the following:
Run time: About 3 times the retention time of cyclandelate, *Sample solution*
Analysis
Samples: *Standard solution 1* and *Sample solution*

USP Monographs

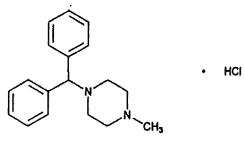
www.webofpharma.com

Acceptance criteria: The total area of all the peaks from the *Sample solution*, other than the peak of cyclandelate, is NMT the peak area of cyclandelate from *Standard solution 1*.
Total impurities: NMT 3.0%

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
Sample: 1 g
Analysis: Dry the *Sample* over silica gel for 24 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store below 40°, preferably between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Cyclandelate RS

Cyclizine Hydrochloride



$C_{18}H_{22}N_2 \cdot HCl$ 302.84
Piperazine, 1-(diphenylmethyl)-4-methyl-, monohydrochloride;
1-(Diphenylmethyl)-4-methylpiperazine monohydrochloride [303-25-3].

DEFINITION
Cyclizine Hydrochloride contains NLT 98.0% and NMT 100.5% of cyclizine hydrochloride ($C_{18}H_{22}N_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY
- **PROCEDURE**
[NOTE—In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the endpoint has been reached.]
Sample: 120 mg
Analysis: Dissolve the *Sample* in 15 mL of anhydrous formic acid. Add 40 mL of acetic anhydride, and titrate with 0.1 N perchloric acid VS. Perform a blank determination (see *Titrimetry* ⟨541⟩), and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 15.14 mg of cyclizine hydrochloride ($C_{18}H_{22}N_2 \cdot HCl$).
Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**
[NOTE—Prepare solutions immediately before use.]
Standard solution: 0.05 mg/mL of USP Cyclizine Hydrochloride RS in methanol
Impurity standard solution: 0.25 mg/mL each of USP Cyclizine Hydrochloride RS, USP Cyclizine Related Compound A RS, and USP Benzhydrol RS in methanol
Sample solution: Prepare a solution containing 50 mg/mL of Cyclizine Hydrochloride by dissolving a suitable amount first in methanol, using 80% of the final volume, and then diluting with 1 N sodium hydroxide to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.33-mm × 25-m; coated with a 0.5-µm film of phase G27
Temperatures
Injection port: 250°
Detector: 290°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	10	240	0
240	15	270	14

Carrier gas: Helium
Flow rate: 1 mL/min
Injection volume: 1 µL
Injection type: Split ratio, 1:25
System suitability
Sample: *Impurity standard solution*
Suitability requirements
Peak-to-valley ratio: NLT 50 between cyclizine related compound A and methanol
Analysis
Samples: *Standard solution*, *Impurity standard solution*, and *Sample solution*
Calculate the percentage of cyclizine related compound A and benzhydrol in the portion of Cyclizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each related compound from the *Sample solution*
r_S = peak response of the corresponding related compound from the *Impurity standard solution*
C_S = concentration of the corresponding related compound in the *Impurity standard solution* (mg/mL)
C_U = concentration of Cyclizine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Cyclizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of cyclizine from the *Standard solution*
C_S = concentration of USP Cyclizine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Cyclizine Hydrochloride in the *Sample solution* (mg/mL)

USP Monographs

www.webofpharma.com

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
1-Methylpiperazine (cyclizine related compound A)	0.2	0.5
Benzhydrol	0.7	0.5
Cyclizine	1.0	—
Any other individual impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: 20 mg/mL in a mixture of alcohol and water (2:3)
Acceptance criteria: 4.5–5.5

• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample at 120° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

• **USP Reference Standards** ⟨11⟩
USP Cyclizine Hydrochloride RS
USP Cyclizine Related Compound A RS
1-Methylpiperazine.
$C_5H_{12}N_2$ 100.16
USP Benzhydrol RS
Diphenylmethanol.
$C_{13}H_{12}O$ 184.23

Cyclizine Hydrochloride Tablets

DEFINITION

Cyclizine Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of cyclizine hydrochloride ($C_{18}H_{22}N_2 \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Extract a quantity of powdered Tablets containing 100 mg of cyclizine hydrochloride with 10 mL of ethanol. Filter, evaporate to dryness, and use the dried residue.
Acceptance criteria: Meet the requirements

ASSAY

• **Procedure**
Analysis: Proceed with Tablets as directed in *Salts of Organic Nitrogenous Bases* ⟨501⟩. Dilute the *Standard Preparation* and the *Assay Preparation*, respectively, with an equal volume of dilute sulfuric acid (1 in 100), and determine the absorbance at the wavelength of maximum absorbance at about 264 nm.
Calculate the percentage of the labeled amount of cyclizine hydrochloride ($C_{18}H_{22}N_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Assay Preparation*
A_S = absorbance of the *Standard Preparation*
C_S = concentration of USP Cyclizine Hydrochloride RS in the *Standard Preparation* (mg/mL)
C_U = nominal concentration of cyclizine hydrochloride in the *Assay Preparation* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **Dissolution,** *Procedure for a Pooled Sample* ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Analysis: Determine the amount of cyclizine hydrochloride ($C_{18}H_{22}N_2 \cdot HCl$) dissolved by proceeding as directed in the *Assay*, making any necessary modifications.
Tolerances: NLT 75% (*Q*) of the labeled amount of cyclizine hydrochloride ($C_{18}H_{22}N_2 \cdot HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

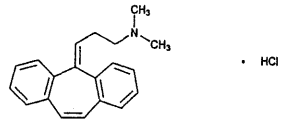
• **Organic Impurities**
Diluent: Methanol
Standard solution 1: 0.05 mg/mL of USP Cyclizine Hydrochloride RS in *Diluent*
Standard solution 2: 0.05 mg/mL of USP Cyclizine Related Compound A RS in *Diluent*
System suitability solution: 1 mg/mL of USP Cyclizine Hydrochloride RS and 1 mg/mL of USP Hydroxyzine Hydrochloride RS in *Diluent*
Sample solution: Triturate a quantity of powdered Tablets containing 100 mg of cyclizine hydrochloride with 10 mL of methanol, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: Mix methylene chloride, methanol, and ammonium hydroxide (90:8:2). Allow the layers to separate, and use the lower layer.
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: The chromatogram shows two clearly visible and separated spots.
Analysis
Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*
Proceed as directed in *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*. Air-dry the plate for several min, expose it to iodine vapor for 20 min, and examine the plate under short-wavelength UV light.
Acceptance criteria
Cyclizine related compound A: The spot corresponding to cyclizine related compound A in the *Sample solution* is not more intense than the principal spot obtained from *Standard solution 2* (NMT 0.5%).
Any unspecified impurity: Any other secondary spot in the chromatogram from the *Sample solution* is not more intense than the principal spot obtained from *Standard solution 1* (NMT 0.5%).

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cyclizine Hydrochloride RS
  USP Cyclizine Related Compound A RS
  1-Methylpiperazine.
  $C_5H_{12}N_2$ 100.16
  USP Hydroxyzine Hydrochloride RS

Cyclobenzaprine Hydrochloride



$C_{20}H_{21}N \cdot HCl$ 311.85

1-Propanamine, 3-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-*N,N*-dimethyl-, hydrochloride;

N,N-Dimethyl-5*H*-dibenzo[*a,d*]cycloheptene-$\Delta^{5,\gamma}$-propylamine hydrochloride [6202-23-9].

DEFINITION

Cyclobenzaprine Hydrochloride contains NLT 98.0% and NMT 102.0% of cyclobenzaprine hydrochloride ($C_{20}H_{21}N \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*
  Sample solution: 20 mg/mL of Cyclobenzaprine Hydrochloride in water
  Acceptance criteria: Meets the requirements

ASSAY

Change to read:

- **PROCEDURE**
  Mobile phase: Dissolve 2.0 g of ammonium acetate in 350 mL of water. Add 650 mL of methanol, and adjust with 25% ammonium hydroxide to a pH of 8.9.
  Standard solution: 0.2 mg/mL of USP Cyclobenzaprine Hydrochloride RS in *Mobile phase*
  Sample solution: 0.2 mg/mL of Cyclobenzaprine Hydrochloride in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 226 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Column temperature: 30°
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  Run time: ▲NLT▲ 2S (USP41) 2 times the retention time of cyclobenzaprine
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 1.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of cyclobenzaprine hydrochloride ($C_{20}H_{21}N \cdot HCl$) in the portion of Cyclobenzaprine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Cyclobenzaprine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Delete the following:

- **▲• HEAVY METALS,** *Method II* ⟨231⟩: NMT 10 ppm▲ (Official 1-Jan-2018)

Change to read:

- **ORGANIC IMPURITIES**
  ▲Buffer: 5.7 g/L of ammonium acetate in water. Adjust with 25% ammonium hydroxide to a pH of 7.2.
  Mobile phase: Methanol and *Buffer* (65:35)
  System suitability solution: 0.5 mg/mL of USP Cyclobenzaprine Hydrochloride RS and 0.75 µg/mL each of USP Cyclobenzaprine Related Compound A RS and USP Cyclobenzaprine Related Compound B RS in *Mobile phase*
  Standard solution: 0.5 µg/mL of USP Cyclobenzaprine Hydrochloride RS in *Mobile phase*
  Sample solution: 500 µg/mL of Cyclobenzaprine Hydrochloride in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 240 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L7
  Column temperature: 30°
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  Run time: NLT 3.5 times the retention time of cyclobenzaprine
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—See *Table 1* for relative retention times.]
  Suitability requirements
  Resolution: NLT 1.5 between cyclobenzaprine related compound A and cyclobenzaprine related compound B, *System suitability solution*
  Tailing factor: NMT 2.0 for the cyclobenzaprine peak, *System suitability solution*
  Relative standard deviation: NMT 5.0% for cyclobenzaprine, *Standard solution*
  Analysis
  Samples: *Sample solution* and *Standard solution*
  Calculate the percentage of each specified impurity and any individual unspecified impurity in the portion of Cyclobenzaprine Hydrochloride taken:

USP Monographs

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of cyclobenzaprine from the *Standard solution*
C_S = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Cyclobenzaprine Hydrochloride in the *Sample solution* (µg/mL)
F = relative response factor for each impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%, w/w)
Cyclobenzaprine related compound A	0.51	0.35	0.15
Cyclobenzaprine related compound B	0.57	1.0	0.15
Cyclobenzaprine *N*-oxide[a]	0.74	0.53	0.15
Dibenzocycloheptenol[b]	0.87	0.45	0.1
Cyclobenzaprine	1.0	—	—
Amitriptyline[c]	1.3	0.48	0.15
Dibenzocycloheptenone[d]	1.6	0.33	0.15
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 3-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*,*N*-dimethyl-1-propanamine *N*-oxide.
[b] 5*H*-Dibenzo[*a*,*d*]cycloheptene-5-ol.
[c] 10,11-Dihydro-*N*,*N*-dimethyl-5*H*-dibenzo[*a*,*d*]cycloheptene-Δ$^{5,\gamma}$-propylamine.
[d] Dibenzo[*a*,*d*]cyclohepten-5-one. ▲2S (USP41)

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° to constant weight.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨11⟩
  USP Cyclobenzaprine Hydrochloride RS
  USP Cyclobenzaprine Related Compound A RS
  5-[3-(Dimethylamino)propyl]-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-ol.
  $C_{20}H_{23}NO$ 293.40
  USP Cyclobenzaprine Related Compound B RS
  3-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*-methyl-1-propanamine hydrochloride.
  $C_{19}H_{19}N \cdot HCl$ 297.82

Cyclobenzaprine Hydrochloride Extended-Release Capsules

DEFINITION

Cyclobenzaprine Hydrochloride Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of cyclobenzaprine hydrochloride ($C_{20}H_{21}N \cdot HCl$).

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197A**▲ (CN 1-May-2020)
  Standard: Transfer 90 mg of USP Cyclobenzaprine Hydrochloride RS to a 25-mL volumetric flask. Add 10 mL of water and sonicate for 30 s. Transfer the solution to a 40-mL vial. Add 10 mL of methylene chloride. Cap the vial and shake. Centrifuge, if necessary, and transfer the lower methylene chloride layer to a watch glass. Evaporate the solvent, and use the residue. [Note—The use of a glass vial and a centrifuge speed of NMT 2000 rpm may be suitable. An infrared heat lamp may be used to evaporate the solvent.]
  Sample: Transfer a portion of the contents of NLT 10 Capsules containing nominally 90 mg of cyclobenzaprine hydrochloride to a 25-mL volumetric flask. Add 10 mL of water and sonicate for 30 s. Transfer the solution to a 40-mL vial. Add 10 mL of methylene chloride. Cap the vial and shake. Centrifuge, and transfer the lower methylene chloride layer to a watch glass. Evaporate the solvent, and use the residue. [Note—The use of a glass vial and a centrifuge speed of NMT 2000 rpm may be suitable. An infrared heat lamp may be used to evaporate the solvent.]
  Analysis
  Samples: *Standard* and *Sample*
  Acceptance criteria: The maxima of the IR spectrum from the *Sample* correspond to those from the *Standard*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**
  Buffer: Add 3.7 mL of methanesulfonic acid per 1 L of water and adjust with diethylamine to a pH of 3.6.
  Mobile phase: Acetonitrile, methanol, and *Buffer* (25.5: 21.0: 53.5)
  Diluent: Acetonitrile and water (50:50)
  Standard solution: 0.12 mg/mL of USP Cyclobenzaprine Hydrochloride RS in *Diluent*
  Sample solution: Nominally 0.12 mg/mL of cyclobenzaprine hydrochloride from Capsules prepared as follows. Remove the contents of NLT 20 Capsules, and transfer a suitable portion of the contents to a volumetric flask. Add 80% of the final flask volume of *Diluent*. Stir and sonicate, if necessary. Dilute with *Diluent* to volume, and centrifuge. Use the supernatant.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 290 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0

USP Monographs

www.webofpharma.com

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cyclobenzaprine hydrochloride ($C_{20}H_{21}N \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cyclobenzaprine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm, wire helix sinkers

Times: 2, 4, 8, and 16 h

Solution A: Transfer 10 empty Capsule shells to a 1-L volumetric flask. Add 80% of the final flask volume of *Medium*, which has been warmed to 37°, and stir until the Capsule shells have dissolved. Allow to cool, and dilute with *Medium* to volume.

Standard solution: (L/900) mg/mL of USP Cyclobenzaprine Hydrochloride RS in *Medium*, where L is the label claim of cyclobenzaprine hydrochloride in mg/Capsule

Sample solution: Centrifuge a portion of the solution under test. Use the supernatant.

Instrumental conditions

Mode: UV

Analytical wavelength: 290 nm

Cell: 1 cm

Blank: A mixture of *Solution A* and *Medium* prepared as follows. Transfer 10% of the final flask volume of *Solution A* to a suitable volumetric flask. Dilute with *Medium* to volume, centrifuge, and use the supernatant.

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Correct the instrument by using the *Blank*.

Calculate the concentration (C_i) of cyclobenzaprine hydrochloride ($C_{20}H_{21}N \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S$$

A_U = absorbance from the *Sample solution* at time point i
A_S = absorbance from the *Standard solution*
C_S = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of cyclobenzaprine hydrochloride ($C_{20}H_{21}N \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of cyclobenzaprine hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (i)	Time (h)	Amount Dissolved (%)
1	2	NMT 40
2	4	43–63
3	8	66–86
4	16	NLT 80

The percentage of the labeled amount of cyclobenzaprine hydrochloride ($C_{20}H_{21}N \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer: Dissolve 10 mL of *n*-butylamine in 900 mL of water, and adjust with acetic acid to a pH of 6.0. Dilute to 1 L.

Mobile phase: Acetonitrile, methanol, and *Buffer* (15:29:56)

Sensitivity solution: 0.001 mg/mL of USP Cyclobenzaprine Hydrochloride RS in *Mobile phase*

Standard solution: 0.02 mg/mL of USP Cyclobenzaprine Hydrochloride RS and 0.01 mg/mL of USP Amitriptyline Hydrochloride RS in *Mobile phase*

Sample solution: Nominally 2 mg/mL of cyclobenzaprine hydrochloride from Capsules prepared as follows. Remove the contents of NLT 20 Capsules, and transfer a suitable portion of the contents to a volumetric flask. Add 70% of the final flask volume of *Mobile phase*. Stir and sonicate, if necessary. Dilute with *Mobile phase* to volume, and centrifuge. Use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

Run time: NLT 3.5 times the retention time of cyclobenzaprine

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[NOTE—The relative retention times for cyclobenzaprine and amitriptyline are 1.0 and 1.3, respectively. For other relative retention times, see *Table 2*.]

Suitability requirements

Resolution: NLT 2.5 between cyclobenzaprine and amitriptyline, *Standard solution*

Relative standard deviation: NMT 2.0% for cyclobenzaprine, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of each unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of cyclobenzaprine from the *Standard solution*
C_S = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cyclobenzaprine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cyclobenzaprine related compound A[a, b]	0.5	—
Cyclobenzaprine	1.0	—
Cyproheptadine related compound B[b, c]	2.9	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

[a] 5-[3-(Dimethylamino)propyl]-5*H* -dibenzo[a,d]-cycloheptene-5-ol.
[b] This is a process impurity that is included in the table for identification purposes only. It is controlled in the drug substance and is not to be reported or included in the total impurities for the drug product.
[c] Dibenzo[*a*,*d*]cycloheptene-5-one (also known as dibenzocycloheptenone).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amitriptyline Hydrochloride RS
  USP Cyclobenzaprine Hydrochloride RS

Cyclobenzaprine Hydrochloride Tablets

DEFINITION

Cyclobenzaprine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cyclobenzaprine hydrochloride ($C_{20}H_{21}N \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
  Sample: Transfer an amount equivalent to 50 mg of cyclobenzaprine hydrochloride from a quantity of finely powdered Tablets to a small flask. Add 10 mL of methylene chloride, swirl to dissolve, and filter. Evaporate the clear filtrate to about 5 mL, transfer to a centrifuge tube, and add 1–2 mL of ether. Evaporate with the aid of a current of air to about 1 mL, and agitate until crystallization occurs. Wash the crystals with several portions of ether, and air-dry.
  Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 11.4 g/L of ammonium acetate in water. Adjust with ammonium hydroxide to a pH of 7.2.
  Mobile phase: Methanol and *Buffer* (65:35)
  Standard solution: 0.2 mg/mL of USP Cyclobenzaprine Hydrochloride RS in *Mobile phase*. Sonication may be used to aid in dissolution.
  Sample solution: Nominally 0.2 mg/mL of cyclobenzaprine hydrochloride from NLT 20 finely powdered Tablets in *Mobile phase* prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 60% of the flask volume of *Mobile phase*, and sonicate for 30 min. Allow the solution to cool to room temperature, and then dilute with *Mobile phase* to volume. Centrifuge the solution, and use the supernatant.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 226 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L7
  Column temperature: 30°
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 0.85%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of cyclobenzaprine hydrochloride ($C_{20}H_{21}N \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cyclobenzaprine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Medium: 0.1 N hydrochloric acid; 900 mL
  Apparatus 1: 50 rpm
  Time: 30 min
  Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium* if necessary.
  Standard solution: USP Cyclobenzaprine Hydrochloride RS in *Medium* with a concentration similar to the one expected in the *Sample solution*
  Instrumental conditions
  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
  Mode: UV
  Analytical wavelength: 290 nm
  Analysis
  Samples: *Sample solution* and *Standard solution*
  Calculate the percentage of the labeled amount of cyclobenzaprine hydrochloride ($C_{20}H_{21}N \cdot HCl$) dissolved:

USP Monographs

www.webofpharma.com

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of cyclobenzaprine hydrochloride ($C_{20}H_{21}N \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer and **Mobile phase:** Proceed as directed in the *Assay*.

Standard solution: 0.6 µg/mL each of USP Cyclobenzaprine Hydrochloride RS, USP Cyclobenzaprine Related Compound A RS, and USP Cyclobenzaprine Related Compound B RS in *Mobile phase*

Sample solution: Nominally 400 µg/mL of cyclobenzaprine hydrochloride from NLT 20 finely powdered Tablets in *Mobile phase* prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 75% of the flask volume of *Mobile phase*, and sonicate for 30 min. Allow the solution to cool to room temperature, and then dilute with *Mobile phase* to volume. Centrifuge the solution, and use the supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 3 times the retention time of cyclobenzaprine

System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between the cyclobenzaprine related compound A and cyclobenzaprine related compound B peaks
Relative standard deviation: NMT 2.0% for the cyclobenzaprine peak

Analysis
Sample: *Standard solution* and *Sample solution*
Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*
r_S = peak response of cyclobenzaprine from the *Standard solution*
C_S = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cyclobenzaprine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria NMT (%)
Cyclobenzaprine related compound A[a]	0.51	—
Cyclobenzaprine related compound B[a]	0.59	—
Cyclobenzaprine *N*-oxide[b]	0.74	0.2
Cyclobenzaprine	1.0	—
Amitriptyline[a, c]	1.3	—
Dibenzocycloheptenone[a, d]	1.6	—
Any individual unspecified degradation product	—	0.1
Total degradation products	—	2.0

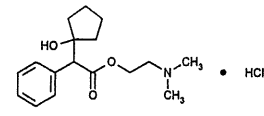
[a] Process impurity included for identification only and not included in the calculation of total degradation products.
[b] 3-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*,*N*-dimethyl-1-propanamine *N*-oxide.
[c] 10,11-Dihydro-*N*,*N*-dimethyl-5*H*-dibenzo[*a*,*d*]cycloheptene-$\Delta^{5,\gamma}$,-propylamine.
[d] Dibenzo[*a*,*d*]cyclohepten-5-one.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cyclobenzaprine Hydrochloride RS
USP Cyclobenzaprine Related Compound A RS
5-[3-(Dimethylamino)propyl]-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-ol.
$C_{20}H_{23}NO$ 293.40
USP Cyclobenzaprine Related Compound B RS
3-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*-methyl-1-propanamine hydrochloride.
$C_{19}H_{19}N \cdot HCl$ 297.82

Cyclopentolate Hydrochloride



$C_{17}H_{25}NO_3 \cdot HCl$ 327.85
Benzeneacetic acid, α-(1-hydroxycyclopentyl)-, 2-(dimethylamino)ethyl ester, hydrochloride, (±)-;
2-(Dimethylamino)ethyl (±)-1-hydroxy-α-phenylcyclopentaneacetate hydrochloride [5870-29-1].

DEFINITION

Cyclopentolate Hydrochloride contains NLT 98.0% and NMT 102.0% of cyclopentolate hydrochloride ($C_{17}H_{25}NO_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

www.webofpharma.com

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*
Sample solution: 2 mg/mL
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Buffer solution: Dissolve 660 mg of *dibasic ammonium phosphate* in 1000 mL of water. Adjust with *phosphoric acid* to a pH of 3.0 ± 0.1.
Mobile phase: *Acetonitrile* and *Buffer solution* (7:3)
Standard solution: 0.1 mg/mL of USP Cyclopentolate Hydrochloride RS in *water*
Sample solution: 0.1 mg/mL of Cyclopentolate Hydrochloride in *water*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing *L15*
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cyclopentolate hydrochloride ($C_{17}H_{25}NO_3 \cdot HCl$) in the portion of Cyclopentolate Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cyclopentolate Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Cyclopentolate Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.05%
• **ORGANIC IMPURITIES**
Buffer solution, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*, except for the *Run time*.
Run time: NLT twice the retention time of cyclopentolate
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Cyclopentolate Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the responses of all of the peaks, excluding the solvent peak, from the *Sample solution*

Acceptance criteria
Individual impurity: NMT 1.0%
Total impurities: NMT 2.0%

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 4.5–5.5
• **LOSS ON DRYING** ⟨731⟩
Sample: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a cold place.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cyclopentolate Hydrochloride RS

Cyclopentolate Hydrochloride Ophthalmic Solution

» Cyclopentolate Hydrochloride Ophthalmic Solution is a sterile, aqueous solution of Cyclopentolate Hydrochloride. It may contain suitable buffers and other additives. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{25}NO_3 \cdot HCl$.

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Cyclopentolate Hydrochloride RS
Identification—Place in a 125-mL separator a volume of Ophthalmic Solution, equivalent to about 50 mg of cyclopentolate hydrochloride, and place in a second separator about 50 mg of USP Cyclopentolate Hydrochloride RS dissolved in 5 mL of water. Treat each solution as follows. Add 1 g of potassium carbonate, and extract with two 10-mL portions of ether. Pass the ether extracts through ether-washed filter paper, collect the filtrate in a small beaker, and evaporate to dryness: the residue so obtained responds to *IDENTIFICATION* test *IDENTIFICATION* under *Cyclopentolate Hydrochloride*.
Sterility Tests ⟨71⟩ **:** meets the requirements.
pH ⟨791⟩: between 3.0 and 5.5.

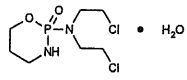
Assay—
Buffer solution, Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *ASSAY* under *Cyclopentolate Hydrochloride*.
Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of cyclopentolate hydrochloride, to a 100-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Proceed as directed in the *ASSAY* under *Cyclopentolate Hydrochloride*. Calculate the quantity, in mg, of cyclopentolate hydrochloride ($C_{17}H_{25}NO_3 \cdot HCl$) in each mL of the Ophthalmic Solution taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which V is the volume, in mL, of Ophthalmic Solution taken, and the other terms are as defined therein.

www.webofpharma.com

Cyclophosphamide



$C_7H_{15}Cl_2N_2O_2P \cdot H_2O$ 279.10

$C_7H_{15}Cl_2N_2O_2P$ 261.09

2*H*-1,3,2-Oxazaphosphorin-2-amine, *N*,*N*-bis(2-chloroethyl) tetrahydro-, 2-oxide, monohydrate, (±);
(±)-2-[Bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide monohydrate [6055-19-2].
Anhydrous [50-18-0].

DEFINITION

Cyclophosphamide contains NLT 97.0% and NMT 103.0% of $C_7H_{15}Cl_2N_2O_2P$, calculated on the anhydrous basis.
[**CAUTION**—Great care should be taken in handling Cyclophosphamide, as it is a potent cytotoxic agent.]

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: Acetonitrile and water (3:7)
Ethylparaben solution: Dissolve 185 mg of ethylparaben in 250 mL of alcohol in a 1000-mL volumetric flask, and dilute with water to volume.
System suitability solution: Transfer USP Cyclophosphamide RS, equivalent to 25 mg of anhydrous cyclophosphamide, to a 50-mL volumetric flask, add 25 mL of water, and shake to dissolve the USP Reference Standard. Add 5.0 mL of *Ethylparaben solution*, and dilute with water to volume.
Standard solution: 0.5 mg/mL of USP Cyclophosphamide RS in water
Sample solution: 0.5 mg/mL of Cyclophosphamide in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 195 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 25 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for cyclophosphamide and ethylparaben are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2 between cyclophosphamide and ethylparaben
Relative standard deviation: NMT 2% from six replicate injections, cyclophosphamide peak
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_7H_{15}Cl_2N_2O_2P$ in the Cyclophosphamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cyclophosphamide RS in the *Standard solution* (mg/mL) ▲▲ (ERR 1-Oct-2018)
C_U = concentration of Cyclophosphamide in the *Sample solution* (mg/mL) ▲▲ (ERR 1-Oct-2018)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

INORGANIC IMPURITIES

ORGANIC IMPURITIES

- **Procedure 1: Limit of Propanolamine**

Diluent: Methylene chloride and dehydrated alcohol (17:3)
Standard solution: 12.5 µg/mL of USP Propanolamine RS in *Diluent*. [NOTE—Propanolamine in the *Standard solution* is 0.025% of Cyclophosphamide in the *Sample solution*.]
Sample solution: 50 mg/mL of Cyclophosphamide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.1-mm layer of chromatographic silica gel
Application volume: 2 µL
Developing solvent system A: Toluene, methylene chloride, and methanol (5:5:1). Prepare at time of use.
Developing solvent system B: Methanol and glacial acetic acid (9:1)
Solution A: Hydrochloric acid and water (7:18)
Solution B: 5 g/L of potassium permanganate in water
Reagent A: *Solution A* and *Solution B* (1:1). [NOTE—Mix in a small beaker at the time of use under a fume hood to generate chlorine gas, and immediately place the beaker with solution into closed TLC chamber (placed in a fume hood).]
Reagent B: 100 mg of tetramethylbenzidine in 2.5 mL of methylene chloride, and diluted with cyclohexane to 100 mL
Analysis
Samples: *Standard solution* and *Sample solution*
Develop with *Developing solvent system A* over a path of 7 cm followed by air drying for 15 min. Develop again in *Developing solvent system B* over a path of 2 cm followed by air drying for NLT 10 min. [NOTE—Transfer *Developing solvent system B* to the chamber 15 min before development.] Dry the plate at 45° under a vacuum for 50 min. Place the plate in a closed chromatography tank (placed in a fume hood) containing *Reagent A*, and leave the plate in the tank for 10 min. Remove the plate and place it in a fume hood for 10 min to remove the excess chlorine. Stain the plate by dipping it into *Reagent B*. Remove it from *Reagent B* and wait for 15 min, evaluate it with a suitable densitometer, equipped with a filter having its maximum transmittance at 375 nm, and locate and scan the spot produced by propanolamine from the *Standard solution* and any spot from the *Sample solution* having the same R_F as that produced by propanolamine from the *Standard solution*.
Acceptance criteria
Propanolamine: The spot of propanolamine from the *Sample solution* is not more intense than the spot of propanolamine from the *Standard solution* (0.025%).

- **Procedure 2: Limit of Degradation Products**

Diluent: Methanol and water (1:1)

USP Monographs

www.webofpharma.com

Standard solution A: 12 µg/mL of USP Cyclophosphamide Related Compound A RS in *Diluent*
Standard solution B: 12 µg/mL of USP Cyclophosphamide Related Compound B RS in *Diluent*
Standard solution C: 12 µg/mL of USP Cyclophosphamide Related Compound C RS in *Diluent*
Standard solution D: 15 µg/mL of USP Cyclophosphamide Related Compound D RS in *Diluent*.
[NOTE—Cyclophosphamide related compound D is free base (M_r = 260.66) and USP Cyclophosphamide Related Compound D RS is available as dihydrochloride salt (M_r = 333.58).]
Standard solution E: 12 µg/mL of USP Cyclophosphamide RS in *Diluent*
Sample solution: 20 mg/mL of Cyclophosphamide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture containing a fluorescent indicator
Application volume: 20 µL
Developing solvent system: Methylene chloride, glacial acetic acid, methanol, and water (50:25:15:12)
Reagent A: 3.16 g/L solution of potassium permanganate in water and 10% hydrochloric acid (1:1). [NOTE—Mix in a small beaker at the time of use under a fume hood to generate chlorine gas, and immediately place the beaker with solution into closed TLC chamber (placed in a fume hood).]
Reagent B: Dissolve 250 mg of tetramethylbenzidine in 50 mL of dehydrated alcohol, and dilute with cyclohexane to 200 mL.
Analysis
Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, *Standard solution D*, *Standard solution E*, and *Sample solution*
[NOTE—Apply *Standard solution E* after the plate development in the *Developing solvent system*. Proceed as directed in the *Analysis* below.]
Develop with *Developing solvent system* over a path of 10 cm followed by drying at room temperature for 15 min in a fume hood. Develop again in the fresh portion of the *Developing solvent system* over a path of 10 cm followed by drying at room temperature for 15 min in a fume hood. Apply *Standard solution E* at the starting point of the plate. Dry the plate in an oven at 50° under a vacuum for 20 min or using a TLC heating plate at 50° for 20 min in a fume hood. Allow the plate to stand at room temperature for 5 min. Place the plate in a closed chromatography tank (placed in a fume hood) containing *Reagent A*, and leave the plate in the tank for at least 15 min. Remove the plate and place it in a fume hood for 15 min to remove the excess chlorine. Stain the plate by dipping it into *Reagent B* or spraying it with *Reagent B*. Examine the plate by visual evaluation.
Acceptance criteria
The spot of cyclophosphamide related compound A from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound A from *Standard solution A* (0.06%).
The spot of cyclophosphamide related compound B from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound B from *Standard solution B* (0.06%).
The spot of cyclophosphamide related compound C from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound C from *Standard solution C* (0.06%).
The spot of cyclophosphamide related compound D from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound D from *Standard solution D* (0.06%).
The spot of any individual unspecified impurity in the *Sample solution* is not more intense than the spot of cyclophosphamide from *Standard solution E* (0.06%).
Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Retardation Factor	Acceptance Criteria, NMT (%)
Cyclophosphamide related compound D[a]	0.15	0.06
Cyclophosphamide related compound C[b]	0.20	0.06
Cyclophosphamide related compound B[c]	0.43	0.06
Cyclophosphamide related compound A[d]	0.90	0.06
Any unspecified impurity	—	0.06

[a] 3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate.
[b] 3-Aminopropyl dihydrogen phosphate.
[c] 3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.
[d] Bis(2-chloroethyl)amine hydrochloride.

SPECIFIC TESTS

• **LIMIT OF CHLORIDE**
Sample solution: Dissolve 2.0 g of Cyclophosphamide in 30 mL of water, and add 80 mL of isopropyl alcohol and 5 mL of 10% nitric acid.
Analysis: Titrate potentiometrically with 0.01 N silver nitrate VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each 1.0 mL of 0.01 N silver nitrate equals 0.355 mg of chloride ion.
Calculate the percentage of chloride in the portion of Cyclophosphamide taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/[TN \times W \times (100 - A)/100]$$

V = sample titrant volume (mL)
B = blank titrant volume (mL)
N = titrant normality
F = equivalence factor, 0.355 mg of chloride ion/mL of TN
TN = theoretical normality, 0.01 N
W = sample weight (mg)
A = assay correction for water

Acceptance criteria: NMT 0.033%

• **LIMIT OF PHOSPHATE**
Diluent: 0.2 g/mL of hydrochloric acid in water
Solution A: Heat 20 g of tin with 85 mL of hydrochloric acid until no more hydrogen is released. Allow to cool. Transfer 1.0 mL of this solution into a 10-mL volumetric flask, and dilute with *Diluent* to volume.
Standard stock solution: 0.72 g/L of monobasic potassium phosphate. Transfer 1.0 mL of this solution into a 100-mL volumetric flask, and dilute with water to volume. Prepare immediately before use.
Standard solution: *Standard stock solution* and water (1:49). Prepare immediately before use. [NOTE—This solution contains 100 µg/L of PO_4.]
Sample solution: Dissolve 100 mg of Cyclophosphamide in water, and dilute to 100 mL.

USP Monographs

www.webofpharma.com

Analysis: To the *Sample solution* add 4 mL of sulfomolybdic acid TS. Shake and add 0.1 mL of *Solution A*. Prepare a standard in the same manner using the *Standard solution*. After 10 min, compare the colors using 20 mL of each solution in color comparison tubes in diffused daylight, viewing vertically against a white background.

Acceptance criteria: Any color from the *Sample solution* is not more intense than that from the *Standard solution* (NMT 0.01%).

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Cyclophosphamide is sterile, it contains NMT 0.0625 USP Endotoxin Unit/mg of cyclophosphamide.
- **STERILITY TESTS** ⟨71⟩: Where the label states that Cyclophosphamide is sterile, it meets the requirements.
- **pH** ⟨791⟩: 3.9–7.1, in a solution (1 in 100), determined 30 min after its preparation
- **WATER DETERMINATION,** *Method I* ⟨921⟩: 5.7%–6.8%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers at a temperature between 2° and 30°.
- **LABELING:** Where the label states that Cyclophosphamide is sterile, the tests for *Bacterial Endotoxins Test* ⟨85⟩ and *Sterility Tests* ⟨71⟩ should be performed.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Cyclophosphamide RS

USP Cyclophosphamide Related Compound A RS
Bis(2-chloroethyl)amine hydrochloride.
$C_4H_9Cl_2N \cdot HCl$ 178.49

USP Cyclophosphamide Related Compound B RS
3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.
$C_7H_{16}ClN_2O_3P$ 242.64

USP Cyclophosphamide Related Compound C RS
3-Aminopropyl dihydrogen phosphate.
$C_3H_{10}NO_4P$ 155.09

USP Cyclophosphamide Related Compound D RS
3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate dihydrochloride.
$C_7H_{18}ClN_2O_4P \cdot 2HCl$ 333.58

▲▲ (CN 1-May-2018)

USP Propanolamine RS
3-Aminopropan-1-ol.
C_3H_9NO 75.11

Cyclophosphamide for Injection

DEFINITION

Change to read:

Cyclophosphamide for Injection is a sterile mixture of Cyclophosphamide with or without a suitable ▲excipient. The sterile powder formulation contains NLT 90.0% and NMT 105.0% of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$). The lyophilized formulation contains NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$). ▲ (USP 1-Aug-2019)

IDENTIFICATION

Delete the following:

▲• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 20 mg/mL of USP Cyclophosphamide RS in chloroform

Sample solution: 20 mg/mL of cyclophosphamide in chloroform

Chromatographic system

Application volume: 5 µL

Developing solvent system: Chloroform, methanol, and ammonium hydroxide (75:20:5)

Analysis

Samples: *Standard solution* and *Sample solution*

Analysis: Place the plate in an iodine chamber, and visualize the spots.

Acceptance criteria: Meets the requirements. ▲ (USP 1-Aug-2019)

Change to read:

▲• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

If labeled as sterile powder formulation: Proceed as directed in the chapter.

If labeled as lyophilized formulation: Prepare the *Sample* as follows.

Sample: Suspend 100 mg of the lyophilized formulation in 25 mL of methylene chloride, sonicate for 10 min and filter. Remove the solvent from the filtrate, and dissolve the resulting clear and colorless oil in 10 mL of diethylether that is saturated with water. Cyclophosphamide crystallizes from this solution after a few minutes. Remove diethylether by evaporation.

Acceptance criteria: Meets the requirements▲ (USP 1-Aug-2019)

Change to read:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, ▲▲ (USP 1-Aug-2019)as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Mobile phase: Acetonitrile and water (30:70)
▲▲ (USP 1-Aug-2019)

Standard solution: ▲0.5 mg/mL of USP Cyclophosphamide RS in water▲ (USP 1-Aug-2019)

Sample solution: Nominally equivalent to 0.5 mg/mL of anhydrous cyclophosphamide ▲in water▲ (USP 1-Aug-2019)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 195 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*
▲▲ (USP 1-Aug-2019)

Suitability requirements

▲Tailing factor: NMT 2.0▲ (USP 1-Aug-2019)

Relative standard deviation: ▲NMT 1.0%▲ (USP 1-Aug-2019)

www.webofpharma.com

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$) in the portion of Cyclophosphamide for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cyclophosphamide from the *Sample solution*

r_S = peak response of cyclophosphamide from the *Standard solution* (USP 1-Aug-2019)

C_S = concentration of USP Cyclophosphamide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of anhydrous cyclophosphamide in the *Sample solution* (mg/mL)

Acceptance criteria

For the lyophilized formulation: (USP 1-Aug-2019) 90.0%–110.0%

For the sterile powder formulation: 90.0%–105.0% (USP 1-Aug-2019)

IMPURITIES

Add the following:

• ORGANIC IMPURITIES: PROCEDURE FOR THE STERILE POWDER FORMULATION

Diluent: Methanol and water (1:1)

Standard solution A: 30 µg/mL of USP Cyclophosphamide Related Compound A RS in *Diluent*

Standard solution B: 30 µg/mL of USP Cyclophosphamide Related Compound B RS in *Diluent*

Standard solution C: 30 µg/mL of USP Cyclophosphamide Related Compound C RS in *Diluent*

Standard solution D: 38.4 µg/mL of USP Cyclophosphamide Related Compound D RS in *Diluent*. [NOTE—Cyclophosphamide related compound D is free base (molecular weight = 260.66) and USP Cyclophosphamide Related Compound D RS is available as dihydrochloride salt (molecular weight = 333.60).]

Standard solution E: 22 µg/mL of USP Cyclophosphamide RS in *Diluent*

Sample solution: Nominally equivalent to 20 mg/mL of anhydrous cyclophosphamide in *Diluent*, from Cyclophosphamide for Injection

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Methylene chloride, glacial acetic acid, methanol, and water (50:25:15:12)

Reagent A: 3.16 g/L solution of potassium permanganate in water and 10% hydrochloric acid (1:1). [NOTE—Mix in a small beaker at the time of use under a fume hood to generate chlorine gas, and immediately place the beaker with solution into a closed TLC chamber (placed in a fume hood).]

Reagent B: Dissolve 250 mg of tetramethylbenzidine in 50 mL of dehydrated alcohol, and dilute with cyclohexane to 200 mL.

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, *Standard solution D*, *Standard solution E*, and *Sample solution*. [NOTE—Apply *Standard solution E* after the plate development in the *Developing solvent system*. Proceed as directed in *Procedure* as follows.]

Procedure: Develop with *Developing solvent system* over a path of 10 cm followed by drying at room temperature for 15 min in a fume hood. Develop again in a fresh portion of the *Developing solvent system* over a path of 10 cm followed by drying at room temperature for 15 min in a fume hood. Apply *Standard solution E* at the starting point of the plate. Dry the plate in an oven at 50° under vacuum for 20 min or using a TLC heating plate at 50° for 20 min in a fume hood. Allow the plate to stand at room temperature for 5 min. Place the plate in a closed chromatography tank (placed in a fume hood) containing *Reagent A*, and leave the plate in the tank for at least 15 min. Remove the plate and place it in a fume hood for 15 min to remove the excess chlorine. Stain the plate by dipping it into *Reagent B* or spraying it with *Reagent B*. Examine the plate by visual evaluation.

Acceptance criteria: See *Table 1*.

- The spot of cyclophosphamide related compound A in the *Sample solution* is not more intense than the spot of cyclophosphamide related compound A from *Standard solution A*.
- The spot of cyclophosphamide related compound B in the *Sample solution* is not more intense than the spot of cyclophosphamide related compound B from *Standard solution B*.
- The spot of cyclophosphamide related compound C in the *Sample solution* is not more intense than the spot of cyclophosphamide related compound C from *Standard solution C*.
- The spot of cyclophosphamide related compound D in the *Sample solution* is not more intense than the spot of cyclophosphamide related compound D from *Standard solution D*.
- The spot of any individual unspecified impurity in the *Sample solution* is not more intense than the spot of cyclophosphamide from *Standard solution E*.

Table 1

Name	Retardation Factor	Acceptance Criteria, NMT (%)
Cyclophosphamide related compound D[a]	0.15	0.15
Cyclophosphamide related compound C[b]	0.20	0.15
Cyclophosphamide related compound B[c]	0.43	0.15
Cyclophosphamide related compound A[d]	0.90	0.15
Any unspecified impurity	—	0.11

[a] 3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate.
[b] 3-Aminopropyl dihydrogen phosphate.
[c] 3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.
[d] Bis(2-chloroethyl)amine hydrochloride. (USP 1-Aug-2019)

USP Monographs

www.webofpharma.com

Add the following:

▲• **ORGANIC IMPURITIES: PROCEDURE FOR THE LYOPHILIZED FORMULATION**

Use plastic containers to prepare the solutions containing cyclophosphamide and its related substances.

Mobile phase: 0.2 mL of 85% phosphoric acid in 1 L of water. Adjust to a pH of 2.6.

Diluent: 7.5 mg/mL of mannitol in water

Standard stock solution A: 0.36 mg/mL of USP Cyclophosphamide Related Compound A RS in water

Standard stock solution B: 0.28 mg/mL of USP Cyclophosphamide Related Compound B RS in water

Standard stock solution D: 0.44 mg/mL of USP Cyclophosphamide Related Compound D RS in water

System suitability solution: 0.036 mg/mL of USP Cyclophosphamide Related Compound A RS, 0.028 mg/mL of USP Cyclophosphamide Related Compound B RS, and 0.044 mg/mL of USP Cyclophosphamide Related Compound D RS in *Diluent*, from *Standard stock solution A*, *Standard stock solution B*, and *Standard stock solution D*, respectively

Standard solution A: 0.036 mg/mL of USP Cyclophosphamide Related Compound A RS in *Diluent*, from *Standard stock solution A*

Standard solution B: 0.028 mg/mL of USP Cyclophosphamide Related Compound B RS in *Diluent*, from *Standard stock solution B*

Standard solution D: 0.044 mg/mL of USP Cyclophosphamide Related Compound D RS in *Diluent*, from *Standard stock solution D*

Sample solution: Nominally equivalent to 10 mg/mL of anhydrous cyclophosphamide in water, from Cyclophosphamide for Injection

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detectors: UV at 200 nm and conductivity in series

Polarity: Negative

Cell temperature: 45°

Column: 4.6-mm × 12.5-cm; packing L76

Autosampler temperature: 5°

Flow rate: 1.2 mL/min

Injection volume: 10 µL

Run time: NLT 3 times the retention time of cyclophosphamide related compound D

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2 between cyclophosphamide related compound A and cyclophosphamide related compound D

Relative standard deviation: NMT 5% for each peak

Analysis

Samples: *Standard solutions* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cyclophosphamide for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of the corresponding USP Reference Standard from the *Standard solution* (see *Table 2*)

C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of anhydrous cyclophosphamide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.02%.

Table 2

Name	Relative Retention Time[a]	Detection Mode	External Reference Standard	Acceptance Criteria, NMT (%)
Impurity 1	0.23	Conductivity	—	Disregard
Cyclophosphamide related compound B[b]	0.49	UV	USP Cyclophosphamide Related Compound B RS	0.25
Piperazinylpropyl pentahydroxyhexyl phosphate[c]	0.60–0.72	Conductivity	USP Cyclophosphamide Related Compound D RS	0.50
Chlorodiazinonyl pentahydroxyhexyl phosphate[d]	0.77–0.86	Conductivity	USP Cyclophosphamide Related Compound D RS	0.30
Dihydroxycyclophosphamide[e]	0.80–0.91	Conductivity	USP Cyclophosphamide Related Compound D RS	1.0
Impurity 2	0.84	Conductivity	—	Disregard
Cyclophosphamide	1.0	UV	—	—
Cyclophosphamide related compound D[f]	1.0	Conductivity	USP Cyclophosphamide Related Compound D RS	2.0
Chlorodiazinonyl phosphamide[g]	1.19–1.31	Conductivity	USP Cyclophosphamide Related Compound D RS	0.50
Cyclophosphamide related compound A[h]	1.40–1.70	Conductivity	USP Cyclophosphamide Related Compound A RS	2.0

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time[a]	Detection Mode	External Reference Standard	Acceptance Criteria, NMT (%)
Cyclophosphamide pyrophosphate analog[i]	1.46–1.67	Conductivity	USP Cyclophosphamide Related Compound D RS	0.50
Any individual unspecified impurity	—	Conductivity	USP Cyclophosphamide Related Compound D RS	0.20
Total impurities[j]	—	—	—	5.0

[a] The relative retention times are measured with respect to cyclophosphamide for UV detection and to cyclophosphamide related compound D for conductivity detection.
[b] 3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.
[c] 2,3,4,5,6-Pentahydroxyhexyl [3-(piperazin-1-yl)propyl] hydrogen phosphate.
[d] 3-({2-[(2-Chloroethyl)amino]ethyl}amino)propyl (2,3,4,5,6-pentahydroxyhexyl) hydrogen phosphate.
[e] 2-[Bis(2-hydroxyethyl)amino]-1,3,2-oxazaphosphinane 2-oxide.
[f] 3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate.
[g] (2-Chloroethyl){2-[(3-hydroxypropyl)amino]ethyl}phosphoramidic acid.
[h] Bis(2-chloroethyl)amine hydrochloride.
[i] 3-[2-(2-Chloroethylamino)ethylamino]propyl 3-aminopropyl dihydrogen diphosphate.
[j] The total impurities are the sum of the impurities in this table and cyclophosphamide related compound C in the *Limit of Cyclophosphamide Related Compound C: Procedure for the Lyophilized Formulation* test. ▲ (USP 1-Aug-2019)

Add the following:

▲• **Limit of Cyclophosphamide Related Compound C: Procedure for the Lyophilized Formulation**

Solution A: 10 mM ammonium acetate in water. Adjust with acetic acid to a pH of 4.6.
Solution B: Methanol
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7.5	100	0
7.7	85	15
10.0	85	15
12.0	15	85
20.0	15	85
22.5	100	0
30.0	100	0

Diluent: 7.5 mg/mL of mannitol in water
Standard stock solution: 0.44 mg/mL of USP Cyclophosphamide Related Compound C RS in water
Sensitivity solution: 0.007 mg/mL of USP Cyclophosphamide Related Compound C RS in *Diluent*, from *Standard stock solution*
Standard solutions C1–C5: Prepare solutions at concentrations of 0.0088 mg/mL (C1), 0.022 mg/mL (C2), 0.044 mg/mL (C3), 0.088 mg/mL (C4), and 0.132 mg/mL (C5) in *Diluent*, from *Standard stock solution*.
Sample solution: Nominally equivalent to 10 mg/mL of anhydrous cyclophosphamide in water, from Cyclophosphamide for Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Evaporative light scattering
Nitrogen gas flow: 1.5 mL/min
Nitrogen pressure: 3.5 bar
Column: 4.6-mm × 25-cm; 5-µm packing L109
Temperatures
Autosampler: 5°
Column: 20°
Detector: 55°
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Samples: *Sensitivity solution* and *Standard solution C3*
Suitability requirements
Relative standard deviation: NMT 10%, *Standard solution C3*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solutions C1–C5* and *Sample solution*
Calibration curve: Perform one injection for each concentration of the *Standard solutions*. Plot the logarithm of the peak areas of *Standard solutions C1–C5* versus the logarithm of their concentrations, in mg/mL. The linear regression coefficient is NLT 0.99.
Perform one injection for each *Sample solution*. Determine the concentration (C_S) of cyclophosphamide related compound C in the *Sample solution* from *Calibration curve*.
Calculate the percentage of cyclophosphamide related compound C in the portion of Cyclophosphamide for Injection taken:

$$\text{Result} = (C_S/C_U) \times 100$$

C_S = concentration of cyclophosphamide related compound C in the *Sample solution* (mg/mL)
C_U = nominal concentration of anhydrous cyclophosphamide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0% for cyclophosphamide related compound C ▲ (USP 1-Aug-2019)

PERFORMANCE TESTS

• **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements

SPECIFIC TESTS

Add the following:

▲• **Limit of Chloride for the Lyophilized Formulation**

Sample solution: Dissolve an amount equivalent to 2.0 g of anhydrous cyclophosphamide, from Cyclophosphamide for Injection, in 30 mL of water. Add 80 mL of isopropyl alcohol and 5 mL of 10% nitric acid.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.01 N silver nitrate VS
Endpoint detection: Potentiometric

USP Monographs

www.webofpharma.com

Analysis: Titrate potentiometrically with *Titrant*. Perform a blank determination, and make any necessary correction. Each 1.0 mL of 0.01 N silver nitrate equals 0.355 mg of chloride ion.

Calculate the percentage of chloride in the portion of Cyclophosphamide for Injection taken:

$$\text{Result} = [(V_S - V_B) \times N_A \times F \times 100]/[N_T \times W \times (100 - A)/100]$$

V_S = *Titrant* volume consumed by the sample (mL)
V_B = *Titrant* volume consumed by the blank (mL)
N_A = actual normality of the *Titrant*
F = equivalency factor, 0.355 mg of chloride ion/mL of N_T
N_T = theoretical normality of the *Titrant*, 0.01 N
W = sample weight (mg)
A = assay correction for water

Acceptance criteria: NMT 1.4%▲ (USP 1-Aug-2019)

Change to read:

• **pH** ⟨791⟩
Sample solution: Nominally 20 mg/mL of anhydrous cyclophosphamide, determined 30 min after preparation
Acceptance criteria
▲**For the sterile powder formulation:**▲ (USP 1-Aug-2019) 3.0–9.0, but the range does not exceed 3 pH units
▲**For the lyophilized formulation:** 3.0–6.4▲ (USP 1-Aug-2019)

Add the following:

▲• **Water Determination** ⟨921⟩, *Method I*
Sample for sterile powder formulation: Proceed as directed in the chapter.
Sample solution for lyophilized formulation: 10 mg/mL of anhydrous cyclophosphamide prepared as follows. Transfer an appropriate amount of the drug product in anhydrous methanol. Shake or sonicate for 15 min, and allow the suspension to rest. Use 10 mL of the supernatant.
Acceptance criteria: 6.0%–6.8% for the sterile powder formulation; NLT 6.4% for the lyophilized formulation based on the anhydrous cyclophosphamide in the drug product.▲ (USP 1-Aug-2019)

Change to read:

• **Bacterial Endotoxins Test** ⟨85⟩: NMT ▲0.0625▲ (USP 1-Aug-2019) USP Endotoxin Units/mg of cyclophosphamide
• **Sterility Tests** ⟨71⟩: Meets the requirements
• **Constituted Solution:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions.*
• **Other Requirements:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for Constitution.* Storage at a temperature not exceeding 25° is recommended. It will withstand brief exposure to temperatures up to 30° but is to be protected from temperatures above 30°.

Add the following:

▲• **Labeling:** The labeling should indicate that it is either the sterile powder formulation or the lyophilized formulation.▲ (USP 1-Aug-2019)

Change to read:

• **USP Reference Standards** ⟨11⟩
USP Cyclophosphamide RS
▲USP Cyclophosphamide Related Compound A RS
Bis(2-chloroethyl)amine hydrochloride.
$C_4H_9Cl_2N \cdot HCl$ 178.49
USP Cyclophosphamide Related Compound B RS
3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.
$C_7H_{16}ClN_2O_3P$ 242.64
USP Cyclophosphamide Related Compound C RS
3-Aminopropyl dihydrogen phosphate.
$C_3H_{10}NO_4P$ 155.09
USP Cyclophosphamide Related Compound D RS
3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate dihydrochloride.
$C_7H_{18}ClN_2O_4P \cdot 2HCl$ 333.58▲ (USP 1-Aug-2019)

Add the following:

▲Cyclophosphamide Compounded Oral Suspension

DEFINITION

Cyclophosphamide Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$).

Prepare Cyclophosphamide Compounded Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Cyclophosphamide capsules,[a] equivalent to	2 g of cyclophosphamide
Ora-Blend,[b] a sufficient quantity to make	100 mL

[a] Cyclophosphamide 50-mg capsules, Roxane Laboratories, Inc., Columbus, OH.
[b] Perrigo, Allegan, MI.

Empty the *Cyclophosphamide capsules* into a suitable container and triturate to a fine powder. Add a small amount of *Ora-Blend* and mix well to form a smooth paste. Add a sufficient amount of *Ora-Blend* to make the container contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the remainder of the *Ora-Blend*. Add sufficient *Ora-Blend* to bring to final volume. Mix well.

[**Caution**—Cyclophosphamide is a hazardous drug and must be handled accordingly.]

ASSAY

• **Procedure**
Mobile phase: Add 2.34 g of monobasic potassium phosphate to 780 mL of water. Adjust with 6 N potassium hydroxide to a pH of 7.0. Add 220 mL of acetonitrile. Pass the solution through a filter of 0.22-µm pore size.

www.webofpharma.com

Standard solution: 0.4 mg/mL of cyclophosphamide prepared from USP Cyclophosphamide RS in water
Sample solution: Transfer 1.0 mL of Oral Suspension into a 50-mL volumetric flask and bring to final volume with water. Pass through a filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 3.0-mm × 15-cm; 3-µm packing L1
Temperatures
Autosampler: 5°
Column: 45°
Flow rate: 0.5 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for cyclophosphamide is about 8.1 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cyclophosphamide from the *Sample solution*
r_S = peak response of cyclophosphamide from the *Standard solution*
C_S = concentration of USP Cyclophosphamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cyclophosphamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 3.6–4.6

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator.
• **BEYOND-USE DATE:** NMT 30 days after the date on which it was compounded when stored in a refrigerator
• **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cyclophosphamide RS▲ 2S (USP41)

Cyclophosphamide Tablets

DEFINITION
Cyclophosphamide Tablets contain Cyclophosphamide equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
Sample: Extract a portion of finely powdered Tablets, equivalent to 50 mg of cyclophosphamide, with 25 mL of chloroform. Filter about 2 mL of the chloroform solution, mix the filtrate with 500 mg of potassium bromide, evaporate the chloroform, carefully removing the last trace of solvent in a small vacuum flask, and use the residue to prepare a potassium bromide dispersion.
Acceptance criteria: The IR absorption spectrum of the *Sample* exhibits maxima, between 6.5 and 14 µm, only at the same wavelengths as those of a similar preparation of USP Cyclophosphamide RS.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (30:70)
Ethylparaben solution: Dissolve 185 mg of ethylparaben in 250 mL of alcohol in a 1000-mL volumetric flask, and dilute with water to volume.
System suitability solution: 0.5 mg/mL of USP Cyclophosphamide RS and 0.0185 mg/mL of ethylparaben in water prepared as follows. Transfer a quantity of USP Cyclophosphamide RS to a suitable volumetric flask, add water equivalent to 50% of the final volume, and shake to dissolve. Add *Ethylparaben solution* equivalent to 10% of the final volume, and dilute with water to volume.
Standard solution: 0.5 mg/mL of USP Cyclophosphamide RS in water
Sample solution: Nominally equivalent to 0.5 mg/mL of anhydrous cyclophosphamide prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask. Fill about half full with water, shake for 30 min, and dilute with water to volume. Pass through fast, fluted filter paper, and discard the first 40–50 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 195 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 25 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for cyclophosphamide and ethylparaben are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2 between cyclophosphamide and ethylparaben, *System suitability solution*
Relative standard deviation: NMT 2% from six replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cyclophosphamide from the *Sample solution*

www.webofpharma.com

r_S = peak response of cyclophosphamide from the *Standard solution*
C_S = concentration of USP Cyclophosphamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of anhydrous cyclophosphamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL, deaerated
Apparatus 1: 100 rpm
Time: 45 min
Mobile phase: Acetonitrile and water (30:70)
Standard solution: USP Cyclophosphamide RS in water at a concentration similar to that of the *Sample solution*
Sample solution: Pass a portion of solution under test through a suitable filter of 0.8-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 195 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of cyclophosphamide from the *Sample solution*
r_S = peak response of cyclophosphamide from the *Standard solution*
C_S = concentration of USP Cyclophosphamide RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Storage at a temperature not exceeding 25° is recommended. Tablets will withstand brief exposure to temperatures up to 30° but are to be protected from temperatures above 30°.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cyclophosphamide RS

Cyclopropane

△

C_3H_6 42.08
Cyclopropane.
Cyclopropane [75-19-4].

» Cyclopropane contains not less than 99.0 percent, by volume, of C_3H_6.
[**CAUTION**—Cyclopropane is highly flammable. Do not use where it may be ignited.]

Packaging and storage—Preserve in cylinders. [NOTE—Maintain cylinders of Cyclopropane at 25 ± 2° for not less than 6 hours prior to withdrawing specimens for the tests and assay, and correct the results to 25° and 760 mm of mercury.]
Labeling—The label bears a warning that cyclopropane is highly flammable and is not to be used where it may be ignited.
Acidity or alkalinity—Add 0.3 mL of methyl red TS and 0.3 mL of bromothymol blue TS to 400 mL of boiling water, and boil the solution for 5 minutes. Pour 100 mL of the boiling solution into each of three color-comparison tubes marked *A*, *B*, and *C*, respectively. To tube *B* add 0.20 mL of 0.012 N hydrochloric acid, and to tube *C* add 0.40 mL of 0.012 N hydrochloric acid. Insert the stopper in each of the tubes, and cool them to room temperature. Pass 2000 mL of Cyclopropane through the solution in tube *B* at a rate requiring about 30 minutes for the passage of the gas: the color of the solution in tube *B* is no deeper orange-red than that in tube *C* and no deeper yellow-green than that in tube *A*.
[NOTE—The various detector tubes called for in the respective tests are listed under *Reagents* in the section *Reagents, Indicators, and Solutions*.]
Carbon dioxide—Place the container so that when its valve is opened, the gaseous phase can be sampled. Connect one end of a carbon dioxide detector tube to the container valve, and the other end to a gas flow meter. Pass 1000 mL of the Cyclopropane through the tube at a suitable rate: the indicator change corresponds to not more than 0.03%.
Halogens—Provide a 500-mL flask with a tightly fitting two-hole stopper. Through one opening pass a delivery tube bent at right angles and extending just beyond the lower surface of the stopper. Through the other opening insert a capillary tube bent at right angles and having a bore of 1 ± 0.2 mm, in the same manner. Place in a 50-mL cylinder, having an internal diameter of 2 ± 0.25 cm, 40 mL of a solution containing 850 mg of sodium carbonate in 1000 mL of water. Provide the cylinder with a two-hole stopper, and through one opening pass a right-angle delivery tube, having a bore of 3 ± 0.5 mm, to within 2 mm of the bottom of the cylinder. The end of the delivery tube that extends out of the cylinder is provided with an enlargement 8 ± 0.5 cm long having an internal diameter of 2 ± 0.25 cm. Through the other opening in the stopper pass another right-angle delivery tube, having it extend just below the surface of the stopper. Collect 500 mL of Cyclopropane in the flask. By means of hydrostatic pressure, applied through the delivery tube, force the gas through the capillary tube, the water used being previously saturated with Cyclopropane. Ignite the gas, place the enlarged end of the delivery tube, connected with the cylinder, around the flame, extending the flame one-third of the way into the enlargement. Apply suction to the shorter delivery tube connected with the cylinder, thus drawing the spent gases through the sodium carbonate solution, the period of ignition

USP Monographs

www.webofpharma.com

of the 500 mL of Cyclopropane requiring approximately 30 minutes. Make any necessary correction for the amount of halogen in the volume of air used for the ignition of the gas. Transfer the sodium carbonate solution to a 500-mL volumetric flask, and rinse the cylinder thoroughly, collecting the rinsings in the flask. Dilute the solution with water to volume, and mix. To a 50-mL aliquot add sufficient nitric acid to make it acid to litmus paper, and then add 1 mL of acid in excess. Prepare a blank containing 0.50 mL of 0.0012 N hydrochloric acid and 4 mL of the sodium carbonate solution in 46 mL of water, acidify to litmus with nitric acid, then add 1 mL of acid in excess and 1 mL of silver nitrate TS to each solution: after 5 minutes any opalescence in the solution representing the Cyclopropane does not exceed that in the blank (0.02% as chloride).

Propylene, allene, and other unsaturated hydrocarbons—Place the container so that when its valve is opened, the gaseous phase can be sampled. Connect one end of an olefin detector tube to the container valve, and the other end to a gas flow meter. Pass the Cyclopropane through the detector tube at a suitable rate: the color of the indicating layer of the tube contents matches the color standard after the passage of not less than 400 mL of Cyclopropane (0.9% as propylene).

Assay—Place the container so that when its valve is opened, the gaseous phase can be sampled. Withdraw 100 mL of Cyclopropane, accurately measured in a gas buret previously filled with mercury and equipped with a leveling bulb at the lower end. Connect one arm of the buret stopcock to a pipet that previously has been filled with sulfuric acid. By appropriate manipulation of the stopcock and the leveling bulb, transfer the gas between the pipet and the buret, bringing about sufficient contact of the gas with the acid to reduce the volume of unabsorbed gas to a minimum as measured in the buret. Not more than 1.0 mL of gas remains.

Cycloserine



$C_3H_6N_2O_2$ 102.09

3-Isoxazolidinone, 4-amino-, (*R*)-.
(+)-4-Amino-3-isoxazolidinone [68-41-7].

» Cycloserine has a potency of not less than 900 µg of $C_3H_6N_2O_2$ per mg.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Cycloserine RS

Identification—Dissolve about 1 mg in 10 mL of 0.1 N sodium hydroxide. To 1 mL of the resulting solution add 3 mL of 1 N acetic acid and 1 mL of a mixture, prepared 1 hour before use, of equal parts of sodium nitroprusside solution (1 in 25) and 4 N sodium hydroxide: a blue color gradually develops.

Condensation products—Its absorptivity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) at 285 nm, determined in a 0.1 N sodium hydroxide solution containing 0.40 mg per mL is not more than 0.80.

Specific rotation ⟨781S⟩: between 108° and 114°.
Test solution: 50 mg per mL, in 2 N sodium hydroxide.

Crystallinity ⟨695⟩: meets the requirements.

pH ⟨791⟩: between 5.5 and 6.5, in a solution (1 in 10).

Loss on drying ⟨731⟩—Dry about 100 mg in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.5%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

Assay—
pH 6.8 Phosphate buffer—Prepare as directed in *Buffer Solutions* under *Solutions* in the section *Reagents, Indicators, and Solutions.*
Mobile phase—Dissolve 0.5 g of sodium 1-decanesulfonate in 800 mL of water, add 50 mL of acetonitrile and 5 mL of glacial acetic acid, and mix. Adjust with 1 N sodium hydroxide to a pH of 4.4. Filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Cycloserine RS in *pH 6.8 Phosphate buffer* to obtain a solution having a known concentration of about 0.4 mg per mL.
Assay preparation—Transfer about 20 mg of Cycloserine, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *pH 6.8 Phosphate buffer* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 219-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at about 30°. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for cycloserine. Calculate the quantity, in µg, of $C_3H_6N_2O_2$ in each mg of Cycloserine taken by the formula:

$$50{,}000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cycloserine RS in the *Standard preparation; W* is the quantity, in mg, of Cycloserine taken to prepare the *Assay preparation;* and r_U and r_S are the peak responses for cycloserine obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cycloserine Capsules

» Cycloserine Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cycloserine ($C_3H_6N_2O_2$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Cycloserine RS

Identification—Shake a quantity of the contents of Capsules, equivalent to about 10 mg of cycloserine, with 100 mL of 0.1 N sodium hydroxide, and filter: 1 mL of the filtrate so obtained responds to the *Identification* test under *Cycloserine*

Dissolution ⟨711⟩—
Medium: pH 6.8 Phosphate buffer (see *Buffer Solutions* under *Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

USP Monographs

www.webofpharma.com

Apparatus 1: 100 rpm.
Time: 30 minutes.
Determine the amount of $C_3H_6N_2O_2$ dissolved by employing the following method.
pH 6.8 Phosphate buffer, Mobile phase, and *Chromatographic system*—Proceed as directed in the *Assay.*
Standard solution—Quantitatively dissolve an accurately weighed quantity of USP Cycloserine RS in *pH 6.8 Phosphate buffer* to obtain a solution having a known concentration of about 0.25 mg per mL.
Test solution—Use a filtered portion of the solution under test.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for cycloserine. Calculate the quantity, in mg, of cycloserine ($C_3H_6N_2O_2$) dissolved by the formula:

$$900C(r_U/r_S)$$

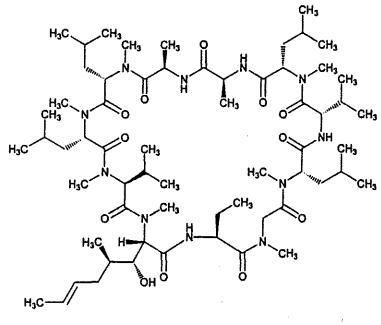
in which C is the concentration, in mg per mL, of USP Cycloserine RS in the *Standard solution;* and r_U and r_S are the peak responses for cycloserine obtained from the *Test solution* and the *Standard solution,* respectively.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_3H_6N_2O_2$ is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Loss on drying ⟨731⟩—Dry about 100 mg of the contents of Capsules in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

Assay—
pH 6.8 Phosphate buffer, Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cycloserine*
Assay preparation—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of cycloserine, to a 250-mL volumetric flask, dilute with *pH 6.8 Phosphate buffer* to volume, mix, and filter.
Procedure—Proceed as directed in the *Assay* under *Cycloserine* Calculate the quantity, in mg, of cycloserine ($C_3H_6N_2O_2$) in the portion of Capsules taken by the formula:

$$250C(r_U/r_S)$$

in which the terms are as defined therein.

Cyclosporine



$C_{62}H_{111}N_{11}O_{12}$ 1202.61

Cyclo[[(*E*)-(2*S*,3*R*,4*R*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-aminobutyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl];
[*R*-[*R**,*R**-(*E*)]]-Cyclic(L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-3-hydroxy-*N*,4-dimethyl-L-2-amino-6-octenoyl-L-α-aminobutyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl) [59865-13-3].

DEFINITION
Cyclosporine contains NLT 97.0% and NMT 101.5% of cyclosporine A ($C_{62}H_{111}N_{11}O_{12}$), calculated on the dried basis.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, *tert*-butyl methyl ether, water, and phosphoric acid (430:50:520:1)
Diluent: Acetonitrile and water (1:1)
System suitability solution: 1.25 mg/mL of USP Cyclosporine Resolution Mixture RS in *Diluent*
Standard solution: 1.25 mg/mL of USP Cyclosporine RS in *Diluent*
Sample solution: 1.25 mg/mL of Cyclosporine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 210 nm
Column: 4-mm × 25-cm; 3- to 5-µm packing L1; with 0.25-mm × 1-m stainless steel tubing connected to the column inlet
Column temperature: 80°. The tubing and column are maintained at 80°, to ensure that the *Mobile phase* entering the column is heated to 80°.
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for cyclosporine U and cyclosporine are 0.95 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.0 between cyclosporine U and cyclosporine, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$) in the portion of Cyclosporine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak area of cyclosporine from the *Sample solution*
r_S = peak area of cyclosporine from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of cyclosporine in USP Cyclosporine RS (mg/mg)

Acceptance criteria: 97.0%–101.5% on the dried basis

USP Monographs

www.webofpharma.com

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.01 mg/mL of USP Cyclosporine RS in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cyclosporine U and cyclosporine are 0.95 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.0 between cyclosporine U and cyclosporine, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cyclosporine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak area of an individual impurity from the *Sample solution*

r_S = peak area of cyclosporine from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of cyclosporine in USP Cyclosporine RS (mg/mg)

Acceptance criteria

Reporting threshold is 0.05%.

Any individual impurity: NMT 0.7%

Total impurities: NMT 1.5%

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Sample: 100 mg

Analysis: Dry in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Cyclosporine RS

USP Cyclosporine Resolution Mixture RS

This is a 100:1 (w/w) mixture of cyclosporine and cyclosporine U. The chemical name for cyclosporine U is given below.

Cyclo[[(*E*)-(2*S*,3*R*,4*R*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-aminobutyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl].

$C_{61}H_{109}N_{11}O_{12}$ 1188.58

Cyclosporine Capsules

» Cyclosporine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$).

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Dissolution ⟨711⟩—

WHERE CAPSULES CONTAIN LIQUID—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 15 minutes.

Procedure—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

Tolerances—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

WHERE CAPSULES CONTAIN POWDER—

Medium: 0.1 N hydrochloric acid containing 0.5% of sodium lauryl sulfate; 1000 mL.

Apparatus 1: 150 rpm.

Time: 90 minutes.

Determine the amount of $C_{62}H_{111}N_{11}O_{12}$ dissolved by employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, methanol, and phosphoric acid (900: 450: 50: 0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Quantitatively dissolve an accurately weighed quantity of USP Cyclosporine RS in *Dissolution Medium* to obtain a solution having a known concentration of about 0.001*L* mg per mL, *L* being the labeled quantity, in mg, of cyclosporine in each Capsule. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix. This solution contains about 0.0005*L* mg of USP Cyclosporine RS per mL.

Test solution—Filter a portion of the solution under test. Transfer 5.0 mL of the filtrate to a 10-mL volumetric flask, dilute with acetonitrile to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains packing L1 and is maintained at a constant temperature of about 80°. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution,* and record the peak areas as directed for *Procedure:* the column efficiency is not less than 700 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the solution estimated to contain 0.1 mg of cyclosporine per mL, or 40 µL of the solution estimated to contain 0.025 mg of cyclosporine per mL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{62}H_{111}N_{11}O_{12}$ dissolved by the formula:

$$2000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard solution;* and r_U and r_S are the cyclosporine peak areas obtained from the *Test solution* and the *Standard solution,* respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{62}H_{111}N_{11}O_{12}$ is dissolved in 90 minutes.

www.webofpharma.com

Uniformity of dosage units ⟨905⟩: meet the requirements.
Water Determination, *Method I* ⟨921⟩ —For Capsules that contain powder, not more than 3.5% is found, using finely ground Capsule contents.

Assay—

WHERE CAPSULES CONTAIN LIQUID—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Cyclosporine Injection.*
Standard preparation—Dissolve an accurately weighed quantity of USP Cyclosporine RS in dehydrated alcohol to obtain a solution having a known concentration of about 1 mg per mL. Use this solution promptly after preparation.
Assay preparation—Using a sharp blade, carefully cut open not fewer than 20 Capsules, and with the aid of dehydrated alcohol transfer the contents of the Capsules to a suitable volumetric flask. Wash the blade with dehydrated alcohol, and transfer the washings to the volumetric flask. Dilute the contents of the volumetric flask with dehydrated alcohol to volume, and mix. Quantitatively dilute an accurately measured volume of this solution with dehydrated alcohol to obtain a solution having a concentration of about 1 mg of cyclosporine per mL.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$) in each Capsule taken by the formula:

$$(L/D)(CP/1000)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of cyclosporine in each Capsule taken; D is the concentration, in mg per mL, of the *Assay preparation,* based on the labeled quantity of cyclosporine in the Capsules taken and the extent of dilution; C is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard preparation; P* is the purity, in µg per mg, of USP Cyclosporine RS; and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

WHERE CAPSULES CONTAIN POWDER—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, methanol, and phosphoric acid (605: 400: 50: 0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluting solvent—Prepare a mixture of acetonitrile, tetrahydrofuran, and dehydrated alcohol (9:5:4).
Standard preparation—Transfer about 25 mg of USP Cyclosporine RS, accurately weighed, to a 25-mL volumetric flask. Add 2.5 mL of water, and sonicate for 10 minutes. Add about 10 mL of *Diluting solvent,* sonicate for 5 minutes, dilute with *Diluting solvent* to volume, and mix.
Assay stock preparation—Transfer the contents of 20 Capsules to a volumetric flask of such capacity, V, in mL, to make a final concentration of 10 mg of cyclosporine per mL. Add $0.1V$ mL of water to the flask, and sonicate for 10 minutes. Add $0.4V$ mL of *Diluting solvent* to the flask, and sonicate for 5 minutes. Dilute with *Diluting solvent* to volume, and mix.
Assay preparation—Transfer 5.0 mL of *Assay stock preparation* to a 50-mL volumetric flask, add 5 mL of water, dilute with *Diluting solvent* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains packing L13 and is maintained at a constant temperature of about 70°. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the column efficiency is not less than 700 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$) in each Capsule taken by the formula:

$$10CV(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard preparation; V* is the volume, in mL, of the volumetric flask used to prepare the *Assay stock preparation;* and r_U and r_S are the cyclosporine peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cyclosporine Injection

DEFINITION

Cyclosporine Injection is a sterile solution of Cyclosporine in a suitable vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Solution A: 17 mg/mL of bismuth subnitrate in 20% acetic acid
Solution B: 400 mg/mL of potassium iodide
Standard solution: 0.5 mg/mL of USP Cyclosporine RS in methanol
Sample solution: Nominally 0.5 mg/mL of cyclosporine from Injection in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system 1: Ethyl ether
Developing solvent system 2: Ethyl acetate, methyl ethyl ketone, water, and formic acid (60:40:2:1)
Spray reagent 1: Mix 5 mL of *Solution A* with 5 mL of *Solution B* and 20 mL of glacial acetic acid, and dilute with water to 100 mL. Prepare freshly.
Spray reagent 2: Hydrogen peroxide TS
Analysis
Samples: *Standard solution* and *Sample solution*
Apply the *Standard solution* and the *Sample solution* to the plate. Allow the spots to dry in a current of air, place the plate in a suitable chromatographic chamber, and develop the chromatogram, using *Developing solvent system 1,* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow it to dry. Place the plate in a second chromatographic chamber, and develop the chromatogram in *Developing solvent system 2* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow it to dry. Spray the plate with *Spray reagent 1.* Immediately again spray the plate with *Spray reagent 2.* Cyclosporine appears as a brown spot having an R_F value of about 0.45.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution.* Disregard any spots at the origin.

USP Monographs

www.webofpharma.com

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, methanol, water, and phosphoric acid (550: 50: 400: 0.5)

Standard solution: 0.5 mg/mL of USP Cyclosporine RS in methanol. Use this solution promptly after preparation.

Sample solution 1 (where it is represented as being in a single-dose container): Nominally 0.5 mg/mL of cyclosporine from Injection in methanol, prepared as follows. Using a suitable hypodermic needle and syringe, withdraw all of the withdrawable contents from 1 container of Injection, and dilute with methanol. Use this solution promptly after preparation.

Sample solution 2 (where the label states the quantity of cyclosporine in a given volume): Nominally 0.5 mg/mL of cyclosporine from Injection in methanol, prepared as follows. Dilute a suitable aliquot of Injection with methanol. Use this solution promptly after preparation.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L16
Column temperature: 70°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor: NLT 3–NMT 10
Column efficiency: NLT 700 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.5%

Analysis
Samples: *Standard solution* and *Sample solution 1* or *Sample solution 2*
Calculate the percentage of the labeled amount of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from *Sample solution 1* or *Sample solution 2*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of *Sample solution 1* or *Sample solution 2* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **CONTENT OF ALCOHOL** (where present)

Internal standard solution: *n*-Propyl alcohol and butyl alcohol (3:50)

Standard stock solution: 64 mg/mL of dehydrated alcohol in butyl alcohol

Standard solution: 12.8 mg/mL of alcohol, prepared as follows. Transfer a suitable aliquot of *Standard stock solution* to a suitable volumetric flask. Add *Internal standard solution*, using 24% of the final volume, and dilute with butyl alcohol to volume.

Sample solution: Nominally 12.8 mg/mL of alcohol from Injection, prepared as follows. Transfer a suitable aliquot of Injection to a suitable volumetric flask. Add *Internal standard solution*, using 24% of the final volume, and dilute with butyl alcohol to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 2-mm × 2-m glass; packed with support S3
Temperatures
Injection port: 280°
Detector: 290°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
145	0	145	8
145	32	270	0

Carrier gas: Nitrogen
Flow rate: 35 mL/min
Injection volume: 1 µL. [NOTE—Make adjustments, if necessary, to obtain satisfactory chromatography.]

System suitability
Sample: *Standard solution*
Suitability requirements: [NOTE—The elution order is alcohol, *n*-propyl alcohol, and butyl alcohol.]
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alcohol (C_2H_5OH) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of alcohol to *n*-propyl alcohol from the *Sample solution*
R_S = peak area ratio of alcohol to *n*-propyl alcohol from the *Standard solution*
C_S = concentration of alcohol in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 80.0%–120.0% of the labeled amount

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩

Sample solution: Make a 1:10 dilution of the Injection with Water for Injection.

Analysis: Add 0.1 mL of *Sample solution* and 0.1 mL of appropriately constituted LAL reagent to a suitable pyrogen-free test tube. Mix on a vortex mixer for about 5 s.

Acceptance criteria: NMT 0.84 USP Endotoxin Unit/mg of cyclosporine

• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers.

• **LABELING:** Label it to indicate that it is to be diluted with a suitable parenteral vehicle before intravenous infusion.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cyclosporine RS

USP Monographs

www.webofpharma.com

Cyclosporine Oral Solution

DEFINITION

Cyclosporine Oral Solution is a solution of Cyclosporine in a suitable vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Solution A: 17 mg/mL of bismuth subnitrate in 20% acetic acid

Solution B: 400 mg/mL of potassium iodide

Diluent: Methanol and chloroform (4:1)

Standard solution: 1 mg/mL of USP Cyclosporine RS in *Diluent*

Sample solution: Nominally 1 mg/mL of cyclosporine from Oral Solution in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system 1: Ethyl ether

Developing solvent system 2: Ethyl acetate, methyl ethyl ketone, water, and formic acid (60:40:2:1)

Spray reagent 1: Mix 5 mL of *Solution A* with 5 mL of *Solution B* and 20 mL of glacial acetic acid, and dilute with water to 100 mL. Prepare freshly.

Spray reagent 2: Hydrogen peroxide TS

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* to the plate. Allow the spots to dry in a current of air, place the plate in a suitable chromatographic chamber, and develop the chromatogram, using *Developing solvent system 1*, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow it to dry. Place the plate in a second chromatographic chamber, and develop the chromatogram in *Developing solvent system 2* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow it to dry. Spray the plate with *Spray reagent 1*. Immediately again spray the plate with *Spray reagent 2*. Cyclosporine appears as a brown spot having an R_F value of about 0.45.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. Disregard any spots at the origin.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, methanol, water, and phosphoric acid (550: 50: 400: 0.5)

Diluent: Methanol and chloroform (4:1)

Standard solution: 1 mg/mL of USP Cyclosporine RS in *Diluent*. Use this solution promptly after preparation.

Sample solution: Nominally 1 mg/mL of cyclosporine from Oral Solution in *Diluent*. Use this solution promptly after preparation.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; packing L16

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor: 3–10

Column efficiency: NLT 700 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **CONTENT OF ALCOHOL** (where present)

Internal standard solution: *n*-Propyl alcohol and butyl alcohol (3:50)

Standard stock solution: 50 mg/mL of dehydrated alcohol in butyl alcohol

Standard solution: 10 mg/mL of alcohol, prepared as follows. Transfer a suitable aliquot of *Standard stock solution* to a suitable volumetric flask. Add *Internal standard solution*, using 24% of the final volume, and dilute with butyl alcohol to volume.

Sample solution: Nominally 10 mg/mL of alcohol from Oral Solution, prepared as follows. Transfer a suitable aliquot of Oral Solution to a suitable volumetric flask. Add *Internal standard solution*, using 24% of the final volume, and dilute with butyl alcohol to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 2-m glass; packed with support S3

Temperatures

Injection port: 280°

Detector: 290°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
145	0	145	8
145	32	270	0

Carrier gas: Nitrogen

Flow rate: 35 mL/min

Injection volume: 1 µL. [NOTE—Make adjustments if necessary to obtain satisfactory chromatography.]

System suitability

Sample: *Standard solution*. [NOTE—The elution order is alcohol, *n*-propyl alcohol, and butyl alcohol.]

Suitability requirements

Relative standard deviation: NMT 2.0%

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of alcohol (C_2H_5OH) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of alcohol to *n*-propyl alcohol from the *Sample solution*
R_S = peak area ratio of alcohol to *n*-propyl alcohol from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 80.0%–120.0% of the labeled amount

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements for oral solution packaged in single-unit containers
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements for oral solution packaged in multiple-unit containers

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cyclosporine RS

Cyclosporine Compounded Ophthalmic Solution, Veterinary

DEFINITION
Cyclosporine Compounded Ophthalmic Solution, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$).
Prepare Cyclosporine Compounded Ophthalmic Solution, Veterinary 10 mg/mL as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Cyclosporine Oral Solution[a] equivalent to	100 mg of cyclosporine
Corn Oil, *NF*, a sufficient quantity to make	10 mL

[a] Sandimmune Oral Solution 100 mg/mL, Novartis Pharmaceuticals Corporation, East Hanover, NJ.

Mix the *Cyclosporine Oral Solution* with sufficient *Corn Oil* to bring to final volume and mix thoroughly. Pass the solution through a compatible sterile membrane filter of 0.22-µm pore size into a sterile ophthalmic container. Replace the tip and cap, and mix well. [NOTE—Cyclosporine Oral Solution Modified is not interchangeable and should not be used.]

ASSAY
- **PROCEDURE**
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Acetonitrile (%)	Water (%)
0	60	40
40	90	10
45	90	10
45.05	60	40
55	60	40

Standard solution: 0.5 mg/mL of cyclosporine prepared from USP Cyclosporine RS in acetonitrile. Mix well to dissolve.
Sample solution: Transfer 0.5 mL of Ophthalmic Solution, Veterinary into a 10-mL volumetric flask, dilute with acetonitrile to volume, and mix well. Allow the oil to separate from the solution. Once the top layer appears clear, transfer about 1 mL of the top layer into an amber HPLC vial.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV-Vis 208 nm
Column: 4.6-mm × 10-cm; 2.6-µm packing L1
Column temperature: 60°
Flow rate: 0.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for cyclosporine is about 34.0 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$) in the portion of Ophthalmic Solution, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cyclosporine from the *Sample solution*
r_S = peak response of cyclosporine from the *Standard solution*
C_S = concentration of USP Cyclosporine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cyclosporine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements.
- **PARTICULATE MATTER IN OPHTHALMIC SOLUTIONS** ⟨789⟩**:** It meets the requirements.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in sterile ophthalmic dropper bottles, protected from light. Store at controlled room temperature.

Change to read:

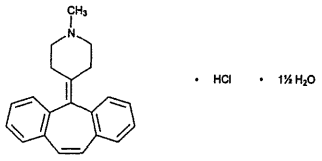
- **BEYOND-USE DATE:** ▲In the absence of passing a sterility test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test is passed, NMT 180 days after the date on which it was compounded when stored at controlled room temperature. ▲ (CN 1-May-2020)
- **LABELING:** Label it to indicate that it is for veterinary use only. State that it is intended for use in the eye and to not use if a precipitate is present. State the *Beyond-Use Date*.

USP Monographs

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cyclosporine RS

Cyproheptadine Hydrochloride



$C_{21}H_{21}N \cdot HCl \cdot 1½H_2O$ 350.88

$C_{21}H_{21}N \cdot HCl$ 323.87

Piperidine, 4-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-methyl-, hydrochloride, sesquihydrate;
4-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride sesquihydrate [41354-29-4].
Anhydrous [969-33-5].

DEFINITION
Cyproheptadine Hydrochloride contains NLT 98.0% and NMT 102.0% of cyproheptadine hydrochloride ($C_{21}H_{21}N \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the cyproheptadine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 1 mL/L of trifluoroacetic acid in water
Solution B: Acetonitrile and *Solution A* (38:62)
Solution C: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0.0	100	0
6.0	100	0
6.1	15	85
9.0	15	85
9.1	100	0
12	100	0

Diluent: Acetonitrile and water (38:62)
Standard solution: 40 µg/mL of USP Cyproheptadine Hydrochloride RS in *Diluent*. Sonication may be used to aid dissolution.
Sample solution: 40 µg/mL of Cyproheptadine Hydrochloride in *Diluent*. Sonication may be used to aid dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 266 nm
Column: 4.6-mm × 15-cm; 2.7-µm packing L60
Flow rate: 1 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cyproheptadine hydrochloride ($C_{21}H_{21}N \cdot HCl$) in the portion of Cyproheptadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cyproheptadine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Cyproheptadine Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **ORGANIC IMPURITIES**
Solution A, Solution B, Solution C, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 50 µg/mL each of USP Cyproheptadine Hydrochloride RS, USP Cyproheptadine Related Compound A RS, USP Amitriptyline Related Compound A RS, and USP Cyproheptadine Related Compound C RS, prepared as follows. Transfer a suitable quantity of each Reference Standard to an appropriate volumetric flask. Add 38% of the total flask volume of acetonitrile to dissolve, and then dilute with water to volume.
Standard solution: 0.75 µg/mL each of USP Cyproheptadine Hydrochloride RS, USP Cyproheptadine Related Compound A RS, USP Amitriptyline Related Compound A RS, and USP Cyproheptadine Related Compound C RS from the *Standard stock solution* in *Diluent*
Sensitivity solution: 0.25 µg/mL each of USP Cyproheptadine Hydrochloride RS, USP Cyproheptadine Related Compound A RS, USP Amitriptyline Related Compound A RS, and USP Cyproheptadine Related Compound C RS from the *Standard stock solution* in *Diluent*
Sample solution: 500 µg/mL of Cyproheptadine Hydrochloride in *Diluent*. Sonication may be used to aid dissolution.
System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between amitriptyline related compound A and cyproheptadine related compound A, *Standard solution*
Relative standard deviation: NMT 5.0% for cyproheptadine, cyproheptadine related compound A, amitriptyline related compound A, and cyproheptadine related compound C, *Standard solution*
Signal-to-noise ratio: NLT 10 for cyproheptadine hydrochloride, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of cyproheptadine related compound A, amitriptyline related compound A, and cyproheptadine related compound C in the portion of Cyproheptadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each related compound from the *Sample solution*
r_S = peak response of each related compound from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (µg/mL)
C_U = concentration of Cyproheptadine Hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unknown impurity in the portion of Cyproheptadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unknown impurity from the *Sample solution*
r_S = peak response of cyproheptadine hydrochloride from the *Standard solution*
C_S = concentration of USP Cyproheptadine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Cyproheptadine Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cyproheptadine related compound C	0.7	0.15
Cyproheptadine	1.0	—
Amitriptyline related compound A	2.5	0.15
Cyproheptadine related compound A	2.6	0.15
Any individual unknown impurity	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS
- **WATER DETERMINATION** ⟨921⟩, *Method I*: 7.0%–9.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Amitriptyline Related Compound A RS
  Dibenzosuberone.
  $C_{15}H_{12}O$ 208.26
  USP Cyproheptadine Hydrochloride RS
  USP Cyproheptadine Related Compound A RS
  5*H*-Dibenzo[*a*,*d*]cycloheptene.
  $C_{15}H_{12}$ 192.26
  USP Cyproheptadine Related Compound C RS
  5-(1-Methyl-piperidin-4-yl)-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ol.
  $C_{21}H_{23}NO$ 305.41

Cyproheptadine Hydrochloride Oral Solution

DEFINITION
Cyproheptadine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of cyproheptadine hydrochloride ($C_{21}H_{21}N \cdot HCl$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 1 mL/L of trifluoroacetic acid in water
  Solution B: Acetonitrile and *Solution A* (38:62)
  Solution C: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0.0	100	0
6.0	100	0
6.1	15	85
9.0	15	85
9.1	100	0
12	100	0

Diluent: Acetonitrile and water (38:62)
Standard solution: 40 µg/mL of USP Cyproheptadine Hydrochloride RS in *Diluent*. Sonication may be used to aid dissolution.
Sample solution: Nominally 40 µg/mL of cyproheptadine hydrochloride from Oral Solution in *Diluent* prepared as follows. Transfer an appropriate volume of Oral Solution to a suitable volumetric flask and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 266 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.
Column: 4.6-mm × 15-cm; 2.7-µm packing L60
Flow rate: 1 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of cyproheptadine hydrochloride ($C_{21}H_{21}N \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Cyproheptadine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cyproheptadine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Solution C, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 50 µg/mL each of USP Cyproheptadine Hydrochloride RS, USP Cyproheptadine Related Compound A RS, USP Amitriptyline Related Compound A RS, and USP Cyproheptadine Related Compound C RS, prepared as follows. Transfer a suitable quantity of each Reference Standard to an appropriate volumetric flask. Add 38% of the total flask volume of acetonitrile to dissolve, and then dilute with water to volume.

Standard solution: 0.6 µg/mL each of USP Cyproheptadine Hydrochloride RS, USP Cyproheptadine Related Compound A RS, USP Amitriptyline Related Compound A RS, and USP Cyproheptadine Related Compound C RS from the *Standard stock solution* in *Solution B*

Sample solution: Oral Solution

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between amitriptyline related compound A and cyproheptadine related compound A

Relative standard deviation: NMT 5.0% for cyproheptadine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified degradation product from the *Sample solution*
r_S = peak response of cyproheptadine hydrochloride from the *Standard solution*
C_S = concentration of USP Cyproheptadine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cyproheptadine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.10%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cyproheptadine related compound C[a]	0.7	—
Cyproheptadine	1.0	—
Amitriptyline related compound A[a]	2.5	—
Cyproheptadine related compound A[a]	2.6	—
Any individual unspecified degradation product	—	0.20
Total degradation products	—	0.5

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities for the drug product.

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.5–4.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Amitriptyline Related Compound A RS
Dibenzosuberone.
$C_{15}H_{12}O$ 208.26

USP Cyproheptadine Hydrochloride RS

USP Cyproheptadine Related Compound A RS
5*H*-Dibenzo[*a*,*d*]cycloheptene.
$C_{15}H_{12}$ 192.26

USP Cyproheptadine Related Compound C RS
5-(1-Methyl-piperidin-4-yl)-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ol.
$C_{21}H_{23}NO$ 305.41

Cyproheptadine Hydrochloride Tablets

» Cyproheptadine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{21}N \cdot HCl$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Cyproheptadine Hydrochloride RS

Identification—Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{21}H_{21}N \cdot HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 285 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Cyproheptadine Hydrochloride RS in the same *Medium*.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{21}H_{21}N \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Methanesulfonic acid solution—Prepare a solution of methanesulfonic acid in water (3:1000).
Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, isopropyl alcohol, and *Methanesulfonic acid solution* (20:15:65); while mixing adjust with triethylamine

USP Monographs

www.webofpharma.com

to a pH of 4.0 ± 0.05. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Cyproheptadine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.08 mg per mL.

Assay preparation—Transfer a number of Tablets, accurately weighed, equivalent to 80 mg of cyproheptadine hydrochloride, to a 1-liter volumetric flask, dissolve by sonication in 500 mL of *Mobile phase* for 15 minutes, and agitate for 30 minutes. Dilute with *Mobile phase* to volume, and mix. Pass through a filter having a 0.45-µm or finer porosity.

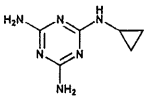
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 285-nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{21}N \cdot HCl$ in each of the Tablets taken by the formula:

$$1000(C/N)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cyproheptadine Hydrochloride RS in the *Standard preparation; N* is the number of Tablets taken for the *Assay preparation*; and r_U and r_S are the cyproheptadine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cyromazine



$C_6H_{10}N_6$ 166.18

N-Cyclopropyl-1,3,5-triazine-2,4,6-triamine.
2-Cyclopropylamino-4,6-diamino-*s*-triazine [66215-27-8].

» Cyromazine contains not less than 98.0 percent and not more than 102.0 percent of $C_6H_{10}N_6$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Cyromazine RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Melting range ⟨741⟩**:** between 219° and 226°.
Loss on drying ⟨731⟩—Dry it at 105° to a constant weight: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩**:** not more than 0.1%.

Assay—

Mobile phase—Mix 930 mL of water, 3.72 g of dibasic potassium phosphate, and 6.48 g of monobasic potassium phosphate. Add 50 mL of methanol and 20 mL of acetonitrile, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Cyromazine RS in methanol to obtain a solution having a known concentration of about 0.50 mg per mL. Dilute an aliquot of the resulting solution with *Mobile phase* to obtain a solution having a known concentration of about 10 µg per mL.

Assay preparation—Dissolve an accurately weighed quantity of Cyromazine in methanol to obtain a solution having a known concentration of about 0.50 mg per mL. Dilute an aliquot of the resulting solution with *Mobile phase* to obtain a solution having a known concentration of about 10 µg per mL.

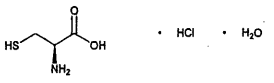
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 3000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the content, in percentage, of $C_6H_{10}N_6$ in the portion of Cyromazine taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in µg per mL, of USP Cyromazine RS in the *Standard preparation; C_U* is the concentration, in µg per mL, of Cyromazine in the *Assay preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cysteine Hydrochloride



$C_3H_7NO_2S \cdot HCl \cdot H_2O$ 175.63
$C_3H_7NO_2S \cdot HCl$ 157.62

L-Cysteine hydrochloride monohydrate [7048-04-6].
Anhydrous [52-89-1].

DEFINITION

Cysteine Hydrochloride is L-cysteine hydrochloride monohydrate and contains NLT 98.5% and NMT 101.5% of L-cysteine hydrochloride ($C_3H_7NO_2S \cdot HCl$), calculated on the dried basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Sample: 250 mg of Cysteine Hydrochloride

Blank: Proceed as directed in the *Analysis* without the *Sample*.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Residual titration

Titrant: 0.1 N iodine VS

Back-titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to an iodine flask. Add 20.0 mL of water and 4 g of potassium iodide, and mix. Cool the solution in an ice bath, and add 5 mL of 3 N hydrochloric acid and 25.0 mL of 0.1 N iodine VS. Insert the stopper, and allow to stand in the dark for 20 min, while remaining in the ice bath. Titrate the excess iodine with the *Back titrant*. Add 3 mL of starch TS as the endpoint is approached. Perform the *Blank* determination.

Calculate the percentage of cysteine hydrochloride ($C_3H_7NO_2S \cdot HCl$) in the portion of Cysteine Hydrochloride taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F]/W\} \times 100$$

V_B = *Back-titrant* volume consumed by the *Blank* (mL)

V_S = *Back-titrant* volume consumed by the *Sample* (mL)

N = actual normality of the *Back-titrant* (mEq/mL)

F = equivalency factor, 157.6 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.4%

• **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.33 g of Cysteine Hydrochloride

Acceptance criteria: NMT 0.03%

• **IRON** ⟨241⟩: NMT 30 ppm

• **RELATED COMPOUNDS**

***N*-Ethylmaleimide solution:** 40 mg/mL of N-ethylmaleimide in alcohol

Standard stock solution: Dissolve 20 mg of USP L-Cysteine Hydrochloride RS in 10.0 mL of water. Add 10.0 mL of *N-Ethylmaleimide solution*, and mix. Allow the solution to stand for 5 min before using.

Standard solution: 0.05 mg/mL from *Standard stock solution* in water. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]

System suitability solution: Transfer 10 mg of USP L-Tyrosine RS and 10 mL of the *Standard stock solution* to a 25-mL volumetric flask. Dilute with water to volume.

Sample stock solution: Transfer 0.2 g of Cysteine Hydrochloride to a 10-mL volumetric flask. Dissolve and dilute with water to volume.

Sample solution: To 5.0 mL of the *Sample stock solution* add 5.0 mL of *N-Ethylmaleimide solution*, and mix. Allow the solution to stand for 5 min before using.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)

Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

System suitability

Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

Analysis

Samples: *Standard solution*, *System suitability solution*, and *Sample solution*

After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.

Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 80 mg/mL in 6 N hydrochloric acid

Acceptance criteria: +5.7° to +6.8°

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at room temperature for 24 h in a vacuum desiccator using a suitable desiccant and maintaining a pressure of NMT 5 mm of mercury (Hg).

Acceptance criteria: 8.0%–12.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP L-Cysteine Hydrochloride RS

USP L-Tyrosine RS

Cysteine Hydrochloride Injection

» Cysteine Hydrochloride Injection is a sterile solution of Cysteine Hydrochloride in Water for Injection. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of $C_3H_7NO_2S \cdot HCl \cdot H_2O$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

USP Reference standards ⟨11⟩—

USP L-Cysteine Hydrochloride RS

Identification—

A: To 2 mL of Injection add 3 mL of water, and mix. Add 10 mL of cupric sulfate TS: a bluish-gray precipitate is formed.

B: To 2 mL of Injection add 3 mL of water, and mix. Add 2 mL of 3 N sodium hydroxide and 2 drops of sodium nitroferricyanide solution (1 in 20): a red-purple color is produced, and it rapidly changes to yellow.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.7 USP Endotoxin Unit per mg of cysteine hydrochloride.

pH ⟨791⟩: between 1.0 and 2.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

www.webofpharma.com

Assay—

Standard stock preparation—Dissolve an accurately weighed quantity of USP L-Cysteine Hydrochloride RS in nitrogen-saturated water to obtain a solution having a known concentration of about 1 mg per mL.

Standard preparation—Transfer 20.0 mL of *Standard stock preparation* to a 200-mL volumetric flask, dilute with nitrogen-saturated 1.0 N sodium hydroxide to volume, and mix.

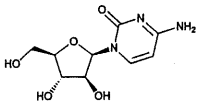
Assay preparation—Dilute an accurately measured volume of Injection, equivalent to about 250 mg of cysteine hydrochloride, quantitatively and stepwise with nitrogen-saturated 1.0 N sodium hydroxide, to obtain a solution having a concentration of about 0.1 mg per mL.

Procedure—Transfer a suitable amount of *Standard preparation* to a polarographic cell. With mercury dropping from the electrode, lower the dropping mercury electrode of a polarograph so that the end is submerged in the liquid. Bubble oxygen-free, water-saturated nitrogen through the liquid for 15 minutes. Record the polarogram from −0.2 volt to −1.10 volts, using a saturated calomel electrode as the reference electrode. In a similar manner, record the polarograms obtained using portions of the *Assay preparation* and of the nitrogen-saturated 1.0 N sodium hydroxide. Determine the height of the diffusion current wave at −0.4 volt. Calculate the quantity, in mg, of $C_3H_7NO_2S \cdot HCl \cdot H_2O$ in each mL of the Injection taken by the formula:

$$2500(C/V)[(i_d)_U/(i_d)_S]$$

in which C is the concentration, in mg per mL, of USP L-Cysteine Hydrochloride RS. In the *Standard preparation*, V is the volume, in mL, of Injection taken; and $(i_d)_U$ and $(i_d)_S$ are the observed diffusion currents, corrected for the diffusion current of the 0.1 N sodium hydroxide, of the *Assay preparation* and the *Standard preparation*, respectively.

Cytarabine



$C_9H_{13}N_3O_5$ 243.22

2(1*H*)-Pyrimidinone, 4-amino-1-β-D-arabinofuranosyl-;

1-β-D-Arabinofuranosylcytosine [147-94-4].

DEFINITION

Cytarabine contains NLT 98.0% and NMT 102.0% of cytarabine ($C_9H_{13}N_3O_5$), calculated on the dried basis.

Change to read:

IDENTIFICATION

▲• **A. Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197M▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Solution A: 0.73 g/L of monobasic sodium phosphate and 1.4 g/L of dibasic sodium phosphate in water

Mobile phase: Methanol and *Solution A* (5:95)

Standard solution: 0.1 mg/mL of USP Cytarabine RS in water

System suitability solution: 0.1 mg/mL each of USP Uracil Arabinoside RS and USP Cytarabine RS in water, prepared as follows. Dissolve USP Uracil Arabinoside RS in *Standard solution*.

Sample solution: 0.1 mg/mL of Cytarabine in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 µL

[Note—After chromatography has been completed, flush the column with a mixture of water and methanol (7:3).]

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for cytarabine and uracil arabinoside are about 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 2.5 between cytarabine and uracil arabinoside, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cytarabine ($C_9H_{13}N_3O_5$) in the portion of Cytarabine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Cytarabine RS in the *Standard solution* (mg/mL)

C_U = concentration of Cytarabine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.5%

• **Organic Impurities**

Buffer A: 0.02 M monobasic sodium phosphate in water

Buffer B: 0.02 M dibasic sodium phosphate in water

Buffer: *Buffer A* and *Buffer B* (1:1). Adjust with 0.1 M sodium hydroxide or 0.1 M phosphoric acid to a pH of 7.0.

Solution A: Methanol and *Buffer* (2:98)

Solution B: Methanol and *Buffer* (30:70)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
20	0	100
25	0	100
30	100	0
50	100	0

USP Monographs

www.webofpharma.com

System suitability solution: 0.02 mg/mL of USP Uridine RS, 0.02 mg/mL of USP Uracil Arabinoside RS, and 5.0 mg/mL of USP Cytarabine RS in water
Standard solution: 4 µg/mL of USP Cytarabine RS in water
Sample solution: 5 mg/mL of Cytarabine in water. Prepare this solution fresh daily.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.25 between cytarabine and uridine, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Cytarabine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of cytarabine from the *Standard solution*
C_S = concentration of USP Cytarabine RS in the *Standard solution* (mg/mL)
C_U = concentration of Cytarabine in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cyclocytidine[a]	0.38	1.5	0.10
Cytosine[b]	0.43	1.5	0.10
Uracil[c]	0.55	2.5	0.10
Uridine[d]	1.14	1.5	0.10
Uracil arabinoside	1.62	1.34	0.30
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.30

[a] (2*R*,3*R*,3a*S*,9a*R*)-2-(Hydroxymethyl)-6-imino-2,3,3a,9a-tetrahydro-6*H*-furo[2′,3′:4,5]oxazolo[3,2-a]pyrimidin-3-ol.
[b] 6-Amino-2(1*H*)-pyramidinone.
[c] Pyrimidine-2,4(1*H*,3*H*)-dione.
[d] 1-β-D-Ribofuranosylpyrimidine-2,4(1*H*,3*H*)-dione.

SPECIFIC TESTS
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL in water
Acceptance criteria: +154° to +160°
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.
Acceptance criteria: NMT 1.0%
- **STERILITY TESTS** ⟨71⟩: Where the label states that Cytarabine is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Cytarabine is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.07 USP Endotoxin Units/mg of cytarabine.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Cytarabine RS
USP Uracil Arabinoside RS
2,4(1*H*,3*H*)-Pyrimidinedione, 1-β-D-arabinofuranosyl-.
$C_9H_{12}N_2O_6$ 244.20
USP Uridine RS
1-β-D-Ribofuranosylpyrimidine-2,4(1*H*,3*H*)-dione.
$C_9H_{12}N_2O_6$ 244.20

Cytarabine for Injection

DEFINITION
Cytarabine for Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of cytarabine ($C_9H_{13}N_3O_5$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Solution A: 0.73 g/L of monobasic sodium phosphate and 1.4 g/L of dibasic sodium phosphate in water
Mobile phase: Methanol and *Solution A* (5:95)
Standard solution: 0.1 mg/mL of USP Cytarabine RS in water
System suitability solution: 0.1 mg/mL each of USP Uracil Arabinoside RS and USP Cytarabine RS in water prepared as follows. Dissolve USP Uracil Arabinoside RS in *Standard solution*.
Sample solution: 0.1 mg/mL of cytarabine in water prepared as follows. Separately constitute 5 vials of Cytarabine for Injection in a volume of water corresponding to the volume specified in the labeling. Pool and mix the constituted solutions in a suitable container. Transfer a volume of the constituted solution, nominally equivalent to 100 mg of cytarabine, into a 100-mL volumetric flask, and dilute with water to volume. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
After chromatography has been completed, flush the column with a mixture of water and methanol (7:3).
System suitability
Samples: *Standard solution* and *System suitability solution*

USP Monographs

www.webofpharma.com

[NOTE—The relative retention times for cytarabine and uracil arabinoside are about 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 2.5 between cytarabine and uracil arabinoside, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cytarabine ($C_9H_{13}N_3O_5$) in the portion of Cytarabine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Cytarabine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cytarabine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

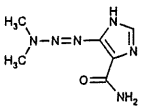
SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample solution: 10 mg/mL of cytarabine
  Acceptance criteria: 4.0–6.0
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 3.0%
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.07 USP Endotoxin Unit/mg of cytarabine.
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **OTHER REQUIREMENTS:** The drug substance in the vial meets the requirements for *Cytarabine.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging.*
- **LABELING:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cytarabine RS
  USP Uracil Arabinoside RS
  2,4(1*H*,3*H*)-Pyrimidinedione, 1-beta-D-arabinofuranosyl-.
  $C_9H_{12}N_2O_6$ 244.20

Dacarbazine



$C_6H_{10}N_6O$ 182.18

1*H*-Imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazenyl)-.

5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide [4342-03-4].

» Dacarbazine contains not less than 97.0 percent and not more than 102.0 percent of $C_6H_{10}N_6O$. [**CAUTION**—Great care should be taken in handling Dacarbazine, as it is a potent cytotoxic agent.]

Packaging and storage—Preserve in tight, light-resistant containers, in a refrigerator.

USP Reference standards ⟨11⟩—
USP Dacarbazine RS
USP Dacarbazine Related Compound A RS
5-Aminoimidazole-4-carboxamide hydrochloride.
USP Dacarbazine Related Compound B RS
2-Azahypoxanthine.
$C_4H_3N_5O \cdot H_2O$ 155.12

Identification—The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Dacarbazine RS.

Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—Dissolve an accurately weighed quantity of Dacarbazine in 0.1 N hydrochloric acid to obtain a solution having a concentration of 40 mg per mL, and apply 5 µL of the solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Apply, separately, 5 µL of a methanolic solution containing 0.40 mg of USP Dacarbazine Related Compound A RS per mL, and 5 µL of an aqueous solution containing 0.40 mg of USP Dacarbazine Related Compound B RS per mL. Develop the chromatogram in a mixture of butanol, water, and acetic acid (5:2:1), until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: any spots obtained from the test solution are not greater in size or intensity than the spots, occurring at the respective R_F values, produced by the Standard solutions, corresponding to not more than 1.0% of dacarbazine related compound A and not more than 1.0% of dacarbazine related compound B.

Assay— [NOTE—Throughout this procedure, avoid exposing Dacarbazine and its solutions to light.]

Standard preparations—Transfer about 30 mg of USP Dacarbazine RS, accurately weighed, to a 50-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix (*Standard stock solution*). Dilute a portion of *Standard stock solution* quantitatively and stepwise with 0.1 N hydrochloric acid to obtain an *Acidic standard preparation* having a known concentration of about 6 µg per mL. Dilute a portion of *Standard stock solution* quantitatively and stepwise with pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) to obtain a *Neutral standard preparation* having a known concentration of about 6 µg per mL.

Assay preparations—Prepare as directed under *Standard preparations*, except to use about 30 mg of Dacarbazine, accurately weighed.

Procedure—Concomitantly determine the absorbances of the *Acidic standard preparation* and the *Acidic assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 323 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Concomitantly determine the absorbances of the *Neutral standard preparation* and the *Neutral assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 329 nm, using pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) as the blank. Calculate the quantity, in mg, of $C_6H_{10}N_6O$ in the portion of Dacarbazine taken by the formula:

www.webofpharma.com

$$5C[(A_{323} + A_{329})_U/(A_{323} + A_{329})_S]$$

in which C is the concentration, in µg per mL, of USP Dacarbazine RS in the *Standard preparations*, and the parenthetic expressions are the sums of the absorbances of the *Assay preparations* (*U*) and the *Standard preparations* (*S*), respectively, measured at the wavelengths indicated by the subscripts.

Dacarbazine for Injection

DEFINITION

Dacarbazine for Injection is a sterile, freeze-dried mixture of Dacarbazine and suitable buffers or diluents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of dacarbazine ($C_6H_{10}N_6O$).

[**CAUTION**—Great care should be taken to prevent inhaling particles of Dacarbazine for Injection and exposing the skin to it.]

IDENTIFICATION

• **A.**

Standard solution: 10 mg/mL each of USP Dacarbazine RS and citric acid in water

Sample solution: Equivalent to 10 mg/mL of dacarbazine in water from Dacarbazine for Injection

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 1 µL

Developing solvent system: Isopropyl alcohol and 1 N ammonium hydroxide (3:1)

Spray reagent: Freshly prepared solution containing 1% of ferric chloride and 1% of potassium ferricyanide (prepared by mixing 5 mL of a 10% aqueous solution of ferric chloride with 5 mL of a 10% aqueous solution of potassium ferricyanide, and diluting with water to 50 mL)

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved three-quarters of the length of the plate. Allow the solvent to evaporate. Spray the plate evenly with *Spray reagent*. Dacarbazine appears as an intense blue spot on a light yellow background.

Acceptance criteria: The R_F value of the spot from the *Sample solution* corresponds to that from the *Standard solution*.

• **B.**

Sample solution: An aqueous solution (1 in 100)

Analysis: To 1 mL of the *Sample solution* in a test tube add a few crystals of periodic acid and 4 drops of methanol. Shake, and after 1 min add 5 mL of a 0.2% acetylacetone reagent solution (prepared by mixing 15.0 g of ammonium acetate, 0.30 mL of glacial acetic acid, and 0.20 mL of acetylacetone in a 100-mL volumetric flask, diluting with water to volume, and mixing). Shake, and place in a water bath maintained at a temperature of 60°.

Acceptance criteria: An intense yellow color develops in a few min (presence of mannitol).

• **C.**

Sample solution: An aqueous solution (1 in 100)

Analysis: To 2 drops of the *Sample solution* in a 15-mL test tube add 10 mL of a solution prepared by mixing 10 mL of acetic anhydride with 30 mL of pyridine.

Acceptance criteria: An intense yellow color is produced immediately and, after a few min, becomes red-violet (presence of citric acid).

ASSAY

• **PROCEDURE**

Standard solution: 3.2 µg/mL of USP Dacarbazine RS in 0.1 N hydrochloric acid

Sample solution: Equivalent to 3.2 µg/mL of dacarbazine in 0.1 N hydrochloric acid prepared as follows. Dissolve the contents of NLT 10 containers of Dacarbazine for Injection in 0.1 N hydrochloric acid. Transfer and combine the solutions into a suitable volumetric flask, rinsing as necessary with 0.1 N hydrochloric acid, and dilute with 0.1 N hydrochloric acid to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 323 nm

Cell: 1 cm

Blank: 0.1 N hydrochloric acid

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dacarbazine ($C_6H_{10}N_6O$) in the portion of Dacarbazine for Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Dacarbazine RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of dacarbazine in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **LIMIT OF 2-AZAHYPOXANTHINE**

The *Mobile phase* used in this procedure is corrosive. The system should be rinsed well with methanol after completion of the analysis.

Mobile phase: Transfer 2.2 g of docusate sodium to a 1000-mL volumetric flask, dissolve in a mixture of 100 mL of water and 15 mL of glacial acetic acid, and dilute with water to volume. Prepare this solution fresh daily.

Standard solution: 0.04 mg/mL of USP Dacarbazine Related Compound B RS in water

Sample solution: Constitute the contents of 1 vial of Dacarbazine for Injection, and dilute quantitatively with water to obtain a solution containing 4 mg/mL of dacarbazine.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0% (5 replicate injections)

Analysis

Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of 2-azahypoxanthine in the portion of Dacarbazine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

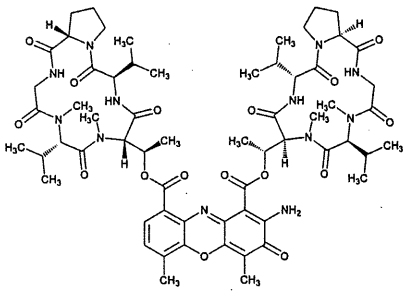
r_U = peak response of 2-azahypoxanthine from the *Sample solution*
r_S = peak response of 2-azahypoxanthine from the *Standard solution*
C_S = concentration of USP Dacarbazine Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dacarbazine in the *Sample solution*

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS
- **pH** ⟨791⟩
  Sample solution: Equivalent to 10 mg/mL of dacarbazine in water from Dacarbazine for Injection
  Acceptance criteria: 3.0–4.0
- **Water Determination,** *Method I* ⟨921⟩**:** NMT 1.5%
- **Bacterial Endotoxins Test** ⟨85⟩**:** It contains NMT 0.52 USP Endotoxin Unit/mg of dacarbazine.
- **Sterility Tests** ⟨71⟩**:** Meets the requirements
- **Completeness of Solution:** When dissolved as directed in the labeling, it yields a clear, pale yellow to yellow solution.
- **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*, preferably of Type I glass, protected from light.
- **Labeling:** Meets requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*
- **USP Reference Standards** ⟨11⟩
  USP Dacarbazine RS
  USP Dacarbazine Related Compound B RS
  2-Azahypoxanthine.
  $C_4H_3N_5O \cdot H_2O$ 155.12

Dactinomycin



$C_{62}H_{86}N_{12}O_{16}$ 1255.42
Actinomycin D [50-76-0].

DEFINITION
Dactinomycin contains NLT 950 µg/mg and NMT 1030 µg/mg of dactinomycin ($C_{62}H_{86}N_{12}O_{16}$), calculated on the dried basis.

[**Caution**—Great care should be taken to prevent inhaling particles of Dactinomycin and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Standard solution: 25 µg/mL of dactinomycin from USP Dactinomycin RS in methanol
  Sample solution: 25 µg/mL in methanol
  Analytical wavelengths: 240 and 445 nm
  Acceptance criteria
  Absorptivity: The absorptivity, ▲calculated on the dried basis,▲ (ERR 1-Jun-2019) of the *Sample solution* at 445 nm is NLT 95.0% and NMT 103.0% that of the *Standard solution.*
  Ratio: A_{240}/A_{445}, 1.30–1.50, *Sample solution*
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY
- **Procedure**
  Mobile phase: Acetonitrile, 0.04 M sodium acetate, and 0.07 M acetic acid (46:25:25). Pass through a filter of 1-µm or finer pore size. The acetonitrile concentration may be varied to provide appropriate chromatographic system performance and a suitable elution time.
  Standard solution: 1.2 mg/mL of USP Dactinomycin RS in *Mobile phase.* Use a freshly prepared solution, and protect it from light.
  Sample solution: 1.2 mg/mL of Dactinomycin in *Mobile phase.* Use a freshly prepared solution, and protect it from light.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: UV 254 nm
  Column: 3.9-mm × 30-cm; packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  [Note—The retention time for dactinomycin is about 25 min.]
  Suitability requirements
  Relative standard deviation: NMT 1.0%, dactinomycin (3 replicate injections)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the potency, in µg/mg, of dactinomyin ($C_{62}H_{86}N_{12}O_{16}$) in the Dactinomycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dactinomycin RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of dactinomycin in USP Dactinomycin RS (mg/mg)
F = conversion factor, 1000 µg/mg

Acceptance criteria: 950–1030 µg/mg on the dried basis

SPECIFIC TESTS
- **Optical Rotation,** *Specific Rotation* ⟨781S⟩
  Sample solution: 1 mg/mL in methanol

USP Monographs

www.webofpharma.com

Acceptance criteria: −293° to −329° (t = 20°)
- **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.
  Acceptance criteria: NMT 5.0%
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 100 USP Endotoxin Units/mg.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dactinomycin RS

Dactinomycin for Injection

DEFINITION
Dactinomycin for Injection is a sterile mixture of Dactinomycin and Mannitol. It contains NLT 90.0% and NMT 120.0% of the labeled amount of $C_{62}H_{86}N_{12}O_{16}$.
[**CAUTION**—Great care should be taken to prevent inhaling particles of Dactinomycin and exposing the skin to it.]

IDENTIFICATION
- **A. PROCEDURE**
  Standard solution: 25 µg/mL of USP Dactinomycin RS in methanol
  Sample solution: 25 µg/mL of dactinomycin in methanol
  Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as the *Standard solution*, concomitantly measured.
  Ratio: A_{240}/A_{445}, 1.30–1.50
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  [NOTE—Use freshly prepared *Standard solution* and *Sample solution*, protected from light.]
  Mobile phase: Acetonitrile and water (3:2)
  Standard solution: 250 µg/mL of USP Dactinomycin RS in *Mobile phase*
  Sample solution: 250 µg/mL of dactinomycin from Dactinomycin for Injection diluted with *Mobile phase*. Filter, if necessary, to obtain a clear solution. [NOTE—Prepare the solution by adding a suitable aliquot of *Mobile phase* to one container of Dactinomycin for Injection.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 3.9-mm × 30-cm; packing L1
  Flow rate: 2.5 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The retention time for dactinomycin is 6 min.]
  Suitability requirements
  Column efficiency: NLT 1200 theoretical plates
  Tailing factor: NMT 2
  Relative standard deviation: NMT 3.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{62}H_{86}N_{12}O_{16}$ in the portion of Dactinomycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dactinomycin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dactinomycin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 5.5–7.5, in the solution constituted as directed in the labeling
- **LOSS ON DRYING** ⟨731⟩**:** Dry a portion in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 4.0% of its weight.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 100.0 USP Endotoxin Units/mg of dactinomycin.
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined, Membrane Filtration*, each container being constituted aseptically by injecting Sterile Water for Injection through the stopper, and the entire contents of all the containers being collected aseptically with the aid of 200 mL of *Fluid A* before filtering.
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions* and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*; protect from light.
- **LABELING:** Label it to include the statement "Protect from light."
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dactinomycin RS

Dalfampridine



$C_5H_6N_2$ 94.11
4-Pyridinamine;
4-Aminopyridine [504-24-5].

DEFINITION
Dalfampridine contains NLT 98.0% and NMT 102.0% of dalfampridine ($C_5H_6N_2$).

IDENTIFICATION
- **A. INFRARED ABSORPTION** ⟨197⟩**:** [NOTE—Methods described in ⟨197K⟩ or ⟨197A⟩ may be used.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: 3.03 g/L of sodium 1-heptanesulfonate, 1.36 g/L of monobasic potassium phosphate, and 1.15 g/L of phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
5.0	90	10
15.0	80	20
20.0	80	20
20.1	90	10
25.0	90	10

Diluent: *Solution A* and *Solution B* (90:10)

System suitability stock solution: 0.04 mg/mL of USP Dalfampridine Related Compound A RS prepared as follows. Transfer a suitable quantity of USP Dalfampridine Related Compound A RS to an appropriate volumetric flask and add 20% of the total flask volume of *Diluent*. Sonicate for about 5 min. Allow to cool to room temperature and dilute with *Diluent* to volume. Use within 24 h.

System suitability solution: 0.0002 mg/mL of USP Dalfampridine Related Compound A RS from *System suitability stock solution* and 0.2 mg/mL of USP Dalfampridine RS in *Diluent* prepared as follows. Transfer a suitable quantity of USP Dalfampridine RS to an appropriate volumetric flask, add an appropriate volume of *System suitability stock solution*, and add 25% of the total flask volume of *Diluent*. Sonicate for NLT 5 min. Allow to cool to room temperature and dilute with *Diluent* to volume. Pass the resulting solution through a suitable filter, discard NLT the first 2 mL, and use the filtrate.

Standard solution: 0.2 mg/mL of USP Dalfampridine RS in *Diluent* prepared as follows. Transfer a suitable quantity of USP Dalfampridine RS to an appropriate volumetric flask and add 25% of the total flask volume of *Diluent*. Sonicate for NLT 5 min. Allow to cool to room temperature and dilute with *Diluent* to volume. Pass the resulting solution through a suitable filter and use the filtrate.

Sample solution: 0.2 mg/mL of Dalfampridine in *Diluent* prepared as follows. Transfer a suitable quantity of Dalfampridine to an appropriate volumetric flask and add 25% of the total flask volume of *Diluent*. Sonicate for NLT 5 min. Allow to cool to room temperature and dilute with *Diluent* to volume. Pass the resulting solution through a suitable filter and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 10.0-cm; 3.5-µm packing L1

Column temperature: 30°

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between dalfampridine and dalfampridine related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dalfampridine ($C_5H_6N_2$) in the portion of Dalfampridine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Dalfampridine RS in the *Standard solution* (mg/mL)

C_U = concentration of Dalfampridine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩

Ignition temperature: 800°

Acceptance criteria: NMT 0.3%

• **LIMIT OF DALFAMPRIDINE RELATED COMPOUND B AND DALFAMPRIDINE RELATED COMPOUND C**

[NOTE—This test should be conducted if dalfampridine related compound B and dalfampridine related compound C are possible from the manufacturing process.]

Solution A, Solution B, Mobile phase, and **Diluent:** Prepare as directed in the *Assay*.

Standard solution: 2 µg/mL each of USP Dalfampridine Related Compound B RS and USP Dalfampridine Related Compound C RS in *Diluent*

Sensitivity solution: 0.1 µg/mL each of USP Dalfampridine Related Compound B RS and USP Dalfampridine Related Compound C RS from *Standard solution* in *Diluent*

Sample solution: 2000 µg/mL of Dalfampridine in *Diluent* prepared as follows. Transfer a suitable quantity of Dalfampridine to an appropriate volumetric flask and add 25% of the total flask volume of *Diluent*. Sonicate for NLT 5 min. Allow to cool to room temperature and dilute with *Diluent* to volume.

Chromatographic system: Proceed as directed in the *Assay*, except use an *Injection volume* of 30 µL.

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Relative standard deviation: NMT 10% each for dalfampridine related compound B and dalfampridine related compound C, *Standard solution*

Signal-to-noise ratio: NLT 10 each for dalfampridine related compound B and dalfampridine related compound C, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dalfampridine related compound B and dalfampridine related compound C in the portion of Dalfampridine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dalfampridine related compound B or dalfampridine related compound C from the *Sample solution*

r_S = peak response of dalfampridine related compound B or dalfampridine related compound C from the *Standard solution*

USP Monographs

www.webofpharma.com

C_S = concentration of USP Dalfampridine Related Compound B RS or USP Dalfampridine Related Compound C RS in the *Standard solution* (µg/mL)
C_U = concentration of Dalfampridine in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.0075% each for dalfampridine related compound B and dalfampridine related compound C

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and **System suitability solution:** Prepare as directed in the *Assay.*

Standard solution: 2 µg/mL of USP Dalfampridine RS in *Diluent*

Sensitivity solution: 0.1 µg/mL of USP Dalfampridine RS from *Standard solution* in *Diluent*

Sample solution: 200 µg/mL of Dalfampridine in *Diluent* prepared as follows. Transfer a suitable quantity of Dalfampridine to an appropriate volumetric flask and add 25% of the total flask volume of *Diluent.* Sonicate for NLT 5 min. Allow to cool to room temperature and dilute with *Diluent* to volume. Pass the resulting solution through a suitable filter and use the filtrate.

Chromatographic system: Proceed as directed in the *Assay,* except use a *Detector* wavelength of 265 nm for isonicotinamide.

System suitability

Samples: *System suitability solution, Standard solution,* and *Sensitivity solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between dalfampridine and dalfampridine related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for dalfampridine, *System suitability solution*

Relative standard deviation: NMT 5.0% for dalfampridine, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of isonicotinamide in the portion of Dalfampridine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of isonicotinamide at 265 nm from the *Sample solution*
r_S = peak response of dalfampridine at 275 nm from the *Standard solution*
C_S = concentration of USP Dalfampridine RS in the *Standard solution* (µg/mL)
C_U = concentration of Dalfampridine in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Calculate the percentage of dalfampridine related compound A and any other unspecified impurity in the portion of Dalfampridine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of dalfampridine related compound A or any unspecified impurity at 275 nm from the *Sample solution*
r_S = peak response of dalfampridine at 275 nm from the *Standard solution*
C_S = concentration of USP Dalfampridine RS in the *Standard solution* (µg/mL)
C_U = concentration of Dalfampridine in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2.* Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Isonicotinamide	0.64	0.45	0.15
Dalfampridine	1.0	—	—
Dalfampridine related compound A	1.2	1.6	0.10
Dalfampridine related compound B[a]	2.4	—	—
Dalfampridine related compound C[a]	6.4	—	—
Any individual unspecified impurity	—	1.0	0.10
Total impurities[b]	—	—	0.50

[a] This impurity is quantified using the *Limit of Dalfampridine Related Compound B and Dalfampridine Related Compound C* test.
[b] The sum of all impurities from the test for *Organic Impurities* and the *Limit of Dalfampridine Related Compound B and Dalfampridine Related Compound C* test.

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia:* NMT 0.3%

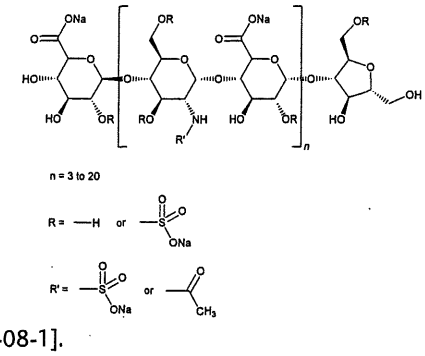
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Dalfampridine RS
USP Dalfampridine Related Compound A RS
4-Aminopyridine 1-oxide.
$C_5H_6N_2O$ 110.11
USP Dalfampridine Related Compound B RS
3,5-Dibromopyridin-4-amine.
$C_5H_4Br_2N_2$ 251.91
USP Dalfampridine Related Compound C RS
1,3-Di(pyridin-4-yl)urea.
$C_{11}H_{10}N_4O$ 214.23

Dalteparin Sodium



[9041-08-1].

www.webofpharma.com

DEFINITION

Dalteparin Sodium is the sodium salt of a low molecular weight heparin obtained by nitrous acid depolymerization of heparin from porcine intestine or intestinal mucosa. Heparin source material used in the manufacture of Dalteparin Sodium complies with the compendial requirements stated in the Heparin Sodium monograph. Dalteparin Sodium is produced by a validated manufacturing and purification procedure under conditions shown to minimize the presence of species containing the N–NO group. The majority of the components have a 2-*O*-sulfo-α-L-idopyranosuronic acid structure at the non-reducing end and a 6-*O*-sulfo-2,5-anhydro-D-mannitol structure at the reducing end of their chains. The weight-average molecular weight (M_w) ranges between 5600 Da and 6400 Da, with a characteristic value of about 6000 Da. The percentage of chains lower than molecular weight 3000 Da is NMT 13.0%, and the percentage of chains higher than molecular weight 8000 Da ranges between 15.0% and 25.0%. The degree of sulfation is NLT 1.8/disaccharide unit. The potency is NLT 110 and NMT 210 Anti-Factor X_a International Units (IU)/mg of activity, calculated on the dried basis. The anti-factor IIa activity is NLT 35 IU/mg and NMT 100 IU/mg, calculated on the dried basis. The ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.9 and 3.2.

IDENTIFICATION

• **A. ^{1}H NMR SPECTRUM**

Standard solution: Dissolve 15 mg of USP Dalteparin Sodium RS in 0.7 mL of deuterium oxide with deuterated trimethylsilylpropionic (TSP) acid sodium salt. The sample is freeze-dried to remove exchangeable protons. Redissolve the sample and repeat the freeze-drying step twice more before transferring the sample into an NMR tube.

Sample solution: Dissolve 15 mg of Dalteparin Sodium in 0.7 mL of deuterium oxide (99.9%) with deuterated TSP. The sample is freeze-dried to remove exchangeable protons. Redissolve the sample and repeat the freeze-drying step twice more before transferring the sample into an NMR tube.

Instrumental conditions

(See *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩.)

Mode: NMR, pulsed (Fourier transform)

Frequency: NLT 500 MHz for ^{1}H

Temperature: 30°

System suitability

Samples: *Standard solution* and *Sample solution*

Transfer the *Standard solution* and the *Sample solution* to NMR tubes of 5 mm in diameter. Using a pulsed (Fourier transform) NMR spectrometer operating at NLT 500 MHz for ^{1}H, acquire a free induction decay (FID) with NLT 32 scans using a 90° pulse, an acquisition time of NLT 2 s, and at least a 10-s delay. For each sample, an initial short spectrum is collected (1 scan), and the water resonance is then suppressed by selective irradiation during the relaxation delay. Final spectra are recorded over 32 scans. For all samples, the TSP methyl signal should be set to 0.00 ppm. Record the ^{1}H NMR spectrum of the *Standard solution*. Collect the ^{1}H NMR spectrum with a spectral window of at least 10 to −2 ppm and without spinning. The *Standard solution* shall be run at least daily when the *Sample solution* is being run. All spectra are phased, and linear baseline correction is applied to all spectra before peak identification.

Suitability requirements

Chemical shift: The TSP methyl signal should be set to 0.00 ppm for all samples.

Chemical shifts for system suitability: The ppm values for the methyl group of *N*-acetyl, the H-2 of *N*-sulfo glucosamine, the H-2 of glucuronic acid plus 3-*O*-sulfo glucosamine, the H-1 of iduronic acid, and the H-1 of 3-*O* sulfo glucosamine of dalteparin in the *Standard solution* are present at 2.05, 3.28, 3.39, 5.01, and 5.51, respectively. Two additional signals, corresponding to the H-1 of the 2-*O*-sulfo iduronic acid linked to the terminal 2, 5-anhydromannitol and the H-1 of 2-*O*-sulfo iduronic acid are located at 5.18–5.22 ppm. The ppm values of these signals do not differ by more than ±0.03 ppm, *Standard solution*.

[NOTE—Depending on specific sample makeup and instrument parameters, including the field strength of the NMR instrument, the two signals associated with the H-1 of 2-*O*-sulfo iduronic acid at 5.18–5.22 ppm may appear well separated or as a main signal with a shoulder.]

Analysis

Sample: *Sample solution*

Record the ^{1}H NMR spectra of the *Sample solution*.

Acceptance criteria: The ppm values for the methyl group of *N*-acetyl, the H-2 of *N*-sulfo glucosamine, the H-2 of glucuronic acid plus 3-*O*-sulfo glucosamine, the H-1 of iduronic acid and the H-1 of the 2-*O*-sulfo iduronic acid linked to the terminal anhydromannitol, the H-1 of 2-*O*-sulfo iduronic acid and the H-1 of 3-*O* sulfo glucosamine of dalteparin in the *Sample solution* are present at 2.05, 3.28, 3.39, 5.01, 5.18–5.22, and 5.51, respectively. The ppm values of these signals do not differ by more than ±0.03 ppm.

• **B. MOLECULAR WEIGHT DISTRIBUTION AND WEIGHT-AVERAGE MOLECULAR WEIGHT**

(See *Low Molecular Weight Heparin Molecular Weight Determinations* ⟨209⟩.)

Acceptance criteria: The weight-average molecular weight (M_w) ranges between 5600 Da and 6400 Da, with a characteristic value of about 6000 Da. The percentage of chains lower than the molecular weight 3000 Da (M_{3000}) is NMT 13.0%, and the percentage of chains higher than the molecular weight 8000 Da (M_{8000}) ranges between 15.0% and 25.0%.

• **C. ANTI-FACTOR Xa TO ANTI-FACTOR IIa RATIO**

(See *Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated and Low Molecular Weight Heparins* ⟨208⟩, *Anti-Factor Xa and Anti-Factor IIa Assays for Low Molecular Weight Heparins*.)

Acceptance criteria: The ratio of the numerical value of the anti-factor Xa activity, in Anti-Factor X_a IU/mg, to the numerical value of the anti-factor IIa activity, in Anti-Factor II_a IU/mg, as determined by the *Anti-Factor Xa Activity* and *Anti-Factor IIa Activity* assays, is NLT 1.9 and NMT 3.2, respectively.

• **D. IDENTIFICATION TESTS—GENERAL:** Meets the requirements for *Sodium Content*

ASSAY

• **ANTI-FACTOR Xa ACTIVITY**

(See *Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated and Low Molecular Weight Heparins* ⟨208⟩, *Anti-Factor Xa and Anti-Factor IIa Assays for Low Molecular Weight Heparins, Anti-Factor Xa Activity for Low Molecular Weight Heparin*.)

Analysis: Proceed as directed in the chapter.

Acceptance criteria: The potency is NLT 110 and NMT 210 Anti-Factor X_a IU/mg on the dried basis.

OTHER COMPONENTS

• **NITROGEN DETERMINATION,** *Method II* ⟨461⟩**:** 1.5%–2.5% on the dried basis

• **SODIUM CONTENT**

Cesium chloride solution: 1.27 mg/mL of cesium chloride in 0.1 M hydrochloric acid

www.webofpharma.com

Standard solution A: 0.0025% of sodium chloride in *Cesium chloride solution*
Standard solution B: 0.0050% of sodium chloride in *Cesium chloride solution*
Standard solution C: 0.0075% of sodium chloride in *Cesium chloride solution*
Sample solution: Transfer 50.0 mg of Dalteparin Sodium to a 100-mL volumetric flask, and dissolve in and dilute with *Cesium chloride solution* to volume.
Analysis
Samples: *Cesium chloride solution, Standard solution A, Standard solution B, Standard solution C,* and *Sample solution*
Concomitantly determine the absorbances of the *Cesium chloride solution* (blank), the *Sample solution,* and the *Standard solutions* at 330.3 nm, using a sodium hollow-cathode lamp and an air–acetylene flame. Using the absorbances of *Standard solutions A, B,* and *C,* determine the sodium content in the *Sample solution* after an appropriate blank correction.
Acceptance criteria: 10.5%–13.5% on the dried basis

IMPURITIES

• **Limit of Nitrites**
Mobile phase: Dissolve 13.6 g of sodium acetate trihydrate in 900 mL of water in a 1000-mL volumetric flask. Adjust with orthophosphoric acid to a pH of 4.3, and dilute with water to 1000 mL. Filter through a 0.45-µm membrane.
Nitrite stock standard solution: Dissolve 0.075 g of sodium nitrite in a 1000-mL volumetric flask with carbon dioxide-free water (0.05 g/L of nitrite).
Nitrite standard solution: Dilute 1 mL of *Nitrite stock standard solution* in a 100-mL volumetric flask with carbon dioxide-free water (500 ng/mL of nitrite).
Calibration standard solutions: Dilute *Nitrite standard solution* in carbon dioxide-free water to prepare four solutions with the final nitrite concentrations of 2.5, 5, 15, and 25 ng/mL.
Sample solution: Weigh 80.0 mg of Dalteparin Sodium into a 20-mL volumetric flask, and dissolve in carbon dioxide-free water.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: Electrochemical detector containing a working electrode (glassy carbon type) with the potential of +1.00 V against a silver–silver chloride reference electrode
Column: 3-mm × 15-cm; 5-µm packing L92
Column temperature: 30 ± 5°
Column regeneration: 1 M sodium chloride (NaCl) at 0.5 mL/min for about 1 h. After regeneration, wash the column with water and re-equilibrate with *Mobile phase.*
Flow rate: 0.5 mL/min
Injection volume: 25 µL
Run time: 10 min
System suitability
Samples: *Calibration standard solutions* and *Sample solution*
Suitability requirements
Column efficiency: NLT 4000 theoretical plates for the nitrite peak for all *Calibration solutions* and *Sample solution* runs
Tailing factor: Between 0.8 and 1.2 for all *Calibration solutions* and *Sample solution* runs
Relative standard deviation: Inject *Calibration standard solutions* with 25 ng/mL concentration at least six times. Calculate the relative standard deviation % (%RSD) of the nitrite peak areas of the last six injections. The %RSD is NMT 2%.
Analysis
Samples: *Calibration standard solutions* and *Sample solution*
Plot the areas of the nitrite peaks from the chromatograms of the *Calibration standard solutions* against respective concentrations of nitrite. Draw a best-fit regression line through the points. The correlation coefficient is NLT 0.995. Calculate the concentration of nitrite from the areas of the nitrite peak in the chromatogram of the *Sample solution.*
Acceptance criteria: NMT 5 ppm

• **Boron**
[Note—Use only plastic labware, avoid glass.]
Blank: 1% (v/v) solution of nitric acid in water
Calibration solution: Prepare a 11.4-µg/mL solution of USP Boric Acid RS in the *Blank.*
Standard solution A: Dissolve 0.2500 g of USP Low Molecular Weight Heparin for Boron Analysis RS in about 2 mL of water, add 100 µL of nitric acid, and dilute with the *Blank* to 10.00 mL.
Standard solution B: Dissolve 0.2500 g of USP Low Molecular Weight Heparin for Boron Analysis RS in about 2 mL of *Blank,* add 10 µL of a 5.7-mg/mL solution of USP Boric Acid RS, and dilute with the *Blank* to 10.00 mL. This solution contains 1 µg/mL of boron.
Sample solution: Dissolve 0.2500 g of Dalteparin Sodium in about 2 mL of water, add 100 µL of nitric acid, and dilute with the *Blank* to 10.00 mL.
Analysis
Samples: *Blank, Calibration solution, Standard solution A, Standard solution B,* and *Sample solution*
Boron is determined by measurement of the emission from inductively coupled plasma (ICP) at 249.733 nm or a suitable wavelength. Use an appropriate apparatus with settings that have been optimized as directed by the manufacturer.
Calculate the content of boron in Dalteparin Sodium using the following correction factor:

$$F = (r_{SB} - r_{SA}) \times 2/(r_C - r_B)$$

r_{SB} = response of boron from *Standard solution B*
r_{SA} = response of boron from *Standard solution A*
r_C = response of boron from the *Calibration solution*
r_B = response of boron from the *Blank*

Acceptance criteria: NMT 1 ppm

SPECIFIC TESTS

• **Anti-Factor IIa Activity**
(See *Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated and Low Molecular Weight Heparins,* ⟨208⟩, *Anti-Factor Xa and Anti-Factor IIa Assays for Low Molecular Weight Heparins, Anti-Factor IIa Activity for Low Molecular Weight Heparin.*)
Acceptance criteria: NLT 35 and NMT 100 Anti-Factor II_a IU/mg on the dried basis

• **Molar Ratio of Sulfate to Carboxylate**
Mobile phase: Carbon dioxide-free water
Sample solution: 50 mg of Dalteparin Sodium in 10 mL of carbon dioxide-free water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: Ion
Column: Two columns: one 1.5-cm × 2.5-cm column, packed with an anion-exchange resin L64 packing, and one 1.5-cm × 7.5-cm column, packed with a

USP Monographs

www.webofpharma.com

cation-exchange resin L65 packing.[1] The outlet of the anion-exchange column is connected to the inlet of the cation-exchange column.

Flow rate: 1 mL/min

Analysis

Sample: *Sample solution*

[NOTE—Regenerate the anion-exchange column and the cation-exchange column with 1 N sodium hydroxide and 1 N hydrochloric acid, respectively, between two injections.]

With the valve in the inject position, inject the *Sample solution* into the anion-exchange column, and collect the eluate from the cation-exchange column in a beaker at the outlet until the ion detector reading returns to the baseline value. Quantitatively transfer the eluate to a titration vessel containing a magnetic stirring bar, and dilute with carbon dioxide-free water to about 60 mL. Position the titration vessel on a magnetic stirrer, and immerse the electrodes. Note the initial conductivity reading, and titrate with approximately 0.1 N sodium hydroxide added in 100-µL portions. [NOTE—Prepare the sodium hydroxide solution in carbon dioxide-free water.] Record the buret reading and the conductivity meter reading after each addition of the sodium hydroxide solution.

Plot the conductivity measurements on the *y*-axis against the volumes of sodium hydroxide added on the *x*-axis. The graph will have three linear sections—an initial downward slope, a middle slight rise, and a final rise. For each of these sections, draw the best-fit straight lines using linear regression analysis. At the points where the first and second straight lines intersect and where the second and third lines intersect, draw perpendiculars to the *x*-axis to determine the volumes of sodium hydroxide taken up by the sample at those points. The point where the first and second lines intersect corresponds to the volume of sodium hydroxide taken up by the sulfate groups (V_S). The point where the second and third lines intersect corresponds to the volume of sodium hydroxide consumed by the sulfate and the carboxylate groups together (V_T).

Calculate the molar ratio of sulfate to carboxylate:

$$\text{Result} = V_S/(V_T - V_S)$$

Acceptance criteria: The molar ratio of sulfate to carboxylate is NLT 1.8.

- **pH** ⟨791⟩**:** 5.5–8.0 for a 1.0% solution in water
- **Loss on Drying** ⟨731⟩

Sample: 1 g

Analysis: Dry the *Sample* under vacuum at 70° for 6 h.

Acceptance criteria: NMT 10%

- **Bacterial Endotoxins Test** ⟨85⟩**:** It contains NMT 0.01 USP Endotoxin Unit/IU of anti-factor Xa activity.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store below 40°, preferably at room temperature.
- **Labeling:** Label to state the number of Anti-factor X_a International Units of activity per mg.
- **USP Reference Standards** ⟨11⟩

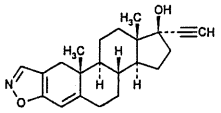
USP Boric Acid RS

USP Dalteparin Sodium RS

USP Low Molecular Weight Heparin for Boron Analysis RS

[1] The procedure is based on analyses performed with two columns: one 1.5-cm × 2.5-cm packed with anion-exchange resin Dowex 1X8 (200–400 mesh) and the other 1.5-cm × 7.5-cm packed with cation-exchange resin Dowex 50WX2 (100–200 mesh).

Danazol



$C_{22}H_{27}NO_2$ 337.46

Pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-ol, (17α)-.

17α-Pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-ol [17230-88-5].

» Danazol contains not less than 97.0 percent and not more than 102.0 percent of $C_{22}H_{27}NO_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Danazol RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—

Solution: prepared as directed in the *Assay*.

Specific rotation ⟨781S⟩: between +21° and +27°.

Test solution: 10 mg per mL, in chloroform.

Loss on drying ⟨731⟩—Dry it at a pressure not exceeding 5 mm of mercury at 60° to constant weight: it loses not more than 2.0% of its weight.

Chromatographic purity—

Solvent—Prepare a mixture of chloroform and methanol (9:1).

Standard solutions—Dissolve an accurately weighed quantity of USP Danazol RS in *Solvent* to obtain a solution having a known concentration of 1 mg per mL. Dilute quantitatively with *Solvent* to obtain *Standard solutions* having the following compositions:

Standard solution	Dilution	Concentration (µg RS per mL)	Percentage (%, for comparison with test specimen)
A	(1 in 2)	500	1.0
B	(1 in 4)	250	0.5
C	(1 in 10)	100	0.2
D	(1 in 20)	50	0.1

Test solution—Dissolve an accurately weighed quantity of Danazol in *Solvent* to obtain a solution containing 50 mg per mL.

Procedure—Apply separately 5 µL of the *Test solution* and 5 µL of each *Standard solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber and develop the chromatograms in a solvent system consisting of a mixture

www.webofpharma.com

of cyclohexane and ethyl acetate (7:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in warm, circulating air. Examine the plate under short-wavelength UV light. Expose the plate to iodine vapors for 5 minutes. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions:* the sum of the intensities of secondary spots obtained from the *Test solution* corresponds to not more than 1.0% of related compounds, with no single impurity corresponding to more than 0.5%.

Assay—Dissolve about 100 mg of Danazol, accurately weighed and previously dried, in about 50 mL of alcohol in a 100-mL volumetric flask, swirl until dissolved, dilute with alcohol to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with alcohol to volume, and mix. Similarly, dissolve an accurately weighed quantity of USP Danazol RS in alcohol to obtain a Standard solution having a known concentration of about 20 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 285 nm, using alcohol as the blank. Calculate the quantity, in mg, of $C_{22}H_{27}NO_2$ in the portion of Danazol taken by the formula:

$$5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Danazol RS in the Standard solution; and A_U and A_S are the absorbances of the solution of Danazol and the Standard solution, respectively.

Danazol Capsules

» Danazol Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{22}H_{27}NO_2$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Danazol RS

Identification—Shake the contents of a sufficient number of Capsules, equivalent to about 50 mg of Danazol, with 50 mL of chloroform, and filter. Evaporate the filtrate on a steam bath with the aid of a stream of nitrogen to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue, previously dried, exhibits maxima at the same wavelengths as that of a similar preparation of USP Danazol RS.

Dissolution ⟨711⟩—
Medium: 0.75% sodium lauryl sulfate solution; 900 mL.
Apparatus 2: 75 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{22}H_{27}NO_2$ dissolved as follows. Remove an aliquot from the solution under test at a point midway between the stirring shaft and the wall of the vessel and approximately midway in depth. Measure the amount in solution in filtered portions of the *Dissolution Medium*, suitably diluted with the *Dissolution Medium*, at the wavelength of maximum absorbance at about 286 nm, with a suitable spectrophotometer, in comparison with a solution of known concentration of USP Danazol RS prepared as follows. Transfer 10 mg of USP Danazol RS, accurately weighed, to a 10-mL volumetric flask, and dissolve in isopropyl alcohol. Transfer 2.0 mL to a 100-mL volumetric flask, dilute with *Dissolution Medium* to volume, and mix.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{22}H_{27}NO_2$ is dissolved in 30 minutes.

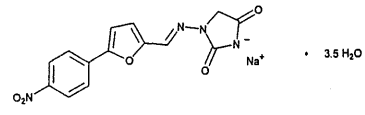
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, methanol, and water (4:3:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Danazol RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.
Assay preparation—Accurately weigh the contents of not less than 20 Capsules. Mix the contents, and transfer an accurately weighed portion of the powder, equivalent to about 100 mg of danazol, to a 100-mL volumetric flask. Add about 50 mL of *Mobile phase*, and shake by mechanical means for about 10 minutes. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 5 mL of the filtrate. Pipet 5 mL of the filtrate into a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.45-µm porosity filter, discarding the first 5 mL of the filtrate.
Chromatographic system (see *System Suitability* under *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 270-nm detector and a 3.9-mm × 15-cm column that contains 4-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of danazol in the portion of Capsules taken by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Danazol RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dantrolene Sodium



$C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$ 399.29
2,4-Imidazolidinedione, 1-[[[5-(4-nitrophenyl)-2-furanyl] methylene]amino]-, sodium salt, hydrate (2:7);
1-[[5-(*p*-Nitrophenyl)furfurylidene]amino]hydantoin sodium salt hydrate [24868-20-0].

DEFINITION
Dantrolene Sodium contains NLT 90.0% and NMT 96.0% of dantrolene ($C_{14}H_{10}N_4O_5$), the free acid form of Dantrolene Sodium, calculated on the anhydrous basis.

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*

Sample solution: To 0.1 g of Dantrolene Sodium, add 20 mL of water and 2 drops of acetic acid, shake well, and pass the resulting solution through a suitable filter. Use 2 mL of the filtrate.

Analysis

Sample: *Sample solution*

Acceptance criteria: Meets the requirements

- **D.**

Solution A: Dissolve 2.7 g of methoxyphenylacetic acid in 6 mL of tetramethylammonium hydroxide TS, and add 20 mL of dehydrated alcohol.

Solution B: 158 mg/mL of ammonium carbonate in water

Sample solution: To 0.1 g of Dantrolene Sodium, add 20 mL of water and 2 drops of acetic acid, shake well, and pass the resulting solution through a suitable filter. Use the filtrate.

Analysis

Sample: *Sample solution*

Part 1: To 0.5 mL of the *Sample solution* in a suitable container, add 1.5 mL of *Solution A*, and cool in ice water for 30 min.

Part 2: Transfer the container from *Part 1* to a water bath at 20°, and stir for 5 min.

Part 3: Add 1 mL of ammonia TS to the container from *Part 2*.

Part 4: Add 1 mL of *Solution B* to the container from *Part 3*.

Acceptance criteria: The requirements for *Part 1*, *Part 2*, *Part 3*, and *Part 4* must all be met.

Part 1: A voluminous, white, crystalline precipitate is formed.

Part 2: The precipitate does not disappear.

Part 3: The precipitate dissolves completely.

Part 4: No precipitate is formed.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 3.85 g of ammonium acetate in 1.0 L of water; adjusted with glacial acetic acid to a pH of 4.5 ± 0.1.

Solution A: Acetonitrile, *Buffer*, and water (10:20:70)

Solution B: Acetonitrile and *Buffer* (80:20)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	60	40
20	10	90
25	10	90
25.1	90	10
35	90	10

Diluent: Acetonitrile and water (50:50)

System suitability stock solution A: 1.25 mg/mL of USP Dantrolene Sodium RS prepared as follows. Transfer a suitable amount of USP Dantrolene Sodium RS to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

System suitability stock solution B: 0.125 mg/mL each of USP Dantrolene Related Compound B RS and USP Dantrolene Related Compound C RS prepared as follows. Transfer suitable amounts of USP Dantrolene Related Compound B RS and USP Dantrolene Related Compound C RS to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

System suitability solution: 125 µg/mL of USP Dantrolene Sodium RS from *System suitability stock solution A* and 2.5 µg/mL each of USP Dantrolene Related Compound B RS and USP Dantrolene Related Compound C RS from *System suitability stock solution B* in *Diluent*

Standard stock solution: 1.0 mg/mL of USP Dantrolene RS prepared as follows. Transfer a suitable amount of USP Dantrolene RS to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

Standard solution: 100 µg/mL of USP Dantrolene RS from *Standard stock solution* in *Diluent*

Sample stock solution: 1.25 mg/mL of Dantrolene Sodium prepared as follows. Transfer a suitable amount of Dantrolene Sodium to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

Sample solution: 125 µg/mL of Dantrolene Sodium from *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for dantrolene related compound B, dantrolene, and dantrolene related compound C are 0.68, 1.0, and 1.24, respectively.]

Suitability requirements

Resolution: NLT 8 between dantrolene related compound C and dantrolene, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dantrolene ($C_{14}H_{10}N_4O_5$) in the portion of Dantrolene Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dantrolene from the *Sample solution*

r_S = peak response of dantrolene from the *Standard solution*

C_S = concentration of USP Dantrolene RS in the *Standard solution* (µg/mL)

C_U = concentration of Dantrolene Sodium in the *Sample solution* (µg/mL)

USP Monographs

www.webofpharma.com

Acceptance criteria: 90.0%–96.0% on the anhydrous basis

IMPURITIES

• **LIMIT OF DANTROLENE RELATED COMPOUND A**

Mobile phase: Acetonitrile and water (80:20)

Standard stock solution: 17.5 µg/mL of USP Dantrolene Related Compound A RS and 50 µg/mL of USP Dantrolene Sodium RS in dimethylformamide

Standard solution: 0.35 µg/mL of USP Dantrolene Related Compound A RS and 1 µg/mL of USP Dantrolene Sodium RS from *Standard stock solution* in acetonitrile

Sample stock solution: 1.25 mg/mL of Dantrolene Sodium prepared as follows. Transfer a suitable amount of Dantrolene Sodium to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

Sample solution: 175 µg/mL of Dantrolene Sodium from *Sample stock solution* in acetonitrile

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The dantrolene peak elutes at void volume at approximately 1.5 min.]

Suitability requirements

Tailing factor: NMT 1.5 for dantrolene related compound A

Relative standard deviation: NMT 5% for dantrolene related compound A

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dantrolene related compound A in the portion of Dantrolene Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dantrolene related compound A from the *Sample solution*

r_S = peak response of dantrolene related compound A from the *Standard solution*

C_S = concentration of USP Dantrolene Related Compound A RS in the *Standard solution* (µg/mL)

C_U = concentration of Dantrolene Sodium in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.15%

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability stock solution B, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution A: Use the *Standard solution* from the *Assay*.

Standard solution B: 0.25 µg/mL each of USP Dantrolene Related Compound B RS and USP Dantrolene Related Compound C RS from *System suitability stock solution B* in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution A*

[NOTE—The relative retention times for dantrolene related compound B, dantrolene, and dantrolene related compound C are 0.68, 1.0, and 1.24, respectively.]

Suitability requirements

Resolution: NLT 8 between dantrolene related compound C and dantrolene, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution A*

Relative standard deviation: NMT 1.0%, *Standard solution A*

Analysis

Samples: *Sample solution* and *Standard solution B*

Calculate the percentage of dantrolene related compound B and dantrolene related compound C in the portion of Dantrolene Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dantrolene related compound B or dantrolene related compound C from the *Sample solution*

r_S = peak response of dantrolene related compound B or dantrolene related compound C from *Standard solution B*

C_S = concentration of USP Dantrolene Related Compound B RS or USP Dantrolene Related Compound C RS in *Standard solution B* (µg/mL)

C_U = concentration of Dantrolene Sodium in the *Sample solution* (µg/mL)

Acceptance criteria

Dantrolene related compound B: NMT 0.50%

Dantrolene related compound C: NMT 0.30%

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method Ia*: 14.5%–17.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Dantrolene RS

1-({[5-(4-Nitrophenyl)furan-2-yl]methylene}amino) imidazolidine-2,4-dione.

$C_{14}H_{10}N_4O_5$ 314.25

USP Dantrolene Sodium RS

USP Dantrolene Related Compound A RS

1,2-Bis{[5-(4-nitrophenyl)furan-2-yl]methylene} hydrazine;

Also known as 5-(4-Nitrophenyl)furaldehyde azine.

$C_{22}H_{14}N_4O_6$ 430.38

USP Dantrolene Related Compound B RS

N-Carbamoyl-*N*-({[5-(4-nitrophenyl)furan-2-yl] methylene}amino)glycine;

Also known as 5-(4-Nitrophenyl)-2-furaldehyde-2-carboxymethyl semicarbazone.

$C_{14}H_{12}N_4O_6$ 332.27

USP Dantrolene Related Compound C RS

5-(4-Nitrophenyl)furan-2-carbaldehyde.

$C_{11}H_7NO_4$ 217.18

Dantrolene Sodium Capsules

» Dantrolene Sodium Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dantrolene sodium ($C_{14}H_9N_4NaO_5 \cdot 3½H_2O$).

Packaging and storage—Preserve in tight containers.

www.webofpharma.com

USP Reference standards ⟨11⟩—
USP Dantrolene RS
USP Dantrolene Related Compound B RS
5-(4-Nitrophenyl)-2-furaldehyde-2-carboxymethyl semicarbazone.
$C_{14}H_{12}N_4O_6$
USP Dantrolene Sodium RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K.*▲ (CN 1-May-2020)

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: 0.5% methylbenzethonium chloride in water, pH 6.8 (adjusted with 0.1 N potassium hydroxide or 0.1 N hydrochloric acid); 900 mL, deaerated.
Apparatus 1: 100 rpm.
Time: 40 minutes.
Determine the amount of $C_{14}H_9N_4NaO_5 \cdot 3½H_2O$ dissolved by employing the following method.
Standard solution 1 (for Capsules labeled to contain 100 mg)—Transfer 25 mg of USP Dantrolene RS, accurately weighed, to a 250-mL volumetric flask. Dissolve in 5.0 mL of dimethylformamide. Add 200 mL of *Medium* and 10.0 mL of 0.1 N potassium hydroxide. Mix, dilute with *Medium* to volume, and mix. Pass through a 0.45-µm polytetrafluoroethylene (PTFE) filter, previously wetted with a few drops of isopropyl alcohol, discarding the first 5 mL.
Standard solution 2 (for Capsules labeled to contain 50 mg)—Transfer 25.0 mL of *Standard solution 1* to a 50-mL volumetric flask containing 0.5 mL of 0.1 N potassium hydroxide. Dilute with *Medium* to volume, and mix. Pass through a 0.45-µm PTFE filter, previously wetted with a few drops of isopropyl alcohol, discarding the first 5 mL.
Standard solution 3 (for Capsules labeled to contain 25 mg)—Transfer 25.0 mL of *Standard solution 1* to a 100-mL volumetric flask containing 1.0 mL of 0.1 N potassium hydroxide. Dilute with *Medium* to volume, and mix. Pass through a 0.45-µm PTFE filter, previously wetted with a few drops of isopropyl alcohol, discarding the first 5 mL.
Test solution—Withdraw 10 mL of the solution under test. Pass through a 0.45-µm PTFE filter, previously wetted with a few drops of isopropyl alcohol. Discard the first 5 mL. Collect the filtered solution in a tube that contains 1 drop of 1 N potassium hydroxide, and mix.
System suitability— [NOTE—All absorbance values should be obtained on solutions within 2 hours of their preparation.] Using a 0.1-cm cell, measure the absorbance of the *Medium*, using water as the blank, and measure the absorbance of each of the three *Standard solutions* using *Medium* as the blank, at the wavelength of maximum absorbance at about 395 nm. The system is considered suitable for use if the following criteria are met: the absorbance of the *Medium* is less than 10% of the absorbance of *Standard solution 1;* the absorbance of *Standard solution 2* is between 0.3 and 0.5; and the ratio of the absorbance of *Standard solution 1* to that of *Standard solution 3* is 4.00 ± 0.10.
Determine the amount of $C_{14}H_9N_4NaO_5 \cdot 3½H_2O$ dissolved by measuring the absorbance of the *Test solution* at the wavelength of maximum absorbance at about 395 nm in comparison with the appropriate *Standard solution,* using a 0.1-cm cell and *Medium* as the blank. All absorbance values are obtained on solutions within 2 hours of their preparation.
Calculate the percentage of $C_{14}H_9N_4NaO_5 \cdot 3½H_2O$ dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times 0.79186 \times LC}$$

in which A_U and A_S are the absorbances obtained from the *Test solution* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of dantrolene in the *Standard solution;* 900 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; 0.79186 is the correction for water of hydration and sodium contained in the dantrolene sodium monohydrate form of the drug, assuming that the bulk drug contains 15% of water and 6.84% of sodium; and *LC* is the Capsule label claim, in mg.
Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{14}H_9N_4NaO_5 \cdot 3½H_2O$ is dissolved in 40 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Related compounds—
Diluent, Solution A, Solution B, Mobile phase, and *Chromatographic system*—Proceed as directed in the *Assay.*
Standard solution—Transfer 5 mg, accurately weighed, of USP Dantrolene Related Compound B RS into a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume. The final concentration is about 0.1 mg per mL. Quantitatively dilute this solution with *Diluent* to obtain a solution having a known concentration of about 0.0005 mg per mL of dantrolene related compound B.
Test solution—Use the *Assay preparation.*
Procedure—Inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of dantrolene related compound B in the portion of Capsules taken by the formula:

$$100(r_U/r_S)(C_S/C_T)$$

in which r_U is the individual peak response for dantrolene related compound B obtained from the *Test solution;* r_S is the response of the corresponding peak in the *Standard solution;* C_S is the concentration, in mg per mL, of dantrolene related compound B in the *Standard solution;* and C_T is the concentration, in mg per mL, of dantrolene sodium in the *Test solution:* not more than 2% of dantrolene related compound B is found.

Assay—
Diluent—Prepare a solution of acetonitrile and water (70:30).
Buffer solution—Dissolve 3.3 g of ammonium acetate in 1 L of water.
Solution A—Prepare a filtered and degassed mixture of *Buffer solution,* acetonitrile, and glacial acetic acid (120:76:7).
Solution B—Prepare a filtered and degassed mixture of acetonitrile and water (70:30).
Mobile phase—Use variable mixtures of *Solution A* and *Solution B,* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Use the *Standard solution,* prepared as directed in the test for *Related compounds.*
Standard preparation—Transfer 40 mg, accurately weighed, of USP Dantrolene RS to a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume. The final concentration is about 0.8 mg per mL. Quantitatively dilute

www.webofpharma.com

this solution with *Diluent* to obtain a solution having a known concentration of about 0.08 mg per mL of dantrolene.

Assay preparation—Mix the combined contents of not fewer than 20 Capsules, and transfer an accurately weighed portion, equivalent to the average weight of one Capsule, to a 50-mL volumetric flask. Add 10 mL of dimethylformamide, and sonicate for 15 minutes to dissolve. Add 5 mL of glacial acetic acid, and dilute with acetone to volume. Quantitatively dilute this solution with *Diluent* to obtain a solution having 0.1 mg per mL of dantrolene sodium, and pass through a 0.45-µm nylon filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 365-nm detector and a 4.6-mm ×15-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–8	100	0	isocratic
8–8.1	100→0	0→100	linear gradient
8.1–13	0	100	isocratic
13–13.1	0→100	100→0	linear gradient
13.1–20	100	0	re-equilibration

Separately inject the *System suitability solution* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections for dantrolene is not more than 1.0%.

[NOTE—For the purpose of peak identification, the approximate relative retention times are 0.68 for dantrolene related compound B and 1.0 for dantrolene.]

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dantrolene peaks. Calculate the percentage of dantrolene sodium ($C_{14}H_9N_4NaO_5 \cdot 3½H_2O$) in the portion of Capsules taken by the formula:

$$100(399.29/314.25)(r_U/r_S)(C_S/C_U)$$

in which 399.29 is the molecular weight of dantrolene sodium; 314.25 is the molecular weight of dantrolene; r_U and r_S are the peak responses for dantrolene obtained from the *Assay preparation* and the *Standard preparation,* respectively; C_S is the concentration, in mg per mL, of dantrolene in the *Standard preparation;* and C_U is the concentration, in mg per mL, of dantrolene sodium ($C_{14}H_9N_4NaO_5 \cdot 3½H_2O$) in the *Assay preparation.*

Dantrolene Sodium for Injection

» Dantrolene Sodium for Injection is a sterile, non-pyrogenic, lyophilized formulation containing Dantrolene Sodium, and one or more suitable buffering or sequestering agents. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{14}H_9N_4NaO_5 \cdot 3½H_2O$.

Packaging and storage—Preserve in tight containers. Store at controlled room temperature, protected from light.

USP Reference standards ⟨11⟩—
USP Dantrolene RS
USP Dantrolene Related Compound B RS
5-(4-Nitrophenyl)-2-furaldehyde-2-carboxymethyl semicarbazone.
$C_{14}H_{12}N_4O_6$

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—
Test specimen—To 0.5 g of Dantrolene Sodium for Injection, add 10 mL of 0.1 N hydrochloric acid and 10 mL of ethyl acetate, and mix. Allow the phases to separate, and transfer the upper ethyl acetate phase to a suitable glass container. Evaporate the solvent, dry the residue at 105° for 10 minutes, and use the residue.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.5 USP Endotoxin Unit per mg of dantrolene sodium.

Sterility Tests ⟨71⟩ **:** meets the requirements.

Uniformity of dosage units ⟨905⟩**:** meets the requirements.

pH ⟨791⟩—Dissolve the contents of 1 vial in 60 mL of USP Water for Injection: the pH is between 8.8 and 11.0.

Water Determination, *Method Ia* ⟨921⟩ **:** not more than 3.0%.

Related compounds—

Mobile phase and *Diluent*—Proceed as directed in the *Assay.*

Standard solution—Transfer 10 mg of USP Dantrolene Related Compound B RS, accurately weighed, into a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume to obtain a solution having a known concentration of about 0.2 mg per mL. Dilute with *Diluent* to obtain a solution having a known concentration of about 0.002 mg per mL of dantrolene related compound B.

Test solution—Use the *Assay preparation.*

Chromatographic system—Proceed as directed in the *Assay.* Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections for dantrolene related compound B is not more than 5.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of dantrolene related compound B in the portion of Dantrolene Sodium for Injection taken by the formula:

$$100(r_U/r_S)(C_S/C_T)$$

in which r_U is the peak response for dantrolene related compound B obtained from the *Test solution;* r_S is the corresponding peak response in the *Standard solution;* C_S is the concentration, in mg per mL, of dantrolene related compound B in the *Standard solution;* and C_T is the concentration, in mg per mL, of dantrolene sodium hydrate in the *Test solution.* Not more than 8% of dantrolene related compound B is found.

Other requirements: meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

USP Monographs

www.webofpharma.com

Assay—

Buffer—Dissolve 3.3 g of ammonium acetate in 1 L of water, and adjust with acetic acid to a pH of 4.5 ± 0.1.

Solution A—Prepare a filtered and degassed mixture of *Buffer*, acetonitrile, and glacial acetic acid (120:80:7).

Solution B—Prepare a filtered and degassed mixture of acetonitrile and water (70:30).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B*, as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of acetonitrile and water (60:40).

Standard preparation—Transfer 40 mg of USP Dantrolene RS, accurately weighed, into a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume to obtain a solution having a known concentration of about 0.8 mg per mL. Dilute this solution with *Diluent* to obtain a solution having a known concentration of about 0.08 mg per mL of dantrolene.

Assay preparation—Using 70 mL of water for each vial, transfer the entire contents of the required number of vials to a suitable flask necessary to obtain a solution having a known concentration of about 0.1 mg of dantrolene sodium hydrate per mL. Sonicate for 2 to 5 minutes to dissolve the sample. Dilute with *Diluent* to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 365-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–8	100	0	isocratic
8–8.1	100→0	0→100	linear gradient
8.1–13	0	100	isocratic
13–13.1	0→100	100→0	linear gradient
13.1–20	100	0	re-equilibration

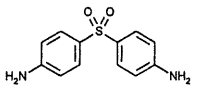
Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections for dantrolene is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for dantrolene. Calculate the percentage of $C_{14}H_9N_4NaO_5 \cdot 3½H_2O$ in the portion of Dantrolene Sodium for Injection taken by the formula:

$$(399.29/314.25)(r_U/r_S)(C_S/C_U)$$

in which 399.29 and 314.25 are the molecular weights of dantrolene sodium hydrate and dantrolene, respectively; r_U and r_S are the peak responses for dantrolene obtained from the *Assay preparation* and the *Standard preparation*, respectively; C_S is the concentration, in mg per mL, of dantrolene in the *Standard preparation*; and C_U is the concentration, in mg per mL, of dantrolene sodium hydrate in the *Assay preparation*.

Dapsone



$C_{12}H_{12}N_2O_2S$ 248.30

Benzenamine, 4,4′-sulfonylbis-;
4,4′-Sulfonyldianiline [80-08-0].

DEFINITION

Dapsone contains NLT 98.0% and NMT 102.0% of dapsone ($C_{12}H_{12}N_2O_2S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Transfer 100 mL of isopropyl alcohol, 100 mL of acetonitrile, and 100 mL of ethyl acetate to a 1000-mL volumetric flask. Add hexane to volume without mixing, then mix, and allow the mixture to cool to room temperature.

Standard solution: 25 µg/mL of USP Dapsone RS in *Mobile phase*

Sample solution: 25 µg/mL of Dapsone in the *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; 10-µm diameter packing L3

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: Two times the retention time of Dapsone

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dapsone ($C_{12}H_{12}N_2O_2S$) in the portion of Dapsone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*

r_S = peak response of the *Standard solution*

C_S = concentration of USP Dapsone RS in the *Standard solution* (µg/mL)

C_U = concentration of Dapsone in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **SELENIUM** ⟨291⟩

Sample: 100-mg sample mixed with 100 mg of magnesium oxide

Acceptance criteria: NMT 30 ppm

USP Monographs

www.webofpharma.com

• **ORGANIC IMPURITIES**
Standard solution A: 12.5 mg/mL of USP Dapsone RS in methanol
Standard solution B: 62.5 µg/mL of USP Dapsone RS in methanol from *Standard solution A*
Sample solution: 12.5 mg/mL of Dapsone in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
Mode: TLC
Adsorbent: 150- to 200-µm layer of chromatographic silica gel
Application volume: 4 µL
Developing solvent system: Acetone, chloroform, *n*-butyl alcohol, and formic acid (15:60:15:10). Prepare the solvent system fresh daily. Equilibrate the chromatographic chamber with the solvent system for 30 min prior to developing the chromatographic plate.
Spray reagent: 0.1% (w/v) solution of 4-dimethylaminocinnamaldehyde in glacial acetic acid and water (1:1)
Analysis
Samples: *Standard solution A* and *Standard solution B* and *Sample solution*
Position the plate in a chromatographic chamber, and develop the chromatograms in the developing solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber and air-dry. Spray the plate lightly with *Spray reagent.* Examine the spots that are developed immediately, and compare the intensities of any secondary spots observed in the *Sample solution* with those of the principal spots of the *Standard solutions.*
Acceptance criteria: No secondary spot from the chromatogram of the *Sample solution* is larger or more intense than the principal spot of *Standard solution B* (0.5%), and the sum of the intensities of all the secondary spots of the *Sample solution* corresponds to NMT 1.0%.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dapsone RS

Dapsone Compounded Oral Suspension

DEFINITION
Dapsone Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of dapsone ($C_{12}H_{12}N_2O_2S$).
Prepare Dapsone Compounded Oral Suspension 2 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Dapsone tablets[a] equivalent to	200 mg of dapsone
Vehicle: a 1:1 mixture of Ora-Sweet[b] and Ora-Plus[b], a sufficient quantity to make	100 mL

[a] Dapsone 25-mg tablets, Jacobus Pharmaceutical Company, Princeton, NJ.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Dapsone tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a dapsone liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY
• **PROCEDURE**
Solution A: 50 mM ammonium phosphate adjusted to a pH of 4.6
Mobile phase: Acetonitrile and *Solution A* (12:88). Filter and degas.
Internal standard solution: 1.0 mg/mL of diazoxide in methanol
Standard stock solution: 2.0 mg/mL of USP Dapsone RS in methanol
Standard solution: Pipet 2.5 mL of *Standard stock solution* into a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution,* and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 50 µg/mL of dapsone and 50 µg/mL of diazoxide. Centrifuge.
Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 2.5 mL of Oral Suspension into a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution,* and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 50 µg/mL of dapsone and 50 µg/mL of diazoxide. Centrifuge.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 295 nm
Column: 3.0-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 0.7 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention times for dapsone and diazoxide are about 8.9 and 12.9 min, respectively.]
Suitability requirements
Relative standard deviation: NMT 2.3% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dapsone ($C_{12}H_{12}N_2O_2S$) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of dapsone to the internal standard from the *Sample solution*
R_S = peak response ratio of dapsone to the internal standard from the *Standard solution*
C_S = concentration of USP Dapsone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dapsone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 3.8–4.8

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.

www.webofpharma.com

- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator or at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date.*
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dapsone RS

Dapsone Tablets

DEFINITION

Dapsone Tablets contain NLT 92.5% and NMT 107.5% of the labeled amount of dapsone ($C_{12}H_{12}N_2O_2S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample solution: Transfer a quantity of finely powdered Tablets, equivalent to 100 mg of dapsone, to a suitable container, add 5 mL of acetone, shake for 5 min, filter, and evaporate the filtrate to dryness. Dry this residue at 105° for 1 h.
  Acceptance criteria: Meets the requirements
- **B. ULTRAVIOLET ABSORPTION** ⟨197U⟩
  Sample solution: Triturate a quantity of finely powdered Tablets, equivalent to 100 mg of dapsone, with 50 mL of methanol, and filter. Dilute a portion of the filtrate with methanol to make approximately a 1 in 200,000 solution.
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
  Mobile phase: Transfer 100 mL of isopropyl alcohol, 100 mL of acetonitrile, and 100 mL of ethyl acetate to a 1000-mL volumetric flask. Add hexane to volume without mixing, then mix, and allow the mixture to cool to room temperature.
  Standard solution: 25 µg/mL of USP Dapsone RS in *Mobile phase*
  Sample solution: Nominally 25 µg/mL of dapsone prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to 50 mg of dapsone, to a 200-mL volumetric flask. Add 150 mL of methanol, and place the flask in an ultrasonic bath at a temperature of 35° for 15 min, with occasional shaking. Allow to cool to room temperature, and add methanol to volume. Centrifuge a portion of the mixture until clear. Transfer 5.0 mL of the clear supernatant to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4-mm × 30-cm; 10-µm diameter, packing L3
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution* (chromatograph a sufficient number)
  Suitability requirements
  Relative standard deviation: NMT 2%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of dapsone ($C_{12}H_{12}N_2O_2S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dapsone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dapsone in the *Sample solution* (µg/mL)

Acceptance criteria: 92.5%–107.5%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Medium: Dilute hydrochloric acid (2 in 100); 1000 mL
  Apparatus 1: 100 rpm
  Time: 60 min
  Standard solution: USP Dapsone RS of a known concentration in *Medium*
  Sample solution: Withdraw and filter a portion of the *Sample solution*. Transfer a portion of the filtrate estimated to contain 0.2 mg of dapsone to a 25-mL volumetric flask, add 5 mL of 1 N sodium hydroxide, and dilute with water to volume.
  Instrumental conditions
  Mode: UV
  Analytical wavelength: 290 nm
  Tolerances: NLT 75% (*Q*) of the labeled amount of dapsone ($C_{12}H_{12}N_2O_2S$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  Procedure for content uniformity
  Standard solution: 8 µg/mL of USP Dapsone RS in methanol
  Sample solution: Nominally 8 µg/mL of dapsone prepared as follows. To 1 Tablet in a 100-mL volumetric flask add 2.0 mL of water, and allow to stand for 30 min, swirling occasionally. Add 70 mL of methanol, and place the flask in an ultrasonic bath until the specimen is completely dispersed. Add methanol to volume, and centrifuge a portion of the mixture. Quantitatively dilute a measured volume of the clear supernatant with methanol.
  Instrumental conditions
  Mode: UV
  Analytical wavelength: 296 nm
  Cell: 1 cm
  Blank: Methanol
  Analysis
  Samples: *Standard solution* and *Sample solution*.
  Calculate the percentage of the labeled amount of dapsone ($C_{12}H_{12}N_2O_2S$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Dapsone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dapsone in the *Sample solution* (µg/mL)

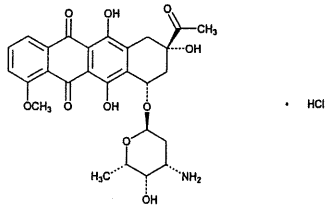
Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dapsone RS

www.webofpharma.com

Daunorubicin Hydrochloride



$C_{27}H_{29}NO_{10} \cdot HCl$ 563.98

5,12-Naphthacenedione, 8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-, (8*S*-*cis*)-, hydrochloride.

(1*S*,3*S*)-3-Acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthacenyl 3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside hydrochloride [23541-50-6].

» Daunorubicin Hydrochloride has a potency equivalent to not less than 842 µg and not more than 1030 µg of $C_{27}H_{29}NO_{10}$ per mg. [**CAUTION**—Great care should be taken to prevent inhaling particles of Daunorubicin Hydrochloride and exposing the skin to it.]

Packaging and storage—Preserve in tight containers, protected from light and excessive heat.

USP Reference standards ⟨11⟩—
USP Daunorubicin Hydrochloride RS

Identification—

A: The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Daunorubicin Hydrochloride RS.

B: The retention time of the main peak obtained with the *Assay preparation* corresponds to that obtained with the *Standard preparation* as directed in the *Assay*.

Crystallinity ⟨695⟩: meets the requirements.

pH ⟨791⟩: between 4.5 and 6.5, in a solution containing 5 mg per mL.

Water Determination, *Method I* ⟨921⟩: not more than 3.0%.

Assay—

Mobile phase—Mix 62 volumes of water and 38 volumes of acetonitrile, and adjust with phosphoric acid to a pH of 2.2 ± 0.2. The acetonitrile concentration may be varied to meet system suitability requirements and to provide a suitable elution time for daunorubicin. Filter the solution through a membrane filter (1 µm or finer porosity), and degas.

Standard preparation—Dissolve an accurately weighed quantity of USP Daunorubicin Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 250 µg of daunorubicin per mL.

Resolution solution—Prepare a solution of doxorubicin hydrochloride in the *Standard preparation* containing about 250 µg per mL.

Assay preparation—Transfer about 25 mg of Daunorubicin Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The chromatograph is equipped with a 254-nm detector and a 4.6-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for doxorubicin and 1.0 for daunorubicin; and the resolution, *R*, between the doxorubicin peak and the daunorubicin peak is not less than 3. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the potency, in µg of $C_{27}H_{29}NO_{10}$ per mg, taken by the formula:

$$100(C/W)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of daunorubicin in the *Standard preparation; W* is the weight, in mg, of Daunorubicin Hydrochloride taken; and r_U and r_S are the daunorubicin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Daunorubicin Hydrochloride for Injection

» Daunorubicin Hydrochloride for Injection is a sterile mixture of Daunorubicin Hydrochloride and Mannitol. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{27}H_{29}NO_{10}$.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*; protect from light.

USP Reference standards ⟨11⟩—
USP Daunorubicin Hydrochloride RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

Identification—The retention time of the main peak obtained with the *Assay preparation* corresponds to that obtained with the *Standard preparation* as directed in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 4.3 USP Endotoxin Units per mg of daunorubicin.

pH ⟨791⟩: between 4.5 and 6.5, in the solution constituted as directed in the labeling.

Water Determination, *Method I* ⟨921⟩: not more than 3.0%, the *Test Preparation* being prepared as directed for a hygroscopic specimen.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Daunorubicin Hydrochloride*.

Assay preparation—Transfer the contents of 1 vial of Daunorubicin Hydrochloride for Injection with the aid of *Mobile phase* to an appropriate volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution containing about 0.25 mg of daunorubicin per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Daunorubicin Hydrochloride*. Calculate the quantity,

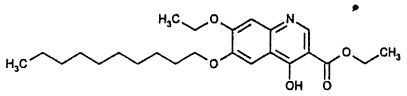
www.webofpharma.com

in mg, of $C_{27}H_{29}NO_{10}$ in the vial of Daunorubicin Hydrochloride for Injection taken by the formula:

$$(CV/1000)(r_U/r_S)$$

in which V is the volume, in mL, of the *Assay preparation*, and the other terms are as defined therein.

Decoquinate



$C_{24}H_{35}NO_5$ 417.54

3-Quinolinecarboxylic acid, 6-(decyloxy)-7-ethoxy-4-hydroxy-, ethyl ester.

Ethyl 6-(decyloxy)-7-ethoxy-4-hydroxy-3-quinolinecarboxylate [18507-89-6].

» Decoquinate contains not less than 99.0 percent and not more than 101.0 percent of $C_{24}H_{35}NO_5$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Decoquinate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Transfer about 40 mg of it, accurately weighed, to a 100-mL volumetric flask, add 10 mL of hot chloroform, swirl to dissolve, and while still warm add about 60 mL of dehydrated alcohol. Allow to cool, dilute with dehydrated alcohol to volume, and mix. Promptly transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with dehydrated alcohol to volume, and mix. Transfer 10.0 mL of this solution to a third 100-mL volumetric flask, add 10 mL of 0.1 N hydrochloric acid, dilute with dehydrated alcohol to volume, and mix: the UV absorption spectrum of this solution exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Decoquinate RS, concurrently measured, and the respective absorptivities, calculated on the dried basis, at the wavelength of maximum absorption at about 265 nm do not differ by more than 2.5%.

Loss on drying ⟨731⟩—Dry it at 105° to constant weight: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Ordinary impurities ⟨466⟩—

Test solution: chloroform, prepared with the aid of heat.

Standard solution: chloroform, using dilutions of the *Test solution*.

Eluant: a mixture of chloroform, dehydrated alcohol, and anhydrous formic acid (85:10:5).

Visualization: 1.

Tolerances: no impurity exceeds 1%, and the total does not exceed 2%.

Assay—Dissolve about 1000 mg of Decoquinate, accurately weighed, in 100 mL of glacial acetic acid, with the aid of gentle heat. Allow to cool, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 41.76 mg of $C_{24}H_{35}NO_5$.

Decoquinate Type A Medicated Article

(Title for this monograph not to change until December 1, 2017)

(Prior to December 1, 2017, the current practice of labeling the article of commerce with the name Decoquinate Premix may be continued. Use of the name Decoquinate Type A Medicated Article will be permitted as of June 1, 2017; however, the use of this name will not be mandatory until December 1, 2017.)

DEFINITION

Decoquinate Type A Medicated Article contains NLT 90.0% and NMT 110.0% of the labeled amount of decoquinate ($C_{24}H_{35}NO_5$), the labeled amount being between 1 g/100 g and 10 g/100 g of Decoquinate Type A Medicated Article.

IDENTIFICATION

• **A.**

Standard solution: 2.5 mg/mL of USP Decoquinate RS in chloroform

Sample solution: To a weighed quantity of sample, equivalent to 100 mg of decoquinate, add 40 mL of chloroform, and heat under a reflux condenser on a water bath for 20 min, cool, and filter. Use the filtrate as the *Sample solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Chloroform and alcohol (70:30)

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Standard solution* and *Sample solution* to the starting line of the chromatographic plate. Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and allow to dry in a current of air. Locate the spots under short-wavelength UV light.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Standard solution: 0.005 mg/mL of USP Decoquinate RS prepared as follows. Transfer 50 mg of USP Decoquinate RS to a 100-mL volumetric flask, add 10 mL of hot chloroform, swirl to dissolve, and while still warm, slowly add 70 mL of dehydrated alcohol. Allow to cool, and dilute with dehydrated alcohol to volume. Immediately transfer 10.0 mL of this solution to a second 100-mL volumetric flask, and dilute with dehydrated alcohol to volume. Transfer 10.0 mL of this solution to a third 100-mL

www.webofpharma.com

volumetric flask, add 10 mL of 0.1 N hydrochloric acid, and dilute with dehydrated alcohol to volume.

Sample solution: Extract an accurately weighed quantity of Decoquinate Type A Medicated Article, equivalent to 200 mg of decoquinate, with 50 mL of chloroform in a small continuous extraction apparatus for eight reflux cycles. Transfer the extract to a 100-mL volumetric flask with the aid of chloroform, cool, and dilute with chloroform to volume. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, and dilute with dehydrated alcohol to volume. Transfer 5.0 mL of this solution to a third 100-mL volumetric flask, add 10 mL of 0.1 N hydrochloric acid, and dilute with dehydrated alcohol to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 265 nm

Blank: Chloroform, dehydrated alcohol, and 0.1 N hydrochloric acid (0.25: 90: 10)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of decoquinate ($C_{24}H_{35}NO_5$) in the portion of Decoquinate Type A Medicated Article taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

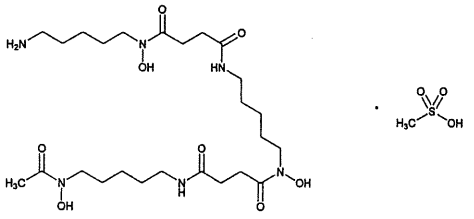
A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Decoquinate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of decoquinate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Decoquinate RS

Deferoxamine Mesylate



$C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$ 656.79

Butanediamide, *N'*-[5-[[4-[[5-(acetylhydroxyamino)pentyl]amino]-1,4-dioxobutyl]hydroxyamino]pentyl]-*N*-(5-aminopentyl)-*N*-hydroxy-, monomethanesulfonate;
N-[5-[3-[(5-Aminopentyl)hydroxycarbamoyl]propionamido]pentyl]-3-[[5-(*N*-hydroxyacetamido)pentyl]carbamoyl]propionohydroxamic acid monomethanesulfonate (salt) [138-14-7].

DEFINITION

Deferoxamine Mesylate contains NLT 93.0% and NMT 102.0% of deferoxamine mesylate ($C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 1.32 g/L of dibasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile and *Solution A* (1:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	88	12
20	80	20
35	57.5	42.5
35.1	88	12
40	88	12

Diluent: Acetonitrile and water (6:94)

Standard solution: 1.0 mg/mL of USP Deferoxamine Mesylate RS in *Diluent*

Sample solution: 1.0 mg/mL of Deferoxamine Mesylate in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1

Temperatures

Column: 32°

Autosampler: 5°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of deferoxamine mesylate ($C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$) in the portion of Deferoxamine Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of deferoxamine from the *Sample solution*
r_S = peak response of deferoxamine from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–102.0% on the anhydrous basis

USP Monographs

www.webofpharma.com

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%, 2.0 g being used
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩: NMT 0.012%; a 1.2-g portion shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid.
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩: NMT 0.04%; a 0.5-g portion shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid.
- **ORGANIC IMPURITIES**

Solution A, Solution B, Diluent, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: Use the *Standard solution*, prepared as directed in the *Assay*. [NOTE—USP Deferoxamine Mesylate RS contains impurity A as a minor component.]

Standard solution: 0.01 mg/mL of USP Deferoxamine Mesylate RS in *Diluent* from the *Standard stock solution*

System suitability

Samples: *Standard stock solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the impurity A and deferoxamine peaks, *Standard stock solution*

Relative standard deviation: NMT 5.0% for the deferoxamine peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Deferoxamine Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of deferoxamine from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

[NOTE—The reporting level for impurities is 0.04%.]

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Impurity A[a, b]	0.85–0.87	3.0[c]
Deferoxamine	1.0	—
Any unspecified impurity	—	1.0
Total impurities eluting before deferoxamine	—	5.0
Total impurities eluting after deferoxamine	—	2.0

[a] Des-methylene impurity (desferrioxamine A_1 and/or other desferrioxamines).

[b] All des-methylene impurities that elute in the 0.85–0.87 range should be treated as a single impurity. Where the cluster of unresolved peaks in this range is present, it should be integrated together as one peak to determine compliance.

[c] The acceptance criterion of NMT 3.0% applies to the sum of the peaks in the specified range.

SPECIFIC TESTS

- **PH** ⟨791⟩: 4.0–6.0, in a solution (1 in 100)
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 2.0%
- **STERILITY TESTS** ⟨71⟩: Where the label states that Deferoxamine Mesylate is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Deferoxamine Mesylate is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.33 USP Endotoxin Unit/mg of deferoxamine mesylate.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Deferoxamine Mesylate RS

Deferoxamine Mesylate for Injection

DEFINITION

Deferoxamine Mesylate for Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of deferoxamine mesylate ($C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$).

IDENTIFICATION

- **A.**

Sample: 5 mg of Deferoxamine Mesylate for Injection

Analysis: Dissolve the *Sample* in 5 mL of water, add 2 mL of tribasic sodium phosphate solution (1 in 200), mix, then add 10 drops of β-naphthoquinone-4-sodium sulfonate solution (1 in 40).

Acceptance criteria: A blackish brown color is produced.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 6.7 g of ferric chloride in dilute hydrochloric acid (1 in 100) in a 100-mL volumetric flask. Add dilute hydrochloric acid (1 in 100) to volume.

Standard solution: 1 mg/mL of USP Deferoxamine Mesylate RS

Sample solution: Nominally 1 mg/mL, pepared as follows. Constitute the contents of 1 vial in water, and dilute with water to volume.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Vis

Analytical wavelength: 485 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Standard solution, Sample solution,* and *Blank*

Pipet 2 mL each of the *Standard solution, Sample solution,* and *Blank* into separate 25-mL volumetric flasks. To each flask add 3 mL of *Solution A*, and dilute with water to volume. Concomitantly determine the absorbances.

Calculate the percentage of the labeled amount of deferoxamine mesylate ($C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$) in the portion of Deferoxamine Mesylate for Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Deferoxamine Mesylate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of deferoxamine mesylate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

USP Monographs

www.webofpharma.com

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

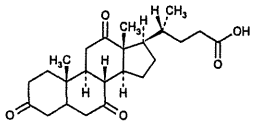
SPECIFIC TESTS
- **PH** ⟨791⟩: 4.0–6.0, in a solution (1 in 100)
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.5%
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.33 USP Endotoxin Unit/mg of deferoxamine mesylate
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Deferoxamine Mesylate RS

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 231°–242°, but the range between beginning and end of melting does not exceed 3°.
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩: +29.0° to +32.5°
  Sample solution: 20 mg/mL in dioxane
- **LOSS ON DRYING** ⟨731⟩: Dry at 105° for 2 h: it loses NMT 1.0% of its weight.
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the test for absence of *Salmonella* species.
- **ODOR ON BOILING:** Boil 2 g with 100 mL of water for 2 min: the mixture is odorless.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dehydrocholic Acid RS

Dehydrocholic Acid



$C_{24}H_{34}O_5$ 402.52
Cholan-24-oic acid, 3,7,12-trioxo-, (5β)-;
3,7,12-Trioxo-5β-cholan-24-oic acid [81-23-2].

DEFINITION
Dehydrocholic Acid contains NLT 98.5% and NMT 101.0% of $C_{24}H_{34}O_5$, calculated on the dried basis. Dehydrocholic Acid for parenteral use melts between 237° and 242°.

IDENTIFICATION

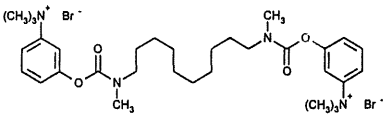
Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

ASSAY
- **PROCEDURE**
  Sample solution: Transfer 500 mg of Dehydrocholic Acid to a 300-mL conical flask, add 60 mL of neutralized alcohol, and warm on a steam bath until dissolved. Allow to cool.
  Analysis: Add phenolphthalein TS and 20 mL of water to the *Sample solution*. Titrate with 0.1 N sodium hydroxide VS, adding 100 mL of water shortly before the endpoint is reached. Each mL of 0.1 N sodium hydroxide is equivalent to 40.25 mg of $C_{24}H_{34}O_5$.
  Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%
- **BARIUM**
  Sample: 2 g
  Analysis: Boil the *Sample* with 100 mL of water for 2 min. Add 2 mL of hydrochloric acid, and again boil for 2 min. Cool, filter, and wash the filter with water until the filtrate measures 100 mL. To 10 mL of the filtrate add 1 mL of 2 N sulfuric acid.
  Acceptance criteria: No turbidity is produced.

Demecarium Bromide



$C_{32}H_{52}Br_2N_4O_4$ 716.59
Benzenaminium, 3,3′-[1,10-decanediylbis[(methylimino) carbonyloxy]]bis[*N,N,N*-trimethyl]-, dibromide.
(*m*-Hydroxyphenyl)trimethylammonium bromide decamethylenebis[methylcarbamate] (2:1) [56-94-0].

» Demecarium Bromide contains not less than 95.0 percent and not more than 100.5 percent of $C_{32}H_{52}Br_2N_4O_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Demecarium Bromide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K▲* (CN 1-May-2020).

B: Dissolve about 100 mg in 50 mL of 1 N sodium hydroxide, and reflux for 15 minutes. Cool, and add 3 mL of the refluxed solution to 25 mL of saturated sodium bicarbonate solution. Add, with mixing, 4 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride solution (1.5 in 10,000) and 2 mL of sodium hypochlorite solution (1.5 in 20,000): a violet-blue color is produced.

C: Dissolve about 50 mg in 20 mL of water, add 10 mL of a 1 in 50 solution of ammonium reineckate in methanol, and allow to stand for 30 minutes with occasional swirling: a pink reineckate of demecarium forms, and it melts between 131° and 136°, with decomposition.

D: A solution of it responds to the tests for *Bromide* ⟨191⟩.
pH ⟨791⟩: between 5.0 and 7.0, in a solution (1 in 100).
Water Determination, *Method I* ⟨921⟩: not more than 2.0%.
Residue on ignition ⟨281⟩: not more than 0.1%.

www.webofpharma.com

Limit of *m*-trimethylammoniophenol bromide—
Control solution—Dissolve 100 mg of *m*-dimethylaminophenol in 10 mL of alcohol in a 1000-mL volumetric flask, dilute with water to volume, and mix. Pipet 1 mL of this solution into a 500-mL volumetric flask, dilute with water to volume, and mix.
Test solution—Transfer 100 mg of Demecarium Bromide to a 100-mL volumetric flask, add water to volume, and mix.
Procedure—Pipet 25 mL of the *Test solution* into a glass-stoppered, 50-mL centrifuge tube, and pipet 25 mL of the *Control solution* into a second, similar tube. To each tube add 3 mL of pH 7.0 phosphate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*), 1 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride solution (1.5 in 10,000), 5 mL of isobutyl alcohol, and 1 mL of sodium hypochlorite solution (1.5 in 20,000). Insert the stoppers in the tubes, shake the mixtures for 5 minutes, and centrifuge: any blue color produced in the upper layer obtained from the *Test solution* is not more intense than that obtained from the *Control solution*.
Assay—Dissolve about 0.8 g of Demecarium Bromide, accurately weighed, in a mixture of 75 mL of glacial acetic acid and 15 mL of mercuric acetate TS, warming slightly, if necessary, to effect solution. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 35.83 mg of $C_{32}H_{52}Br_2N_4O_4$.

Demecarium Bromide Ophthalmic Solution

» Demecarium Bromide Ophthalmic Solution is a sterile, aqueous solution of Demecarium Bromide. It contains not less than 92.0 percent and not more than 108.0 percent of the labeled amount of $C_{32}H_{52}Br_2N_4O_4$. It contains a suitable antimicrobial agent.

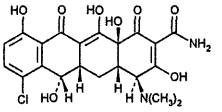
Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Demecarium Bromide RS

Identification—
A: Mix about 10 mL with 12.5 mL of 1 N sodium hydroxide, and proceed as directed in *Identification*— test *Identification*— under *Demecarium Bromide*, beginning with "reflux for 15 minutes": a violet-blue color is produced.
B: To 10 mL add 5 mL of a 1 in 50 solution of ammonium reineckate in methanol, and allow to stand for 30 minutes with occasional swirling: a pink reineckate of demecarium forms, and it melts between 131° and 136°, with decomposition.
C: It responds to the tests for *Bromide* ⟨191⟩.
Sterility Tests ⟨71⟩: meets the requirements.
Assay—Into each of two 50-mL volumetric flasks marked *1* and *2* pipet a volume of Ophthalmic Solution, equivalent to about 2.5 mg of demecarium bromide. Into each of two additional 50-mL volumetric flasks, marked *3* and *4*, pipet 5.0 mL of a freshly prepared Standard solution made by dissolving about 50 mg of USP Demecarium Bromide RS, accurately weighed, in 100.0 mL of water. To flasks *1* and *3*, add 1 N sodium hydroxide to volume, and mix. Transfer 10.0 mL of each of these solutions to separate glass-stoppered tubes, insert the stoppers loosely, heat the tubes in a water bath for 15 minutes, then cool to room temperature. The concentration of USP Demecarium Bromide RS in the Standard solution is about 50 µg per mL. To flasks *2* and *4*, add pH 10.0 alkaline borate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*) to volume, and mix. Concomitantly determine the absorbances of the two sodium hydroxide solutions at the wavelength of maximum absorbance at about 292 nm, with a suitable spectrophotometer, using the corresponding borate buffer solutions as the respective solvent blanks. Calculate the quantity, in mg, of $C_{32}H_{52}Br_2N_4O_4$ in each mL of the Ophthalmic Solution taken by the formula:

$$(50C/V)(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Demecarium Bromide RS in the Standard solution; V is the volume, in mL, of Ophthalmic Solution taken; and A_U and A_S are the absorbances of the sodium hydroxide solution from the Ophthalmic Solution and the Standard solution, respectively.

Demeclocycline



$C_{21}H_{21}ClN_2O_8$ 464.85
2-Naphthacenecarboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-, [4*S*-(4α,4aα,5aα,6β,12aα)]-.
7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-2-naphthacenecarboxamide [127-33-3].
Sesquihydrate 491.88
[13215-10-6].

» Demeclocycline has a potency equivalent to not less than 970 µg of demeclocycline hydrochloride ($C_{21}H_{21}ClN_2O_8 \cdot HCl$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Demeclocycline Hydrochloride RS

Identification—
A: Transfer about 40 mg, accurately weighed, to a 250-mL volumetric flask, add 2 mL of 0.1 N hydrochloric acid to dissolve, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add about 75 mL of water and 5.0 mL of 5 N sodium hydroxide, dilute with water to volume, and mix: the UV absorption spectrum of this solution, measured at 6 minutes, accurately timed, after the addition of the sodium hydroxide, exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Demeclocycline Hydrochloride RS, concomitantly measured, and the absorptivity, calculated on the anhydrous basis, at the wavelength of maximum absorbance at about 380 nm is between 103.5% and 111.3% of that of the USP

www.webofpharma.com

Demeclocycline Hydrochloride RS, the potency of the Reference Standard being taken into account.

B: Transfer 40 mg, accurately weighed, to a 200-mL volumetric flask, add 100 mL of 0.1 N hydrochloric acid, shake to dissolve, dilute with 0.1 N hydrochloric acid to volume, and mix. Transfer 5.0 mL of this solution into each of two 50-mL volumetric flasks (*Solutions 1* and *2*). Prepare similar solutions of USP Demeclocycline Hydrochloride RS (*Solutions 3* and *4*). To *Solutions 1* and *3*, add 10 mL of 6 N hydrochloric acid, and to *Solutions 2* and *4*, add 10 mL of 3 N hydrochloric acid. Heat the four flasks in a water bath for 20 minutes, cool, dilute the contents with water to volume, and mix. Determine the absorbances of *Solutions 1* and *3* at the wavelength of maximum absorbance at about 430 nm, with a suitable spectrophotometer, using *Solutions 2* and *4*, respectively, as the blanks. Determine the absorbances of *Solutions 2* and *4* at the wavelength of maximum absorbance at about 368 nm, using *Solutions 1* and *3*, respectively, as the blanks. Calculate the ratio:

$$(W_S P/1000)(A_{368} + A_{430})_U / W_U(A_{368} + A_{430})_S$$

in which W_S is the weight, in mg, of USP Demeclocycline Hydrochloride RS taken, calculated on the dried basis; P is the potency, in µg per mg, of the USP Demeclocycline Hydrochloride RS; W_U is the weight, in mg, of specimen taken, calculated on the anhydrous basis; and the final two parenthetic expressions are the absorbances of the four solutions at the wavelengths indicated by the subscripts for the specimen (U) and the Standard (S): the ratio is between 0.97 and 1.17.

Crystallinity ⟨695⟩: meets the requirements.

pH ⟨791⟩: between 4.0 and 5.5, in a solution containing 10 mg per mL.

Water Determination, *Method I* ⟨921⟩: between 4.3% and 6.7%.

Assay—

Mobile phase—Transfer 80 g of tertiary butyl alcohol to a 1000-mL volumetric flask with the aid of 200 mL of water, add 100 mL of 0.2 M pH 9.0 phosphate buffer (prepared by mixing appropriate volumes of 0.2 M dibasic potassium phosphate and 0.2 M monobasic potassium phosphate), 150 mL of 0.02 M tetrabutylammonium hydrogen sulfate (adjusted with sodium hydroxide TS to a pH of 9.0), and 100 mL of 0.01 M edetate disodium (adjusted with sodium hydroxide TS to a pH of 9.0). Dilute with water to volume, mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). Decreasing the amount of tertiary butyl alcohol increases the resolution.

Standard preparation—Dissolve an accurately weighed quantity of USP Demeclocycline Hydrochloride RS in 0.01 N hydrochloric acid to obtain a solution having a known concentration of about 1 mg per mL.

Assay preparation—Transfer about 45 mg of Demeclocycline, accurately weighed, to a 50-mL volumetric flask, dissolve in 0.01 N hydrochloric acid, dilute with 0.01 N hydrochloric acid to volume, and mix.

Resolution solution—Prepare a solution of USP Demeclocycline Hydrochloride RS in 0.01 N hydrochloric acid, and allow to stand for 3 hours.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column containing packing L21 and maintained at 60 ± 0.5°. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure:* the relative retention times are about 0.7 for epidemethylchlortetracycline and 1.0 for demeclocycline; and the resolution between the epidemethylchlortetracycline peak and the demeclocycline peak is not less than 3.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

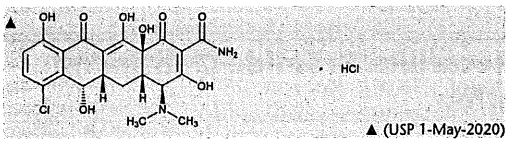
Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of demeclocycline hydrochloride ($C_{21}H_{21}ClN_2O_8 \cdot HCl$) equivalent in each mg of the Demeclocycline taken by the formula:

$$50(CE/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Demeclocycline Hydrochloride RS in the *Standard preparation*; E is the demeclocycline hydrochloride equivalent, in µg per mg, of USP Demeclocycline Hydrochloride RS; W is the quantity, in mg, of the Demeclocycline taken; and r_U and r_S are the demeclocycline peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Demeclocycline Hydrochloride

Change to read:



▲ (USP 1-May-2020)

$C_{21}H_{21}ClN_2O_8 \cdot HCl$ 501.31

2-Naphthacenecarboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-, monohydrochloride, 4*S*-(4α,4aα,5aα,6β,12aα)-;
7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [64-73-3].

DEFINITION

Demeclocycline Hydrochloride has a potency of NLT 900 µg/mg of demeclocycline hydrochloride ($C_{21}H_{21}ClN_2O_8 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* ⟨197K⟩ or ⟨197A⟩▲ (USP 1-May-2020)

Change to read:

- **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

ASSAY

Change to read:

- **PROCEDURE**

Solution A: Mix appropriate volumes of 0.2 M dibasic potassium phosphate and 0.2 M monobasic potassium phosphate to prepare a buffer with a pH of 9.0.

www.webofpharma.com

Solution B: 0.02 M tetrabutylammonium hydrogen sulfate. Adjust with sodium hydroxide TS to a pH of 9.0.
Solution C: 0.01 M edetate disodium. Adjust with sodium hydroxide TS to a pH of 9.0.
Mobile phase: Transfer 80 g of tertiary butyl alcohol to a 1000-mL volumetric flask with the aid of 200 mL of water. Add 100 mL of *Solution A*, 150 mL of *Solution B*, and 100 mL of *Solution C*. Dilute with water to volume.
▲ (USP 1-May-2020)
Diluent: 0.01 N hydrochloric acid
System suitability solution: ▲1 mg/mL of▲ (USP 1-May-2020) USP Demeclocycline Hydrochloride RS in *Diluent*. Allow to stand for 3 h.
Standard solution: 1 mg/mL of USP Demeclocycline Hydrochloride RS in *Diluent*
Sample solution: 1 mg/mL of Demeclocycline Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; ▲8-µm▲ (USP 1-May-2020) packing L21
Column temperature: 60 ± 0.5°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for epidemethylchlortetracycline and demeclocycline are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the epidemethylchlortetracycline peak and the demeclocycline peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of demeclocycline hydrochloride ($C_{21}H_{21}ClN_2O_8 \cdot HCl$) in the portion of Demeclocycline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response of demeclocycline from the *Sample solution*
r_S = peak response of demeclocycline from the *Standard solution*
C_S = concentration of USP Demeclocycline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Demeclocycline Hydrochloride in the *Sample solution* (mg/mL)
P = potency of demeclocycline hydrochloride in USP Demeclocycline Hydrochloride RS (µg/mg)

Acceptance criteria: NLT 900 µg/mg on the dried basis

SPECIFIC TESTS
• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
• **PH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 2.0–3.0
• **LOSS ON DRYING** ⟨731⟩
Sample: 100 mg
Analysis: Dry the *Sample* at 60° for 3 h.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Demeclocycline Hydrochloride RS

Demeclocycline Hydrochloride Tablets

DEFINITION
Demeclocycline Hydrochloride Tablets contain NLT 90.0% and NMT 125.0% of the labeled amount of demeclocycline hydrochloride ($C_{21}H_{21}ClN_2O_8 \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

ASSAY

Change to read:

• **PROCEDURE**
Solution A: Mix appropriate volumes of 0.2 M dibasic potassium phosphate and 0.2 M monobasic potassium phosphate to prepare a buffer with a pH of 9.0.
Solution B: 0.02 M tetrabutylammonium hydrogen sulfate. Adjust with sodium hydroxide TS to a pH of 9.0.
Solution C: 0.01 M edetate disodium. Adjust with sodium hydroxide TS to a pH of 9.0.
Mobile phase: Transfer 80 g of tertiary butyl alcohol to a 1000-mL volumetric flask with the aid of 200 mL of water. Add 100 mL of *Solution A*, 150 mL of *Solution B*, and 100 mL of *Solution C*. Dilute with water to volume, and degas.
Diluent: 0.01 N hydrochloric acid
System suitability solution: ▲1 mg/mL of▲ (USP 1-May-2020) USP Demeclocycline Hydrochloride RS in *Diluent*. Allow to stand for 3 h.
Standard solution: 1 mg/mL of USP Demeclocycline Hydrochloride RS in *Diluent*
Sample solution: Nominally 1 mg/mL of demeclocycline hydrochloride prepared as follows. Weigh and finely powder NLT 10 Tablets. Transfer a portion, containing nominally 50 mg of demeclocycline hydrochloride, to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Sonicate for 5 min, and centrifuge for 5 min. Pass a portion of the supernatant through a suitable filter of 1.5-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2020)
Column: 4.6-mm × 25-cm; 8-µm packing L21
Column temperature: 60 ± 0.5°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*

USP Monographs

www.webofpharma.com

[NOTE—The relative retention times for epidemethylchlortetracycline and demeclocycline are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the epidemethylchlortetracycline and demeclocycline peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of demeclocycline hydrochloride ($C_{21}H_{21}ClN_2O_8 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Demeclocycline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of demeclocycline hydrochloride in the *Sample solution* (mg/mL)
P = potency of demeclocycline in USP Demeclocycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–125.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 45 min

Standard solution: Prepare a solution with a known concentration of USP Demeclocycline Hydrochloride RS in *Medium*.

Sample solution: Sample per *Dissolution* ⟨711⟩. A filtered portion of the solution under test suitably diluted with *Medium*

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 274 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of demeclocycline ($C_{21}H_{21}ClN_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times P \times F \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Demeclocycline Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL
P = potency of demeclocycline in USP Demeclocycline Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Tolerances: NLT 75% (Q) of the labeled amount of demeclocycline ($C_{21}H_{21}ClN_2O_8$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. ▲Store at controlled room temperature.▲ (USP 1-May-2020)

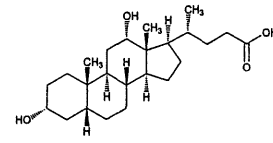
• **USP REFERENCE STANDARDS** ⟨11⟩

USP Demeclocycline Hydrochloride RS

Deoxycholic Acid

(Title for this monograph—to become official December 1, 2021)

(Prior to December 1, 2021, the current practice of labeling the article of commerce with the name Desoxycholic Acid may be continued. Use of the name Deoxycholic Acid will be permitted as of December 1, 2016; however, the use of this name will not be mandatory until December 1, 2021. The 60-month extension will provide the time needed by manufacturers and users to make necessary changes.)



$C_{24}H_{40}O_4$ 392.58

Cholan-24-oic acid, 3,12-dihydroxy-, (3α,5β,12α)-; 3α,12α-Dihydroxy-5β-cholan-24-oic acid [83-44-3].

DEFINITION

Deoxycholic Acid contains NLT 97.0% and NMT 103.0% of deoxycholic acid ($C_{24}H_{40}O_4$), calculated on the anhydrous basis. Deoxycholic Acid can be of animal or synthetic origin. FDA has not approved the use of animal-derived Deoxycholic Acid as a drug substance.

Change to read:

IDENTIFICATION

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

• **B.** The retention time of the deoxycholic acid peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Methanol and water (80:20)

Solution A: 0.1% formic acid in water

Solution B: 0.1% formic acid in acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	75.0	25.0
2.0	55.0	45.0
14.0	42.0	58.0
24.0	0.0	100.0
35.0	0.0	100.0

USP Monographs

www.webofpharma.com

Standard solution: 0.01 mg/mL of USP Deoxycholic Acid RS in *Diluent*
Sample solution: 0.01 mg/mL of Deoxycholic Acid in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Charged aerosol
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 25 µL
Run time: 35 min
System suitability
Sample: *Standard solution*
[NOTE—The retention time of deoxycholic acid is about 13.0 min.]
Suitability requirements
Relative standard deviation: NMT 3.0% for six injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of deoxycholic acid ($C_{24}H_{40}O_4$) in the portion of Deoxycholic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area of deoxycholic acid from the *Sample solution*
r_S = peak area of deoxycholic acid from the *Standard solution*
C_S = concentration of the USP Deoxycholic Acid RS in the *Standard solution*
C_U = concentration of Deoxycholic Acid in the *Sample solution*
P = labeled purity of USP Deoxycholic Acid RS (%)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Sample: 1.0 g
Acceptance criteria: NMT 0.2%

• **LIMIT OF LEAD**
[NOTE—This test and *Acceptance criteria* are only applicable to Deoxycholic Acid of animal origin. Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 50% nitric acid followed by water.]
Standard solutions: [NOTE—Prepare these solutions on the day of use.] Transfer 10.0 and 50.0 mL of standard lead solution TS into two separate 100-mL volumetric flasks, add 10 mL of 3 N hydrochloric acid to each, and dilute with water to volume. The third standard, 10.0 µg/mL, is taken directly from standard lead solution TS.
Sample solution: Transfer 10.0 g of Deoxycholic Acid, weighed to the nearest 0.1 mg, into an evaporating dish. Add 5 mL of 25% sulfuric acid (made by adding 25 mL of sulfuric acid to 75 mL of water), and distribute the 25% sulfuric acid solution uniformly. Within a hood, place the dish on a steam bath to evaporate most of the water. Place the dish on a burner, and slowly pre-ash the remaining *Sample solution* by expelling most of the sulfuric acid. Place the dish in a muffle furnace that has been set at 525°, and ash the contents of the dish until the residue appears free from carbon. Cool, and cautiously wash down the inside of the evaporation dish with water. Add 5 mL of 1 N hydrochloric acid. Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 N hydrochloric acid and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer to a 10-mL volumetric flask, dilute with water to volume, and mix.
Sample blank: Prepare by ashing 5 mL of 25% sulfuric acid solution. Cool, and cautiously wash down the inside of the evaporation dish with water. Add 5 mL of 1 N hydrochloric acid. Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 N hydrochloric acid and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer to a 10-mL volumetric flask, dilute with water to volume, and mix.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption
Analytical wavelength: 283.3 nm
Lamp: Lead electrodeless discharge
Flame: Air–acetylene
Slit width: 0.7 nm
Instrument blank: Water
Standard curve
Samples: *Standard solutions* and *Sample blank*
Plot: Corrected absorbance values versus their corresponding concentration (µg/mL). [NOTE—Determine corrected absorbance values by subtracting the absorbance of the *Sample blank* from the absorbance of the *Standard solutions*.]
Analysis
Samples: *Sample solution* and *Sample blank*
[NOTE—Determine corrected absorbance values by subtracting the absorbance of the *Sample blank* from the absorbance of the *Sample solution*.]
From the *Standard curve*, determine the lead concentration in the *Sample solution*.
Calculate the lead content in the portion of Deoxycholic Acid taken:

$$\text{Result} = (C_S \times V)/W$$

C_S = concentration of lead from the *Standard curve* (µg/mL)
V = final volume of the sample (mL)
W = weight of the sample taken (g)

Acceptance criteria: NMT 4 µg/g

• **ORGANIC IMPURITIES**
Diluent, Mobile phase, Standard solution, and **Chromatographic system:** Proceed as described in the procedure for *Assay*.
Sensitivity solution: 0.5 µg/mL of USP Deoxycholic Acid RS in *Diluent*
Cholic acid solution: 0.01 mg/mL of USP Cholic Acid RS in *Diluent*
Sample stock solution: 1.0 mg/mL of Deoxycholic Acid in *Diluent*
Sample solution: 0.01 mg/mL of Deoxycholic Acid in *Diluent* prepared from the *Sample stock solution*
System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—The retention time of deoxycholic acid is about 13.0 min. The relative retention times for cholic acid and deoxycholic acid are 0.56 and 1, respectively. The relative retention times for other impurities are listed in *Table 2*.]
Suitability requirements
Relative standard deviation: NMT 3.0% for six injections, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

www.webofpharma.com

Analysis
Samples: *Cholic acid solution*, *Sample solution*, and *Sample stock solution*
Calculate the percentage of each impurity in the portion of Deoxycholic Acid taken:

$$\text{Result} = [r_U/(r_S \times 100 + r_T)] \times 100$$

r_U = peak area of individual impurity from the *Sample stock solution*
r_S = peak area of deoxycholic acid from the *Sample solution*
r_T = sum of peak areas of all impurities from the *Sample stock solution*

Acceptance criteria
Deoxycholic Acid of animal origin
Cholic acid: NMT 1.0%
Total impurities: NMT 2.0%
Deoxycholic Acid of synthetic origin: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria NMT (%)
3α,12β-Dihydroxy-5β-cholan-24-oic acid	0.69	0.15
3α,12α-Dihydroxy-5β-chol-9(11)-en-24-oic acid	0.87	0.15
Ethyl 3α,12α-dihydroxy-5β-cholan-24-oate	1.61	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

SPECIFIC TESTS

• **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 1% (w/v) solution in ethanol
Acceptance criteria
Deoxycholic Acid of animal origin: NLT +55°
Deoxycholic Acid of synthetic origin: +50° to +60°

• **Water Determination** ⟨921⟩, *Method I, Method Ic*: NMT 1.0%

• **Clarity of Solution**
[Note—If intended for use in preparing parenteral dosage forms, it meets the requirements of the *Clarity of Solution* test.]
Hydrazine solution: 10 mg/mL of hydrazine sulfate. Allow to stand 4–6 h before use.
Methenamine solution: Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.
Primary opalescent suspension: To the flask containing *Methenamine solution* add 25.0 mL of the *Hydrazine solution*, mix, and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.
Opalescence standard: Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. Use this suspension within 24 h after preparation.
Reference suspension: Transfer 5.0 mL of the *Opalescence standard* to a 100-mL volumetric flask, and dilute with water to volume.
Sample solution: 5 mg/mL of Deoxycholic Acid in 0.1 N sodium hydroxide
Analysis: Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer a portion of the *Reference suspension* to a separate matching test tube. Compare the *Sample solution* and the *Reference suspension* in diffused daylight, viewing vertically against a black background (see *Visual Comparison* ⟨630⟩) for 5 min after preparation of the *Reference suspension*.
Acceptance criteria: The *Sample solution* is not more opalescent than the *Reference suspension*.

• **Color of Solution**
[Note—If intended for use in preparing parenteral dosage forms, it meets the requirements of the *Color of Solution* test.]
Diluent: 27.5 mL of hydrochloric acid in 1000 mL of water
Standard stock solutions: Prepare two solutions, A and B, containing, respectively, the following parts of ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and *Diluent*.
Standard stock solution A: 2.4: 0.6: 0: 7.0
Standard stock solution B: 2.4: 1.0: 0.4: 6.2
Standard solutions
[Note—Prepare and use these solutions immediately.]
Standard solution A: Transfer 2.5 mL of *Standard stock solution A* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.
Standard solution B: Transfer 2.5 mL of *Standard stock solution B* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.
Sample solution: 5 mg/mL of Deoxycholic Acid in 0.1 N sodium hydroxide
Analysis: Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm, to obtain a depth of 40 mm. Similarly transfer portions of *Standard solution A* and *Standard solution B* to separate matching test tubes. Compare the color of the *Sample solution* with that of *Standard solution A* and *Standard solution B* in diffused daylight, viewing vertically against a white background (see *Visual Comparison* ⟨630⟩).
Acceptance criteria: The *Sample solution* is not more intensely colored than *Standard solution A* and *Standard solution B*.

• **Bacterial Endotoxins Test** ⟨85⟩: If labeled for use in preparing parenteral dosage forms, it meets the requirements of NMT 240 Endotoxin Units/g.

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: If labeled for use in preparing parenteral dosage forms, the total aerobic microbial count does not exceed 100 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. No storage requirements specified.

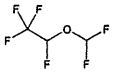
• **Labeling:** Label it to indicate whether Deoxycholic Acid is derived from an animal or synthetic source. Deoxycholic Acid intended for use in preparing parenteral dosage forms is so labeled.

• **USP Reference Standards** ⟨11⟩
USP Cholic Acid RS
USP Deoxycholic Acid RS
USP Endotoxin RS

USP Monographs

www.webofpharma.com

Desflurane



$C_3H_2F_6O$ 168.04

Ethane, 2-(difluoromethoxy)-1,1,1,2-tetrafluoro-, (±)-; (±)-2-Difluoromethyl 1,2,2,2-tetrafluoroethyl ether [57041-67-5].

DEFINITION

Desflurane contains NLT 99.7% and NMT 100.0% of $C_3H_2F_6O$.

IDENTIFICATION

- The IR absorption spectrum of Desflurane obtained using a gas cell exhibits maxima only at the same wavelengths as that of a similar preparation of USP Desflurane RS.

ASSAY

- **PROCEDURE**

Using the results from the *Organic Impurities* procedure, calculate the percentage of $C_3H_2F_6O$ in the sample of Desflurane taken by subtracting the sum of all impurities found from 100.0%.

Acceptance criteria: 99.7%–100.0%

IMPURITIES

INORGANIC IMPURITIES

- **Limit of Nonvolatile Residue:** Transfer 20.0 mL of Desflurane to an evaporating dish, and evaporate with a stream of nitrogen to dryness: the weight of the residue does not exceed 2.0 mg (0.01%).
- **Limit of Antimony**

Diluent A: Nitric acid and water (1:1)

Diluent B: Nitric acid and hydrochloric acid (9:1)

Standard solutions: Transfer 0.1 mL (234 mg) of antimony pentachloride to a 50-mL volumetric flask, dilute with *Diluent B* to volume, and mix. This stock solution contains about 1906 µg of antimony/mL. Dilute a portion of this solution quantitatively and stepwise with *Diluent B* to obtain *Standard solutions* containing 2.5, 5.0, and 10.0 µg of antimony/mL.

Sample solution: Weigh a stoppered stock bottle containing a quantity of Desflurane at ambient temperature, and then cool it in powdered dry ice. Using a cold syringe, transfer 5–7 mL of Desflurane from the cold bottle to a separator containing 20 mL of *Diluent A*. Allow the stock bottle containing the remaining Desflurane to come to ambient temperature, weigh it, and calculate the quantity, in g, of Desflurane taken for the test. Allow the Desflurane in the separator to evaporate, and with the aid of a few mL of *Diluent A*, transfer the acid solution to a clean, dry beaker. Add 1 mL of hydrochloric acid to the solution in the beaker, and reduce the volume to 8 mL by evaporating on a hot plate. Transfer this solution to a 10-mL volumetric flask, and dilute with *Diluent B* to volume.

Spectrometric conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometer

Analytical wavelength: Antimony emission line at 217.6 nm

Lamp: Antimony hollow-cathode

Flame: Air–acetylene

Blank: *Diluent B*

Analysis

Samples: *Standard solutions* and *Sample solution*

Calculation: Plot the absorbances of the *Standard solutions* versus the concentration (µg/mL) of antimony, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration of antimony in the *Sample solution*. Calculate the quantity, in µg/g, of antimony in the portion of Desflurane taken:

$$\text{Result} = (C/W) \times V$$

C = concentration of antimony in the *Sample solution* (µg/mL)

W = weight of Desflurane taken to prepare the *Sample solution* (g)

V = volume of *Sample solution*

Acceptance criteria: NMT 3 µg/g

- **Limit of Fluoride**

[NOTE—Store all solutions except *Solution A* in plastic containers.]

Solution A: 57 mL of glacial acetic acid, 58 g of sodium chloride, and 4 g of (1,2-cyclohexylenedinitrilo)tetraacetic acid in 500 mL of water. Adjust with 5 N sodium hydroxide to a pH of 5.25 ± 0.25, and dilute with water to 1000 mL. An equivalent commercial preparation may be used.

Standard stock solution: 2210 µg/mL of USP Sodium Fluoride RS in water. Each mL of this solution contains 1000 µg of fluoride/mL.

Standard solutions: Dilute volumes of *Standard stock solution* with *Solution A* to obtain solutions with concentrations of 0.1, 0.3, 0.5, 1.0, 3.0, and 5.0 µg of fluoride/mL.

Sample solution: Transfer 20.0 mL of Desflurane to a 60-mL separator, add 20.0 mL of water, shake for 1 min, and allow the layers to separate. Drain the lower organic layer and a small portion of the aqueous layer into a beaker, and discard. Transfer 10.0 mL of the aqueous phase remaining in the separator to a plastic cup, and add 10.0 mL of *Solution A*.

Analysis

Samples: *Standard solutions* and *Sample solution*

Concomitantly measure the potentials (see *pH* ⟨791⟩), in mV, of the *Samples* with a pH meter capable of a minimum reproducibility of ±0.2 mV and equipped with a fluoride-specific ion-indicating electrode and a calomel reference electrode.

[NOTE—When taking measurements, immerse the electrodes in the solution, stir with a polytef-coated stirring bar and a magnetic stirrer having an insulated top until equilibrium is attained (about 1–2 min), and record the potential. Rinse the electrodes with *Solution A*, and dry, taking care to avoid damaging the crystal of the specific-ion electrode.]

Plot the logarithms of the fluoride concentrations (µg/mL) of the *Standard solutions* versus the potential, in mV. From the measured potential of the *Sample solution* and the standard response line, determine the concentration, C (µg/mL), of fluoride in the *Sample solution*. Multiply C by 0.0002 to obtain the percentage of fluoride in the portion of Desflurane taken.

Acceptance criteria: NMT 0.001%

ORGANIC IMPURITIES

- **Procedure**

Standard stock solution: To a suitable tared vial, fitted with a septum, add 20 mL (29.4 g) of Desflurane. Seal and re-weigh the vial to determine the weight of Desflurane added. To this vial sequentially add 20 µL of USP Desflurane Related Compound A RS, 23 µL of dichloromethane, 20 µL of chloroform, 38 µL of acetone, and 21 µL of USP Isoflurane RS. Record the weight after the addition of each impurity and determine the total weight.

Calculate the percentage of each impurity in the *Standard stock solution*:

$$\text{Result} = W_I/W_T \times P_I$$

USP Monographs

www.webofpharma.com

W_I = weight of each impurity added (g)
W_T = total weight of the *Standard stock solution* (g)
P_I = purity of each impurity added (%)

Standard solution: To a suitable tared vial, fitted with a septum, add 10.2 mL (15 g) of Desflurane. Seal and re-weigh the vial to determine the weight of Desflurane added. To this vial add 250 µL of the *Standard stock solution*, and record the weight to determine the weight of the *Standard stock solution* added and the final weight of the *Standard solution*. Calculate the spiked concentration (C_S) of each impurity in the *Standard solution*:

$$\text{Result} = W_I/W_T \times C_I$$

W_I = weight of *Standard stock solution* added (g)
W_T = total weight of the *Standard solution* (g)
C_I = concentration of each impurity in the *Standard stock solution* (%)

System suitability solution: To a suitable vial, fitted with a septum, add 10.2 mL (15 g) of Desflurane. Seal the vial. To this vial add 100 µL of the *Standard stock solution*.
Sample: Desflurane
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 105-m capillary column coated with 1.5-µm film of G6
Carrier gas: Helium
Autosampler/Syringe temperature: 2°–5°
Flow rate: 3 mL/min
Split flow: 25 mL/min
Temperature
Injection port: 150°
Detector: 200°
Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
30	—	30	11
30	20	50	13

Injection size: 3 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between isoflurane and dichloromethane, *Standard solution*
Tailing factor: NMT 1.5 for isoflurane, *Standard solution*
Relative standard deviation: NMT 5% for each impurity, *Standard solution*
Signal-to-noise ratio: NLT 40 for isoflurane, *System suitability solution*
Analysis
[NOTE—Injections of Desflurane used to prepare the *Standard solution* must be made to estimate the amount of known impurities that may be present in the solvent. The final concentration of each impurity is equal to the concentration of the impurity added plus the concentration inherent in the matrix.]
Samples: *Standard solution* and *Sample solution*
Calculate the final concentration of each impurity in the *Standard solution*:

$$\text{Result} = r_U/(r_S - r_U) \times C_S + C_S$$

r_U = peak response of each impurity from the Desflurane used as the solvent
r_S = peak response of each impurity from the *Standard solution*
C_S = spiked concentration of each impurity in the *Standard solution* (%)

Calculate the percentage of each impurity observed in the *Sample solution* that is also present in the *Standard solution*:

$$\text{Result} = (r_U/r_S) \times C_F$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of each impurity from the *Standard solution*
C_F = final concentration of each impurity in the *Standard solution* (%)

Calculate the percentage of all other impurities:

$$\text{Result} = (r_U/r_S) \times C_S \times 1/F$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of Isoflurane from the *Standard solution*
C_S = concentration of USP Isoflurane RS in the *Standard solution* (%)
F = relative response factor relative to isoflurane (see *Impurity Table 1*)

Acceptance criteria: See *Impurity Table 1*.
Total impurities: NMT 0.3%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desflurane[a]	1.0	—	—
Dichlorofluoromethane	1.04	0.43	0.01
Trichlorofluoromethane	1.07	0.15	0.001
Desflurane related compound A[b, a]	1.12	—	0.10
Trichlorotrifluoroethane	1.35	1.3	0.001
Dichloromethane[a]	1.44	—	0.001
Isoflurane[a]	1.55	1.0	0.20
Chloroform[a]	1.88	—	0.006
Acetone[a]	2.12	—	0.010

[a] These impurities are present in the *Standard solution* and are quantified by external standards.
[b] Bis-(1,2,2,2-tetrafluoroethyl)ether.

SPECIFIC TESTS

• **ACIDITY OR ALKALINITY**
Bromocresol purple solution: 0.5 mg/mL of bromocresol purple. Prepared by dissolving 50 mg of bromocresol purple in 0.92 mL of 0.1 M sodium hydroxide and 20 mL of ethanol, and then diluting with water to 100 mL.
Sample solution: Transfer 20 mL of Desflurane to a separatory funnel, and add 20 mL of carbon dioxide-free water. Shake for 3 min, allow the layers to separate, and

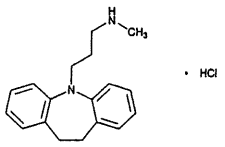
USP Monographs

www.webofpharma.com

discard the lower organic layer. Collect the upper layer, and add 0.2 mL of *Bromocresol purple solution*.
Acceptance criteria: NMT 0.1 mL of 0.01 M sodium hydroxide or 0.6 mL of 0.01 M hydrochloric acid is required to change the color of the indicator.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature. Replace the cap securely after each use.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Desflurane RS
  USP Desflurane Related Compound A RS
  Bis-(1,2,2,2-tetrafluoroethyl)ether.
  $C_4H_2F_8O$ 218.05
  USP Isoflurane RS
  USP Sodium Fluoride RS

Desipramine Hydrochloride



$C_{18}H_{22}N_2 \cdot HCl$ 302.84
5*H*-Dibenz[*b*,*f*]azepine-5-propanamine, 10,11-dihydro-*N*-methyl-, monohydrochloride;
10,11-Dihydro-5-[3-(methylamino)propyl]-5*H*-dibenz[*b*,*f*]azepine monohydrochloride [58-28-6].

DEFINITION
Desipramine Hydrochloride contains NLT 98.0% and NMT 102.0% of desipramine hydrochloride ($C_{18}H_{22}N_2 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
  Sample solution: 50 mg/mL of Desipramine Hydrochloride in alcohol
  Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**
  Buffer: 3.4 g/L of sodium acetate trihydrate in water adjusted with glacial acetic acid to a pH of 5.0
  Mobile phase: Acetonitrile, methanol, and *Buffer* (35:20:45)
  Diluent: 0.1 M hydrochloric acid
  System suitability solution: 0.02 mg/mL each of USP Desipramine Hydrochloride RS and USP Imipramine Hydrochloride RS in *Diluent*. Sonication may be used to promote dissolution.
  Standard solution: 0.1 mg/mL of USP Desipramine Hydrochloride RS in *Diluent*. Sonication may be used to promote dissolution.
  Sample solution: 0.1 mg/mL of Desipramine Hydrochloride in *Diluent*. Sonication may be used to promote dissolution.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 250 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L10
  Flow rate: 1 mL/min
  Injection volume: 25 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for desipramine and imipramine are 1.0 and 1.1, respectively.]
  Suitability requirements
  Resolution: NLT 1.5 between desipramine and imipramine, *System suitability solution*
  Tailing factor: NMT 2, *Standard solution*
  Relative standard deviation: NMT 0.73%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of desipramine hydrochloride ($C_{18}H_{22}N_2 \cdot HCl$) in the portion of Desipramine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Desipramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Desipramine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Buffer: 5.2 g/L of dibasic potassium phosphate in water. Add 1 mL of triethylamine per L, and adjust with phosphoric acid to a pH of 6.4.
  Solution A: Acetonitrile and methanol (55:45)
  Solution B: *Solution A* and *Buffer* (25:75)
  Solution C: *Solution A* and *Buffer* (62.5: 37.5)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	85	15
35	0	100
50	0	100
50.1	85	15
60	85	15

Standard stock solution: 0.25 mg/mL of USP Desipramine Hydrochloride RS prepared as follows. Transfer a suitable quantity of USP Desipramine Hydrochloride RS to an appropriate volumetric flask, and add 50% of the final flask volume of *Solution B*. Sonicate for NLT 2 min, and allow the solution to equilibrate to room temperature. Dilute with *Solution B* to volume.
Standard solution: 0.005 mg/mL of USP Desipramine Hydrochloride RS from *Standard stock solution* in *Solution B*
System suitability solution: 0.01 mg/mL each of USP Imipramine Hydrochloride RS and USP Iminodibenzyl RS in *Standard stock solution*

www.webofpharma.com

Sensitivity solution: 0.3 µg/mL of USP Desipramine Hydrochloride RS from *Standard solution* in *Solution B*. Use within 24 h.
Sample solution: 0.5 mg/mL of Desipramine Hydrochloride prepared as follows. Transfer a suitable quantity of Desipramine Hydrochloride to an appropriate volumetric flask, and add 50% of the final flask volume of *Solution B*. Sonicate for NLT 2 min, and allow the solution to equilibrate to room temperature. Dilute with *Solution B* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 4-µm or 5-µm packing L1
Column temperature: 60°
Flow rate: 1.4 mL/min
Injection volume: 40 µL
System suitability
Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between imipramine and iminodibenzyl, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Desipramine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of desipramine from the *Standard solution*
C_S = concentration of USP Desipramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Desipramine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desipramine	1.0	—	—
Imipramine	1.6	1.0	0.15
Iminodibenzyl	2.1	0.55	0.1
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from light.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Desipramine Hydrochloride RS
USP Iminodibenzyl RS
10,11-Dihydro-5*H*-dibenzo[*b*,*f*]azepine.
$C_{14}H_{13}N$ 195.28
USP Imipramine Hydrochloride RS

Desipramine Hydrochloride Tablets

DEFINITION
Desipramine Hydrochloride Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of desipramine hydrochloride ($C_{18}H_{22}N_2 \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 3.4 g/L of sodium acetate in water. Adjust with glacial acetic acid to a pH of 5.0.
Mobile phase: Acetonitrile, methanol, and *Buffer* (30:20:50)
Diluent: 0.1 N hydrochloric acid
System suitability solution: 0.02 mg/mL each of USP Desipramine Hydrochloride RS and USP Imipramine Hydrochloride RS in *Diluent*. Sonication may be used to promote dissolution.
Standard solution: 0.02 mg/mL of USP Desipramine Hydrochloride RS in *Diluent*. Sonication may be used to promote dissolution.
Sample stock solution: Nominally 1–1.5 mg/mL of desipramine hydrochloride from Tablets prepared as follows. Transfer NLT 20 Tablets into a suitable volumetric flask. Add 50% of the final flask volume of *Diluent*. Sonicate the flask for NLT 15 min. Shake the flask for NLT 15 min. Dilute with *Diluent* to volume.
Sample solution: Nominally 0.02 mg/mL of desipramine hydrochloride prepared as follows. Transfer a suitable volume of *Sample stock solution* to an appropriate volumetric flask. Add 50% of the final flask volume of *Diluent*. Shake the flask for NLT 5 min and dilute with *Diluent* to volume. Pass through a suitable filter and discard the first 5 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L10
Flow rate: 1.5–2.0 mL/min
Injection volume: 25 µL
Run time: NLT 1.2 times the retention time of the imipramine peak
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for desipramine and imipramine are 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between desipramine and imipramine, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*

www.webofpharma.com

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desipramine hydrochloride ($C_{18}H_{22}N_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Desipramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of desipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Standard solution: USP Desipramine Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. If necessary, dilute with *Medium* to a concentration that is similar to that of the *Standard solution.*

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at 251 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desipramine hydrochloride ($C_{18}H_{22}N_2 \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Desipramine Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of desipramine hydrochloride ($C_{18}H_{22}N_2 \cdot HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**

Buffer: 5.2 g/L of dibasic potassium phosphate in water. To each L of solution, add 1 mL of triethylamine and adjust with phosphoric acid to a pH of 6.4.

Solution A: Acetonitrile and methanol (55:45)

Solution B: *Solution A* and *Buffer* (25:75)

Solution C: *Solution A* and *Buffer* (62.5: 37.5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	85	15
35	0	100
50	0	100
50.1	85	15
60	85	15

Standard stock solution: 0.25 mg/mL of USP Desipramine Hydrochloride RS prepared as follows. Transfer a suitable quantity of USP Desipramine Hydrochloride RS to an appropriate volumetric flask. Add 50% of the final flask volume of *Solution B*. Sonicate for NLT 2 min. Allow the solution to equilibrate to room temperature. Dilute with *Solution B* to volume.

Standard solution: 0.005 mg/mL of USP Desipramine Hydrochloride RS from *Standard stock solution* in *Solution B*

System suitability solution: 0.01 mg/mL each of USP Imipramine Hydrochloride RS and USP Iminodibenzyl RS in *Standard stock solution*

Sensitivity solution: 0.3 µg/mL of USP Desipramine Hydrochloride RS from *Standard solution* in *Solution B*. Use within 24 h.

Sample solution: Nominally 0.5 mg/mL of desipramine hydrochloride from Tablets prepared as follows. Finely powder NLT 20 Tablets. Transfer a suitable portion of this powder, equivalent to 50 mg of desipramine hydrochloride, to a 100-mL volumetric flask with the aid of *Solution B*. Add *Solution B* to about 50% of the flask volume, and sonicate the flask with occasional shaking for NLT 10 min. Allow the solution to equilibrate to room temperature. Dilute with *Solution B* to volume. Pass through a suitable filter, and discard NLT the first 2 mL of filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 4-µm or 5-µm packing L1

Column temperature: 60°

Flow rate: 1.4 mL/min

Injection volume: 40 µL

System suitability

Samples: *Standard solution, System suitability solution,* and *Sensitivity solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between imipramine and iminodibenzyl, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of desipramine from the *Standard solution*
C_S = concentration of USP Desipramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of desipramine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

USP Monographs

www.webofpharma.com

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

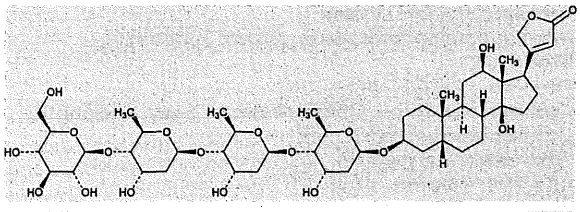
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desipramine	1.0	—	—
Imipramine	1.6	1.0	0.2
Iminodibenzyl	2.1	0.55	0.5
Any unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	2.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Desipramine Hydrochloride RS
  USP Iminodibenzyl RS
  10,11-Dihydro-5*H*-dibenzo[*b*,*f*]azepine.
  $C_{14}H_{13}N$ 195.28
  USP Imipramine Hydrochloride RS

Delete the following:

▲Deslanoside



$C_{47}H_{74}O_{19}$ 943.08

Card-20(22)-enolide, 3-[(*O*-β-D-glucopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12,14-dihydroxy-, (3β,5β,12β)-;
Deacetyllanatoside C [17598-65-1].

DEFINITION

Deslanoside contains NLT 95.0% and NMT 103.0% of deslanoside ($C_{47}H_{74}O_{19}$), calculated on the dried basis.
[**CAUTION**—Handle Deslanoside with exceptional care, because it is highly potent.]

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 4 mg/mL of USP Deslanoside RS in methanol
Sample solution: 4 mg/mL in methanol
Chromatographic system
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 5 µL
Developing solvent system: Methylene chloride, methanol, and water (130:36:3)
Spray reagent: Dilute perchloric acid (1 in 20)
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent has moved three-fourths of the length of the plate. Remove the plate, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent* and heating at about 100° for 3 min. Cool, and examine under UV light.
Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

- **PROCEDURE**

Standard solution: 0.2 mg/mL of USP Deslanoside RS in alcohol
Sample solution: 0.2 mg/mL of Deslanoside in alcohol
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 590 nm (λ max)
Cell: 1 cm
Blank: Alcohol
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Transfer 3.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to separate 25-mL conical flasks. Evaporate each with gentle warming and with the aid of a current of air just to dryness, and cool in a vacuum desiccator for 30 min. Add 15.0 mL of acid ferric chloride TS to each flask, and mix by swirling. Allow the mixtures to stand protected from light, swirling them frequently, at a temperature not exceeding 30°, for 15 min. Pass each solution through separate fine glass wool filters. Concomitantly determine the absorbances of the solutions, using the *Blank* to set the instrument. Repeat the measurements at 2-min intervals until maximum absorbance readings have been obtained.
Calculate the percentage of deslanoside ($C_{47}H_{74}O_{19}$) in the portion of Deslanoside taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = maximum absorbance of the *Sample solution*
A_S = maximum absorbance of the *Standard solution*
C_S = concentration of USP Deslanoside RS in the *Standard solution* (mg/mL)
C_U = concentration of Deslanoside in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 20 mg/mL in anhydrous pyridine
Acceptance criteria: +7.0° to +8.5°

- **LOSS ON DRYING** ⟨731⟩

Sample: 0.5 g
Analysis: Dry the *Sample* under vacuum at 100° to constant weight.
Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Deslanoside RS▲ (USP 1-May-2020)

www.webofpharma.com

Delete the following:

▲Deslanoside Injection

DEFINITION

Deslanoside Injection is a sterile solution of Deslanoside in a suitable solvent. It contains NLT 90.0% and NMT 110.0% of the labeled amount of deslanoside ($C_{47}H_{74}O_{19}$). It may contain Glycerin.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 4 mg/mL of USP Deslanoside RS in methanol

Sample solution: Transfer a volume of Injection, nominally equivalent to 2 mg of deslanoside, to a small separator, and extract with 25 mL of a mixture of chloroform and alcohol (7:3). Transfer the extract to a 10-mL conical flask, evaporate on a steam bath to dryness, and dissolve the residue in 500 µL of methanol.

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Methylene chloride, methanol, and water (130:36:3)

Spray reagent: Dilute perchloric acid (1 in 20)

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent has moved three-fourths of the length of the plate. Remove the plate, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent* and heating at about 100° for 3 min. Cool, and examine under UV light.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Standard solution: 0.2 mg/mL of USP Deslanoside RS in alcohol

Sample solution: Transfer a volume of Injection, nominally equivalent to 600 µg of deslanoside, to a separator, and add 50 mL of water and 1 mL of 2 N sulfuric acid. Extract four 30-mL portions of a mixture of chloroform and *n*-propyl alcohol (5:1), washing each portion in a second separator containing 5 mL of water and filtering through cotton that has previously been moistened with chloroform. Combine the extracts, and evaporate on a steam bath, with the aid of a current of air, just to dryness. Transfer the residue, with the aid of a small volume of the chloroform–*n*-propyl alcohol mixture (5:1), to a 25-mL conical flask.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 590 nm (λ max)

Cell: 1 cm

Blank: Alcohol

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Transfer 3.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to separate 25-mL conical flasks. Evaporate each with gentle warming and with the aid of a current of air just to dryness, and cool in a vacuum desiccator for 30 min. Add 15.0 mL of acid ferric chloride TS to each flask, and mix by swirling. Allow the mixtures to stand protected from light, swirling them frequently, at a temperature not exceeding 30°, for 15 min. Pass each solution through separate fine glass wool filters. Concomitantly determine the absorbances of the solutions, using the *Blank* to set the instrument. Repeat the measurements at 2-min intervals until maximum absorbance readings have been obtained.

Calculate the percentage of deslanoside ($C_{47}H_{74}O_{19}$) in the portion of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = maximum absorbance of the *Sample solution*

A_S = maximum absorbance of the *Standard solution*

C_S = concentration of USP Deslanoside RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of deslanoside in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 5.5–7.0

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

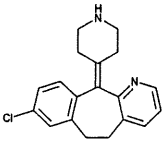
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Deslanoside RS▲ (USP 1-May-2020)

Desloratadine



$C_{19}H_{19}ClN_2$ 310.82

Benzo[5,6]cyclohepta[1,2-*b*]pyridine, 8-chloro-6,11-dihydro-11-(4-piperidinylidene)-, 5*H*-;

8-Chloro-6,11-dihydro-11-(piperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine [100643-71-8].

DEFINITION

Desloratadine contains NLT 98.0% and NMT 102.0% of desloratadine ($C_{19}H_{19}ClN_2$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 0.865 g of sodium dodecyl sulfate in water, add 0.5 mL of trifluoroacetic acid, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (43:57)

USP Monographs

www.webofpharma.com

System suitability solution: 0.08 mg/mL of USP Desloratadine RS and 0.2 µg/mL of USP Desloratadine Related Compound B RS in *Mobile phase*
Standard solution: 0.08 mg/mL of USP Desloratadine RS in *Mobile phase*
Sample solution: 0.08 mg/mL of Desloratadine in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 4-µm packing L1
Column temperature 35°
Flow rate: 1 mL/min
Injection volume: 100 µL
Run time: NLT 2 times the retention time of desloratadine
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between desloratadine related compound B and desloratadine, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of desloratadine ($C_{19}H_{19}ClN_2$) in the portion of Desloratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)
C_U = concentration of Desloratadine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

• **ORGANIC IMPURITIES, PROCEDURE 1**
Use *Organic Impurities, Procedure 1*, when the impurity profile includes desloratadine related compound B or fluorodesloratadine.
Buffer, Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.08 µg/mL of USP Desloratadine RS in *Mobile phase*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between desloratadine related compound B and desloratadine, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Desloratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of desloratadine from the *Standard solution*
C_S = concentration of USP Desloratadine RS in the *Standard solution* (µg/mL)
C_U = concentration of Desloratadine in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fluorodesloratadine[a]	0.8	0.6	0.2
Desloratadine related compound B	0.9	0.6	0.3
Desloratadine	1.0	—	—
Any other individual unspecified impurity	—	1.00	0.10
Total impurities	—	—	0.4

[a] 8-Chloro-11-fluoro-11-(piperidin-4-yl)-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

• **ORGANIC IMPURITIES, PROCEDURE 2**
Use *Organic Impurities, Procedure 2*, when the impurity profile includes dechloro desloratadine, desloratadine related compound A, or dehydrodesloratadine.
Buffer: 1.36 g/L of monobasic potassium phosphate in water. Add 10 mL of triethylamine per L of the solution, and adjust with dilute phosphoric acid (1 in 10) to a pH of 2.0.
Solution A: Acetonitrile, methanol, and *Buffer* (10:10:80)
Solution B: Acetonitrile, tetrahydrofuran, and *Buffer* (70:5:30)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
15	90	10
20	70	30
25	60	40
30	50	50
38	50	50
40	100	0
45	100	0

System suitability solution: 0.15 µg/mL of USP Desloratadine Related Compound A RS and 100 µg/mL of USP Desloratadine RS in *Solution A*
Standard solution: 0.5 µg/mL of USP Desloratadine RS in *Solution A*
Sample solution: 500 µg/mL of Desloratadine in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm

www.webofpharma.com

Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 60 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between desloratadine and desloratadine related compound A, *System suitability solution*
Tailing factor: NMT 3.0 for desloratadine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Desloratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of desloratadine from the *Standard solution*
C_S = concentration of USP Desloratadine RS in the *Standard solution* (µg/mL)
C_U = concentration of Desloratadine in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dechloro desloratadine[a]	0.38	0.90	0.15
Desloratadine	1.0	—	—
Desloratadine related compound A	1.30	0.86	0.15
Dehydro desloratadine[b]	1.59	1.00	0.15
Loratadine[c]	2.25	0.79	0.20
Any other individual unspecified impurity	—	1.00	0.10
Total impurities	—	—	0.40

[a] 6,11-Dihydro-11-(piperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.
[b] 8-Chloro-11-(piperidin-4-ylidene)benzo[5,6]cyclohepta[1,2-*b*]pyridine.
[c] 8-Chloro-6,11-dihydro-11-(1-ethoxycarbonylpiperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ic* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Desloratadine RS
USP Desloratadine Related Compound A RS
8-Bromo-6,11-dihydro-11-(piperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.
$C_{19}H_{19}BrN_2$ 355.27
USP Desloratadine Related Compound B RS
8-Chloro-11-(1,2,3,6-tetrahydropyridin-4-yl)-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine hydrochloride.
$C_{19}H_{20}Cl_2N_2$ 347.28

Desloratadine Tablets

DEFINITION
Desloratadine Tablets contain NLT 93.0% and NMT 105.0% of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV absorption spectra of the desloratadine peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Use amber, low-actinic glassware.
Buffer: Dissolve 4.35 g of dibasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Methanol and *Buffer* (20:80)
Diluent: Methanol and water (90:10)
Standard solution: 0.02 mg/mL of USP Desloratadine RS in *Diluent*
Sample stock solution: Nominally 0.2 mg/mL of desloratadine, prepared as follows. Transfer NLT 20 Tablets into a suitable volumetric flask, add water to fill 10% of the flask volume, and allow the Tablets to disperse. Add methanol, about 50% of the flask volume, and stir for NLT 60 min. Allow the solution to cool to room temperature and dilute with methanol to volume. Centrifuge a portion of this solution and use the supernatant.
Sample solution: Nominally 0.02 mg/mL of desloratadine in *Diluent*, from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Assay: UV 241 nm
Identification B: Diode array; UV 230–400 nm
Column: 4.6-mm × 15-cm; 5-µm packing L10
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 4.2 times the retention time of desloratadine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

USP Monographs

www.webofpharma.com

C_S = concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of desloratadine in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: 0.01 mg/mL of USP Desloratadine RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard the first 5 mL of the filtrate.
Instrumental conditions
Mode: UV
Analytical wavelength: 282 nm
Cell: 1.0 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
▲Protect all solutions containing desloratadine from light.▲ (ERR 1-May-2018)
Solution A: Dissolve 4.35 g of dibasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 3.2.
Solution B: Acetonitrile
Solution C: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	70	15	15
12	70	15	15
30	40	30	30
45	40	30	30
47	70	15	15
55	70	15	15

Diluent: Methanol and water (90:10)
Standard solution: 0.002 mg/mL each of USP Desloratadine RS and USP Desloratadine Related Compound F RS in *Diluent*
Sensitivity solution: 0.1 µg/mL of USP Desloratadine RS in *Diluent*
Sample solution: Nominally 0.2 mg/mL of desloratadine, prepared as follows. Transfer NLT 20 Tablets into a suitable volumetric flask, add 10% of the flask volume of water, and allow the Tablets to disperse. Add methanol, about 50% of the flask volume, and stir for at least 60 min. Allow the solution to cool to room temperature, and dilute with methanol to volume. Centrifuge a portion of this solution and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 241 nm
Column: 4.6-mm × 15-cm; 3-µm packing L7
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Column efficiency: NLT 1500 theoretical plates for desloratadine, *Standard solution*
Relative standard deviation: NMT 5.0% for desloratadine, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution*, *Sensitivity solution*, and *Sample solution*
Calculate the percentage of desloratadine related compound F in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desloratadine related compound F from the *Sample solution*
r_S = peak response of desloratadine related compound F from the *Standard solution*
C_S = concentration of USP Desloratadine Related Compound F RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of desloratadine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of desloratadine from the *Standard solution*
C_S = concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of desloratadine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dechloro desloratadine[a]	0.37	—[b]
Desloratadine	1.00	—
Dehydro desloratadine[c]	1.4	—[b]

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desloratadine related compound F	1.8	0.30
Loratadine[d]	2.7	—[b]
Any unspecified degradation product	—	0.2
Total impurities	—	0.50

[a] 6,11-Dihydro-11-(piperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.
[b] Process impurity controlled in the drug substance monograph. Provided for information only; the content is not calculated and not reported.
[c] 8-Chloro-11-(piperidin-4-ylidene)benzo[5,6]cyclohepta[1,2-*b*]pyridine.
[d] 8-Chloro-6,11-dihydro-11-(1-ethoxycarbonylpiperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine (loratadine).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Desloratadine RS
  USP Desloratadine Related Compound F RS
  8-Chloro-6,11-dihydro-11-(*N*-formyl-4-piperidinylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.
  $C_{20}H_{19}ClN_2O$ 338.83

Desloratadine Orally Disintegrating Tablets

DEFINITION

Desloratadine Orally Disintegrating Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Standard solution and **Sample solution:** Proceed as directed in the *Assay*.
  Instrumental conditions
  Mode: UV
  Wavelength range: 230–330 nm
  [NOTE—Alternatively, a diode array detector may be used in the *Assay* to obtain the spectra.]
  Acceptance criteria: The UV spectrum of the *Sample solution* corresponds to that of the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 6.8 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.
  Mobile phase: Acetonitrile, methanol, and *Buffer* (28:7:65)
  Diluent: Methanol and 0.1 N hydrochloric acid (40:60)
  Standard solution: 0.05 mg/mL of USP Desloratadine RS in *Diluent*. Sonication may be used to aid dissolution.
  Sample stock solution: Nominally 0.25 mg/mL of desloratadine, prepared as follows. Transfer 10 Tablets to a suitable volumetric flask, add water to 15% of the flask volume, and shake until the Tablets disintegrate completely. Add 75% of the flask volume of *Diluent* and sonicate for 30 min with intermittent shaking, and dilute with *Diluent* to volume. Centrifuge a portion of this solution. Use the supernatant.
  Sample solution: Nominally 0.05 mg/mL of desloratadine from the *Sample stock solution* in *Diluent*; centrifugate
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 258 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of desloratadine in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩: NMT 30 s
- **DISSOLUTION** ⟨711⟩
  Medium: 0.1 N hydrochloric acid (degassed); 900 mL
  Apparatus 2: 50 rpm
  Time: 10 min
  Buffer: 6.8 g/L of monobasic potassium phosphate
  Solution A: Acetonitrile and methanol (80:20)
  Mobile phase: *Solution A* and *Buffer* (40:60)
  Standard stock solution: 0.28 mg/mL of USP Desloratadine RS in methanol. Sonication may be used to aid dissolution.
  Standard solution: (*L*/900) mg/mL of USP Desloratadine RS from the *Standard stock solution* in *Medium*, where *L* is the label claim (mg/Tablet)
  Sample solution: Pass a portion of the solution under test through a suitable filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 258 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 40 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

www.webofpharma.com

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of desloratadine ($C_{19}H_{19}ClN_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Buffer: Add 10 mL/L of triethylamine to a 1.36 g/L solution of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.5.

Solution A: Methanol, acetonitrile, and *Buffer* (15:5:80)

Solution B: Acetonitrile, tetrahydrofuran, and *Buffer* (70:5:30)

Solution C: Dilute 8.5 mL of hydrochloric acid with methanol to 1 L.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
40	50	50
50	50	50
52	100	0
65	100	0

Diluent: *Solution C* and *Buffer* (30:70)

System suitability stock solution: 0.05 mg/mL each of USP Desloratadine Related Compound A RS and USP Desloratadine Related Compound F RS in methanol,

System suitability solution: 0.5 mg/mL of USP Desloratadine RS, 1.0 µg/mL each of USP Desloratadine Related Compound A RS and USP Desloratadine Related Compound F RS, prepared as follows. Transfer 50 mg of USP Desloratadine RS into a 100-mL volumetric flask, add 70 mL of *Diluent*, and sonicate to dissolve. Add 2 mL of the *System suitability stock solution* and dilute with *Diluent* to volume.

Standard solution: 0.0025 mg/mL of USP Desloratadine RS and 0.001 mg/mL of USP Desloratadine Related Compound F RS in *Diluent*

Sample solution: Nominally 0.5 mg/mL of desloratadine from NLT 40 Tablets, prepared as follows. Transfer an amount of powder to a suitable volumetric flask to obtain the nominal concentration of desloratadine. Add 70% of the flask volume of *Diluent* and sonicate for 20 min with intermittent shaking. Dilute with *Diluent* to volume. Centrifuge a portion of the solution and use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 40 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—Relative retention times are given in *Table 2*.]

Suitability requirements

Resolution: NLT 2.0 between desloratadine and desloratadine related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for desloratadine and desloratadine related compound F, *Standard solution*

Relative standard deviation: NMT 10.0% desloratadine related compound F, *Standard solution*

Signal-to-noise ratio: NLT 10 for desloratadine peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the impurities using the relative retention times given in *Table 2*.

Calculate the percentage of desloratadine related compound F in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desloratadine related compound F from the *Sample solution*
r_S = peak response of desloratadine related compound F from the *Standard solution*
C_S = concentration of USP Desloratadine Related Compound F RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of desloratadine in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified degradation product from the *Sample solution*
r_S = peak response of desloratadine from the *Standard solution*
C_S = concentration of USP Desloratadine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of desloratadine in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Compound	Relative Retention Time	Acceptance Criteria, NMT (%)
Dechloro desloratadine[a, b]	0.42	—
Desloratadine	1.00	—
Desloratadine related compound A[b]	1.09	—
Dehydro desloratadine[b, c]	1.33	—
Desloratadine related compound F	1.37	0.2
Loratadine[b, d]	1.89	—
Any other individual unspecified degradation product	—	▲0.2▲ (RB 1-Dec-2018)

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Compound	Relative Retention Time	Acceptance Criteria, NMT (%)
Total degradation products	—	0.5

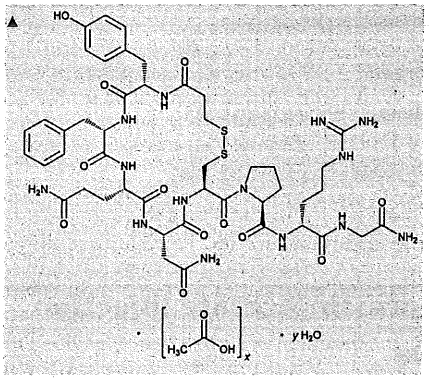
[a] 6,11-Dihydro-11-(piperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.
[b] This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.
[c] 8-Chloro-11-(piperidin-4-ylidene)benzo[5,6]cyclohepta[1,2-*b*]pyridine.
[d] 8-Chloro-6,11-dihydro-11-(1-ethoxycarbonylpiperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Desloratadine RS
  USP Desloratadine Related Compound A RS
  8-Bromo-6,11-dihydro-11-(piperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.
  $C_{19}H_{19}BrN_2$ 355.27
  USP Desloratadine Related Compound F RS
  8-Chloro-6,11-dihydro-11-(*N*-formyl-4-piperidinylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.
  $C_{20}H_{19}ClN_2O$ 338.83

Desmopressin Acetate

Change to read:



$C_{46}H_{64}N_{14}O_{12}S_2 \cdot xC_2H_4O_2 \cdot yH_2O$ 1069.22 (anhydrous, free base)
Vasopressin, 1-(3-mercaptopropanoic acid)-8-D-arginine-, acetate (salt) hydrate;
1-(3-Mercaptopropionic acid)-8-D-arginine-vasopressin, acetate (salt) hydrate.
x(acetate), *y*(water).
Monoacetate trihydrate [62357-86-2].
Monoacetate anhydrous [62288-83-9]. ▲(USP 1-Dec-2019)

DEFINITION

Desmopressin Acetate is a synthetic octapeptide hormone having the property of antidiuresis. It is a synthetic analog of vasopressin. It contains NLT 95.0% and NMT 105.0% of desmopressin ($C_{46}H_{64}N_{14}O_{12}S_2$), calculated on the anhydrous, acetic acid-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲The monoisotopic mass by *Mass Spectrometry* ⟨736⟩ is 1068.4 ± 0.5 mass units.▲ (USP 1-Dec-2019)
- **B.**

Buffer solution, Mobile phase, Standard solution, and **Sample solution:** Prepare as directed in the *Assay*.
Identity sample solution: 10 µg/mL each of USP Desmopressin Acetate RS and Desmopressin Acetate in *Mobile phase*
Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. The major peaks of the *Identity sample solution* coelute.

ASSAY

Change to read:

- **PROCEDURE**

Buffer solution: Dissolve 3.4 g of monobasic potassium phosphate and 2.0 g of sodium 1-heptanesulfonic acid in 1000 mL of water. Adjust with phosphoric acid or sodium hydroxide to a pH of 4.50 ± 0.05, as needed. Pass through a filter of 0.45-µm pore size.
Mobile phase: Mix acetonitrile and *Buffer solution* (22:78), and degas. Make adjustments, if necessary (see *Chromatography* ⟨621⟩, *System Suitability*). [NOTE—The retention time of desmopressin is very sensitive to the composition of the *Mobile phase*.]
Standard solution: 20 µg/mL of USP Desmopressin Acetate RS in *Mobile phase*
Sample solution: 20 µg/mL of Desmopressin Acetate in *Mobile phase*
System suitability solution: Dissolve about 1 mg of ▲USP Oxytocin RS,▲ (USP 1-Dec-2019) accurately weighed, in a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL each of the resulting solution and the *Sample solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between desmopressin and oxytocin, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0% for the desmopressin peak area for replicate injections, *Standard solution*
Chromatogram similarity: The desmopressin peak elutes before the oxytocin peak, *System suitability solution*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of desmopressin ($C_{46}H_{64}N_{14}O_{12}S_2$) in the portion of Desmopressin Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

www.webofpharma.com

r_S = peak response from the *Standard solution*
C_S = concentration of USP Desmopressin Acetate RS▲(calculated▲ (USP 1-Dec-2019) on the anhydrous, acetic acid-free basis) in the *Standard solution* (mg/mL)
C_U = concentration of Desmopressin Acetate (calculated on the anhydrous, acetic acid-free basis) in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the anhydrous, acetic acid-free basis

IMPURITIES

Change to read:

• **DESMOPRESSIN-RELATED IMPURITIES**
Mobile phase and **System suitability solution:** Prepare as directed in the *Assay*.
Standard solution: 1 µg/mL of USP Desmopressin Acetate RS in *Mobile phase*, ▲prepared by diluting 0.5 mL of the *Standard solution* from the *Assay* with *Mobile phase* to 10 mL▲ (USP 1-Dec-2019)
Sample solution: 200 µg/mL of Desmopressin Acetate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 200 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between desmopressin and oxytocin, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0% for the desmopressin peak area for replicate injections, *Standard solution*
Chromatogram similarity: The desmopressin peak elutes before the oxytocin peak, *System suitability solution*.
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms, and measure the response for each peak, except for the main desmopressin peak of the *Sample solution*.
Calculate the percentage of each individual impurity in the portion of Desmopressin Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of desmopressin from the *Standard solution*
C_S = concentration of USP Desmopressin Acetate RS▲(calculated▲ (USP 1-Dec-2019) on the anhydrous, acetic acid-free basis) in the *Standard solution* (mg/mL)
C_U = concentration of Desmopressin Acetate (calculated on the anhydrous, acetic acid-free basis) in the *Sample solution* (mg/mL)

Acceptance criteria
Any individual impurity: NMT 0.5%
Total impurities: NMT 1.5%

OTHER COMPONENTS
• **ACETIC ACID IN PEPTIDES** ⟨503⟩: 3.0%–8.0%

SPECIFIC TESTS

Change to read:

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIC MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed ▲10^2▲ (USP 1-Dec-2019) cfu/g.
• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ic*: NMT 6.0%

Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲The level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Desmopressin Acetate is used can be met. Where the label states Desmopressin Acetate must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Desmopressin Acetate is used can be met.▲ (USP 1-Dec-2019)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight containers, preferably of Type I glass, protected from light ▲and moisture.▲ (USP 1-Dec-2019) Store at a temperature not exceeding 25°, preferably between 2° and 8°.

Delete the following:

▲• **LABELING:** Label it to state the strength, in mcg per mL, of desmopressin.▲ (USP 1-Dec-2019)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Desmopressin Acetate RS
▲USP Oxytocin RS▲ (USP 1-Dec-2019)

Desmopressin Acetate Injection

DEFINITION
Desmopressin Acetate Injection is a sterile solution of Desmopressin Acetate in a suitable diluent. It may contain suitable preservatives. It possesses, in each mL, an activity of NLT 90.0% and NMT 110.0% of the labeled amount of desmopressin ($C_{46}H_{64}N_{14}O_{12}S_2$), calculated on the anhydrous, acetic acid-free basis.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer solution: Dissolve 4.9 g of phosphoric acid, accurately weighed, in water. Dilute with water to 1000 mL, and adjust with triethylamine to a pH of 3.5.

USP Monographs

www.webofpharma.com

Solution A: Transfer 9 g of sodium chloride, accurately weighed, to a 1000-mL flask, and dissolve in and dilute with water to volume. Adjust with hydrochloric acid to a pH between 3.5 and 5.0.
Solution B: Transfer 9 g of sodium chloride, accurately weighed, to a 1000-mL flask, dissolve in water, and add 5 g of chlorobutanol. Dilute with water to volume, and adjust with hydrochloric acid to a pH between 3.5 and 5.0.
Mobile phase: Acetonitrile and *Buffer solution* (16.5: 83.5). Filter and degas. Make adjustments, if necessary (see *Chromatography* ⟨621⟩, *System Suitability*).
Sample solution: For injections with concentrations of desmopressin between 4 µg/mL and 0.1 mg/mL, use undiluted Injection. For injections with concentrations exceeding 0.1 mg/mL and without preservatives, dilute 1000 µL of Injection, accurately measured, with 10 mL of *Solution A*. For injections with concentrations exceeding 0.1 mg/mL and containing preservatives, dilute 1000 µL of Injection, accurately measured, with 10 mL of *Solution B*.
Standard solution: 1 mg/mL of USP Desmopressin Acetate RS in water. Dilute with *Solution A* or *Solution B*, as directed in *Sample solution*, to obtain a solution with a concentration of desmopressin equivalent to that of the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 100 µL; 50 µL for *System suitability*
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.4
Relative standard deviation: NMT 5.0% for replicate injections
Analysis
Samples: *Sample solution* and *Standard solution*
Separately inject the *Sample solution* and the *Standard solution*, both freshly prepared, and record the chromatograms for a total time of NLT 2.5 times the retention time of the desmopressin peak.
Calculate the quantity of desmopressin ($C_{46}H_{64}N_{14}O_{12}S_2$), in mg, in the volume of Injection taken:

$$\text{Result} = C \times D \times (r_U / r_S)$$

C = concentration of USP Desmopressin Acetate RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

Acceptance criteria: 90.0%–110.0% on the anhydrous, acetic acid-free basis

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.5–6.0
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 10 USP Endotoxin Units/µg of desmopressin
- **Sterility Tests** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements for small-volume injections
- **Other Requirements:** It meets the requirements in *Injections* ⟨1⟩, *Container Content*.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers, protected from light. Store at a temperature between 2° and 8°.
- **Labeling:** Label it to state the potency, in mg, of desmopressin.
- **USP Reference Standards** ⟨11⟩
USP Desmopressin Acetate RS

Desmopressin Nasal Spray Solution

DEFINITION
Desmopressin Nasal Spray Solution is a solution of Desmopressin Acetate in a suitable diluent. It is supplied in a form suitable for nasal administration and contains suitable preservatives. It contains NLT 90.0% and NMT 110.0% of the labeled amount of desmopressin ($C_{46}H_{64}N_{14}O_{12}S_2$), calculated on the anhydrous, acetic acid-free basis.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**
Buffer solution: Dissolve 4.9 g of phosphoric acid, accurately weighed, in water. Dilute with water to 1000 mL, and adjust with triethylamine to a pH of 3.5.
Solution A: Transfer 9 g of sodium chloride, accurately weighed, to a 1000-mL flask. Dissolve in and dilute with water to volume. Adjust with hydrochloric acid to a pH of between 3.5 and 5.0.
Solution B: Transfer 9 g of sodium chloride, accurately weighed, to a 1000-mL flask, dissolve in water, and add 5 g of chlorobutanol. Dilute with water to volume, and adjust with hydrochloric acid to a pH of between 3.5 and 5.0.
Mobile phase: Acetonitrile and *Buffer solution* (16.5: 83.5). Filter and degas. Make adjustments, if necessary (see *Chromatography* ⟨621⟩, *System Suitability*).
Sample solution: For nasal spray solutions with concentrations of desmopressin between 4 µg/mL and 0.1 mg/mL, use undiluted Nasal Spray Solution. For nasal spray solutions with concentrations exceeding 0.1 mg/mL and without preservatives, dilute 1000 µL of Nasal Spray Solution, accurately measured, with 10 mL of *Solution A*. For nasal spray solutions with concentrations exceeding 0.1 mg/mL and containing preservatives, dilute 1000 µL of Nasal Spray Solution, accurately measured, with 10 mL of *Solution B*.
Standard solution: 1 mg/mL of USP Desmopressin Acetate RS in water. Dilute with *Solution A* or *Solution B*, as directed in the *Sample solution*, to obtain a solution with a concentration of desmopressin equivalent to that of the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 100 µL; 50 µL for *System suitability*
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.4

USP Monographs

www.webofpharma.com

Relative standard deviation: NMT 5.0% for replicate injections

Analysis

Samples: *Sample solution* and *Standard solution*

Separately inject the *Sample solution* and the *Standard solution*, both freshly prepared, and record the chromatograms for a total of NLT 2.5 times the retention time of the desmopressin peak.

Calculate the quantity of desmopressin ($C_{46}H_{64}N_{14}O_{12}S_2$), in mg, in the volume of Nasal Spray Solution taken:

$$\text{Result} = C \times D \times (r_U/r_S)$$

C = concentration of USP Desmopressin Acetate RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

Acceptance criteria: 90.0%–110.0% on the anhydrous, acetic acid-free basis

SPECIFIC TESTS

- **pH** ⟨791⟩: 3.5–6.0
- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total aerobic microbial count does not exceed 100 cfu/mL, the total combined molds and yeasts count does not exceed 10 cfu/mL, and it meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in containers suitable for administering the contents by spraying into the nasal cavities in a controlled, individualized dosage. Protect from light, and store at a temperature between 2° and 8°.
- **Labeling:** Label it to indicate that it is for intranasal administration only and to state the total number of discharges. Label it also to state that the dosage regulation is described in the package insert.
- **Uniformity of Unit Spray Weight and Total Number of Discharges per Container**

Samples: Three Nasal Spray Solution units

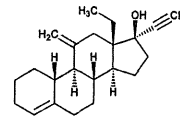
Analysis: Prime each spray pump as directed on the label, but NMT 5 times. Accurately weigh, by difference, 10 individual deliveries from each unit, weighing the first 3 discharges immediately after priming, 4 discharges from the middle of each unit, and 3 discharges close to the end of each unit. Continue to discharge until the unit is empty. For each unit, determine the total number of discharges, including the number of priming deliveries, and calculate the mean weight delivered per discharge.

Acceptance criteria: Each unit contains NLT the number of discharges stated on the label; the mean weight delivered per discharge is within 10% of the labeled weight per discharge; and NLT 9 tested discharges for each unit are between 85% and 125% of the labeled weight per discharge.

- **USP Reference Standards** ⟨11⟩

USP Desmopressin Acetate RS

Desogestrel



$C_{22}H_{30}O$ 310.47

18,19-Dinorpregn-4-en-20-yn-17-ol, 13-ethyl-11-methylene-, (17α)-;

13-Ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol [54024-22-5].

DEFINITION

Desogestrel contains NLT 98.0% and NMT 102.0% of desogestrel ($C_{22}H_{30}O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Mobile phase: Acetonitrile and water (73:27)

System suitability solution: 400 µg/mL of USP Desogestrel RS and 0.4 µg/mL each of USP Desogestrel Related Compound A RS and USP Desogestrel Related Compound D RS prepared as follows. Dissolve the material in acetonitrile equivalent to 50% of the volume of a suitable volumetric flask and dilute with water to volume.

Standard solution: 400 µg/mL of USP Desogestrel RS prepared as follows. Dissolve the material in acetonitrile equivalent to 50% of the volume of a suitable volumetric flask and dilute with water to volume.

Sample solution: 400 µg/mL of Desogestrel prepared as directed in the *Standard solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 15 µL

Run time: 1.5 times the retention time of desogestrel

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 1.3 between desogestrel and desogestrel related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Peak-to-valley ratio: NLT 2.0 between desogestrel and desogestrel related compound D, *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of desogestrel ($C_{22}H_{30}O$) in the portion of Desogestrel taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desogestrel from the *Sample solution*
r_S = peak response of desogestrel from the *Standard solution*
C_S = concentration of USP Desogestrel RS in the *Standard solution* (µg/mL)
C_U = concentration of Desogestrel in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, Chromatographic system, and **Sample solution:** Proceed as directed in the *Assay*.

Diluent: Acetonitrile and water (50:50)

Standard stock solution: 0.04 mg/mL each of USP Desogestrel RS, USP Desogestrel Related Compound B RS, and USP Desogestrel Related Compound C RS, and 0.08 mg/mL each of USP Desogestrel Related Compound A RS and USP Desogestrel Related Compound D RS prepared as follows. Dissolve the materials in acetonitrile equivalent to 50% of the volume of a suitable volumetric flask and dilute with water to volume.

Standard solution: 0.4 µg/mL each of USP Desogestrel RS, USP Desogestrel Related Compound B RS, and USP Desogestrel Related Compound C RS, and 0.8 µg/mL each of USP Desogestrel Related Compound A RS and USP Desogestrel Related Compound D RS in *Diluent*, from *Standard stock solution*

System suitability

Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 1.3 between desogestrel and desogestrel related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0% for each corresponding peak present in the *Standard solution*

Peak-to-valley ratio: NLT 2.0 between desogestrel and desogestrel related compound D, *System suitability solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of desogestrel related compound A, desogestrel related compound B, desogestrel related compound C, or desogestrel related compound D in the portion of Desogestrel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desogestrel related compound A, desogestrel related compound B, desogestrel related compound C, or desogestrel related compound D from the *Sample solution*
r_S = peak response of the corresponding Reference Standard from the *Standard solution*
C_S = concentration of USP Desogestrel Related Compound A RS, USP Desogestrel Related Compound B RS, USP Desogestrel Related Compound C RS, or USP Desogestrel Related Compound D RS in the *Standard solution* (µg/mL)
C_U = concentration of Desogestrel in the *Sample solution* (µg/mL)

Calculate the percentage of 11-methylene lynestrenol and any other individual unspecified impurity in the portion of Desogestrel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 11-methylene lynestrenol or any other individual unspecified impurity from the *Sample solution*
r_S = peak response of desogestrel from the *Standard solution*
C_S = concentration of USP Desogestrel RS in the *Standard solution* (µg/mL)
C_U = concentration of Desogestrel in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desogestrel related compound B	0.16	0.10
Desogestrel related compound C	0.19	0.1
11-Methylene lynestrenol[a]	0.71	0.2
Desogestrel related compound A	0.96	0.2
Desogestrel	1.0	—
Desogestrel related compound D	1.06	0.2
Any other individual unspecified impurity	—	0.10
Total impurities	—	0.5

[a] 11-Methylene-19-nor-17α-pregn-4-en-20-yn-17-ol.

SPECIFIC TESTS

• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL of Desogestrel in absolute alcohol
Acceptance criteria: +53° to +57° (dried substance)

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at a pressure not exceeding 15 mm of mercury at room temperature to constant weight.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Desogestrel RS
USP Desogestrel Related Compound A RS
13-Ethyl-11-methylene-18,19-dinor-5α,17α-pregn-3-en-20-yn-17-ol;
Desogestrel Δ3-isomer.
$C_{22}H_{30}O$ 310.47
USP Desogestrel Related Compound B RS
13-Ethyl-3-hydroxy-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol.
$C_{22}H_{30}O_2$ 326.48
USP Desogestrel Related Compound C RS
13-Ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol-3-one.
$C_{22}H_{28}O_2$ 324.46

www.webofpharma.com

USP Desogestrel Related Compound D RS
13-Ethyl-11-methylenegon-4-en-17-one.
$C_{20}H_{28}O$ 284.44

Desogestrel and Ethinyl Estradiol Tablets

DEFINITION
Desogestrel and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of desogestrel ($C_{22}H_{30}O$) and ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION
• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩
Standard solution: 0.15 mg/mL of USP Desogestrel RS and 0.03 mg/mL of USP Ethinyl Estradiol RS in ether
Sample solution: Transfer a number of Tablets, equivalent to 1.5 mg desogestrel and 0.3 mg ethinyl estradiol, to a suitable container, add 50 mL of water, and sonicate until the Tablets disintegrate (if necessary, remove any coating with water before sonication). Place the sample in a separatory funnel, add 25 mL of ether, and shake well to extract the actives. Using a glass pipet, transfer the ether layer to a clean beaker, and evaporate to about 10 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Application volume: 30 µL
Developing solvent system: Chloroform and alcohol (96:4)
Spray reagent: Methanol and sulfuric acid (1:1)
Analysis: Proceed as directed in the chapter, and then air-dry. Spray the plate with the *Spray reagent*, place in an oven at 105° for about 5 min, and examine the plate.
Acceptance criteria: Meet the requirements
• **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Buffer: 20 mM potassium phosphate buffer, pH 6.0
Mobile phase: Acetonitrile and *Buffer* (1:1)
Diluent: Acetonitrile and water (1:1)
Standard stock solution A: 0.3 mg/mL of USP Desogestrel RS in methanol
Standard stock solution B: 0.3 mg/mL of USP Ethinyl Estradiol RS in methanol
Standard solution: 0.6 µg/mL of USP Desogestrel RS and 0.12 µg/mL of USP Ethinyl Estradiol RS in *Diluent*, prepared by diluting appropriate aliquots of *Standard stock solution A* and *Standard stock solution B* with *Diluent*
Sample solution: Transfer 20 Tablets into a 200-mL volumetric flask. Add about 120 mL of *Diluent*, and shake for about 30 min. Dilute with *Diluent* to volume, and mix. Centrifuge a portion of the sample, and dilute with *Diluent* to obtain a solution nominally containing 0.6 µg/mL of desogestrel.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Desogestrel analysis: UV 210 nm
Ethinyl estradiol analysis: Spectrofluorometric detector, excitation at 285 nm and emission at 310 nm
Columns
Guard: 4.6-mm × 12.5-mm; packing L11
Analytical: 4.6-mm × 15-cm; packing L11
Flow rate: 2 mL/min
Injection volume: 200 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ethinyl estradiol and desogestrel are about 0.2 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0 for both ethinyl estradiol and desogestrel
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of desogestrel ($C_{22}H_{30}O$) and ethinyl estradiol ($C_{20}H_{24}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the relevant analyte from the *Sample solution*
r_S = peak response of the relevant analyte from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the relevant analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Test 1
Medium: 0.05% sodium lauryl sulfate with an assay content of NLT 95%; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
Buffer: 20 mM potassium phosphate buffer, pH 6.0
Mobile phase: Acetonitrile and *Buffer* (1:1)
Standard stock solution A: 0.005 mg/mL of USP Desogestrel RS in *Medium* prepared as follows. Dissolve a sufficient quantity of USP Desogestrel RS in methanol to obtain a solution containing 0.25 mg/mL of USP Desogestrel RS. Dilute 1.0 mL of this solution with *Medium* to 50.0 mL.
Standard stock solution B: 0.005 mg/mL of USP Ethinyl Estradiol RS in *Medium* prepared as follows. Dissolve a sufficient quantity of USP Ethinyl Estradiol RS in methanol to obtain a solution containing 0.25 mg/mL of USP Ethinyl Estradiol RS. Dilute 1.0 mL of this solution with *Medium* to 50.0 mL.
Standard solution: 0.3 µg/mL of USP Desogestrel RS and 0.06 µg/mL of USP Ethinyl Estradiol RS in *Medium*, from *Standard stock solution A* and *Standard stock solution B*
Sample solution: Sample per *Dissolution* ⟨711⟩. Centrifuge a portion of the dissolution sample, and use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Desogestrel analysis: UV 210 nm
Ethinyl estradiol analysis: Spectrofluorometric detector, excitation at 285 nm and emission at 310 nm
Columns
Guard: 4.6-mm × 12.5-mm; packing L11
Analytical: 4.6-mm × 15-cm; packing L11

USP Monographs

www.webofpharma.com

Flow rate: 2 mL/min
Injection volume: 200 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ethinyl estradiol and for desogestrel are about 0.2 and 1.0, respectively.]
Suitability requirements
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amounts of desogestrel ($C_{22}H_{30}O$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of the relevant analyte from the *Sample solution*
r_S = peak response of the relevant analyte from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of medium, 500 mL

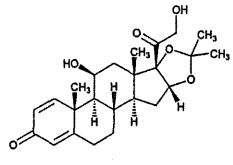
Tolerances: NLT 80% (Q) of each of the labeled amounts of desogestrel ($C_{22}H_{30}O$) and ethinyl estradiol ($C_{20}H_{24}O_2$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.3% sodium lauryl sulfate; 500 mL
Apparatus 2: 100 rpm
Time: 30 min
Analysis: Determine the amounts of desogestrel ($C_{22}H_{30}O$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved by the chromatographic method used in *Test 1*.
Tolerances: NLT 80% (Q) of each of the labeled amounts of desogestrel ($C_{22}H_{30}O$) and ethinyl estradiol ($C_{20}H_{24}O_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements for *Content Uniformity* for both desogestrel and ethinyl estradiol

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Desogestrel RS
USP Ethinyl Estradiol RS

Desonide



$C_{24}H_{32}O_6$ 416.51
Pregna-1,4-diene-3,20-dione, 11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (11β,16α)-;
11β,16α,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone [638-94-8].

DEFINITION

Desonide contains NLT 98.0% and NMT 102.0% of the labeled amount of desonide ($C_{24}H_{32}O_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
Use low-actinic glassware for all solutions containing desonide.
Solution A: 0.1% Phosphoric acid in water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
4	68	32
5	68	32
9	65	35
13	30	70
16	30	70

Diluent: *Solution A* and acetonitrile (60:40)
System suitability solution: 0.7 mg/mL of USP Desonide Impurities Mixture RS in *Diluent*
Standard solution: 0.7 mg/mL of USP Desonide RS in *Diluent*
Sample solution: 0.7 mg/mL of Desonide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 2.6-µm packing L7
Temperatures
Column: 20° (15°–22° was shown to be acceptable)
Autosampler: 20°
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between desonide glyoxal and deoxyprednisolone-16-ene; NLT 2.0 between desonide and dihydrodesonide, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of desonide ($C_{24}H_{32}O_6$) in the portion of Desonide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

USP Monographs

www.webofpharma.com

C_S = concentration of USP Desonide RS in the *Standard solution* (mg/mL)
C_U = concentration of Desonide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.10%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity solution: 0.28 µg/mL of USP Desonide RS in *Diluent* from the *Standard solution*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0 between desonide glyoxal and deoxyprednisolone-16-ene; NLT 2.0 between desonide and dihydrodesonide, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Desonide taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_T = total peak area from the *Sample solution*
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

[NOTE—Disregard peaks that are less than 0.04% of the desonide peak from the *Standard solution*.]

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
16α-Hydroxyprednisolone[a]	0.31	1.0	0.15
Prednisolone[b]	0.48	1.0	0.15
Desonide glyoxal[c]	0.76	1.0	0.50
Deoxyprednisolone-16-ene[d]	0.82	1.7	0.50
Sum of desonide glyoxal and deoxyprednisolone-16-ene	—	—	0.50
Desonide	1.00	—	—
Dihydrodesonide[e]	1.07	0.84	0.50
Epoxydesonide[f]	1.18	1.0	0.50
Sum of dihydrodesonide and epoxydesonide	—	—	0.50
Bromodesonide[g]	1.43	0.68	0.15
Acetyldesonide[h]	1.74	0.82	0.15
Any other impurity	—	1.0	0.10

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	1.0

[a] 11β,16α,17,21-Tetrahydroxy-3,20-dioxopregna-1,4-diene.
[b] 11β,17,21-Trihydroxy-3,20-dioxopregna-1,4-diene.
[c] 11β-Hydroxy-16α,17-[(1-methylethylidene)bis(oxy)]-3,20-dioxopregna-1,4-dien-21-al.
[d] 11β,21-Dihydroxypregna-1,4,16-triene-3,20-dione.
[e] 11β,21-Dihydroxy-16α,17-[(1-methylethylidene)bis(oxy)]pregn-4-ene-3,20-dione.
[f] 9,11β-Epoxy-21-hydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione.
[g] 9α-Bromo-11β,21-dihydroxy-16α,17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-dione.
[h] 11β-Hydroxy-16α,17-[(1-methylethylidene)bis(oxy)]-3,20-dioxopregna-1,4-dien-21-yl acetate.

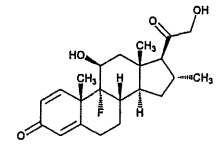
SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
  Sample: 1.0 g
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 0.5%
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample: 10 mg/mL in dioxane
  Acceptance criteria: +104.0° to +110.0°, calculated on the dried basis

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature. Protect from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Desonide RS
  USP Desonide Impurities Mixture RS
  This is a mixture that contains desonide and may contain about 1% each of 16α-hydroxyprednisolone, prednisolone, desonide glyoxal, deoxyprednisolone-16-ene, dihydrodesonide, epoxydesonide, bromodesonide, and acetyldesonide.

Desoxymetasone



$C_{22}H_{29}FO_4$ 376.46

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-dihydroxy-16-methyl-, (11β,16α)-;
9-Fluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione [382-67-2].

DEFINITION

Desoxymetasone contains NLT 97.0% and NMT 103.0% of desoxymetasone ($C_{22}H_{29}FO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020) ▲or ⟨197A⟩▲ (USP 1-Dec-2019)

www.webofpharma.com

Delete the following:

▲• **B. THIN-LAYER CHROMATOGRAPHY**
Diluent: Chloroform and alcohol (3:1)
Standard solution: 10 mg/mL of USP Desoximetasone RS in *Diluent*
Sample solution: 10 mg/mL of desoximetasone in *Diluent*
Chromatographic system
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: Chloroform and ethyl acetate (1:1)
Spray reagent: 200 mg/mL of *p*-toluenesulfonic acid in alcohol
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry, and develop the chromatogram in a saturated chamber containing the *Developing solvent system*. Allow the solvent front to move 10 cm beyond the application point. After drying, examine the plate under UV light at 254 nm. Spray the dried plate with *Spray reagent*.
Acceptance criteria: The major spot from the *Sample solution* corresponds in R_F value (0.25) and appearance to that obtained from the *Standard solution*.▲ (USP 1-Dec-2019)

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

• **PROCEDURE**
▲**Buffer:** 1.36 g/L of monobasic potassium phosphate
Solution A: Acetonitrile, tetrahydrofuran, and *Buffer* (23.3: 1.5: 75). Adjust with 10% phosphoric acid to a pH of 4.0.
Solution B: Acetonitrile and *Buffer* (49.6: 50). Adjust with 10% phosphoric acid to a pH of 4.0.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
25	5	95
30	5	95
31	95	5
35	95	5

System suitability stock solution: 0.12 mg/mL of USP Desoximetasone Related Compound A RS prepared as follows. Transfer a suitable amount of USP Desoximetasone Related Compound A RS to a suitable volumetric flask. Add about 1 mL of tetrahydrofuran and swirl to dissolve. Dilute with *Solution A* to volume. Sonication may be used to aid dissolution.
System suitability solution: 1 mg/mL of USP Desoximetasone RS and 1.2 µg/mL of USP Desoximetasone Related Compound A RS prepared as follows. Transfer a suitable amount of USP Desoximetasone RS to a suitable volumetric flask. Add about 1 mL of tetrahydrofuran and swirl to dissolve. Add 1% of the flask volume of the *System suitability stock solution* and dilute with *Solution A* to volume. Sonication may be used to aid dissolution.
Standard solution: 0.1 mg/mL of USP Desoximetasone RS prepared as follows. Transfer a suitable amount of USP Desoximetasone RS to a suitable volumetric flask. Add about 1 mL of tetrahydrofuran and swirl to dissolve. Dilute with *Solution A* to volume. Sonication may be used to aid dissolution.
Sample solution: 0.1 mg/mL of Desoximetasone prepared as follows. Transfer a suitable amount of Desoximetasone to a suitable volumetric flask. Add about 1 mL of tetrahydrofuran and swirl to dissolve. Dilute with *Solution A* to volume. Sonication may be used to aid dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between desoximetasone and desoximetasone related compound A, *System suitability solution*
Relative standard deviation: NMT 1.10%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of desoximetasone ($C_{22}H_{29}FO_4$) in the portion of Desoximetasone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Desoximetasone RS in the *Standard solution* (mg/mL)
C_U = concentration of Desoximetasone in the *Sample solution* (mg/mL)▲ (USP 1-Dec-2019)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

Add the following:

▲• **ORGANIC IMPURITIES**
Buffer, Solution A, Solution B, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.001 mg/mL of USP Desoximetasone RS prepared as follows. Transfer a suitable amount of USP Desoximetasone RS to a suitable volumetric flask. Add about 1 mL of tetrahydrofuran and swirl to dissolve. Dilute with *Solution A* to volume. Sonication may be used to aid dissolution.
Sensitivity solution: 0.2 µg/mL of USP Desoximetasone RS in *Solution A* from the *Standard solution*
Sample solution: 1 mg/mL of Desoximetasone prepared as follows. Transfer a suitable amount of Desoximetasone to a suitable volumetric flask. Add about 1 mL of

www.webofpharma.com

tetrahydrofuran and swirl to dissolve. Dilute with *Solution A* to volume. Sonication may be used to aid dissolution.

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between desoximetasone and desoximetasone related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Desoximetasone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of desoximetasone from the *Standard solution*

C_S = concentration of USP Desoximetasone RS in the *Standard solution* (mg/mL)

C_U = concentration of Desoximetasone in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.02%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desoximetasone diacetal[a]	0.88	0.15
Desoximetasone	1.00	—
Desoximetasone related compound A	1.10	0.15
Desoximetasone acid[b]	1.18	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

[a] 9-Fluoro-11β,21,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione.
[b] 9-Fluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid. ▲(USP 1-Dec-2019)

SPECIFIC TESTS

Delete the following:

▲• **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 206°–218°; but the range between beginning and end of melting does not exceed 4° ▲(USP 1-Dec-2019)

Change to read:

• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 5 mg/mL of Desoximetasone in ▲alcohol▲ (USP 1-Dec-2019)

Acceptance criteria: ▲+123° to +129°▲ (USP 1-Dec-2019)

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° to constant weight.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Desoximetasone RS

▲USP Desoximetasone Related Compound A RS

Dihydrodesoximetasone;

9-Fluoro-11β,21-dihydroxy-16α-methylpregna-4-ene-3,20-dione.

$C_{22}H_{31}FO_4$ 378.48▲ (USP 1-Dec-2019)

Desoximetasone Cream

DEFINITION

Desoximetasone Cream is Desoximetasone in an emollient cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of desoximetasone ($C_{22}H_{29}FO_4$).

IDENTIFICATION

• **A.** The retention time of the desoximetasone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol, glacial acetic acid, and water (65:1:35)

Internal standard solution: 0.04 mg/mL of ethylparaben in methanol

Standard stock solution: 0.4 mg/mL of USP Desoximetasone RS in methanol

Standard solution: 0.05 mg/mL of USP Desoximetasone RS and 0.01 mg/mL of ethylparaben from the *Standard stock solution* and *Internal standard solution*, respectively, in methanol

Sample solution: Transfer an amount of Cream, nominally equivalent to 2 mg of desoximetasone, to a 50-mL centrifuge tube, and add a few 3-mm glass beads. Add 10.0 mL of *Internal standard solution* and 30 mL of methanol. Tightly cap the centrifuge tube, and immerse it for 10 min in a bath maintained at 65°. Remove the tube from the bath, and immediately vortex at high speed for 30 s. Return the tube to the hot water bath for 5 min, remove it from the bath, and immediately vortex for 30 s. Repeat the procedure, then cool the tube in an ice-bath held at 10° until no further flocculent precipitation occurs. Centrifuge, and use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ethylparaben and desoximetasone are about 1 and 2, respectively.]

Suitability requirements

Resolution: NLT 2.0 between desoximetasone and ethylparaben

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of desoximetasone ($C_{22}H_{29}FO_4$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of desoximetasone to ethylparaben from the *Sample solution*
R_S = peak response ratio of desoximetasone to ethylparaben from the *Standard solution*
C_S = concentration of USP Desoximetasone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of desoximetasone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Minimum Fill** ⟨755⟩: Meets the requirements

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: Add 15 mL of boiling water to 3.5 g of Cream in a 50-mL centrifuge tube, cap the tube, shake vigorously until the Cream is uniformly dispersed, then place the tube in a steam bath until the water and oil layers separate completely. Cool and separate the layers.
Analysis: Determine the pH of the aqueous phase.
Acceptance criteria: 4.0–8.0

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in collapsible tubes, at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Desoximetasone RS

Desoximetasone Gel

DEFINITION
Desoximetasone Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of desoximetasone ($C_{22}H_{29}FO_4$).

IDENTIFICATION
• **A.** The retention time of the desoximetasone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: Methanol, glacial acetic acid, and water (65:1:35). Adjust the ratio, if necessary, so that the retention time of desoximetasone is about 8 min.
Diluent: 15 mg/mL of calcium chloride in methanol
Standard stock solution: 0.5 mg/mL of USP Desoximetasone RS in methanol
Standard solution: 0.025 mg/mL of USP Desoximetasone RS from the *Standard stock solution* in *Diluent*
Sample solution: Nominally 0.025 mg/mL of desoximetasone in *Diluent* prepared as follows. Transfer an adequate amount of Gel to a suitable volumetric flask. Add about 60% of the flask volume of *Diluent* and sonicate to disperse the Gel. Dilute with *Diluent* to volume and centrifuge. Use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of desoximetasone ($C_{22}H_{29}FO_4$) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desoximetasone from the *Sample solution*
r_S = peak response of desoximetasone from the *Standard solution*
C_S = concentration of USP Desoximetasone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of desoximetasone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS
• **Alcohol Content**
Sample solution: 50 mg/mL of Gel in methanol
Analysis: Determine the alcohol content of the *Sample solution* by proceeding as directed in *Alcohol Determination* ⟨611⟩, *Method II—Gas Chromatographic Method*, using isopropyl alcohol as the internal standard and methanol in place of water as the solvent.
Acceptance criteria: 18.0%–24.0% (w/w)

PERFORMANCE TESTS
• **Minimum Fill** ⟨755⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in collapsible tubes, at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Desoximetasone RS

Desoximetasone Ointment

DEFINITION
Desoximetasone Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of desoximetasone ($C_{22}H_{29}FO_4$).

IDENTIFICATION
• **A.** The retention time of the desoximetasone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Mobile phase: Methanol, glacial acetic acid, and water (65:1:35)
Diluent: Methanol and spectrophotometric acetonitrile saturated with *n*-heptane (1:1)
Standard stock solution: 0.4 mg/mL of USP Desoximetasone RS in methanol
Standard solution: 0.04 mg/mL of USP Desoximetasone RS from the *Standard stock solution* in *Diluent*
Sample solution: Nominally 0.04 mg/mL of desoximetasone in *Diluent* prepared as follows. Transfer an amount of Ointment nominally equivalent to 2 mg of

USP Monographs

www.webofpharma.com

desoximetasone to a 50-mL centrifuge tube. Add 20 mL of *n*-heptane that has been previously saturated with spectrophotometric acetonitrile, and heat gently with occasional shaking until the Ointment is completely dispersed. Allow to cool slightly, and extract with a 10-mL portion of spectrophotometric acetonitrile. Shake vigorously, centrifuge, remove the bottom layer of spectrophotometric acetonitrile with a syringe and needle, and transfer to a 50-mL volumetric flask. Using the same needle and syringe, extract the desoximetasone with successive 10- and 8-mL portions of spectrophotometric acetonitrile, combining all spectrophotometric acetonitrile layers in the 50-mL flask. Dilute with methanol nearly to volume, and allow the solution to reach room temperature. Dilute with methanol to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between the desoximetasone and solvent peaks
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of desoximetasone ($C_{22}H_{29}FO_4$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desoximetasone from the *Sample solution*
r_S = peak response of desoximetasone from the *Standard solution*
C_S = concentration of USP Desoximetasone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of desoximetasone in the *Sample solution* (mg/mL)

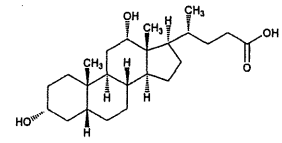
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Minimum Fill** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in collapsible tubes at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Desoximetasone RS

Desoxycholic Acid

(Title for this monograph—not to change until December 1, 2021)
(Prior to December 1, 2021, the current practice of labeling the article of commerce with the name Desoxycholic Acid may be continued. Use of the name Deoxycholic Acid will be permitted as of December 1, 2016; however, the use of this name will not be mandatory until December 1, 2021. The 60-month extension will provide the time needed by manufacturers and users to make necessary changes.)



$C_{24}H_{40}O_4$ 392.58
Cholan-24-oic acid, 3,12-dihydroxy-, (3α,5β,12α)-; 3α,12α-Dihydroxy-5β-cholan-24-oic acid [83-44-3].

DEFINITION
Desoxycholic Acid contains NLT 97.0% and NMT 103.0% of desoxycholic acid ($C_{24}H_{40}O_4$), calculated on the anhydrous basis. Desoxycholic Acid can be of animal or synthetic origin. FDA has not approved the use of animal-derived Desoxycholic Acid as a drug substance.

Change to read:

IDENTIFICATION
• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
• **B.** The retention time of the desoxycholic acid peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Diluent: Methanol and water (80:20)
Solution A: 0.1% formic acid in water
Solution B: 0.1% formic acid in acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	75.0	25.0
2.0	55.0	45.0
14.0	42.0	58.0
24.0	0.0	100.0
35.0	0.0	100.0

Standard solution: 0.01 mg/mL of USP Deoxycholic Acid RS in *Diluent*
Sample solution: 0.01 mg/mL of Desoxycholic Acid in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Charged aerosol
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 25 µL
Run time: 35 min

System suitability
Sample: *Standard solution*
[Note—The retention time of desoxycholic acid is about 13.0 min.]
Suitability requirements
Relative standard deviation: NMT 3.0% for six injections

Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of desoxycholic acid ($C_{24}H_{40}O_4$) in the portion of Desoxycholic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area of desoxycholic acid from the *Sample solution*
r_S = peak area of deoxycholic acid (desoxycholic acid) from the *Standard solution*
C_S = concentration of the USP Deoxycholic Acid RS in the *Standard solution*
C_U = concentration of Desoxycholic Acid in the *Sample solution*
P = labeled purity of USP Deoxycholic Acid RS (%)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Sample: 1.0 g
Acceptance criteria: NMT 0.2%

• **LIMIT OF LEAD**
[NOTE—This test and *Acceptance criteria* are only applicable to Desoxycholic Acid of animal origin. Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 50% nitric acid followed by water.]
Standard solutions: [NOTE—Prepare these solutions on the day of use.] Transfer 10.0 and 50.0 mL of standard lead solution TS into two separate 100-mL volumetric flasks, add 10 mL of 3 N hydrochloric acid to each, and dilute with water to volume. The third standard, 10.0 µg/mL, is taken directly from standard lead solution TS.
Sample solution: Transfer 10.0 g of Desoxycholic Acid, weighed to the nearest 0.1 mg, into an evaporating dish. Add 5 mL of 25% sulfuric acid (made by adding 25 mL of sulfuric acid to 75 mL of water), and distribute the 25% sulfuric acid solution uniformly. Within a hood, place the dish on a steam bath to evaporate most of the water. Place the dish on a burner, and slowly pre-ash the remaining *Sample solution* by expelling most of the sulfuric acid. Place the dish in a muffle furnace that has been set at 525°, and ash the contents of the dish until the residue appears free from carbon. Cool, and cautiously wash down the inside of the evaporation dish with water. Add 5 mL of 1 N hydrochloric acid. Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 N hydrochloric acid and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer to a 10-mL volumetric flask, dilute with water to volume, and mix.
Sample blank: Prepare by ashing 5 mL of 25% sulfuric acid solution. Cool, and cautiously wash down the inside of the evaporation dish with water. Add 5 mL of 1 N hydrochloric acid. Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 N hydrochloric acid and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer to a 10-mL volumetric flask, dilute with water to volume, and mix.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption
Analytical wavelength: 283.3 nm
Lamp: Lead electrodeless discharge
Flame: Air–acetylene
Slit width: 0.7 nm
Instrument blank: Water
Standard curve
Samples: *Standard solutions* and *Sample blank*
Plot: Corrected absorbance values versus their corresponding concentration (µg/mL). [NOTE—Determine corrected absorbance values by subtracting the absorbance of the *Sample blank* from the absorbance of the *Standard solutions*.]
Analysis
Samples: *Sample solution* and *Sample blank*
[NOTE—Determine corrected absorbance values by subtracting the absorbance of the *Sample blank* from the absorbance of the *Sample solution*.]
From the *Standard curve*, determine the lead concentration in the *Sample solution*.
Calculate the lead content in the portion of Desoxycholic Acid taken:

$$\text{Result} = (C_S \times V)/W$$

C_S = concentration of lead from the *Standard curve* (µg/mL)
V = final volume of the sample (mL)
W = weight of the sample taken (g)

Acceptance criteria: NMT 4 µg/g

• **ORGANIC IMPURITIES**
Diluent, Mobile phase, Standard solution, and **Chromatographic system:** Proceed as described in the procedure for *Assay*.
Sensitivity solution: 0.5 µg/mL of USP Deoxycholic Acid RS in *Diluent*
Cholic acid solution: 0.01 mg/mL of USP Cholic Acid RS in *Diluent*
Sample stock solution: 1.0 mg/mL of Desoxycholic Acid in *Diluent*
Sample solution: 0.01 mg/mL of Desoxycholic Acid in *Diluent* prepared from the *Sample stock solution*
System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—The retention time of desoxycholic acid is about 13.0 min. The relative retention times for cholic acid and desoxycholic acid are 0.56 and 1, respectively. The relative retention times for other impurities are listed in *Table 2*.]
Suitability requirements
Relative standard deviation: NMT 3.0% for six injections, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Cholic acid solution, Sample solution,* and *Sample stock solution*
Calculate the percentage of each impurity in the portion of Desoxycholic Acid taken:

$$\text{Result} = [r_U/(r_S \times 100 + r_T)] \times 100$$

r_U = peak area of individual impurity from the *Sample stock solution*
r_S = peak area of desoxycholic acid from the *Sample solution*
r_T = sum of peak areas of all impurities from the *Sample stock solution*

Acceptance criteria
Desoxycholic Acid of animal origin
Cholic acid: NMT 1.0%
Total impurities: NMT 2.0%
Desoxycholic Acid of synthetic origin: See *Table 2*.

www.webofpharma.com

Table 2

Name	Relative Retention Time	Acceptance Criteria NMT (%)
3α,12β-Dihydroxy-5β-cholan-24-oic acid	0.69	0.15
3α,12α-Dihydroxy-5β-chol-9(11)-en-24-oic acid	0.87	0.15
Ethyl 3α,12α-dihydroxy-5β-cholan-24-oate	1.61	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 1% (w/v) solution in ethanol
  Acceptance criteria
  Desoxycholic Acid of animal origin: NLT +55°
  Desoxycholic Acid of synthetic origin: +50° to +60°
- **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ic*: NMT 1.0%
- **CLARITY OF SOLUTION**
  [NOTE—If intended for use in preparing parenteral dosage forms, it meets the requirements of the *Clarity of Solution* test.]
  Hydrazine solution: 10 mg/mL of hydrazine sulfate. Allow to stand 4–6 h before use.
  Methenamine solution: Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.
  Primary opalescent suspension: To the flask containing *Methenamine solution* add 25.0 mL of the *Hydrazine solution*, mix, and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.
  Opalescence standard: Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. Use this suspension within 24 h after preparation.
  Reference suspension: Transfer 5.0 mL of the *Opalescence standard* to a 100-mL volumetric flask, and dilute with water to volume.
  Sample solution: 5 mg/mL of Desoxycholic Acid in 0.1 N sodium hydroxide
  Analysis: Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer a portion of the *Reference suspension* to a separate matching test tube. Compare the *Sample solution* and the *Reference suspension* in diffused daylight, viewing vertically against a black background (see *Visual Comparison* ⟨630⟩) for 5 min after preparation of the *Reference suspension*.
  Acceptance criteria: The *Sample solution* is not more opalescent than the *Reference suspension*.
- **COLOR OF SOLUTION**
  [NOTE—If intended for use in preparing parenteral dosage forms, it meets the requirements of the *Color of Solution* test.]
  Diluent: 27.5 mL of hydrochloric acid in 1000 mL of water
  Standard stock solutions: Prepare two solutions, A and B, containing, respectively, the following parts of ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and *Diluent*.
  Standard stock solution A: 2.4: 0.6: 0: 7.0
  Standard stock solution B: 2.4: 1.0: 0.4: 6.2
  Standard solutions
  [NOTE—Prepare and use these solutions immediately.]
  Standard solution A: Transfer 2.5 mL of *Standard stock solution A* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.
  Standard solution B: Transfer 2.5 mL of *Standard stock solution B* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.
  Sample solution: 5 mg/mL of Desoxycholic Acid in 0.1 N sodium hydroxide
  Analysis: Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm, to obtain a depth of 40 mm. Similarly transfer portions of *Standard solution A* and *Standard solution B* to separate matching test tubes. Compare the color of the *Sample solution* with that of *Standard solution A* and *Standard solution B* in diffused daylight, viewing vertically against a white background (see *Visual Comparison* ⟨630⟩).
  Acceptance criteria: The *Sample solution* is not more intensely colored than *Standard solution A* and *Standard solution B*.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: If labeled for use in preparing parenteral dosage forms, it meets the requirements of NMT 240 Endotoxin Units/g.
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: If labeled for use in preparing parenteral dosage forms, the total aerobic microbial count does not exceed 100 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. No storage requirements specified.
- **LABELING:** Label it to indicate whether Desoxycholic Acid is derived from an animal or synthetic source. Desoxycholic Acid intended for use in preparing parenteral dosage forms is so labeled.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cholic Acid RS
  USP Deoxycholic Acid RS
  USP Endotoxin RS

Desoxycorticosterone Pivalate

$C_{26}H_{38}O_4$ 414.58

Pregn-4-ene-3,20-dione, 21-(2,2-dimethyl-1-oxopropoxy)-.
11-Deoxycorticosterone pivalate [808-48-0].

» Desoxycorticosterone Pivalate contains not less than 97.0 percent and not more than 103.0 percent of $C_{26}H_{38}O_4$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Desoxycorticosterone Pivalate RS

www.webofpharma.com

Identification—

Change to read:

A: ▲Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197K▲ (CN 1-May-2020)

Change to read:

B: ▲Spectroscopic Identification Tests ⟨197⟩, Ultraviolet-Visible Spectroscopy: 197U▲ (CN 1-May-2020)
Solution: 20 µg per mL.
Medium: methanol.
Absorptivities at 241 nm, calculated on the dried basis, do not differ by more than 3.0%.
Melting range ⟨741⟩**:** between 200° and 206°.
Specific rotation ⟨781S⟩**:** between +155° and +163°.
Test solution: 10 mg per mL, in dioxane.
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of methanol and water (4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Transfer about 100 mg of desoxycorticosterone acetate to a 50-mL volumetric flask, add methanol to volume, and mix.
Standard preparation—Transfer about 12.5 mg of USP Desoxycorticosterone Pivalate RS, accurately weighed, to a 25-mL volumetric flask, add 20 mL of methanol, and mix. Add 2.5 mL of *Internal standard solution,* dilute with methanol to volume, and mix to obtain a solution having a known concentration of about 0.5 mg of USP Desoxycorticosterone Pivalate RS per mL.
Assay preparation—Transfer about 50 mg of Desoxycorticosterone Pivalate, accurately weighed, to a 100-mL volumetric flask, add 80 mL of methanol, and mix. Add 10.0 mL of *Internal standard solution,* dilute with methanol to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the analyte and internal standard peaks is not less than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.
Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for desoxycorticosterone acetate and 1.0 for desoxycorticosterone pivalate. Calculate the quantity, in mg, of $C_{26}H_{38}O_4$ in the portion of Desoxycorticosterone Pivalate taken by the formula:

$$100C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Desoxycorticosterone Pivalate RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Desoxycorticosterone Pivalate Injectable Suspension

DEFINITION
Desoxycorticosterone Pivalate Injectable Suspension is a sterile suspension of Desoxycorticosterone Pivalate in an aqueous medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of desoxycorticosterone pivalate ($C_{26}H_{38}O_4$).

IDENTIFICATION
• **A.**
Sample: Centrifuge a portion of Injectable Suspension, decant the supernatant, wash the residue by stirring with several successive portions of water, centrifuging and decanting each time, and finally dry the residue at 105°. The residue so obtained meets the following requirements.
Analysis 1: Melting point
Acceptance criteria 1: Melts at 198°–206°
Analysis 2: Dissolve 5 mg in 2 mL of sulfuric acid.
Acceptance criteria 2: The solution is yellowish, with a greenish fluorescence.
Analysis 3: Dilute the solution obtained from *Analysis 2* with 2 mL of water.
Acceptance criteria 3: The color changes to a dark red-blue, and on further dilution with 2 mL of water, the color is discharged.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol and water (4:1)
Internal standard solution: 2 mg/mL of desoxycorticosterone acetate in methanol
Standard solution: 0.5 mg/mL of USP Desoxycorticosterone Pivalate RS in methanol, prepared as follows. Transfer 12.5 mg of USP Desoxycorticosterone Pivalate RS to a 25-mL volumetric flask, and add 20 mL of methanol. Add 2.5 mL of the *Internal standard solution,* and dilute with methanol to volume.
Sample solution: Nominally 0.5 mg/mL of desoxycorticosterone pivalate in methanol, prepared as follows. Transfer a nominal equivalent of 125 mg of desoxycorticosterone pivalate from Injectable Suspension to a 250-mL volumetric flask. Add 200 mL of methanol, and sonicate to dissolve. Add 25.0 mL of *Internal standard solution,* and dilute with methanol to volume. Centrifuge a 20-mL portion at high speed for 5 min. Filter the supernatant through a 5-µm disk, discarding the first 5 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1.5 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for desoxycorticosterone acetate and for desoxycorticosterone pivalate are about 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between desoxycorticosterone acetate and desoxycorticosterone pivalate
Relative standard deviation: NMT 1.5% for the peak response ratio of desoxycorticosterone pivalate to the internal standard
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of the labeled amount of desoxycorticosterone pivalate ($C_{26}H_{38}O_4$) in the portion of Injectable Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of desoxycorticosterone pivalate to the internal standard from the *Sample solution*
R_S = peak response ratio of desoxycorticosterone pivalate to the internal standard from the *Standard solution*
C_S = concentration of USP Desoxycorticosterone Pivalate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of desoxycorticosterone pivalate in the *Sample solution* (mg/mL)

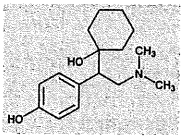
Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 5.0–7.0
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.
- **Bacterial Endotoxins Test** ⟨85⟩: It contains NMT 2.78 USP Endotoxin Units/mg of desoxycorticosterone pivalate.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.
- **Labeling:** Label Suspension to indicate that it is for veterinary use only.
- **USP Reference Standards** ⟨11⟩
  USP Desoxycorticosterone Pivalate RS

Add the following:

▲Desvenlafaxine



$C_{16}H_{25}NO_2$ 263.38
4-[2-(Dimethylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol;
1-[2-(Dimethylamino)-1-(4-hydroxyphenyl)ethyl] cyclohexanol [93413-62-8].

DEFINITION
Desvenlafaxine contains NLT 98.0% and NMT 102.0% of desvenlafaxine ($C_{16}H_{25}NO_2$), calculated on the dried basis.

IDENTIFICATION
- **A. Infrared Absorption** ⟨197⟩: [Note—Methods described in ⟨197K⟩ or ⟨197A⟩ may be used.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**

Solution A: 4 g/L of ammonium bicarbonate in water adjusted with phosphoric acid to a pH of 7.0
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	55	45
25	35	65
30	35	65
32	55	45
40	55	45

Diluent: Acetonitrile and water (90:10)
System suitability solution: 0.701 mg/mL of USP Desvenlafaxine RS, 0.001 mg/mL of USP Desvenlafaxine Related Compound A RS, and 0.0035 mg/mL of USP Venlafaxine Hydrochloride RS in *Diluent*
Standard solution: 0.7 mg/mL of USP Desvenlafaxine RS in *Diluent*
Sample solution: 0.7 mg/mL of Desvenlafaxine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm x 25-cm; 5.0-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 3.5 between desvenlafaxine related compound A and venlafaxine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of desvenlafaxine ($C_{16}H_{25}NO_2$) in the portion of Desvenlafaxine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Desvenlafaxine RS in the *Standard solution* (mg/mL)
C_U = concentration of Desvenlafaxine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.20%
- **Organic Impurities**

Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.0007 mg/mL of USP Desvenlafaxine RS, 0.001 mg/mL of USP Desvenlafaxine Related Compound A RS, and 0.0011 mg/mL of USP Venlafaxine Hydrochloride RS (equivalent to 0.001 mg/mL of venlafaxine) in *Diluent*
Sensitivity solution: 0.35 µg/mL of USP Desvenlafaxine RS from the *Standard solution* in *Diluent*

www.webofpharma.com

System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 3.5 between desvenlafaxine related compound A and venlafaxine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of desvenlafaxine related compound A in the portion of Desvenlafaxine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desvenlafaxine related compound A from the *Sample solution*
r_S = peak response of desvenlafaxine related compound A from the *Standard solution*
C_S = concentration of USP Desvenlafaxine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Desvenlafaxine in the *Sample solution* (mg/mL)

Calculate the percentage of venlafaxine in the portion of Desvenlafaxine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of venlafaxine from the *Sample solution*
r_S = peak response of venlafaxine from the *Standard solution*
C_S = concentration of USP Venlafaxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Desvenlafaxine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of venlafaxine, 277.40
M_{r2} = molecular weight of venlafaxine hydrochloride, 313.86

Calculate the percentage of any individual impurity in the portion of Desvenlafaxine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of desvenlafaxine from the *Standard solution*
C_S = concentration of USP Desvenlafaxine RS in the *Standard solution* (mg/mL)
C_U = concentration of Desvenlafaxine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

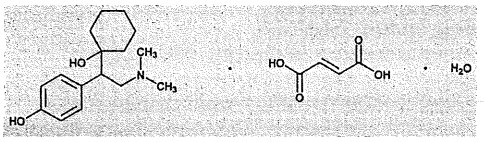
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desvenlafaxine	1.0	—
Desvenlafaxine related compound A	1.8	0.15
Venlafaxine	2.4	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	0.50

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.50%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Desvenlafaxine RS
USP Desvenlafaxine Related Compound A RS
4-[1-(Cyclohex-1-en-1-yl)-2-(dimethylamino)ethyl] phenol.
$C_{16}H_{23}NO$ 245.37
USP Venlafaxine Hydrochloride RS▲ (USP 1-Aug-2019)

Add the following:

▲Desvenlafaxine Fumarate



$C_{16}H_{25}NO_2 \cdot C_4H_4O_4 \cdot H_2O$ 397.46
$C_{16}H_{25}NO_2 \cdot C_4H_4O_4$ 379.45
trans-Butenedioic acid, compound with 4-[2-(dimethylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol (1:1), monohydrate;
1-[2-(Dimethylamino)-1-(4-hydroxyphenyl)ethyl] cyclohexanol hydrogen (*E*)-but-2-enedioate monohydrate [313471-75-9].
Anhydrous [93414-04-1].

DEFINITION
Desvenlafaxine Fumarate contains NLT 98.0% and NMT 102.0% of desvenlafaxine fumarate ($C_{16}H_{25}NO_2 \cdot C_4H_4O_4$), calculated on the anhydrous basis.

IDENTIFICATION
• **A. INFRARED ABSORPTION** ⟨197⟩: [NOTE—Methods described in ⟨197K⟩ or ⟨197A⟩ may be used.]
• **B.** The retention times of the desvenlafaxine and fumaric acid peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: To each liter of water, add 1 mL of triethylamine and adjust with phosphoric acid to a pH of 3.0.
Solution A: Acetonitrile and *Buffer* (10:90)
Solution B: Acetonitrile and *Buffer* (60:40)

www.webofpharma.com

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
4	70	30
4.1	10	90
4.5	10	90
4.6	80	20
7	80	20

Diluent: *Solution A* and *Solution B* (50:50)
Standard solution: 0.05 mg/mL of USP Desvenlafaxine Fumarate RS in *Diluent*. Sonication may be used to promote dissolution.
Sample solution: 0.05 mg/mL of Desvenlafaxine Fumarate in *Diluent*. Sonication may be used to promote dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 2.1-mm × 15-cm; 1.7-µm packing L1
Column temperature: 55°
Flow rate: 0.3 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for fumaric acid and desvenlafaxine are 0.48 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of desvenlafaxine fumarate ($C_{16}H_{25}NO_2 \cdot C_4H_4O_4$) in the portion of Desvenlafaxine Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desvenlafaxine from the *Sample solution*
r_S = peak response of desvenlafaxine from the *Standard solution*
C_S = concentration of USP Desvenlafaxine Fumarate RS in the *Standard solution* (mg/mL)
C_U = concentration of Desvenlafaxine Fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	95	5
5	90	10
15	55	45
27	50	50
27.1	95	5
30	95	5

System suitability solution: 1 mg/mL of USP Desvenlafaxine Fumarate RS and 0.0015 mg/mL of USP Desvenlafaxine Related Compound B RS in *Diluent*
Sensitivity solution: 0.5 µg/mL of USP Desvenlafaxine Fumarate RS, equivalent to 0.3 µg/mL of desvenlafaxine, in *Diluent*
Standard solution: 0.001 mg/mL of USP Desvenlafaxine Fumarate RS, equivalent to 0.0006 mg/mL of desvenlafaxine, in *Diluent*
Sample solution: 1 mg/mL of Desvenlafaxine Fumarate in *Diluent*
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[NOTE—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 0.8 between desvenlafaxine related compound B and desvenlafaxine, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Desvenlafaxine Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of desvenlafaxine fumarate from the *Standard solution*
C_S = concentration of USP Desvenlafaxine Fumarate RS in the *Standard solution* (mg/mL)
C_U = concentration of Desvenlafaxine Fumarate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. The reporting threshold is 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fumaric acid[a]	0.28	—	—
Desvenlafaxine related compound B	0.95	1.2	0.15
Desvenlafaxine	1.0	—	—
Desvenlafaxine benzyl ether[b]	4.9	1.7	0.15

www.webofpharma.com

Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] Included for identification only. This peak is due to the fumarate counterion; hence it is not an impurity.
[b] 1-(1-[4-(Benzyloxy)phenyl]-2-(dimethylamino)ethyl)cyclohexan-1-ol.

SPECIFIC TESTS

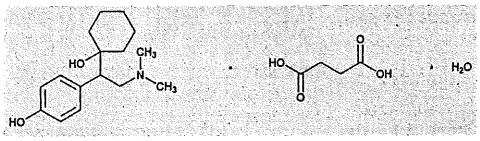
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 10 mg/mL of Desvenlafaxine Fumarate in methanol
  Acceptance criteria: −0.5° to 0.5° on the anhydrous basis
- **pH** ⟨791⟩
  Sample solution: 10 mg/mL of Desvenlafaxine Fumarate in water
  Acceptance criteria: 3.0–5.0
- **WATER DETERMINATION** ⟨921⟩, *Method I:* 4.0%–6.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Desvenlafaxine Fumarate RS
  USP Desvenlafaxine Related Compound B RS
  4-[1-(1-Hydroxycyclohexyl)-2-(methylamino)ethyl] phenol.
  $C_{15}H_{23}NO_2$ 249.35▲ (USP 1-Aug-2019)

Add the following:

▲Desvenlafaxine Succinate



$C_{16}H_{25}NO_2 \cdot C_4H_6O_4 \cdot H_2O$ 399.48

$C_{16}H_{25}NO_2 \cdot C_4H_6O_4$ 381.47

Butanedioic acid, compound with 4-[2-(dimethylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol (1:1), monohydrate;
1-[2-(Dimethylamino)-1-(4-hydroxyphenyl)ethyl] cyclohexanol hydrogen butanedioate monohydrate [386750-22-7].
Anhydrous [448904-47-0].

DEFINITION

Desvenlafaxine Succinate contains NLT 98.0% and NMT 102.0% of desvenlafaxine succinate ($C_{16}H_{25}NO_2 \cdot C_4H_6O_4$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨197⟩: [NOTE—Methods described in ⟨197K⟩ or ⟨197A⟩ may be used.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 2.7 g/L of monobasic potassium phosphate in water
  Solution A: Acetonitrile and *Buffer* (18:82)
  Mobile phase: To each liter of *Solution A*, add 5.4 mL of triethylamine, and adjust with phosphoric acid to a pH of 4.0.
  Standard solution: 0.05 mg/mL of USP Desvenlafaxine Succinate RS in *Mobile phase*. Sonication may be used to promote dissolution.
  Sample solution: 0.05 mg/mL of Desvenlafaxine Succinate in *Mobile phase*. Sonication may be used to promote dissolution.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 225 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 10 µL
  Run time: NLT 1.6 times the retention time of desvenlafaxine
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 0.73%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of desvenlafaxine succinate ($C_{16}H_{25}NO_2 \cdot C_4H_6O_4$) in the portion of Desvenlafaxine Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Desvenlafaxine Succinate RS in the *Standard solution* (mg/mL)
C_U = concentration of Desvenlafaxine Succinate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Buffer: 7.1 g/L of anhydrous dibasic sodium phosphate in water
  Solution A: Acetonitrile and *Buffer* (10:90); adjusted with phosphoric acid to a pH of 3.0
  Solution B: Acetonitrile and *Buffer* (60:40); adjusted with phosphoric acid to a pH of 3.0
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
30	50	50
35	35	65
37	100	0

www.webofpharma.com

System suitability solution: 1 mg/mL of USP Desvenlafaxine Succinate RS and 0.0015 mg/mL of USP Desvenlafaxine Related Compound B RS in *Solution A*. Sonication may be used to promote dissolution.
Standard solution: 0.001 mg/mL of USP Desvenlafaxine Succinate RS, equivalent to 0.0007 mg/mL of desvenlafaxine, in *Solution A*. Sonication may be used to promote dissolution.
Sensitivity solution: 0.5 µg/mL of USP Desvenlafaxine Succinate RS, equivalent to 0.35 µg/mL of desvenlafaxine, from the *Standard solution* in *Solution A*
Sample solution: 1 mg/mL of Desvenlafaxine Succinate in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 3-µm packing L1
Temperatures
Autosampler: 10°
Column: 50°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between desvenlafaxine related compound B and desvenlafaxine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Desvenlafaxine Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of desvenlafaxine succinate from the *Standard solution*
C_S = concentration of USP Desvenlafaxine Succinate RS in the *Standard solution* (mg/mL)
C_U = concentration of Desvenlafaxine Succinate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

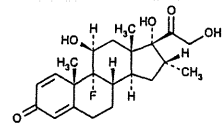
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fumaric acid	0.16	1.7	0.10
Succinic acid[a]	0.17	—	—
4-(Dimethylaminoethyl) phenol[b]	0.20	1.1	0.15
Didesmethyl desvenlafaxine[c]	0.91	0.94	0.15
Desvenlafaxine related compound B	0.96	0.80	0.15
Desvenlafaxine	1.0	—	—
Desvenlafaxine related compound A[d]	1.46	1.0	0.15
Venlafaxine[e]	1.55	0.97	0.15
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] Included for identification only. This peak is due to the succinate counterion; hence it is not an impurity.
[b] 4-[2-(Dimethylamino)ethyl]phenol.
[c] 4-[2-Amino-1-(1-hydroxycyclohexyl)ethyl]phenol.
[d] 4-[1-(Cyclohex-1-en-1-yl)-2-(dimethylamino)ethyl]phenol.
[e] 1-[2-(Dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexan-1-ol.

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*: 4.0%–5.2%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Desvenlafaxine Succinate RS
USP Desvenlafaxine Related Compound B RS
4-[1-(1-Hydroxycyclohexyl)-2-(methylamino)ethyl] phenol.
$C_{15}H_{23}NO_2$ 249.35▲ (USP 1-Aug-2019)

Dexamethasone



$C_{22}H_{29}FO_5$ 392.46
Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17,21-trihydroxy-16-methyl-, (11β,16α)-;
9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione [50-02-2].

DEFINITION
Dexamethasone contains NLT 97.0% and NMT 102.0% of dexamethasone ($C_{22}H_{29}FO_5$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 3.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

www.webofpharma.com

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	76	24
10	76	24
15	45	55
16	10	90
16.1	76	24
20.0	76	24

Diluent: Acetonitrile and water (56:44)
System suitability solution: 0.3 mg/mL of USP Dexamethasone RS and 20 µg/mL of USP Betamethasone RS in *Diluent*. Sonicate to dissolve as needed.
Standard solution: 0.3 mg/mL of USP Dexamethasone RS in *Diluent*. Sonicate to dissolve as needed.
Sample solution: 0.3 mg/mL of Dexamethasone in *Diluent*. Sonicate to dissolve as needed.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 35°
Flow rate: 0.4 mL/min
Injection volume: 2 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between betamethasone and dexamethasone, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dexamethasone ($C_{22}H_{29}FO_5$) in the portion of Dexamethasone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dexamethasone RS in the *Standard solution* (mg/mL)
C_U = concentration of Dexamethasone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Sample: 250 mg
Analysis: Use a platinum crucible.
Acceptance criteria: NMT 0.2%

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 4.0 µg/mL of USP Dexamethasone RS, 6.0 µg/mL each of USP Betamethasone RS and USP Desoximetasone RS, and 12.0 µg/mL of USP Dexamethasone Acetate RS in *Diluent*
Sample solution: 4.0 mg/mL of Dexamethasone in *Diluent*. Sonicate to dissolve as needed.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between betamethasone and dexamethasone, *System suitability solution*
Relative standard deviation: NMT 5.0% for betamethasone, dexamethasone, desoximetasone, and dexamethasone acetate, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of betamethasone, desoximetasone, and dexamethasone acetate in the portion of Dexamethasone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of betamethasone, desoximetasone, or dexamethasone acetate from the *Sample solution*
r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = concentration of Dexamethasone in the *Sample solution* (mg/mL)

Calculate the percentage of 16α-methylprednisone, dexamethasone 7,9-diene, and any individual unspecified impurity in the portion of Dexamethasone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of 16α-methylprednisone, dexamethasone 7,9-diene, or any individual unspecified impurity from the *Sample solution*
r_S = peak response of dexamethasone from the *Standard solution*
C_S = concentration of USP Dexamethasone RS in the *Standard solution* (mg/mL)
C_U = concentration of Dexamethasone in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
16α-Methylprednisone[a]	0.86	1.0	0.15
Betamethasone	0.94	—	0.15
Dexamethasone	1.00	—	—
Dexamethasone 7,9-diene[b]	1.40	1.7	0.10
Desoximetasone	1.58	—	0.15
Dexamethasone acetate	1.74	—	0.30
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] 17,21-Dihydroxy-16α-methylpregna-1,4-diene-3,11,20-trione.
[b] 17,21-Dihydroxy-16α-methylpregna-1,4,7,9(11)-tetraene-3,20-dione.

www.webofpharma.com

SPECIFIC TESTS
- **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 10 mg/mL of Dexamethasone in dioxane
  Acceptance criteria: +72° to +80°
- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨11⟩
  USP Betamethasone RS
  USP Desoximetasone RS
  USP Dexamethasone RS
  USP Dexamethasone Acetate RS

Dexamethasone Elixir

» Dexamethasone Elixir contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{22}H_{29}FO_5$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Dexamethasone RS
Identification—Evaporate 9 mL of the *Assay preparation*, prepared as directed in the *Assay*, on a steam bath just to dryness, and dissolve the residue in 2 mL of a mixture of methylene chloride and methanol (1:1). Apply separately 5 µL of this solution and 5 µL of a solution of USP Dexamethasone RS in the mixture of methylene chloride and methanol (1:1), containing 0.5 mg per mL, to a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* ⟨621⟩). Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, acetone, and glacial acetic acid (80:40:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots by viewing under short-wavelength UV light: the R_F value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.
Alcohol Determination, *Method II* ⟨611⟩: between 3.8% and 5.7% of C_2H_5OH, *n*-propyl alcohol being used as the internal standard.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Dexamethasone RS in dilute methanol (1 in 2), and dilute quantitatively, and stepwise if necessary, with dilute methanol (1 in 2) to obtain a solution having a known concentration of about 0.1 mg per mL.
Assay preparation—Transfer an accurately measured volume of Elixir, freshly mixed and free from air bubbles, equivalent to about 1 mg of dexamethasone, to a 10-mL volumetric flask, dilute with water to volume, mix, and filter through a suitable membrane filter.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute.
Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the relative standard deviation for replicate injections is not more than 3.0%.
Procedure—Separately inject equal volumes (between 5 µL and 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{22}H_{29}FO_5$ in each mL of the Elixir taken by the formula:

$$10(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*, V is the volume, in mL, of Elixir taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dexamethasone Injection

» Dexamethasone Injection is a sterile solution of Dexamethasone in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$).

Packaging and storage—Preserve in light-resistant single-dose or multiple-dose containers, preferably of Type I glass.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Dexamethasone RS

Identification—
A: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Test solution*—Transfer a quantity of Injection, equivalent to about 5 mg of dexamethasone, to a 50-mL separator, add 10 mL of water, and extract with two 20-mL portions of chloroform. Filter the lower layers through chloroform-saturated cotton into a 50-mL conical flask, and evaporate to dryness. Dissolve the residue in 10 mL of chloroform.
Developing solvent system: a mixture of methylene chloride and methanol (180:16).
Procedure—Visualize the spots using a 1 in 5 solution of *p*-toluenesulfonic acid in a mixture of alcohol and propylene glycol (9:1), followed by heat.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 21.0 USP Endotoxin Units per mg of dexamethasone.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 4.0 and 5.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

www.webofpharma.com

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (70:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare a solution in *Mobile phase* containing in each mL about 0.3 mg of USP Dexamethasone RS, 1.35 mg of benzyl alcohol, 0.27 mg of methylparaben, and 0.03 mg of propylparaben.

Standard preparation—Quantitatively dissolve an accurately weighed amount of USP Dexamethasone RS in methanol to obtain a stock solution having a known concentration of about 7.5 mg per mL. Transfer 4.0 mL to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.3 mg of USP Dexamethasone RS per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 30 mg of dexamethasone, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.4 for benzyl alcohol, 0.5 for methylparaben, 1.0 for dexamethasone, and 1.4 for propylparaben; and the resolution, *R*, between the neighboring peaks for benzyl alcohol and methylparaben, methylparaben and dexamethasone, and dexamethasone and propylparaben is not less than 3. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for dexamethasone. Calculate the quantity, in mg, of dexamethasone ($C_{22}H_{29}FO_5$) in each mL of the Injection taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dexamethasone Ophthalmic Suspension

DEFINITION

Dexamethasone Ophthalmic Suspension is a sterile, aqueous suspension of dexamethasone containing a suitable antimicrobial preservative. It may contain suitable buffers, stabilizers, and suspending and viscosity agents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$).

IDENTIFICATION

Delete the following:

▲• **THIN-LAYER CHROMATOGRAPHY**

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Standard solution: 500 µg/mL of USP Dexamethasone RS in chloroform

Sample solution: Transfer a volume of Ophthalmic Suspension, equivalent to about 2.5 mg of dexamethasone, to a test tube, add 5 mL of chloroform, shake, and centrifuge.

Application volume: 10 µL of the chloroform layer

Developing solvent system: *Single-Steroid Assay* ⟨511⟩, *Solvent A* as directed under *Single-Steroid Assay* ⟨511⟩

Analysis: Develop the chromatogram, mark the solvent front, and locate the spots on the plate by spraying with a 1 in 5 solution of *p*-toluenesulfonic acid in a mixture of 9 volumes of alcohol and 1 volume of propylene glycol, and heating until spots appear.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. ▲ (USP 1-May-2019)

Add the following:

▲• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲ (USP 1-May-2019)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**

▲**Solution A:** 3.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	76	24
10	76	24
15	45	55
16	10	90
16.1	76	24
20.0	76	24

Diluent: Acetonitrile and water (20:80)

System suitability solution: 0.06 mg/mL of USP Dexamethasone RS and 4 µg/mL of USP Betamethasone RS in *Diluent*. Sonicate to dissolve as needed.

Standard solution: 0.06 mg/mL of USP Dexamethasone RS in *Diluent*. Sonicate to dissolve as needed.

Sample solution: Prior to sampling, sonicate the drug product in the original container to achieve optimum

USP Monographs

www.webofpharma.com

homogeneity of the sample to be tested. Nominally 0.06 mg/mL of dexamethasone from Ophthalmic Suspension in *Diluent*. Vortex for 10–15 s and then sonicate for 2 min to dissolve.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 35°
Flow rate: 0.4 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between betamethasone and dexamethasone, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$) in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dexamethasone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dexamethasone in the *Sample solution* (mg/mL) ▲(USP 1-May-2019)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.6 µg/mL of USP Dexamethasone RS in *Diluent*. Sonicate to dissolve as needed.
Sample solution: Prior to sampling, sonicate the drug product in the original container to achieve optimum homogeneity of the sample to be tested. Nominally 300 µg/mL of dexamethasone from Ophthalmic Suspension in *Diluent*. Vortex for 10–15 s and then sonicate for 2 min to dissolve.
System suitability
[NOTE—See *Table 2* for the relative retention times.]
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between betamethasone and dexamethasone, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual degradation product in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*
r_S = peak response of dexamethasone from the *Standard solution*
C_S = concentration of USP Dexamethasone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dexamethasone in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
16α-Methylprednisone[a]	0.87	—[b]
Betamethasone	0.94	—[b]
Dexamethasone	1.00	—[b]
Dexamethasone 7,9-diene[c]	1.39	—[b]
Desoximetasone	1.55	—[b]
Dexamethasone acetate	1.72	—[b]
Any unspecified degradation product	—	0.2
Total degradation products	—	0.5

[a] 17,21-Dihydroxy-16α-methylpregna-1,4-diene-3,11,20-trione.
[b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total degradation products for the drug product.
[c] 17,21-Dihydroxy-16α-methylpregna-1,4,7,9(11)-tetraene-3,20-dione.▲(USP 1-May-2019)

SPECIFIC TESTS

- **pH** ⟨791⟩: 5.0–6.0
- **STERILITY TESTS** ⟨71⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers. ▲Store upright at 8°–27°.▲ (USP 1-May-2019)

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
▲USP Betamethasone RS▲ (USP 1-May-2019)
USP Dexamethasone RS

Dexamethasone Oral Solution

DEFINITION

Dexamethasone Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$).

USP Monographs

www.webofpharma.com

IDENTIFICATION

• **A.** The retention time of the dexamethasone peak from the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and water (1:1)

Internal standard solution: 0.1 mg/mL of USP Prednisolone RS in methanol

System suitability stock solution: 0.6 mg/mL of USP Methylparaben RS and 0.075 mg/mL of USP Propylparaben RS in methanol

System suitability solution: 0.24 mg/mL of USP Methylparaben RS, 0.03 mg/mL of USP Propylparaben RS, and 0.01 mg/mL of USP Prednisolone RS prepared as follows. To an amount of *System suitability stock solution* equivalent to 40% of the final volume, add an amount of *Internal standard solution* equivalent to 10% of the final volume. Dilute with water to volume.

Standard stock solution: 0.2 mg/mL of USP Dexamethasone RS in *Mobile phase*

Standard solution: 0.02 mg/mL of USP Dexamethasone RS and 0.01 mg/mL of USP Prednisolone RS in *Mobile phase* prepared by diluting suitable volumes of *Standard stock solution* and *Internal standard solution*

Sample solution: Nominally equivalent to 0.02 mg/mL of dexamethasone from a volume of Oral Solution and 0.01 mg/mL of USP Prednisolone RS in *Mobile phase* from the *Internal standard solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.4 mL/min

Injection volume: 10 µL

System suitability

[NOTE—The relative retention times for methylparaben, prednisolone, propylparaben, and dexamethasone are about 0.43, 0.71, 0.88, and 1.0, respectively.]

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between methylparaben and prednisolone; NLT 2.0 between propylparaben and prednisolone, *System suitability solution*

Tailing factor: NMT 2.0 for each peak, *System suitability solution* and *Standard solution*

Relative standard deviation: NMT 2.0% for the peak height ratio of dexamethasone to prednisolone, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio of dexamethasone to prednisolone from the *Sample solution*

R_S = peak height ratio of dexamethasone to prednisolone from the *Standard solution*

C_S = concentration of USP Dexamethasone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dexamethasone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩ (if present): 27.0%–33.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements for oral solution packaged in single-unit containers

• **DELIVERABLE VOLUME** ⟨698⟩: Meets the requirements for oral solution packaged in multiple-unit containers

SPECIFIC TESTS

• **PH** ⟨791⟩: 2.7–4.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **LABELING:** Label concentrated Oral Solution to state that the term **"Concentrate"** is to appear apart from and immediately after the official title in prominent boldface type. Label concentrated Oral Solution also to indicate that it is to be diluted to appropriate strength with a suitable diluent prior to administration unless produced for dispensing with instructions for administration by a calibrated dropper or syringe.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Dexamethasone RS
USP Methylparaben RS
USP Prednisolone RS
USP Propylparaben RS

Dexamethasone Tablets

» Dexamethasone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{22}H_{29}FO_5$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—

USP Dexamethasone RS

Identification—Evaporate 10 mL of the methanol extract of Tablets obtained as directed under *Assay preparation* in the *Assay* on a steam bath just to dryness, and dissolve the residue in 1 mL of chloroform. Apply 10 µL of this solution and 20 µL of a solution of Dexamethasone RS in chloroform containing 500 µg per mL on a thin-layer chromatographic plate (see *System Suitability* under *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in *Solvent A* as directed under *Single-Steroid Assay* ⟨511⟩. Mark the solvent front, and locate the spots on the plate by visualizing under short-wavelength UV light: the R_f value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

Dissolution ⟨711⟩—

Medium: dilute hydrochloric acid (1 in 100); 500 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Standard solution—Prepare as directed for *Standard Preparation* under *Assay for Steroids* ⟨351⟩, using USP Dexamethasone RS.

Procedure—Extract a filtered aliquot of *Dissolution Medium,* equivalent to about 200 µg of dexamethasone, with three 15-mL portions of chloroform. Evaporate the combined chloroform extracts on a steam bath just to dryness, cool, and dissolve the residue in 20 mL of alcohol. Proceed as directed

www.webofpharma.com

for *Procedure* under *Assay for Steroids* ⟨351⟩, except to allow to stand in the dark for 45 minutes. Calculate the portion, in mg, of $C_{22}H_{29}FO_5$ dissolved by the formula:

$$10(C/V)(A_U/A_S)$$

in which V is the volume, in mL, of the aliquot extracted with chloroform.

Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{22}H_{29}FO_5$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—

Standard solution—Prepare as directed for *Standard Preparation* under *Assay for Steroids* ⟨351⟩, using USP Dexamethasone RS.

Test solution—Place 1 Tablet in a separator with 15 mL of water, and swirl to disintegrate the Tablet completely. Extract with four 10-mL portions of chloroform, filtering each portion through chloroform-washed cotton into a 50-mL volumetric flask, add chloroform to volume, and mix. Pipet a volume of this solution, equivalent to about 200 µg of dexamethasone into a glass-stoppered, 50-mL conical flask, evaporate the chloroform on a steam bath just to dryness, cool, and dissolve the residue in 20.0 mL of alcohol. Use this where *Assay Preparation* is specified in the *Procedure.*

Procedure—Proceed as directed for *Procedure* under *Assay for Steroids* ⟨351⟩, except to allow to stand in the dark for 45 minutes. Calculate the quantity, in mg, of total steroids, as $C_{22}H_{29}FO_5$, in the Tablet by the formula:

$$(C/V)(A_U/A_S)$$

in which V is the volume, in mL, of the aliquot taken to prepare the *Test solution.*

Assay—

Mobile solvent—Prepare a suitable aqueous solution of acetonitrile, approximately 1 in 3, such that the retention time of dexamethasone is between 3 minutes and 6 minutes.

Standard preparation—Dissolve an accurately weighed quantity of USP Dexamethasone RS in dilute methanol (1 in 2) to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Weigh and finely powder not fewer than 10 Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of dexamethasone, transfer to a 50-mL volumetric flask, and add 30 mL of dilute methanol (1 in 2). Sonicate the flask for about 2 minutes, shake by mechanical means for 30 minutes, and dilute with the same solvent to volume. Filter a portion of the mixture through a suitable filter to obtain a clear filtrate.

Procedure—Introduce equal volumes (between 5 µL and 25 µL) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* ⟨621⟩) operated at room temperature, by means of a loop injector, adjusting the specimen size and other operating parameters such that the peak obtained with the *Standard preparation* is about 0.6 full scale. Typically, the apparatus is fitted with a 4.6-mm × 30-cm column packed with packing L1 and is equipped with an UV detector capable of monitoring absorption at 254 nm and a suitable recorder. In a suitable chromatogram, the coefficient of variation for five replicate injections of a single specimen is not more than 3.0%. Measure the responses of the peaks, at identical retention times, obtained with the *Assay preparation* and the *Standard preparation.* Calculate the quantity, in mg, of $C_{22}H_{29}FO_5$ in the portion of Tablets taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dexamethasone Acetate

$C_{24}H_{31}FO_6.H_2O$ 452.51

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-9-fluoro-11,17-dihydroxy-16-methyl-, (11β,16α)-monohydrate.

9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-acetate monohydrate [55812-90-3].

Anhydrous 434.51 [1177-87-3].

» Dexamethasone Acetate contains one molecule of water of hydration or is anhydrous. It contains not less than 97.0 percent and not more than 102.0 percent of $C_{24}H_{31}FO_6$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

Labeling—Label it to indicate whether it is hydrous or anhydrous.

USP Reference standards ⟨11⟩—
USP Dexamethasone Acetate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020)

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)

Solution: 15 µg per mL.

Medium: methanol.

Absorptivities at 239 nm, calculated on the dried basis, do not differ by more than 3.0%.

Specific rotation ⟨781S⟩: between +82° and +88°.

Test solution: 10 mg per mL, in dioxane.

Loss on drying ⟨731⟩—Dry it in vacuum at 105° for 3 hours: the hydrous form loses between 3.5% and 4.5%, and the anhydrous form not more than 0.4%, of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—

Format buffer—Dissolve 1.32 g of ammonium formate in 1 L of water, adjust with formic acid to a pH of 3.6, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Formate buffer* and acetonitrile (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Test solution—Transfer about 200 mg of Dexamethasone Acetate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix. Transfer about 40 mL of this solution to a 100-mL volumetric flask, dilute with *Formate buffer* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector

USP Monographs

www.webofpharma.com

and a 4.6-mm × 25-cm column that contains packing L11. The flow rate is about 1 mL per minute. Chromatograph the *Test solution,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 5400 theoretical plates.

Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Dexamethasone Acetate taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (550:450). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

pH 6.0 Buffer solution—Transfer 3 mL of 1 N sodium hydroxide, 138 mL of 0.5 N potassium chloride, and 50 mL of 0.5 M monobasic potassium phosphate to a 1-L volumetric flask, dilute with water to volume, and mix.

Diluent—Prepare a mixture of acetonitrile and *pH 6.0 Buffer solution* (1:1).

Standard preparation—Transfer about 25 mg of USP Dexamethasone Acetate RS, accurately weighed, to a 250-mL volumetric flask. Add 100 mL of *Diluent,* and sonicate until a clear solution is obtained. Dilute with *Diluent* to volume, and mix.

Assay preparation—Transfer about 25 mg of Dexamethasone Acetate, accurately weighed, to a 250-mL volumetric flask. Add 100 mL of *Diluent,* and sonicate until a clear solution is obtained. Dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column containing 10-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the capacity factor, *k′,* is not less than 2.0; the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* (before and after injections of the *Assay preparation*) and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{24}H_{31}FO_6$ in the portion of Dexamethasone Acetate taken by the formula:

$$250C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dexamethasone Acetate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dexamethasone Acetate Injectable Suspension

» Dexamethasone Acetate Injectable Suspension is a sterile suspension of Dexamethasone Acetate in Water for Injection. It contains an amount of dexamethasone acetate monohydrate ($C_{24}H_{31}FO_6 \cdot H_2O$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

USP Reference standards ⟨11⟩—
USP Dexamethasone Acetate RS

Change to read:

▲ ***Identification, Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: ***197M*** ▲(CN 1-May-2020)—Obtain the test specimen as follows. Transfer the contents of a well-shaken container of Injectable Suspension to a fine-porosity, sintered-glass vacuum filter, filter, and wash with several 10-mL portions of water. Remove the powder from the filter and allow to air-dry. [NOTE—Do not use heat to dry the specimen. Total or partial dehydration may occur. Use a similar undried preparation of USP Dexamethasone Acetate RS.]

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 21.7 USP Endotoxin Units per mg of dexamethasone acetate.

pH ⟨791⟩: between 5.0 and 7.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase, pH 6.0 Buffer solution, Diluent, and *Chromatographic system*—Proceed as directed in the *Assay*—under *Dexamethasone Acetate.*

Standard preparation—Dissolve an accurately weighed quantity of USP Dexamethasone Acetate RS in *Diluent* to obtain a solution having a known concentration of about 0.09 mg per mL.

Assay preparation—Transfer an accurately measured volume of well-shaken Injectable Suspension, equivalent to about 40 mg of dexamethasone, to a 100-mL volumetric flask. Add 75 mL of *Diluent,* and sonicate until a clear solution is obtained. Dilute with *Diluent* to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* (before and after injections of the *Assay preparation*) and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of dexamethasone ($C_{22}H_{29}FO_5$) in each mL of the Injectable Suspension taken by the formula:

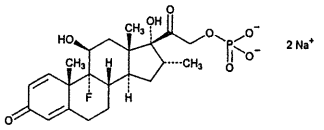
$$(392.47/434.51)(500C/V)(r_U/r_S)$$

in which 392.47 and 434.51 are the molecular weights of dexamethasone and anhydrous dexamethasone acetate, respectively; C is the concentration, in mg per mL, of USP Dexamethasone Acetate RS in the *Standard preparation; V* is the volume, in mL, of Injectable Suspension taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

USP Monographs

www.webofpharma.com

Dexamethasone Sodium Phosphate



$C_{22}H_{28}FNa_2O_8P$ 516.40

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(phosphonooxy)-, disodium salt, (11β,16α)-;
9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-(dihydrogen phosphate) disodium salt [2392-39-4].

DEFINITION

Dexamethasone Sodium Phosphate contains NLT 97.0% and NMT 102.0% of dexamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)**:** If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the Reference Standard separately in a minimum of alcohol, evaporate on a water bath to dryness, and repeat the test on the residues.
- **B. IDENTIFICATION TESTS—GENERAL, *Phosphate* ⟨191⟩:** The residue from its ignition meets the requirements.
- **C. IDENTIFICATION TESTS—GENERAL, *Sodium* ⟨191⟩:** The residue from its ignition meets the requirements.

ASSAY

- **PROCEDURE**

Mobile phase: Mix 520 mL of water with 2 mL of phosphoric acid. Bring the temperature to 20°, and adjust with sodium hydroxide to a pH of 2.6. Mix this solution with 36 mL of tetrahydrofuran and 364 mL of methanol.

System suitability stock solution: 0.02 mg/mL each of USP Dexamethasone Sodium Phosphate RS and USP Dexamethasone RS, prepared as follows. Dissolve 2 mg of each compound in 2 mL of tetrahydrofuran, and dilute with *Mobile phase* to 100 mL.

System suitability solution: 2 µg/mL each of USP Dexamethasone Sodium Phosphate RS and USP Dexamethasone RS in *Mobile phase* from the *System suitability stock solution*

Standard solution: 0.06 mg/mL of USP Dexamethasone Sodium Phosphate RS in *Mobile phase*

Sample solution: 0.06 mg/mL of Dexamethasone Sodium Phosphate in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 7-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: 3 times the retention time of the dexamethasone sodium phosphate peak

System suitability
[NOTE—The relative retention times of the dexamethasone sodium phosphate and dexamethasone peaks are 1.0 and 2.0, respectively.]
Samples: *System suitability solution* and *Standard solution*

Suitability requirements
Resolution: NLT 6.0 between dexamethasone sodium phosphate and dexamethasone peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dexamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$) in the portion of Dexamethasone Sodium Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dexamethasone Sodium Phosphate RS in the *Standard solution* (mg/mL)
C_U = concentration of Dexamethasone Sodium Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **LIMIT OF PHOSPHATE IONS**

Solution A: 50 mg/mL of ammonium molybdate in 1 N sulfuric acid

Solution B: Dissolve 350 mg of *p*-methylaminophenol sulfate in 50 mL of water, add 20 g of sodium bisulfite, mix to dissolve, and dilute with water to 100 mL.

Standard stock solution: 0.14 mg/mL of dried monobasic potassium phosphate in water. This solution contains the equivalent of 0.10 mg/mL of phosphate (PO_4) ion.

Standard solution: In a 25-mL volumetric flask, mix 5.0 mL of *Standard stock solution*, 10 mL of water, and 5 mL of 2 N sulfuric acid. Add 1 mL each of *Solution A* and *Solution B*, dilute with water to volume, and allow to stand at room temperature for 30 min. Prepare concomitantly with the *Sample solution*.

Sample solution: In a 25-mL volumetric flask, dissolve 50 mg of Dexamethasone Sodium Phosphate in a mixture of 10 mL of water and 5 mL of 2 N sulfuric acid, by warming if necessary. Add 1 mL each of *Solution A* and *Solution B*, dilute with water to volume, and allow to stand at room temperature for 30 min.

Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Visible
Analytical wavelength: 730 nm
Cell: 1 cm
Blank: Water

Analysis
Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution*. The limit is 1.0% of phosphate (PO_4).

- **LIMIT OF ALCOHOL**

Internal standard solution: 0.4 mg/mL of acetonitrile, prepared as follows. Dilute 5.0 mL of USP Alcohol Determination–Acetonitrile RS with water to 200.0 mL.

Standard stock solution: 0.20 mg/mL of alcohol, prepared as follows. Dilute 25.0 mL of USP Alcohol Determination–Alcohol RS with water to 2000.0 mL.

Standard solution: 0.08 mg/mL of alcohol, prepared as follows. Dilute 10.0 mL of the *Standard stock solution* and 5.0 mL of the *Internal standard solution* with water to 25.0 mL.

USP Monographs

www.webofpharma.com

Sample solution: 5.0 mg/mL of Dexamethasone Sodium Phosphate, prepared as follows. Transfer 125 mg of Dexamethasone Sodium Phosphate to a 25-mL volumetric flask, add 5.0 mL of the *Internal standard solution*, and dilute with water to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m capillary coated with 3-µm film of G43
Carrier gas: Hydrogen
Linear velocity: 36 cm/s
Split ratio: 2:1
Temperatures
Injection port: 210°
Detector: 280°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	—	50	5
50	50	200	4

Injection volume: 0.4 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between alcohol and acetonitrile
Relative standard deviation: NMT 3.0% for alcohol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of alcohol in the portion of Dexamethasone Sodium Phosphate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the alcohol to the internal standard from the *Sample solution*
R_S = peak response ratio of the alcohol to the internal standard from the *Standard solution*
C_S = concentration of alcohol in the *Standard solution* (mg/mL)
C_U = concentration of Dexamethasone Sodium Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.5%

• **ORGANIC IMPURITIES**
Buffer: 7.0 g/L of ammonium acetate in water
Solution A: Mix 300 mL of *Buffer* and 350 mL of water, adjust with 5 M acetic acid to a pH of 3.8, and then add 350 mL of methanol.
Solution B: Adjust 300 mL of *Buffer* with 5 M acetic acid to a pH of 4.0, and then add 700 mL of methanol.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3.5	90	10
23.5	60	40
34.5	5	95
50	5	95

System suitability solution: 0.02 mg/mL each of USP Dexamethasone Sodium Phosphate RS and USP Betamethasone Sodium Phosphate RS in *Solution A*
Standard solution: 1 µg/mL of USP Dexamethasone Sodium Phosphate RS in *Solution A*
Sample solution: 1 mg/mL of Dexamethasone Sodium Phosphate in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between dexamethasone sodium phosphate and betamethasone sodium phosphate peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Dexamethasone Sodium Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of dexamethasone phosphate from the *Standard solution*
C_S = concentration of USP Dexamethasone Sodium Phosphate RS in the *Standard solution* (µg/mL)
C_U = concentration of Dexamethasone Sodium Phosphate in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any peak below 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
16(17)a-Homodexamethasone sodium phosphate[a]	0.5	1.0	0.2
16(17)a-Homobetamethasone sodium phosphate[b]	0.6	1.0	0.2
16(17)a-17*R*-Homodexamethasone sodium phosphate[c]	0.8	1.0	0.2
13(17)a-Homodexamethasone sodium phosphate[d]	0.92	1.0	0.2
Betamethasone sodium phosphate	0.95	1.0	0.2
Dexamethasone sodium phosphate	1.00	—	—

www.webofpharma.com

Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dexamethasone ethyl ester[e]	1.20	1.0	0.3
Dexamethasone	1.37	1.2	0.5
Fluoroandrostadiene carboxylic acid[f]	1.41	1.0	0.3
Dexamethasone sodium phosphate diester[g]	2.10	1.0	0.1
Any other individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 9-Fluoro-11β,17,21-trihydroxy-16α-methyl-16(17)a-homopregna-1,4-diene-3,16a,20-trione 21-(dihydrogen phosphate) disodium salt.
[b] 9-Fluoro-11β,17,21-trihydroxy-16β-methyl-16(17)a-homopregna-1,4-diene-3,16a,20-trione 21-(dihydrogen phosphate) disodium salt.
[c] 9-Fluoro-11β,17a,21-trihydroxy-16β-methyl-16(17)a-homopregna-1,4-diene-3,16a,20-trione 21-(dihydrogen phosphate) disodium salt.
[d] 9-Fluoro-11β,17,21-trihydroxy-16α-methyl-13(17)a-homopregna-1,4-diene-3,13a,20-trione 21-(dihydrogen phosphate) disodium salt.
[e] Ethyl 11β,17α-dihydroxy-9-fluoro-16α-methylandrostane-1,4-diene-3-one-17-ylcarboxylate.
[f] 9-Fluoro-11β,17α-dihydroxy-16α-methylandrosta-1,4-diene-3-one-17β-carboxylic acid.
[g] Sodium bis[9-fluoro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-yl]phosphate.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL in water
  Acceptance criteria: +74° to +82°, calculated on the anhydrous and solvent-free basis
- **PH** ⟨791⟩
  Sample solution: 10 mg/mL
  Acceptance criteria: 7.5–10.5
- **WATER DETERMINATION,** *Method I* ⟨921⟩
  Acceptance criteria: NMT 10.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Alcohol Determination–Acetonitrile RS
  USP Alcohol Determination–Alcohol RS
  USP Betamethasone Sodium Phosphate RS
  USP Dexamethasone RS
  USP Dexamethasone Sodium Phosphate RS

Dexamethasone Sodium Phosphate Injection

DEFINITION

Dexamethasone Sodium Phosphate Injection is a sterile solution of Dexamethasone Sodium Phosphate in Water for Injection. It contains NLT 90.0% and NMT 115.0% of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$), present as the disodium salt.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**
  Solution A: Mix 3.1 g of boric acid and 500 mL of water in a 1-L volumetric flask. Add 21 mL of 1 N sodium hydroxide and 10 mL of 0.1 M magnesium chloride, and dilute with water to volume.
  Solution B: 1 mg/mL of alkaline phosphatase enzyme in *Solution A*
  Standard solution: 300 µg/mL of USP Dexamethasone RS in methylene chloride
  Sample solution: Pipet a volume of Injection, equivalent to 10 mg of dexamethasone phosphate, into a 100-mL volumetric flask, and dilute with water to volume. Pipet 5 mL of this solution into a 125-mL separator, and wash with two 10-mL portions of water-washed methylene chloride, discarding the washings. Transfer the solution to a glass-stoppered, 50-mL tube, and add 5 mL of *Solution B*. Allow to stand at 37° for 45 min, and extract with 25 mL of methylene chloride. Evaporate 15 mL of the methylene chloride extract on a steam bath to dryness, and dissolve the residue in 1 mL of methylene chloride.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture (20- × 20-cm plate)
  Application volume: 5 µL
  Developing solvent system: Chloroform, acetone, and water (50:50:1)
  Spray reagent: Dilute sulfuric acid (1 in 2)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Allow the spots to dry, and develop the chromatogram using the *Developing solvent system* in a tank completely lined with filter paper, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing tank, mark the solvent front, and allow the spots to dry. Spray the plate with *Spray reagent*, and heat at 105° until brown or black spots appear.
  Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: 0.01 M monobasic potassium phosphate in a mixture of methanol and water (1:1)
  Standard solution: 0.09 mg/mL of freshly prepared USP Dexamethasone Sodium Phosphate RS in *Mobile phase*
  Sample solution: Nominally 0.08 mg/mL of dexamethasone phosphate from Injection, in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4-mm × 30-cm; packing L1
  Flow rate: 1.6 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The retention time for dexamethasone phosphate is about 5 min.]
  Suitability requirements
  Relative standard deviation: NMT 1.5%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) in the portion of Injection taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r22}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dexamethasone Sodium Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dexamethasone phosphate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dexamethasone phosphate, 472.44
M_{r2} = molecular weight of dexamethasone sodium phosphate, 516.40

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 7.0–8.5
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 31.3 USP Endotoxin Units/mg of dexamethasone phosphate
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.

Change to read:

- **USP Reference Standards** ⟨11⟩
  USP Dexamethasone RS
  USP Dexamethasone Sodium Phosphate RS

▲▲ (ERR 1-Aug-2018)

Dexamethasone Sodium Phosphate Compounded Injection

DEFINITION
Dexamethasone Sodium Phosphate Compounded Injection contains NLT 90.0% and NMT 115.0% of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$), present as the disodium salt. It contains no bacteriostat or other preservative.
Prepare Dexamethasone Sodium Phosphate Compounded Injection, 24 mg/mL, as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Dexamethasone phosphate (as Dexamethasone Sodium Phosphate)	240 mg (262.3 mg[a])
Sodium Citrate Dihydrate	100 mg
Sodium Bisulfite	10 mg
Sodium Hydroxide, 10% solution	To adjust the pH to 7.4
Sterile Water for Injection, a sufficient quantity to make	10 mL

[a] Calculate the amount of dexamethasone sodium phosphate based on the water content stated on the certificate of analysis.

Dissolve the *Dexamethasone Sodium Phosphate*, *Sodium Citrate Dihydrate*, and *Sodium Bisulfite* in about 8 mL of *Sterile Water for Injection*. Adjust with *Sodium Hydroxide 10% solution* to a pH of 7.4. Add sufficient *Sterile Water for Injection* to bring to final volume and mix well. Pass through a sterile filter of 0.22-µm pore size into sterile containers.

ASSAY
- **Procedure**

Solution A: Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water and adjust with 6 N potassium hydroxide to a pH of 9.
Solution B: Acetonitrile and methanol (50:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
3.0	70	30
16.0	65	35
16.1	70	30
25.0	70	30

Diluent: Acetonitrile and water (25:75)
Standard solution: 0.12 mg/mL of dexamethasone phosphate prepared from USP Dexamethasone Sodium Phosphate RS in *Diluent*
Sample solution: Transfer 0.5 mL of the Injection to a 100-mL volumetric flask and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
[Note—The retention time for dexamethasone phosphate is about 15.4 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dexamethasone phosphate from the *Sample solution*
r_S = peak response of dexamethasone phosphate from the *Standard solution*
C_S = concentration of dexamethasone phosphate in the *Standard solution* (mg/mL)
C_U = nominal concentration of dexamethasone phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 7.0–8.0
- **Sterility Tests** ⟨71⟩**:** Meets the requirements
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 31.3 USP Endotoxin Units/mg of dexamethasone phosphate
- **Particulate Matter in Injections** ⟨788⟩**:** Meets the requirements

USP Monographs

www.webofpharma.com

Change to read:

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in light-resistant single-dose containers. Store at controlled room temperature.
- **BEYOND-USE DATE:** ▲In the absence of passing a sterility test and endotoxins test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test and endotoxins test is passed, NMT 90 days after the date on which it was compounded when stored at controlled room temperature. ▲ (CN 1-May-2020)
- **LABELING:** Label it to indicate that it is a single-dose container and to state the *Beyond-Use Date*. Label to state that it should be protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dexamethasone Sodium Phosphate RS

Dexamethasone Sodium Phosphate Ophthalmic Solution

DEFINITION

Dexamethasone Sodium Phosphate Ophthalmic Solution is a sterile, aqueous solution of Dexamethasone Sodium Phosphate. It contains an amount of dexamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$) equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

  Solution A: Dissolve 3.1 g of boric acid, 203 mg of magnesium chloride, and 860 mg of sodium hydroxide in enough water to make 1000 mL.

  Solution B: 1 mg/mL of alkaline phosphatase enzyme in *Solution A*

  Standard solution: 300 µg/mL of USP Dexamethasone RS in methylene chloride

  Sample solution: Transfer 5 mL of *Solution B* to a glass-stoppered, 50-mL tube containing 5 mL of the *Sample solution* from the *Assay*. Incubate at 37° for 45 min, then add 25 mL of methylene chloride and shake for 2 min. Evaporate 15 mL of the methylene chloride extract on a steam bath to dryness, and dissolve the residue in 1 mL of methylene chloride.

  Chromatographic system

  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture (20- × 20-cm plate)

  Application volume: 5 µL

  Developing solvent system: Chloroform, acetone, and water (50:50:1)

  Spray reagent: Dilute sulfuric acid (1 in 2)

  Analysis

  Samples: *Standard solution* and *Sample solution*

  Allow the spots to dry, and develop the chromatogram using the *Developing solvent system* in a tank completely lined with filter paper until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing tank, mark the solvent front, and allow the spots to dry. Spray the plate with *Spray reagent*, and heat at 105° until brown or black spots appear.

  Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

  Mobile phase: 0.01 M monobasic potassium phosphate in a mixture of methanol and water (1:1)

  Standard solution: 0.09 mg/mL of freshly prepared USP Dexamethasone Sodium Phosphate RS in *Mobile phase*

  Sample solution: Nominally 0.08 mg/mL of dexamethasone phosphate from Ophthalmic Solution in *Mobile phase*

  Chromatographic system

  (See *Chromatography* ⟨621⟩, *System Suitability*.)

  Mode: LC

  Detector: UV 254 nm

  Column: 4-mm × 30-cm; packing L1

  Flow rate: 1.6 mL/min

  Injection volume: 20 µL

  System suitability

  Sample: *Standard solution*

  [NOTE—The retention time for dexamethasone phosphate is about 5 min.]

  Suitability requirements

  Relative standard deviation: NMT 1.5%

  Analysis

  Samples: *Standard solution* and *Sample solution*

  Calculate the percentage of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U	= peak response from the *Sample solution*
r_S	= peak response from the *Standard solution*
C_S	= concentration of USP Dexamethasone Sodium Phosphate RS in the *Standard solution* (mg/mL)
C_U	= nominal concentration of dexamethasone phosphate in the *Sample solution* (mg/mL)
M_{r1}	= molecular weight of dexamethasone phosphate, 472.44
M_{r2}	= molecular weight of dexamethasone sodium phosphate, 516.40

Acceptance criteria: 90.0%–115.0%

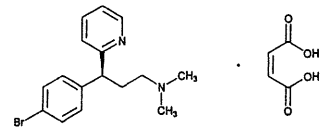
SPECIFIC TESTS

- **PH** ⟨791⟩**:** 6.6–7.8
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 15° and 25°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dexamethasone RS
  USP Dexamethasone Sodium Phosphate RS

Dexbrompheniramine Maleate



$C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ 435.32

USP Monographs

www.webofpharma.com

2-Pyridinepropanamine, γ-(4-bromophenyl)-*N*,*N*-dimethyl-, (*S*)-, (*Z*)-2-butenedioate (1:1);
(+)-2-[*p*-Bromo-α-[2-(dimethylamino)ethyl]benzyl]pyridine maleate (1:1) [2391-03-9].

DEFINITION

Dexbrompheniramine Maleate contains NLT 98.0% and NMT 102.0% of dexbrompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197M▲** (CN 1-May-2020)
- **B.** The retention times of the maleic acid and dexbrompheniramine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 5.44 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0 ± 0.1.
Solution B: Acetonitrile
Diluent: Acetonitrile and *Solution A* (5:95)
System suitability stock solution: 0.02 mg/mL each of USP Pheniramine Maleate RS, USP Chlorpheniramine Maleate RS, and USP Chlorpheniramine Related Compound B RS in *Diluent*. Sonicate for 1 min.
System suitability solution: 0.5 mg/mL of USP Dexbrompheniramine Maleate RS and 2 µg/mL each of USP Pheniramine Maleate RS, USP Chlorpheniramine Maleate RS, and USP Chlorpheniramine Related Compound B RS in *Diluent*, prepared as follows. Transfer 5.0 mg of USP Dexbrompheniramine Maleate RS to a 10-mL volumetric flask, add 5.0 mL of *Diluent* and 1.0 mL of the *System suitability stock solution*, and dilute with *Diluent* to volume.
Standard solution: 0.5 mg/mL of USP Dexbrompheniramine Maleate RS in *Diluent*. Sonicate for 1 min.
Sample solution: 0.5 mg/mL of Dexbrompheniramine Maleate in *Diluent*. Sonicate for 1 min.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
1	95	5
20	70	30
30	70	30
31	95	5
40	95	5

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
[NOTE—The relative retention times for maleic acid and dexbrompheniramine are 0.18 and 1.0, respectively.]
Resolution: NLT 1.5 between chlorpheniramine and dexbrompheniramine; NLT 2.0 between chlorpheniramine related compound B and pheniramine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dexbrompheniramine maleate ($C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$) in the portion of Dexbrompheniramine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dexbrompheniramine Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of Dexbrompheniramine Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.2%
- **ORGANIC IMPURITIES**

Solution A, Diluent, System suitability solution, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 2.7 µg/mL of USP Dexbrompheniramine Maleate RS in *Diluent*, equivalent to 2.0 µg/mL of dexbrompheniramine. Sonicate for 1 min.
Sensitivity solution: 0.74 µg/mL of USP Pheniramine Maleate RS in *Diluent*
Sample solution: 0.5 mg/mL of Dexbrompheniramine Maleate in *Diluent*. Sonicate for 1 min.
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between chlorpheniramine and dexbrompheniramine; NLT 2.0 between chlorpheniramine related compound B and pheniramine, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Sample: *Sample solution*
[NOTE—Exclude the maleic acid peak at the relative retention time 0.18.]
Calculate the percentage of each impurity in the portion of Dexbrompheniramine Maleate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity
r_T = sum of the responses of all the peaks

Acceptance criteria
Total impurities: NMT 2.0%

SPECIFIC TESTS

- **LOSS ON DRYING ⟨731⟩**

Analysis: Dry at 65° for 4 h.

USP Monographs

www.webofpharma.com

Acceptance criteria: NMT 0.5%

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 50 mg/mL, in dimethylformamide
  Acceptance criteria: +35.0° to +38.5°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Chlorpheniramine Maleate RS
  USP Chlorpheniramine Related Compound B RS
  Di(pyridin-2-yl)amine.
  $C_{10}H_9N_3$ 171.20
  USP Dexbrompheniramine Maleate RS
  USP Pheniramine Maleate RS

Dexbrompheniramine Maleate and Pseudoephedrine Sulfate Oral Solution

» Dexbrompheniramine Maleate and Pseudoephedrine Sulfate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of dexbrompheniramine maleate ($C_{10}H_{15}BrN_2 \cdot C_4H_4O_4$) and pseudoephedrine sulfate [$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$].

USP Reference standards ⟨11⟩—
USP Dexbrompheniramine Maleate RS
USP Pseudoephedrine Sulfate RS

Identification—

A: The retention time of the major peak for dexbrompheniramine maleate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

B: The retention time of the major peak for pseudoephedrine sulfate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

C: A solution of it responds to the test for *Sulfate* ⟨191⟩.

D: Transfer a volume of Oral Solution, equivalent to about 6 mg of dexbrompheniramine maleate, to a separatory funnel, add 0.5 mL of ammonium hydroxide and 5 mL of methylene chloride, shake for 1 minute, and allow the layers to separate. Use the clear, lower layer as the test solution. Prepare a Standard solution in methanol containing 1.2 mg of USP Dexbrompheniramine Maleate RS and a second Standard solution in methanol containing 9 mg of USP Pseudoephedrine Sulfate RS per mL. Separately apply 5 µL of the test solution and each Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl ether, methanol, and ammonium hydroxide (16:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the R_F values of the two principal spots obtained from the test solution correspond to those obtained from the respective Standard solutions.

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Assay—

Mobile phase—Prepare a mixture of water, acetonitrile, methanol, and tetrahydrofuran (55:32:8:5). Transfer 0.1 mL of phosphoric acid, followed by 0.433 g of sodium lauryl sulfate, to each 100 mL of this mixture, and mix. Adjust with ammonium hydroxide to a pH of 3.50 ± 0.05, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). [NOTE—The pH of the *Mobile phase* is critical, and may cause differences of 1 to 4 minutes in the retention times of the internal standard and dexbrompheniramine.]

Internal standard solution—Dissolve an accurately weighed quantity of naphazoline hydrochloride in *Mobile phase* to obtain a solution containing 0.5 mg per mL.

Dexbrompheniramine standard solution—Dissolve an accurately weighed quantity of USP Dexbrompheniramine Maleate RS in *Mobile phase* to obtain a solution having a known concentration of about 6000/ µg per mL, *J* being the ratio of the labeled amount, in mg, of dexbrompheniramine maleate to the labeled amount, in mg, of pseudoephedrine sulfate per mL of the Oral Solution.

Standard preparation—Transfer about 30 mg of USP Pseudoephedrine Sulfate RS, accurately weighed, to a 25-mL volumetric flask, add 5.0 mL each of *Dexbrompheniramine standard solution* and *Internal standard solution,* dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having known concentrations of about 1.2/ mg of USP Dexbrompheniramine Maleate RS per mL and about 1.2 mg of USP Pseudoephedrine Sulfate RS per mL.

Assay preparation—Using a "to contain" pipet, transfer an accurately measured volume of Oral Solution, equivalent to about 30 mg of pseudoephedrine sulfate, to a 25-mL volumetric flask. Rinse the pipet with about 5 mL of *Mobile phase,* collecting the rinsing in the volumetric flask. Add 5.0 mL of *Internal standard solution,* dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for pseudoephedrine, 1.5 for naphazoline, and 2.5 for dexbrompheniramine; the resolution, *R,* between the pseudoephedrine and naphazoline peaks is not less than 3; the resolution, *R,* between the dexbrompheniramine and naphazoline peaks is not less than 3; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg per mL, of dexbrompheniramine maleate ($C_{10}H_{15}BrN_2 \cdot C_4H_4O_4$) and pseudoephedrine sulfate [$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$] in the portion of Oral Solution taken by the formula:

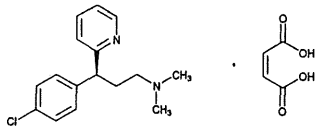
$$25CV(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation; V* is the volume, in mL, of Oral Solution taken; and R_U and R_S are the ratios of the peak responses of the

www.webofpharma.com

corresponding analyte to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dexchlorpheniramine Maleate



$C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ 390.86

2-Pyridinepropanamine, γ-(4-chlorophenyl)-*N*,*N*-dimethyl-, (*S*)-, (*Z*)-2-butenedioate (1:1);
(+)-2-[*p*-Chloro-α-[2-(dimethylamino)ethyl]benzyl]pyridine maleate (1:1) [2438-32-6].

DEFINITION

Dexchlorpheniramine Maleate, dried at 65° for 4 h, contains NLT 98.0% and NMT 102.0% of dexchlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$).

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention times of the maleic acid and dexchlorpheniramine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Solution A: 5.44 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0 ± 0.1.
Solution B: Acetonitrile
Diluent: Acetonitrile and *Solution A* (5:95)
System suitability stock solution: 0.02 mg/mL each of USP Pheniramine Maleate RS, USP Chlorpheniramine Related Compound B RS, and USP Chlorpheniramine Related Compound C RS in *Diluent*. Sonicate for 1 min.
System suitability solution: 0.5 mg/mL of USP Dexchlorpheniramine Maleate RS and 2 µg/mL each of USP Pheniramine Maleate RS, USP Chlorpheniramine Related Compound B RS, and USP Chlorpheniramine Related Compound C RS in *Diluent*, prepared as follows. Transfer 5.0 mg of USP Dexchlorpheniramine Maleate RS to a 10-mL volumetric flask, add 5 mL of *Diluent* and 1.0 mL of the *System suitability stock solution*, and dilute with *Diluent* to volume. Sonicate for 1 min.
Standard solution: 0.5 mg/mL of USP Dexchlorpheniramine Maleate RS in *Diluent*. Sonicate for 1 min.
Sample solution: 0.5 mg/mL of Dexchlorpheniramine Maleate in *Diluent*. Sonicate for 1 min.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
1	95	5
20	70	30
30	70	30
31	95	5
40	95	5

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times of maleic acid, chlorpheniramine related compound C, and dexchlorpheniramine are 0.18, 0.94, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between chlorpheniramine related compound C and dexchlorpheniramine; NLT 2.0 between chlorpheniramine related compound B and pheniramine, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dexchlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in the portion of Dexchlorpheniramine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for dexchlorpheniramine from the *Sample solution*
r_S = peak response for dexchlorpheniramine from the *Standard solution*
C_S = concentration of USP Dexchlorpheniramine Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of Dexchlorpheniramine Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%, previously dried at 65° for 4 h

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.2%
- **Organic Impurities**

Solution A, Solution B, Diluent, System suitability solution, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 2.8 µg/mL of USP Dexchlorpheniramine Maleate RS in *Diluent*, equivalent to 2.0 µg/mL of dexchlorpheniramine. Sonicate for 1 min.
Sensitivity solution: 0.74 µg/mL of USP Pheniramine Maleate RS in *Diluent*
Sample solution: 0.5 mg/mL of Dexchlorpheniramine Maleate in *Diluent*. Sonicate for 1 min.
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between chlorpheniramine related compound C and dexchlorpheniramine; NLT 2.0

USP Monographs

www.webofpharma.com

between chlorpheniramine related compound B and pheniramine, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Dexchlorpheniramine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of dexchlorpheniramine from the *Standard solution*
C_S = concentration of dexchlorpheniramine in the *Standard solution* (mg/mL)
C_U = concentration of Dexchlorpheniramine Maleate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks having areas less than 0.05% of dexchlorpheniramine.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Maleic acid[a]	0.18	—	—
Chlorpheniramine related compound B[b]	0.49	—	—
Pheniramine	0.57	0.40	0.4
Chlorpheniramine related compound C[c]	0.97	—	—
Dexchlorpheniramine	1.0	—	—
Any other unspecified impurity	—	1.0	0.10
Total impurities	—	—	1

[a] Salt counter ion is included in the table for identification purposes only.
[b] Di(pyridin-2-yl)amine. Used only to establish system suitability.
[c] 3-(4-Chlorophenyl-*N*-methyl-3-(pyridin-2-yl)propan-1-amine. Used only to establish system suitability.

• **ENANTIOMERIC PURITY**
System suitability solution: 0.7 mg/mL of chlorpheniramine in 2-propanol prepared as follows. Dissolve 10.0 mg of USP Chlorpheniramine Maleate RS in 3 mL of water. Make the solution basic by adding a few drops of concentrated ammonium hydroxide, and shake with 5 mL of methylene chloride. Separate the layers and evaporate the lower, methylene chloride layer on a water bath until an oily residue is obtained. Dissolve the residue, and dilute with 2-propanol to 10.0 mL.
Standard stock solution: 0.7 mg/mL of dexchlorpheniramine in 2-propanol prepared as follows. Dissolve 10.0 mg of USP Dexchlorpheniramine Maleate RS in 3 mL of water. Make the solution basic by adding a few drops of concentrated ammonium hydroxide, and shake with 5 mL of methylene chloride. Separate the layers and evaporate the lower, methylene chloride layer on a water bath until an oily residue is obtained. Dissolve the residue, and dilute with 2-propanol to 10.0 mL.
Standard solution: 0.014 mg/mL of dexchlorpheniramine in 2-propanol from the *Standard stock solution*
Sample solution: 0.7 mg/mL of dexchlorpheniramine in 2-propanol prepared as follows. Dissolve 10.0 mg of Dexchlorpheniramine Maleate in 3 mL of water. Make the solution basic by adding a few drops of concentrated ammonium hydroxide, and shake with 5 mL of methylene chloride. Separate the layers and evaporate the lower, methylene chloride layer on a water bath until an oily residue is obtained. Dissolve the residue, and dilute with 2-propanol to 10.0 mL.
Mobile phase: *n*-Hexane, 2-propanol, and diethylamine (980:20:3)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 10-µm packing L51
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
[NOTE—Under these conditions the dexchlorpheniramine (*S*-enantiomer) elutes first.]
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between the *R*-enantiomer and dexchlorpheniramine (*S*-enantiomer)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the *R*-enantiomer in the portion of dexchlorpheniramine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *R*-enantiomer from the *Sample solution*
r_S = peak response of dexchlorpheniramine from the *Standard solution*
C_S = concentration of dexchlorpheniramine in the *Standard solution* (mg/mL)
C_U = concentration of dexchlorpheniramine in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2%

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 50 mg/mL, in dimethylformamide
Acceptance criteria: +39.5° to +43.0°
• **pH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 4.0–5.0

ADDITIONAL REQUIREMENTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at 65° for 4 h.
Acceptance criteria: NMT 0.5%
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Chlorpheniramine Maleate RS
USP Chlorpheniramine Related Compound B RS
Di(pyridin-2-yl)amine.
$C_{10}H_9N_3$ 171.20
USP Chlorpheniramine Related Compound C RS
3-(4-Chlorophenyl-*N*-methyl-3-(pyridin-2-yl)propan-1-amine maleate.
$C_{15}H_{17}ClN_2 \cdot C_4H_4O_4$ 376.83
USP Dexchlorpheniramine Maleate RS
USP Pheniramine Maleate RS

USP Monographs

www.webofpharma.com

Dexchlorpheniramine Maleate Oral Solution

» Dexchlorpheniramine Maleate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexchlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Dexchlorpheniramine Maleate RS

Identification—
A: Evaporate the remaining extract from the *Assay* on a steam bath to a small volume, then transfer it to a smaller, more suitable vessel, and evaporate just to the point where hexane vapors are no longer perceptible. Transfer the oily residue, with the aid of four 3-mL portions of dimethylformamide, to a suitable glass-stoppered graduated cylinder, dilute with dimethylformamide to 15.0 mL, and mix: the optical rotation of the solution so obtained, in a 100-mm tube, after correcting for the blank, is between +0.06° and +0.11° (*distinction from chlorpheniramine maleate*).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020): *Assay preparation* compared to *Standard preparation* from *Assay*.
Alcohol Determination ⟨611⟩: between 5.0% and 7.0% of C_2H_5OH.

Assay—
Standard preparation—Transfer about 40 mg of USP Dexchlorpheniramine Maleate RS, accurately weighed, to a 100-mL volumetric flask, add water to volume, and mix. Transfer 10.0 mL of this solution to a separator, adjust with 1 N sodium hydroxide to a pH of 11, and cool. Extract with two 50-mL portions of solvent hexane, shaking each portion for 2 minutes before separating the phases, and combining the hexane extracts in a second separator. Extract the hexane solution with two 40-mL portions of dilute hydrochloric acid (1 in 120), combine the acid extracts in a 100-mL volumetric flask, add dilute hydrochloric acid (1 in 120) to volume, and mix. Filter the solution into a glass-stoppered conical flask, discarding the first few mL of the filtrate. The concentration of USP Dexchlorpheniramine Maleate RS in the *Standard preparation* is about 40 µg per mL.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 40 mg of dexchlorpheniramine maleate, to a 250-mL separator, using a pipet calibrated "to contain" the required volume. Rinse the pipet with small portions of water, add the rinsings to the separator, adjust with 1 N sodium hydroxide to a pH of 11, and cool. Extract with five 70-mL portions of solvent hexane, combine the hexane extracts in a 500-mL separator, and wash the hexane solution with two 10-mL portions of sodium hydroxide solution (1 in 250). Extract the combined alkaline washings with two 20-mL portions of solvent hexane, and add these extracts to the bulk of the alkali-washed hexane solution. Filter the hexane solution through a pledget of cotton that previously has been saturated with solvent hexane into a 500-mL volumetric flask, rinse the separator with portions of solvent hexane, pass the rinsings through the filter to add to volume, and mix. Transfer 50.0 mL of this solution to a separator (retain the remaining extract for *Identification* test *A*), and proceed as directed for *Standard preparation*, beginning with "Extract the hexane solution."
Procedure—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 264 nm, using dilute hydrochloric acid (1 in 120) as the blank. Calculate the quantity, in mg, of dexchlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in each mL of the Oral Solution taken by the formula:

$$(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Dexchlorpheniramine Maleate RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Dexchlorpheniramine Maleate Tablets

DEFINITION
Dexchlorpheniramine Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of dexchlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$).

IDENTIFICATION
• **A. Identification—Organic Nitrogenous Bases** ⟨181⟩:
Meet the requirements
• **B.**
Analysis: Shake a quantity of finely powdered Tablets, equivalent to 150 mg of dexchlorpheniramine maleate, with 100 mL of 1 N acetic acid for 10 min, and filter through a sintered-glass funnel into a suitable vessel. Adjust the filtrate with sodium hydroxide solution (1 in 10) to a pH of 11, and extract the solution with six 100-mL portions of solvent hexane, filtering each hexane extract using suitable means to separate the hexane layer from the aqueous layer. Concentrate the combined extracts on a steam bath to a small volume, transfer to a smaller, more suitable vessel, and evaporate just to the point where hexane vapors are no longer perceptible. Transfer the oily residue, with the aid of four 3-mL portions of dimethylformamide, to a suitable glass-stoppered graduated cylinder, dilute with dimethylformamide to 15.0 mL, mix, and centrifuge if necessary.
Acceptance criteria: The optical rotation of the solution so obtained in a 100-mm tube after correcting for the blank is between +0.24° and +0.35° (distinction from chlorpheniramine maleate).

ASSAY
• **Procedure**
Diluent: Dilute hydrochloric acid (1 in 120)
Standard stock solution: 0.4 mg/mL of USP Dexchlorpheniramine Maleate RS in water
Standard solution: 40 µg/mL of USP Dexchlorpheniramine Maleate RS, prepared as follows. Transfer 10.0 mL of the *Standard stock solution* to a separator, adjust with 1 N sodium hydroxide to a pH of 11, and cool. Extract with two 50-mL portions of solvent hexane, shaking each portion for 2 min before separating the phases, and combining the hexane extracts in a second separator. Extract the hexane solution with two 40-mL portions of *Diluent*, combine the *Diluent* extracts in a 100-mL volumetric flask, and add *Diluent* to volume. Filter

www.webofpharma.com

the solution into a glass-stoppered conical flask, discarding the first few mL of the filtrate.

Sample solution: Nominally 40 µg/mL of dexchlorpheniramine maleate, prepared as follows. Transfer an equivalent to 8 mg of dexchlorpheniramine maleate, from NLT 20 finely powdered Tablets, to a 250-mL separator. Mix with 50 mL of water for 10 min, adjust with sodium hydroxide solution (1 in 10) to a pH of 11, and cool to room temperature. Extract the mixture with two 75-mL portions of solvent hexane, and combine the extracts in a second separator. Extract the solvent hexane solution with three 50-mL portions of *Diluent*, combining the *Diluent* extracts in a 200-mL volumetric flask. Add *Diluent* to volume.

Instrumental conditions

Analytical wavelength: Maximum absorbance at about 264 nm

Cell: 1 cm

Blank: *Diluent*

Analysis

Samples: *Standard solution* and *Sample solution*

Concomitantly determine the absorbance of the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of dexchlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Dexchlorpheniramine Maleate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of dexchlorpheniramine maleate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 45 min

Solution A: Sodium hydroxide solution (1 in 2)

Internal standard solution: 90 µg/mL of dexbrompheniramine maleate in water

Standard stock solution: 12.5 µg/mL of USP Dexchlorpheniramine Maleate RS in water

Standard solution: Pipet 5 mL of the *Standard stock solution* into a 50-mL centrifuge tube, and add 10.0 mL water and 1.0 mL *Internal standard solution*. Adjust with *Solution A* to a pH of 11 ± 0.1, and add 3.0 mL of chromatographic hexane. Insert the stopper in the tube, shake by mechanical means for 3 min, centrifuge, and use the clear supernatant hexane layer.

Sample solution: Pipet 15 mL of a portion of the solution under test into a 50-mL centrifuge tube, and add 1.0 mL of *Internal standard solution*. Adjust with *Solution A* to a pH of 11 ± 0.1, and add 3.0 mL of chromatographic hexane. Insert the stopper in the tube, shake by mechanical means for 3 min, centrifuge, and use the clear supernatant hexane layer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m; contains a packing consisting of 1.2% phase G16 and 0.5% potassium hydroxide on support S1AB

Temperatures

Column: 205°

Injector: 250°

Detector: 250°

Flow rate: 60 mL/min

Carrier gas: Helium

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for dexchlorpheniramine and dexbrompheniramine are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.9 between dexchlorpheniramine and dexbrompheniramine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity of dexchlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) dissolved by comparing the peak response ratios.

Tolerances: NLT 75% (*Q*) of the labeled amount of dexchlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

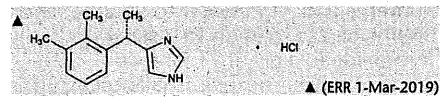
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Dexchlorpheniramine Maleate RS

Change to read:

Dexmedetomidine Hydrochloride



▲ (ERR 1-Mar-2019)

$C_{13}H_{16}N_2 \cdot HCl$ 236.74

1*H*-Imidazole, 4-[1-(2,3-dimethylphenyl)ethyl]-, monohydrochloride, (*S*)-;

4-[(*S*)-α,2,3-Trimethylbenzyl]imidazole monohydrochloride [145108-58-3].

DEFINITION

Dexmedetomidine Hydrochloride contains NLT 98.0% and NMT 102.0% of dexmedetomidine hydrochloride ($C_{13}H_{16}N_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Enantiomeric Purity*.

• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: Meets the requirements

ASSAY

• **PROCEDURE**

Buffer: 0.89 g/L dibasic sodium phosphate dihydrate solution prepared as follows. Dissolve a suitable amount of

www.webofpharma.com

dibasic sodium phosphate dihydrate with 90% of total volume of water, adjust with 16 g/L of monobasic sodium phosphate dihydrate solution in water to a pH of 7.0, and dilute with water to volume.
Mobile phase: Methanol and *Buffer* (60:40)
Standard solution: 0.2 mg/mL of USP Dexmedetomidine Hydrochloride RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Dexmedetomidine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4-mm × 12.5-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dexmedetomidine hydrochloride ($C_{13}H_{16}N_2 \cdot HCl$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dexmedetomidine from the *Sample solution*
r_S = peak response of dexmedetomidine from the *Standard solution*
C_S = concentration of USP Dexmedetomidine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Dexmedetomidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%
• **Organic Impurities**
Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
Standard solution: 2 µg/mL of USP Dexmedetomidine Hydrochloride RS in *Mobile phase*
Sensitivity solution: 0.8 µg/mL of USP Dexmedetomidine Hydrochloride RS in *Mobile phase*
Sample solution: 2 mg/mL of sample in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay* except for the following.
Run time: NLT 9 times the retention time of the dexmedetomidine peak
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times of known impurities are given in *Table 1*.]

Table 1

Name	Relative Retention Time
Hydroxymedetomidine[a]	0.36
Dexmedetomidine	1.00
N-Benzyl hydroxymedetomidine[b]	2.22
Ethylmedetomidine[c]	2.47
N-Benzyl medetomidine[d]	6.25
N-Benzyl vinyl analog[e]	6.31

[a] 1-(2,3-Dimethylphenyl)-1-(1*H*-imidazol-5-yl)ethanol.
[b] 1-(1-Benzyl-1*H*-imidazol-5-yl)-1-(2,3-dimethylphenyl)ethanol.
[c] 5-[1-(2,3-Dimethylphenyl)ethyl]-1-ethyl-1*H*-imidazole.
[d] 1-Benzyl-5-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole.
[e] 1-Benzyl-5-[1-(2,3-dimethylphenyl)vinyl]-1*H*-imidazole.

Calculate the percentage of each impurity in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of dexmedetomidine in the *Standard solution*
C_S = concentration of USP Dexmedetomidine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Dexmedetomidine Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: Disregard peaks below 0.04%.
Individual impurities: NMT 0.10%
Total impurities: NMT 0.3%
• **Enantiomeric Purity**
Buffer: To 1 L of 5.34 g/L dibasic sodium phosphate dihydrate solution, adjust with a suitable amount (about 700–800 mL) of 4.08 g/L monobasic potassium phosphate solution to a pH of 7.0.
Mobile phase: Acetonitrile and *Buffer* (35:165)
System suitability solution: 1 µg/mL of USP Levomedetomidine RS and 50 µg/mL of USP Dexmedetomidine Hydrochloride RS in *Mobile phase*
Standard solution: 0.5 µg/mL of USP Levomedetomidine RS in *Mobile phase*
Sensitivity solution: 0.05 µg/mL of USP Levomedetomidine RS in *Mobile phase*
Sample solution: 50 µg/mL of sample in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4-mm × 10-cm; 5-µm packing L41
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between levomedetomidine and dexmedetomidine, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levomedetomidine hydrochloride in the portion of sample taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak height of levomedetomidine in the *Sample solution*
r_S = peak height of levomedetomidine in the *Standard solution*
C_S = concentration of USP Levomedetomidine RS in the *Standard solution* (µg/mL)
C_U = concentration of Dexmedetomidine Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levomedetomidine[a]	0.69	1.0
Dexmedetomidine	1.00	—

[a] (*R*)-4-[1-(2,3-Dimethylphenyl)ethyl]-1*H*-imidazole hydrochloride.

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Sample: 1.0 g
Analysis: Dry the *Sample* at 105° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Store at controlled room temperature in tight containers in a dry place.
• **USP Reference Standards** ⟨11⟩
USP Dexmedetomidine Hydrochloride RS
USP Levomedetomidine RS
(*R*)-4-[1-(2,3-Dimethylphenyl)ethyl]-1*H*-imidazole hydrochloride.
$C_{13}H_{16}N_2 \cdot HCl$ 236.74

Dexmedetomidine Injection

DEFINITION
Dexmedetomidine Injection is a sterile solution containing Dexmedetomidine Hydrochloride and sodium chloride, suitable for dilution prior to intravenous administration. It contains NLT 90.0% and NMT 110.0% of the labeled amount of dexmedetomidine free base ($C_{13}H_{16}N_2$). [Note—This monograph is not applicable to veterinary articles described under 21 CFR 522.558.]

IDENTIFICATION
• **A.** The retention time of the dexmedetomidine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV absorption spectrum of the major peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Buffer: Dissolve 2.4 g of disodium hydrogen phosphate heptahydrate and 0.14 g of monobasic sodium phosphate in 1 L of water. Adjust with 0.1 N phosphoric acid or 0.1 N sodium hydroxide to a pH of 7.8, if needed.
Mobile phase: Methanol and *Buffer* (600:400)
Diluent: 0.9% sodium chloride in water
Standard solution: 4.8 µg/mL of USP Dexmedetomidine Hydrochloride RS in water
Sample solution: Nominally equivalent to 4 µg/mL of dexmedetomidine in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm. For *Identification B*, use UV diode array, 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: NLT 1.8 times the retention time of dexmedetomidine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dexmedetomidine ($C_{13}H_{16}N_2$) as free base in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dexmedetomidine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dexmedetomidine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of dexmedetomidine free base, 200.28
M_{r2} = molecular weight of dexmedetomidine hydrochloride, 236.74

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **Organic Impurities**
Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
Butylparaben stock solution: 0.25 mg/mL of butylparaben prepared as follows. To a suitable amount of butylparaben in a suitable volumetric flask, add methanol, 5% of total volume, and dilute with water to volume.
Butylparaben solution: 0.0025 mg/mL of butylparaben in water from *Butylparaben stock solution*
System suitability solution: Equivalent to 0.18 µg/mL of dexmedetomidine free base from USP Dexmedetomidine Hydrochloride RS and 0.25 µg/mL of butylparaben from *Butylparaben solution* in water
Standard solution: Equivalent to 0.2 µg/mL of dexmedetomidine free base from USP Dexmedetomidine Hydrochloride RS in water
Sensitivity solution: Equivalent to 0.04 µg/mL of dexmedetomidine free base from USP Dexmedetomidine Hydrochloride RS in water from the *Standard solution*
Sample solution: Use the neat Injection as is.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 25 µL

USP Monographs

www.webofpharma.com

Run time: NLT 3 times the retention time of dexmedetomidine

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 4.0 between butylparaben and dexmedetomidine, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of dexmedetomidine from the *Standard solution*

C_S = concentration of USP Dexmedetomidine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of dexmedetomidine in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of dexmedetomidine free base, 200.28

M_{r2} = molecular weight of dexmedetomidine hydrochloride, 236.74

Acceptance criteria: Disregard peaks below 0.02%.

Any individual impurity: NMT 0.5%

Total impurities: NMT 0.7%

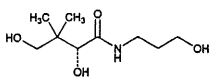
SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 2.5 USP Endotoxin Units/µg of dexmedetomidine free base
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **pH** ⟨791⟩**:** 4.5–7.0
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose, clear, glass vials. Store at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be diluted with 0.9% Sodium Chloride Injection USP for intravenous use.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dexmedetomidine Hydrochloride RS

Dexpanthenol



$C_9H_{19}NO_4$ 205.25

Butanamide, 2,4-dihydroxy-*N*-(3-hydroxypropyl)-3,3-dimethyl-, (*R*)-;

D-(+)-2,4-Dihydroxy-*N*-(3-hydroxypropyl)-3,3-dimethyl butyramide [81-13-0].

DEFINITION

Dexpanthenol contains NLT 98.0% and NMT 102.0% of dexpanthanol ($C_9H_{19}NO_4$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F▲** (CN 1-May-2020)
- **B.**

Sample solution: 100 mg/mL of Dexpanthenol

Analysis: To 1 mL of the *Sample solution* add 5 mL of 1 N sodium hydroxide and 1 drop of cupric sulfate TS, and shake vigorously.

Acceptance criteria: A deep blue color develops.

- **C.**

Sample solution: 10 mg/mL of Dexpanthenol

Analysis: To 1 mL of the *Sample solution* add 1 mL of 1 N hydrochloric acid, and heat on a steam bath for 30 min. Cool, add 100 mg of hydroxylamine hydrochloride, and add 5 mL of 1 N sodium hydroxide. Allow to stand for 5 min, then adjust with 1 N hydrochloric acid to a pH of 2.5–3.0, and add 1 drop of ferric chloride TS.

Acceptance criteria: A purplish red color develops.

ASSAY

- **PROCEDURE**

0.1 M potassium biphthalate: Transfer 20.42 g of potassium biphthalate into a 1000-mL volumetric flask, and add sufficient glacial acetic acid to dissolve. If necessary, warm the mixture on a steam bath to achieve complete solution, observing precautions against absorption of moisture. Cool to room temperature, and dilute with glacial acetic acid to volume.

Sample: 400 mg of Dexpanthenol

Blank: Proceed as directed in the *Analysis*, omitting the *Sample*.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Residual titration

Titrant: 0.1 N perchloric acid VS

Back-titrant: 0.1 M potassium biphthalate

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 300-mL flask fitted to a reflux condenser by means of a standard-taper glass joint, add 50.0 mL of *Titrant*, and reflux for 5 h. Cool, observing precautions to prevent atmospheric moisture from entering the condenser, and rinse the condenser with glacial acetic acid, collecting the rinsings in the flask. To the flask, add 5 drops of crystal violet TS, and titrate with the *Back-titrant* to a blue-green endpoint. Perform the *Blank* determination. Calculate the percentage of dexpanthenol ($C_9H_{19}NO_4$) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times M \times F]/W\} \times 100$$

V_B = *Back-titrant* volume consumed by the *Blank* (mL)

V_S = *Back-titrant* volume consumed by the *Sample* (mL)

M = actual molarity of the *Back-titrant* (mM/mL)

F = equivalency factor, 205.3 mg/mM

W = *Sample* weight (mg)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

USP Monographs

www.webofpharma.com

• **LIMIT OF AMINOPROPANOL**
Sample: 5 g of Dexpanthenol
Blank: 10 mL of water
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N sulfuric acid VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 10 mL of water, and add bromothymol blue TS. Titrate with the *Titrant* to a yellow endpoint. Perform the *Blank* determination.
Calculate the percentage of aminopropanol in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 75.11 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 50 mg/mL in water
Acceptance criteria: +29.0° to +31.5°
• **REFRACTIVE INDEX** ⟨831⟩**:** 1.495–1.502, at 20°
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dexpanthenol RS

Dexpanthenol Preparation

DEFINITION
Dexpanthenol Preparation contains NLT 94.5% and NMT 98.5% of dexpanthenol ($C_9H_{19}NO_4$), and NLT 2.7% and NMT 4.2% of pantolactone, both calculated on the anhydrous basis.

IDENTIFICATION
• **A.** The IR absorption spectrum of a thin film of Preparation exhibits maxima only at the same wavelengths as that of a similar preparation of USP Dexpanthenol RS, except that there is an additional maximum at 5.6 µm due to pantolactone.
• **B.**
Sample solution: 100 mg/mL of Preparation
Analysis: To 1 mL of the *Sample solution* add 5 mL of 1 N sodium hydroxide and 1 drop of cupric sulfate TS, and shake vigorously.
Acceptance criteria: A deep blue color develops.

COMPOSITION
• **CONTENT OF DEXPANTHENOL**
0.1 M potassium biphthalate: Transfer 20.42 g of potassium biphthalate into a 1000-mL volumetric flask, and add sufficient glacial acetic acid to dissolve. If necessary, warm the mixture on a steam bath to achieve complete solution, observing precautions against absorption of moisture. Cool to room temperature, and dilute with glacial acetic acid to volume.
Sample: 400 mg of Preparation
Blank: Proceed as directed in the *Analysis*, omitting the *Sample*.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 0.1 N perchloric acid VS
Back-titrant: 0.1 M potassium biphthalate
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 300-mL flask fitted to a reflux condenser by means of a standard-taper glass joint, add 50.0 mL of *Titrant*, and reflux for 5 h. Cool, observing precautions to prevent atmospheric moisture from entering the condenser, and rinse the condenser with glacial acetic acid, collecting the rinsings in the flask. To the flask add 5 drops of crystal violet TS, and titrate with the *Back-titrant* to a blue-green endpoint. Perform the *Blank* determination.
Calculate the percentage of dexpanthenol ($C_9H_{19}NO_4$) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times M \times F]/W\} \times 100$$

V_B = *Back-titrant* volume consumed by the *Blank* (mL)
V_S = *Back-titrant* volume consumed by the *Sample* (mL)
M = actual molarity of the *Back-titrant* (mM/mL)
F = equivalency factor, 205.3 mg/mM
W = weight of the *Sample* (mg)

Acceptance criteria: 94.5%–98.5% on the anhydrous basis
• **CONTENT OF PANTOLACTONE**
Internal standard solution: 100 mg/mL of 2,6-dimethylphenol in toluene
Standard stock solution: 10 mg/mL of USP Pantolactone RS in methylene chloride
Standard solution: Pipet 0.4 mL of *Standard stock solution* into a suitable small vial. Evaporate the solvent by means of a steady stream of dry air, and add 50.0 µL of *Internal standard solution.* Add 1 mL of a mixture of pyridine, hexamethyldisilazane, and trimethylchlorosilane (9:3:1), immediately close the vial, and shake vigorously for 30 s.
Sample solution: Transfer 100 mg of Preparation to a suitable small vial, and proceed as directed for the *Standard solution,* beginning with "add 50.0 µL of *Internal standard solution*".
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: GC
Detector: Flame ionization
Column: 2.0-mm × 1.8-m, packed with 5% liquid phase G2 on support S1A
Temperature
Column: 170°, isothermal
Injector: 180°
Flow rate: Using a suitable carrier gas, adjust the flow rate so that the derivatized pantolactone elutes in 4 min.
Injection size: 0.5 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention times of the derivatized pantolactone and derivatized internal standard are 0.75 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the derivatized pantolactone and derivatized internal standard
Relative standard deviation: NMT 2.0% for the ratios of the derivatized pantolactone peak response to the derivatized internal standard peak response

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pantolactone in the portion of Preparation taken:

$$\text{Result} = (R_U/R_S) \times (V/W) \times C_S \times 100$$

R_U = internal standard ratio (peak response of derivatized pantolactone/peak response of derivatized internal standard) from the *Sample solution*
R_S = internal standard ratio (peak response of derivatized pantolactone/peak response of derivatized internal standard) from the *Standard solution*
V = volume of the *Standard stock solution* taken to prepare the *Standard solution*, 0.4 mL
W = weight of Preparation taken to prepare the *Sample solution* (mg)
C_S = concentration of USP Pantolactone RS in the *Standard stock solution* (mg/mL)

Acceptance criteria: 2.7%–4.2% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF AMINOPROPANOL**

Sample: 5 g of Preparation
Blank: 10 mL of water
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N sulfuric acid VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 10 mL of water, and add bromothymol blue TS. Titrate with the *Titrant* to a yellow endpoint. Perform the *Blank* determination.
Calculate the percentage of aminopropanol in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 75.11 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 50 mg/mL in water
Acceptance criteria: +27.5° to +30.0°
- **REFRACTIVE INDEX** ⟨831⟩: 1.495–1.502, at 20°
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Dexpanthenol RS
USP Pantolactone RS

Dextran 1

DEFINITION
Dextran 1 is a low molecular weight fraction of dextran, consisting of a mixture of isomaltooligosaccharides. It is obtained by controlled hydrolysis and fractionation of dextrans produced by fermentation of *Leuconostoc mesenteroides* (strain NRRL B-512; CIP 78.59, or its sub-strains, for example *L. mesenteroides* B-512F; NCTC, 10817), in the presence of sucrose. It is a glucose polymer in which the linkages between glucose units are almost exclusively α-1,6. Its weight-average molecular weight is about 1000.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020): To 1–2 mg each of USP Dextran 1 RS and the sample, add 1–2 drops of water, grind in an agate mortar for 1–2 min, add 300 mg of potassium bromide, and mix to a slurry. [NOTE—Do not grind.] Dry under vacuum at 40° for 15 min, and if it is not dry, continue drying for another 15 min. Crush the residue, prepare a disk, and run the IR spectrum with a blank potassium bromide disk in the reference beam.
- **B.** It meets the requirements of the test for *Molecular Weight Distribution* and *Average Molecular Weight*.

IMPURITIES
- **LIMIT OF SODIUM CHLORIDE**

Sample: 5 g
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 100 mL of water. Add 0.2 mL of potassium chromate TS, and titrate with *Titrant*. Each mL of *Titrant* is equivalent to 5.844 mg of sodium chloride.
Acceptance criteria: NMT 1.5%
- **LIMIT OF NITROGENOUS IMPURITIES** (where it is labeled as intended for use in the preparation of injectables)

(See *Nitrogen Determination* ⟨461⟩.)
Sulfate solution: To 1000 mL of sulfuric acid add 5 g of anhydrous cupric sulfate and 500 g of potassium sulfate. Dissolve by heating, and store at 60°. [NOTE—If storage at 60° is not possible, prepare a smaller quantity of *Sulfate solution* on the day of use, adjusting proportions accordingly.]
Indicator: Dilute a mixture of 20 mL of a 0.1% solution of bromocresol green in alcohol and 4 mL of methyl red TS with water to 100 mL.
Sample solution: Transfer 0.2 g of Dextran 1, accurately weighed, to a micro-Kjeldahl flask. Add 4 mL of *Sulfate solution*.
Analysis
Sample: *Sample solution*
Heat the *Sample solution* until the solution exhibits a clear green color and the sides of the flask are free from carbonaceous material. Cool, cautiously add 30 mL of water, and transfer the solution to a steam distillation unit. Rinse the Kjeldahl flask with three 5-mL portions of water, adding the washings to the solution. Add 15 mL of 45% sodium hydroxide solution, immediately close the distillation apparatus, and commence steam distillation without delay. Receive the distillate in 1 mL of *Indicator* and sufficient water to cover the end of the condensing tube. Upon completion of the distillation, remove the receiving flask, and rinse the end of the condensing tube with a small quantity of water, adding the rinse to the distillate. Titrate the distillate with 0.010 N hydrochloric acid until the color changes from blue to reddish violet.

www.webofpharma.com

Perform a blank determination, and make any necessary correction.

Acceptance criteria: The corrected volume of 0.010 N hydrochloric acid required to change the color does not exceed 0.15 mL (110 ppm of nitrogen).

• **LIMIT OF ALCOHOL AND RELATED IMPURITIES**

Standard solution: To 25.0 mL of the *Sample solution* add 0.5 mL of a 2.5% (w/v) solution of *n*-propyl alcohol.

Sample solution: Dissolve without heating 5.0 g of Dextran 1 in 100 mL of water, and distill the solution, collecting the first 45 mL of the distillate. Dilute the distillate with water to 50.0 mL, and mix.

Chromatographic system

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m; packed with S3

Temperatures

Column: 160°

Injection port: 240°

Detector: 210°

Carrier gas: Nitrogen

Flow rate: 25 mL/min. [NOTE—Injector seals may deteriorate after multiple injections of the *Standard solution* and *Sample solution*. Inspect the seals before making a series of injections.]

Injection volume: 1 µL

Analysis

Samples: *Standard solution*, *Sample solution*, and a 0.05% (w/v) solution of *n*-propyl alcohol and water

Acceptance criteria: After corrections for any impurities in the *n*-propyl alcohol solution and water, the total area of peaks from impurities in the *Sample solution* does not exceed the area of the *n*-propyl alcohol solution peak.

SPECIFIC TESTS

• **ULTRAVIOLET-VISIBLE SPECTROSCOPY** ⟨857⟩

Sample solution: 15%

Instrumental conditions

Mode: UV

Analytical wavelength: 375 nm

Blank: Water

Acceptance criteria: NMT 0.12

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: A solution in water at 20°, on the dried basis (dried at 70° under vacuum to constant weight), corrected for sodium chloride content

Acceptance criteria: +148° to +164°

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/g, determined by plate-count, and the total combined molds and yeasts count does not exceed 10 cfu/g.

• **pH** ⟨791⟩

Sample solution: A 15% solution in water

Acceptance criteria: 4.5–7.0

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 100°–105° for 5 h.

Acceptance criteria: NMT 5.0%

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩ (where it is labeled as intended for use in the preparation of injectables)**:** NMT 25.0 USP Endotoxin Units/g

• **MOLECULAR WEIGHT DISTRIBUTION** and **AVERAGE MOLECULAR WEIGHT**

Mobile phase: 2.9 mg/mL of sodium chloride, filtered and degassed

Calibration solution: 0.45 mg of isomaltotriose (3 glucose units) and 0.60 mg of sodium chloride per mL

Standard solution: 6.0–6.5 mg/mL of USP Dextran 1 RS in *Mobile phase*

Sample solution: 6.0–6.5 mg/mL solution of Dextran 1 in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Differential refractive index

Column: Two 10-mm × 30-cm columns in series; packing L54

Column temperature: 20°–25°

Flow rate: 0.07–0.08 mL/min, maintained constant to ±1%

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements: The M_w and the amounts of the fractions obtained for the *Standard solution* are within the values stated in the data sheet that accompanies USP Dextran 1 RS.

Analysis

Samples: *Calibration solution* and *Sample solution*

Using the retention times in the chromatogram of the *Calibration solution*, identify the peaks due to isomaltotriose and sodium chloride in the chromatograms of the *Standard solution* and the *Sample solution*. Disregard the peak due to sodium chloride in the *Standard solution* and the *Sample solution*.

Calculate the weight-average molecular weight, M_w:

$$\text{Result} = \Sigma W_i M_i$$

W_i = weight proportion of oligosaccharide i

M_i = molecular weight of oligosaccharide i

Use the molecular weight values for calculation from *Table 1*.

Table 1

Oligosaccharide	Molecular Weight
Glucose	180
Isomaltose	342
Isomaltotriose	504
Isomaltotetraose	666
Isomaltopentaose	828
Isomaltohexaose	990
Isomaltoheptaose	1152
Isomaltooctaose	1314
Isomaltononaose	1476
Isomaltodecaose	1638
Isomaltoundecaose	1800
Isomaltododecaose	1962
Isomaltotridecaose	2124
Isomaltotetradecaose	2286
Isomaltopentadecaose	2448
Isomaltohexadecaose	2610
Isomaltoheptadecaose	2772
Isomaltooctadecaose	2934
Isomaltononadecaose	3096

USP Monographs

www.webofpharma.com

Calculate the amounts of the fractions with fewer than 3 and with more than 9 glucose units for the *Standard solution* and the *Sample solution*.

Acceptance criteria: The M_w of Dextran 1 is 850–1150. The fraction with fewer than 3 units of glucose is less than 15%, and the fraction with more than 9 units of glucose is less than 20%.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in well-closed containers at a temperature between 4° and 30°.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dextran 1 RS

Dextran 40

Dextrans [9004-54-0].

DEFINITION

Dextran 40 is derived by controlled hydrolysis and fractionation of polysaccharides elaborated by the fermentative action of certain strains of *Leuconostoc mesenteroides* (NRRL, B.512 F; NCTC, 10817) on a sucrose substrate. It is a glucose polymer in which the linkages between glucose units are almost entirely of the α-1:6 type. Its weight average molecular weight is in the 35,000–45,000 range.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.**

Sample solutions: Prepare four solutions of Dextran 40 in water, in such a manner that the concentrations are accurately known and approximately evenly distributed in the range of 2%–0.5%.

Analysis: Using a capillary tube viscometer having dimensions such that the flow time of water is NLT 100 s, measure the flow times of water and of the *Sample solution* at 20°.

Calculate the viscosity numbers of each of the *Sample solutions*:

$$\text{Result} = \{\ln[(R_D) \times (t/t_0)]\}/C$$

R_D = ratio of the density of the individual *Sample solution* to that of water
t = *Sample solution* flow time
t_0 = water flow time
C = concentration of Dextran 40 in the *Sample solution* (g/mL)

Plot the viscosity numbers of each of the *Sample solutions* against their respective concentrations, draw the straight line of best fit through the points, and extrapolate to zero concentration.

Acceptance criteria: The value of the intercept is 18–23 mL/g.

IMPURITIES

- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩**:** A 1.5-g portion of Dextran 40 shows NMT 0.03%, which corresponds to 0.45 mL of 0.020 N sulfuric acid.
- **LIMIT OF NITROGENOUS IMPURITIES** (where it is labeled as intended for use in the preparation of injectables)

Sulfate solution: To 1000 mL of sulfuric acid add 5 g of anhydrous cupric sulfate and 500 g of potassium sulfate. Dissolve by heating, and store at 60°. [NOTE—If storage at 60° is not possible, prepare a smaller quantity of *Sulfate solution* on the day of use, adjusting the proportions accordingly.]

Indicator: Dilute a mixture of 20 mL of a 0.1% solution of bromocresol green in alcohol and 4 mL of methyl red TS with water to 100 mL.

Sample solution: Transfer 0.2 g of Dextran 40, accurately weighed, to a micro-Kjeldahl flask. Add 4 mL of *Sulfate solution*.

Analysis

Sample: *Sample solution*

Heat until the solution exhibits a clear green color and the sides of the flask are free from carbonaceous material. Cool, and transfer the solution to a steam distillation unit. Rinse the Kjeldahl flask three times with 5 mL of water, adding the washings to the solution. Add 15 mL of 45% sodium hydroxide solution, immediately close the distillation apparatus, and commence steam distillation without delay. Receive the distillate in 1 mL of *Indicator* in a 100-mL flask, keeping the end of the condensing tube below the liquid surface for 5 min and above the liquid surface for 1 min. Upon completing the distillation, remove the receiving flask, and rinse the end of the condensing tube with a small quantity of water, adding the rinse to the distillate. Titrate the distillate with 0.010 N hydrochloric acid until the color changes from blue to reddish violet. Perform a blank determination, and make any necessary correction.

Acceptance criteria: The corrected volume of 0.010 N hydrochloric acid titrated does not exceed 0.14 mL (0.01%, as N).

- **LIMIT OF ALCOHOL AND RELATED IMPURITIES**

Standard solution: To 25.0 mL of the *Sample solution* add 0.5 mL of a 2.5% (w/v) solution of *n*-propyl alcohol.

Sample solution: Dissolve without heating 5.0 g of Dextran 40 in 100 mL of water, and distill the solution, collecting the first 45 mL of the distillate. Dilute the distillate with water to 50.0 mL.

Chromatographic system

Mode: GC
Detector: Flame ionization
Column: 2-mm × 1.8-m; packed with S3
Temperatures
Column: 160°
Injection port: 240°
Detector: 210°
Carrier gas: Nitrogen
Flow rate: 25 mL/min

[NOTE—Injector seals may deteriorate after multiple injections of the *Standard solution* and *Sample solution*. Inspect the seals before making a series of injections.]

Injection volume: 1 µL

Analysis

Samples: *Standard solution*, *Sample solution*, and a 0.05% (w/v) solution of *n*-propyl alcohol and water

Acceptance criteria: After corrections for any impurities in the *n*-propyl alcohol solution and water, the total area of peaks from impurities in the *Sample solution* does not exceed the area of the *n*-propyl alcohol solution peak.

USP Monographs

www.webofpharma.com

• **ANTIGENIC IMPURITIES** (where it is labeled as intended for use in the preparation of injectables)
Sample solution: Prepare a sterile solution containing 100 mg/mL of Dextran 40 in sodium chloride injection.
Analysis: At intervals of about 48 h, inject three 0.5-mL doses into the peritoneal cavities of each of six guinea pigs. At 14 days after the first intraperitoneal injection, inject 0.20 mL intravenously into each of three of the guinea pigs, and at 21 days treat the other three guinea pigs similarly. Observe the animals for 30 min after each intravenous injection and again 24 h later.
Acceptance criteria: The animals exhibit no evidence of anaphylactoid reactions, such as coughing, bristling of hair, or respiratory distress.

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 20 mg/mL of Dextran 40, heated, if necessary, on a water bath to dissolve
Acceptance criteria: +195° to +203°

• **PH** ⟨791⟩
Sample solution: A solution (1 in 10)
Acceptance criteria: 4.5–7.0

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 5 h.
Acceptance criteria: NMT 7.0%

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩ (where it is labeled as intended for use in the preparation of injectables)**:** When tested in sodium chloride injection (1 in 10), it contains NMT 1.0 USP Endotoxin Unit/mL.

• **COLOR OF SOLUTION**
Sample solution: A solution in water (1 in 10)
Blank solution: Water
Analysis: Absorbance at 375 nm in a 4-cm cell
Acceptance criteria: NMT 0.20

• **SAFETY:** Inject intravenously 1.0 mL of a sterile solution (1 in 10) of 10% Dextran 40 in saline TS into each of five mice weighing 18–20 g. The injection period is 10–15 s. If there are no deaths within 72 h, it meets the requirements of the test. If one or more animals die, continue the test using 10 mice weighing 20 ± 0.5 g. If all animals survive for 72 h, the requirements of the test are met.

• **MOLECULAR WEIGHT DISTRIBUTION** and **WEIGHT AND NUMBER AVERAGE MOLECULAR WEIGHTS**
Mobile phase: 7.1 g/L of anhydrous sodium sulfate in water, filtered and degassed
Calibration solutions: Separately prepare 20 mg/mL in *Mobile phase*, USP Dextran 4 Calibration RS, USP Dextran 10 Calibration RS, USP Dextran 40 Calibration RS, USP Dextran 70 Calibration RS, and USP Dextran 250 Calibration RS
Marker solution: 3 mg/mL each of dextrose and USP Dextran V0 Marker RS in *Mobile phase*
System suitability solution: 20 mg/mL of USP Dextran 40 System Suitability RS in *Mobile phase*
Sample solution: 20 mg/mL of Dextran 40 in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: Three 7.5-mm × 30-cm columns; packing L38
Column temperature: Constant temperature
Injection volume: 50 µL
System suitability
Samples: *Calibration solutions*, *Marker solution*, and *System suitability solution*
Suitability requirements
Elution profile: Profile shows two peaks: the first due to the V_0 marker, the second due to dextrose, *Marker solution*.
Tailing factor: NMT 1.3, for the dextrose peak, *Marker solution*
Relative standard deviation of the ratio V_0:V_T: Determine the void volume, V_0, of the system as the inflection point of the ascending part of the first peak. Determine the total volume, V_T, of the system as the maximum of the second peak. The relative standard deviation of the ratio V_0:V_T is NMT 1%, *Marker solution*.
Weight average molecular weight: Chromatograph each of the *Calibration solutions* separately. Divide each profile into at least 60 vertical sections of equal volume increments. (The actual number of sections is represented by the variable a in the equations below.) Record y_i, the height above the baseline, corresponding to each value of v_i, the volume eluted at that section. For each value of v_i, calculate the distribution coefficient, K_i:

$$\text{Result} = (v_i - V_0)/(V_T - V_0)$$

Find appropriate values of b_1, b_2, b_3, b_4, and b_5, using a suitable method[1] that, when substituted in the equation:

$$M_i = b_5 + e^{\left(b_4 + b_1K_i + b_2K_i^2 + b_3K_i^3\right)}$$

and the resulting values of M_i substituted, along with their corresponding values of y_i, in the equation:

$$\overline{M}_W = \sum_{i=1}^{a}\left(y_iM_i\right)/\sum_{i=1}^{a} y_i$$

give values of weight average molecular weight, $\overline{M}_w$, within 5% of the labeled values for each of the *Calibration solutions* and 180 ± 2 for dextrose. Using the *System suitability solution*, calculate $\overline{M}_w$ of the total molecular weight distribution using the same method, but inserting the now known values of b_1, b_2, b_3, b_4, and b_5. It is between 39,000 and 46,000.
Weight average molecular weight, high-fraction: Calculate $\overline{M}_w$ of the high-fraction dextran eluted through section n:

$$\sum_{i=1}^{n}\left(y_iM_i\right)/\sum_{i=1}^{n} y_i$$

in which n is defined by the relations:

$$\sum_{i=1}^{n} y_i \le 0.1\left(\sum_{i=1}^{a} y_i\right) \quad \text{and}$$

$$\sum_{i=1}^{n+1} y_i > 0.1\left(\sum_{i=1}^{a} y_i\right)$$

It is between 111,000 and 135,000, *System suitability solution*.
Weight average molecular weight, low-fraction: Calculate $\overline{M}_w$ of the low-fraction dextran eluted in and after section m:

[1] The Gauss-Newton method, modified by Hartley [see D. Hartley *Technometrics*, **3** (1961)], and the G. Nilsson and K. Nilsson method [see G. Nilsson and K. Nilsson J. *Chromat.*, **101**, 137 (1974)] are suitable methods. A curve-fitting program capable of nonlinear regression may be used.

www.webofpharma.com

$$\sum_{i=m}^{a}(y_i M_i) / \sum_{i=m}^{a} y_i$$

in which m is defined by:

$$\sum_{i=m}^{a} y_i \le 0.1\left(\sum_{i=1}^{a} y_i\right) \quad \text{and}$$

$$\sum_{i=m-1}^{a} y_i > 0.1\left(\sum_{i=1}^{a} y_i\right)$$

It is between 6000 and 9000, *System suitability solution.*

Analysis

Sample: *Sample solution*

Calculate the weight average molecular weight, $\overline{M}_w$, for the total molecular weight distribution, the high-fraction dextran, and the low-fraction dextran. With the values of b_1, b_2, b_3, b_4, and b_5, obtained with the *Calibration solutions* in *System suitability*, calculate the number average molecular weight, $\overline{M}_n$, of the total molecular weight distribution of the *Sample solution* by substituting the corresponding values of M_i, along with their corresponding values of y_i, in the equation:

$$\overline{M}_n = \sum_{i=1}^{a} y_i / \sum_{i=1}^{a} (y_i / M_i)$$

Acceptance criteria: The weight average molecular weight, $\overline{M}_w$, of the total molecular weight distribution is 35,000–45,000. The weight average molecular weight, $\overline{M}_w$, of the high-fraction dextran and of the low-fraction dextran are NMT 120,000 and NLT 5,000, respectively. The number average molecular weight, $\overline{M}_n$, is 16,000–30,000. Where Dextran 40 is labeled as intended for use in the preparation of injectables, the ratio $\overline{M}_w$:$\overline{M}_n$ is in the 1.4–1.9 range.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°; excursions are permitted between 15° and 30°.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dextran 40 RS
  USP Dextran 4 Calibration RS
  USP Dextran 10 Calibration RS
  USP Dextran 40 Calibration RS
  USP Dextran 70 Calibration RS
  USP Dextran 250 Calibration RS
  USP Dextran 40 System Suitability RS
  USP Dextran Vo Marker RS

Dextran 40 in Dextrose Injection

DEFINITION

Dextran 40 in Dextrose Injection is a sterile solution of Dextran 40 and Dextrose in Water for Injection. It contains in each 100 mL NLT 9.0 g and NMT 11.0 g of Dextran 40 and NLT 4.5 g and NMT 5.5 g of dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$). It contains no bacteriostatic agents.

IDENTIFICATION

- **A.**

Diluent: Dextrose (4.5 in 100)

Sample solution: Injection, diluted with *Diluent* to 10 mg/mL of dextran 40

Analysis: Using a capillary tube viscometer having dimensions such that the flow time of water is NLT 100 s, measure the flow times of the *Diluent* and of the *Sample solution* at 20°.

Calculate the intrinsic viscosity:

$$\text{Result} = \{\ln[R_D \times (t/t_0)]\}/C$$

R_D = ratio of the density of the *Sample solution* to that of the *Diluent*
t = flow time of the *Sample solution*
t_0 = flow time of the *Diluent*
C = concentration of dextran 40 in the *Sample solution* (g/mL)

Acceptance criteria: 18–23 mL/g

ASSAY

- **DEXTROSE**

Mobile phase: 0.01 N sulfuric acid, filtered and degassed

System suitability solution: 5 mg/mL each of dextrose and xylitol in water

Standard solution: USP Dextrose RS, diluted to 5 mg/mL of dextrose monohydrate in water

Sample solution: Transfer an accurately measured volume of Injection, equivalent to about 250 mg of dextrose monohydrate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: Refractive index

Column: 7.8-mm × 30-cm; packing L17

Temperatures: Column and, if necessary, detector are maintained at a constant temperature of about 40°.

Flow rate: 0.6 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.5 between the dextrose and xylitol peaks, *System suitability solution*

Relative standard deviation: NMT 1.5% for dextrose, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration of dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$), in g/100 mL, in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times C \times D \times (M_{r1}/M_{r2})$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C = concentration of USP Dextrose RS in the *Standard solution* (g/100 mL)
D = dilution factor for the *Sample solution*
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of dextrose, 180.16

Acceptance criteria: 4.5–5.5 g/100 mL of dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$)

USP Monographs

www.webofpharma.com

• **DEXTRAN 40**
Sample solution: To 25 mL of Injection add 1 drop of 5 N ammonium hydroxide.
Analysis: Determine the optical rotation (see *Optical Rotation* ⟨781⟩).
Calculate the concentration, in g/100 mL, of dextran 40 in the volume of Injection taken:

$$\text{Result} = (1/Av_1) \times \{[(F \times a)/l] - [Av_2 \times C_d \times (M_{r2}/M_{r1})]\}$$

Av_1 = average value for the specific rotation of dextran 40, 197.5
F = conversion factor for 100 mL, 100
a = observed optical rotation (°)
l = length of the polarimeter tube (dm)
Av_2 = average value for the specific rotation of dextrose, 52.75
C_d = concentration of dextrose monohydrate as determined in the *Assay* for *Dextrose* (g/100 mL)
M_{r2} = molecular weight of dextrose, 180.16
M_{r1} = molecular weight of dextrose monohydrate, 198.17

Acceptance criteria: 9.0–11.0 g/100 mL

IMPURITIES
• **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**
Sample solution: Dilute Injection with water to 2.0 mg/mL of dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$).
Instrumental conditions
Analytical wavelength: 284 nm
Cell: 1 cm
Blank: Water
Analysis
Samples: *Sample solution* and *Blank*
Acceptance criteria: Absorbance NMT 0.25

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 3.0–7.0
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 1.0 USP Endotoxin Unit/mL
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*
• **COLOR OF SOLUTION:** Absorbance, determined at 375 nm against a water blank, is NMT 0.06.
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections* ⟨1⟩ and *Particulate Matter in Injections* ⟨788⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers.
• **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dextrose RS

Dextran 40 in Sodium Chloride Injection

DEFINITION
Dextran 40 in Sodium Chloride Injection is a sterile solution of Dextran 40 and Sodium Chloride in Water for Injection. It contains in each 100 mL NLT 9.0 g and NMT 11.0 g of Dextran 40; and NLT 0.81 g and NMT 0.99 g of sodium chloride. It contains no bacteriostatic agents.

IDENTIFICATION
• **A.**
Diluent: Sodium chloride solution (0.9 in 100)
Sample solution: Injection, diluted with *Diluent* to 10 mg/mL of Dextran 40
Analysis: Using a capillary tube viscometer with a flow time for water of NLT 100 s, measure the flow times of the *Diluent* and of the *Sample solution* at 20°.
Calculate the intrinsic viscosity:

$$\text{Result} = \{\ln[(R_D) \times (t/t_0)]\}/C$$

R_D = ratio of the density of the *Sample solution* to that of the *Diluent*
t = *Sample solution* flow time
t_0 = *Diluent* flow time
C = concentration of Dextran 40 in the *Sample solution* (g/mL)

Acceptance criteria: 18–23 mL/g

ASSAY
• **SODIUM CHLORIDE**
Sample solution: Pipet a volume of Injection, equivalent to 90 mg of chloride, into a porcelain casserole, and add 100 mL of water and 1 mL of dichlorofluorescein TS.
Analysis: Titrate with 0.1 N silver nitrate VS until the silver chloride flocculates and the mixture acquires a faint pink color. Each mL of 0.1 N silver nitrate VS is equivalent to 5.844 mg of sodium chloride.
Acceptance criteria: 0.81–0.99 g of sodium chloride per 100 mL
• **DEXTRAN 40**
Sample solution: To 25 mL of Injection add 1 drop of 5 N ammonium hydroxide.
Analysis: Determine the optical rotation (see *Optical Rotation* ⟨781⟩).
Calculate the concentration, in g per 100 mL, of Dextran 40 in the portion of Injection taken:

$$\text{Result} = (a/l) \times (1/A_v) \times 100$$

a = observed optical rotation (°)
l = length of the polarimeter tube (dm)
A_v = average value for the specific rotation of Dextran 40, 197.5

Acceptance criteria: 9.0–11.0 g of Dextran 40 per 100 mL

IMPURITIES

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 3.5–7.0
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 1.0 USP Endotoxin Unit/mL.
• **STERILITY TESTS** ⟨71⟩**:** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
• **COLOR OF SOLUTION:** Its absorbance, determined at 375 nm against a water blank, is NMT 0.05.
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections* ⟨1⟩ and *Particulate Matter in Injections* ⟨788⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers.
• **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the

www.webofpharma.com

label alternatively may state the total osmolar concentration in mOsmol/mL.

Dextran 70

Dextrans.

DEFINITION

Dextran 70 is derived by controlled hydrolysis and fractionation of polysaccharides elaborated by the fermentative action of certain appropriate strains of *Leuconostoc mesenteroides* (NRRL, B-512F; NCTC, 10817) on a sucrose substrate. It is a glucose polymer in which the linkages between glucose units are almost entirely of the α-1:6 type. Its weight average molecular weight is in the 63,000–77,000 range.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.**

Sample solutions: Prepare four solutions of Dextran 70 in water in such a manner that the concentrations are accurately known and approximately evenly distributed in the range of 2%–0.5%.

Analysis: Using a capillary tube viscometer having dimensions such that the flow time of water is NLT 100 s, measure the flow times of water and of the *Sample solution* at 20°.

Calculate the viscosity numbers of each of the *Sample solutions*:

$$\text{Result} = \{\ln[(R_D) \times (t/t_0)]\}/C$$

R_D = ratio of the density of the individual *Sample solution* to that of water
t = *Sample solution* flow time
t_0 = water flow time
C = concentration of Dextran 70 in the *Sample solution* (g/mL)

Plot the viscosity numbers of each of the *Sample solutions* against their respective concentrations, draw the straight line of best fit through the points, and extrapolate to zero concentration.

Acceptance criteria: The value of the intercept is 24–29 mL/g.

IMPURITIES

- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩**:** A 1.5-g portion of Dextran 70 shows NMT 0.03%, which corresponds to 0.45 mL of 0.020 N sulfuric acid.
- **LIMIT OF NITROGENOUS IMPURITIES** (where it is labeled as intended for use in the preparation of injectables)

Sulfate solution: To 1000 mL of sulfuric acid add 5 g of anhydrous cupric sulfate and 500 g of potassium sulfate. Dissolve by heating, and store at 60°. [NOTE—If storage at 60° is not possible, prepare a smaller quantity of *Sulfate solution* on the day of use, adjusting the proportions accordingly.]

Indicator: Dilute a mixture of 20 mL of a 0.1% solution of bromocresol green in alcohol and 4 mL of methyl red TS with water to 100 mL.

Sample solution: Transfer 0.2 g of Dextran 70, accurately weighed, to a micro-Kjeldahl flask. Add 4 mL of *Sulfate solution*.

Analysis

Sample: *Sample solution*

Heat until the solution exhibits a clear green color and the sides of the flask are free from carbonaceous material. Cool, and transfer the solution to a steam distillation unit. Rinse the Kjeldahl flask three times with 5 mL of water, adding the washings to the solution. Add 15 mL of 45% sodium hydroxide solution, immediately close the distillation apparatus, and commence steam distillation without delay. Receive the distillate in 1 mL of *Indicator* in a 100-mL flask, keeping the end of the condensing tube below the liquid surface for 5 min and above the liquid surface for 1 min. Upon completing the distillation, remove the receiving flask, and rinse the end of the condensing tube with a small quantity of water, adding the rinse to the distillate. Titrate the distillate with 0.010 N hydrochloric acid until the color changes from blue to reddish violet. Perform a blank determination, and make any necessary correction.

Acceptance criteria: The corrected volume of 0.010 N hydrochloric acid titrated does not exceed 0.14 mL (0.01%, as N).

- **LIMIT OF ALCOHOL AND RELATED IMPURITIES**

Standard solution: To 25.0 mL of the *Sample solution* add 0.5 mL of a 2.5% (w/v) solution of *n*-propyl alcohol.

Sample solution: Dissolve without heating 5.0 g of Dextran 70 in 100 mL of water, and distill the solution, collecting the first 45 mL of the distillate. Dilute the distillate with water to 50.0 mL, and mix.

Chromatographic system

Mode: GC
Detector: Flame ionization
Column: 2-mm × 1.8-m; packed with S3
Temperatures
Column: 160°
Injection port: 240°
Detector: 210°
Carrier gas: Nitrogen
Flow rate: 25 mL/min

[NOTE—Injector seals may deteriorate after multiple injections of the *Standard solution* and *Sample solution*. Inspect the seals before making a series of injections.]

Injection volume: 1 µL

Analysis

Samples: *Standard solution, Sample solution*, and a 0.05% (w/v) solution of *n*-propyl alcohol and water

Acceptance criteria: After corrections for any impurities in the *n*-propyl alcohol solution and water, the total area of peaks from impurities in the *Sample solution* does not exceed the area of the *n*-propyl alcohol solution peak.

- **ANTIGENIC IMPURITIES** (where it is labeled as intended for use in the preparation of injectables)

Sample solution: Prepare a sterile solution containing 60 mg/mL of Dextran 70 in sodium chloride injection.

Analysis: At intervals of about 48 h, inject three 0.5-mL doses into the peritoneal cavities of each of six guinea pigs. At 14 days after the first intraperitoneal injection, inject 0.20 mL intravenously into each of three of the guinea pigs, and at 21 days treat the other three guinea pigs similarly. Observe the animals for 30 min after each intravenous injection and again 24 h later.

Acceptance criteria: The animals exhibit no evidence of anaphylactoid reactions, such as coughing, bristling of hair, or respiratory distress.

www.webofpharma.com

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 20 mg/mL of Dextran 70, heated, if necessary, on a water bath to dissolve
  Acceptance criteria: +195° to +203°
- **pH** ⟨791⟩
  Sample solution: A solution (6 in 100)
  Acceptance criteria: 4.5–7.0
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 5 h.
  Acceptance criteria: NMT 7.0%
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩ (where it is labeled as intended for use in the preparation of injectables)**:** When tested in sodium chloride injection (0.6 in 10), it contains NMT 0.5 USP Endotoxin Unit/mL.
- **COLOR OF SOLUTION**
  Sample solution: A solution in water (6 in 100)
  Blank solution: Water
  Analysis: Absorbance at 375 nm in a 4-cm cell
  Acceptance criteria: NMT 0.15
- **SAFETY:** Inject intravenously 1.0 mL of a sterile solution of 6% Dextran 70 in saline TS into each of five mice weighing 18–20 g. The injection period is 10–15 s. If there are no deaths within 72 h, it meets the requirements of the test. If one or more animals die, continue the test using 10 mice weighing 20 ± 0.5 g. If all animals survive for 72 h, the requirements of the test are met.
- **MOLECULAR WEIGHT DISTRIBUTION** and **WEIGHT AND NUMBER AVERAGE MOLECULAR WEIGHTS**
  Mobile phase: 7.1 g/L of anhydrous sodium sulfate in water, filtered and degassed
  Calibration solutions: Separately prepare 20 mg/mL in *Mobile phase*, USP Dextran 4 Calibration RS, USP Dextran 10 Calibration RS, USP Dextran 40 Calibration RS, USP Dextran 70 Calibration RS, and USP Dextran 250 Calibration RS
  Marker solution: 3 mg/mL each of dextrose and USP Dextran V0 Marker RS in *Mobile phase*
  System suitability solution: 20 mg/mL of USP Dextran 70 System Suitability RS in *Mobile phase*
  Sample solution: 20 mg/mL of Dextran 70 in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: Refractive index
  Column: Three 7.5-mm × 30-cm columns; packing L38
  Column temperature: Constant temperature
  Injection volume: 50 µL
  System suitability
  Samples: *Calibration solutions*, *Marker solution*, and *System suitability solution*
  Suitability requirements
  Elution profile: Profile shows two peaks: the first due to the V_0 marker, the second due to dextrose, *Marker solution*
  Tailing factor: NMT 1.3 for the dextrose peak, *Marker solution*
  Relative standard deviation of the ratio V_0:V_T: Determine the void volume, V_0, of the system as the inflection point of the ascending part of the first peak. Determine the total volume, V_T, of the system as the maximum of the second peak. The relative standard deviation of the ratio V_0:V_T is NMT 1%, *Marker solution*
  Weight average molecular weight: Chromatograph each of the *Calibration solutions* separately. Divide each profile into at least 60 vertical sections of equal volume increments. (The actual number of sections is represented by the variable a in the equations below.) Record y_i, the height above the baseline, corresponding to each value of v_i, the volume eluted at that section. For each value of v_i, calculate the distribution coefficient, K_i:

$$\text{Result} = (v_i - V_0)/(V_T - V_0)$$

Find appropriate values of b_1, b_2, b_3, b_4, and b_5 using a suitable method[1] that, when substituted in the equation:

$$M_i = b_5 + e^{\left(b_4 + b_1K_i + b_2K_i^2 + b_3K_i^3\right)}$$

and the resulting values of M_i substituted, along with their corresponding values of y_i, in the equation:

$$\overline{M}_W = \sum_{i=1}^{a}\left(y_iM_i\right) / \sum_{i=1}^{a} y_1$$

give values of weight average molecular weight, $\overline{M}_w$, within 5% of the labeled values for each of the *Calibration solutions* and 180 ± 2 for dextrose. Using the *System suitability solution*, calculate $\overline{M}_w$ of the total molecular weight distribution using the same method, but inserting the now known values of b_1, b_2, b_3, b_4, and b_5. It is between 65,000 and 74,000.

Weight average molecular weight, high-fraction: Calculate $\overline{M}_w$ of the high-fraction dextran eluted through section n:

$$\sum_{i=1}^{n}(y_iM_i) / \sum_{i=1}^{n} y_i$$

in which n is defined by the relations:

$$\sum_{i=1}^{n} y_i \le 0.1\left(\sum_{i=1}^{a} y_i\right) \text{ and } \sum_{i=1}^{n+1} y_i > 0.1\left(\sum_{i=1}^{a} y_i\right)$$

It is between 180,000 and 240,000, *System suitability solution*.

Weight average molecular weight, low-fraction: Calculate $\overline{M}_w$ of the low-fraction dextran eluted in and after section m:

$$\sum_{i=m}^{a}(y_iM_i) / \sum_{i=m}^{a} y_i$$

in which m is defined by:

$$\sum_{i=m}^{a} y_i \le 0.1\left(\sum_{i=1}^{a} y_i\right) \text{ and } \sum_{i=m-1}^{a} y_i > 0.1\left(\sum_{i=1}^{a} y_i\right)$$

It is between 7,000 and 11,000, *System suitability solution*.

Analysis
Sample: *Sample solution*

[1] The Gauss-Newton method, modified by Hartley [see D. Hartley *Technometrics*, **3** (1961)], and the G. Nilsson and K. Nilsson method [see G. Nilsson and K. Nilsson J. *Chromat*, **101**, 137 (1974)] are suitable methods. A curve-fitting program capable of nonlinear regression may be used.

www.webofpharma.com

Calculate the weight average molecular weight, $\overline{M}_w$, for the total molecular weight distribution, the high-fraction dextran, and the low-fraction dextran. With the values of b_1, b_2, b_3, b_4, and b_5 obtained with the *Calibration solutions* in *System suitability*, calculate the number average molecular weight, $\overline{M}_n$, of the total molecular weight distribution of the *Sample solution* by substituting the corresponding values of M_i, along with their corresponding values of y_i, in the equation:

$$\overline{M}_n = \sum_{i=1}^{a} y_i / \sum_{i=1}^{a} (y_i/M_i)$$

Acceptance criteria: The weight average molecular weight, $\overline{M}_w$, of the total molecular weight distribution is 63,000–77,000. The weight average molecular weights, $\overline{M}_w$, of the high-fraction dextran and of the low-fraction dextran are NMT 195,000 and NLT 13,000, respectively. The number average molecular weight, $\overline{M}_n$, is 34,000–48,000. Where Dextran 70 is labeled as intended for use in the preparation of injectables, the ratio $\overline{M}_w$:$\overline{M}_n$ is in the 1.4–1.9 range.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°; excursions are permitted between 15° and 30°.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dextran 70 RS
  USP Dextran 4 Calibration RS
  USP Dextran 10 Calibration RS
  USP Dextran 40 Calibration RS
  USP Dextran 70 Calibration RS
  USP Dextran 250 Calibration RS
  USP Dextran 70 System Suitability RS
  USP Dextran V0 Marker RS

Dextran 70 in Dextrose Injection

DEFINITION

Dextran 70 in Dextrose Injection is a sterile solution of Dextran 70 and Dextrose in Water for Injection. It contains in each 100 mL NLT 9.0 g and NMT 11.0 g of Dextran 70 and NLT 4.5 g and NMT 5.5 g of dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$). It contains no bacteriostatic agents.

IDENTIFICATION

- **A.**

Diluent: Dextrose (4.5 in 100)

Sample solution: Injection, diluted with *Diluent* to 10 mg/mL of dextran 70

Analysis: Using a capillary tube viscometer having dimensions such that the flow time of water is NLT 100 s, measure the flow times of the *Diluent* and of the *Sample solution* at 20°.

Calculate the intrinsic viscosity:

$$\text{Result} = \{\ln[R_D \times (t/t_0)]\}/C$$

R_D = ratio of the density of the *Sample solution* to that of the *Diluent*
t = flow time of the *Sample solution*
t_0 = flow time of the *Diluent*
C = concentration of dextran 70 in the *Sample solution* (g/mL)

Acceptance criteria: 24–29 mL/g

ASSAY

- **DEXTROSE**

Mobile phase: 0.01 N sulfuric acid, filtered and degassed

System suitability solution: 5 mg/mL each of dextrose and xylitol in water

Standard solution: USP Dextrose RS, diluted to 5 mg/mL of dextrose monohydrate in water

Sample solution: 10 mL of Injection in 25 mL of water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 7.8-mm × 30-cm; packing L17

Temperatures: Column and, if necessary, detector are maintained at a constant temperature of about 40°.

Flow rate: 0.6 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.5 between the dextrose and xylitol peaks, *System suitability solution*

Relative standard deviation: NMT 1.5% for dextrose, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration of dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$), in g/100 mL, in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times C \times D \times (M_{r1}/M_{r2})$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C = concentration of USP Dextrose RS in the *Standard solution* (g/100 mL)
D = dilution factor for the *Sample solution*
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of dextrose, 180.16

Acceptance criteria: 4.5–5.5 g/100 mL of dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$)

- **DEXTRAN 70**

Sample solution: To 25 mL of Injection add 1 drop of 5 N ammonium hydroxide.

Analysis: Determine the optical rotation (see *Optical Rotation* ⟨781⟩).

Calculate the concentration, in g/100 mL, of dextran 70 in the volume of Injection taken:

$$\text{Result} = (1/Av_1) \times \{[(F \times a)/l] - [Av_2 \times C_d \times (M_{r2}/M_{r1})]\}$$

Av_1 = average value for the specific rotation of dextran 70, 197.5
F = conversion factor for 100 mL, 100
a = observed optical rotation (°)
l = length of the polarimeter tube (dm)
Av_2 = average value for the specific rotation of dextrose, 52.75
C_d = concentration of dextrose monohydrate as determined in the *Assay* for *Dextrose* (g/100 mL)
M_{r2} = molecular weight of dextrose, 180.16

USP Monographs

www.webofpharma.com

M_{r1} = molecular weight of dextrose monohydrate, 198.17

Acceptance criteria: 5.4–6.6 g/100 mL

IMPURITIES

- **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Sample solution: Dilute Injection with water to 2.0 mg/mL of dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$).

Instrumental conditions

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Acceptance criteria: Absorbance NMT 0.25

SPECIFIC TESTS

- **pH** ⟨791⟩: 3.5–7.0
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Unit/mL
- **STERILITY TESTS** ⟨71⟩: Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **COLOR OF SOLUTION:** Absorbance, determined at 375 nm against a water blank, is NMT 0.05.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections* ⟨1⟩ and *Particulate Matter in Injections* ⟨788⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers.
- **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Dextrose RS

Dextran 70 in Sodium Chloride Injection

DEFINITION

Dextran 70 in Sodium Chloride Injection is a sterile solution of Dextran 70 and Sodium Chloride in Water for Injection. It contains, in each 100 mL, NLT 5.4 g and NMT 6.6 g of Dextran 70, and NLT 0.81 g and NMT 0.99 g of sodium chloride. It contains no bacteriostatic agents.

IDENTIFICATION

- **A.**

Diluent: Sodium chloride solution (0.9 in 100)

Sample solution: Injection, diluted with *Diluent* to 10 mg/mL of Dextran 70

Analysis: Using a capillary tube viscometer having dimensions such that the flow time of water is NLT 100 s, measure the times of the *Sample solution* and *Diluent* at 20°. Calculate the intrinsic viscosity:

$$\text{Result} = \{\ln[(R_D) \times (t/t_0)]\}/C$$

R_D = ratio of the density of the *Sample solution* to that of the *Diluent*

t = *Sample solution* flow time

t_0 = *Diluent* flow time

C = concentration of Dextran 70 in the *Sample solution* (g/mL)

Acceptance criteria: 24–29 mL/g

ASSAY

- **SODIUM CHLORIDE**

Sample solution: Pipet a volume of Injection, equivalent to 90 mg of chloride, into a porcelain casserole, and add 100 mL of water and 1 mL of dichlorofluorescein TS.

Analysis: Titrate with 0.1 N silver nitrate VS until the silver chloride flocculates and the mixture acquires a faint pink color. Each mL of 0.1 N silver nitrate VS is equivalent to 5.844 mg of sodium chloride.

Acceptance criteria: 0.81–0.99 g of sodium chloride per 100 mL

- **DEXTRAN 70**

Sample solution: To 25 mL of Injection add 1 drop of 5 N ammonium hydroxide.

Analysis: Determine the optical rotation (see *Optical Rotation* ⟨781⟩).

Calculate the concentration, in g per 100 mL, of Dextran 70 in the portion of Injection taken:

$$\text{Result} = (a/l) \times (1/A_v) \times 100$$

a = observed optical rotation (°)

l = length of the polarimeter tube (dm)

A_v = average value for the specific rotation of Dextran 70, 197.5

Acceptance criteria: 5.4–6.6 g of Dextran 70 per 100 mL

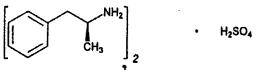
SPECIFIC TESTS

- **pH** ⟨791⟩: 4.0–7.0
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.5 USP Endotoxin Unit/mL.
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration.*
- **COLOR OF SOLUTION:** Its absorbance, determined at 375 nm against a water blank, is NMT 0.04.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections* ⟨1⟩ and *Particulate Matter in Injections* ⟨788⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers.
- **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.

Dextroamphetamine Sulfate



$(C_9H_{13}N)_2 \cdot H_2SO_4$ 368.49

Benzeneethanamine, α-methyl-, (*S*)-, sulfate (2:1); (+)-α-Methylphenethylamine sulfate (2:1) [51-63-8].

DEFINITION

Dextroamphetamine Sulfate, the dextrorotatory isomer of amphetamine sulfate, contains NLT 98.0% and NMT 102.0% of dextroamphetamine sulfate $[(C_9H_{13}N)_2 \cdot H_2SO_4]$, calculated on the dried basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Add 5.0 mL of trifluoroacetic acid to 900 mL of water, adjust with ammonium hydroxide to a pH of 2.2 ± 0.1, and add 100 mL of acetonitrile.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	65	35
20	0	100
22	0	100
23	100	0
30	100	0

Standard solution: 2.0 mg/mL of USP Dextroamphetamine Sulfate RS in *Solution A*
System suitability solution: Transfer about 40 mL of the *Standard solution* to a 50-mL volumetric flask. Using a microliter syringe, add 1 µL each of USP Dextroamphetamine Related Compound A RS and USP Dextroamphetamine Related Compound B RS. Dilute with *Standard solution* to volume.
Sample solution: 2.0 mg/mL of Dextroamphetamine Sulfate in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between dextroamphetamine related compound A and dextroamphetamine related compound B, *System suitability solution*
Tailing factor: NMT 3.0 for dextroamphetamine, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dextroamphetamine sulfate $[(C_9H_{13}N)_2 \cdot H_2SO_4]$ in the portion of Dextroamphetamine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Dextroamphetamine Sulfate in the *Sample solution* (mg/mL)

Acceptance criteria 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Dextroamphetamine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of dextroamphetamine from the *Standard solution*
C_S = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Dextroamphetamine Sulfate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cathinone	0.81	55.6	0.25
Dextroamphetamine	1.0	1.0	—
Benzaldehyde	1.73	105.3	0.25
Dextroamphetamine related compound A	1.88	1.5	0.25
Dextroamphetamine related compound B	2.05	1.8	0.25
Individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	1.0

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 40 mg/mL of Dextroamphetamine Sulfate in water
Acceptance criteria: +20° to +23.5°

- **pH** ⟨791⟩

Sample solution: 50 mg/mL of Dextroamphetamine Sulfate in water
Acceptance criteria: 5.0–6.0

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

USP Monographs

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dextroamphetamine Related Compound A RS
1-Phenyl-2-propanol.
$C_9H_{12}O$ 136.19
USP Dextroamphetamine Related Compound B RS
Phenyl acetone.
$C_9H_{10}O$ 134.18
USP Dextroamphetamine Sulfate RS

Dextroamphetamine Sulfate Tablets

DEFINITION
Dextroamphetamine Sulfate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of dextroamphetamine sulfate $[(C_9H_{13}N)_2 \cdot H_2SO_4]$.

IDENTIFICATION
• **A.**
Sample: Nominally 50 mg of dextroamphetamine sulfate from a portion of finely ground Tablets
Analysis: Transfer the *Sample* to a suitable centrifuge tube. Add 25 mL of water, shake vigorously, and centrifuge until clear. Decant the clear solution into a 250-mL separator, add 5 mL of 2.5 N sodium hydroxide, and extract with 60 mL of ether. Wash the ether extract with two 5-mL portions of 0.25 N sodium hydroxide, and discard the washings. Filter the ether extract through a pledget of cotton, previously saturated with ether, into a 100-mL beaker, and evaporate on a steam bath in a current of air to 1 mL. Dissolve the residue in 3 mL of alcohol, and transfer to a glass-stoppered, 125-mL conical flask containing 25 mL of water. Rinse the beaker with 3 mL of alcohol, and transfer to the flask. Cool to 15°, add 3 mL of 1 N sodium hydroxide, then add 1 mL of a mixture of 1 volume of benzoyl chloride and 2 volumes of anhydrous ethyl ether, and shake for 2 min. Filter the precipitate, wash with 15 mL of cold water, and recrystallize twice from diluted alcohol to obtain the benzoyl derivative of dextroamphetamine. Dry the crystals at 105° for 1 h.
Acceptance criteria: The crystals melt between 154° and 160°.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Solution A: Glacial acetic acid in water (14 in 100)
Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 525 mL of water. Add 25 mL of *Solution A* and 450 mL of methanol. Adjust dropwise, if necessary, with glacial acetic acid to a pH of 3.3 ± 0.1. Pass through a membrane filter of 0.5-µm pore size.
Diluent: 0.12 N phosphoric acid
Standard solution: 0.3 mg/mL of USP Dextroamphetamine Sulfate RS in *Diluent*
Sample solution: Nominally 0.3 mg/mL of dextroamphetamine sulfate from NLT 20 Tablets prepared as follows. Finely powder NLT 20 Tablets, and transfer a portion of the powder, equivalent to 15 mg of dextroamphetamine sulfate, to a 50-mL volumetric flask. Add 40 mL of *Diluent,* and sonicate for 15 min. Dilute with *Diluent* to volume. Pass through a membrane filter of 0.5-µm pore size, discarding the first 20 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 3
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dextroamphetamine sulfate $[(C_9H_{13}N)_2 \cdot H_2SO_4]$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dextroamphetamine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Procedure for a pooled sample
Medium: Water; 500 mL
Apparatus 1: 100 rpm
Time: 45 min
Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 575 mL of water. Add 25 mL of dilute glacial acetic acid (14 in 100) and 400 mL of methanol. Adjust by the dropwise addition of glacial acetic acid to a pH of 3.3 ± 0.1, if necessary.
Standard solution: USP Dextroamphetamine Sulfate RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 210 nm
Column: 3.9-mm × 30-cm; packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dextroamphetamine sulfate $[(C_9H_{13}N)_2 \cdot H_2SO_4]$ dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dextroamphetamine sulfate in the *Sample solution* (mg/mL)
V = volume of *Medium,* 500 mL
L = label claim (mg/Tablet)

USP Monographs

www.webofpharma.com

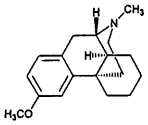
Tolerances: NLT 75% (*Q*) of the labeled amount of dextroamphetamine sulfate $[(C_9H_{13}N)_2 \cdot H_2SO_4)]$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dextroamphetamine Sulfate RS

Dextromethorphan



$C_{18}H_{25}NO$ 271.40

Morphinan, 3-methoxy-17-methyl-, (9α,13α,14α)-;
3-Methoxy-17-methyl-9α,13α,14α-morphinan [125-71-3].

DEFINITION

Dextromethorphan contains NLT 98.0% and NMT 101.0% of dextromethorphan ($C_{18}H_{25}NO$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.**

Buffer: 1.54 g of ammonium acetate in 1 L of water, adjusted with phosphoric acid to a pH of 4.1
Mobile phase: Methanol and *Buffer* (90:10)
Diluent: Methanol and water (90:10)
System suitability solution: 10 µg/mL of levomethorphan from USP Levomethorphan Solution RS and 10 mg/mL of USP Dextromethorphan RS in *Diluent*
Standard solution: 10 µg/mL of USP Dextromethorphan RS in *Diluent*
Sample solution: 10.0 mg/mL of Dextromethorphan in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L8
Flow rate: 1 mL/min
Injection volume: 4 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for dextromethorphan and levomethorphan are 1.0 and 1.28, respectively.]
Suitability requirements
Resolution: NLT 2.0 between dextromethorphan and levomethorphan, *System suitability solution*
Relative standard deviation: NMT 5.0% for dextromethorphan, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levomethorphan in the portion of Dextromethorphan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levomethorphan from the *Sample solution*
r_S = peak response of dextromethorphan from the *Standard solution*
C_S = concentration of USP Dextromethorphan RS in the *Standard solution* (mg/mL)
C_U = concentration of Dextromethorphan in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.10%

ASSAY

- **PROCEDURE**

Sample solution: Dissolve 700 mg of Dextromethorphan in 60 mL of glacial acetic acid, warming slightly, if necessary, to dissolve.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Visual
Analysis: Add 2 drops of crystal violet TS to the *Sample solution* and titrate with *Titrant* to a blue-green endpoint. Perform a blank determination and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.14 mg of dextromethorphan ($C_{18}H_{25}NO$).
Acceptance criteria: 98.0%–101.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF PHENOLIC COMPOUNDS**

Sample: 10 mg of Dextromethorphan
Analysis: Dissolve the *Sample* in 2 mL of 3 N hydrochloric acid, add 2 drops of ferric chloride TS, and mix. Add 2 drops of potassium ferricyanide TS, and observe after 2 min.
Acceptance criteria: No blue-green color develops.

- **LIMIT OF *N,N*-DIMETHYLANILINE**

Standard solution: Transfer 50 mg of *N,N*-dimethylaniline to a 100-mL volumetric flask, add 70.0 mL of water, insert the stopper tightly, shake for 20 min using a mechanical wrist-action shaker or equivalent, and dilute with water to volume. Transfer 1.0 mL to a 100-mL volumetric flask, and dilute with water to volume. Transfer 1.0 mL of the resulting solution to a 25-mL volumetric flask, and add 19 mL of water.
Sample solution: Transfer 500 mg of Dextromethorphan to a 25-mL volumetric flask, add 19 mL of water and 1 mL of 3 N hydrochloric acid, dissolve by warming on a steam bath, and cool.
Analysis: Add 2 mL of 1 N acetic acid and 1 mL of sodium nitrite solution (1 in 100) to the *Sample solution* and dilute with water to volume. This solution shows no more color than the straw yellow to greenish yellow color of the *Standard solution* similarly treated.
Acceptance criteria: NMT 0.001% of *N,N*-dimethylaniline

SPECIFIC TESTS

- **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia*: NMT 0.5%

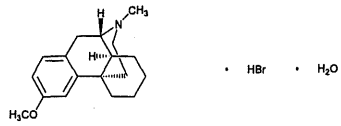
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dextromethorphan RS
  USP Levomethorphan Solution RS
  3-Methoxy-17-methylmorphinan.
  $C_{18}H_{25}NO$ 271.40
  This solution contains 0.1 mg/mL of levomethorphan in methanol.

USP Monographs

www.webofpharma.com

Dextromethorphan Hydrobromide



$C_{18}H_{25}NO \cdot HBr \cdot H_2O$ 370.32
Morphinan, 3-methoxy-17-methyl-, (9α,13α,14α)-, hydrobromide, monohydrate;
3-Methoxy-17-methyl-9α,13α,14α-morphinan hydrobromide monohydrate [6700-34-1].
Anhydrous [125-69-9]. 352.32

DEFINITION
Dextromethorphan Hydrobromide contains NLT 98.0% and NMT 102.0% of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
  Sample: Dry under vacuum over silica for 4 h.
  Acceptance criteria: Meets the requirements
- **B.**
  Buffer: 1.54 g of ammonium acetate in 1 L of water, adjusted with phosphoric acid to a pH of 4.1
  Mobile phase: Methanol and *Buffer* (90:10)
  Diluent: Methanol and water (90:10)
  System suitability solution: 10 µg/mL of levomethorphan from USP Levomethorphan Solution RS and 10 mg/mL of USP Dextromethorphan Hydrobromide RS in *Diluent*
  Standard solution: 10 µg/mL of USP Dextromethorphan Hydrobromide RS in *Diluent*
  Sample solution: 10.0 mg/mL of Dextromethorphan Hydrobromide in *Diluent*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: 225 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L88
  Flow rate: 1 mL/min
  Injection volume: 4 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for dextromethorphan and levomethorphan are 1.0 and 1.28, respectively.]
  Suitability requirements
  Resolution: NLT 2.0 between dextromethorphan and levomethorphan, *System suitability solution*
  Relative standard deviation: NMT 5.0% for dextromethorphan, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of levomethorphan in the portion of Dextromethorphan Hydrobromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of levomethorphan from the *Sample solution*
r_S = peak response of dextromethorphan from the *Standard solution*
C_S = concentration of USP Dextromethorphan Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Dextromethorphan Hydrobromide in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dextromethorphan, 271.40
M_{r2} = molecular weight of dextromethorphan hydrobromide, 352.32

Acceptance criteria: NMT 0.10%

ASSAY
- **PROCEDURE**
  Mobile phase: 0.007 M docusate sodium and 0.007 M ammonium nitrate in acetonitrile and water (70:30), filtered and degassed. Dissolve the docusate sodium in the acetonitrile and water mixture before adding the ammonium nitrate. Adjust the solution with glacial acetic acid to a pH of 3.4.
  Standard stock solution: 1 mg/mL of USP Dextromethorphan Hydrobromide RS in water
  Standard solution: 0.1 mg/mL of USP Dextromethorphan Hydrobromide RS from *Standard stock solution* in *Mobile phase*
  Sample stock solution: 1 mg/mL of Dextromethorphan Hydrobromide in water
  Sample solution: 0.1 mg/mL of Dextromethorphan Hydrobromide from *Sample stock solution* in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.5
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr$) in the portion of Dextromethorphan Hydrobromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dextromethorphan Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Dextromethorphan Hydrobromide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **LIMIT OF PHENOLIC COMPOUNDS**
  Sample: 5 mg of Dextromethorphan Hydrobromide
  Analysis: To the *Sample* add 1 drop of 3 N hydrochloric acid, 1 mL of water, and 2 drops of ferric chloride TS. Add 2 drops of potassium ferricyanide TS, and observe after 2 min.
  Acceptance criteria: No blue-green color develops.

USP Monographs

www.webofpharma.com

• **LIMIT OF *N,N*-DIMETHYLANILINE**
Standard solution: Transfer 50 mg of *N,N*-dimethylaniline to a 100-mL volumetric flask. Add 70.0 mL of water, insert the stopper tightly, shake for 20 min using a mechanical wrist-action shaker or equivalent, and dilute with water to volume. Transfer 1.0 mL to a 100-mL volumetric flask, and dilute with water to volume. Transfer 1.0 mL of the resulting solution to a 25-mL volumetric flask, and add 19 mL of water.
Sample solution: Transfer 500 mg of Dextromethorphan Hydrobromide to a 25-mL volumetric flask. Add 19 mL of water and 1 mL of 3 N hydrochloric acid. Dissolve by warming on a steam bath, and cool.
Analysis: Add 2 mL of 1 N acetic acid and 1 mL of sodium nitrite solution (1 in 100) to the *Sample solution*, and dilute with water to volume. This solution shows no more color than the straw yellow to greenish yellow color of the *Standard solution* similarly treated.
Acceptance criteria: NMT 0.001% of *N,N*-dimethylaniline

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 5.2–6.5
• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia*: 3.5%–5.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dextromethorphan Hydrobromide RS
USP Levomethorphan Solution RS
3-Methoxy-17-methylmorphinan.
$C_{18}H_{25}NO$ 271.40
This solution contains 0.1 mg/mL of levomethorphan in methanol.

Dextromethorphan Hydrobromide Oral Solution

» Dextromethorphan Hydrobromide Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Dextromethorphan Hydrobromide RS

Identification—
A: Transfer about 50 mL of Oral Solution to a 250-mL separator, add 20 mL of water, 5 mL of 2.5 N sodium hydroxide, and 40 mL of solvent hexane, and shake thoroughly. Remove the solvent hexane layer, and filter through anhydrous sodium sulfate into a 150-mL beaker. Repeat the solvent hexane extraction, using two 40-mL portions and collecting the extracts in the beaker after filtering. Evaporate the combined extracts at 50° under nitrogen to dryness, and dissolve the residue in, and dilute with, 10 mL of chloroform: the solution is dextrorotatory (see *Optical Rotation* ⟨781⟩). Retain the chloroform solution for *Identification* test *B*.
B: Evaporate the chloroform solution from *Identification* test *A* on a steam bath to dryness, dissolve the residue in 2 mL of 2 N sulfuric acid, and add 1 mL of a freshly prepared solution of mercuric nitrate (prepared by dissolving 700 mg of mercuric nitrate in 4 mL of water, adding 100 mg of sodium nitrate, mixing, and filtering): no red color is produced immediately, but after heating, a yellow to red color develops in about 15 minutes.

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

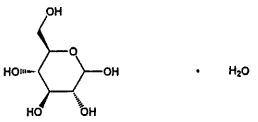
Assay—
Mobile phase and *Standard preparation*—Prepare as directed in the *Assay* under *Dextromethorphan Hydrobromide*.
Assay preparation—Pipet, using a to-contain pipet, a volume of Oral Solution, equivalent to about 10 mg of dextromethorphan hydrobromide, into a 100-mL volumetric flask, dilute with water to volume, and mix.
Chromatographic system and *Procedure* (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay* under *Dextromethorphan Hydrobromide*. Calculate the quantity, in mg, of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$) in the volume of Oral Solution taken by the formula:

$$(370.32/352.32)(100C)(r_U/r_S)$$

in which 370.32 and 352.32 are the molecular weights of dextromethorphan hydrobromide and anhydrous dextromethorphan hydrobromide, respectively; *C* is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS, on the anhydrous basis, in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dextrose

Portions of this monograph that are national USP text, and are not part of the harmonized text, are marked with symbols (•,•) to specify this fact.



$C_6H_{12}O_6 \cdot H_2O$ 198.17
$C_6H_{12}O_6$ 180.16
D-Glucose monohydrate [77938-63-7].
Anhydrous [50-99-7].

DEFINITION
Dextrose is (+)-D-glucopyranose and is derived from starch. It contains one molecule of water of hydration or is anhydrous. It contains NLT 97.5% and NMT 102.0%, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• •**A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197K▲ (CN 1-May-2020)

USP Monographs

www.webofpharma.com

Sample: Dry at 70° under vacuum for at least 2 h to constant weight.
Acceptance criteria: Meets the requirements♦

• **B.**
Analysis: Examine the chromatograms from the *Assay*.
Acceptance criteria: The principal peak from the *Sample solution* is similar in retention time and size to the principal peak from *Standard solution A*.

• **C. WATER DETERMINATION** ⟨921⟩, *Method I*
Samples
Anhydrous: 0.50 g
Monohydrate: 0.25 g
Acceptance criteria
Anhydrous: NMT 1.0%
Monohydrate: 7.5%–9.5%

ASSAY

• **PROCEDURE**
Mobile phase: Water
System suitability solution: Dissolve 5 mg of USP Maltose Monohydrate RS, 5 mg of USP Maltotriose RS, and 5 mg of USP Fructose RS in water and dilute with water to 50.0 mL.
Standard solution A: 30 mg/mL of USP Dextrose RS
Sample solution: 30 mg/mL, determined on the anhydrous basis
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 7.8-mm × 30-cm; 9-µm packing L19[1]
Temperatures
Column: 85 ± 1°
Detector: 40°
Flow rate: 0.3 mL/min
Injection volume: 20 µL
Run time: About 1.5 times the retention time of dextrose
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for maltotriose, maltose, isomaltose, dextrose, and fructose are 0.7, 0.8, 0.8, 1.0, and 1.3, respectively. The retention time for dextrose is about 21 min.]
Suitability requirements
Resolution: NLT 1.3 between maltotriose and maltose
Analysis
Samples: *Standard solution A* and *Sample solution*
Calculate the percentage, on the anhydrous basis, of dextrose ($C_6H_{12}O_6$) in the portion of Dextrose taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dextrose from the *Sample solution*
r_S = peak response of dextrose from *Standard solution A*
C_S = concentration of USP Dextrose RS in *Standard solution A* (mg/mL)
C_U = concentration of the *Sample solution* on the anhydrous basis (mg/mL)

Acceptance criteria: 97.5%–102.0% on the anhydrous basis

IMPURITIES

• **RELATED SUBSTANCES**
Mobile phase, System suitability solution, Standard solution A, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sample solution: 30 mg/mL, determined on the anhydrous basis
Standard solution B: Dilute 1.0 mL of the *Sample solution* with water to 250.0 mL.
Standard solution C: Dilute 25.0 mL of *Standard solution B* with water to 200.0 mL.
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for maltotriose, maltose, isomaltose, dextrose, and fructose are 0.7, 0.8, 0.8, 1.0, and 1.3, respectively. The retention time for dextrose is about 21 min.]
Suitability requirements
Resolution: NLT 1.3 between maltotriose and maltose
Analysis
Samples: *Standard solution A, Sample solution, Standard solution B,* and *Standard solution C*
Disregard any peak with an area less than the principal peak from *Standard solution C* (0.05%).
Acceptance criteria
Maltose and isomaltose: NMT 0.4%. The sum is NMT the area of the principal peak from *Standard solution B*.
Maltotriose: NMT 0.2%. NMT 0.5 times the area of the principal peak from *Standard solution B*.
Fructose: NMT 0.15%. NMT 3 times the area of the principal peak from *Standard solution C*.
Unspecified: NMT 0.10%. NMT twice the area of the principal peak from *Standard solution C*.
Total impurities: NMT 0.5%. NMT 1.25 times the area of the principal peak from *Standard solution B*.

SPECIFIC TESTS

• **COLOR AND CLARITY OF SOLUTION**
Reference solution: To 2.5 mL of cobaltous chloride CS, 6.0 mL of ferric chloride CS, and 1.0 mL of cupric sulfate CS add hydrochloric acid [10 g/L of hydrogen chloride (HCl)] to make 1000.0 mL.
Hydrazine sulfate solution: Dissolve 1.0 g of hydrazine sulfate in water and dilute to 100.0 mL. Allow to stand for 4–6 h.
Hexamethylenetetramine solution: In a 100-mL ground-glass-stoppered flask, dissolve 2.5 g of hexamethylenetetramine in 25.0 mL of water.
Primary opalescent suspension: To the *Hexamethylenetetramine solution* in the flask add 25.0 mL of the *Hydrazine sulfate solution*. Mix and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.
Standard of opalescence: Dilute 15.0 mL of the *Primary opalescent suspension* with water to 1000.0 mL. This suspension is freshly prepared and may be stored for up to 24 h.
Reference suspension: To 5.0 mL of *Standard of opalescence* add 95.0 mL of water. Mix and shake before use.
Sample solution: Dissolve 10.0 g in 15 mL of water using a bath of boiling water. Allow to cool.
Analysis: Make the comparison by viewing the solutions downward in matched color–comparison tubes against a white surface (see *Visual Comparison* ⟨630⟩).
Acceptance criteria: The *Sample solution* is clear (its clarity is the same as that of water or its opalescence is not more pronounced than that of the *Reference suspension*) and not more intensely colored than the *Reference solution*.

• **CONDUCTIVITY**
Sample solution: Dissolve 20.0 g in carbon dioxide-free water prepared from distilled water and dilute with the same solvent to 100.0 mL.

[1] Aminex HPX-87C from Biorad is suitable.

USP Monographs

www.webofpharma.com

Analysis: Measure the conductivity of the solution while gently stirring with a magnetic stirrer.
Acceptance criteria: NMT 20 µS/cm at 25°

• **DEXTRIN**
Sample: 1 g, finely powdered
Analysis: Reflux the *Sample* with 20 mL of alcohol.
Acceptance criteria: The *Sample* dissolves completely.

• **SOLUBLE STARCH, SULFITES**
Sample solution: Dissolve the Dextrose sample (6.7 g of anhydrous or 7.4 g of monohydrate) in 15 mL of water using a bath of boiling water. Allow to cool.
Analysis: To the *Sample solution* add 25 µL of iodine TS.
Acceptance criteria: The resulting solution is yellow (NMT 15 ppm).

ADDITIONAL REQUIREMENTS

• ♦**PACKAGING AND STORAGE:** Preserve in well-closed containers.♦

• ♦**LABELING:** Label to indicate whether it is hydrous or anhydrous.♦

• ♦**USP REFERENCE STANDARDS** ⟨11⟩
USP Dextrose RS
USP Fructose RS
USP Maltose Monohydrate RS
USP Maltotriose RS
♦

Dextrose Injection

DEFINITION
Dextrose Injection is a sterile solution of Dextrose in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). Dextrose Injection contains no antimicrobial agents.

IDENTIFICATION

• **A.**
Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water
Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.
Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

ASSAY

• **PROCEDURE**
Sample solution: Nominally 20–50 mg/mL of dextrose prepared as follows. Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.
Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).
Analysis: Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution*, in degrees
l = length of the polarimeter tube, in decimeters
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
C_U = nominal concentration of dextrose in *Sample solution*, g/100 mL
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**
Sample solution: Nominally 4 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) from a volume of Injection equivalent to 1.0 g of dextrose in water
Instrumental conditions
Mode: UV
Analytical wavelength: 284 nm
Cell: 1 cm
Blank: Water
Analysis
Samples: *Sample solution* and *Blank*
Acceptance criteria: The absorbance of the *Sample solution* is NMT 0.25.

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: Dilute a suitable volume of Injection, if necessary, with water to NMT 5% of dextrose.
Analysis: Add 0.30 mL of saturated potassium chloride to 100 mL of *Sample solution*, and measure the pH.
Acceptance criteria: 3.2–6.5

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩
[NOTE—Before analysis, dilute Injections containing more than 10% of dextrose to a concentration of 10% of dextrose.]
Acceptance criteria: NMT 0.5 USP Endotoxin Unit/mL for Injection containing less than 5% dextrose; NMT 10.0 USP Endotoxin Units/g for Injection containing 5%–70% dextrose

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass. Store at room temperature.

• **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol/mL.

Dextrose and Sodium Chloride Injection

DEFINITION
Dextrose and Sodium Chloride Injection is a sterile solution of Dextrose and Sodium Chloride in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) and of sodium chloride (NaCl). It contains no antimicrobial agents.

IDENTIFICATION

• **A.**
Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water
Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.
Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

• **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩ and *Chloride* ⟨191⟩**:** Meets the requirements

www.webofpharma.com

ASSAY

• **DEXTROSE**

Sample solution: Nominally 2–5 g of dextrose per 100 mL, prepared as follows. Transfer a volume of Injection, containing 2–5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis

Sample: *Sample solution*

Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* (°)

l = length of the polarimeter tube (dm)

α = midpoint of the specific rotation for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 95.0%–105.0%

• **SODIUM CHLORIDE**

Sample solution: A volume of Injection equivalent to 90 mg of sodium chloride

Analysis: To the *Sample solution* add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride (NaCl).

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Sample solution: Nominally 2 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) prepared from a volume of Injection containing the equivalent of 1.0 g of dextrose and water

Instrumental conditions

Mode: UV

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Acceptance criteria: The absorbance of the *Sample solution* is NMT 0.25.

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: Nominally 5% dextrose prepared by diluting, if necessary, a suitable volume of Injection with water

Acceptance criteria: 3.2–6.5

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 10.0 USP Endotoxin Units/g of dextrose.

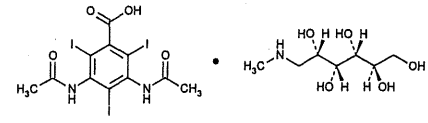
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

• **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol/mL.

Diatrizoate Meglumine



$C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ 809.13

Benzoic acid, 3,5-bis(acetylamino)-2,4,6-triiodo-, compd. with 1-deoxy-1-(methylamino)-D-glucitol (1:1).

1-Deoxy-1-(methylamino)-D-glucitol 3,5-diacetamido-2,4,6-triiodobenzoate (salt) [131-49-7].

» Diatrizoate Meglumine contains not less than 98.0 percent and not more than 102.0 percent of $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—

USP Diatrizoic Acid RS

USP Diatrizoic Acid Related Compound A RS

5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.

$C_9H_7I_3N_2O_3$ 571.88

Identification—

A: It responds to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, the test solution and the Standard solution of USP Diatrizoic Acid RS being prepared at a concentration of 1 mg per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

B: Heat about 500 mg in a suitable crucible: violet vapors are evolved.

Specific rotation ⟨781S⟩**:** between −5.65° and −6.37°.

Test solution: 100 mg per mL, in water.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Iodine and iodide—

Test preparation—Transfer 2.0 g to a 50-mL centrifuge tube provided with a stopper, dilute with water to 24 mL, and shake to dissolve.

Procedure—Add 5 mL of toluene and 5 mL of 2 N sulfuric acid, shake, and centrifuge: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), shake, and centrifuge: any red color in the toluene layer is not darker than that obtained when a mixture of 2.0 mL of potassium iodide solution (1 in 4000) and 22 mL of water is substituted for the solution under test (0.02% of iodide).

Free aromatic amine—Transfer 1.0 g to a 50-mL volumetric flask, and add 5 mL of water and 10 mL of 0.1 N sodium hydroxide. To a second 50-mL volumetric flask transfer 4 mL

USP Monographs

www.webofpharma.com

of water, 10 mL of 0.1 N sodium hydroxide, and 1.0 mL of a Standard solution prepared by dissolving a suitable quantity of USP Diatrizoic Acid Related Compound A RS in 0.1 N sodium hydroxide. Use 0.2 mL of 0.1 N sodium hydroxide for each 5.0 mg of Standard, and dilute with water to obtain a known concentration of 500 µg per mL. To a third 50-mL volumetric flask add 5 mL of water and 10 mL of 0.1 N sodium hydroxide to provide a blank.

Treat each flask as follows. Add 25 mL of dimethyl sulfoxide, insert the stopper, and mix by swirling gently. Chill in an ice bath in the dark for 5 minutes. [NOTE—In conducting the following steps, keep the flasks in the ice bath and in the dark as much as possible until all of the reagents have been added.] Slowly add 2 mL of hydrochloric acid, mix, and allow to stand for 5 minutes. Add 2 mL of sodium nitrite solution (1 in 50), mix, and allow to stand for 5 minutes. Add 1 mL of sulfamic acid solution (2 in 25), shake, and allow to stand for 5 minutes. [**CAUTION**—Considerable pressure is produced.] Add 2 mL of a 1 in 1000 solution of *N*-(1-naphthyl)-ethylenediamine dihydrochloride in dilute propylene glycol (7 in 10), and mix.

Remove the flasks from the ice bath and from storage in the dark, and allow to stand in a water bath at 22° to 25° for 10 minutes. Shake gently and occasionally during this period, releasing the pressure by loosening the stopper. Dilute with water to volume, and mix.

Within 5 minutes from the time of diluting the solutions in all three flasks to 50 mL, concomitantly determine the absorbances of the solution from the substance under test and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 465 nm, with a suitable spectrophotometer, versus the prepared blank. The absorbance of the solution from the Diatrizoate Meglumine is not greater than that of the Standard solution (0.05%).

Assay—Transfer about 400 mg of Diatrizoate Meglumine, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 13.49 mg of $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$.

Diatrizoate Meglumine Injection

» Diatrizoate Meglumine Injection is a sterile solution of Diatrizoate Meglumine in Water for Injection, or a sterile solution of Diatrizoic Acid in Water for Injection prepared with the aid of Meglumine. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of diatrizoate meglumine ($C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Diatrizoate Meglumine Injection intended for intravascular use contains no antimicrobial agents.

Packaging and storage—Preserve Injection intended for intravascular injection either in single-dose containers, preferably of Type I or Type III glass, protected from light or, where intended for administration with a pressure injector through a suitable transfer connection, in similar glass 500-mL or 1000-mL bottles, protected from light. Injection packaged for other than intravascular use may be packaged in 100-mL multiple-dose containers, preferably of Type I or Type III glass, protected from light.

Labeling—Label containers of Injection intended for intravascular injection, where packaged in single-dose containers, to direct the user to discard any unused portion remaining in the container or, where packaged in bulk bottles to state, "Bulk Container—only for sterile filling of pressure injectors," to state that it contains no antimicrobial preservatives, and to direct the user to discard any unused portion remaining in the container after 6 hours. Indicate also in the labeling of bulk bottles that a pressure injector is to be charged with a dose just prior to administration of the Injection. Label containers of Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.

USP Reference standards ⟨11⟩—
USP Diatrizoic Acid RS
USP Diatrizoic Acid Related Compound A RS
5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.
$C_9H_7I_3N_2O_3$ 571.88

Identification—

A: Dilute a volume of Injection, if necessary, with a 0.8 in 1000 solution of sodium hydroxide in methanol to obtain a test solution having a concentration of 1 mg per mL. The test solution responds to the *Thin-layer Chromatographic Identification Test* ⟨201⟩, the Standard solution being prepared at a concentration of 1 mg of USP Diatrizoic Acid RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

B: Evaporate a volume of Injection, equivalent to about 500 mg of diatrizoate meglumine, to dryness, and heat the residue so obtained in a suitable crucible: violet vapors are evolved.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.1 USP Endotoxin Units per mL for Injections containing less than 50% of diatrizoate meglumine, and less than 5.0 USP Endotoxin Units per mL for Injections containing 50% or more of diatrizoate meglumine.

pH ⟨791⟩: between 6.0 and 7.7.

Iodine and iodide—Dilute a volume of Injection, equivalent to 2.0 g of diatrizoate meglumine, with 24 mL of water in a 50-mL centrifuge tube provided with a stopper. Add 5 mL of toluene and 5 mL of 2 N sulfuric acid, shake, and centrifuge: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), shake, and centrifuge: any red color in the toluene layer is not darker than that obtained when a volume of potassium iodide solution (1 in 4000), containing a quantity of iodide corresponding to 0.02% of the weight of diatrizoate meglumine in the volume of Injection taken, is diluted with water to 24 mL and substituted for the solution under test (0.02% of iodide).

Free aromatic amine—Transfer a volume of Injection, accurately measured and equivalent to 1 g of diatrizoate meglumine, to a glass-stoppered, 50-mL volumetric flask. Dilute with water to 5 mL, and add 10 mL of 0.1 N sodium hydroxide. Into a second 50-mL volumetric flask pipet a known volume of a Standard solution prepared by dissolving a suitable quantity of USP Diatrizoic Acid Related Compound A RS in 0.1 N sodium hydroxide. Use 0.2 mL of 0.1 N sodium hydroxide for each 5.0 mg of Standard, and dilute with water to obtain a known concentration of 500 µg per mL. The

www.webofpharma.com

volume of Standard solution used contains a quantity of free aromatic amine corresponding to 0.05% of the weight of diatrizoate meglumine in the volume of Injection taken. Dilute with water to 5 mL, and add 10 mL of 0.1 N sodium hydroxide. Proceed as directed in the test for *Free aromatic amine*— under *Diatrizoate Meglumine,* beginning with "To a third 50-mL volumetric flask add 5 mL of water".

Meglumine content—Determine the angular rotation (see *Optical Rotation* ⟨781⟩) of the Injection, using a 10-cm cell and a suitable polarimeter. Calculate the content, in mg per mL, of meglumine in the Injection by the formula:

$$1000a/24.9$$

in which a is the observed angular rotation, in degrees, corrected for the blank, and the factor, 24.9, is the average specific rotation, in degrees, of meglumine. The meglumine content is between 22.9% and 25.3% of the labeled amount of diatrizoate meglumine.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—Pipet a volume of Injection, or a suitable dilution of it, equivalent to about 600 mg of diatrizoate meglumine, into a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the filter and the flask thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 13.49 mg of $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$.

Diatrizoate Meglumine and Diatrizoate Sodium Injection

» Diatrizoate Meglumine and Diatrizoate Sodium Injection is a sterile solution of Diatrizoate Meglumine and Diatrizoate Sodium in Water for Injection, or a sterile solution of Diatrizoic Acid in Water for Injection prepared with the aid of Sodium Hydroxide and Meglumine. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of diatrizoate meglumine ($C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$) and of iodine (I). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Diatrizoate Meglumine and Diatrizoate Sodium Injection intended for intravascular use contains no antimicrobial agents.

Packaging and storage—Preserve either in single-dose containers, preferably of Type I or Type III glass, protected from light or, where intended for administration with a pressure injector through a suitable transfer connection, in similar glass 500-mL or 1000-mL bottles, protected from light.

Labeling—Label containers of Injection intended for intravascular injection, where packaged in single-dose containers, to direct the user to discard any unused portion remaining in the container or, where packaged in bulk bottles to state, "Bulk Container—only for sterile filling of pressure injectors," to state that it contains no antimicrobial preservatives, and to direct the user to discard any unused portion remaining in the container after 6 hours. Indicate also in the labeling of bulk bottles that a pressure injector is to be charged with a dose just prior to administration of the Injection. Label containers of Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.

USP Reference standards ⟨11⟩—
USP Diatrizoic Acid RS
USP Diatrizoic Acid Related Compound A RS
5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.
$C_9H_7I_3N_2O_3$ 571.88

Identification—

A: Dilute a volume of Injection, if necessary, with a 0.8 in 1000 solution of sodium hydroxide in methanol to obtain a test solution having a concentration of 1 mg per mL. The test solution responds to the *Thin-layer Chromatographic Identification Test* ⟨201⟩, the Standard solution being prepared at a concentration of 1 mg of USP Diatrizoic Acid RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

B: Evaporate a volume of Injection, equivalent to about 500 mg of diatrizoate meglumine and diatrizoate sodium, to dryness, and heat the residue so obtained in a suitable crucible: violet vapors are evolved.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.8 USP Endotoxin Units per mL for Injections containing less than 60% of diatrizoate meglumine, and not more than 3.6 USP Endotoxin Units for Injections containing 60% or more of diatrizoate meglumine.

pH ⟨791⟩: between 6.0 and 7.7.

Free aromatic amine—Transfer an accurately measured volume of Injection, equivalent to about 1 g of diatrizoate meglumine and diatrizoate sodium, to a 50-mL volumetric flask. Dilute with water to 5 mL, and add 10 mL of 0.1 N sodium hydroxide. To a second 50-mL volumetric flask transfer 4 mL of water, 10 mL of 0.1 N sodium hydroxide, and 1.0 mL of a Standard solution prepared by dissolving a suitable quantity of USP Diatrizoic Acid Related Compound A RS in 0.1 N sodium hydroxide. Use 0.2 mL of 0.1 N sodium hydroxide for each 5.0 mg of Standard, and dilute with water to obtain a known concentration of 500 µg per mL. Proceed as directed in the test for *Free aromatic amine*— under *Diatrizoate Meglumine,* beginning with "To a third 50-mL volumetric flask add 5 mL of water".

Iodine and iodide—Transfer an accurately measured volume of Injection, equivalent to about 2.0 g of the total of diatrizoate meglumine and diatrizoate sodium, to a 50-mL centrifuge tube provided with a stopper. Dilute with water to 24 mL. Add 5 mL of toluene and 5 mL of 2 N sulfuric acid, shake, and centrifuge: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), shake, and centrifuge: any red color in the toluene layer is not darker than that obtained when a volume of potassium iodide solution (1 in 4000), containing a quantity of iodide corresponding to 0.02% of the weight of diatrizoate meglumine and diatrizoate sodium in the volume of Injection taken is diluted with water to 24 mL and substituted for the solution under test (0.02% of iodide).

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for diatrizoate meglumine—Pipet 5 mL of Injection into a 10-mL volumetric flask, add water to volume, and mix. Determine the angular rotation (see *Optical Rotation* ⟨781⟩) of the diluted Injection, using a 100-mm tube. Calculate the

USP Monographs

www.webofpharma.com

content, in mg per mL, of $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ in the Injection by the formula:

$$2000a/6.01$$

in which a is the observed angular rotation, in degrees, corrected for the blank, and the factor 6.01 is the specific rotation, in degrees, of diatrizoate meglumine.

Assay for iodine—Transfer an accurately measured volume of Injection, equivalent to about 4 g of the total of diatrizoate meglumine and diatrizoate sodium, to a 50-mL volumetric flask, dilute with water to volume, and mix. Pipet 5 mL of this solution into a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 6.345 mg of iodine.

Diatrizoate Meglumine and Diatrizoate Sodium Solution

» Diatrizoate Meglumine and Diatrizoate Sodium Solution is a solution of Diatrizoic Acid in Purified Water prepared with the aid of Meglumine and Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of diatrizoate meglumine ($C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$) and of iodine (I). It may contain small amounts of suitable buffers, Edetate Disodium, and flavoring agents.

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—Label the container to indicate that the contents are not intended for parenteral use.

USP Reference standards ⟨11⟩—
USP Diatrizoic Acid RS

Identification—

A: Dilute a volume of Solution, if necessary, with an 0.8 in 1000 solution of sodium hydroxide in methanol, to obtain a test solution having a concentration of 1 mg per mL. The test solution responds to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, the Standard solution being prepared at a concentration of 1 mg of USP Diatrizoic Acid RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

B: Evaporate a volume of Solution, equivalent to about 500 mg of diatrizoate meglumine and diatrizoate sodium, to dryness, and heat the residue so obtained in a crucible: violet vapors are evolved.

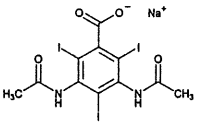
pH ⟨791⟩: between 6.0 and 7.6.

Iodine and iodide—Using as the *Test preparation* a volume of Solution equivalent to about 2 g of diatrizoate meglumine and diatrizoate sodium and diluting it with water to 24 mL in a 50-mL centrifuge tube provided with a stopper, proceed as directed for *Procedure* in the test for *Iodine and iodide*— under *Diatrizoate Meglumine.*

Assay for diatrizoate meglumine—Pipet a volume of Solution, equivalent to about 1.5 g of diatrizoate meglumine and diatrizoate sodium, into a 250-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of the solution so obtained into a glass-stoppered, 250-mL flask. Add 4 mL of 2 N sulfuric acid and 20 mL of sodium metaperiodate solution (1 in 200). Insert the stopper, and set aside in the dark for 1 hour. Add 50 mL of water, mix, and add 10 mL of potassium iodide TS. Insert the stopper quickly, mix by swirling for 20 seconds, and immediately titrate with 0.1 N sodium thiosulfate VS, using 3 mL of starch TS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 10.11 mg of $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$.

Assay for iodine—Transfer an accurately measured volume of Solution, equivalent to about 4 g of the total of diatrizoate meglumine and diatrizoate sodium, to a 50-mL volumetric flask, dilute with water to volume, and mix. Pipet 5 mL of this solution into a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 6.345 mg of iodine.

Diatrizoate Sodium



$C_{11}H_8I_3N_2NaO_4$ 635.90

Benzoic acid, 3,5-bis(acetylamino)-2,4,6-triiodo-, monosodium salt;

Monosodium 3,5-diacetamido-2,4,6-triiodobenzoate [737-31-5].

DEFINITION

Diatrizoate Sodium contains NLT 98.0% and NMT 102.0% of diatrizoate sodium ($C_{11}H_8I_3N_2NaO_4$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩

Solution A: Sodium hydroxide in methanol (0.8 in 1000)

Standard solution: 1 mg/mL of USP Diatrizoic Acid RS in *Solution A*

Sample solution: 1 mg/mL of Diatrizoate Sodium in *Solution A*

Developing solvent system: Methanol, chloroform, and ammonium hydroxide (10:20:2)

Analysis

Samples: *Standard solution* and *Sample solution*

Locate the spots using short-wavelength UV light.

Acceptance criteria: Meets the requirements

www.webofpharma.com

• **B.** A 5-mg/mL solution in water imparts an intense yellow color to a nonluminous flame confirming the presence of sodium.

ASSAY

• **PROCEDURE**

Sample solution: Transfer 300 mg of Diatrizoate Sodium to a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 h. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.05 N silver nitrate VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with the *Titrant* until the yellow precipitate just turns green.

Calculate the percentage of diatrizoate sodium ($C_{11}H_8I_3N_2NaO_4$) in the portion of Diatrizoate Sodium taken:

$$\text{Result} = [(V \times N \times F)/W] \times 100$$

V = sample *Titrant* volume (mL)
N = *Titrant* normality (mEq/mL)
F = equivalent weight of diatrizoate sodium, 212 mg/mEq
W = weight of Diatrizoate Sodium (mg)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Change to read:

• **LIMIT OF FREE AROMATIC AMINE**

Solution A: 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in a mixture of propylene glycol and water (70:30)

Standard stock solution: 0.5 mg/mL of USP Diatrizoic Acid Related Compound A RS▲prepared as follows. Transfer 5 mg of USP Diatrizoic Acid Related Compound A RS to a 10-mL volumetric flask. Add 0.2 mL of 0.1 N sodium hydroxide. Dilute with water to volume.▲ (USP 1-Dec-2019)

Standard solution: Transfer 4 mL of water, 10 mL of 0.1 N sodium hydroxide, and 1.0 mL of *Standard stock solution* to a 50-mL volumetric flask.

Sample solution: Transfer 1.0 g of Diatrizoate Sodium to a 50-mL volumetric flask, and add 5 mL of water and 10 mL of 0.1 N sodium hydroxide.

Blank solution: Transfer 5 mL of water and 10 mL of 0.1 N sodium hydroxide to a 50-mL volumetric flask.

Instrumental conditions

Mode: Vis

Analytical wavelength: 465 nm

Cell: 1 cm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank solution*

Treat the *Samples* as follows. Add 25 mL of dimethylsulfoxide. Place the flask in an ice bath for 5 min. Add 2 mL of hydrochloric acid, and allow to stand in the ice bath for 5 min. Add 2.0 mL of sodium nitrite solution (20 mg/mL), and allow to stand in the ice bath for 5 min. Remove the flask from the ice bath, add 1.0 mL of sulfamic acid solution (80 mg/mL), and allow to stand in the ice bath for 5 min. [**CAUTION**—Considerable pressure is produced.] Add 2.0 mL of *Solution A*. Remove the flasks from the ice bath and allow to stand in a water bath at room temperature. Shake occasionally, releasing the pressure by loosening the stopper. Dilute with water to volume. Within 5 min, measure the absorbances of the *Standard solution* and *Sample solution* against the blank.

Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (NMT 0.05% of free aromatic amine).

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 10.0%

• **LIMIT OF IODINE AND IODIDE**

Standard solution: Dissolve 0.5 mg of potassium iodide in 24 mL of water in a 50-mL stoppered centrifuge tube.

Sample solution: Transfer 2.0 g to a 50-mL stoppered centrifuge tube, dilute with water to 24 mL, and shake to dissolve.

Analysis

Samples: *Standard solution* and *Sample solution*

Treat the *Samples* as follows. Add 5 mL of toluene and 5 mL of 2 N sulfuric acid, shake, and centrifuge. The toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), shake, and centrifuge.

Acceptance criteria

1. The *Sample solution* does not show any red color in the toluene layer prior to addition of the sodium nitrite solution.
2. The red color of the toluene layer in the *Sample solution* after the addition of sodium nitrite solution is not darker than the *Standard solution* (NMT 0.02% of iodide).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Diatrizoic Acid RS

USP Diatrizoic Acid Related Compound A RS

5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.

$C_9H_7I_3N_2O_3$ 571.88

Diatrizoate Sodium Injection

» Diatrizoate Sodium Injection is a sterile solution of Diatrizoate Sodium in Water for Injection, or a sterile solution of Diatrizoic Acid in Water for Injection prepared with the aid of Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of diatrizoate sodium ($C_{11}H_8I_3N_2NaO_4$). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Diatrizoate Sodium Injection intended for intravascular use contains no antimicrobial agents.

Packaging and storage—Preserve Injection intended for intravascular injection in single-dose containers, preferably of Type I or Type III glass, protected from light. Injection intended for other than intravascular use may be packaged in 100-mL

USP Monographs

www.webofpharma.com

multiple-dose containers, preferably of Type I or Type III glass, protected from light.
Labeling—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container. Label containers of Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.
USP Reference standards ⟨11⟩—
USP Diatrizoic Acid RS
USP Diatrizoic Acid Related Compound A RS
5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.
$C_9H_7I_3N_2O_3$ 571.88

Identification—
A: Dilute a volume of Injection, if necessary, with a 0.8 in 1000 solution of sodium hydroxide in methanol to obtain a test solution having a concentration of 1 mg per mL. The test solution responds to the *Thin-layer Chromatographic Identification Test* ⟨201⟩, the Standard solution being prepared at a concentration of 1 mg of USP Diatrizoic Acid RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.
B: Evaporate a volume of Injection, equivalent to about 500 mg of diatrizoate sodium, to dryness, and heat the residue so obtained in a crucible: violet vapors are evolved.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 5.6 USP Endotoxin Units per mL for Injections containing 20% of diatrizoate sodium; not more than 1.3 USP Endotoxin Units per mL for Injections containing 25% of diatrizoate sodium; and not more than 5.0 USP Endotoxin Units per mL for Injections containing 50% of diatrizoate sodium.
pH ⟨791⟩: between 6.0 and 7.7.
Iodine and iodide—Using a volume of Injection equivalent to 2.0 g of diatrizoate sodium, and diluting it with water to 24 mL in a 50-mL centrifuge tube provided with a stopper, proceed as directed for *Procedure* in the test for *Iodine and iodide*— under *Diatrizoate Meglumine.*
Free aromatic amine—Transfer a volume of Injection, equivalent to 1.0 g of diatrizoate sodium, to a 50-mL volumetric flask, dilute with water to 5 mL, and add 10 mL of 0.1 N sodium hydroxide. Proceed as directed in the test for *Free aromatic amine*— under *Diatrizoate Meglumine,* beginning with "To a second 50-mL volumetric flask transfer 4 mL of water."
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Pipet into a glass-stoppered 125-mL conical flask a volume of Injection, equivalent to about 500 mg of diatrizoate sodium. Add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, and reflux the mixture for 1 hour. Cool to room temperature, wash the condenser with 20 mL of water, and filter the mixture. Wash the flask and the filter with small portions of water, adding the washings to the filtrate. Add to the filtrate 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the color of the yellow precipitate just changes to green. Each mL of 0.05 N silver nitrate is equivalent to 10.60 mg of $C_{11}H_8I_3N_2NaO_4$.

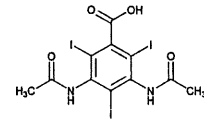
Diatrizoate Sodium Solution

» Diatrizoate Sodium Solution is a solution of Diatrizoate Sodium in Purified Water, or a solution of Diatrizoic Acid in Purified Water prepared with the aid of Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of diatrizoate sodium ($C_{11}H_8I_3N_2NaO_4$). It may contain a suitable preservative.

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Label the container to indicate that the contents are not intended for parenteral use.
USP Reference standards ⟨11⟩—
USP Diatrizoic Acid RS

Identification—
A: Dilute a volume of Solution, if necessary, with a 0.8 in 1000 solution of sodium hydroxide in methanol to obtain a test solution having a concentration of 1 mg per mL. The test solution responds to the *Thin-layer Chromatographic Identification Test* ⟨201⟩, the Standard solution being prepared at a concentration of 1 mg of USP Diatrizoic Acid RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.
B: Evaporate a volume of Solution, equivalent to about 500 mg of diatrizoate sodium, to dryness, and heat the residue so obtained in a suitable crucible: violet vapors are evolved.
pH ⟨791⟩: between 4.5 and 7.5.
Iodine and iodide—Using as the *Test preparation* a volume of Solution equivalent to 2.0 g of diatrizoate sodium and diluting it with water to 24 mL in a 50-mL centrifuge tube provided with a stopper, proceed as directed for *Procedure* in the test for *Iodine and iodide*— under *Diatrizoate Meglumine.*
Assay—Pipet a volume of Solution, equivalent to about 400 mg of diatrizoate sodium, into a 125-mL conical flask. Add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 10.60 mg of $C_{11}H_8I_3N_2NaO_4$.

Diatrizoic Acid



$C_{11}H_9I_3N_2O_4$ (anhydrous) 613.91
$C_{11}H_9I_3N_2O_4 \cdot 2H_2O$ 649.95
Benzoic acid, 3,5-bis(acetylamino)-2,4,6-triiodo-; 3,5-Diacetamido-2,4,6-triiodobenzoic acid [117-96-4]. Dihydrate [50978-11-5].

DEFINITION
Diatrizoic Acid is anhydrous or contains two molecules of water of hydration. It contains NLT 98.0% and NMT 102.0% of diatrizoic acid ($C_{11}H_9I_3N_2O_4$), calculated on the anhydrous basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩

Solution A: Sodium hydroxide in methanol (0.8 in 1000)
Standard solution: 1 mg/mL in *Solution A*
Sample solution: 1 mg/mL in *Solution A*
Developing solvent system: Chloroform, methanol, and ammonium hydroxide (20:10:2)
Analysis: Use short-wavelength UV light to locate the spots.
Acceptance criteria: Meets the requirements

• **B.**

Sample: 500 mg
Analysis: Heat the *Sample* in a suitable crucible.
Acceptance criteria: Violet vapors are evolved.

ASSAY

• **Procedure**

Sample: 300 mg
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.05 N silver nitrate VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 1 h. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS. Titrate the *Sample* with *Titrant* until the yellow precipitate just turns green. Each mL of *Titrant* is equivalent to 10.23 mg of diatrizoic acid ($C_{11}H_9I_3N_2O_4$).
Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

• **Free Aromatic Amine**

Standard stock solution: Dissolve a suitable quantity of USP Diatrizoic Acid Related Compound A RS in 0.1 N sodium hydroxide. Use 0.2 mL of 0.1 N sodium hydroxide for each 5.0 mg of Standard, and dilute with water to obtain 500 µg/mL.
Standard solution: Transfer 4 mL of water, 10 mL of 0.1 N sodium hydroxide, and 1.0 mL of a *Standard stock solution* to a 50-mL volumetric flask.
Sample solution: Transfer 1.0 g to a 50-mL volumetric flask, and add 12.5 mL of water and 2.5 mL of 0.1 N sodium hydroxide.
Blank: Transfer 5 mL of water and 10 mL of 0.1 N sodium hydroxide to a 50-mL volumetric flask.
Analysis: Treat each flask as follows. Add 25 mL of dimethyl sulfoxide, insert the stopper, and mix by swirling gently. Chill in an ice bath in the dark for 5 min. In conducting the following steps, keep the flasks in the ice bath and in the dark as much as possible until all of the reagents have been added.
Slowly add 2 mL of hydrochloric acid, and allow to stand for 5 min. Add 2 mL of sodium nitrite solution (20 mg/mL), and allow to stand for 5 min. Add 1 mL of sulfamic acid solution (80 mg/mL), shake, and allow to stand for 5 min. [Note—Considerable pressure is produced.] Add 2 mL of a solution (1 mg/mL) of *N*-(1-naphthyl)-ethylenediamine dihydrochloride in dilute propylene glycol (700 mg/mL). Remove the flasks from the ice bath and from storage in the dark, and allow to stand in a water bath at 22°–25° for 10 min. Shake gently and occasionally during this period, releasing the pressure by loosening the stopper. Dilute with water to volume.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Vis
Analytical wavelength: 465 nm
Cell: 1 cm
Analysis: Within 5 min from the time of diluting the solutions in all three flasks to 50 mL, concomitantly determine the absorbances of the solutions.
Acceptance criteria: 0.05%; the absorbance of the *Sample solution* is NMT that of the *Standard solution*.

SPECIFIC TESTS

• **Water Determination,** *Method I* ⟨921⟩: NMT 1.0% (anhydrous form), and between 4.5% and 7.0% (hydrous form)

• **Iodine and Iodide**

Sample solution: Suspend 10.0 g in 10 mL of water, and add in small portions, with stirring, 1.5 mL of sodium hydroxide solution (400 mg/mL). When the solution is complete, adjust to a pH between 7.0 and 7.5 with a dilute solution (8 mg/mL) of sodium hydroxide or hydrochloric acid, and dilute with water to 20 mL.
Analysis: Dilute 4.0 mL of *Sample solution* with 20 mL of water in a 50-mL centrifuge tube provided with a stopper, add 5 mL of toluene and 5 mL of 2 N sulfuric acid, shake, and centrifuge: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (20 mg/mL), shake, and centrifuge.
Acceptance criteria: NMT 0.02% of iodide; any red color in the toluene layer is not darker than that obtained when a mixture of 2.0 mL of potassium iodide solution (0.25 mg/mL) and 22 mL of water is substituted for the solution under test.

ADDITIONAL REQUIREMENTS

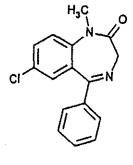
• **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.

• **Labeling:** Label it to indicate whether it is anhydrous or hydrous.

• **USP Reference Standards** ⟨11⟩

USP Diatrizoic Acid RS
USP Diatrizoic Acid Related Compound A RS
5-Acetamido-3-amino-2,4,6-triiodobenzoic acid.
$C_9H_7I_3N_2O_3$ 571.88

Diazepam



$C_{16}H_{13}ClN_2O$ 284.74
2*H*-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-1-methyl-5-phenyl-.
7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one [439-14-5].

» Diazepam contains not less than 95.0 percent and not more than 105.0 percent of $C_{16}H_{13}ClN_2O$, calculated on the dried basis.

www.webofpharma.com

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Diazepam RS
USP Diazepam Related Compound A RS
2-Methylamino-5-chlorobenzophenone.
$C_{14}H_{12}ClNO$ 245.71
USP Diazepam Related Compound B RS
3-Amino-6-chloro-1-methyl-4-phenylcarbostyril.
$C_{16}H_{13}ClN_2O$ 284.74
USP Nordazepam RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.
$C_{15}H_{11}ClN_2O$ 270.72

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Thin-Layer Chromatographic Identification Test ⟨201⟩—
Test solution: 5 mg per mL, in acetone.
Developing solvent system: a mixture of ethyl acetate and *n*-heptane (1:1).
Procedure—Proceed as directed in the chapter except use an unsaturated developing chamber.

Melting range, *Class I* ⟨741⟩**:** between 131° and 135°.

Loss on drying ⟨731⟩—Dry it in vacuum over phosphorus pentoxide at 60° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Related compounds—
Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay.*
Standard solution—Dissolve accurately weighed quantities of USP Diazepam Related Compound B RS, USP Diazepam Related Compound A RS, and USP Nordazepam RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having known concentrations of about 1 µg per mL, 0.1 µg per mL, and 3 µg per mL, respectively.
Test solution—Transfer about 10 mg of Diazepam, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of diazepam related compound B, diazepam related compound A, and nordazepam in the portion of Diazepam taken by the formula:

$$(C_R/W)(r_U/r_S)$$

in which C_R is the concentration, in µg per mL, of USP Diazepam Related Compound B RS, USP Diazepam Related Compound A RS, or USP Nordazepam RS in the *Standard solution; W* is the weight, in mg, of Diazepam taken to prepare the *Test solution;* and r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.01% of diazepam related compound A, not more than 0.1% of diazepam related compound B, and not more than 0.3% of nordazepam are found.
Calculate the percentage of any other impurity in the portion of Diazepam taken by the formula:

$$(C_S/W)(r_i/r_S)$$

in which C_S is the concentration, in µg per mL, of USP Diazepam Related Compound B RS in the *Standard solution; r_i* is the peak response for any other impurity obtained from the *Test solution;* and r_S is the peak response of USP Diazepam Related Compound B RS obtained from the *Standard solution:* not more than 0.1% of any other impurity is found; and not more than 1.0% of the total impurities is found.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and methanol (2:2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve suitable quantities of USP Nordazepam RS and USP Diazepam RS in methanol, using sonication if necessary, to obtain a solution containing about 0.1 mg of each per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Diazepam RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.1 mg per mL.
Assay preparation—Transfer about 10 mg of Diazepam, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.76 for nordazepam and 1.0 for diazepam; the resolution, *R,* between nordazepam and diazepam is not less than 4; the column efficiency is not less than 5000 theoretical plates for the diazepam peak; the tailing factor for diazepam is not more than 2.0; and the relative standard deviation for the diazepam peak for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{16}H_{13}ClN_2O$ in the portion of Diazepam taken by the formula:

$$100C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Diazepam RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Diazepam Injection

» Diazepam Injection is a sterile solution of Diazepam in a suitable medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{16}H_{13}ClN_2O$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Diazepam RS

USP Monographs

www.webofpharma.com

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

B: Transfer a volume of Injection, equivalent to about 10 mg of diazepam, to a separator, add 20 mL of water, and shake. Add 20 mL of chloroform, and shake vigorously for 2 minutes. Filter the chloroform layer through about 5 g of anhydrous granular sodium sulfate into a beaker. Wash the sodium sulfate with 20 mL of chloroform, collecting the washing in the beaker. Evaporate the chloroform extract on a steam bath with the aid of a current of air to a volume of about 5 mL. Remove the beaker from the steam bath, and evaporate the chloroform extract with the aid of a current of air to dryness. Dissolve the residue in 20 mL of anhydrous ether, filter, and evaporate the filtrate to dryness using a current of air. Vigorously scrape the resulting oily film with a spatula, and dry in vacuum over phosphorus pentoxide at 60° for 4 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue exhibits maxima only at the same wavelengths as that of a similar preparation of USP Diazepam RS.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 11.6 USP Endotoxin Units per mg of diazepam.

pH ⟨791⟩: between 6.2 and 6.9.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of methanol and water (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution— [NOTE—Prepare fresh daily.] Prepare a solution of *p*-tolualdehyde in methanol containing about 0.3 µL per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Diazepam RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 1 mg per mL. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.2 mg of USP Diazepam RS per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of diazepam, to a 50-mL volumetric flask. Pipet 10 mL of *Internal standard solution* into the flask, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.4 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.5 for *p*-tolualdehyde and 1.0 for diazepam, the tailing factor for the diazepam peak is not more than 2.5, the resolution, *R*, between the *p*-tolualdehyde and diazepam peaks is not less than 3.5, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (between 10 µL and 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{16}H_{13}ClN_2O$ in each mL of the Injection taken by the formula:

$$50(C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Diazepam RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and R_U and R_S are the ratios of the peak responses of diazepam to that of *p*-tolualdehyde obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Diazepam Tablets

» Diazepam Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of diazepam ($C_{16}H_{13}ClN_2O$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Diazepam RS
USP Nordazepam RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.
$C_{15}H_{11}ClN_2O$ 270.72

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

B: Accurately weigh an amount of Tablet mass, equivalent to 10 mg of diazepam, place in a 50-mL centrifuge tube, and add 2 mL of acetone. Place the centrifuge tube in an ultrasonic bath for 5 minutes, and centrifuge. Using 100 µL of the supernatant as the test solution, 100 µL of a solution of USP Diazepam RS in acetone containing 5 mg per mL as the Standard solution, and a solvent system consisting of equal volumes of ethyl acetate and *n*-heptane, proceed as directed in *Identification* test *B* under *Diazepam*. The specified result is observed.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 1: 100 rpm.
Time: 30 minutes.
Procedure—Determine the amount of $C_{16}H_{13}ClN_2O$ dissolved by employing UV absorption at a wavelength of about 242 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Diazepam RS in the same *Medium*.
Tolerances—Not less than 85% *(Q)* of the labeled amount of $C_{16}H_{13}ClN_2O$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase, System suitability solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Diazepam*.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of diazepam, to a 100-mL volumetric flask. Add about 50 mL of methanol, sonicate for 5 minutes, shake by mechanical means for 5 minutes, dilute with methanol to volume, mix, and filter, discarding the first few mL of the filtrate.

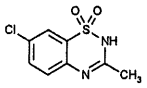
Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Diazepam*. Calculate the quantity, in mg, of diazepam ($C_{16}H_{13}ClN_2O$) in the portion of Tablets taken by the formula:

www.webofpharma.com

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Diazepam RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Diazoxide



$C_8H_7ClN_2O_2S$ 230.67

2H-1,2,4-Benzothiadiazine, 7-chloro-3-methyl-, 1,1-dioxide; 7-Chloro-3-methyl-*2H*-1,2,4-benzothiadiazine 1,1-dioxide [364-98-7].

DEFINITION

Diazoxide contains NLT 97.0% and NMT 102.0% of diazoxide ($C_8H_7ClN_2O_2S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the *Internal standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, glacial acetic acid, and 0.01 M sodium 1-pentanesulfonate (20:1:80)
Diluent: Methanol and water (1:4)
Internal standard solution: 2 mg/mL of hydrochlorothiazide in methanol
Standard stock solution: 1 mg/mL of USP Diazoxide RS in methanol
Standard solution: 50 µg/mL of USP Diazoxide RS and 40 µg/mL of hydrochlorothiazide prepared as follows. Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.
Sample stock solution: 1 mg/mL of Diazoxide in methanol
Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L11
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times of hydrochlorothiazide and diazoxide are about 0.4 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 5 between the diazoxide and hydrochlorothiazide peaks
Relative standard deviation: NMT 1.5%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of diazoxide ($C_8H_7ClN_2O_2S$) in the portion of Diazoxide taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of diazoxide to the internal standard from the *Sample solution*
R_S = peak response ratio of diazoxide to the internal standard from the *Standard solution*
C_S = concentration of USP Diazoxide RS in the *Standard solution* (µg/mL)
C_U = concentration of Diazoxide in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 105° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Diazoxide RS

Delete the following:

▲Diazoxide Capsules

DEFINITION

Diazoxide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of diazoxide ($C_8H_7ClN_2O_2S$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Sample solution: Equivalent to 2 mg/mL of diazoxide prepared as follows. Place a portion of Capsule contents equivalent to about 50 mg of diazoxide in a suitable centrifuge tube, and add 25 mL of 0.1 N sodium hydroxide. Shake for 15 min, and centrifuge. Use the supernatant.
Developing solvent system: Methanol, ethyl acetate, and ammonium hydroxide (40:170:30)
Acceptance criteria: Meet the requirements

- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the *Internal standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, glacial acetic acid, and 0.01 M sodium 1-pentanesulfonate (20:1:80)
Diluent: Methanol and water (1:4)
Internal standard solution: 2 mg/mL of hydrochlorothiazide in methanol
Standard stock solution: 1 mg/mL of USP Diazoxide RS in methanol
Standard solution: 50 µg/mL of USP Diazoxide RS and 40 µg/mL of hydrochlorothiazide prepared as follows. Transfer 5.0 mL of the *Standard stock solution* to a 100-mL

USP Monographs

www.webofpharma.com

volumetric flask, add 2.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.

Sample stock solution: Nominally 1 mg/mL of diazoxide prepared as follows. Remove, as completely as possible, the contents of NLT 20 Capsules. Transfer an amount equivalent to 100 mg of diazoxide to a 100-mL volumetric flask. Add 15 mL of water, and shake by mechanical means for 5 min. Add 60 mL of methanol, shake by mechanical means for 15 min, and dilute with methanol to volume.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L11

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for hydrochlorothiazide and diazoxide are about 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5 between the diazoxide and hydrochlorothiazide peaks

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diazoxide ($C_8H_7ClN_2O_2S$) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of diazoxide to the internal standard from the *Sample solution*

R_S = peak response ratio of diazoxide to the internal standard from the *Standard solution*

C_S = concentration of USP Diazoxide RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of diazoxide in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

Mode: UV

Analytical wavelength: 268 nm

Analysis: Determine the amount of diazoxide ($C_8H_7ClN_2O_2S$) dissolved from UV maximum absorbances of the *Sample solution* compared to a similarly prepared solution of USP Diazoxide RS.

Tolerances: NLT 75% (Q) of the labeled amount of diazoxide ($C_8H_7ClN_2O_2S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Diazoxide RS▲ (USP 1-May-2020)

Delete the following:

▲Diazoxide Injection

DEFINITION

Diazoxide Injection is a sterile solution of Diazoxide in Water for Injection, prepared with the aid of Sodium Hydroxide. It contains NLT 90.0% and NMT 110.0% of the labeled amount of diazoxide ($C_8H_7ClN_2O_2S$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 1 mg/mL of USP Diazoxide RS in methanol

Sample solution: Equivalent to 1 mg/mL of diazoxide from a suitable portion of Injection, in methanol

Developing solvent system: Methanol, ethyl acetate, and ammonium hydroxide (40:170:30)

Acceptance criteria: Meets the requirements

• **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the *Internal standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol, glacial acetic acid, and 0.01 M sodium 1-pentanesulfonate (20:1:80)

Diluent: Methanol and water (1:4)

Internal standard solution: 2 mg/mL of hydrochlorothiazide in methanol

Standard stock solution: 1 mg/mL of USP Diazoxide RS in methanol

Standard solution: 50 µg/mL of USP Diazoxide RS and 40 µg/mL of hydrochlorothiazide prepared as follows. Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.

Sample stock solution: Transfer a volume of Injection equivalent to 45 mg of diazoxide to a 50-mL volumetric flask, and dilute with methanol to volume.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L11

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times of hydrochlorothiazide and diazoxide are about 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5 between the diazoxide and hydrochlorothiazide peaks

USP Monographs

www.webofpharma.com

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diazoxide ($C_8H_7ClN_2O_2S$) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of diazoxide to the internal standard from the *Sample solution*

R_S = peak response ratio of diazoxide to the internal standard from the *Standard solution*

C_S = concentration of USP Diazoxide RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of diazoxide in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 11.2–11.9
- **Bacterial Endotoxins Test** ⟨85⟩**:** It contains NMT 0.5 USP Endotoxin Unit/mg of diazoxide.
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass, protected from light.
- **USP Reference Standards** ⟨11⟩

USP Diazoxide RS▲ (USP 1-May-2020)

Diazoxide Oral Suspension

DEFINITION

Diazoxide Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of diazoxide ($C_8H_7ClN_2O_2S$).

IDENTIFICATION

- **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩

Sample solution: Equivalent to 1 mg/mL of diazoxide prepared as follows. Place a portion of Oral Suspension equivalent to 50 mg of diazoxide in a 50-mL volumetric flask. Add 30 mL of 0.1 N sodium hydroxide, shake for 30 min, and dilute with 0.1 N sodium hydroxide to volume.

Developing solvent system: Methanol, ethyl acetate, and ammonium hydroxide (40:170:30)

Acceptance criteria: Meets the requirements

- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the *Internal standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Mobile phase: Methanol, glacial acetic acid, and 0.01 M sodium 1-pentanesulfonate (20:1:80)

Diluent: Methanol and water (1:4)

Internal standard solution: 2 mg/mL of hydrochlorothiazide in methanol

Standard stock solution: 1 mg/mL of USP Diazoxide RS in methanol

Standard solution: 50 µg/mL of USP Diazoxide RS and 40 µg/mL of hydrochlorothiazide prepared as follows. Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.

Sample stock solution: Transfer an amount equivalent to about 100 mg of diazoxide from freshly mixed Oral Suspension to a 50-mL centrifuge tube. Add 2 mL of water and 35 mL of methanol, shake for 15 min, and centrifuge for 5 min. Transfer the supernatant to a 200-mL volumetric flask. Repeat the extraction process two times, beginning with the addition of 2 mL of water, combine the extracts in the 200-mL volumetric flask, and dilute with methanol to volume.

Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 2.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L11

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times of hydrochlorothiazide and diazoxide are about 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5 between the diazoxide and hydrochlorothiazide peaks

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diazoxide ($C_8H_7ClN_2O_2S$) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of diazoxide to the internal standard from the *Sample solution*

R_S = peak response ratio of diazoxide to the internal standard from the *Standard solution*

C_S = concentration of USP Diazoxide RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of diazoxide in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** ⟨905⟩**:** Meets the requirements for Oral Suspension packaged in single-unit containers
- **Deliverable Volume** ⟨698⟩**:** Meets the requirements for Oral Suspension packaged in multiple-unit containers

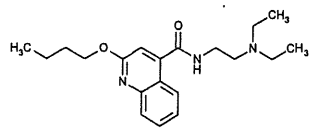
ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩

USP Diazoxide RS

www.webofpharma.com

Dibucaine



$C_{20}H_{29}N_3O_2$ 343.46

4-Quinolinecarboxamide, 2-butoxy-*N*-[2-(diethylamino) ethyl]-.

2-Butoxy-*N*-[2-(diethylamino)ethyl]cinchoninamide [85-79-0].

» Dibucaine contains not less than 97.0 percent and not more than 102.5 percent of $C_{20}H_{29}N_3O_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Dibucaine Hydrochloride RS

Identification—

A: The IR absorption spectrum of a mineral oil dispersion of it, previously dried, exhibits maxima only at the same wavelengths as that of a similar dispersion of the residue prepared by dissolving 30 mg of USP Dibucaine Hydrochloride RS in 5 mL of 0.5 N sodium hydroxide, extracting the resulting solution with 5 mL of ether, evaporating the ether, and drying the residue over phosphorus pentoxide.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Melting range ⟨741⟩**:** between 62.5° and 66.0°, determined after drying.

Loss on drying ⟨731⟩—Dry it over phosphorus pentoxide for 16 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.2%.

Chromatographic purity—Proceed as directed for *Chromatographic purity* under *Dibucaine Hydrochloride,* except to use a *Test solution* containing 36.2 mg of Dibucaine per mL: the principal spot obtained from the *Test solution* corresponds in R_F value, color, and intensity to that obtained from the *Standard solution;* the sum of the intensities of any secondary spots, if present in the chromatogram of the *Test solution,* corresponds to not more than 2.0% of that of the principal spot in the chromatogram of the *Standard solution* on the basis of comparison with the spots obtained from the *Comparison solutions.*

Assay—

Mobile phase—Dissolve 1.20 g of sodium lauryl sulfate, 0.20 g of sodium acetate, and 2.0 mL of triethylamine in 300 mL of water. Adjust with glacial acetic acid to a pH of 5.6, add 700 mL of methanol, mix, and pass through a suitable filter having a 0.5-µm or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Solvent mixture—Prepare a mixture of methanol and water (70:30).

Standard preparation—Dissolve an accurately weighed quantity of USP Dibucaine Hydrochloride RS in *Solvent mixture* to obtain a solution having a known concentration of about 1 mg per mL. Pass through a suitable filter having a 0.5-µm or finer porosity.

Assay preparation—Transfer about 90 mg of Dibucaine, accurately weighed, to a 100-mL volumetric flask, add *Solvent mixture* to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency, determined from the analyte peak, is not less than 1500 theoretical plates; the tailing factor for the analyte peak is not more than 3.0; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_{20}H_{29}N_3O_2$ in the portion of Dibucaine taken by the formula:

$$(343.46/379.93)(100C)(r_U/r_S)$$

in which 343.46 and 379.93 are the molecular weights of dibucaine and dibucaine hydrochloride, respectively; C is the concentration, in mg per mL, of USP Dibucaine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the responses of the dibucaine peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dibucaine Cream

» Dibucaine Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dibucaine ($C_{20}H_{29}N_3O_2$) in a suitable cream base.

Packaging and storage—Preserve in collapsible tubes or in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Dibucaine Hydrochloride RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Minimum fill ⟨755⟩**:** meets the requirements.

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Dibucaine.*

Standard preparation—Dissolve an accurately weighed quantity of USP Dibucaine Hydrochloride RS in an amount of 0.1 N hydrochloric acid equivalent to 20% of the flask's volume, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.25 mg per mL.

Assay preparation—Weigh accurately a portion of Cream, equivalent to about 22 mg of dibucaine, transfer to a separator containing 25 mL of ether, and mix to dissolve. Extract successively with two 9-mL portions of 0.1 N hydrochloric acid, combining the extracts in a 100-mL volumetric flask. Extract the ether phase in the separator with 2 mL of water, collecting the aqueous extract in the 100-mL volumetric flask.

www.webofpharma.com

Dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of dibucaine ($C_{20}H_{29}N_3O_2$) in the portion of Cream taken by the formula:

$$(343.46/379.93)(100C)(r_U/r_S)$$

in which 343.46 and 379.93 are the molecular weights of dibucaine and dibucaine hydrochloride, respectively; *C* is the concentration, in mg per mL, of USP Dibucaine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the area responses of the dibucaine peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dibucaine Ointment

» Dibucaine Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dibucaine ($C_{20}H_{29}N_3O_2$) in a suitable ointment base.

Packaging and storage—Preserve in collapsible tubes or in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Dibucaine Hydrochloride RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Minimum fill ⟨755⟩**:** meets the requirements.

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Dibucaine.*

Standard preparation—Transfer about 55 mg of USP Dibucaine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 24.0 mL of 0.1 N hydrochloric acid, and swirl to dissolve. Dilute with a mixture of methanol and 1.0 N hydrochloric acid (13:12) to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity.

Assay preparation—Weigh accurately a portion of Ointment, equivalent to about 50 mg of dibucaine, transfer to a separator containing 50 mL of ether, and mix to dissolve. Extract successively with 50-mL, 40-mL, and 30-mL portions of 0.1 N hydrochloric acid, combining the extracts in a 250-mL volumetric flask. Dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of dibucaine ($C_{20}H_{29}N_3O_2$) in the portion of Ointment taken by the formula:

$$(343.46/379.93)(250C)(r_U/r_S)$$

in which 343.46 and 379.93 are the molecular weights of dibucaine and dibucaine hydrochloride, respectively; *C* is the concentration, in mg per mL, of USP Dibucaine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the area responses of the dibucaine peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dibucaine Hydrochloride

$C_{20}H_{29}N_3O_2 \cdot HCl$ 379.92

4-Quinolinecarboxamide, 2-butoxy-*N*-[2-(diethylamino)ethyl]-, monohydrochloride.

2-Butoxy-*N*-[2-(diethylamino)ethyl]cinchoninamide monohydrochloride [61-12-1].

» Dibucaine Hydrochloride contains not less than 97.0 percent and not more than 100.5 percent of $C_{20}H_{29}N_3O_2 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Dibucaine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

C: A solution of it responds to tests *A* or *B* in *Identification Tests—General* ⟨191⟩, *Chloride*

Loss on drying ⟨731⟩—Dry it at 80° for 5 hours: it loses not more than 2.0% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Chromatographic purity—Dissolve a suitable quantity of Dibucaine Hydrochloride, accurately weighed, in chloroform to obtain a *Test solution* having a concentration of 40.0 mg per mL. Dissolve a suitable quantity of USP Dibucaine Hydrochloride RS, accurately weighed, in chloroform to obtain a *Standard solution* having a known concentration of about 40 mg per mL. Dilute portions of this solution quantitatively and stepwise with chloroform to obtain three *Comparison solutions* having concentrations of 40, 120, and 200 µg per mL (0.1%, 0.3%, and 0.5%) of the *Standard solution,* respectively. Apply separate 5-µL portions of the five solutions to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of toluene, acetone, methanol, and ammonium hydroxide (50:30:5:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and air-dry. Spray the plate heavily with a 1 in 200 solution of potassium dichromate in dilute sulfuric acid (1 in 5). Place the plate in an oven at 140° for 10 minutes, and view under short-wavelength UV light: the principal spot obtained from the *Test solution* corresponds in R_F value, color, and intensity to that obtained from the *Standard solution;* the sum of the intensities of any secondary spots, if present in the chromatogram of the *Test solution,*

www.webofpharma.com

corresponds to not more than 1.0%, and the intensity of any secondary spot does not exceed 0.5% of that of the principal spot in the chromatogram of the *Standard solution* on the basis of comparison with the spots obtained from the *Comparison solutions.*

Assay—

Mobile phase, Solvent mixture, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Dibucaine.*

Assay preparation—Transfer about 100 mg of Dibucaine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, add *Solvent mixture* to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Dibucaine.* Calculate the quantity, in mg, of $C_{20}H_{29}N_3O_2 \cdot HCl$ in the portion of Dibucaine Hydrochloride taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dibucaine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the responses of the dibucaine peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dibucaine Hydrochloride Injection

» Dibucaine Hydrochloride Injection is a sterile solution of Dibucaine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of dibucaine hydrochloride ($C_{20}H_{29}N_3O_2 \cdot HCl$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and protect from light.

USP Reference standards ⟨11⟩—
USP Dibucaine Hydrochloride RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

B: Place a volume of Injection, equivalent to about 30 mg of dibucaine hydrochloride, in a suitable evaporating dish, and concentrate on a steam bath to a volume of about 10 mL. Transfer the solution to a separator, render distinctly alkaline with 1 N sodium hydroxide, and extract with four 20-mL portions of ether. Wash the combined ether extracts with 5 mL of water, discarding the washing. Evaporate the ether extracts with the aid of a current of air to dryness, and dry the residue over phosphorus pentoxide for 3 hours: the dibucaine so obtained melts between 62° and 65°.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 35.7 USP Endotoxin Units per mg of dibucaine hydrochloride.

pH ⟨791⟩: between 4.5 and 7.0.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Dibucaine.*

Standard preparation—Transfer about 50 mg of USP Dibucaine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add an accurately measured volume of water, equivalent to the volume of Injection taken to prepare the *Assay preparation,* dilute with methanol to volume, and mix. Where the *Assay preparation* is prepared in a 50-mL volumetric flask, transfer about 25 mg of USP Dibucaine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add an accurately measured volume of water, equivalent to twice the volume of Injection taken to prepare the *Assay preparation,* dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity.

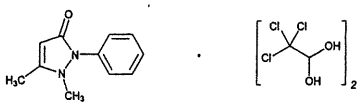
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of dibucaine, to a 100-mL volumetric flask. Dilute with methanol to volume, and mix. Where the Injection is labeled to contain 1 mg or less of dibucaine hydrochloride per mL, transfer an accurately measured volume of Injection, equivalent to about 13 mg of dibucaine hydrochloride, to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5-µm or finer porosity.

Procedure—Separately inject equal volumes (about 10 µL, or 20 µL where the concentration of dibucaine hydrochloride is about 0.25 mg per mL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of dibucaine hydrochloride ($C_{20}H_{29}N_3O_2 \cdot HCl$) in each mL of the Injection taken by the formula:

$$C(v/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dibucaine Hydrochloride RS in the *Standard preparation; v* is the volume, in mL, of the *Assay preparation; V* is the volume, in mL, of Injection taken; and r_U and r_S are the area responses of the dibucaine peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dichloralphenazone



$C_{15}H_{18}Cl_6N_2O_5$ 519.03

1,2-Dihydro-1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one, compound with 2,2,2-trichloro-1,1-ethanediol (1:2); Antipyrine, compound with chloral hydrate (1:2) [480-30-8].

DEFINITION

Dichloralphenazone contains NLT 97.0% and NMT 100.5% of dichloralphenazone ($C_{15}H_{18}Cl_6N_2O_5$), determined by both *Assay* procedures.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

www.webofpharma.com

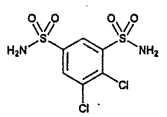
• **B.**
Sample: 0.1 g of Dichloralphenazone
Analysis: Dissolve the *Sample* in 10 mL of water containing 0.1 g of sodium nitrite. Add 1 mL of 2 N sulfuric acid.
Acceptance criteria: A green color is produced.

ASSAY
• **PROCEDURE 1**
Sample: 1500 mg of Dichloralphenazone
Titrimetric system
Titrant A: 0.5 N sulfuric acid VS
Titrant B: 0.1 N silver nitrate VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a beaker. Add 10 mL of water and 20.0 mL of 0.5 N sodium hydroxide VS. Allow to stand for 2 min, and titrate with *Titrant A* to a colorless phenolphthalein endpoint. Titrate this neutralized solution with *Titrant B*, using potassium chromate TS as the indicator. To the volume of *Titrant A* used to reach the first endpoint add four-fifteenths of the volume of *Titrant B* used to reach the second endpoint. Subtract the sum so obtained from the volume of 0.5 N sodium hydroxide added. Each mL of 0.5 N sodium hydroxide represented by the difference is equivalent to 129.8 mg of dichloralphenazone ($C_{15}H_{18}Cl_6N_2O_5$).
Acceptance criteria: 97.0%–100.5%
• **PROCEDURE 2**
Solution A: Sodium acetate in water (1 in 10)
Sample: 400 mg of Dichloralphenazone
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 0.1 N sodium thiosulfate VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 125-mL conical flask. Add 20 mL of *Solution A*, and swirl to dissolve. Add 25.0 mL of 0.1 N iodine VS, and allow to stand in the dark for 20 min, gently swirling occasionally. Add 10 mL of chloroform, rinsing the walls of the flask, and swirl until the precipitate is dissolved. Titrate the excess iodine with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a blank titration. The difference between the titrations represents the amount of iodine consumed. Each mL of 0.1 N iodine consumed is equivalent to 25.95 mg of dichloralphenazone ($C_{15}H_{18}Cl_6N_2O_5$).
Acceptance criteria: 97.0%–100.5%; the difference between the percentages calculated from *Procedure 1* and *Procedure 2* is NMT 1.5%.

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dichloralphenazone RS

Dichlorphenamide



$C_6H_6Cl_2N_2O_4S_2$ 305.16
1,3-Benzenedisulfonamide, 4,5-dichloro-;
4,5-Dichloro-*m*-benzenedisulfonamide [120-97-8].

DEFINITION
Dichlorphenamide contains NLT 98.0% and NMT 101.0% of dichlorphenamide ($C_6H_6Cl_2N_2O_4S_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: 197U▲ (CN 1-May-2020)
Sample solution: 0.1 mg/mL of Dichlorphenamide in 0.4 mg/mL of sodium hydroxide solution
Analysis: To 10 mL of the *Sample solution* add 0.1 mL of hydrochloric acid.
Acceptance criteria: It exhibits absorption maxima at 295 ± 2 nm and at 286 ± 2 nm. The ratio A_{295}/A_{286} is between 0.90 and 1.00.

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197M▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Solution A: 2.4 g/L of monobasic sodium phosphate and 2.8 g/L of dibasic sodium phosphate in water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	65	35
6	65	35
23	40	60
23.1	65	35
30	65	35

Diluent: Acetonitrile and water (1:1)
Standard solution: 0.5 mg/mL of USP Dichlorphenamide RS in *Diluent*
Sample solution: 0.5 mg/mL of Dichlorphenamide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 2.7-µm packing L1
Flow rate: 0.4 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dichlorphenamide ($C_6H_6Cl_2N_2O_4S_2$) in the portion of Dichlorphenamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

USP Monographs

www.webofpharma.com

r_S = peak response from the *Standard solution*
C_S = concentration of USP Dichlorphenamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Dichlorphenamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

• **ORGANIC IMPURITIES**

Solution A, Solution B, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.002 mg/mL of USP Dichlorphenamide RS in *Diluent*
Sample solution: 2 mg/mL of Dichlorphenamide in *Diluent*
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.5
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Dichlorphenamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of dichlorphenamide from the *Standard solution*
C_S = concentration of USP Dichlorphenamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Dichlorphenamide in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dichlorphenamide	1.0	1.0	—
3,4-Dichlorobenzene-sulfonamide	2.0	0.55	0.15
Any individual impurity	—	1.0	0.10
Total impurities	—	—	1.0

• **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*
Standard solution: 0.28 mL of 0.020 N hydrochloric acid
Sample solution: To 100 mg of Dichlorphenamide add 25 mL of 8 N nitric acid, and warm on a steam bath to dissolve. Cool to room temperature.
Acceptance criteria: 0.20%; the *Sample solution* shows no more chloride than the *Standard solution*.

• **SELENIUM** ⟨291⟩
Sample: A 100-mg specimen mixed with 100 mg of magnesium oxide
Acceptance criteria: NMT 30 ppm

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at a pressure of NMT 5 mm of mercury at 100° to constant weight.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dichlorphenamide RS

Dichlorphenamide Tablets

DEFINITION

Dichlorphenamide Tablets contain NLT 92.0% and NMT 108.0% of the labeled amount of dichlorphenamide ($C_6H_6Cl_2N_2O_4S_2$).

IDENTIFICATION

Change to read:

• **A.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

Change to read:

• ▲**C.**▲ 2S (USP41) **IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sulfite*
Sample: Fuse a quantity of powdered Tablets, equivalent to 200 mg of dichlorphenamide, with 1 pellet of sodium hydroxide.
Acceptance criteria: The ammonia fumes produced cause moistened red litmus paper to turn blue. The fusion mixture meets the requirements of the test for *Sulfite*.

ASSAY

Change to read:

• **PROCEDURE**
▲**Solution A:** 2.4 g/L of monobasic sodium phosphate and 2.8 g/L of dibasic sodium phosphate in water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	65	35
6	65	35
23	40	60
23.1	65	35
30	65	35

Diluent: Acetonitrile and water (1:1)
Standard solution: 0.5 mg/mL of USP Dichlorphenamide RS in *Diluent*. Sonicate if necessary.
Sample stock solution: Nominally 1 mg/mL of dichlorphenamide in *Diluent* prepared as follows. Transfer NLT 20 finely powdered Tablets, equivalent to 100 mg of dichlorphenamide, to a 100-mL volumetric flask. Fill the

USP Monographs

www.webofpharma.com

flask with 80 mL of *Diluent*, sonicate for 5 min, and mechanically shake for at least 30 min. Dilute with *Diluent* to volume, and centrifuge for 10 min.
Sample solution: Nominally 0.5 mg/mL of dichlorphenamide in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. For *Identification B*, use a diode array detector in the range of 220–400 nm.
Column: 4.6-mm × 15-cm; 2.7-µm packing L1
Flow rate: 0.4 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dichlorphenamide ($C_6H_6Cl_2N_2O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dichlorphenamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dichlorphenamide in the *Sample solution* (mg/mL)▲ 2S (USP41)

Acceptance criteria: 92.0%–108.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩
Medium: 0.1 M pH 8.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL
Apparatus 2: 75 rpm
Time: 60 min
Standard solution: USP Dichlorphenamide RS in *Medium*
Sample solution: Use filtered portions of the solution under test, suitably diluted with *Medium* in comparison with the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 285 nm
Analysis
▲**Samples:** *Standard solution* and *Sample solution*▲ 2S (USP41)
Calculate the percentage of the labeled amount of dichlorphenamide ($C_6H_6Cl_2N_2O_4S_2$) dissolved:

$$\text{▲Result} = (A_U/A_S) \times C_S \times D \times (V/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)▲ 2S (USP41)

Tolerances: NLT 80% (Q) of the labeled amount of dichlorphenamide ($C_6H_6Cl_2N_2O_4S_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

Add the following:

▲• **Organic Impurities**
Solution A, Solution B, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.004 mg/mL of USP Dichlorphenamide RS in *Diluent*
Sample solution: Nominally 2 mg/mL of dichlorphenamide in *Diluent* prepared as follows. Transfer NLT 20 finely powdered Tablets, equivalent to 50 mg of dichlorphenamide, to a 25-mL volumetric flask. Fill the flask with 20 mL of *Diluent*. Sonicate for 5 min and mechanically shake for at least 30 min. Dilute with *Diluent* to volume, and centrifuge for 10 min.
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*
r_S = peak response of dichlorphenamide from the *Standard solution*
C_S = concentration of USP Dichlorphenamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dichlorphenamide in the *Sample solution* (mg/mL)

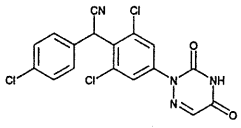
Acceptance criteria
Individual degradation product: NMT 0.20%
Total degradation products: NMT 1.0%▲ 2S (USP41)

ADDITIONAL REQUIREMENTS

Change to read:

• **Packaging and Storage:** Preserve in well-closed containers. ▲Store at controlled room temperature.▲ 2S (USP41)
• **USP Reference Standards** ⟨11⟩
USP Dichlorphenamide RS

Diclazuril



$C_{17}H_9Cl_3N_4O_2$ 407.64
Benzeneacetonitrile, 2,6-dichloro-α-(4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3*H*)-yl)-; (*p*-Chlorophenyl)[2,6-dichloro-4-(4,5-dihydro-3,5-dioxo-*as*-triazin-2(3*H*)-yl)phenyl]acetonitrile [101831-37-2].

www.webofpharma.com

DEFINITION

Diclazuril contains NLT 97.0% and NMT 101.0% of $C_{17}H_9Cl_3N_4O_2$, calculated on the dried basis.

IDENTIFICATION

Change to read:

▲• **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Buffer: Dissolve 6.3 g of ammonium formate in 800 mL of water, adjust with anhydrous formic acid to a pH of 4.0, and add 200 mL of water.

Solution A: Acetonitrile, water, and *Buffer* (3:15:2)

Solution B: Acetonitrile, water, and *Buffer* (85:5:10)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	0	100
25	0	100
26	100	0
36	100	0

Standard solution: 0.5 mg/mL of USP Diclazuril RS in dimethylformamide

System suitability solution: 0.5 mg/mL of USP Diclazuril System Suitability Mixture RS in dimethylformamide

Sample solution: 0.5 mg/mL of Diclazuril in dimethylformamide

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 10-cm; base-deactivated 3-µm packing L1

Flow rate: 1 mL/min

Column temperature: 35°

Injection size: 5 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 1.9 between diclazuril and diclazuril ketone peaks, *System suitability solution*

Tailing factor: NMT 1.4, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{17}H_9Cl_3N_4O_2$ in the portion of Diclazuril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Diclazuril RS in the *Standard solution* (mg/mL)

C_U = concentration of Diclazuril in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–101.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

• **Procedure 1: Residual Solvents** ⟨467⟩

Acceptance criteria: NMT 4000 ppm of *N*,*N*-dimethylformamide

• **Procedure 2**

Buffer, Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the area percentage of each impurity, relative to diclazuril, in the portion of Diclazuril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of diclazuril from the *Standard solution*

C_S = concentration of USP Diclazuril RS in the *Standard solution* (mg/mL)

C_U = concentration of Diclazuril in the *Sample solution* (mg/mL)

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria

[NOTE—Disregard any peak observed in the blank. The reporting level for impurities is 0.05%.]

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 1.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
6-Carboxylic acid[a]	0.62	0.85	0.50
6-Carboxamide[b]	0.80	0.92	0.50
Diclazuril	1.00	—	—
Ketone[c]	1.03	0.52	0.10
4-Amino derivative[d]	1.09	0.81	0.50
Des-cyano derivative[e]	1.16	1.1	0.50
Trichlorodiphenyl acetonitrile[f]	1.24	0.71	0.50
Any other individual impurity	—	1.0	0.20

[a] (*RS*)-2-[3,5-Dichloro-4-[(4-chlorophenyl)cyanomethyl]phenyl]-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-carboxylic acid.

[b] (*RS*)-2-[3,5-Dichloro-4-[(4-chlorophenyl)cyanomethyl]phenyl]-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-carboxamide.

[c] 2-[3,5-Dichloro-4-(4-chlorobenzoyl)phenyl]-1,2,4-triazine-3,5(2*H*,4*H*)-dione.

[d] (*RS*)-2-(4-Amino-2,6-dichlorophenyl)-2-(4-chlorophenyl)acetonitrile.

[e] 2-[3,5-Dichloro-4-(4-chlorobenzyl)phenyl]-1,2,4-triazine-3,5(2*H*,4*H*)-dione.

[f] (*RS*)-2-(4-Chlorophenyl)-2-(2,6-dichlorophenyl)acetonitrile.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩: Dry a sample at between 100° and 105° under a vacuum for 4 h: it loses NMT 0.5% of its weight.

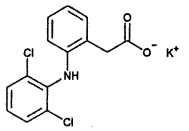
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.

www.webofpharma.com

- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Diclazuril RS
  USP Diclazuril System Suitability Mixture RS
  Contains diclazuril and specified impurities.

Diclofenac Potassium



$C_{14}H_{10}Cl_2KNO_2$ 334.24
Benzeneacetic acid, 2-[(2,6-dichlorophenyl)amino]-, monopotassium salt;
Potassium [*o*-(2,6-dichloroanilino)phenyl]acetate [15307-81-0].

DEFINITION
Diclofenac Potassium contains NLT 99.0% and NMT 101.0% of diclofenac potassium ($C_{14}H_{10}Cl_2KNO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Solution: 0.01 mg/mL in *Medium*
  Medium: Methanol
  Acceptance criteria: Meets the requirements
- **C.**
  Sample solution: Suspend 0.5 g of Diclofenac Potassium in 10 mL of water. Stir and add water until the substance is dissolved. Add 2 mL of 7 N hydrochloride acid, stir for 1 h, and filter with the aid of a vacuum. Neutralize with 5 N sodium hydroxide.
  Analysis: To 1 mL of *Sample solution*, add 1 mL of 2 N acetic acid and 1 mL of a freshly prepared 100-g/L solution of sodium cobaltinitrite.
  Acceptance criteria: A yellow or orange yellow precipitate is formed immediately.

ASSAY
- **PROCEDURE**
  Sample solution: Dissolve about 300 mg of Diclofenac Potassium in 50 mL of glacial acetic acid.
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Titrant: 0.1 N perchloric acid VS
  Analysis: Titrate with *Titrant*, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 33.424 mg of diclofenac potassium ($C_{14}H_{10}Cl_2KNO_2$).
  Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES
- **ORGANIC IMPURITIES**
  Solution A: 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate (1:1). If necessary, adjust with additional portions of the appropriate components to a pH of 2.5 ± 0.2.
  Mobile phase: Methanol and *Solution A* (70:30)
  Diluent: Methanol and water (70:30)
  System suitability solution: 40 µg/mL of diethyl phthalate, 0.5 mg/mL of USP Diclofenac Potassium RS, and 22.5 µg/mL of USP Diclofenac Related Compound A RS in *Diluent*
  Standard stock solution: 0.25 mg/mL of USP Diclofenac Related Compound A RS in methanol
  Standard solution: 1.5 µg/mL of USP Diclofenac Related Compound A RS in *Diluent* from *Standard stock solution*
  Sample solution: 0.5 mg/mL of Diclofenac Potassium in *Diluent*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 25-cm; packing L7
  Flow rate: 1 mL/min
  Injection volume: 30 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for diethyl phthalate, diclofenac related compound A, and diclofenac are about 0.5, 0.7, and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 4.0 between diethyl phthalate and diclofenac related compound A, *System suitability solution*
  Relative standard deviation: NMT 5.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of diclofenac related compound A in the portion of Diclofenac Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diclofenac related compound A from the *Sample solution*
r_S = peak response of diclofenac related compound A from the *Standard solution*
C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (µg/mL)
C_U = concentration of Diclofenac Potassium in the *Sample solution* (µg/mL)

Calculate the percentage of each other impurity in the portion of Diclofenac Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of diclofenac related compound A from the *Standard solution*
C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (µg/mL)
C_U = concentration of Diclofenac Potassium in the *Sample solution* (µg/mL)

Acceptance criteria
Diclofenac related compound A: NMT 0.1%
Each other individual impurity: NMT 0.1%

USP Monographs

www.webofpharma.com

Total impurities: NMT 0.3%

SPECIFIC TESTS
- **pH** ⟨791⟩
  Sample solution: 1% aqueous solution
  Acceptance criteria: 7.0–8.5
- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° under vacuum for 3 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Diclofenac Potassium RS
  USP Diclofenac Related Compound A RS
  N-(2,6-Dichlorophenyl)indolin-2-one.
  $C_{14}H_9Cl_2NO$ 278.14

Add the following:

▲Diclofenac Potassium for Oral Solution

DEFINITION
Diclofenac Potassium for Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of diclofenac potassium ($C_{14}H_{10}Cl_2NKO_2$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**
  Solution A: Dissolve 1.56 g of monobasic sodium phosphate dihydrate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.5.
  Solution B: Dissolve 6.8 g of monobasic potassium phosphate and 0.88 g of sodium hydroxide in 1000 mL of water. Adjust with 1 N sodium hydroxide to a pH of 6.8.
  Mobile phase: Acetonitrile and *Solution A* (40:60)
  Diluent: Methanol and *Solution B* (10:90)
  Standard solution: 0.2 mg/mL of USP Diclofenac Potassium RS in *Diluent*. Sonicate to dissolve, if needed.
  Sample stock solution: Nominally 0.5 mg/mL of diclofenac potassium prepared as follows. Transfer a portion of finely powdered contents of NLT 5 packets of Diclofenac Potassium for Oral Solution, equivalent to 100 mg of diclofenac potassium, to a 200-mL volumetric flask. Add 140 mL of *Diluent*, and sonicate for about 10 min. Dilute with *Diluent* to volume. Centrifuge a portion of the solution. [Note—A centrifuge speed of 3500 rpm for 10 min may be suitable.]
  Sample solution: Nominally 0.2 mg/mL of diclofenac potassium in *Diluent* from the *Sample stock solution*. Pass through a suitable filter of 0.45-µm pore size.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Column temperature: 40°
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  Run time: NLT 1.4 times the retention time of diclofenac
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of diclofenac potassium ($C_{14}H_{10}Cl_2NKO_2$) in the portion of Diclofenac Potassium for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diclofenac from the *Sample solution*
r_S = peak response of diclofenac from the *Standard solution*
C_S = concentration of USP Diclofenac Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac potassium in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
- **Dissolution** ⟨711⟩
  Medium: pH 6.8 phosphate buffer (19 g/L of trisodium phosphate dodecahydrate in 0.1 N hydrochloric acid, adjusted with 1 N sodium hydroxide to a pH of 6.8); 400 mL
  Apparatus 2: 75 rpm
  Time: 5 min
  Solution A and **Chromatographic system:** Proceed as directed in the *Assay*.
  Mobile phase: Acetonitrile and *Solution A* (50:50)
  Standard solution: 0.125 mg/mL of USP Diclofenac Potassium RS prepared as follows. Transfer a quantity of USP Diclofenac Potassium RS to a suitable volumetric flask. Add methanol to 5% of the flask volume and sonicate to dissolve. Dilute with *Medium* to volume.
  Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of diclofenac potassium ($C_{14}H_{10}Cl_2NKO_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of diclofenac from the *Sample solution*
r_S = peak response of diclofenac from the *Standard solution*
C_S = concentration of USP Diclofenac Potassium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 400 mL
L = label claim of diclofenac potassium (mg/packet)

Tolerances: NLT 80% (*Q*) of the labeled amount of diclofenac potassium ($C_{14}H_{10}Cl_2NKO_2$) is dissolved.

USP Monographs

www.webofpharma.com

• **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements

IMPURITIES

• **Organic Impurities**

Solution A: Prepare as directed in the *Assay*.

Solution B: Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water. Adjust with 1 N sodium hydroxide to a pH of 6.8.

Mobile phase: Methanol and *Solution A* (66:34)

Diluent: Methanol and *Solution B* (10:90)

Sensitivity solution: 0.5 µg/mL of USP Diclofenac Potassium RS in *Diluent*

Standard stock solution: 0.15 mg/mL of USP Diclofenac Related Compound A RS in methanol

Standard solution: 1 µg/mL of USP Diclofenac Related Compound A RS and 2.5 µg/mL of USP Diclofenac Potassium RS, prepared as follows. Transfer a volume of *Standard stock solution* and a quantity of USP Diclofenac Potassium RS to a suitable volumetric flask, and add *Diluent* to dissolve. Sonicate if needed. Dilute with *Diluent* to volume.

Sample solution: Nominally 500 µg/mL of diclofenac potassium in *Diluent*, prepared as follows. Transfer a portion of finely powdered contents of NLT 5 packets of Diclofenac Potassium for Oral Solution, equivalent to 50 mg of diclofenac potassium, to a 100-mL volumetric flask. Add 70 mL of *Diluent*, and sonicate for 10 min. Dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 25 µL

Run time: NLT 2.2 times the retention time of diclofenac

System suitability

Samples: *Sensitivity solution* and *Standard solution*

[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Relative standard deviation: NMT 5.0% for diclofenac and diclofenac related compound A, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of diclofenac related compound A in the portion of Diclofenac Potassium for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diclofenac related compound A from the *Sample solution*

r_S = peak response of diclofenac related compound A from the *Standard solution*

C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of diclofenac potassium in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Diclofenac Potassium for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified degradation product from the *Sample solution*

r_S = peak response of diclofenac from the *Standard solution*

C_S = concentration of USP Diclofenac Potassium RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of diclofenac potassium in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Diclofenac related compound A	0.6	0.2
Diclofenac	1.0	—
Any unspecified degradation product	—	0.2
Total degradation products	—	1.0

SPECIFIC TESTS

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total aerobic microbial count is NMT 10^3 cfu/g. The total combined yeasts and molds count is NMT 10^2 cfu/g. It meets the requirements of the test for absence of *Escherichia coli*.

• **pH** ⟨791⟩

Sample: Dissolve the contents of a unit dosage of Diclofenac Potassium for Oral Solution in 30 mL of water.

Acceptance criteria: 7.0–9.0

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩

USP Diclofenac Potassium RS

USP Diclofenac Related Compound A RS

1-(2,6-Dichlorophenyl)indolin-2-one.

$C_{14}H_9Cl_2NO$ 278.13▲ (USP 1-May-2020)

Diclofenac Potassium Tablets

DEFINITION

Diclofenac Potassium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diclofenac potassium ($C_{14}H_{10}Cl_2KNO_2$).

IDENTIFICATION

• **A.** The retention time of the diclofenac peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.** The UV (190–400 nm) spectrum of the diclofenac peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: 0.7708 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 5.3. Pass through a suitable filter of 0.2-µm pore size.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.00	70	30
0.50	70	30
8.50	5	95
10.00	5	95
10.01	70	30
14.00	70	30

Diluent: Acetonitrile and water (50:50)

Standard solution: 0.2 mg/mL of USP Diclofenac Potassium RS in *Diluent*

Sample solution: Nominally 0.2 mg/mL of diclofenac potassium in *Diluent* prepared as follows. Transfer a suitable portion of diclofenac potassium to a suitable volumetric flask from NLT 20 finely powdered Tablets. Add *Diluent* equivalent to 80% of the flask volume. Dissolve and fill with *Diluent* to volume. Pass the solution through a suitable filter of 0.22-µm pore size.

[NOTE—Protect the *Standard solution* and *Sample solution* from light.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm. For *Identification* test *B*, use a photo-diode array detector.

Column: 10-cm × 2.0-mm; 1.9-µm packing L1

Column temperature: 35°

Flow rate: 0.3 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Tailing factor: NMT 1.2

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diclofenac potassium ($C_{14}H_{10}Cl_2KNO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diclofenac from the *Sample solution*

r_S = peak response of diclofenac from the *Standard solution*

C_S = concentration of USP Diclofenac Potassium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diclofenac potassium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Simulated intestinal fluid (without enzyme); 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Standard solution: A known concentration of USP Diclofenac Potassium RS in *Medium*. Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute with *Medium* if necessary.

Sample solution: Sample as per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: Maximum absorbance at about UV 276 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diclofenac potassium ($C_{14}H_{10}Cl_2KNO_2$) dissolved:

$$\frac{A_U \times C_S \times V \times 100}{A_S \times L}$$

A_U = absorbance of the *Sample solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

A_S = absorbance of the *Standard solution*

L = label claim of diclofenac potassium (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of diclofenac potassium ($C_{14}H_{10}Cl_2KNO_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase and **Diluent:** Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL each of USP Diclofenac Potassium RS and USP Diclofenac Related Compound A RS in *Diluent*

Sample solution: Nominally 1.0 mg/mL of diclofenac potassium in *Diluent* prepared as follows. Transfer a suitable portion of diclofenac potassium to a suitable volumetric flask from NLT 20 finely powdered Tablets. Add *Diluent* equivalent to 80% of the flask volume. Dissolve and fill with *Diluent* to volume. Pass the solution through a suitable filter of 0.22-µm pore size.

Chromatographic system: Proceed as directed in the *Assay* except for the *Detector*.

Detector: UV 254 nm

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5%

Signal-to-noise ratio: NLT 10

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of diclofenac related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diclofenac related compound A from the *Sample solution*

www.webofpharma.com

r_S = peak response of diclofenac related compound A from the *Standard solution*
C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac potassium in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of diclofenac from the *Standard solution*
C_S = concentration of USP Diclofenac Potassium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac potassium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

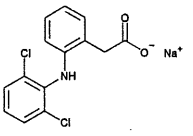
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxindole[a, b]	0.4	—
Diclofenac	1.0	—
Diclofenac related compound D (diclofenac bromo analog)[c, b]	1.04	—
Diclofenac related compound A	1.48	0.5
Diclofenac alcohol analog[d, b]	1.55	—
Diclofenac benzaldehyde analog[e, b]	1.81	—
Any individual unspecified impurity	—	0.5
Total impurities[b]	—	1.5

[a] 1,3-Dihydro-2*H*-indol-2-one.
[b] Process-related impurities, not to be counted in total impurities.
[c] 2-{2-[(2-Bromo-6-chlorophenyl)amino]phenyl}acetic acid.
[d] {2-[(2,6-Dichlorophenyl)amino]phenyl}methanol.
[e] 2-[(2,6-Dichlorophenyl)amino]benzaldehyde.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Diclofenac Potassium RS
  USP Diclofenac Related Compound A RS
  1-(2,6-Dichlorophenyl)indolin-2-one (diclofenac lactam).
  $C_{14}H_9Cl_2NO$ 278.13

Diclofenac Sodium



$C_{14}H_{10}Cl_2NNaO_2$ 318.13

Benzeneacetic acid, 2-[(2,6-dichlorophenyl)amino]-, monosodium salt;
Sodium [*o*-(2,6-dichloroanilino)phenyl]acetate [15307-79-6].

DEFINITION

Diclofenac Sodium contains NLT 99.0% and NMT 101.0% of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the diclofenac peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Organic Impurities*.
- **C.** The residue obtained by igniting it imparts an intense yellow color to a nonluminous flame.

ASSAY

- **PROCEDURE**

Sample solution: Dissolve about 450 mg of Diclofenac Sodium in 25 mL of glacial acetic acid.
Titrimetric system
Titrant: 0.1 N perchloric acid VS
Analysis: Titrate with *Titrant*, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.81 mg of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$).
Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate (1:1). If necessary, adjust with additional portions of the appropriate components to a pH of 2.5 ± 0.2.
Mobile phase: Methanol and *Solution A* (70:30)
Diluent: Methanol and water (70:30)
System suitability solution: 20 µg/mL of diethyl phthalate, 7.5 µg/mL of USP Diclofenac Related Compound A RS, and 0.75 mg/mL of USP Diclofenac Sodium RS in *Diluent*
Standard stock solution: 0.75 mg/mL of USP Diclofenac Related Compound A RS in methanol
Standard solution: 1.5 µg/mL of USP Diclofenac Related Compound A RS in *Diluent* from *Standard stock solution*
Sample solution: 0.75 mg/mL of Diclofenac Sodium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L7 (end-capped)
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 2.5 times the retention time of diclofenac
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for diethyl phthalate, diclofenac related compound A, and diclofenac are 0.5, 0.6, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.2 between diethyl phthalate and diclofenac related compound A; NLT 6.5 between diclofenac related compound A and diclofenac, *System suitability solution*

USP Monographs

www.webofpharma.com

Relative standard deviation: NMT 5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of diclofenac related compound A in the portion of Diclofenac Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diclofenac related compound A from the *Sample solution*

r_S = peak response of diclofenac related compound A from the *Standard solution*

C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Diclofenac Sodium in the *Sample solution* (mg/mL)

Calculate the percentage of each other impurity in the portion of Diclofenac Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of diclofenac related compound A from the *Standard solution*

C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Diclofenac Sodium in the *Sample solution* (mg/mL)

Acceptance criteria

Diclofenac related compound A: NMT 0.2%

Each other individual impurity: NMT 0.2%

Total impurities: NMT 0.5%

SPECIFIC TESTS

- **COLOR OF SOLUTION:** A solution (1 in 20) of Diclofenac Sodium in methanol is colorless to faintly yellow, and the absorbance of the solution, determined in a 1-cm cell at 440 nm, is NMT 0.050, methanol being used as the blank.
- **CLARITY OF SOLUTION:** The solution prepared as directed under *Color of Solution* is not significantly less clear than an equal volume of methanol contained in a similar vessel and examined similarly.
- **PH** ⟨791⟩

Sample solution: A solution (1 in 100)

Acceptance criteria: 7.0–8.5

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105°–110° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Diclofenac Sodium RS

USP Diclofenac Related Compound A RS

N-(2,6-Dichlorophenyl)indolin-2-one.

$C_{14}H_9Cl_2NO$ 278.14

Diclofenac Sodium Delayed-Release Tablets

DEFINITION

Diclofenac Sodium Delayed-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$).

IDENTIFICATION

- **A.** The retention time of the diclofenac peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV (190–400 nm) spectrum of the diclofenac peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.7708 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 5.3. Pass through a suitable filter of 0.2-µm pore size.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.00	70	30
0.50	70	30
8.50	5	95
10.00	5	95
10.01	70	30
14.00	70	30

Diluent: Acetonitrile and water (50:50)

Standard solution: 0.2 mg/mL of USP Diclofenac Sodium RS in *Diluent*

Sample solution: Nominally 0.2 mg/mL of diclofenac sodium in *Diluent* prepared as follows. Transfer a suitable portion of diclofenac sodium to a suitable volumetric flask from NLT 20 finely powdered Tablets. Add *Diluent* equivalent to 50% of the flask volume. Dissolve with sonication for 20 min at 40° and fill with *Diluent* to volume. Pass the solution through a suitable filter of 0.22-µm pore size.

[NOTE—Protect the *Standard solution* and *Sample solution* from light.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm. For *Identification* test *B*, use a photo-diode array detector.

Column: 10-cm × 2.0-mm; 1.9-µm packing L1

Column temperature: 35°

Flow rate: 0.3 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Tailing factor: NMT 1.2

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the percentage of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diclofenac from the *Sample solution*
r_S = peak response of diclofenac from the *Standard solution*
C_S = concentration of USP Diclofenac Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Proceed as directed in *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B* to determine the amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) dissolved.

Acid stage

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm, paddles constructed of (or coated with) polytef being used
Time: 2 h
Detector: UV, maxima at about 276 nm
Standard solution: Transfer 68 mg of USP Diclofenac Sodium RS to a 100-mL volumetric flask, add 10.0 mL of 0.1 N sodium hydroxide, and dilute with water to volume. Transfer 2.0 mL of this solution to a second 100-mL volumetric flask, dilute with a mixture of 0.1 N hydrochloric acid and 5 N sodium hydroxide (900:20) to volume, and mix. This *Standard solution* contains 13.6 µg/mL of USP Diclofenac Sodium RS.
Sample solution: At the end of 2 h, remove each Tablet, or the major portion thereof if the Tablet is not intact, from the individual vessels, and subject them to the test under *Buffer stage*. To the 0.1 N hydrochloric acid remaining in each vessel, add 20.0 mL of 5 N sodium hydroxide, and stir for 5 min.

Buffer stage

Medium: pH 6.8 phosphate buffer; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Detector: UV, maxima at about 276 nm
Solution A: 76 mg/mL of tribasic sodium phosphate
pH 6.8 phosphate buffer: *Solution A* and 0.1 N hydrochloric acid (1:3), adjusted with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8 ± 0.05, if necessary
Standard solution: Transfer 68 mg of USP Diclofenac Sodium RS to a 100-mL volumetric flask. Add 10.0 mL of 0.1 N sodium hydroxide, dilute with water to volume, and mix. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, dilute with *Buffer stage Medium* to volume, and mix. The final concentration is about 0.0204 mg/mL of diclofenac sodium.
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.
Tolerances: NLT 75% (*Q*) of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**

Mobile phase and **Diluent:** Proceed as directed in the *Assay*.
Standard solution: 0.001 mg/mL each of USP Diclofenac Sodium RS and USP Diclofenac Related Compound A RS in *Diluent*
Sample solution: Nominally 1.0 mg/mL of diclofenac sodium in *Diluent* prepared as follows. Transfer a suitable portion of diclofenac sodium to a suitable volumetric flask from NLT 20 finely powdered Tablets. Add *Diluent* equivalent to 50% of the flask volume. Dissolve with sonication for 20 min at 40° and fill with *Diluent* to volume. Pass the solution through a suitable filter of 0.22-µm pore size.
Chromatographic system: Proceed as directed in the *Assay*, except for the *Detector*.
Detector: UV 254 nm
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5%
Signal-to-noise ratio: NLT 10
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of diclofenac related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diclofenac related compound A from the *Sample solution*
r_S = peak response of diclofenac related compound A from the *Standard solution*
C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of diclofenac from the *Standard solution*
C_S = concentration of USP Diclofenac Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxindole[a, b]	0.4	—
Diclofenac	1.0	—
Diclofenac related compound D (diclofenac bromo analog)[c, b]	1.04	—
Diclofenac related compound A	1.48	0.5
Diclofenac alcohol analog[d, b]	1.55	—

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Diclofenac benzaldehyde analog[e, b]	1.81	—
Any individual unspecified impurity	—	0.5
Total impurities[b]	—	1.5

[a] 1,3-Dihydro-2*H*-indol-2-one.
[b] Process-related impurities, not to be counted in total impurities.
[c] 2-{2-[(2-Bromo-6-chlorophenyl)amino]phenyl}acetic acid.
[d] {2-[(2,6-Dichlorophenyl)amino]phenyl}methanol.
[e] 2-[(2,6-Dichlorophenyl)amino]benzaldehyde.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature. Protect from moisture.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Diclofenac Sodium RS
  USP Diclofenac Related Compound A RS
  1-(2,6-Dichlorophenyl)indolin-2-one.
  $C_{14}H_9Cl_2NO$ 278.13

Diclofenac Sodium Extended-Release Tablets

DEFINITION

Diclofenac Sodium Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$).

IDENTIFICATION

- **A.** The retention time of the diclofenac peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV (190–400 nm) spectrum of the diclofenac peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.7708 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 5.3. Pass through a suitable filter of 0.2-µm pore size.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.00	70	30
0.50	70	30
8.50	5	95
10.00	5	95
10.01	70	30
14.00	70	30

Diluent: Acetonitrile and water (50:50)
Standard solution: 0.2 mg/mL of USP Diclofenac Sodium RS in *Diluent*
Sample solution: Nominally 0.2 mg/mL of diclofenac sodium in *Diluent* prepared as follows. Transfer a suitable portion of diclofenac sodium to a suitable volumetric flask from NLT 20 finely powdered Tablets. Add *Diluent* equivalent to 70% of the flask volume. Dissolve with the aid of sonication for 25 min at 45° and fill with *Diluent* to volume. Pass the solution through a suitable filter of 0.22-µm pore size.
[NOTE—Protect the *Standard solution* and *Sample solution* from light.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm. For *Identification* test *B*, use a photo-diode array detector.
Column: 10-cm × 2.0-mm; 1.9-µm packing L1
Column temperature: 35°
Flow rate: 0.3 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Tailing factor: NMT 1.2
Relative standard deviation: NMT 2.8%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diclofenac from the *Sample solution*
r_S = peak response of diclofenac from the *Standard solution*
C_S = concentration of USP Diclofenac Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1
Medium: 0.05 M phosphate buffer, pH 7.5; 900 mL
Apparatus 2: 50 rpm; use wire sinkers.
Times: 1, 5, 10, 16, and 24 h
Detector: UV 276 nm
Standard solution: USP Diclofenac Sodium RS in *Medium*
Analysis: Pass portions of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.
Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved
1	15%–35%
5	45%–65%
10	65%–85%
16	75%–95%
24	NLT 80%

www.webofpharma.com

The percentages of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Apparatus 2, and **Analysis:** Proceed as directed for *Dissolution Test 1*.

Times: 1, 2, 4, 6, and 10 h

Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved
1	NMT 28%
2	20%–40%
4	35%–60%
6	50%–80%
10	NLT 65%

The percentages of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium and **Analysis:** Proceed as directed for *Dissolution Test 1*.

Apparatus 1: 100 rpm

Times: 2, 4, 8, and 16 h

Tolerances: See *Table 4*.

Table 4

Time (h)	Amount Dissolved
2	22%–42%
4	34%–61%
8	52%–82%
16	NLT 73%

The percentages of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium and **Analysis:** Proceed as directed for *Dissolution Test 1*.

Apparatus 1: 100 rpm

Times: 2, 4, 8, and 16 h

Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved
2	20%–40%
4	35%–55%
8	60%–85%
16	NLT 85%

The percentages of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**

Mobile phase and **Diluent:** Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL each of USP Diclofenac Sodium RS and USP Diclofenac Related Compound A RS in *Diluent*

Sample solution: Nominally 1.0 mg/mL of diclofenac sodium in *Diluent* prepared as follows. Transfer a suitable portion of diclofenac sodium to a suitable volumetric flask from NLT 20 finely powdered Tablets. Add *Diluent* equivalent to 70% of the flask volume. Dissolve with the aid of sonication for 25 min at 45° and fill with *Diluent* to volume. Pass the solution through a suitable filter of 0.22-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 10-cm × 2.0-mm; 1.9-µm packing L1

Column temperature: 35°

Flow rate: 0.3 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5%

Signal-to-noise ratio: NLT 10

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of diclofenac related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diclofenac related compound A from the *Sample solution*

r_S = peak response of diclofenac related compound A from the *Standard solution*

C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of diclofenac from the *Standard solution*

C_S = concentration of USP Diclofenac Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 6*. Disregard any impurity peak less than 0.05%.

Table 6

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxindole[a,b]	0.4	—
Diclofenac	1.0	—

USP Monographs

www.webofpharma.com

Table 6 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Diclofenac related compound D (diclofenac bromo analog)[c, b]	1.04	—
Diclofenac related compound A	1.48	0.5
Diclofenac alcohol analog[d, b]	1.55	—
Diclofenac benzaldehyde analog[e, b]	1.81	—
Any individual unspecified impurity	—	0.5
Total impurities[b]	—	1.5

[a] 1,3-Dihydro-2*H*-indol-2-one.
[b] Process-related impurities, not to be counted in total impurities.
[c] 2-{2-[(2-Bromo-6-chlorophenyl)amino]phenyl}acetic acid.
[d] {2-[(2,6-Dichlorophenyl)amino]phenyl}methanol.
[e] 2-[(2,6-Dichlorophenyl)amino]benzaldehyde.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature, and protect from light and moisture.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Diclofenac Sodium RS
  USP Diclofenac Related Compound A RS
  1-(2,6-Dichlorophenyl)indolin-2-one.
  $C_{14}H_9Cl_2NO$ 278.13

Add the following:

▲Diclofenac Sodium Topical Solution

DEFINITION

Diclofenac Sodium Topical Solution is a solution of Diclofenac Sodium in a suitable vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Phosphoric acid and water (0.62: 1000)
Solution B: 1.86 g of monobasic sodium phosphate dihydrate in 1000 mL of water
Solution C: *Solution A* and *Solution B* (50:50)
Mobile phase: Methanol and *Solution C* (70:30)
Diluent: Methanol and water (70:30)
Standard stock solution: 0.6 mg/mL of USP Diclofenac Sodium RS prepared as follows. Transfer a quantity of USP Diclofenac Sodium RS to a suitable volumetric flask, add 20% of the flask volume of methanol, sonicate to dissolve, and dilute with *Diluent* to volume.
Standard solution: 0.06 mg/mL of USP Diclofenac Sodium RS from *Standard stock solution* diluted with *Diluent*
Sample solution: Nominally 0.06 mg/mL of diclofenac sodium prepared as follows. Transfer a quantity of Topical Solution to a suitable volumetric flask, add 20% of the flask volume of methanol, sonicate for about 10 min, and dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of diclofenac
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diclofenac from the *Sample solution*
r_S = peak response of diclofenac from the *Standard solution*
C_S = concentration of USP Diclofenac Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Solution B, Solution C, and **Diluent:** Prepare as directed in the *Assay*.
Mobile phase A: Use *Solution C*.
Mobile phase B: Methanol and acetonitrile (90:10)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	40	60
30	40	60
48	25	75
60	25	75
62	40	60
70	40	60

Standard stock solution: 0.3 mg/mL of USP Diclofenac Sodium RS prepared as follows. Transfer a quantity of USP Diclofenac Sodium RS to a suitable volumetric flask, add 20% of the flask volume of methanol, sonicate to dissolve, and dilute with *Diluent* to volume.
Standard solution: 0.003 mg/mL of USP Diclofenac Sodium RS from *Standard stock solution* diluted with *Diluent*

USP Monographs

www.webofpharma.com

Sensitivity solution: 0.3 µg/mL of USP Diclofenac Sodium RS in *Diluent* from *Standard solution*
Sample solution: Nominally 0.6 mg/mL of diclofenac sodium prepared as follows. Transfer a suitable quantity of the Topical Solution to a suitable volumetric flask, add 20% of the flask volume of methanol, and sonicate to disperse. Add 50% of the flask volume of *Diluent*, sonicate for about 15 min, and dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified and any unspecified degradation product in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each specified or unspecified degradation product from the *Sample solution*
r_S = peak response of diclofenac from the *Standard solution*
C_S = concentration of USP Diclofenac Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Diclofenac keto analog[a]	0.3	1.7	0.2
Diclofenac lactam (diclofenac related compound A)[b]	0.5	1.4	0.5
Diclofenac	1.0	—	—
Unidentified degradation product A	2.2	1.3	0.2
Any unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.0

[a] 2-[(2,6-Dichlorophenyl)amino]phenyl-2-oxoacetic acid.
[b] 1-(2,6-Dichlorophenyl)indolin-2-one.

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count is NMT 10^2 cfu/mL, and the total combined yeasts and molds count is NMT 10 cfu/mL. It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
• **PH** ⟨791⟩: 8.0–10.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Diclofenac Sodium RS▲ (USP 1-Aug-2019)

Diclofenac Sodium and Misoprostol Delayed-Release Tablets

DEFINITION
Diclofenac Sodium and Misoprostol Delayed-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) and NLT 90.0% and NMT 110.0% of the labeled amount of misoprostol ($C_{22}H_{38}O_5$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Misoprostol
Diluent: Methanol and water (4:1)
Standard solution: 16 µg/mL of USP Misoprostol RS in *Diluent*. [NOTE—If outer misoprostol layers of the Tablets contain hypromellose, the *Standard solution* should also contain hypromellose at the same concentration as in the *Sample solution*.]
Sample solution: Gently break up one by one a quantity of Tablets equivalent to 0.4 mg of misoprostol, and remove the inner diclofenac layers. [NOTE—Keep the diclofenac layers for *Identification A, Diclofenac sodium*.] Transfer the outer misoprostol layers to a 25-mL volumetric flask. Add about 15 mL of *Diluent*, shake for 30 min, dilute with *Diluent* to volume, and mix well. Transfer a portion of the solution into a glass centrifuge tube, and centrifuge for 10 min under refrigerated conditions (10°). Use the supernatant.
Blank: *Diluent*
Cell: 1 cm
Acceptance criteria: Meet the requirements
Diclofenac sodium
Standard solution: 0.1 mg/mL of USP Diclofenac Sodium RS in methanol
Sample solution: Transfer the diclofenac inner layers reserved from *Identification A, Misoprostol*, to a 100-mL volumetric flask. Add about 60 mL of methanol, shake for 10 min, dilute with methanol to volume, and mix well. Further dilute a suitable volume of the solution to obtain a solution containing about 0.1 mg/mL of diclofenac sodium, based on the label claim. Pass a portion of the solution through a polytetrafluoroethylene (PTFE) with glass microfiber (GMF) filter of 0.45-µm pore size.[1] Discard the first few milliliters of the filtrate, and use the filtrate.
Blank: Methanol
Cell: 0.05 cm
Acceptance criteria: Meet the requirements

[1] A suitable filter is available as GD/X Syringe Filter, Whatman, www.whatman.com, catalog no. 6874-2504.

www.webofpharma.com

• **B.**

Misoprostol: The retention time of the misoprostol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Misoprostol.*

Diclofenac sodium: The retention time of the diclofenac peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Diclofenac Sodium.*

ASSAY

• **MISOPROSTOL**

Buffer: Prepare 0.025 M monobasic potassium phosphate, pH 6.5, as follows. Adjust a solution containing 3.4 g/L of monobasic potassium phosphate in water with 1 N sodium hydroxide to a pH of 6.5.

Mobile phase: Acetonitrile and *Buffer* (45:55)

Standard solution: 0.01 mg/mL of USP Misoprostol RS in *Mobile phase*, using sonication as needed

Sample solution: Nominally 0.01 mg/mL of misoprostol prepared as follows. Using a quantity of Tablets equivalent to 5 mg of misoprostol, place 1 Tablet at a time on its edge inside a well-folded piece of weighing paper. Tap very carefully the edge of the Tablet with a pestle to separate the Tablet into the outer and inner layers. Remove the inner core containing diclofenac sodium. Transfer the outer portions of the Tablets, containing misoprostol, into a 500-mL volumetric flask containing a magnetic stir bar, and add 250 mL of acetonitrile. Stir the flask for 1 h. Add 150 mL of water, and stir for an additional 30 min or until the Tablets are completely disintegrated. Remove the stir bar, rinse it inside the flask with water, dilute with water to volume, and mix well. Transfer a portion of the solution into a glass centrifuge tube, and centrifuge for 10 min under refrigerated conditions (10°). Use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 25-cm; 5-µm packing L10

Temperatures

Autosampler: 10°

Column: 35°

Flow rate: 1.0 mL/min

Injection volume: 80 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of misoprostol ($C_{22}H_{38}O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Misoprostol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of misoprostol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

• **DICLOFENAC SODIUM**

Buffer: Mix equal volumes of 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate. If necessary, adjust with additional portions of the appropriate component to a pH of 2.5.

Mobile phase: Methanol and *Buffer* (70:30)

Diluent: Methanol and water (70:30)

System suitability solution: 20 µg/mL of diethyl phthalate, 8 µg/mL of USP Diclofenac Related Compound A RS, and 0.75 mg/mL of USP Diclofenac Sodium RS in *Diluent*

Standard solution: 0.75 mg/mL of USP Diclofenac Sodium RS in *Diluent*, using sonication as needed

Sample stock solution: Transfer a quantity of Tablets, equivalent to 1500 mg of diclofenac sodium, into a 1000-mL volumetric flask containing a magnetic stir bar. Add 700 mL of *Diluent*, and stir for 60 min or until the Tablets are completely disintegrated. Remove the stir bar, rinse it with *Diluent*, and sonicate the sample for 15 min. Allow the sample to cool to room temperature, dilute with *Diluent* to volume, and mix well.

Sample solution: Nominally 0.75 mg/mL of diclofenac sodium prepared as follows. Transfer 10.0 mL of the *Sample stock solution* into a 20-mL volumetric flask, and dilute with *Diluent* to volume. Pass a portion of the solution through a PTFE with GMF filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for diethyl phthalate, diclofenac related compound A, and diclofenac are about 0.6, 0.7, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.2 between the diethyl phthalate and diclofenac related compound A peaks; NLT 6.5 between the diclofenac related compound A and diclofenac peaks, *System suitability solution*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Diclofenac Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Misoprostol

Medium: Water; 500 mL, deaerated

Apparatus 2: 50 rpm

Time: 20 min

Buffer: Prepare as directed in the *Assay* for *Misoprostol.*

Mobile phase: Acetonitrile and *Buffer* (42:58)

Standard stock solution: Transfer 4 mg of USP Misoprostol RS into a 100-mL volumetric flask, add 20 mL of acetonitrile, and shake for about 15 min. If the outer misoprostol layers of the Tablets contain hypromellose, add a suitable amount of hypromellose to the flask to

USP Monographs

www.webofpharma.com

achieve the same final concentration of hypromellose in the *Standard solution* as expected in the *Sample solution*. Add 20 mL of water, and sonicate for about 2 min. Add water up to the neck of the flask, and allow the solution to cool to room temperature before the final dilution to volume.

Standard solution: About 0.0004 mg/mL of USP Misoprostol RS prepared as follows. Dilute 2.0 mL of the *Standard stock solution* with *Medium* to 200 mL.

Sample solution: Pass a portion of the solution under test through a suitable filter of 10-µm pore size.

Chromatographic system: Proceed as directed in the *Assay* for *Misoprostol*, except for *Injection volume*.

Injection volume: 200 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of misoprostol ($C_{22}H_{38}O_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Misoprostol RS in the *Standard solution* (mg/mL)
L = label claim for misoprostol (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of misoprostol ($C_{22}H_{38}O_5$) is dissolved.

Diclofenac sodium

Proceed as directed in *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method A Procedure*.

Acid stage medium: 0.1 N hydrochloric acid; 750 mL, deaerated

Buffer stage medium: After 2 h, add 250 mL of 0.2 M tribasic sodium phosphate to the *Acid stage medium* and, if needed, adjust with either 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8.

Apparatus 2: 100 rpm

Times: 2 h for *Acid stage*; 45 min for *Buffer stage*

Buffer: 0.025 M monobasic potassium phosphate buffer with a pH of 3.0 prepared as follows. Adjust a solution containing 3.4 g/L of monobasic potassium phosphate in water with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (60:40)

Standard stock solution: 0.68 mg/mL of USP Diclofenac Sodium RS, first dissolved in 0.1 N sodium hydroxide using about 10% of the final volume, and then diluted with water to volume

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.0 mL/min

Injection volume: 10 µL

Acid stage

Acid stage standard solution: 13.6 µg/mL of USP Diclofenac Sodium RS prepared as follows. Transfer 2.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with a mixture of 0.1 N hydrochloric acid and 5 N sodium hydroxide (900:20) to volume.

Acid stage sample solution: Run the test in *Acid stage medium* for 2 h. Withdraw a 10-mL aliquot, transfer it to a flask containing 1.0 mL of 1 N sodium hydroxide, and mix well. Pass a portion of this solution through a suitable filter of 10-µm pore size.

System suitability

Sample: *Acid stage standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Acid stage standard solution* and *Acid stage sample solution*

Calculate the percentage of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) dissolved during the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times 100$$

r_U = peak response from the *Acid stage sample solution*
r_S = peak response from the *Acid stage standard solution*
C_S = concentration of USP Diclofenac Sodium RS in the *Acid stage standard solution* (mg/mL)
L = label claim for diclofenac sodium (mg/Tablet)
V = volume of *Acid stage medium*, 750 mL
D = dilution factor for the *Acid stage sample solution*, 1.1

Tolerances: NMT 10% of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) is dissolved. The percentage of the labeled amount of diclofenac sodium dissolved at the time specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 3*.

Buffer stage

Buffer stage standard solution: 13.6 µg/mL of USP Diclofenac Sodium RS prepared as follows. Transfer 2.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with *Buffer stage medium* to volume.

Buffer stage sample solution: Pass a portion of the solution under test through a suitable filter of 10-µm pore size.

System suitability

Sample: *Buffer stage standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Acid stage sample solution, Buffer stage standard solution*, and *Buffer stage sample solution*

Calculate the percentage of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) dissolved during the *Buffer stage*:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (V - V_S) \times 100$$

r_U = peak response from the *Buffer stage sample solution*
r_S = peak response from the *Buffer stage standard solution*
C_S = concentration of USP Diclofenac Sodium RS in the *Buffer stage standard solution* (mg/mL)
L = label claim for diclofenac sodium (mg/Tablet)
V = volume of *Buffer stage medium*, 1000 mL
V_S = volume of the *Acid stage sample solution*, 10 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) is dissolved. The percentage of the labeled amount of diclofenac sodium

www.webofpharma.com

dissolved at the time specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 4*.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*: Meet the requirements for diclofenac sodium and misoprostol

IMPURITIES

• **ORGANIC IMPURITIES: MISOPROSTOL**

Buffer: Prepare as directed in the *Assay* for *Misoprostol.*

Solvent mixture: Acetonitrile and methanol (26:28)

Mobile phase: *Solvent mixture* and *Buffer* (58:42)

Diluent: Acetonitrile and water (50:50)

Standard stock solution: Use the *Standard solution* prepared as directed in the *Assay* for *Misoprostol.*

Standard solution: 0.001 mg/mL of USP Misoprostol RS prepared as follows. Transfer 5 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Sample solution: Nominally 0.1 mg/mL of misoprostol prepared as follows. Using a quantity of Tablets equivalent to 2 mg of misoprostol, place 1 Tablet at a time on its edge inside a well-folded piece of weighing paper. Tap very carefully the edge of the Tablet with a pestle to separate the Tablet into the outer and inner layers. Remove the inner core containing diclofenac sodium. Fold back the outer layers of the Tablets containing misoprostol, and gently grind them. Transfer the ground outer layers into a 20-mL volumetric flask containing a magnetic stir bar, add 10 mL of acetonitrile, and stir the flask for 2 h. Allow the sample to stand for 10 min, transfer a portion of the solution into a glass centrifuge tube, and centrifuge for 10 min under refrigerated conditions (10°). Transfer 2.5 mL of the supernatant into a 5-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 and 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Temperatures

Autosampler: 10°

Column: 35°

Flow rate: 0.6 mL/min

Injection volume: 100 µL

Run time: About 2.5 times the retention time of the misoprostol peak

System suitability

Sample: *Standard solution* at 200 nm

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 8-epimisoprostol, A-type misoprostol, and any other individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response at 200 nm of any impurity from the *Sample solution*

r_S = peak response at 200 nm of misoprostol from the *Standard solution*

C_S = concentration of USP Misoprostol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of misoprostol in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Calculate the percentage of B-type misoprostol in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response at 280 nm of B-type misoprostol from the *Sample solution*

r_S = peak response at 200 nm of misoprostol from the *Standard solution*

C_S = concentration of USP Misoprostol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of misoprostol in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
8-Epimisoprostol[a]	0.87	0.93	2.0[b]
Misoprostol	1.0	1.0	—
B-Type misoprostol[c]	1.5	4.8[d]	0.7
A-Type misoprostol[e]	1.7	1.6	3.5
Any other individual impurity	—	1.0	0.6
Total misoprostol-related impurities	—	—	6.2

[a] Methyl(1*S**,2*R**,3*R**)-3-hydroxy-2-[(*E*)-4-hydroxy-4-methyl-1-octenyl]-5-oxocyclopentaneheptanoate.

[b] 12-Epimisoprostol, which is a process impurity controlled in the drug substance, and 8-epimisoprostol are not separated by this method and should be integrated together to determine conformance.

[c] (*E*)-Methyl 7-[2-(4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopent-1-enyl] heptanoate.

[d] Impurity peak response determined at 280 nm, quantitated against the misoprostol peak response determined at 200 nm.

[e] Methyl 7-[(1*R**,2*S**)-2-[(*E*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopent-3-enyl]heptanoate.

• **ORGANIC IMPURITIES: DICLOFENAC SODIUM**

Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay* for *Diclofenac Sodium.*

Standard solution: 0.004 mg/mL of USP Diclofenac Related Compound A RS in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for diethyl phthalate, diclofenac related compound A, and diclofenac are about 0.6, 0.7, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.2 between the diethyl phthalate and diclofenac related compound A peaks; NLT 6.5 between the diclofenac related compound A and diclofenac peaks, *System suitability solution*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of diclofenac related compound A and any other individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

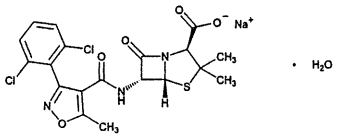
www.webofpharma.com

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Diclofenac Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diclofenac sodium in the *Sample solution* (mg/mL)

Acceptance criteria
Diclofenac related compound A: NMT 0.5%
Any other individual impurity: NMT 0.2%
Total diclofenac-related impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Diclofenac Sodium RS
  USP Diclofenac Related Compound A RS
  N-(2,6-Dichlorophenyl)indolin-2-one.
  $C_{14}H_9Cl_2NO$ 278.14
  USP Misoprostol RS

Dicloxacillin Sodium



$C_{19}H_{16}Cl_2N_3NaO_5S \cdot H_2O$ 510.32
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolyl]carbonyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, monohydrate, [2*S*-(2α,5α,6β)]-;
Monosodium (2*S*,5*R*,6*R*)-6-[3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate [13412-64-1].
Anhydrous
$C_{19}H_{16}Cl_2N_3NaO_5S$ 492.30
[343-55-5].

DEFINITION
Dicloxacillin Sodium contains the equivalent of NLT 850 µg/mg of dicloxacillin ($C_{19}H_{17}Cl_2N_3O_5S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*
  Analysis: Ignite about 100 mg. Proceed as directed in the chapter using a solution (1 in 20) of the residue in acetic acid.
  Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**
  Protect solutions containing dicloxacillin from light.

Solution A: 1.18 g/L of sodium 1-hexanesulfonate monohydrate and 0.8 mL/L of ammonium hydroxide in water; adjusted with phosphoric acid to a pH of 2.9–3.1
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	45	55
2	45	55
2.5	35	65
5	35	65

Return to the original conditions and re-equilibrate the system.
Diluent: Acetonitrile and water (50:50)
System suitability stock solution: 0.1 mg/mL of USP Dicloxacillin Related Compound D RS in *Diluent*. Sonicate as needed to dissolve.
System suitability solution: 0.001 mg/mL of USP Dicloxacillin Related Compound D RS from the *System suitability stock solution* and 0.1 mg/mL of USP Dicloxacillin Sodium RS in *Diluent*. Store this solution at 4°.
Standard solution: 0.1 mg/mL of USP Dicloxacillin Sodium RS in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.
Sample solution: 0.1 mg/mL of Dicloxacillin Sodium in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 4°
Column: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for dicloxacillin and dicloxacillin related compound D are about 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 1.5 between dicloxacillin and dicloxacillin related compound D, *System suitability solution*
Tailing factor: 0.8–1.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of dicloxacillin ($C_{19}H_{17}Cl_2N_3O_5S$) in the portion of Dicloxacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dicloxacillin Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Dicloxacillin Sodium in the *Sample solution* (mg/mL)

USP Monographs

www.webofpharma.com

P = potency of dicloxacillin in USP Dicloxacillin Sodium RS (µg/mg)

Acceptance criteria: NLT 850 µg/mg

IMPURITIES

• **ORGANIC IMPURITIES**

Protect solutions containing dicloxacillin from light.

Solution A, Solution B, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	70	30
10	70	30
30	50	50
35	35	65
45	25	75

Return to the original conditions and re-equilibrate the system.

System suitability stock solution: 0.1 mg/mL of USP Dicloxacillin Related Compound D RS in *Diluent*

System suitability solution: 0.01 mg/mL of USP Dicloxacillin Related Compound D RS from the *System suitability stock solution* and 1 mg/mL of USP Dicloxacillin Sodium RS in *Diluent*. Store this solution at 4°.

Standard solution: 0.01 mg/mL of USP Dicloxacillin Sodium RS in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

Sample solution: 1 mg/mL of Dicloxacillin Sodium in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between dicloxacillin related compound D and dicloxacillin, *System suitability solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 2.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Dicloxacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Dicloxacillin Sodium RS in the *Standard solution* (mg/mL)

C_U = concentration of Dicloxacillin Sodium in the *Sample solution* (mg/mL)

P = potency of dicloxacillin in USP Dicloxacillin Sodium RS (µg/mg)

F_1 = conversion factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 3*)

Acceptance criteria

Individual impurities: NMT 1.0%

Total impurities: NMT 5.0%

See *Table 3* for relative retention times and relative response factors. The reporting threshold is 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor
Amoxicillin related compound A[a]	0.07	0.20
Dicloxacillin penicilloic acid[b, c]	0.23 0.25	1.0
Dicloxacillin glycine analog[d]	0.38	1.0
Dicloxacillin *N*-acetyl penicilloic acid[e]	0.43	1.0
Dicloxacillin penilloic acid[b, f]	0.61 0.69	0.75
Dicloxacillin related compound D[g]	0.90	1.4
Dicloxacillin	1.0	—
Dicloxacillin penicillamide[h]	1.3	1.0

[a] 6-Aminopenicillanic acid; (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[b] The system resolves two isomers.

[c] (4*S*)-2-{Carboxy[3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[d] [3-(2,6-Dichlorophenyl)-5-methylisoxazole-4-carbonyl]glycine.

[e] (2*R*,4*S*)-3-Acetyl-2-{(*R*)-carboxy[3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[f] (4*S*)-2-{[3-(2,6-Dichlorophenyl)-5-methylisoxazole-4-carboxamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[g] 3-(2,6-Dichlorophenyl)-5-methylisoxazole-4-carboxylic acid.

[h] (2*S*,5*R*,6*R*)-6-{(2*S*,5*R*,6*R*)-6-[3-(2,6-Dichlorophenyl)-5-methylisoxazole-4-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

• **DIMETHYLANILINE** ⟨223⟩**:** Meets the requirements

SPECIFIC TESTS

• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements

• **PH** ⟨791⟩

Sample solution: 10 mg/mL in water

Acceptance criteria: 4.5–7.5

• **WATER DETERMINATION** ⟨921⟩, *Method I*: 3.0%–5.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Dicloxacillin Sodium RS

USP Dicloxacillin Related Compound D RS

3-(2,6-Dichlorophenyl)-5-methylisoxazole-4-carboxylic acid.

$C_{11}H_7Cl_2NO_3$ 272.08

Dicloxacillin Sodium Capsules

DEFINITION

Dicloxacillin Sodium Capsules contain NLT 90.0% and NMT 120.0% of the labeled amount of dicloxacillin ($C_{19}H_{17}Cl_2N_3O_5S$).

IDENTIFICATION

• **A.** The retention time of the dicloxacillin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: 2.72 g/L of monobasic potassium phosphate in water, adjusted with 8 N potassium hydroxide to a pH of 5.0 ± 0.1

Mobile phase: Acetonitrile and *Buffer* (50:150)

Standard solution: 1.1 mg/mL of USP Dicloxacillin Sodium RS in *Buffer*. Use the *Standard solution* promptly, or refrigerate and use on the day prepared.

Sample solution: Nominally 1 mg/mL of dicloxacillin in *Buffer*, prepared as follows. Remove, as completely as possible, the contents of NLT 10 Capsules. Transfer a suitable portion of the powder to a volumetric flask, dilute with *Buffer* to volume, and mix for 10 min with the aid of a magnetic stirrer. Pass a portion of the solution through a suitable filter, discarding the first 5 mL of the filtrate. Use the clear filtrate. Use the *Sample solution* promptly, or refrigerate and use on the day prepared.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 700 theoretical plates

Tailing factor: NMT 2.0

Capacity factor: 4–11

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dicloxacillin ($C_{19}H_{17}Cl_2N_3O_5S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Dicloxacillin Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dicloxacillin in the *Sample solution* (mg/mL)

P = potency of dicloxacillin in USP Dicloxacillin Sodium RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: USP Dicloxacillin Sodium RS in *Medium*

Sample solution: Sample per the chapter. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

Tolerances: NLT 75% (*Q*) of the labeled amount of dicloxacillin ($C_{19}H_{17}Cl_2N_3O_5S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 5.0%

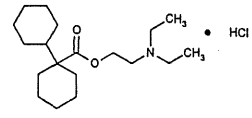
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Dicloxacillin Sodium RS

Dicyclomine Hydrochloride



$C_{19}H_{35}NO_2 \cdot HCl$ 345.95

[Bicyclohexyl]-1-carboxylic acid, 2-(diethylamino)ethyl ester, hydrochloride;

2-(Diethylamino)ethyl [bicyclohexyl]-1-carboxylate hydrochloride [67-92-5].

DEFINITION

Dicyclomine Hydrochloride contains NLT 99.0% and NMT 102.0% of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.**

Sample solution: 2 mg/mL

Analysis: Mix 5 mL of *Sample solution* with 2 mL of 2 N nitric acid, and add 2 mL of silver nitrate TS.

Acceptance criteria: A white precipitate is formed, which is insoluble in nitric acid but soluble in a slight excess of 6 N ammonium hydroxide.

ASSAY

• **PROCEDURE**

Sample: 600 mg

Analysis: Dissolve the *Sample* in 70 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS and 1 drop of crystal violet TS. Titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 34.60 mg of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$).

Acceptance criteria: 99.0%–102.0% on the dried basis

IMPURITIES

• **READILY CARBONIZABLE SUBSTANCES TEST** ⟨271⟩

Sample solution: Dissolve 500 mg in 5 mL of sulfuric acid.

Acceptance criteria: The solution has no more color than *Matching Fluid D*.

SPECIFIC TESTS

• **PH** ⟨791⟩

Sample solution: 10 mg/mL

Acceptance criteria: 5.0–5.5

• **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩: 169°–174°

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dicyclomine Hydrochloride RS

Dicyclomine Hydrochloride Capsules

DEFINITION
Dicyclomine Hydrochloride Capsules contain NLT 93.0% and NMT 107.0% of the labeled amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$).

IDENTIFICATION
• **A.**
Sample: Transfer a portion of the contents of the Capsules, equivalent to 100 mg of dicyclomine hydrochloride, to a separator containing 10 mL of water and 1 mL of hydrochloric acid. Extract the aqueous acid solution with two 30-mL portions of chloroform, transfer the chloroform extracts to a second separator containing 20 mL of water and 1 mL of sodium hydroxide solution (1 in 10), and shake. Filter the chloroform layer through anhydrous sodium sulfate into a suitable container. Add 3 mL of a freshly prepared 1 in 20 solution of acetyl chloride in anhydrous methanol, prepared by cautiously adding acetyl chloride dropwise to anhydrous methanol with stirring. Evaporate under reduced pressure at room temperature until the residue has been thoroughly dried. Use the residue so obtained to prepare a potassium bromide dispersion.
Standard: Use a similarly prepared potassium bromide dispersion of USP Dicyclomine Hydrochloride RS.
Acceptance criteria: The IR absorption spectrum of the *Sample* exhibits maxima and minima at the same wavelengths as those of the *Standard*.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: Dissolve 2.72 g of monobasic potassium phosphate in 900 mL of water, adjust with 10% sodium hydroxide to a pH of 7.5 ± 0.1, and dilute to 1000 mL.
Mobile phase: Acetonitrile and *Buffer* (70:30)
Diluent: Acetonitrile and water (70:30)
Standard solution: 0.4 mg/mL of USP Dicyclomine Hydrochloride RS in *Diluent*. [NOTE—This solution is stable for 2 days.]
Sample solution: Remove, as completely as possible, the contents of NLT 20 Capsules, and mix the contents. Transfer a portion of the powder, equivalent to 20 mg of dicyclomine hydrochloride, to a 50-mL volumetric flask, add 2.0 mL of water, and sonicate for at least 2 min to disperse the sample. Add 35 mL of acetonitrile, sonicate for at least 5 min, and shake by mechanical means for at least 30 min. Add 10 mL of water, allow the solution to equilibrate to room temperature, then dilute with water to volume. Centrifuge a portion of this solution in a 15-mL glass centrifuge tube for at least 5 min. Use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for the analyte peak
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of dicyclomine from the *Sample solution*
r_S = peak area of dicyclomine from the *Standard solution*
C_S = concentration of USP Dicyclomine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dicyclomine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 45 min
Determine the amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$) dissolved by employing the following method.
Buffer: Dissolve 2.72 g of monobasic potassium phosphate in 450 mL of water, adjust with 10% sodium hydroxide to a pH of 7.5 ± 0.1, and dilute to 500 mL.
Mobile phase: Prepare as directed in the *Assay*.
Diluent: Acetonitrile and *Buffer* (1:1)
Standard stock solution: 20 µg/mL of USP Dicyclomine Hydrochloride RS in *Medium*
Standard solution: Mix 25.0 mL of *Standard stock solution* and 25.0 mL of *Diluent*.
Sample solution: Pass a portion of the solution under test through a glass microfiber filter of 0.7-µm pore size. Transfer 5.0 mL of the filtrate to a suitable flask, and add 5.0 mL of *Diluent*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 250 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the analyte peak
Relative standard deviation: 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dicyclomine Hydrochloride RS

www.webofpharma.com

Dicyclomine Hydrochloride Injection

DEFINITION

Dicyclomine Hydrochloride Injection is a sterile, isotonic solution of Dicyclomine Hydrochloride in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$).

IDENTIFICATION

• **A.**

Sample: Transfer a portion of Injection, equivalent to 100 mg of dicyclomine hydrochloride, to a separator containing 10 mL of water and 1 mL of hydrochloric acid. Shake with 25 mL of ether, and discard the ether layer. Extract the aqueous acid solution with two 30-mL portions of chloroform, transfer the chloroform extracts to a second separator containing 20 mL of water and 1 mL of sodium hydroxide solution (1 in 10), and shake. Filter the chloroform layer through anhydrous sodium sulfate into a suitable container. Add 3 mL of a freshly prepared 1 in 20 solution of acetyl chloride in anhydrous methanol, prepared by cautiously adding acetyl chloride dropwise to anhydrous methanol with stirring. Evaporate under reduced pressure at room temperature until the residue has been thoroughly dried. Use the residue so obtained to prepare a potassium bromide dispersion.

Standard: Use a similarly prepared potassium bromide dispersion of USP Dicyclomine Hydrochloride RS.

Acceptance criteria: The IR absorption spectrum of the *Sample* exhibits maxima and minima at the same wavelengths as those of the *Standard*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 2.72 g of monobasic potassium phosphate in 900 mL of water, adjust with 10% sodium hydroxide to a pH of 7.5 ± 0.1, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (70:30)

Diluent: Acetonitrile and water (70:30)

Standard solution: 0.4 mg/mL of USP Dicyclomine Hydrochloride RS in *Diluent*. [NOTE—This solution is stable for 2 days.]

Sample solution: Prepare a composite sample of at least 5 ampules or 2 vials. Transfer a volume of the composite sample, equivalent to 20.0 mg of dicyclomine hydrochloride, to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Flow rate: 1.0 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for the analyte peak

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of dicyclomine from the *Sample solution*

r_S = peak area of dicyclomine from the *Standard solution*

C_S = concentration of USP Dicyclomine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dicyclomine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 17.2 USP Endotoxin Units/mg of dicyclomine hydrochloride.

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Dicyclomine Hydrochloride RS

Dicyclomine Hydrochloride Oral Solution

DEFINITION

Dicyclomine Hydrochloride Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$).

IDENTIFICATION

• **A.**

Sample: Transfer a portion of the Oral Solution, equivalent to 100 mg of dicyclomine hydrochloride, to a separator containing 10 mL of water and 1 mL of hydrochloric acid. Extract with two 30-mL portions of ether, and discard the ether. Extract the aqueous acid solution with two 30-mL portions of chloroform, transfer the chloroform extracts to a second separator containing 20 mL of water and 1 mL of sodium hydroxide solution (1 in 10), and shake. Filter the chloroform layer through anhydrous sodium sulfate into a suitable container. Add 3 mL of a freshly prepared 1 in 20 solution of acetyl chloride in anhydrous methanol, prepared by cautiously adding acetyl chloride dropwise to anhydrous methanol with stirring. Evaporate under reduced pressure at room temperature until the residue has been thoroughly dried. Use the residue so obtained to prepare a potassium bromide dispersion.

Standard: Use a similarly prepared potassium bromide dispersion of USP Dicyclomine Hydrochloride RS.

Acceptance criteria: The IR absorption spectrum of the *Sample* exhibits maxima and minima at the same wavelengths as those of the *Standard*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 2.72 g of monobasic potassium phosphate in 900 mL of water, adjust with 10% sodium hydroxide to a pH of 7.5 ± 0.1, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (70:30)

Diluent: Acetonitrile and *Buffer* (35:65)

Standard solution: 0.1 mg/mL of USP Dicyclomine Hydrochloride RS in *Diluent*. [NOTE—This solution is stable for 2 days.]

www.webofpharma.com

Sample solution: Using a "to contain" pipet, transfer a measured volume of Oral Solution, equivalent to 10.0 mg of dicyclomine hydrochloride, to a 100-mL volumetric flask. Rinse the pipet with several small portions of *Diluent*, and add the rinsings to the volumetric flask. Dilute with *Diluent* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 50 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for the analyte peak
Relative standard deviation: NMT 1.5%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of dicyclomine from the *Sample solution*
r_S = peak area of dicyclomine from the *Standard solution*
C_S = concentration of USP Dicyclomine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dicyclomine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dicyclomine Hydrochloride RS

Dicyclomine Hydrochloride Tablets

DEFINITION

Dicyclomine Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$).

IDENTIFICATION

- **A.**

Sample: Transfer a portion of finely powdered Tablets, equivalent to 100 mg of dicyclomine hydrochloride, to a separator containing 10 mL of water and 1 mL of hydrochloric acid. Extract the aqueous acid solution with two 30-mL portions of chloroform, transfer the chloroform extracts to a second separator containing 20 mL of water and 1 mL of sodium hydroxide solution (1 in 10), and shake. Filter the chloroform layer through anhydrous sodium sulfate into a suitable container. Add 3 mL of a freshly prepared 1 in 20 solution of acetyl chloride in anhydrous methanol, prepared by cautiously adding acetyl chloride dropwise to anhydrous methanol with stirring. Evaporate under reduced pressure at room temperature until the residue has been thoroughly dried. Use the residue so obtained to prepare a potassium bromide dispersion.
Standard: Use a similarly prepared potassium bromide dispersion of USP Dicyclomine Hydrochloride RS.
Acceptance criteria: The IR absorption spectrum of the *Sample* exhibits maxima and minima at the same wavelengths as those of the *Standard*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Dissolve 2.72 g of monobasic potassium phosphate in 900 mL of water, adjust with 10% sodium hydroxide to a pH of 7.5 ± 0.1, and dilute with water to 1000 mL.
Mobile phase: Acetonitrile and *Buffer* (70:30)
Diluent: Acetonitrile and water (70:30)
Standard solution: 0.4 mg/mL of USP Dicyclomine Hydrochloride RS in *Diluent*. [NOTE—This solution is stable for 2 days.]
Sample solution: Transfer NLT 20 Tablets to a tared container, and determine the average Tablet weight. Grind the Tablets to a fine powder using a glass mortar and pestle. Transfer a portion of the powder, equivalent to 20 mg of dicyclomine hydrochloride, to a 50-mL volumetric flask, add 2.0 mL of water, and sonicate for at least 2 min to disperse the sample. Add 35 mL of acetonitrile, sonicate for at least 5 min, and shake by mechanical means for at least 30 min. Add 10 mL of water, allow the solution to equilibrate to room temperature, then dilute with water to volume. Centrifuge a portion of this solution in a 15-mL glass centrifuge tube for at least 5 min. Use the clear supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 50 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for the analyte peak
Relative standard deviation: NMT 1.5%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of dicyclomine from the *Sample solution*
r_S = peak area of dicyclomine from the *Standard solution*
C_S = concentration of USP Dicyclomine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dicyclomine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.01 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 45 min
Determine the amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$) dissolved by employing the following method.

www.webofpharma.com

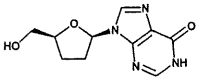
Buffer: Dissolve 2.72 g of monobasic potassium phosphate in 450 mL of water, adjust with 10% sodium hydroxide to a pH of 7.5 ± 0.1, and dilute with water to 500 mL.
Mobile phase: Prepare as directed in the *Assay*.
Diluent: Acetonitrile and *Buffer* (1:1)
Standard stock solution: 40 µg/mL of USP Dicyclomine Hydrochloride RS in *Medium*
Standard solution: Mix 25.0 mL of *Standard stock solution* and 25.0 mL of *Diluent*.
Sample solution: Pass a portion of the solution under test through a glass microfiber filter of 0.7-µm pore size. Transfer 5.0 mL of the filtrate to a suitable flask, and add 5.0 mL of *Diluent*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 250 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the analyte peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of dicyclomine hydrochloride ($C_{19}H_{35}NO_2 \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Dicyclomine Hydrochloride RS

Didanosine



$C_{10}H_{12}N_4O_3$ 236.23
Inosine, 2′,3′-dideoxy-;
2′,3′-Dideoxyinosine [69655-05-6].

DEFINITION

Didanosine contains NLT 98.0% and NMT 102.0% of $C_{10}H_{12}N_4O_3$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.77 g/L of ammonium acetate in water
Mobile phase: Acetonitrile and *Buffer* (1:21)
Standard solution: 0.1 mg/mL of USP Didanosine RS in water
Sample solution: 0.1 mg/mL of Didanosine in water
[NOTE—Mix for 1 h to dissolve completely before use.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time of didanosine is between 7 and 11 min.]
Suitability requirements
Column efficiency: NLT 6000 theoretical plates
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of didanosine ($C_{10}H_{12}N_4O_3$) in the portion of Didanosine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

Change to read:

- **RELATED COMPOUNDS**

Buffer: Prepare as directed in the *Assay*.
Diluent: Adjust the pH of the *Buffer* with sodium hydroxide to 9. Prepare a mixture of acetonitrile and *Buffer* (1:19).
Solution A: Acetonitrile and *Buffer* (1:19)
Solution B: Acetonitrile and *Buffer* (1:3)
System suitability solution: 0.5 mg/mL of ▲▲ (ERR 1-Jun-2019) USP Didanosine System Suitability Mixture RS in *Diluent*
Standard stock solution A: 0.05 mg/mL of USP Didanosine Related Compound A RS in *Diluent*
Standard stock solution B: 0.025 mg/mL of USP Didanosine RS in *Diluent*
Standard stock solution C: 0.025 mg/mL of USP Didanosine Related Compound B RS in *Diluent*
Standard solution: 5 µg/mL of USP Didanosine Related Compound A RS, 1.5 µg/mL of USP Didanosine RS, and 1.5 µg/mL of USP Didanosine Related Compound B RS from a mixture of *Standard stock solution A*, *Standard stock solution B*, and *Standard stock solution C*, respectively, diluted with *Diluent*
Sample solution: 0.5 mg/mL of Didanosine in *Diluent*
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
20	0	100

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
30	0	100
35	100	0
45	100	0

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection size: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—Didanosine elutes between 6 and 7.5 min; the relative retention times for didanosine, didanosine related compound A, and didanosine related compound B are 1.0, 0.28, and 2.11, respectively.]

Suitability requirements
Resolution: NLT 3.0 between didanosine and dideoxydidehydroinosine, *System suitability solution*
Column efficiency: NLT 6000 theoretical plates for dideoxydidehydroinosine, *System suitability solution*
Relative standard deviation: NMT 2.0% for didanosine related compound A, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of didanosine related compound A in the portion of Didanosine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of didanosine related compound A from the *Sample solution*
r_S = peak response of didanosine related compound A from the *Standard solution*
C_S = concentration of USP Didanosine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of didanosine in the *Sample solution* (mg/mL)

Calculate the percentage of all other impurities in the portion of Didanosine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity in the *Sample solution*
r_S = peak response for didanosine from the *Standard solution*
C_S = concentration of USP Didanosine RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Didanosine related compound A	0.28	0.5
Inosine	0.39	0.2
2′-Deoxyinosine	0.45	0.3
3′-Deoxyinosine	0.51	0.2
2′,3′-Anhydroinosine	0.59	0.2
Dideoxydidehydroinosine	0.81	0.2
Didanosine	1.0	—
Didanosine related compound B	2.1	0.2
5′-Deoxydideoxyadenosine	3.1	0.2
Any other individual, unidentified impurity	—	0.1
Total impurities	—	1.0

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in water
Acceptance criteria: −28° to −24°, anhydrous
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 2.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Didanosine RS
USP Didanosine Related Compound A RS
Hypoxanthine.
USP Didanosine Related Compound B RS
2′,3′-Dideoxyadenosine.
USP Didanosine System Suitability Mixture RS

Didanosine Delayed-Release Capsules

DEFINITION
Didanosine Delayed-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of didanosine ($C_{10}H_{12}N_4O_3$).

IDENTIFICATION
• **A.** The retention time of major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
Sample: Empty the contents of a suitable number of Capsules, and grind to a fine powder.

ASSAY
• **PROCEDURE**
Buffer: 0.77 g/L of ammonium acetate in water and pass through a suitable membrane filter of 0.45-µm pore size
Mobile phase: Acetonitrile and *Buffer* (35:965)
Diluent: 34.8 g/L of dibasic potassium phosphate and adjust with phosphoric acid to a pH of 7.5. Use within two weeks of preparation.
Standard solution: 0.1 mg/mL of USP Didanosine RS in water
Sample stock solution: Nominally prepare a solution at 0.6 mg/mL to 2.0 mg/mL of didanosine as follows. Mix a

www.webofpharma.com

composite of the Capsule contents from NLT 20 Capsules in a suitable container, weigh 1 Capsule fill weight, transfer to a suitable size volumetric flask, and add 50% of the final volume of *Diluent*. Stir, shake or sonicate to dissolve. Cool to room temperature, and dilute with *Diluent* to final volume.

Sample solution: Nominally 0.1 mg/mL of didanosine in *Diluent* from the *Sample stock solution* and use within 24 h

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of didanosine ($C_{10}H_{12}N_4O_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of didanosine from the *Sample solution*
r_S = peak response of didanosine from the *Standard solution*
C_S = concentration of USP Didanosine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of didanosine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩**:** Proceed as directed for *Method B* under *Procedure*, *Apparatus 1*, *Apparatus 2*, and *Delayed-Release Dosage Forms*.
Acid stage medium: 0.1 N hydrochloric acid; 1000 mL
Buffer 1: 76 g/L of tribasic sodium phosphate in water
Buffer stage medium: 0.1 N hydrochloric acid and 0.2 M tribasic sodium phosphate (3:1). Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 6.8; 1000 mL
Buffer 2: 1.36 g/L of monobasic potassium phosphate in water
Apparatus 1: 100 rpm
Times
Acid stage: 2 h
Buffer stage: 45 min
Mobile phase: Acetonitrile and *Buffer 2* (2:98)
Sample solution: Run the *Acid stage*. After the time specified, withdraw a portion of the solution under test, and pass it through a suitable filter. To the filtrate, add a volume of 10 N sodium hydroxide equivalent to 1% of the filtrate volume. Raise the basket. Discard the *Acid stage medium* from the vessels. Rinse the vessel with water. Add the *Buffer stage medium* pre-warmed to the vessel. After the time specified, withdraw a portion of the solution under test and pass it through a suitable filter. Store the acid stage and buffer stage filtrates at 5°.
Standard stock solution: 0.8 mg/mL of USP Didanosine RS in water
Didanosine related compound A standard stock solution: 0.1 mg/mL of USP Didanosine Related Compound A RS in water
Standard solution: Accurately transfer portions of the *Standard stock solution* and the *Didanosine related compound A standard stock solution* to a volumetric flask, and dilute with water to volume to obtain a didanosine final concentration of (L/1000) mg/mL where L is the Capsule label claim in mg and 0.01 mg/mL of didanosine related compound A.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 3.9-mm × 5-cm; 5-µm packing L7
Flow rate: 1 mL/min
Sample temperature: 5°
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both the didanosine and didanosine related compound A peaks
Relative standard deviation: NMT 2.0% for the didanosine peak and NMT 5.0% for the didanosine related compound A peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of didanosine as calculated based on response of didanosine related compound A released in the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times (M_{r1}/M_{r2}) \times V \times 100$$

Calculate the percentage of didanosine released in the *Buffer stage*:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

r_U = peak response from the *Sample solution*. [Note—During the *Acid stage*, didanosine is converted to didanosine related compound A so the peak response is the hypoxanthine peak.]
r_S = peak response from the *Standard solution*. [Note—During the *Acid stage*, didanosine is converted to didanosine related compound A so the peak response is the hypoxanthine peak.]
C_S = concentration of USP Didanosine Related Compound A RS in the *Standard solution* for the *Acid stage* or concentration of USP Didanosine RS in the *Standard solution* for the *Buffer stage* (mg/mL)
L = label claim (mg/Capsule)
D = dilution factor of the *Sample solution*, if applicable
M_{r1} = molecular weight of didanosine, 236.2
M_{r2} = molecular weight of didanosine related compound A, 136.11
V = volume of medium, 1000 mL

Tolerances
Acid stage: NMT 10% (Q) of the labeled amount of didanosine ($C_{10}H_{12}N_4O_3$) is dissolved in 2 h.
Buffer stage: NLT 80% (Q) of the labeled amount of didanosine ($C_{10}H_{12}N_4O_3$) is dissolved in 45 min.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Buffer, Mobile phase, Diluent, Sample stock solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

www.webofpharma.com

System suitability solution: 0.1 mg/mL of USP Didanosine RS and 5 µg/mL of USP Didanosine Related Compound A RS in water
Standard solution: 5 µg/mL of USP Didanosine Related Compound A RS in water
System suitability
Sample: *System suitability solution*
Suitability requirements
Tailing factor: NMT 1.5 for the didanosine peak
Relative standard deviation: NMT 2.0% for the didanosine peak and NMT 5.0% for the didanosine related compound A peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of didanosine related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of didanosine related compound A from the *Sample solution*
r_S = peak response of didanosine related compound A from the *Standard solution*
C_S = concentration of USP Didanosine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of didanosine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurities in the portion of Capsules taken:

$$\text{Result} = (r_X/r_{SUM}) \times 100$$

r_X = peak response of each impurity from the *Sample solution*
r_{SUM} = peak response of all peaks from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Didanosine related compound A[a]	0.21	2
Didanosine	1.0	—
Any unspecified impurities	—	0.2
Total unspecified impurities	—	0.5
Total impurities	—	2.5

[a] Hypoxanthine.

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is not more than 10^2 cfu/g. The total yeasts and molds count is not more than 10^2 cfu/g.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Didanosine RS
USP Didanosine Related Compound A RS
6-Hydroxypurine (Hypoxanthine).
$C_5H_4N_4O$ 136.11

Didanosine for Oral Solution

DEFINITION
Didanosine for Oral Solution, when reconstituted as directed in the labeling, yields a 10 mg/mL solution that contains NLT 90.0% and NMT 110.0% of the labeled amount of didanosine ($C_{10}H_{12}N_4O_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**
Solution A: 0.77 mg/mL of ammonium acetate in water
Mobile phase: Acetonitrile and *Solution A* (1:24)
Standard solution: 0.1 mg/mL of USP Didanosine RS in water. [NOTE—Use this solution within 24 h of preparation.]
Sample solution: 0.1 mg/mL obtained by diluting the contents of 1 bottle of Didanosine for Oral Solution in water. [NOTE—Use this solution within 24 h of preparation.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Analytical column: 4-mm × 25-cm; packing L1
Guard column: 4.6-mm × ▲20-mm;▲ (ERR 1-May-2018) packing L1
Flow rate: 2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Retention time: Between 7 and 11 min
Column efficiency: NLT 6000 theoretical plates
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{10}H_{12}N_4O_3$ in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Didanosine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of didanosine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

IMPURITIES
ORGANIC IMPURITIES
- **Procedure**
Solution A, Mobile phase, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

USP Monographs

www.webofpharma.com

Standard solution: 5 µg/mL of USP Didanosine Related Compound A RS in water
[NOTE—Use this solution within 48 h of preparation.]
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of didanosine related compound A in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of didanosine related compound A from the *Sample solution*
r_S = peak response of didanosine related compound A from the *Standard solution*
C_S = concentration of USP Didanosine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of didanosine in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** NMT 3%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 15° and 30°.
• **LABELING:** The label contains directions for constitution of the powder and states the equivalent amount of $C_{10}H_{12}N_4O_3$ in a given volume of Oral Solution obtained after constitution.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Didanosine RS
USP Didanosine Related Compound A RS
Hypoxanthine

Didanosine Tablets for Oral Suspension

DEFINITION
Didanosine Tablets for Oral Suspension contain NLT 90.0% and NMT 110.0% of the labeled amount of didanosine ($C_{10}H_{12}N_4O_3$).

IDENTIFICATION
• **A.** The retention time of the major peak in the *Sample solution* corresponds to that of the major peak in the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 0.77 g/L of ammonium acetate in water
Mobile phase: Methanol and *Buffer* (5:95)
Standard solution: 0.1 mg/mL of USP Didanosine RS in water. [NOTE—Use this solution within 24 h of preparation.]
Sample stock solution: Transfer NLT 5 crushed Tablets for Oral Suspension to a 500-mL volumetric flask. Dissolve in 250 mL of water, dilute with water to volume, and shake for about 10 min.
Sample solution: Nominally 0.1 mg/mL of didanosine from the *Sample stock solution* diluted with water. [NOTE—Use this solution within 72 h of preparation. The *Sample solution* is stable for a longer time than the *Standard solution* because of the buffering agents in the Tablets for Oral Suspension.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Columns
Guard: Matching analytical column
Analytical: 4-mm × 12.5-cm; 5-µm packing L7
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time of didanosine is more than 3.0 min.]
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of didanosine ($C_{10}H_{12}N_4O_3$) in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Didanosine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of didanosine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
Standard stock solution: 0.8 mg/mL of USP Didanosine RS in *Medium*. [NOTE—This solution is stable for 48 h at 5°.]
Standard solution: Dilute the *Standard stock solution* in *Medium* to obtain a concentration of approximately (L/900), where L is the Tablet label claim.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system: Proceed as directed in the *Assay*, except disregard the *Injection volume* requirement, and use equal volumes of the *Standard solution* and *Sample solution*, equivalent to 2 µg of didanosine, as the injection volume, and no guard column is used.
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%.
[NOTE—Didanosine elutes at about 4.8 min.]
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms for at least 7 min.
Calculate the percentage of didanosine ($C_{10}H_{12}N_4O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Didanosine RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

USP Monographs

www.webofpharma.com

Tolerances: NLT 80% (*Q*) of the labeled amount of didanosine ($C_{10}H_{12}N_4O_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer: Proceed as directed in the *Assay*.

Mobile phase: Methanol and *Buffer* (1:99)

Standard stock solution: 0.125 mg/mL of USP Didanosine Related Compound A RS in water. [NOTE—Use this solution within 48 h of preparation.]

Standard solution: 1.5 µg/mL of USP Didanosine Related Compound A RS in water from *Standard stock solution*. [NOTE—Use this solution within 48 h of preparation of the *Standard stock solution*.]

Sample stock solution: Proceed as directed in the *Assay*.

Sample solution: Nominally 0.1 mg/mL from the *Sample stock solution* diluted with *Mobile phase*

Chromatographic system: Proceed as directed in the *Assay*, except use an *Injection volume* of 100 µL.

Run time: 30 min

System suitability

Sample: *Standard solution*

[NOTE—The retention time of didanosine related compound A is 1.5–2.5 min.]

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of didanosine related compound A in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/(C_U) \times 100$$

r_U = peak response from didanosine related compound A in the *Sample solution*

r_S = peak response from didanosine related compound A in the *Standard solution*

C_S = concentration of USP Didanosine Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of didanosine in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity as follows:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response from any other individual impurity in the *Sample solution*

r_T = sum of the responses from all the peaks in the *Sample solution*, including those of didanosine and didanosine related compound A

Acceptance criteria

Individual impurities: NMT 0.7% of didanosine related compound A; NMT 0.2% of any other individual impurity

Total impurities: NMT 1.2%, excluding didanosine related compound A

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Sample: 4 Tablets for Oral Suspension

Analysis: Dry the *Sample* at 130° for 16 h.

Acceptance criteria: The Tablets for Oral Suspension lose NMT 6% of their weight.

• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩**:** NLT 17 mEq/Tablet

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 15° and 30°.

• **LABELING:** The label states that the Tablets are not to be swallowed whole, and that they may be chewed or dispersed in water before administration.

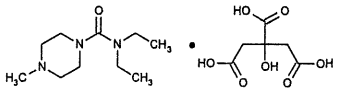
• **USP REFERENCE STANDARDS** ⟨11⟩

USP Didanosine RS

USP Didanosine Related Compound A RS

Hypoxanthine

Diethylcarbamazine Citrate



$C_{10}H_{21}N_3O \cdot C_6H_8O_7$ 391.42

1-Piperazinecarboxamide, *N*,*N*-diethyl-4-methyl-, 2-hydroxy-1,2,3-propanetricarboxylate.

N,*N*-Diethyl-4-methyl-1-piperazinecarboxamide citrate (1:1). [1642-54-2].

» Diethylcarbamazine Citrate contains not less than 98.0 percent and not more than 102.0 percent of $C_{10}H_{21}N_3O \cdot C_6H_8O_7$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Diethylcarbamazine Citrate RS

Identification—

A: It meets the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

B: It meets the requirements of the test for *Citrate* ⟨191⟩.

Water Determination, *Method I* ⟨921⟩**:** not more than 0.5%.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Ordinary impurities ⟨466⟩—

Test solution: methanol.

Standard solution: methanol.

Eluant: a mixture of methanol and ammonium hydroxide (100: 1.5).

Visualization: 16.

Chromatographic purity—

Phosphate buffer, *Mobile phase*, and *Chromatographic system* —Proceed as directed in the *Assay*.

Standard solution—Prepare a solution of USP Diethylcarbamazine Citrate RS in *Phosphate buffer* having a known concentration of about 0.003 mg per mL.

Test solution—Transfer about 300 mg of Diethylcarbamazine Citrate, accurately weighed, to a 100-mL volumetric flask, add 100 mL of *Phosphate buffer*, and mix. Filter or centrifuge, and use the clear filtrate or supernatant.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the percentage of each impurity in the portion of Diethylcarbamazine Citrate taken by the formula:

$$10{,}000(C/W)(r_i/r_S)$$

www.webofpharma.com

in which C is the concentration, in mg per mL, of USP Diethylcarbamazine Citrate RS in the *Standard solution; W* is the weight, in mg, of Diethylcarbamazine Citrate taken to prepare the *Test solution; r_i* is the peak response for each impurity obtained from the *Test solution;* and r_s is the peak response for diethylcarbamazine citrate obtained from the *Standard solution.* Not more than 0.1% of any individual impurity is found.

Assay—

Phosphate buffer—Dissolve 31.24 g of monobasic potassium phosphate in 1000 mL of water.

Mobile phase—Dissolve 10 g of monobasic potassium phosphate in 1000 mL of water. Prepare a filtered and degassed mixture of 900 mL of this solution and 100 mL of methanol.

Standard preparation—Transfer about 5 mg of USP Diethylcarbamazine Citrate RS, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Phosphate buffer* to volume, and mix.

Assay preparation—Transfer about 5 mg of Diethylcarbamazine Citrate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Phosphate buffer* to volume, and mix.

Chromatographic system—The liquid chromatograph is equipped with a 220-nm detector and a 3.9-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{10}H_{21}N_3O \cdot C_6H_8O_7$ in the portion of Diethylcarbamazine Citrate taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Diethylcarbamazine Citrate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Diethylcarbamazine Citrate Tablets

DEFINITION

Diethylcarbamazine Citrate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of diethylcarbamazine citrate ($C_{10}H_{21}N_3O \cdot C_6H_8O_7$).

[NOTE—Diethylcarbamazine Citrate Tablets labeled solely for veterinary use are exempt from the requirements of the test for *Dissolution.*]

IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩**:** Meet the requirements

ASSAY

- **PROCEDURE**

Solution A: 10 g/L of monobasic potassium phosphate in water

Solution B: 31.24 mg/mL of monobasic potassium phosphate in water

Mobile phase: Methanol and *Solution A* (100:900)

Standard solution: 0.1 mg/mL of USP Diethylcarbamazine Citrate RS in *Solution B*

Sample solution: Nominally 0.1 mg/mL of diethylcarbamazine citrate prepared as follows. Transfer an amount of finely powdered Tablets (from NLT 20 Tablets) equivalent to 5 mg of diethylcarbamazine citrate to a 50-mL volumetric flask, and dissolve in and dilute with *Solution B* to volume.

Chromatographic system

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diethylcarbamazine citrate ($C_{10}H_{21}N_3O \cdot C_6H_8O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Diethylcarbamazine Citrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diethylcarbamazine citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩ (for Tablets labeled solely for veterinary use)**:** 30 min
- **DISSOLUTION** ⟨711⟩

Procedure for a pooled sample

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Buffer: 62.48 g/L of monobasic potassium phosphate in water

Sample solutions: Prepare by quantitatively diluting filtered portions of the solution under test with *Buffer* (1:1)

Analysis: Determine the amount of diethylcarbamazine citrate ($C_{10}H_{21}N_3O \cdot C_6H_8O_7$) dissolved as directed in the *Assay.*

Tolerances: NLT 75% (Q) of the labeled amount of diethylcarbamazine citrate ($C_{10}H_{21}N_3O \cdot C_6H_8O_7$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay.*

Citric acid solution: 2 mg/mL of citric acid in *Solution B*

Standard solution: 0.003 mg/mL of USP Diethylcarbamazine Citrate RS in *Solution B*

Sample solution: Transfer an amount of finely powdered Tablets (from NLT 20 Tablets) nominally equivalent to 300 mg of diethylcarbamazine citrate to a 100-mL volumetric flask. Dilute with *Solution B* to volume. Filter or centrifuge, and use the clear filtrate or supernatant.

USP Monographs

www.webofpharma.com

Analysis
Samples: *Citric acid solution*, *Standard solution*, and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Tablets taken:

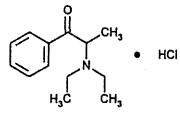
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*. [NOTE—Disregard any peak having a retention time corresponding to that of the main peak of the *Citric acid solution*.]
r_S = peak response of diethylcarbamazine citrate from the *Standard solution*
C_S = concentration of USP Diethylcarbamazine Citrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diethylcarbamazine citrate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Diethylcarbamazine Citrate RS

Diethylpropion Hydrochloride



$C_{13}H_{19}NO \cdot HCl$ 241.76
1-Propanone, 2-(diethylamino)-1-phenyl-, hydrochloride.
2-(Diethylamino)propiophenone hydrochloride [134-80-5].

» Diethylpropion Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of $C_{13}H_{19}NO \cdot HCl$, calculated on the anhydrous basis. It may contain tartaric acid as a stabilizer.

Packaging and storage—Preserve in well-closed, light-resistant containers.
Labeling—The label indicates whether it contains tartaric acid as a stabilizer.
USP Reference standards ⟨11⟩—
USP Diethylpropion Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.
C: A solution (1 in 100) responds to the tests for *Chloride* ⟨191⟩.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%.
Secondary amines—Dissolve 100 mg in 2 mL of methylene chloride in a centrifuge tube. Transfer to a second tube 2 mL of a Standard solution of diethylamine hydrochloride (dried at 105° for 2 hours before being used) in methylene chloride having a known concentration of 250 µg per mL. Treat each solution as follows. Extract with 2 mL of a buffer solution containing 5.7 g of sodium carbonate and 3.0 g of sodium bicarbonate per 100 mL of water. Centrifuge, if necessary, to clarify the upper phase, and immediately transfer 0.5 mL of it to a spot plate. Immediately add 2 drops of acetaldehyde TS, and then, in rapid succession, add 1 drop of sodium nitroferricyanide solution (1 in 100) to each spot. Immediately and simultaneously stir both spots to mix the reagents: any blue color produced within 3 minutes by the test solution is not more intense than that of the Standard solution (not more than 0.5% of secondary amines as diethylamine hydrochloride).
Free bromine—One drop of a solution (1 in 10) produces no discoloration when placed upon starch iodide paper.
Limit of hydrobromic acid and bromide—To 10 mL of a solution (1 in 10) add 1 mL of sodium hydroxide solution (1 in 10), extract with about 25 mL of chloroform, and discard the chloroform extract. Add 1 mL of 6 N hydrochloric acid, 0.5 mL of chloroform, and 0.5 mL of freshly prepared chloramine T solution (1 in 10), and shake vigorously: no yellow or brown-red color is produced in the chloroform layer.

Chromatographic purity—
Phosphate buffer—Dissolve 136.1 g of monobasic potassium phosphate in 900 mL of water, add 3.2 mL of phosphoric acid, dilute with water to 1000 mL, and mix.
Diluent—Prepare a mixture of water, *Phosphate buffer*, and acetonitrile (8:1:1).
Mobile phase—Mix 100 mL of acetonitrile, 100 mL of *Phosphate buffer*, 7.0 mL of diethylamine, and sufficient water to make 1 L. Filter, and degas before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Test preparation—Transfer 100 mg of Diethylpropion Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in about 40 mL of *Diluent*, add *Diluent* to volume, and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Diethylpropion Hydrochloride RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.01 mg per mL.
System suitability solution—Prepare a solution in *Diluent* containing about 25 µg of 2-ethylaminopropiophenone hydrochloride and 50 µg of USP Diethylpropion Hydrochloride RS per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.5 for 2-ethylaminopropiophenone and 1.0 for diethylpropion, and the resolution, *R*, between the 2-ethylaminopropiophenone and diethylpropion peaks is not less than 6.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the peak responses. The sum of all of the peak responses, excluding the solvent peak responses and the diethylpropion response, from the *Test preparation* is not greater than the diethylpropion response from the *Standard preparation* (0.5%).

www.webofpharma.com

Assay—

Phosphate buffer—Dissolve 136.1 g of monobasic potassium phosphate in 900 mL of water, add 4.3 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a suitable mixture of water, acetonitrile, *Phosphate buffer*, and 1.0 M sodium nitrate (730:200: 50:20), filter through a membrane filter (0.7-µm or finer porosity), and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Diethylpropion Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 40 µg per mL.

Assay preparation—Transfer about 100 mg of Diethylpropion Hydrochloride, accurately weighed, to a 250-mL volumetric flask, dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

System suitability preparation—Prepare a solution in *Mobile phase* containing about 200 µg of benzoic acid and 40 µg of USP Diethylpropion Hydrochloride RS per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.5 for diethylpropion hydrochloride and 1.0 for benzoic acid, and the resolution, *R*, between the diethylpropion hydrochloride and benzoic acid peaks is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{13}H_{19}NO \cdot HCl$ in the portion of Diethylpropion Hydrochloride taken by the formula:

$$2.5C(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Diethylpropion Hydrochloride RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Diethylpropion Hydrochloride Tablets

» Diethylpropion Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{13}H_{19}NO \cdot HCl$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Diethylpropion Hydrochloride RS

Identification—

A: The Tablets meet the requirements under *Identification —Organic Nitrogenous Bases* ⟨181⟩.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.

Procedure—Determine the amount of $C_{13}H_{19}NO \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 253 nm of filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid, in comparison with a Standard solution having a known concentration of USP Diethylpropion Hydrochloride RS in the same medium.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{13}H_{19}NO \cdot HCl$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Phosphate buffer, Mobile phase, and *Chromatographic system* —Prepare as directed in the *Assay*— under *Diethylpropion Hydrochloride.*

Standard preparation—Dissolve an accurately weighed quantity of USP Diethylpropion Hydrochloride RS in 0.1 N hydrochloric acid, and dilute quantitatively, and stepwise if necessary, with 0.1 N hydrochloric acid to obtain a stock solution having a known concentration of about 160 µg per mL. Transfer 5.0 mL of this stock solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 8 µg per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of diethylpropion hydrochloride, to a 250-mL volumetric flask. Add 200 mL of 0.1 N hydrochloric acid, and stir with the aid of a stir bar for 45 minutes. Remove the stir bar, dilute with 0.1 N hydrochloric acid to volume, mix, and filter, discarding the first 25 mL of the filtrate. Transfer 5.0 mL of the filtrate to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. If necessary, filter the solution through a 0.7-µm porosity membrane filter.

System suitability preparation—Dissolve benzoic acid in 0.1 N hydrochloric acid to obtain a solution having a concentration of about 1 mg per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of the stock solution prepared as directed for the *Standard preparation*, dilute with *Mobile phase* to volume, and mix.

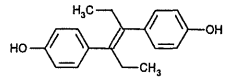
Procedure—Proceed as directed for *Procedure* in the *Assay* —under *Diethylpropion Hydrochloride.* Calculate the quantity, in mg, of $C_{13}H_{19}NO \cdot HCl$ in the portion of Tablets taken by the formula:

$$5C(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Diethylpropion Hydrochloride RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

www.webofpharma.com

Diethylstilbestrol



$C_{18}H_{20}O_2$ 268.35
Phenol 4,4′-(1,2-diethyl-1,2-ethenediyl)bis-, (*E*)-.
α,α′-Diethyl-(*E*)-4,4′-stilbenediol [56-53-1].

» Diethylstilbestrol contains not less than 97.0 percent and not more than 100.5 percent of $C_{18}H_{20}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at room temperature.
USP Reference standards ⟨11⟩—
USP Diethylstilbestrol RS

Identification—

Change to read:

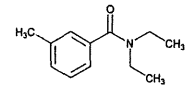
A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: alcohol.
Absorptivities at 230 to 350 nm do not differ by more than 3.0%.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Melting range ⟨741⟩**:** between 169° and 175°, but the range between beginning and end of melting does not exceed 4°.
Acidity or alkalinity—A solution of 100 mg in 5 mL of neutralized 70% alcohol is neutral to litmus.
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩**:** not more than 0.05%.

Assay—
Diluent—Prepare a mixture of alcohol and water (1:1).
Mobile phase—Prepare a filtered and degassed mixture of methanol and water (3:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Diethylstilbestrol RS in *Diluent,* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 20 µg per mL.
System suitability solution—Dissolve 10 mg of USP Diethylstilbestrol RS in 50 mL of chloroform, and allow the solution to stand in the dark for not less than 5 hours. Pipet 5.0 mL of this solution into a 50-mL volumetric flask, and evaporate to dryness under a current of air. Dissolve the residue (the *cis*- and *trans*-isomers of diethylstilbestrol) in *Diluent,* sonicating if necessary. Dilute with *Diluent* to volume, and mix.
Assay preparation—Dissolve an accurately weighed quantity of Diethylstilbestrol in *Diluent,* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a concentration of about 20 µg per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.00 for *trans*-diethylstilbestrol and 1.33 for *cis*-diethylstilbestrol; and the resolution, *R,* between *trans*-diethylstilbestrol and *cis*-diethylstilbestrol is not less than 4.0. Chromatograph the *Standard preparation,* and record the peak responses for the *trans*-isomer as directed for *Procedure:* the column efficiency is not less than 3000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the peak responses for the *cis*- and *trans*-isomers of diethylstilbestrol. Calculate the quantity, in µg, of $C_{18}H_{20}O_2$ in the portion of Diethylstilbestrol taken by the formula:

$$C(r_{t,U} + 1.26r_{c,U})/(r_{t,S} + 1.26r_{c,S})$$

in which C is the concentration, in µg per mL, of USP Diethylstilbestrol RS in the *Standard preparation;* and $r_{t,U}$ and $r_{t,S}$ are the peak responses for the *trans*-isomer obtained from the *Assay preparation* and the *Standard preparation,* respectively; and $r_{c,U}$ and $r_{c,S}$ are the peak responses for the *cis*-isomer obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Diethyltoluamide



$C_{12}H_{17}NO$ 191.27
Benzamide, *N,N*-diethyl-3-methyl-;
N,N-Diethyl-*m*-toluamide [134-62-3].

DEFINITION
Diethyltoluamide contains NLT 95.0% and NMT 103.0% of the *meta*-isomer of diethyltoluamide ($C_{12}H_{17}NO$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
- **PROCEDURE**

Solution A: 1 mL of phosphoric acid in 1000 mL of water
Mobile phase: Methanol and *Solution A* (45:55)
Diluent: Methanol and water (45:55)
System suitability solution: 0.2 mg/mL of USP Diethyltoluamide RS (*m*-isomer) and 0.02 mg/mL of USP Diethyltoluamide Related Compound A RS in *Diluent.* [NOTE—USP Diethyltoluamide Related Compound A RS is the *p*-isomer of diethyltoluamide.]
Standard solution: 0.2 mg/mL of USP Diethyltoluamide RS in *Diluent*
Sample solution: 0.2 mg/mL of Diethyltoluamide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC

Stimuli Solution IRA

www.webofpharma.com

Detector: UV 235 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between diethyltoluamide (*m*-isomer) and diethyltoluamide (*p*-isomer), *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of diethyltoluamide ($C_{12}H_{17}NO$) in the portion of Diethyltoluamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–103.0% on the anhydrous basis

SPECIFIC TESTS
• **SPECIFIC GRAVITY** ⟨841⟩: 0.996–1.002
• **REFRACTIVE INDEX** ⟨831⟩: 1.520–1.524
• **ACIDITY**
Sample solution: Dissolve 10.0 g in 50 mL of neutralized alcohol, and add phenolphthalein TS.
Analysis: Titrate the *Sample solution* with 0.010 N sodium hydroxide.
Acceptance criteria: NMT 4.0 mL of 0.010 N sodium hydroxide is required.
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Diethyltoluamide RS
USP Diethyltoluamide Related Compound A RS
N,N-Diethyl-4-toluamide.
$C_{12}H_{17}NO$ 191.27

Diethyltoluamide Topical Solution

DEFINITION
Diethyltoluamide Topical Solution is a solution of Diethyltoluamide in Alcohol or Isopropyl Alcohol. It contains NLT 92.0% and NMT 108.0% of the labeled amount of the *meta*-isomer of diethyltoluamide ($C_{12}H_{17}NO$). If it contains Alcohol, NLT 95.0% and NMT 105.0% of the labeled amount of alcohol (C_2H_5OH) is present.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)
Sample solution: Nominally 20 mg/mL of diethyltoluamide in carbon disulfide prepared as follows. Transfer a quantity of Topical Solution, equivalent to 200 mg of diethyltoluamide, into a beaker. Place the beaker into a vacuum oven containing silica gel and calcium chloride, and adjusted to a pressure of about 380 mm of mercury, and heat at 35° for 6 h. Transfer the residue with the aid of carbon disulfide to a 10-mL volumetric flask, and add carbon disulfide to volume.
Analysis: Spectral region between 8 and 15 µm
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 1 mL of phosphoric acid in 1000 mL of water
Mobile phase: Methanol and *Solution A* (45:55)
Diluent: Methanol and water (45:55)
System suitability solution: 0.2 mg/mL of USP Diethyltoluamide RS (*m*-isomer) and 0.02 mg/mL of USP Diethyltoluamide Related Compound A RS in *Diluent*. [NOTE—USP Diethyltoluamide Related Compound A RS is the *p*-isomer of diethyltoluamide.]
Standard solution: 0.2 mg/mL of USP Diethyltoluamide RS in *Diluent*
Sample solution: Nominally 0.2 mg/mL of diethyltoluamide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between diethyltoluamide (*m*-isomer) and diethyltoluamide (*p*-isomer), *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of the *m*-isomer of diethyltoluamide ($C_{12}H_{17}NO$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration in the *Standard solution* (mg/mL)
C_U = nominal concentration in the *Sample solution* (mg/mL)

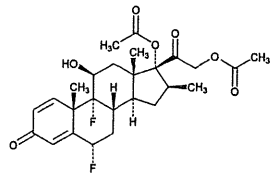
Acceptance criteria: 92.0%–108.0%

OTHER COMPONENTS
• **ALCOHOL DETERMINATION** ⟨611⟩ ***(if present)*:** 29.0%–89.0% of alcohol (C_2H_5OH) is found.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Diethyltoluamide RS
USP Diethyltoluamide Related Compound A RS
N,N-Diethyl-4-toluamide.
$C_{12}H_{17}NO$ 191.27

www.webofpharma.com

Diflorasone Diacetate



$C_{26}H_{32}F_2O_7$ 494.52

Pregna-1,4-diene-3,20-dione, 17,21-bis(acetyloxy)-6,9-difluoro-11-hydroxy-16-methyl-, (6α,11β,16β)-;
6α,9-Difluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-diacetate [33564-31-7].

DEFINITION

Diflorasone Diacetate contains NLT 97.0% and NMT 103.0% of diflorasone diacetate ($C_{26}H_{32}F_2O_7$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**

Mobile phase: Tetrahydrofuran, glacial acetic acid, water-saturated *n*-butyl chloride, and water-saturated methylene chloride (10:15:350:125)

Internal standard solution: 0.04 mg/mL of isoflupredone acetate in water-saturated chloroform

Standard solution: 33 µg/mL of USP Diflorasone Diacetate RS in the *Internal standard solution*

Sample solution: 0.03 mg/mL of Diflorasone Diacetate in the *Internal standard solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 10-cm; 3-µm packing L3

Flow rate: 2.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for diflorasone diacetate and the internal standard are 1.0 and 2.4, respectively.]

Suitability requirements

Resolution: NLT 12 between the analyte and the internal standard peaks

Relative standard deviation: NMT 2.0% for NLT four replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of diflorasone diacetate ($C_{26}H_{32}F_2O_7$) in the portion of Diflorasone Diacetate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of diflorasone diacetate and the internal standard from the *Sample solution*

R_S = peak area ratio of diflorasone diacetate and the internal standard from the *Standard solution*

C_S = concentration of USP Diflorasone Diacetate RS in the *Standard solution* (µg/mL)

C_U = concentration of Diflorasone Diacetate in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%
- **ORGANIC IMPURITIES**

Mobile phase and **System suitability:** Proceed as directed in the *Assay*.

Sample solution: 6 mg/mL of Diflorasone Diacetate in water-saturated chloroform

Chromatographic system: Proceed as directed in the *Assay*, except for the following.

Run time: 5 times the retention time of the major peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Diflorasone Diacetate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area for each impurity

r_T = sum of all the peak areas

Acceptance criteria

Individual impurities: NMT 1.0%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at 60° at a pressure NMT 5 mm of mercury for 16 h.

Acceptance criteria: NMT 0.5%

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 20 mg/mL of undried Diflorasone Diacetate, in chloroform

Acceptance criteria: +58° to +68°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Diflorasone Diacetate RS

Diflorasone Diacetate Cream

» Diflorasone Diacetate Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{26}H_{32}F_2O_7$.

Packaging and storage—Preserve in collapsible tubes, preferably at controlled room temperature.

USP Reference standards ⟨11⟩—

USP Diflorasone Diacetate RS

Identification—The chromatogram of the *Assay preparation* exhibits a major peak for diflorasone diacetate at a retention time that corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained in the *Assay*.

Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Minimum fill ⟨755⟩—meets the requirements.

www.webofpharma.com

Assay—

Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Diflorasone Diacetate.*

Assay preparation—Transfer an accurately weighed amount of Cream, equivalent to about 1 mg of diflorasone diacetate, to a suitable container. Add 30.0 mL of *Internal standard solution,* and shake for about 30 minutes. Centrifuge the solution, and remove and discard the top (excipient) layer. Use the lower, clear chloroform layer.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Diflorasone Diacetate.* Calculate the quantity, in mg, of diflorasone diacetate ($C_{26}H_{32}F_2O_7$) in the portion of Cream taken by the formula:

$$0.03C(R_U/R_S)$$

in which the terms are as defined therein.

Diflorasone Diacetate Ointment

» Diflorasone Diacetate Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{26}H_{32}F_2O_7$.

Packaging and storage—Preserve in collapsible tubes, preferably at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Diflorasone Diacetate RS

Identification—It responds to the *Identification*— under *Diflorasone Diacetate Cream.*

Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—Proceed with Ointment as directed for *Microbial enumeration tests 61 and Tests for specified microorganisms 62*— under *Diflorasone Diacetate Cream.*

Minimum fill ⟨755⟩: meets the requirements.

Assay—

Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Diflorasone Diacetate.*

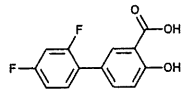
Assay preparation—Using Ointment instead of the Cream, proceed as directed for *Assay preparation* in the *Assay*— under *Diflorasone Diacetate Cream.*

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Diflorasone Diacetate.* Calculate the quantity, in mg, of diflorasone diacetate ($C_{26}H_{32}F_2O_7$) in the portion of Ointment taken by the formula:

$$0.03C(R_U/R_S)$$

in which the terms are as defined therein.

Diflunisal



$C_{13}H_8F_2O_3$ 250.20
[1,1′-Biphenyl]-3-carboxylic acid, 2′,4′-difluoro-4-hydroxy-.
2′,4′-Difluoro-4-hydroxy-3-biphenylcarboxylic acid [22494-42-4].

» Diflunisal contains not less than 98.0 percent and not more than 101.5 percent of $C_{13}H_8F_2O_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Diflunisal RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 40 µg per mL.
Medium: hydrochloric acid in methanol (1 in 120).
Absorptivities at 315 nm, calculated on the dried basis, do not differ by more than 3.0%.

Loss on drying ⟨731⟩—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 4 hours: it loses not more than 0.3% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—Prepare a solution of it in methanol containing about 10 mg per mL. Prepare solutions of USP Diflunisal RS in methanol having concentrations of 10, 0.05, and 0.02 mg per mL, respectively (*Standard solutions A, B,* and *C*). Apply 5-µL portions of all four solutions to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture and previously washed with methanol. Allow the spots to dry, and develop the chromato gram in a freshly prepared solvent system consisting of a mixture of *n*-hexane, dioxane, and glacial acetic acid (85:10:5) in a paper-lined, equilibrated tank, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow to air-dry, and examine the plate under short-wavelength UV light: the chromatograms show principal spots at about the same R_F value. Estimate the concentration of any spot observed in the chromatogram of the test solution, other than the principal spot, by comparison with the spots in the chromatograms of *Standard solutions B* and *C:* the intensity of any individual spot is not greater than that of the principal spot obtained from *Standard solution C* (0.2%), and the sum of all additional spots is not greater than that of the principal spot obtained from *Standard solution B* (0.5%).

Assay—

Mobile phase—Prepare a suitable mixture of water, methanol, acetonitrile, and glacial acetic acid (55:23:10:2) such that the retention time of diflunisal is about 18 minutes. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Diflunisal RS in a mixture of acetonitrile and water (4:1) to obtain a solution having a known concentration of about 1 mg per mL. Dilute an accurately measured volume of this solution with a mixture of acetonitrile and water (1:1) to obtain a solution having a known concentration of about 0.2 mg per mL.

www.webofpharma.com

Assay preparation—Transfer about 50 mg of Diflunisal, accurately weighed, to a 50-mL volumetric flask. Dilute with a mixture of acetonitrile and water (4:1) to volume, and mix. Transfer 5.0 mL of this solution to a 25-mL volumetric flask. Dilute with a mixture of acetonitrile and water (1:1) to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1 and is maintained at a temperature of 40°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the column efficiency determined from the analyte peak is not less than 2500 theoretical plates, the tailing factor is not more than 2.0, the capacity factor is not less than 7.2, and the relative standard deviation for replicate injections is not more than 1%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{13}H_8F_2O_3$ in the portion of Diflunisal taken by the formula:

$$250C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Diflunisal RS in the *Standard preparation*, and r_U and r_S are the peak responses of the major peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Diflunisal Tablets

DEFINITION

Diflunisal Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diflunisal ($C_{13}H_8F_2O_3$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Delete the following:

▲• **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 10 mg/mL of USP Diflunisal RS in methanol and water (80:20)

Sample solution: Nominally 10 mg/mL of diflunisal prepared as follows. Transfer a quantity of finely ground Tablets, equivalent to about 100 mg of diflunisal, to a 10-mL volumetric flask. Add 2 mL of water, and sonicate for 5 min. Dilute with methanol to volume, sonicate for an additional 5 min, and pass through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: *n*-Hexane, glacial acetic acid, and chloroform (17:3:2)

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and examine under long-wavelength UV light.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. ▲ (USP 1-May-2020)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲ (USP 1-May-2020)

ASSAY

Change to read:

• **PROCEDURE**

▲**Mobile phase:** Methanol, acetonitrile, glacial acetic acid, and water (40:17:6:45)

Diluent: Acetonitrile and water (60:40)

Standard solution: 0.1 mg/mL of USP Diflunisal RS in *Diluent*. Sonicate to dissolve as needed.

Sample stock solution: Nominally 1 mg/mL of diflunisal prepared as follows. Finely powder Tablets (NLT 20). Transfer a quantity of the powder, equivalent to about 100 mg of diflunisal, to a 100-mL volumetric flask. Add 5 mL of water and sonicate for 5 min. Add 60 mL of acetonitrile, and sonicate with occasional shaking for 10 min. Dilute with water to volume. Pass through a suitable filter of 0.45-µm pore size. Discard the first few milliliters of the filtrate.

Sample solution: Nominally 0.1 mg/mL of diflunisal in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: NLT 2 times the retention time of diflunisal ▲ (USP 1-May-2020)

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diflunisal ($C_{13}H_8F_2O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diflunisal from the *Sample solution*

r_S = peak response of diflunisal from the *Standard solution*

C_S = concentration of USP Diflunisal RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diflunisal in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

USP Monographs

www.webofpharma.com

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩
Citric acid solution: Dissolve 7 g of anhydrous citric acid in 100 mL of water.
0.1 M tris buffer: Dissolve 121 g of tris(hydroxymethyl) aminomethane (THAM) in 9 L of water. Adjust with *Citric acid solution* to a pH of 7.45, at 25°. Dilute with water to 10.0 L, equilibrate to 37°, and adjust to a pH of 7.20, if necessary.
Medium: *0.1 M tris buffer*, 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: A known concentration of USP Diflunisal RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Instrumental conditions
▲(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)▲ (USP 1-May-2020)
Mode: UV
Analytical wavelength: 306 nm
▲**Analysis**
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of diflunisal ($C_{13}H_8F_2O_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Diflunisal RS in the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)▲ (USP 1-May-2020)

Tolerances: NLT 80% (Q) of the labeled amount of diflunisal ($C_{13}H_8F_2O_3$) is dissolved.

Change to read:

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements
▲▲ (USP 1-May-2020)

IMPURITIES

Add the following:

▲• **Organic Impurities**
Mobile phase: Methanol, acetonitrile, glacial acetic acid, and water (36:15:6:50)
Diluent: Acetonitrile and water (60:40)
Sensitivity solution: 1 µg/mL of USP Diflunisal RS in *Diluent*. Sonicate to dissolve as needed.
Standard solution: 2 µg/mL of USP Diflunisal RS in *Diluent*. Sonicate to dissolve as needed.
Sample solution: Prepare as directed in the *Sample stock solution* in the *Assay*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified degradation product from the *Sample solution*
r_S = peak response of diflunisal from the *Standard solution*
C_S = concentration of USP Diflunisal RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of diflunisal in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Difluorobiphenol[a, b]	0.69	—
Diflunisal	1.0	—
Diflunisal acetophenone analog[a, c]	1.1	—
Diflunisal acetate analog[a, d]	1.3	—
Difluorobiphenyl[a, e]	2.1	—
Any unspecified degradation product	—	0.10
Total degradation products	—	0.5

[a] Process impurity for identification only. It is not to be reported or included in the total degradation products.
[b] 2′,4′-Difluorobiphenyl-4-ol.
[c] 1-(2′,4′-Difluorobiphenyl-4-yl)ethan-1-one.
[d] 2′,4′-Difluorobiphenyl-4-yl acetate.
[e] 2,4-Difluorobiphenyl.▲ (USP 1-May-2020)

ADDITIONAL REQUIREMENTS

Change to read:

• **Packaging and Storage:** Preserve in well-closed containers. ▲ Store at controlled room temperature.▲ (USP 1-May-2020)
• **USP Reference Standards** ⟨11⟩
USP Diflunisal RS

Digitalis

DEFINITION
Digitalis is the dried leaf of *Digitalis purpurea* L. (Fam. Scrophulariaceae). The potency of Digitalis is such that,

USP Monographs

www.webofpharma.com

when assayed as directed, 100 mg is equivalent to NLT 1 USP Digitalis Unit.[1]

[NOTE—When Digitalis is prescribed, Powdered Digitalis is to be dispensed.]

ASSAY

• **PROCEDURE**

Standard solution: Weigh the contents of 1 container of USP Digitalis RS to the nearest milligram, either in the original container or in a weighing bottle, and transfer to a dry, hard-glass, glass-stoppered container or centrifuge tube of at least 50-mL capacity. Complete the weighing within 5 min after opening the ampul. Add a menstruum consisting of 4 volumes of alcohol and 1 volume of water so that the total volume of menstruum added corresponds to 10 mL for each gram of powder. Insert the stopper, the upper third of which is greased lightly with petrolatum. Shake the mixture for 24 ± 2 h at 25 ± 5° by mechanical means, which continuously brings the solid material into fresh contact with the liquid phase. Immediately thereafter transfer, if necessary, to a centrifuge tube, centrifuge, and decant the supernatant tincture into a dry, hard-glass bottle with a tight closure. Preserve under refrigeration, and use within 30 days.

Sample solution: Transfer 5 g of Digitalis, reduced to a fine powder, to a hard-glass, glass-stoppered container or centrifuge tube of at least 50-mL capacity. Prepare as directed in the *Standard solution*, beginning with "Add a menstruum". Preserve under refrigeration, and use within 30 days.

Pigeons: Use adult pigeons free from gross evidence of disease or emaciation, and of such weight that the heaviest weighs less than twice the weight of the lightest. Divide the pigeons into groups as nearly alike as practicable with respect to breed and weight so that the average weight of the group assigned by random choice to the *Standard solution* shall differ by NMT 30% from the average weight of the group assigned to the preparation to be assayed. Withhold food but not water for 16–28 h before use. Preparatory to injection, lightly anesthetize the pigeon with ether, and immobilize it; expose an alar vein, and cannulate with a suitable cannula. Maintain the anesthesia during cannulation and throughout the subsequent injection period at such a level that pain is absent, the pupillary and corneal reflexes are present, and the voluntary musculature is not relaxed beyond permitting the pigeon to make some voluntary movement occasionally.

Preparation of sample dilutions: On the day of the *Assay*, dilute portions of the *Standard solution* and the *Sample solution* with isotonic sodium chloride solution in such a way that the estimated fatal dose of each dilution will be 15 mL/kg of body weight.

Injection of sample dilutions: Arrange to inject the appropriate sample dilution by suitable means such as a small-bore buret calibrated to 0.05 mL. Start the injection after ensuring the absence of air bubbles from the injection apparatus, by infusing, within a few seconds, a volume of the test dilution equivalent to 1 mL/kg of body weight. Repeat this dose at 5-min intervals thereafter until the pigeon dies of cardiac arrest. Use a total of NLT 6 pigeons for the *Standard solution* and NLT 6 pigeons for the preparation to be assayed. If the average number of doses for any given dilution required to produce death is fewer than 13 or greater than 19, or if the larger exceeds the smaller in the same assay by more than 4 doses, regard these data as preliminary. Use them as a guide, and repeat with a fresh, higher or lower dilution. Complete the *Assay* within 30 days for preservation of the *Standard solution* and *Sample solution*.

Calculation of potency: Tabulate and average the number of doses of the *Standard solution*, designating the average $\bar{z}_S$, and likewise obtain the corresponding average, $\bar{z}_U$, for the *Sample solution*.

Calculate the potency in USP Digitalis Units/mL (i.e., per 100 mg) of the *Sample solution* as:

$$\text{Potency} = [\bar{z}_S \times (v_S/v_U)]/\bar{z}_U$$

$\bar{z}_S$ = average number of doses of the *Standard solution*

v_S = number of USP Digitalis Units/mL of *Standard solution* dilution

v_U = volume of *Sample solution* per mL of dilution (mL)

$\bar{z}_U$ = average number of doses of the *Sample solution*

Calculate the confidence interval, *L* [see *Equation* (31) in *Design and Analysis of Biological Assays* ⟨111⟩, *The Confidence Interval and Limits of Potency*]. If *L* exceeds 0.30, repeat the *Assay* or inject more pigeons with one or both preparations until the confidence interval is 0.30 or less.

Acceptance criteria: The potency of Digitalis, calculated from that of the *Sample solution*, is satisfactory if the result is NLT 0.85 USP Digitalis Units/100 mg.

CONTAMINANTS

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Acid-Insoluble Ash*: NMT 5.0%

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Foreign Organic Matter*: The proportion of stems, browned leaves, flowers, and other foreign organic matter does not exceed 2.0%.

• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Pesticide Residue Analysis*: Meets the requirements

SPECIFIC TESTS

• **BOTANICAL CHARACTERISTICS**

Macroscopic: Unground digitalis occurs as more or less crumpled or broken leaves. The leaf blades are ovate, oblong-ovate to ovate-lanceolate, mostly 10–35 cm in length and 4–11 cm in width and contracted into a winged petiole. The apex is obtuse; the margin irregularly crenate or serrate; the lower surface densely pubescent, the upper surface wrinkled and finely hairy. The venation is conspicuously reticulate, the mid-rib and principal veins broad and flat, and the lower veins are continued into the wings of the petiole. The color of the upper surface is dark green, of the lower surface grayish from the dense pubescence, the larger veins often purplish. The odor is slight when dry, peculiar and characteristic when moistened.

Microscopic: Digitalis shows an upper epidermis whose cells possess slightly wavy anticlinal walls, numerous hairs, and no stomata; a lower epidermis with wavy anticlinal walls, numerous oval stomata, and many hairs, and frequently not attached over irregular areas to the cell layer within, especially near the veins; a broad chlorenchyma of a single layer of short palisade cells and several layers of spongy parenchyma; and numerous vascular bundles in the larger veins and petioles, separated by vascular rays one cell in width. On the apex of each marginal tooth, one or two water stomata occur.

Ground digitalis: This is dark green in color. Present are chiefly numerous irregular fragments of epidermis and chlorenchyma; nonglandular hairs that are frequently curved or crooked, up to 500 µm in length, uniseriate, two- to eight-celled, some of the cells collapsed so that the planes of adjoining cells may be at right angles, the terminal

[1] One USP Digitalis Unit represents the potency of 100 mg of USP Digitalis RS.

USP Monographs

www.webofpharma.com

cell pointed or rounded; few, small glandular hairs, usually with a one- or two-celled stalk, and a one- or two-celled head; fragments of veins and petioles with annular, reticulate, spiral, and simple pitted vessels and tracheids. Calcium oxalate is absent.

- **WATER DETERMINATION** ⟨921⟩, *Method III*: NMT 6.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in containers that protect it from absorbing moisture.
- **LABELING:** Digitalis labeled to indicate that it is to be used only in the manufacture of glycosides is exempt from the moisture and storage requirements.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Digitalis RS

Powdered Digitalis

DEFINITION

Powdered Digitalis is Digitalis (*Digitalis purpurea*) dried at a temperature not exceeding 60°, reduced to a fine or a very fine powder, and adjusted, if necessary, to conform to the official potency by admixture with sufficient Lactose, Starch, or exhausted marc of digitalis, or with Powdered Digitalis having either a lower or a higher potency.

The potency of Powdered Digitalis is such that, when assayed as directed, 100 mg is equivalent to 1 USP Digitalis Unit.[1]

[NOTE—When Digitalis is prescribed, Powdered Digitalis is to be dispensed.]

IDENTIFICATION

- **A. BOTANIC CHARACTERISTICS:** The powder is dark green in color. Present chiefly are numerous irregular fragments of epidermis and chlorenchyma; nonglandular hairs that are frequently curved or crooked, up to 500 µm in length, uniseriate, three- to five- (two- to eight-) celled, some of the cells collapsed so that the planes of adjoining cells may be at right angles, the terminal cell pointed or rounded; few, small glandular hairs, usually of two types: one is short (20–40 µm long) with a one-celled stalk and typically two-celled head; a second, less numerous, is long (up to 350 µm) with a uniseriate, multicellular stalk and unicellular head with a one- or two-celled stalk, and a one- or two-celled head; fragments of veins and petioles with annular, reticulate, spiral and simple pitted vessels and tracheids. Calcium oxalate is absent.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution A: Transfer 100 mg of USP Digitalis RS to a 15-mL centrifuge tube containing 2.0 mL of diluted alcohol and 1.0 mL of lead acetate TS, shake, and boil for 2 min. Centrifuge, decant the supernatant into a second 15-mL centrifuge tube, and add 2.0 mL of chloroform. Centrifuge, then remove the lower layer, and filter it through a chloroform-washed small column of anhydrous sodium sulfate (100–300 mg) into a 5-mL centrifuge tube. Evaporate the chloroform solution under a stream of nitrogen to dryness, and dissolve the residue in 100 µL of a mixture of methanol and chloroform (1:1).

Standard solution B: Dissolve USP Digitoxin RS in a mixture of methanol and chloroform (1:1) such that the final concentration is approximately 0.2 mg/mL.

Sample solution: Prepare the *Sample solution* in the same manner as *Standard solution A*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Ethyl acetate, methanol, and water (30:4:3)
Derivatization reagent: Mix 10 mL of chloramine T solution (3 in 100) with 40 mL of a solution (1 in 4) of trichloroacetic acid in alcohol.
[NOTE—Store the mixture in a cool place, and use it within one week.]

Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply *Standard solution A*, *Standard solution B*, and *Sample solution*, each as a narrow band about 15 mm long. Allow the bands to dry. Develop the chromatogram in a saturated chamber until the solvent front has moved about 15 cm from the origin. Remove the plate from the chamber and dry. Treat with *Derivatization reagent*. Heat the plate at 110° for 15–20 min, and examine under long-wavelength UV light. Locate the prominent bands from *Standard solution A* corresponding in R_F value to the band from *Standard solution B*. The chromatogram from the solution under test shows bands corresponding to these, and also shows bands corresponding to the three other most prominent bands from *Standard solution A* but of lower R_F value.

Acceptance criteria: Relative R_F values for the five bands are: 1.0 (digitoxin); 0.8–0.9; 0.6–0.7; 0.4–0.5; and 0.3–0.4.

ASSAY

- **PROCEDURE**

Standard solution: Weigh the contents of one container of USP Digitalis RS to the nearest mg, either in the original container or in a weighing bottle, and transfer to a dry, hard-glass, glass-stoppered container or centrifuge tube of at least 50-mL capacity. Complete the weighing within 5 min of opening the ampul. Add a menstruum consisting of four volumes of alcohol and one volume of water so that the total volume of menstruum added corresponds to 10 mL for each g of powder. Insert the stopper, the upper third of which is greased lightly with petrolatum. Shake the mixture for 24 ± 2 h at 25 ± 5° by mechanical means, which continuously brings the solid material into fresh contact with the liquid phase. Immediately thereafter, transfer to a centrifuge tube if necessary, centrifuge, and decant the supernatant tincture into a dry, hard-glass bottle having a tight closure. Preserve under refrigeration, and use within 30 days.

Sample solution: Transfer 5 g of Powdered Digitalis to a hard-glass, glass-stoppered container or centrifuge tube of at least 50-mL capacity. Prepare as directed under *Standard solution*, beginning with "Add a menstruum." Preserve under refrigeration, and use within 30 days.

Pigeons: Use adult pigeons free from gross evidence of disease or emaciation, and of such weight that the heaviest weighs less than twice the weight of the lightest. Divide the pigeons into groups as nearly alike as practicable with respect to breed and weight so that the average weight of the group assigned by random choice to the *Standard solution* shall not differ by more than 30% from the average weight of the group assigned to the preparation to be assayed. Withhold food but not water for 16–28 h before use. Preparatory to injection, lightly anesthetize the pigeon with ether, and immobilize it; expose an alar vein, and cannulate with a suitable cannula. Maintain the anesthesia

[1] One USP Digitalis Unit represents the potency of 100 mg of USP Digitalis RS.

www.webofpharma.com

during cannulation and throughout the subsequent injection period at such a level that pain is absent, the pupillary and corneal reflexes are present, and the voluntary musculature is not relaxed beyond permitting the pigeon to make some voluntary movement occasionally.

Preparation of sample dilutions: On the day of the *Assay*, dilute portions of the *Standard solution* and the *Sample solution* with isotonic sodium chloride solution in such a way that the estimated fatal dose of each dilution will be 15 mL/kg of body weight.

Injection of sample dilutions: Arrange to inject the appropriate sample dilution by suitable means such as a small-bore buret calibrated to 0.05 mL. Start the injection after ensuring the absence of air bubbles from the injection apparatus, by infusing, within a few s, a volume of the test dilution equivalent to 1 mL/kg of body weight. Repeat this dose at 5-min intervals thereafter until the pigeon dies of cardiac arrest. Use a total of NLT six pigeons for the *Standard solution* and NLT six pigeons for the preparation to be assayed. If the average number of doses for any given dilution required to produce death is less than 13 or greater than 19, or if the larger exceeds the smaller in the same assay by more than 4 doses, regard these data as preliminary. Use them as a guide, and repeat with a fresh, higher or lower dilution. Complete the assay within 30 days for preservation of the *Standard solution* and *Sample solution*.

Calculation of potency: Tabulate and average the number of doses of the *Standard solution*, designating the average, $\bar{z}_S$, and likewise obtain the corresponding average, $\bar{z}_U$, for the *Sample solution*. Compute the potency in USP Digitalis Units/mL (i.e., per 100 mg) of the *Sample solution* as:

$$\text{Potency} = [\bar{z}_S \times (v_S/v_U)]/\bar{z}_U$$

$\bar{z}_S$ = average number of doses of the *Standard solution*
v_S = number of USP Digitalis Units/mL of *Standard solution* dilution
v_U = volume of *Sample solution* per mL of dilution (mL)
$\bar{z}_U$ = average number of doses of the *Sample solution*

Compute the confidence interval, *L* (see *Design and Analysis of Biological Assays* ⟨111⟩, *The Confidence Interval and Limits of Potency*). If *L* exceeds 0.30, repeat the *Assay* or inject more pigeons with one or both preparations until the confidence interval is 0.30 or less.

Acceptance criteria: The potency of Powdered Digitalis, calculated from that of the *Sample solution*, is satisfactory if the result is NLT 0.85 USP Digitalis Units/100 mg and NMT 1.20 USP Digitalis Units/100 mg.

IMPURITIES

- **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Acid-Insoluble Ash*: NMT 5.0%

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements of the tests for absence of *Salmonella* species.
- **WATER DETERMINATION** ⟨921⟩, *Method III (Gravimetric), Procedure for Articles of Botanical Origin*: NMT 5.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. A package of a suitable desiccant may be enclosed in the container.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Digitalis RS
  USP Digitoxin RS

Digitalis Capsules

» Digitalis Capsules contain an amount of Powdered Digitalis equivalent to not less than 85.0 percent and not more than 120.0 percent of the labeled potency.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Digitalis RS
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the test for absence of *Salmonella* species.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Standard preparation—Prepare as directed in the *ASSAY* under *Digitalis*.

Assay preparation—Empty the contents of not less than 20 Capsules into a hard-glass, glass-stoppered container of not less than 50-mL capacity. Add a menstruum consisting of 4 volumes of alcohol and 1 volume of water so that the total volume of menstruum corresponds to 1 mL for each expected USP Digitalis Unit. Insert the stopper, the upper third of which is greased lightly with petrolatum. Shake the mixture at 25 ± 5° for 24 ± 2 hours by mechanical means, which continuously brings the solid material into fresh contact with the liquid phase. Immediately thereafter transfer to a centrifuge tube, centrifuge, and decant the supernatant tincture into a dry, hard-glass bottle having a tight closure. Preserve under refrigeration, and use within 30 days.

Pigeons, Preparation of test dilutions, Injection of test dilutions, and *Calculation of potency*—Proceed as directed in the *ASSAY* under *Digitalis*.

Digitalis Tablets

» Digitalis Tablets contain an amount of Powdered Digitalis equivalent to not less than 85.0 percent and not more than 120.0 percent of the labeled potency.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Digitalis RS
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the test for absence of *Salmonella* species.
Disintegration ⟨701⟩: 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Standard preparation—Prepare as directed in the *ASSAY* under *Digitalis*.

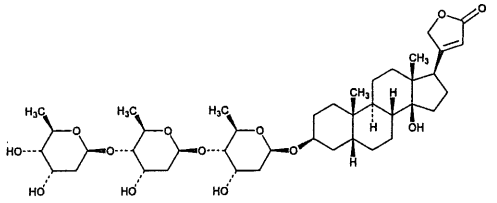
Assay preparation—Weigh and finely powder not fewer than 25 Tablets. Weigh accurately a portion of the powder, equivalent to not less than 20 Tablets. Transfer to a dry, hard-glass, glass-stoppered container of not less than 50-mL capacity. Proceed as directed for the *Assay preparation* in the *Assay*— under *Digitalis Capsules*, beginning with "Add a menstruum."

Pigeons, Preparation of test dilutions, Injection of test dilutions, and *Calculation of potency*—Proceed as directed in the *ASSAY* under *Digitalis*.

Supplement 1

www.webofpharma.com

Digitoxin



$C_{41}H_{64}O_{13}$ 764.94

Card-20(22)-enolide, 3-[(*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl) oxy]-14-hydroxy, (3β,5β)-.

Digitoxin [71-63-6].

» Digitoxin is a cardiotonic glycoside obtained from *Digitalis purpurea* L., *Digitalis lanata* Ehrh. (Fam. Scrophulariaceae), and other suitable species of *Digitalis*. Digitoxin contains not less than 92.0 percent and not more than 103.0 percent of $C_{41}H_{64}O_{13}$, calculated on the dried basis. [**CAUTION**—Handle Digitoxin with exceptional care since it is highly potent.]

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Digitoxin RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Prepare a test solution in methanol containing 1 mg per mL. Apply 1 µL of the test solution and 1 µL of a Standard solution of USP Digitoxin RS in methanol containing 1 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the applications to dry, and develop the chromatogram in a solvent system consisting of a mixture of methylene chloride and methanol (93:7) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 100° to remove the solvent. Spray the plate with a 6 in 10 solution of sulfuric acid in methanol, heat at 105° for 10 minutes, and examine the chromatogram under long-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

C: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the major peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Loss on drying ⟨731⟩—Dry it in vacuum at 105° for 1 hour: it loses not more than 1.5% of its weight.

Residue on ignition ⟨281⟩: negligible, from 100 mg.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Digitoxin RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 40 µg per mL.

Assay preparation—Transfer about 50 mg of Digitoxin, accurately weighed, to a 200-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 4 mL of this solution into a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

System suitability preparation—Prepare a solution in *Mobile phase* containing about 40 µg each of digitoxin and digoxin per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 218-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure*:the relative retention times are about 0.35 for digoxin and 1.0 for digitoxin; the resolution, *R*, between the digoxin and digitoxin peaks is not less than 2.0; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{41}H_{64}O_{13}$ in the portion of Digitoxin taken by the formula:

$$1.25C(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Digitoxin RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Digitoxin Injection

» Digitoxin Injection is a sterile solution of Digitoxin in 5 to 50 percent (v/v) of alcohol, and may contain Glycerin or other suitable solubilizing agents. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{41}H_{64}O_{13}$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Digitoxin RS

Identification—

A: To a portion of Injection, equivalent to about 1 mg of digitoxin, add 10 mL of water, and extract with 10 mL of chloroform. Evaporate the chloroform extract on a steam bath with the aid of a current of air to dryness. Dissolve the residue in 2 mL of a solution prepared by mixing 0.3 mL of ferric chloride TS and 50 mL of glacial acetic acid, and underlay with 2 mL of sulfuric acid: at the zone of contact of the two liquids a brown color, which gradually changes to light green, then to blue, is produced, and finally the entire acetic acid layer acquires a blue color.

B: To a portion of Injection, equivalent to about 0.2 mg of digitoxin, add 10 mL of water, and extract with 10 mL of chloroform. Evaporate the chloroform extract on a steam bath

www.webofpharma.com

with the aid of a current of air to dryness. Add 2 mL of a freshly prepared 1 in 100 solution of *m*-dinitrobenzene in alcohol, and allow to stand for 10 minutes with frequent shaking. Add 2 mL of a 1 in 200 solution of tetramethylammonium hydroxide in alcohol, and mix: a red-violet color develops slowly and then fades.

C: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the major peak in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 111.0 USP Endotoxin Units per mg of digitoxin.

Alcohol Determination ⟨611⟩: between 90.0% and 110.0% of the labeled percentage of C_2H_5OH.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase, Standard preparation, System suitability preparation, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Digitoxin*.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 1 mg of digitoxin, to a 25-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Digitoxin*. Calculate the quantity, in µg, of $C_{41}H_{64}O_{13}$ in each mL of the Injection taken by the formula:

$$25(C/V)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Digitoxin RS in the *Standard preparation*, *V* is the volume, in mL, of Injection taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Digitoxin Tablets

» Digitoxin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{41}H_{64}O_{13}$.

NOTE—Avoid the use of strongly adsorbing substances, such as bentonite, in the manufacture of Digitoxin Tablets.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Digitoxin RS

Identification—

A: Transfer a quantity of finely powdered Tablets, equivalent to not less than 1 mg of digitoxin, to a suitable flask, add 20 mL of chloroform, and sonicate. Filter, and evaporate the filtrate on a steam bath with the aid of a current of air to dryness. Dissolve the residue in 2 mL of a solution prepared by mixing 0.3 mL of ferric chloride TS and 50 mL of glacial acetic acid, and underlay with 2 mL of sulfuric acid: at the zone of contact of the two liquids a brown color, which gradually changes to light green, then to blue, is produced, and finally the entire acetic acid layer acquires a blue color.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the major peak in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

Dissolution ⟨711⟩— [NOTE—Throughout this procedure, use scrupulously clean glassware, which previously has been rinsed successively with hydrochloric acid, water, and alcohol, and carefully dried. Take precautions to prevent contamination from fluorescent particles and from metal and rubber surfaces.]

Medium: dilute hydrochloric acid (3 in 500); 500 mL. [NOTE—Use the same batch of *Medium* throughout the test.]

Apparatus 1: 120 ± 5 rpm.

Times: 30 minutes; 60 minutes.

Standard stock solution—Weigh accurately about 30 mg of USP Digitoxin RS, dissolve in a minimum amount of alcohol in a 500-mL volumetric flask, add dilute alcohol (4 in 5) to volume, and mix.

Standard solutions—Just prior to use, dilute 5.0 mL of the *Standard stock solution* with *Medium* to 500.0 mL, and mix. Transfer aliquots (2.0 to 10.0 mL) of this solution to individual separators to prepare standards equivalent to 20, 40, 60, 80, and 100% of the labeled amount of digitoxin in 500 mL. Add *Medium* to make 10 mL, and proceed as directed for *Procedure*, beginning with "Extract with three 15-mL portions of chloroform."

Procedure—Proceed as directed for *Procedure* under *Dissolution* ⟨711⟩. After 30 minutes, accurately timed, withdraw a suitable aliquot of the solution under test from a point midway between the stirring shaft and the wall of the vessel, and approximately midway in depth. Filter the solution promptly after withdrawal, using a suitable membrane filter of not greater than 0.8-µm porosity, discarding the first 10 mL of the filtrate. Without replacing the *Medium* withdrawn, continue to rotate the basket, and after an additional 30 minutes, accurately timed, similarly withdraw and filter another aliquot. Treat each of these solutions as follows: Assuming dissolution of 100% of the labeled amount of digitoxin, transfer aliquots, equivalent to 6 µg of digitoxin, to suitable separators. Extract with three 15-mL portions of chloroform, and combine the chloroform extracts in glass-stoppered flasks. Evaporate the combined extracts on a steam bath, with the aid of a current of air, to dryness. In a similar manner, prepare a blank using a suitable volume of *Medium*.

Measurement of fluorescence—Begin with the *Standard solutions*, and keep all flasks in the same sequence throughout, so that the elapsed time from addition of reagents to reading of fluorescence is the same for each set. Treat 1 flask at a time as follows: Add 10 mL of a solution freshly prepared by dissolving 35 mg of ascorbic acid in 25 mL of methanol and cautiously adding the solution to 100 mL of hydrochloric acid. Mix, and add 1 mL of a solution freshly prepared by diluting 1 mL of 30 percent hydrogen peroxide with water to 500 mL and diluting 1 volume of the resulting solution with 20 volumes of water. Mix, and insert the stopper in the flask. After 45 minutes, measure the fluorescence at about 575 nm, the excitation wavelength being about 395 nm. Correct each reading for the blank, and plot a standard curve of fluorescence versus percentage dissolution. By calculation from the standard curve, determine the percentage dissolution of digitoxin in each Tablet within 30 minutes and the total percentage dissolution of digitoxin within 60 minutes, taking into account the volume of the solution under test removed after the first 30 minutes of the test.

Tolerances—Not less than 60% of the labeled amount of $C_{41}H_{64}O_{13}$ is dissolved within 30 minutes for each Tablet tested, and not less than 85% of the labeled amount is dissolved within 60 minutes for the average of the Tablets tested.

Uniformity of dosage units ⟨905⟩: meet the requirements.

www.webofpharma.com

Procedure for content uniformity—Place 1 Tablet in a suitable glass-stoppered conical flask. Add an accurately measured volume of *Mobile phase* (prepared as directed in the *Assay*— under *Digitoxin*) sufficient to obtain a solution containing about 10 µg of digitoxin per mL, and shake by mechanical means until the Tablet has completely disintegrated (not less than 30 minutes). Centrifuge, and use the clear supernatant as the test solution. Dissolve an accurately weighed quantity of USP Digitoxin RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a Standard solution having a known concentration of about 10 µg per mL. Proceed as directed in the *Assay*. Calculate the quantity, in mg, of $C_{41}H_{64}O_{13}$ in the Tablet by the formula:

$$(LC/D)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of digitoxin in the Tablet, C is the concentration, in µg per mL, of USP Digitoxin RS in the Standard solution, D is the concentration, in µg per mL, of digitoxin in the test solution based on the labeled quantity in the Tablet and the extent of dilution, and r_U and r_S are the digitoxin peak responses obtained from the test solution and the Standard solution, respectively.

Assay—

Mobile phase, Standard preparation, System suitability preparation, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Digitoxin*.

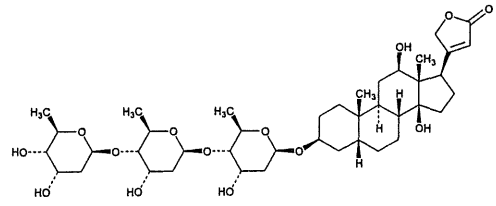
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of powder, equivalent to about 1 mg of digitoxin, to a 25-mL volumetric flask. Add 15 mL of *Mobile phase*, and sonicate. Dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution, discarding the first few mL of the filtrate. The filtrate is the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Digitoxin*. Calculate the quantity, in µg, of $C_{41}H_{64}O_{13}$ in the portion of Tablets taken by the formula:

$$25C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Digitoxin RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Digoxin



$C_{41}H_{64}O_{14}$ 780.94

Card-20(22)-enolide, 3-[(*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl) oxy]-12,14-dihydroxy-, (3β,5β,12β)-;

Digoxin;

3β-[(*O*-2,6-Dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide [20830-75-5].

DEFINITION

Digoxin is a cardiotonic glycoside obtained from the leaves of *Digitalis lanata* Ehrh. (Fam. Plantaginaceae, formerly Scrophulariaceae). It contains NLT 95.0% and NMT 101.0% of digoxin ($C_{41}H_{64}O_{14}$), calculated on the dried basis. [**CAUTION**—Handle Digoxin with exceptional care, because it is extremely poisonous.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and water (13:37)

System suitability solution: 40 µg/mL each of USP Digoxin RS and digoxigenin in diluted alcohol

Standard solution: 0.25 mg/mL of USP Digoxin RS in diluted alcohol. [NOTE—Use a sonic bath to aid dissolution.]

Sample solution: 0.25 mg/mL of Digoxin in diluted alcohol. [NOTE—Dissolve using sonication, before make-up to final volume.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 218 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 25°

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 4.0 between digoxin and digoxigenin

Column efficiency: NLT 1200 theoretical plates for the digoxin peak

Tailing factor: NMT 2.0 for the digoxin peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of digoxin ($C_{41}H_{64}O_{14}$) in the portion of Digoxin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of digoxin from the *Sample solution*

r_S = peak response of digoxin from the *Standard solution*

C_S = concentration of USP Digoxin RS in the *Standard solution* (mg/mL)

C_U = concentration of Digoxin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%, a 100-mg specimen being used
- **RELATED GLYCOSIDES**

Solution A: Acetonitrile and water (10:90)

Solution B: Water and acetonitrile (10:90)

Mobile phase: See *Table 1*.

www.webofpharma.com

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	78	22
5	78	22
15	30	70
16	78	22
30	78	22

Standard stock solution: 0.5 mg/mL of USP Digoxin RS and USP Digitoxin RS in methanol
Standard solution: Dilute 1.0 mL of *Standard stock solution* with methanol to 100 mL.
System suitability solution: Dissolve 50 mg of lanatoside C in methanol, and dilute with methanol to 100 mL. To 1.0 mL of this solution add 1.0 mL of *Standard stock solution*, and dilute with methanol to 20 mL.
Sample solution: Accurately weigh 25 mg of Digoxin, transfer into a 50-mL volumetric flask, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 25°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between the digoxin and lanatoside C peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, determined from the digoxin peak in replicated injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of the peak areas of the total impurities, gitoxin, and digitoxin, against the digoxin peak from the *Standard solution*.
Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Digoxin (standard)	1.00	—
Gitoxin	2.16	0.5
Digitoxin	2.62	0.5
Total impurities	—	3.5

• **Residual Solvents** ⟨467⟩: 2000 µg/g for methylene chloride and for chloroform

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry under vacuum at 105° for 1 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Digoxin RS
USP Digitoxin RS

Digoxin Injection

» Digoxin Injection is a sterile solution of Digoxin in Water for Injection and Alcohol or other suitable solvents. It contains not less than 90.0 percent and not more than 105.0 percent of the labeled amount of $C_{41}H_{64}O_{14}$.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass. Avoid exposure to excessive heat.
USP Reference standards ⟨11⟩—
USP Digoxin RS

Identification—
A: Injection meets the requirements for *Identification* test *A* under *Digoxin Oral Solution*
B: Chloramine T–trichloroacetic acid reagent, Spotting solvent, and *Standard solution*—Proceed as directed for *Identification* test *B* under *Digoxin Oral Solution*.
Test solution—Pipet a volume of Injection, equivalent to 0.5 mg of digoxin, into a separator, and add 5 mL of water. Extract with three 10-mL portions of chloroform, combining the extracts in a conical flask. Evaporate the combined chloroform extracts on a steam bath with the aid of a current of air to dryness. (If traces of water or propylene glycol remain, dry the flask in vacuum at 100° for 30 minutes.) Dissolve the residue in 2 mL of *Spotting solvent*.
Procedure—Apply 10 µL of the *Test solution* and 10 µL of the *Standard solution* on a line parallel to and about 2.5 cm from the bottom edge of a reversed-phase thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture to which is permanently bonded octadecylsilane (C18). Allow the spots to dry, and place the plates in a developing chamber containing a mixture of methanol and water (7:3). Develop the chromatogram until the solvent front has moved about 15 cm above the line of application. Remove the plate, and allow the solvent to evaporate. Spray the plate with *Chloramine T–trichloroacetic acid reagent*, freshly mixed, and heat in an oven at 110° for 10 minutes. Examine the plate under long-wavelength UV light: the R_F value of the principal spot in the chromatogram of the *Test solution* corresponds to that of the *Standard solution*.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 200.0 USP Endotoxin Units per mg of digoxin.
Alcohol Determination ⟨611⟩: between 9.0% and 11.0% of C_2H_5OH.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase—Proceed as directed in the *Assay* under *Digoxin*.
Standard preparation—Dissolve an accurately weighed quantity of USP Digoxin RS in diluted alcohol, and dilute quantitatively with diluted alcohol to obtain a solution having a known concentration of about 250 µg per mL. Use a sonic bath to aid dissolution. If necessary, dilute quantitatively to match, approximately, the concentration of the Injection.
Chromatographic system and *System suitability preparation*—Proceed as directed in the *Assay* under *Digoxin*.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and Injection into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of $C_{41}H_{64}O_{14}$ in each mL of the Injection taken by the formula:

$$C(r_U/r_S)$$

www.webofpharma.com

in which C is the concentration, in µg per mL, of USP Digoxin RS in the *Standard preparation;* and r_U and r_S are the responses for the digoxin peaks obtained from the Injection and the *Standard preparation,* respectively.

Digoxin Oral Solution

» Digoxin Oral Solution contains, in each 100 mL, not less than 4.50 mg and not more than 5.25 mg of digoxin ($C_{41}H_{64}O_{14}$).

Packaging and storage—Preserve in tight containers, and avoid exposure to excessive heat.
USP Reference standards ⟨11⟩—
USP Digoxin RS

Identification—
A: The retention time of the major peak in the chromatogram of Oral Solution corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
B: Chloramine T–trichloroacetic acid reagent—Mix 10 mL of a freshly prepared solution of chloramine T (3 in 100) and 40 mL of a 1 in 4 solution of trichloroacetic acid in dehydrated alcohol.
Spotting solvent—Prepare a mixture of chloroform and methanol (2:1).
Standard solution—Dissolve an accurately weighed quantity of USP Digoxin RS in *Spotting solvent* to obtain a solution containing 0.25 mg per mL.
Test solution—Pipet a volume of Oral Solution, equivalent to 0.5 mg of digoxin, into a separator. Add sufficient water to obtain a final volume of approximately 50 mL. Extract the aqueous layer with three 30-mL portions of chloroform, combining the extracts in a conical flask. Evaporate the combined chloroform extracts on a steam bath with the aid of a current of air to dryness. Add 2 mL of *Spotting solvent* to the residue, and shake for 2 minutes.
Procedure—Apply 10 µL of the *Test solution* and 10 µL of the *Standard solution* on a line parallel to and about 2.5 cm from the bottom edge of a reversed-phase thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture to which is permanently bonded octadecylsilane (C18). Allow the spots to dry, and place the plates in a developing chamber containing a mixture of methanol and water (7:3). Develop the chromatogram until the solvent front has moved about 15 cm above the line of application. Remove the plate, and allow the solvent to evaporate. Spray the plate with *Chloramine T–trichloroacetic acid reagent,* freshly mixed, and heat in an oven at 110° for 10 minutes. Examine the plate under long-wavelength UV light: the R_F value of the principal spot in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution.*
Alcohol Determination ⟨611⟩: between 90.0% and 115.0% of the labeled amount of C_2H_5OH.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and isopropyl alcohol (70: 27.5: 2.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Digoxin RS in diluted alcohol, and dilute quantitatively and stepwise with diluted alcohol to obtain a solution having a known concentration of about 20 µg per mL.
Assay preparation—Transfer an accurately measured volume of 10.0 mL of Oral Solution, equivalent to about 500 µg of digoxin, to a 25-mL volumetric flask, dilute with diluted alcohol to volume, and mix.
System suitability preparation—Prepare as directed in the *Assay* under *Digoxin.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 218-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 0.5 mL per minute. Chromatograph the *System suitability preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between digoxin and digoxigenin bisdigitoxoside is not less than 2.0; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of digoxin ($C_{41}H_{64}O_{14}$) in each mL of the Oral Solution taken by the formula:

$$2.5C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Digoxin RS in the *Standard preparation;* and r_U and r_S are the digoxin peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Digoxin Tablets

» Digoxin Tablets contain not less than 90.0 percent and not more than 105.0 percent of the labeled amount of $C_{41}H_{64}O_{14}$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Digoxin RS

Identification—
A: Chloramine T–trichloroacetic acid reagent—Prepare as directed for *Identification* test *B* under *Digoxin Oral Solution.*
Spotting solvent—Use dehydrated alcohol.
Test solution—Transfer an accurately weighed portion of finely powdered Tablets, equivalent to 0.5 mg of digoxin, to a 10-mL centrifuge tube. Add 2 mL of *Spotting solvent,* sonicate for 10 to 15 minutes, and centrifuge. Decant and use the supernatant.
Standard solution—Dissolve an accurately weighed quantity of USP Digoxin RS in *Spotting solvent* to obtain a solution having a known concentration of about 0.25 mg per mL.
Procedure—Apply 10 µL of the *Test solution* and 10 µL of the *Standard solution* on a line parallel to and about 2.5 cm from the bottom edge of a reversed-phase thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture to which is permanently bonded octadecylsilane (C18). Allow the spots to dry, and place the plates in a developing chamber containing a mixture of methanol and water (7:3). Develop the chromatogram until the solvent front has moved about 15 cm above the line of application. Remove the plate, and allow the solvent to evaporate. Spray the plate with *Chloramine T–trichloroacetic acid reagent,* freshly mixed, and heat in an oven at 110° for 10 minutes. Examine the plate under long-wavelength UV light:

USP Monographs

www.webofpharma.com

the R_F value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution*.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Dissolution ⟨711⟩— [NOTE—Throughout this procedure, use scrupulously clean glassware, which previously has been rinsed successively with hydrochloric acid, water, and alcohol, and carefully dried. Take precautions to prevent contamination from fluorescent particles and from metal and rubber surfaces.]

Medium: 0.1 N hydrochloric acid; 500 mL. [NOTE—Use the same batch of *Dissolution Medium* throughout the test.]

Apparatus 1: 120 rpm.

Time: 60 minutes.

Ascorbic acid–methanol solution—Prepare a solution containing 2 mg of ascorbic acid per mL of methanol.

Hydrogen peroxide–methanol solution—On the day of use, dilute 2.0 mL of recently assayed 30 percent hydrogen peroxide with methanol to 100 mL. Store in a refrigerator. Just prior to use, dilute 2.0 mL of this solution with methanol to 100 mL.

Standard solutions—Transfer about 25 mg of USP Digoxin RS, accurately weighed, to a 500-mL volumetric flask. Dissolve in a minimum amount of alcohol, add dilute alcohol (4 in 5) to volume, and mix. Dilute 10.0 mL of this solution with dilute alcohol (4 in 5) to 100.0 mL, and mix. Just prior to use, dilute suitable aliquots of the resulting solution with *Dissolution Medium* to 50.0 mL to prepare *Standard solutions* equivalent to 20%, 40%, 60%, 80%, and 100%, respectively, of the labeled amount of digoxin in 500 mL.

Test solution—Promptly after withdrawal, pass a portion of the solution under test through a filter having a 0.8-µm or finer porosity, discarding the first 10 mL of the filtrate. This is the *Test solution*.

Procedure—Transfer to individual glass-stoppered flasks duplicate 1.0-mL portions of the *Test solution,* 1.0-mL portions of each of the *Standard solutions,* and 1.0 mL of the *Medium* to provide a blank. Begin with the *Standard solutions,* and keep all flasks in the same sequence throughout, so that the elapsed time from addition of reagents to reading of fluorescence is the same for each flask in the set. Treating one flask at a time, add the following three reagents, in the order named, in as rapid a sequence as possible, swirling after each addition: 1.0 mL of *Ascorbic acid–methanol solution,* 5.0 mL of hydrochloric acid, and 1.0 mL of *Hydrogen peroxide–methanol solution.* Insert the stoppers in the flasks, and after 2 hours, measure the fluorescence at about 485 nm, the excitation wavelength being about 372 nm. To check the stability of the fluorometer, repeat the measurement of fluorescence on one or more treated *Standard solutions*. Correct each reading for the blank, and plot a standard curve of fluorescence versus percentage dissolution. Determine the percentage dissolution of digoxin in the *Test solution* by reading from the standard graph.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{41}H_{64}O_{14}$ is dissolved in 60 minutes. The requirement is met if the quantities dissolved from the Tablets tested conform to the accompanying acceptance table instead of the table shown under *Dissolution* ⟨711⟩.

Acceptance Table

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is not less than Q + 5%.
S_2	6	Average of 12 units (S_1 + S_2) is equal to or greater than Q, and no unit is less than Q − 5%.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase, Chromatographic system, and *System suitability preparation*—Proceed as directed in the *Assay* under *Digoxin*.

Standard preparation—Dissolve an accurately weighed quantity of USP Digoxin RS in diluted alcohol, and dilute quantitatively and stepwise with diluted alcohol to obtain a solution having a known concentration of about 40 µg per mL. Use a sonic bath to aid dissolution.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 mg of digoxin, to a glass-stoppered, 50-mL conical flask. Add 25.0 mL of diluted alcohol with swirling, sonicate for about 30 minutes, and cool. Filter a portion of this solution through a 0.8-µm porosity membrane filter, discarding the first 10 mL of the filtrate.

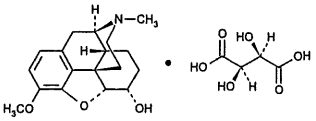
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{41}H_{64}O_{14}$ in the portion of Tablets taken by the formula:

$$25C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Digoxin RS in the *Standard preparation;* and r_U and r_S are the responses for the digoxin peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dihydrocodeine Bitartrate

Change to read:



$C_{18}H_{23}NO_3 \cdot C_4H_6O_6$ 451.47

Morphinan-6-ol, 4,5-epoxy-3-methoxy-17-methyl-, (5α,6α)-2,3-dihydroxybutanedioate (1:1) (salt);
4,5α-Epoxy-3-methoxy-17-methylmorphinan-6α-ol (+)-tartrate (salt) [▲5965-13-9].

Dihydrocodeine (free base)

$C_{18}H_{23}NO_3$ 301.39

[125-28-0▲ (USP 1-May-2019)].

DEFINITION

Dihydrocodeine Bitartrate contains NLT 98.0% and NMT 102.0% of dihydrocodeine bitartrate ($C_{18}H_{23}NO_3 \cdot C_4H_6O_6$), calculated on the dried basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

Change to read:

• **▲B.▲** (USP 1-May-2019) The retention time of the dihydrocodeine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **▲C.▲** (USP 1-May-2019) **IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Tartrate*:Meets the requirements

Delete the following:

▲• C.
Analysis: To a solution of 20 mg in 5 mL of sulfuric acid in a test tube, add 1 drop of ferric chloride TS, and heat in a boiling water bath for 2 min.
Acceptance criteria: Although the solution may darken, no blue color is produced (distinction from codeine and morphine).▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**
▲Buffer: Dissolve 2.0 g of monobasic potassium phosphate and 1.0 g of sodium 1-heptanesulfonate in 1000 mL of water. Adjust with 50% sodium hydroxide to a pH of 7.0.
Solution A: Acetonitrile and *Buffer* (5:195)
Solution B: Acetonitrile, *Buffer*, and water (140:35:10)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
10	65	35
13	40	60
16	40	60
16.5	75	25
23	75	25

Standard solution: 2.25 mg/mL of USP Dihydrocodeine Bitartrate RS in *Solution A*. Sonicate to dissolve, if necessary.
Sample solution: 2.25 mg/mL of Dihydrocodeine Bitartrate in *Solution A*. Sonicate to dissolve, if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 45°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dihydrocodeine bitartrate ($C_{18}H_{23}NO_3 \cdot C_4H_6O_6$) in the portion of Dihydrocodeine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dihydrocodeine from the *Sample solution*
r_S = peak response of dihydrocodeine from the *Standard solution*
C_S = concentration of USP Dihydrocodeine Bitartrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Dihydrocodeine Bitartrate in the *Sample solution* (mg/mL)▲ (USP 1-May-2019)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Delete the following:

▲• ORDINARY IMPURITIES ⟨466⟩
Standard solution and **Sample solution:** Water
Eluant: A mixture of methylene chloride, methanol, and ammonium hydroxide (90:10:1)
Visualization: 17, and view under short-wavelength UV light
Acceptance criteria: Meet the requirements▲ (USP 1-May-2019)

Add the following:

▲• ORGANIC IMPURITIES
Buffer, Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.00113 mg/mL of USP Dihydrocodeine Bitartrate RS in *Solution A*. Sonicate to dissolve, if necessary.
System suitability stock solution A: 0.3 mg/mL each of USP Morphine Sulfate RS and USP Codeine Sulfate RS prepared as follows. Dissolve suitable amounts of USP Morphine Sulfate RS and USP Codeine Sulfate RS with 20% of the final volume of methanol in a suitable volumetric flask. Dilute with *Solution A* to volume. Sonicate to dissolve, if necessary.
System suitability stock solution B: Prepare as directed for the *Standard solution* in the *Assay*.
System suitability solution: 0.015 mg/mL each of USP Morphine Sulfate RS and USP Codeine Sulfate RS, and 2.25 mg/mL of USP Dihydrocodeine Bitartrate RS prepared as follows. Dilute *System suitability stock solution A* with *System suitability stock solution B*.
Standard solution: 0.00225 mg/mL of USP Dihydrocodeine Bitartrate RS in *Solution A*. Sonicate to dissolve, if necessary.
System suitability
Samples: *Sensitivity solution*, *System suitability solution*, and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 2 between morphine and dihydrocodeine; NLT 2 between dihydrocodeine and codeine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Dihydrocodeine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of dihydrocodeine from the *Standard solution*
C_S = concentration of USP Dihydrocodeine Bitartrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Dihydrocodeine Bitartrate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tartaric acid[a]	0.33	—	—
Dihydromorphine[b]	0.52	1.4	0.50
Morphine	0.64	1.3	0.50
Dihydrocodeine bitartrate	1.00	1.0	—
Codeine	1.39	1.4	0.50
Hydrocodone	1.84	1.0	0.50
Tetrahydrothebaine[c]	2.15	1.4	0.15
Individual unspecified impurities	—	1.0	0.10
Total impurities	—	—	1.5

[a] Counter ion; not to be included in the total impurities.
[b] 4,5α-Epoxy-17-methylmorphinan-3,6α-diol.
[c] 4,5α-Epoxy-3,6-dimethoxy-17-methylmorphinan. ▲(USP 1-May-2019)

SPECIFIC TESTS

Delete the following:

▲• **AMMONIUM SALTS**
Analysis: To about 100 mg of Dihydrocodeine Bitartrate in a suitable test tube, add 5 mL of 1 N sodium hydroxide, and heat on a steam bath
Acceptance criteria: The odor of ammonia is not detected ▲(USP 1-May-2019)
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry Dihydrocodeine Bitartrate at 105° for 4 h.
Acceptance criteria: NMT 0.5%

Delete the following:

▲• **MELTING RANGE OR TEMPERATURE** ⟨741⟩, *Class I*: 186°–190°, but the range between beginning and end of melting does not exceed 2.5° ▲(USP 1-May-2019)
• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL of Dihydrocodeine Bitartrate in water
Acceptance criteria: Between –72° and –75°
• **pH** ⟨791⟩
Sample solution: 100 mg/mL of Dihydrocodeine Bitartrate in water
Acceptance criteria: 3.2–4.2

ADDITIONAL REQUIREMENTS

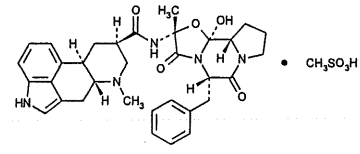
Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight containers. ▲Protect from light. ▲(USP 1-May-2019)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲USP Codeine Sulfate RS ▲(USP 1-May-2019)
USP Dihydrocodeine Bitartrate RS
▲USP Morphine Sulfate RS ▲(USP 1-May-2019)

Dihydroergotamine Mesylate



$C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$ 679.78
Ergotaman-3′,6′,18-trione, 9,10-dihydro-12′-hydroxy-2′-methyl-5′-(phenylmethyl)-, (5′α)-, monomethanesulfonate (salt).
Dihydroergotamine monomethanesulfonate [6190-39-2].

» Dihydroergotamine Mesylate contains not less than 97.0 percent and not more than 103.0 percent of $C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Dihydroergotamine Mesylate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲(CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲(CN 1-May-2020)—
Solution: 50 µg per mL.
Medium: 70% alcohol.

www.webofpharma.com

Absorptivities at 280 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: The principal spot from the *Test preparation* found in the test for *Related alkaloids* corresponds in R_F value to that obtained from the *Standard preparation.*

Specific rotation ⟨781S⟩**:** between −16.7° and −22.7°.

Test solution: 25 mg per mL, in a mixture of chloroform, alcohol, and ammonium hydroxide (10:10:1).

pH ⟨791⟩**:** between 4.4 and 5.4, in a solution (1 in 1000).

Loss on drying ⟨731⟩—Dry it in vacuum at 100° to constant weight: it loses not more than 4.0% of its weight.

Related alkaloids—

Solvent mixture—Mix 10 volumes of chloroform, 10 volumes of methanol, and 1 volume of ammonium hydroxide.

Test solution—Prepare a solution of Dihydroergotamine Mesylate in *Solvent mixture* to contain 20 mg per mL.

Standard solution and *Standard dilutions*—Prepare a solution of USP Dihydroergotamine Mesylate RS in *Solvent mixture* to contain 20 mg per mL *(Standard solution).* Prepare a series of dilutions of the *Standard solution* in *Solvent mixture* to contain 0.40 mg, 0.20 mg, and 0.10 mg per mL *(Standard dilutions).*

Procedure—In a suitable chromatographic chamber arranged for thin-layer chromatography place a volume of a solvent system consisting of a mixture of chloroform and alcohol (9:1) sufficient to develop the chromatogram, cover, and allow to equilibrate for 30 minutes. Apply 5-µL portions of the *Test solution,* the *Standard solution,* and each of the three *Standard dilutions* to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with a solution prepared by dissolving 800 mg of *p*-dimethylaminobenzaldehyde in a cooled mixture of 80 g of alcohol and 20 g of sulfuric acid. The R_F value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution.* Estimate the concentration of any other spots observed in the lane for the *Test solution* by comparison with the *Standard dilutions.* The spots from the 0.40-, 0.20-, and 0.10-mg-per-mL dilutions are equivalent to 2.0%, 1.0%, and 0.50% of impurities, respectively. The sum of the impurities is not greater than 2.0%.

Assay—

Diluent 1—Prepare a solution of 0.1 mL of phosphoric acid in 1000 mL of water.

Diluent 2—Prepare a mixture of *Diluent 1* and acetonitrile (60:40).

Solution A—Prepare a filtered and degassed mixture of water, 25 percent ammonia water, and 98% formic acid (1000:10:5). Adjust the pH to 8.50.

Solution B—Prepare a filtered and degassed mixture of acetonitrile and *Solution A* (80:20).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments to either solution as necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Dihydroergotamine Mesylate RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with *Diluent 1* to obtain a solution having a known concentration of about 0.6 mg per mL.

[NOTE—The final ratio of acetonitrile and *Diluent 1* should be similar to the final ratio obtained in the *Assay preparation.*]

Assay preparation—Transfer about 30 mg of Dihydroergotamine Mesylate, accurately weighed, to a 50-mL volumetric flask, dissolve in 20 mL of acetonitrile, dilute with *Diluent 1* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and 4.0-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	60	40	equilibration
0–12	60→50	40→50	linear gradient
12–20	50→15	50→85	linear gradient
20–24	15	85	isocratic
24–25	15→60	85→40	linear gradient
25–31	60	40	re-equilibration

Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the tailing factor is between 0.8 and 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the analyte peaks. Calculate the quantity, in mg, of $C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$ in the portion of Dihydroergotamine Mesylate taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dihydroergotamine Mesylate RS in the *Standard preparation;* and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dihydroergotamine Mesylate Injection

DEFINITION

Dihydroergotamine Mesylate Injection is a sterile solution of Dihydroergotamine Mesylate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of dihydroergotamine mesylate ($C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$).

IDENTIFICATION

• **A.**

Sample solution: 2 mL of Injection in 25 mL water

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of a similar solution of USP Dihydroergotamine Mesylate RS.

ASSAY

• **PROCEDURE**

Diluent: 10 mg/mL of tartaric acid in water

Solution A: Dissolve 250 mg of *p*-dimethylaminobenzaldehyde in a cooled mixture of 130 mL of sulfuric acid and 70 mL of water, and add 0.40 mL of ferric chloride solution (1 in 20).

Standard solution: 50 µg/mL of USP Dihydroergotamine Mesylate RS in *Diluent*

USP Monographs

www.webofpharma.com

Sample solution: Nominally 50 µg/mL of dihydroergotamine mesylate from a suitable volume of Injection containing NLT 5 mg of dihydroergotamine mesylate in *Diluent*
Blank: *Diluent*
Instrumental conditions
Mode: Vis
Analytical wavelength: 585 nm
Cell: 1 cm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Transfer 5.0 mL each of the *Standard solution*, the *Sample solution*, and the *Blank* to separate 50-mL conical flasks. Add 10.0 mL of *Solution A* to each flask, shake, and allow to stand for 30 min. Measure the absorbance of the resulting *Standard solution* and *Sample solution* against the *Blank*.
Calculate the percentage of the labeled amount of dihydroergotamine mesylate ($C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$) in the portion of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

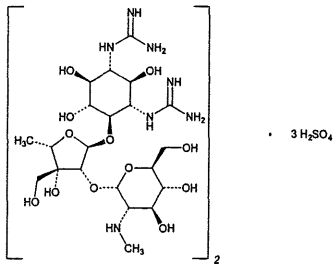
A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Dihydroergotamine Mesylate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dihydroergotamine mesylate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 175.0 USP Endotoxin Units/mg of dihydroergotamine mesylate
- **pH** ⟨791⟩: 3.4–4.9
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, and protect from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Dihydroergotamine Mesylate RS

Dihydrostreptomycin Sulfate



$(C_{21}H_{41}N_7O_{12})_2 \cdot 3H_2SO_4$ 1461.42
Dihydrostreptomycin sulfate (2:3) (salt) [5490-27-7].

» Dihydrostreptomycin Sulfate has a potency equivalent to not less than 650 µg of dihydrostreptomycin ($C_{21}H_{41}N_7O_{12}$) per mg, except that if it is labeled as being crystalline, it has a potency equivalent to not less than 725 µg of dihydrostreptomycin per mg, or if it is labeled as being solely for oral use, it has a potency equivalent to not less than 450 µg of dihydrostreptomycin per mg.

Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate that it is intended for veterinary use only. If it is crystalline, it may be so labeled. If it is intended solely for oral use, it is so labeled. Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Dihydrostreptomycin Sulfate RS
USP Streptomycin Sulfate RS

Identification—
A: To a solution of 4 mg in 2 mL of water, add 0.5 mL of 1 N hydrochloric acid, and heat in a water bath for 20 minutes. Remove the tube from the bath, and add 1.0 mL of a 1 in 200 solution of 1-naphthol in 1 N sodium hydroxide. Heat again for 10 minutes, cool briefly in an ice bath, and add water to make 25 mL: a red color develops, intensifying during about 10 minutes.
B: A solution (1 in 50) responds to the tests for *Sulfate* ⟨191⟩.
Crystallinity ⟨695⟩ (where labeled as being crystalline): meets the requirements.
pH ⟨791⟩: between 4.5 and 7.0, in a solution containing 200 mg of dihydrostreptomycin per mL, except that if it is labeled as being solely for oral use, the pH is between 3.0 and 7.0.
Loss on drying ⟨731⟩—Dry about 100 mg in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 5.0% of its weight, except that if it is labeled as being solely for oral use, it loses not more than 14.0% of its weight.

Streptomycin—
Ferric chloride stock solution—Dissolve 5 g of ferric chloride in 50 mL of 0.1 N hydrochloric acid.
Ferric chloride solution—Dilute 2.5 mL of *Ferric chloride stock solution* with sufficient 0.01 N hydrochloric acid to make 100 mL. Use this solution within 1 day.
Standard solutions—Dissolve an accurately weighed quantity of USP Streptomycin Sulfate RS in water to obtain a stock solution containing 1.0 mg of streptomycin ($C_{21}H_{39}N_7O_{12}$) per mL. Transfer 1.0, 2.0, 3.0, 4.0, and 5.0 mL, respectively, of this stock solution to each of five 25-mL volumetric flasks. Transfer 9.0, 8.0, 7.0, 6.0, and 5.0 mL of water to the flasks, respectively.
Test solution—Transfer about 800 mg of Dihydrostreptomycin Sulfate, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 25-mL volumetric flask.
Procedure—To each of the flasks containing the *Standard solutions* and the *Test solution*, and to a seventh 25-mL volumetric flask containing 10.0 mL of water to provide a blank, add 2.0 mL of 1 N sodium hydroxide, and heat in a water bath for 10 minutes. Cool the flasks in ice water for 3 minutes, and to each add 2.0 mL of 1.2 N hydrochloric acid and 5.0 mL of *Ferric chloride solution*. Dilute with water to volume, and mix. Concomitantly determine the absorbances of the solutions from the *Standard solutions* and the *Test solution* at the wavelength of maximum absorbance at about 550 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. Plot the absorbance values of the solutions from the *Standard solutions* versus concentration, in

USP Monographs

www.webofpharma.com

µg per mL, of streptomycin, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, *C*, in µg per mL, of streptomycin in the solution from the *Test solution*. Calculate the percentage of streptomycin in the portion of Dihydrostreptomycin Sulfate taken by the formula:

$$6250C/WP$$

in which *W* is the weight, in mg, of Dihydrostreptomycin Sulfate taken, and *P* is the potency, in µg of dihydrostreptomycin per mg, of the Dihydrostreptomycin Sulfate taken as determined in the *Assay:* not more than 3.0% is found, except that if it is labeled as being crystalline, not more than 1.0% is found, or if it is labeled as being solely for oral use, not more than 5.0% is found.

Other requirements—Where the label states that Dihydrostreptomycin Sulfate is sterile, it meets the requirements for *Sterility* and *Bacterial Endotoxins Test 85* — under *Dihydrostreptomycin Injection*. Where the label states that Dihydrostreptomycin Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial Endotoxins Test 85* — under *Dihydrostreptomycin Injection*.

Assay—Proceed with Dihydrostreptomycin Sulfate as directed for the turbidimetric assay of dihydrostreptomycin under *Antibiotics—Microbial Assays* ⟨81⟩.

Dihydrostreptomycin Sulfate Boluses

» Dihydrostreptomycin Sulfate Boluses contain the equivalent of not less than 85.0 percent and not more than 120.0 percent of the labeled amount of dihydrostreptomycin ($C_{21}H_{41}N_7O_{12}$).

Packaging and storage—Preserve in tight containers.

Labeling—Label Boluses to indicate that they are intended for veterinary use only.

USP Reference standards ⟨11⟩—
USP Dihydrostreptomycin Sulfate RS

Loss on drying ⟨731⟩—Dry about 100 mg, accurately weighed, of finely ground Boluses in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 10.0% of its weight.

Assay—Proceed as directed for the cylinder-plate assay of dihydrostreptomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using 3 Boluses added to a mixture of 499.0 mL of *Buffer B.3* and 1.0 mL of polysorbate 80 and blended for 5 minutes in a high-speed glass blender jar. Allow to stand for not less than 1 hour, and repeat the blending. While stirring this mixture, withdraw an accurately measured volume of it, and dilute quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Dihydrostreptomycin Injection

» Dihydrostreptomycin Injection contains an amount of Dihydrostreptomycin Sulfate equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of dihydrostreptomycin ($C_{21}H_{41}N_7O_{12}$). It contains one or more suitable preservatives.

Packaging and storage—Preserve in single-dose or multiple-dose containers.

Labeling—Label it to indicate that it is intended for veterinary use only.

USP Reference standards ⟨11⟩—
USP Dihydrostreptomycin Sulfate RS

Identification—It responds to the *Identification* tests under *Dihydrostreptomycin Sulfate*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.5 USP Endotoxin Unit per mg of dihydrostreptomycin.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH ⟨791⟩: between 5.0 and 8.0.

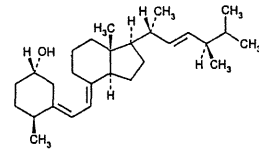
Assay—

Assay preparation 1 (where it is represented as being in a single-dose container)—Withdraw all of the withdrawable contents of Injection, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution containing a convenient quantity of dihydrostreptomycin in each mL.

Assay preparation 2 (where the label states the quantity of dihydrostreptomycin in a given volume of solution)—Dilute an accurately measured volume of Injection quantitatively with water to obtain a solution containing a convenient quantity of dihydrostreptomycin in each mL.

Procedure—Proceed as directed for the turbidimetric assay of dihydrostreptomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of *Assay preparation* diluted quantitatively with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Dihydrotachysterol



$C_{28}H_{46}O$ 398.66

9,10-Secoergosta-5,7,22-trien-3-ol, (3β,5*E*,7*E*,10α,22*E*)-.
Dihydrotachysterol.
9,10-Secoergosta-5,7,22-trien-3β-ol [67-96-9].

» Dihydrotachysterol contains not less than 97.0 percent and not more than 103.0 percent of $C_{28}H_{46}O$.

Packaging and storage—Preserve in light-resistant, hermetic glass containers from which air has been displaced by an inert gas.

USP Reference standards ⟨11⟩—
USP Dihydrotachysterol RS

www.webofpharma.com

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 8 µg per mL.
Medium: alcohol.
Specific rotation ⟨781S⟩: between +100° and +103°.
Test solution: 20 mg per mL, in alcohol.
Residue on ignition ⟨281⟩: not more than 0.1%.

Assay—
Mobile phase—Prepare a degassed and filtered solution of isooctane and isopropyl alcohol (100:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩.
Standard preparation—Dissolve an accurately weighed quantity of USP Dihydrotachysterol RS in *Mobile phase*, and dilute quantitatively and stepwise, if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 10 µg per mL.
Assay preparation—Transfer about 50 mg of Dihydrotachysterol, accurately weighed, to a 500-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 10 mL of the resulting solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
System suitability preparation—Prepare a solution of ergocalciferol in *Mobile phase* having a concentration of about 0.7 mg per mL. Reflux under nitrogen for 20 minutes, and cool to ambient temperature (*Solution A*). Pipet 3 mL of *Solution A* and 2 mL of a solution in *Mobile phase* containing 0.1 mg of dihydrotachysterol per mL into a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.5%. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for pre-ergocalciferol, 0.7 for dihydro tachysterol, and 1.0 for ergocalciferol; and the resolution, *R*, between the pre-ergocalciferol and dihydrotachysterol peaks is not less than 1.5.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{28}H_{46}O$ in the portion of Dihydrotachysterol taken by the formula:

$$5C(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Dihydrotachysterol RS in the *Standard preparation*, and r_U and r_S are the peak responses for dihydrotachysterol obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dihydrotachysterol Capsules

» Dihydrotachysterol Capsules contain a solution of Dihydrotachysterol in a suitable vegetable oil. Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{28}H_{46}O$.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Dihydrotachysterol RS

Identification—
A: Cut open 1 Capsule, and remove the contents: the Capsule contents respond to *Identification* test *A* under *Dihydrotachysterol Oral Solution.*
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay.*
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase , *Standard preparation, System suitability preparation,* and *Chromatographic system*—Prepare as directed in the *Assay*— under *Dihydrotachysterol.*
Assay preparation—Combine the contents of not less than 20 Capsules. Transfer an accurately weighed quantity of Capsule contents, equivalent to about 500 µg of dihydro tachysterol, to a 50-mL volumetric flask, dissolve in 25 mL of *Mobile phase*, and mix. Dilute with *Mobile phase* to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Dihydrotachysterol.* Calculate the quantity, in µg, of $C_{28}H_{46}O$ in the portion of Capsules taken by the formula:

$$50C(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Dihydrotachysterol RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dihydrotachysterol Oral Solution

» Dihydrotachysterol Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dihydrotachysterol ($C_{28}H_{46}O$).

Packaging and storage—Preserve in tight, light-resistant glass containers.
USP Reference standards ⟨11⟩—
USP Dihydrotachysterol RS

Identification—
A: Place 1 drop of Oral Solution on a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Spray the plate with 3.5% phosphomolybdic acid solution (prepared by dissolving 3.5 g of phospho molybdic acid in 100 mL of isopropyl alcohol), and immediately heat the plate over a hot plate: a dark blue spot appears on a yellow background.

www.webofpharma.com

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Assay—
Mobile phase, Standard preparation, System suitability preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Dihydrotachysterol.*

Assay preparation [FOR ORAL SOLUTION IN AN OIL MEDIUM]—Transfer an accurately measured volume of Oral Solution, equivalent to about 500 µg of dihydrotachysterol, by means of a "to contain" pipet to a 50-mL volumetric flask. Rinse the pipet with *Mobile phase,* add the rinsing to the flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation [FOR ORAL SOLUTION IN AN AQUEOUS MEDIUM]—Transfer an accurately measured volume of Oral Solution, equivalent to about 600 µg of dihydrotachysterol, to a separator containing about 30 mL of water. Add about 1 g of sodium chloride, mix, and extract with three 15-mL portions of chloroform, filtering each portion through absorbent cotton into a suitable glass-stoppered conical flask. Wash the cotton with about 5 mL of chloroform, collecting the washing in the glass-stoppered conical flask. Evaporate the chloroform extracts, and wash with the aid of a current of air to dryness. Dissolve the residue in 50.0 mL of *Mobile phase,* and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Dihydrotachysterol.* Calculate the quantity, in µg, of dihydrotachysterol ($C_{28}H_{46}O$) in the volume of Oral Solution taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Dihydrotachysterol RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dihydrotachysterol Tablets

» Dihydrotachysterol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{28}H_{46}O$.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Dihydrotachysterol RS

Identification—
A: Transfer a portion of powdered Tablets, equivalent to about 2 mg of dihydrotachysterol, to a glass-stoppered flask, add about 25 mL of methylene chloride, shake for 15 minutes, and filter. Evaporate the filtrate to dryness, and dissolve the residue in 0.4 mL of methylene chloride. Apply 10 µL of this solution and 10 µL of a Standard solution of USP Dihydrotachysterol RS in methylene chloride containing 5 mg per mL at separate points on a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram using a solvent system consisting of a mixture of ether and cyclo hexane (1:1) until the solvent front has moved about three-fourths of the length of the plate. Air-dry, spray lightly with a 1 in 5 solution of *p*-toluenesulfonic acid in a mixture of alcohol and propylene glycol (9:1), and heat at 80° until reddish brown spots appear (about 10 minutes): the R_F value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* obtained as directed in the *Assay.*
Disintegration ⟨701⟩: 10 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase , Standard preparation, System suitability preparation, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Dihydrotachysterol.*

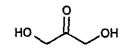
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2.5 mg of dihydrotachysterol, to a suitable container, add 100.0 mL of dehydrated alcohol, shake by mechanical means for 10 minutes, sonicate, and centrifuge. Transfer 20.0 mL of the supernatant to a 50-mL volumetric flask, and evaporate with the aid of a stream of nitrogen to dryness, observing precautions to avoid exposure to moisture and light. Dissolve the residue in *Mobile phase,* dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Dihydrotachysterol.* Calculate the quantity, in µg, of $C_{28}H_{46}O$ in the portion of Tablets taken by the formula:

$$250C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Dihydrotachysterol RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dihydroxyacetone



$C_3H_6O_3$ 90.08
1,3-Dihydroxy-2-propanone [96-26-4].

DEFINITION
Dihydroxyacetone contains NLT 98.0% and NMT 102.0% of dihydroxyacetone ($C_3H_6O_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the test for *Organic Impurities.*

ASSAY
- **PROCEDURE**

Sample solution: Dissolve 0.1 g of Dihydroxyacetone in 20 mL of water, add 20 mL of 0.1 M periodic acid, and allow

USP Monographs

www.webofpharma.com

to stand at room temperature in the dark for 20 min. Add 3 g of sodium bicarbonate, 20 mL of 0.6 M potassium iodide, and 3 mL of starch TS.

Analysis: Titrate with 0.05 M sodium arsenite VS. Perform a blank titration, and make any necessary corrections. Each mL of 0.05 M sodium arsenite is equivalent to 4.504 mg of dihydroxyacetone ($C_3H_6O_3$).

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩**:** NMT 0.1%
- **Iron** ⟨241⟩**:** NMT 0.002%
- **Organic Impurities**

Glycerin solution: 0.25 mg/mL of glycerin in methanol

Standard solution: 50 mg/mL of USP Dihydroxyacetone RS in methanol

Sample solution: 50 mg/mL of Dihydroxyacetone in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 1 µL

Developing solvent system: Acetone and water (19:1)

Spray reagent: Toluene, saturated solution of lead tetraacetate in glacial acetic acid, and 1% solution of dichlorofluorescein in alcohol (190:5:1)

Analysis

Samples: *Glycerin solution*, *Standard solution*, and *Sample solution*

Spray the plate with *Spray reagent*, and dry the plate for 5 min at 105°. Examine the plate under short-wavelength UV light, and compare the intensities of the glycerin spot observed in the *Sample solution* with that of the principal spot of the *Glycerin solution*.

Acceptance criteria: 0.5%; the glycerin spot of the *Sample solution* is not larger or more intense than the principal spot of the *Glycerin solution*, and no other secondary spots are observed in the *Sample solution*.

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: 50 mg/mL in water

Acceptance criteria: 4.0–6.0

- **Water Determination,** *Method I* ⟨921⟩**:** NMT 0.2%
- **Limit of Protein**

Sample solution: 250 mg/mL of Dihydroxyacete in water. Transfer 100 µL of this solution to a 5-mL volumetric flask, dilute with brilliant blue G TS to volume, and mix.

Instrumental conditions

Mode: UV

Analytical wavelength: 595 nm

Blank: 100 µL of water and 5 mL of brilliant blue G TS

Analysis: Determine the absorbance of the *Sample solution*.

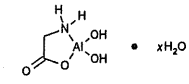
Acceptance criteria: NMT 0.400

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers in a cool place.
- **USP Reference Standards** ⟨11⟩

USP Dihydroxyacetone RS

Dihydroxyaluminum Aminoacetate



$C_2H_6AlNO_4 \cdot xH_2O$

Aluminum, (glycinato-*N*,*O*)dihydroxy-, hydrate.

(Glycinato)dihydroxyaluminum hydrate [41354-48-7].

Anhydrous 135.05

[13682-92-3].

» Dihydroxyaluminum Aminoacetate yields not less than 94.0 percent and not more than 102.0 percent of dihydroxyaluminum aminoacetate ($C_2H_6AlNO_4$), calculated on the dried basis. It may contain small amounts of aluminum oxide and of Aminoacetic Acid.

Packaging and storage—Preserve in well-closed containers.

Identification—Suspend 1 g in 25 mL of water, add hydrochloric acid, dropwise, until a clear solution is formed, and divide it into two equal parts for the following tests.

A: One portion of the solution responds to the tests for *Aluminum* ⟨191⟩.

B: To the other portion of the solution add 1 drop of liquefied phenol and 5 mL of sodium hypochlorite TS: a blue color is produced.

pH ⟨791⟩: between 6.5 and 7.5, in a suspension of 1 g of it, finely powdered, in 25 mL of water.

Loss on drying ⟨731⟩—Dry it at 130° to constant weight: it loses not more than 14.5% of its weight.

Mercury, *Method IIa* ⟨261⟩—Transfer 2.0 g to a 100-mL beaker, and add 35 mL of 1 N sulfuric acid: the limit is 1 ppm.

Isopropyl alcohol—Transfer about 5 g to a flask provided with a reflux condenser, and add 100 mL of potassium permanganate solution (1 in 300) and 10 mL of sulfuric acid. Reflux the mixture for 30 minutes, distill, and collect 10 mL of the distillate. To 1 mL of the distillate add 5 drops of sodium nitroferricyanide TS and 2 mL of 1 N sodium hydroxide, then add a slight excess of 6 N acetic acid: no red color is produced.

Nitrogen—Determine the nitrogen content as directed under *Nitrogen Determination, Method II* ⟨461⟩, using about 100 mg, previously dried and accurately weighed. Each mL of 0.1 N sulfuric acid is equivalent to 1.401 mg of nitrogen. Not less than 9.90% and not more than 10.60% of nitrogen is found.

Assay—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Procedure—Transfer about 2.5 g of Dihydroxyaluminum Aminoacetate, accurately weighed, to a 150-mL beaker, add 15 mL of hydrochloric acid, and warm, if necessary, to dissolve the specimen completely. Transfer the solution with the aid of water to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 20.0 mL of this solution to a 250-mL beaker, and add, with continuous stirring, 25.0 mL of *Edetate disodium titrant* and then 20 mL of acetic acid–ammonium acetate buffer TS. Heat the solution near the boiling point for 5 minutes, cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose pink. Perform a blank determination, substituting 20 mL of water for the assay solution, and make any necessary correction. Each mL of

USP Monographs

www.webofpharma.com

0.05 M *Edetate disodium titrant* is equivalent to 6.753 mg of $C_2H_6AlNO_4$.

Dihydroxyaluminum Aminoacetate Magma

» Dihydroxyaluminum Aminoacetate Magma is a suspension that contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_2H_6AlNO_4$.

Packaging and storage—Preserve in tight containers, and protect from freezing.

Identification—Dilute a volume of Magma, equivalent to about 1 g of dihydroxyaluminum aminoacetate, with water to 25 mL, add hydrochloric acid, dropwise, until a solution results, and then filter: the filtered solution responds to the *Identification*— tests under *Dihydroxyaluminum Aminoacetate*.

Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—The total bacterial count does not exceed 100 cfu per mL, and the test for *Escherichia coli* is negative.

Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than the number of mEq calculated by the formula:

$$0.8(0.0148D)$$

in which 0.0148 is the theoretical acid-neutralizing capacity, in mEq, of $C_2H_6AlNO_4$, and D is the quantity, in mg, of $C_2H_6AlNO_4$ in the specimen tested, based on the labeled quantity.

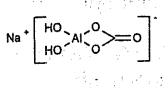
pH ⟨791⟩: between 6.5 and 7.5, in a dilution in water, equivalent to about 1 g of dihydroxyaluminum aminoacetate in 25 mL.

Assay—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Procedure— [NOTE—Shake the container by mechanical means for 1 hour before removing the specimen.] Weigh accurately in a tared beaker a quantity of Dihydroxyaluminum Aminoacetate Magma, equivalent to about 2.5 g of dihydroxyaluminum aminoacetate, add 15 mL of hydrochloric acid, and boil gently for about 5 minutes. Cool, transfer the solution with the aid of water to a 500-mL volumetric flask, dilute with water to volume, mix, and filter, if necessary, to obtain a clear solution. Proceed as directed in the *Assay*— under *Dihydroxyaluminum Aminoacetate*, beginning with "Transfer 20.0 mL of this solution."

Dihydroxyaluminum Sodium Carbonate



$NaAl(OH)_2CO_3$ 143.99

Aluminum, [carbonato(1-)-*O*]dihydroxy-, monosodium salt; Sodium (*T*-4)-[carbonato(2-)-*O*,*O*′]dihydroxyaluminate(1-); Sodium (carbonato)dihydroxyaluminate(1-) [539-68-4; 16482-55-6].

DEFINITION

Dihydroxyaluminum Sodium Carbonate contains NLT 98.3% and NMT 107.9% of dihydroxyaluminum sodium carbonate $[NaAl(OH)_2CO_3]$, calculated on the dried basis.

IDENTIFICATION

- **A.**

  Sample solution: Combine 1 g with 20 mL of 3 N hydrochloric acid.

  Acceptance criteria: The sample dissolves with effervescence.

- **B. IDENTIFICATION TESTS—GENERAL,** *Aluminum* ⟨191⟩

  Sample: *Sample solution* prepared in *Identification* test *A*

  Acceptance criteria: Meets the requirements

- **C.** The *Sample solution*, prepared and tested as directed in the test for *Sodium Content*, exhibits a significant absorption at the sodium emission line at 589.0 nm.

ASSAY

- **PROCEDURE**

  Edetate disodium titrant: Dissolve 18.6 g of edetate disodium in water to make 500 mL, and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.

  Sample: 300 mg undried

  Analysis: Transfer the *Sample* to a 250-mL beaker, add 10 mL of 2 N sulfuric acid, cover the beaker, heat to 80° for 5 min, and boil for 1 min. Add 30.0 mL of 0.1 M edetate disodium VS, again boil for 1 min, cool, and then add 10 mL of acetic acid–ammonium acetate buffer TS, 50 mL of acetone, and 2 mL of dithizone TS. Using a pH meter, adjust with the addition of ammonium hydroxide or dilute sulfuric acid to a pH of 4.5. Titrate with 0.05 M zinc sulfate VS, maintaining the pH of 4.5 by the addition of ammonium hydroxide as necessary, to an orange-pink color. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M *Edetate disodium titrant* is equivalent to 14.40 mg of dihydroxyaluminum sodium carbonate $[NaAl(OH)_2CO_3]$.

  Acceptance criteria: 98.3%–107.9% on the dried basis

IMPURITIES

- **MERCURY,** *Method IIa* ⟨261⟩

  Sample solution: 2.0 g in 35 mL of 1 N sulfuric acid

  Acceptance criteria: NMT 1 ppm

- **ISOPROPYL ALCOHOL**

  Isopropyl alcohol-free dihydroxyaluminum sodium carbonate: Use a portion of Dihydroxyaluminum Sodium Carbonate that has been previously tested as directed in this section and found to be free of isopropyl alcohol.

  Sodium chloride solution: 0.2 g/mL in water

  Standard stock solution: 20 mg/mL of isopropyl alcohol in *Sodium chloride solution*

  Standard solution A: 0.4 mg/mL of isopropyl alcohol in *Sodium chloride solution* from *Standard stock solution*

  Standard solution B: 0.8 mg/mL of isopropyl alcohol in *Sodium chloride solution* from *Standard stock solution*

  Standard solution C: 1.0 mg/mL of isopropyl alcohol in *Sodium chloride solution* from *Standard stock solution*

  Standard solution D: 1.2 mg/mL of isopropyl alcohol in *Sodium chloride solution* from *Standard stock solution*

  Headspace containers: Use suitable 20-mL containers capable of being tightly closed with an inert septum and a metallic crimp cap.

  Standard preparations: To four separate 20-mL *Headspace containers*, add 1.0 g of *Isopropyl alcohol-free dihydroxyaluminum sodium carbonate*. To the containers add, respectively, 10.0 mL of the appropriate *Standard solution*. These containers contain about 4, 8, 10, and 12 mg of isopropyl alcohol, respectively. [NOTE—Keep the

USP Monographs

www.webofpharma.com

containers cool until sealed.] Seal the containers, place in a water bath maintained at 70°, and allow to stand for 1 h.

Sample preparation: Transfer 1.0 g of the Dihydroxyaluminum Sodium Carbonate to a *Headspace container*, and add 10.0 mL of *Sodium chloride solution*. [NOTE—Keep the container cool until sealed.] Seal the container, place in a water bath maintained at 70°, and allow to stand for 1 h.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.9-m × 3-mm; packed with support S3
Temperatures
Column: 180°
Injection port: 200°
Detector: 250°
Injection volume: 1 mL of gaseous phase

System suitability
Samples: *Standard preparations* containing 10 mg per container
Suitability requirements
Relative standard deviation: NMT 4% for replicate injections

Analysis
Samples: *Standard preparations* and *Sample preparation*
[NOTE—Use peak areas where peak responses are indicated.]
Using a gas-tight syringe, separately inject equal volumes of the gaseous headspace of the *Standard preparations* and the *Sample preparation* into the gas chromatograph. Record the chromatograms, and measure the peak responses. Determine, based on a retention time comparison, if isopropyl alcohol is detected in the *Sample preparation*. Plot the responses of the *Standard preparations* versus the content, in mg, of isopropyl alcohol in each container, draw the straight line best fitting the plotted points, and calculate the correlation coefficient for the line. A suitable system is one that yields a line having a correlation coefficient of NLT 0.99. From the graph, determine the total amount, T_U, in mg, of isopropyl alcohol in the *Sample preparation*.
Calculate the percentage of isopropyl alcohol in the Dihydroxyaluminum Sodium Carbonate taken:

$$\text{Result} = 0.1 \times (T_U/W_U)$$

T_U = total amount of isopropyl alcohol in the *Sample preparation* (mg)
W_U = weight of the Dihydroxyaluminum Sodium Carbonate taken (g)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

• **SODIUM CONTENT**
Potassium chloride solution: 38 mg/mL of potassium chloride in water
Sodium chloride stock solution: 25.42 µg/mL of sodium chloride in water (10.0 µg/mL of sodium) from sodium chloride previously dried at 105° for 2 h
Standard solution A: 0.5 µg/mL of sodium from *Sodium chloride stock solution* prepared as follows. On the day of use, transfer 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* to a 100-mL volumetric flask. Add 5.0 mL of *Sodium chloride stock solution* and dilute with water to volume.
Standard solution B: 1.0 µg/mL of sodium from *Sodium chloride stock solution* prepared as follows. On the day of use, transfer 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* to a 100-mL volumetric flask. Add 10.0 mL *Sodium chloride stock solution* and dilute with water to volume.
Sample solution: Transfer 250 mg of Dihydroxyaluminum Sodium Carbonate, previously dried, to a 200-mL volumetric flask. Add 40 mL of 1 N hydrochloric acid, and boil for 1 min. Cool, and dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask containing 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution*, and dilute with water to volume.
Blank solution: Pipet 4 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* into a 100-mL volumetric flask, and dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Analytical wavelength: Sodium emission line at 589.0 nm
Lamp: Sodium hollow-cathode
Flame: Air–acetylene
Blank: *Blank solution*
Analysis
Samples: *Standard solution A*, *Standard solution B*, *Sample solution*, and *Blank solution*
Plot the absorbances of the *Standard solutions* versus the concentrations, in µg/mL of sodium, and draw a straight line between the plotted points. From the graph so obtained, determine the concentration, *C*, in µg/mL of sodium in the *Sample solution*.
Calculate the percentage of sodium in the portion of Dihydroxyaluminum Sodium Carbonate taken:

$$\text{Result} = 4000 \times (C/W)$$

C = concentration of sodium in the *Sample solution* (µg/mL)
W = weight of Dihydroxyaluminum Sodium Carbonate taken (mg)

Acceptance criteria: 15.2%–16.8%
• **ACID-NEUTRALIZING CAPACITY** ⟨301⟩
Sample: 425 mg of undried material
Analysis: Proceed as directed using the *Sample*. Each mg of dihydroxyaluminum sodium carbonate [$NaAl(OH)_2CO_3$] has an expected acid-neutralizing capacity of 0.0278 mEq.
Acceptance criteria: NLT 75.0% of the expected mEq value, calculated in relation to the results of the *Assay*
• **pH** ⟨791⟩**:** 9.2–10.2 in a suspension (1 in 25)
• **LOSS ON DRYING** ⟨731⟩
Sample: Dry at 130° to constant weight.
Acceptance criteria: NMT 14.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Dihydroxyaluminum Sodium Carbonate Chewable Tablets

Former title: Dihydroxyaluminum Sodium Carbonate Tablets

» Dihydroxyaluminum Sodium Carbonate Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of CH_2AlNaO_5.

USP Monographs

www.webofpharma.com

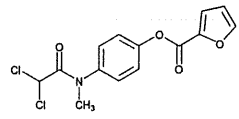
Packaging and storage—Preserve in well-closed containers.
Labeling—Label the Chewable Tablets to indicate that they are to be chewed before swallowing.
Identification—A 1 in 10 suspension of powdered Chewable Tablets in 3 N hydrochloric acid meets the requirements of the tests for *Aluminum* ⟨191⟩ and for *Sodium* ⟨191⟩.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Acid-neutralizing capacity ⟨301⟩—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than the number of mEq calculated by the formula:

$$0.8(0.0278D)$$

in which 0.0278 is the theoretical acid-neutralizing capacity, in mEq, of CH_2AlNaO_5, and D is the quantity, in mg, of CH_2AlNaO_5 in the specimen tested, based on the labeled quantity.

Assay—
Edetate disodium titrant—Dissolve 18.6 g of edetate disodium in water to make 500 mL, and standardize as directed in the *ASSAY* under *Ammonium Alum*.
Procedure—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 300 mg of dihydroxyaluminum sodium carbonate, to a 250-mL beaker, and proceed as directed in the *ASSAY* under *Dihydroxyaluminum Sodium Carbonate*, beginning with "add 10 mL of 2 N sulfuric acid." Each mL of 0.1 M *Edetate disodium titrant* is equivalent to 14.40 mg of CH_2AlNaO_5.

Diloxanide Furoate



$C_{14}H_{11}Cl_2NO_4$ 328.15
4-(*N*-Methyl-2,2-dichloroacetamido)phenyl 2-furoate; 2,2-Dichloroacetamido-4-*N*-methylphenyl 2-furoate [3736-81-0].

DEFINITION
Diloxanide Furoate contains NLT 98.0% and NMT 102.0% of diloxanide furoate ($C_{14}H_{11}Cl_2NO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Analytical wavelength: 258 nm
Sample solution: 14 µg/mL in alcohol
Acceptance criteria: Meets the requirements

- **C.**

Sample: 20 mg
Analysis: Burn the *Sample* by using the method in *Oxygen Flask Combustion* ⟨471⟩ using 10 mL of 1 N sodium hydroxide as the absorbing liquid. When the process is complete, acidify the liquid with nitric acid, and add a solution of silver nitrate (5 in 100).
Acceptance criteria: A white precipitate forms.

ASSAY
- **PROCEDURE**

Sample solution: 300 mg of Diloxanide Furoate in 50.0 mL of dried pyridine
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N tetrabutylammonium hydroxide VS
Endpoint detection: Potentiometric
Analysis: Titrate the *Sample solution* with *Titrant*. Perform a blank determination. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 32.82 mg of diloxanide furoate ($C_{14}H_{11}Cl_2NO_4$).
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Standard solution: Dilute 0.5 mL of *Sample solution* with chloroform to 200 mL.
Sample solution: 100 mg/mL of Diloxanide Furoate in chloroform
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent system: Dichloromethane and methanol (96:4)
Analysis: Examine the plate under short-wavelength UV light.
Acceptance criteria: Any secondary spot obtained from the *Sample solution* is not more intense than the spot obtained from the *Standard solution*.

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 114°–116°
- **ACIDITY**

Sample solution: Shake 3 g of Diloxanide Furoate in 50 mL of water. Filter, wash the residue with three 20-mL portions of water, and combine the filtrate and washings.
Analysis: Titrate the *Sample solution* with 0.1 N sodium hydroxide VS using phenolphthalein TS as the indicator.
Acceptance criteria: NMT 1.3 mL of 0.1 N sodium hydroxide is required for neutralization

- **LOSS ON DRYING** ⟨731⟩

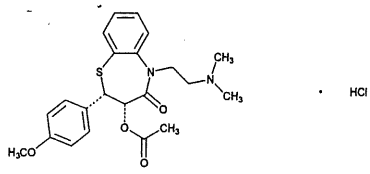
Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Diloxanide Furoate RS

www.webofpharma.com

Diltiazem Hydrochloride



$C_{22}H_{26}N_2O_4S \cdot HCl$ 450.98

1,5-Benzothiazepin-4(5*H*)-one, 3-(acetyloxy)-5-[2-(dimethyl amino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-, monohydrochloride, (+)-*cis*-;

(+)-5-[2-(Dimethylamino)ethyl]-*cis*-2,3-dihydro-3-hydroxy-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one acetate (ester) monohydrochloride [33286-22-5].

DEFINITION

Diltiazem Hydrochloride contains NLT 98.0% and NMT 102.0% of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** *Chloride* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: 1.16 g/L of *d*-10-camphorsulfonic acid in 0.1 M sodium acetate. Adjust with 0.1 N sodium hydroxide to a pH of 6.2.

Mobile phase: Acetonitrile, methanol, and *Buffer* (25:25:50)

System suitability solution: 12 µg/mL each of USP Diltiazem Hydrochloride RS and USP Desacetyl Diltiazem Hydrochloride RS in methanol

Standard solution: 1.2 mg/mL of USP Diltiazem Hydrochloride RS in methanol

Sample solution: 1.2 mg/mL of Diltiazem Hydrochloride in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.6 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for desacetyl diltiazem and diltiazem are about 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3 between desacetyl diltiazem and diltiazem, *System suitability solution*

Column efficiency: NLT 1200 theoretical plates for the diltiazem peak, *System suitability solution*

Relative standard deviation: NMT 2.0% determined from replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Diltiazem Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: Use the *System suitability solution* prepared as directed in the *Assay*.

System suitability: Proceed as directed in the *Assay*, except for the following:

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 10.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of desacetyl diltiazem hydrochloride in the portion of Diltiazem Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desacetyl diltiazem from the *Sample solution*

r_S = peak response of desacetyl diltiazem from the *Standard solution*

C_S = concentration of USP Desacetyl Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Diltiazem Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each impurity peak, other than the main peak and the desacetyl diltiazem peak:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response of each impurity peak from the *Sample solution*

r_S = peak response of desacetyl diltiazem from the *Standard solution*

C_S = concentration of USP Desacetyl Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Diltiazem Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5% of desacetyl diltiazem hydrochloride; NMT 0.5% of each individual impurity; and NMT 1.0% total impurities, including desacetyl diltiazem hydrochloride

SPECIFIC TESTS

- **OPTICAL ROTATION** *Specific Rotation* ⟨781⟩

Sample solution: 10 mg/mL in water

Acceptance criteria: Between +110° and +116°

www.webofpharma.com

• **Loss on Drying** ⟨731⟩
Sample: Dry a sample at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Desacetyl Diltiazem Hydrochloride RS
d-cis-3-Hydroxy-2,3-dihydro-5-[2-dimethylamino)ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one hydrochloride.
$C_{20}H_{24}N_2O_3S \cdot HCl$ 408.95
USP Diltiazem Hydrochloride RS

Diltiazem Hydrochloride Extended-Release Capsules

DEFINITION
Diltiazem Hydrochloride Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$).

IDENTIFICATION
• **A.** The UV-Vis spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Solution A: 0.79 g/L of ammonium bicarbonate in water. Adjust with diluted ammonia solution or acetic acid to a pH of 8.0.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2.0	95	5
5.0	60	40
13.0	60	40
16.0	30	70
20.0	30	70
20.1	95	5
25.0	95	5

Diluent: Acetonitrile and water (40:60)
Standard solution: 0.05 mg/mL of USP Diltiazem Hydrochloride RS in *Diluent*
Sample stock solution: Nominally 0.5 mg/mL of diltiazem hydrochloride from the Capsules in *Diluent* prepared as follows. Transfer a portion of finely powdered contents of NLT 20 Capsules to a suitable volumetric flask. Add *Diluent* equivalent to 80% of the flask volume, mechanically shake for 30 min, and sonicate for 60 min. Dilute with the *Diluent* to volume. Centrifuge and use the supernatant.
Sample solution: Nominally 0.05 mg/mL of diltiazem hydrochloride prepared in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. For *Identification A*, use a diode array detector in the range of 190–400 nm.
Column: 2.1-mm × 15-cm; 1.7-µm packing L1
Flow rate: 0.3 mL/min
Injection volume: 2.0 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diltiazem from the *Sample solution*
r_S = peak response of diltiazem from the *Standard solution*
C_S = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diltiazem hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩
For products labeled for dosing every 12 h
Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.
Medium: Water; 900 mL
Apparatus 2: 100 rpm
Times: 3, 9, and 12 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
3	10–25
9	45–85
12	NLT 70

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: Water; 900 mL

www.webofpharma.com

Apparatus 2: 100 rpm
Times: 4, 8, 12, and 24 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved (%)
4	10–25
8	35–60
12	55–80
24	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: 0.05 M phosphate buffer, pH 7.2; 900 mL
Apparatus 2: 50 rpm
Times: 1, 3, and 8 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 4*.

Table 4

Time (h)	Amount Dissolved (%)
1	NMT 15
3	45–70
8	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 10: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 10*.
Buffer: Dissolve 7.1 g of anhydrous dibasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 6.5.
Medium: *Buffer*; 900 mL
Apparatus 1: 100 rpm
Times: 1, 6, 9, and 24 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved (%)
1	NMT 10
6	10–30
9	34–60
24	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

For products labeled for dosing every 24 h

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 900 mL
Apparatus 2: 100 rpm
Times: 1, 4, 10, and 15 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Dissolved (%)
1	5–20
4	30–50
10	70–90
15	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 100 rpm
Times: 6, 12, 18, 24, and 30 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 7*.

Table 7

Time (h)	Amount Dissolved (%)
6	20–45
12	25–50
18	35–70
24	NLT 70
30	NLT 85

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the

USP Monographs

www.webofpharma.com

times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 2, 4, 8, 12, and 16 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 8*.

Table 8

Time (h)	Amount Dissolved (%)
2	NMT 25
4	25–50
8	60–85
12	NLT 70
16	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.
Buffer: Transfer 115 mL of acetic acid to a 10-L volumetric flask, dilute with water to volume, and mix (*Solution A*). Transfer 165.4 g of anhydrous sodium acetate to a 10-L volumetric flask, dilute with water to volume, and mix (*Solution B*). Mix 4410 mL of *Solution A* with 1590 mL of *Solution B*. Adjust, if necessary, with the addition of *Solution A* or *Solution B* to a pH of 4.2 ± 0.05.
Medium: *Buffer*; 900 mL
Apparatus 2: 100 rpm
Times: 1, 4, 10, and 15 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 9*.

Table 9

Time (h)	Amount Dissolved (%)
1	NMT 10
4	15–35
10	65–85
15	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 8: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.
Medium: Water; 900 mL
Apparatus 2: 100 rpm
Times: 1, 4, 10, and 15 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 10*.

Table 10

Time (h)	Amount Dissolved (%)
1	5–20
4	30–50
10	60–90
15	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 9: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.
[NOTE—Perform the test separately in each of the two media.]
Medium 1: 0.1 N hydrochloric acid; 900 mL
Medium 2: Simulated intestinal fluid TS, prepared without enzyme and adjusted to a pH of 7.5 ± 0.1; 900 mL
Apparatus 2: 75 rpm
Time for Medium 1: 2 h
Times for Medium 2: 2, 12, 18, and 24 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 11*.

Table 11

Time (h)	Amount Dissolved, Medium 1 (%)	Amount Dissolved, Medium 2 (%)
2	0–5	20–45
12	—	35–55
18	—	NLT 60
24	—	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 11: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 11*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 100 rpm
Times: 1, 6, 12, and 18 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

www.webofpharma.com

Tolerances: See *Table 12*.

Table 12

Time (h)	Amount Dissolved (%)
1	NMT 10
6	30–40
12	36–58
18	NLT 85

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 12: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 12*. Proceed as directed for in *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Extended-Release Dosage Forms*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 2, 8, 14, and 24 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 13*.

Table 13

Time (h)	Amount Dissolved (%)
2	NMT 20
8	30–55
14	NLT 65
24	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 13: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 13*. Proceed as directed in *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Extended-Release Dosage Forms*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 2, 8, 14, and 24 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 14*.

Table 14

Time (h)	Amount Dissolved (%)
2	NMT 20
8	30–55
14	60–80

Table 14 *(continued)*

Time (h)	Amount Dissolved (%)
24	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 14: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 14*. Proceed as directed in *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Extended-Release Dosage Forms*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 100 rpm
Times: 6, 12, 18, 24, and 30 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 15*.

Table 15

Time (h)	Amount Dissolved (%)
6	20–45
12	25–50
18	35–70
24	NLT 70
30	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 15: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 15*. Proceed as directed in *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Extended-Release Dosage Forms*.
Medium: 0.05 M phosphate buffer, pH 7.5; 900 mL
Apparatus 2: 75 rpm
Times: 2, 4, 8, 12, and 16 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Tolerances: See *Table 16*.

Table 16

Time (h)	Amount Dissolved (%)
2	NMT 25
4	20–40
8	60–85
12	NLT 70
16	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the

www.webofpharma.com

times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 16: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 16*.
Medium, Apparatus 2, Times, Standard solution, and **Sample solution:** Proceed as directed for *Test 3*.
Detector: UV 238 nm
Tolerances: See *Table 17*.

Table 17

Time (h)	Amount Dissolved (%)
6	20–45
12	30–55
18	40–75
24	NLT 70
30	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 17: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 17*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 100 rpm, with wire helix sinkers
Times: 6, 12, and 30 h
Detector: UV 238 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.
Blank: *Medium*
Tolerances: See *Table 18*.

Table 18

Time (h)	Amount Dissolved (%)
6	20–40
12	35–55
30	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 18: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 18*.
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Times: 2, 4, 8, and 12 h
Detector: UV 237 nm
Standard stock solution: 0.28 mg/mL of USP Diltiazem Hydrochloride RS in *Medium* prepared as follows. To a suitable amount of USP Diltiazem Hydrochloride RS in a suitable volumetric flask, add *Medium* to 50% of the volume of the flask and sonicate for 5 min to dissolve. Dilute with *Medium* to volume.
Standard solution: 0.014 mg/mL of USP Diltiazem Hydrochloride RS in *Medium* from the *Standard stock solution*
Sample solution: At the times specified, withdraw 10 mL of the solution under test. Replace the aliquots withdrawn for analysis with equal volumes of fresh portions of *Medium* maintained at 37°. Pass the solution through a PVDF filter of 0.45-µm pore size. Discard the first 2 mL of filtrate. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) in the sample withdrawn from the vessel at each time point *i*:

$$\text{Result} = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of diltiazem from the *Sample solution* at each time point
A_S = absorbance of diltiazem from the *Standard solution*
C_S = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*

Calculate the percentage of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of diltiazem hydrochloride in the *Sample solution* withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample* withdrawn at each time point (mL)

Tolerances: See *Table 19*.

Table 19

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	NMT 20
2	4	33–58
3	8	68–88
4	12	NLT 80

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 19: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 19*.
Medium: 0.1 N hydrochloric acid; 900 mL
Temperature: 37.0°–37.5°
Apparatus 2: 100 rpm, with a suitable sinker
Times: 1, 4, 12, 18, and 24 h
Detector: UV 238 nm
Cell: 0.5 mm
Standard solution: 0.4 mg/mL of USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: A portion of the solution under test at the time points specified
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Use an automatic dissolution system with an appropriate dissolution software.

Calculate the percentage of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at each time point *i*:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of diltiazem from the *Sample solution* at each time point
A_S = absorbance of diltiazem from the *Standard solution*
C_S = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: See *Table 20*.

Table 20

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	NMT 10
2	4	15–35
3	12	30–50
4	18	50–70
5	24	NLT 85

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 20:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 20*.

Dissolution Test 20 is suitable for products labeled to contain 360 mg of diltiazem hydrochloride.

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 100 rpm
Times: 6, 12, 18, and 24 h
Detector: UV 237 nm
Cell: 0.05 cm
Standard solution: 0.4 mg/mL of USP Diltiazem Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Diltiazem Hydrochloride RS into a suitable volumetric flask, and add methanol to 5% of the total volume of the flask to dissolve. Dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter at the time points specified.
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the concentration of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (A_U/A_S) \times C_S$$

A_U = absorbance of diltiazem from the *Sample solution* at each time point
A_S = absorbance of diltiazem from the *Standard solution*
C_S = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of diltiazem hydrochloride in the *Sample solution* withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample* withdrawn at each time point (mL)

Tolerances: See *Table 21*.

Table 21

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	6	30–50
2	12	35–55
3	18	50–70
4	24	NLT 85

The percentages of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 1-May-2019)

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 2.5 µg/mL each of USP Desacetyl Diltiazem Hydrochloride RS and USP Diltiazem Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of diltiazem hydrochloride from the Capsules in *Diluent* prepared as follows. Transfer a portion of the finely powdered contents of NLT 20 Capsules to a suitable volumetric flask. Add *Diluent* equivalent to 80% of the flask volume, mechanically shake for 30 min, and sonicate for 60 min. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.
System suitability
Sample: *Standard solution*
[Note—For relative retention times see ▲*Table 22*.▲ (RB 1-May-2019)]
Suitability requirements
Resolution: NLT 2.0 between desacetyl diltiazem and diltiazem
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of desacetyl diltiazem hydrochloride in the portion of Capsules taken:

USP Monographs

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desacetyl diltiazem from the *Sample solution*
r_S = peak response of desacetyl diltiazem from the *Standard solution*
C_S = concentration of USP Desacetyl Diltiazem Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of diltiazem hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of diltiazem from the *Standard solution*
C_S = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of diltiazem hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See ▲*Table 22*.▲ (RB 1-May-2019) Disregard limit: 0.05%.

▲Table 22▲ (RB 1-May-2019)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Diltiazem related compound H[a, b]	0.44	—
Diltiazem related compound C[b, c]	0.52	—
Diltiazem related compound C[b, d]	0.58	—
Diltiazem related compound D[b, e]	0.61	—
Diltiazem related compound E[b, f]	0.66	—
Desacetyl diltiazem[g]	0.75	1.5
Diltiazem related compound A[b, h]	0.83	—
Diltiazem related compound B[b, i]	0.89	—
Diltiazem	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities	—	2.0

[a] (2*S*,3*S*)-5-(2-Aminoethyl)-3-hydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine-4(5*H*)-one.
[b] These are impurities related to the drug substance. They are not to be reported for the drug product and should not be included in the total impurities.
[c] (2*S*,3*S*)-3-Hydroxy-2-(3-methoxyphenyl)-5-(2-(methylamino)ethyl)-2,3-dihydrobenzo[*b*][1,4]thiazepin-4(5*H*)-one.
[d] (2*S*,3*S*)-5-[2-(Dimethylamino)ethyl]-2-(4-hydroxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate.
[e] (2*S*,3*S*)-2-(4-Methoxyphenyl)-5-[2-(methylamino)ethyl]-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate.
[f] (2*S*,3*S*)-3-Hydroxy-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepine-4(5*H*)-one.
[g] *d-cis*-3-Hydroxy-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one. The acceptance criteria for this impurity is based on the hydrochloride form.
[h] (2*R*,3*S*)-5-[2-(Dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate.
[i] (2*S*,3*S*)-2-(4-Methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** The labeling indicates the *Dissolution* test with which the product complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Desacetyl Diltiazem Hydrochloride RS
  d-cis-3-Hydroxy-2,3-dihydro-5-[2-(dimethylamino) ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one hydrochloride.
  $C_{20}H_{24}N_2O_3S \cdot HCl$ 408.95
  USP Diltiazem Hydrochloride RS

Diltiazem Hydrochloride Compounded Oral Solution

DEFINITION
Diltiazem Hydrochloride Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$).
Prepare Diltiazem Hydrochloride Compounded Oral Solution 12 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Diltiazem Hydrochloride powder	1.2 g
Vehicle for Oral Solution (regular or sugar-free), *NF*, a sufficient quantity to make	100 mL

Add *Diltiazem Hydrochloride powder* and 10 mL of *Vehicle* to a mortar, and mix. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

ASSAY
- **PROCEDURE**
  Solution A: 1.16 mg/mL of *d*-10-camphorsulfonic acid in 0.1 M sodium acetate. Adjust with 0.1 N sodium hydroxide to a pH of 6.2.
  Mobile phase: Acetonitrile, methanol, and *Solution A* (50:25:25)
  Standard solution: 120 µg/mL of USP Diltiazem Hydrochloride RS in *Mobile phase*
  Sample solution: Agitate the container of Oral Solution for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at −70° until analyzed. At the time of analysis remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the solution to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 240 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The retention time for diltiazem is about 9.6 min.]
  Suitability requirements
  Relative standard deviation: NMT 1.3% for replicate injections

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of diltiazem hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.7–4.7

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
- **Beyond-Use Date:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature, or in a refrigerator
- **Labeling:** Label it to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
USP Diltiazem Hydrochloride RS

Diltiazem Hydrochloride Compounded Oral Suspension

DEFINITION
Diltiazem Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$).
Prepare Diltiazem Hydrochloride Compounded Oral Suspension 12 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Diltiazem Hydrochloride	1.2 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

If using tablets, comminute the tablets to a fine powder in a suitable mortar, or add *Diltiazem Hydrochloride* powder to the mortar. Add 10 mL of *Vehicle*, and mix to a uniform paste. Add *Vehicle* to the mortar in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume.

ASSAY
- **Procedure**
Solution A: 1.16 mg/mL of *d*-10-camphorsulfonic acid in 0.1 M sodium acetate. Adjust with 0.1 N sodium hydroxide to a pH of 6.2.
Mobile phase: Acetonitrile, methanol, and *Solution A* (50:25:25). Filter and degas.
Standard solution: 120 µg/mL of USP Diltiazem Hydrochloride RS in *Mobile phase*
Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[Note—The retention time for diltiazem is about 9.6 min.]
Suitability requirements
Relative standard deviation: NMT 1.3% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of diltiazem hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.7–4.7

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
- **Beyond-Use Date:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature, or in a refrigerator
- **Labeling:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
USP Diltiazem Hydrochloride RS

Diltiazem Hydrochloride Tablets

DEFINITION
Diltiazem Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$).

IDENTIFICATION
- **A.** The UV-Vis spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: 0.79 g/L of ammonium bicarbonate in water. Adjust with diluted ammonia solution to a pH of 8.0.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2.0	95	5
5.0	60	40
13.0	60	40
16.0	30	70
20.0	30	70
20.1	95	5
25.0	95	5

Diluent: Acetonitrile and water (40:60)
Standard solution: 0.05 mg/mL of USP Diltiazem Hydrochloride RS in *Diluent*
Sample stock solution: Nominally 0.5 mg/mL of diltiazem hydrochloride from Tablets in *Diluent* prepared as follows. Transfer an appropriate portion of finely powdered Tablets (NLT 20) to a suitable volumetric flask. Add *Diluent* equivalent to 80% of the flask volume, and sonicate for 60 min. Dilute with *Diluent* to volume. Centrifuge the solution for 20 min. Use the supernatant.
Sample solution: Nominally 0.05 mg/mL of diltiazem hydrochloride in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. For *Identification* test *A*, use a diode-array detector in the range of 190–400 nm.
Column: 2.1-mm × 15-cm; 1.7-µm packing L1
Flow rate: 0.3 mL/min
Injection volume: 2.0 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diltiazem from the *Sample solution*
r_S = peak response of diltiazem from the *Standard solution*
C_S = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diltiazem hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 75 rpm
Time: 30 min and 3 h
Detector: UV 237 nm
Standard solution: USP Diltiazem Hydrochloride RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.
Tolerances: See *Table 2* for the 30-min time point. Use the criteria in *Dissolution* ⟨711⟩, *Acceptance Table 1* for the 3-h time point. NMT 60% (*Q*) of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S \cdot HCl$) is dissolved in 30 min, and NLT 75% (*Q*) is dissolved in 3 h.

Table 2

Stage	Acceptance Criteria
S_1	No unit is more than *Q*.
S_2	Average value is equal to or less than *Q*, and no unit is greater than *Q* + 10%.
S_3	Average value is equal to or less than *Q*, and NMT 2 units are more than *Q* + 10%, and no unit is more than *Q* + 25%.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 2.5 µg/mL each of USP Desacetyl Diltiazem Hydrochloride RS and USP Diltiazem Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of diltiazem hydrochloride from Tablets in *Diluent* prepared as follows. Transfer an appropriate portion of the powdered Tablets (NLT 20) to a suitable volumetric flask. Add *Diluent* equivalent to 80% of the flask volume, and sonicate for 60 min. Dilute with *Diluent* to volume. Centrifuge the solution for 20 min. Use the supernatant.
System suitability
Sample: *Standard solution*
[NOTE—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between desacetyl diltiazem and diltiazem
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of desacetyl diltiazem in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desacetyl diltiazem from the *Sample solution*
r_S = peak response of desacetyl diltiazem from the *Standard solution*
C_S = concentration of USP Desacetyl Diltiazem Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of diltiazem hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of diltiazem from the *Standard solution*
C_S = concentration of USP Diltiazem Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of diltiazem hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 3*. The disregard limit is 0.05%.

Table 3

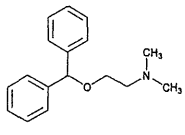
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Diltiazem related compound H[a, h]	0.44	—
Diltiazem related compound G[b, h]	0.52	—
Diltiazem related compound C[c, h]	0.58	—
Diltiazem related compound D[d, h]	0.61	—
Diltiazem related compound E[e, h]	0.66	—
Desacetyl diltiazem	0.75	1.5
Diltiazem related compound A[f, h]	0.83	—
Diltiazem related compound B[g, h]	0.89	—
Diltiazem	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities	—	2.0

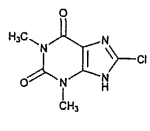
[a] (2*S*,3*S*)-5-(2-Aminoethyl)-3-hydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine-4(5*H*)-one.
[b] (2*S*,3*S*)-3-Hydroxy-2-(3-methoxyphenyl)-5-[2-(methylamino)ethyl]-2,3-dihydrobenzo[*b*][1,4]thiazepin-4(5*H*)-one.
[c] (2*S*,3*S*)-5-[2-(Dimethylamino)ethyl]-2-(4-hydroxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate.
[d] (2*S*,3*S*)-2-(4-Methoxyphenyl)-5-[2-(methylamino)ethyl]-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate.
[e] (2*S*,3*S*)-3-Hydroxy-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepine-4(5*H*)-one.
[f] (2*R*,3*S*)-5-[2-(Dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate.
[g] (2*S*,3*S*)-2-(4-Methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl acetate.
[h] These are impurities related to the drug substance. They are not to be reported for the drug product and should not be included in the total impurities.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Desacetyl Diltiazem Hydrochloride RS
  $C_{20}H_{24}N_2O_3S \cdot HCl$ 408.95
  USP Diltiazem Hydrochloride RS

Dimenhydrinate





$C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$ 469.96

1*H*-Purine-2,6-dione, 8-chloro-3,7-dihydro-1,3-dimethyl-, compd. with 2-(diphenylmethoxy)-*N*,*N*-dimethylethanamine (1:1);
8-Chlorotheophylline, compound with 2-(diphenylmethoxy)-*N*,*N*-dimethylethylamine (1:1) [523-87-5].

DEFINITION

Dimenhydrinate contains NLT 53.0% and NMT 55.5% of diphenhydramine ($C_{17}H_{21}NO$) and NLT 44.0% and NMT 47.0% of 8-chlorotheophylline ($C_7H_7ClN_4O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

- **DIPHENHYDRAMINE**
  Sample: 150 mg of Dimenhydrinate
  Analysis: Dissolve the *Sample* in 75 mL of glacial acetic acid, and titrate with 0.05 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination (see *Titrimetry* ⟨541⟩), and make any necessary corrections. Each mL of 0.05 N perchloric acid is equivalent to 12.77 mg of diphenhydramine ($C_{17}H_{21}NO$).
  Acceptance criteria: 53.0%–55.5% on the dried basis
- **8-CHLOROTHEOPHYLLINE**
  Sample: 800 mg of Dimenhydrinate
  Analysis: Place the *Sample* in a 200-mL volumetric flask, add 50 mL of water, 3 mL of 6 N ammonium hydroxide, and 6 mL of 100 mg/mL ammonium nitrate solution. Warm the mixture on a steam bath for 5 min. Add 25.0 mL of 0.1 N silver nitrate VS, mix, and warm on a steam bath for 15 min with frequent shaking. Cool, dilute with water to volume, and allow the precipitate to settle. Pass through a dry filter paper, discarding the first 20 mL of the filtrate. Pipet 100 mL of the filtrate into a 250-mL flask, acidify with nitric acid, and add an excess of 3 mL of the acid. Add 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N silver nitrate is equivalent to 21.46 mg of 8-chlorotheophylline ($C_7H_7ClN_4O_2$).
  Acceptance criteria: 44.0%–47.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%
- **ORGANIC IMPURITIES**
  Diluent: Acetonitrile and water (18:82)
  Solution A: 10.0 g/L (equivalent to 13.8 mL/L) solution of triethylamine in water, adjusted with phosphoric acid to a pH of 2.5
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	82	18	1.2
2	82	18	1.2
15	50	50	1.2
20	20	80	2.0
32	20	80	2.0

Return to original conditions, and re-equilibrate the system.

www.webofpharma.com

System suitability solution: 0.114 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.1 mg/mL each of USP Diphenhydramine Related Compound A RS, USP Theophylline RS, and USP Dimenhydrinate Related Compound E RS in *Diluent*
Standard solution: 2.28 µg/mL of USP Diphenhydramine Hydrochloride RS in *Diluent*
Sensitivity solution: 0.57 µg/mL of USP Diphenhydramine Hydrochloride RS in *Diluent*, from the *Standard solution*
Sample solution: 1.0 mg/mL of Dimenhydrinate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm end-capped packing L1
Column temperature: 30°
Flow rate: See *Table 1*.
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between diphenhydramine related compound A and diphenhydramine, *System suitability solution*
Signal-to-noise ratio: NLT 10 from the diphenhydramine peak, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Dimenhydrinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of diphenhydramine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Dimenhydrinate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of diphenhydramine, 255.36
M_{r2} = molecular weight of diphenhydramine hydrochloride, 291.82

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline	0.3	0.2
8-Chlorotheophylline	0.47	—
Dimenhydrinate related compound E	0.7	0.15
Diphenhydramine related compound A	0.95	0.2
Diphenhydramine	1.0	—
Any other individual impurity	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample under vacuum over phosphorus pentoxide for 24 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Dimenhydrinate RS
USP Dimenhydrinate Related Compound E RS
8-Chlorocaffeine.
8-Chloro-3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione.
$C_8H_9ClN_4O_2$ 228.64
USP Diphenhydramine Hydrochloride RS
USP Diphenhydramine Related Compound A RS
2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.
$C_{16}H_{19}NO \cdot HCl$ 277.79
USP Theophylline RS

Dimenhydrinate Injection

» Dimenhydrinate Injection is a solution of Dimenhydrinate in a mixture of Propylene Glycol and water. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of dimenhydrinate ($C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass.
USP Reference standards ⟨11⟩—
USP Dimenhydrinate RS
Identification—The relative retention times of the major peaks for 8-chlorotheophylline and diphenhydramine in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
pH ⟨791⟩: between 6.4 and 7.2.

Content of 8-chlorotheophylline—
Mobile phase—Dissolve 0.81 g of *dl*-10-camphorsulfonic acid and 0.70 g of sodium acetate in 700 mL of water. Add 300 mL of methanol, mix, and pass through a membrane filter having a 0.5-µm or finer porosity.
Standard solution—Dissolve an accurately weighed quantity of USP Dimenhydrinate RS in methanol to obtain a Standard stock solution having a known concentration of about 0.5 mg per mL. Retain a portion of this Standard stock solution for use in the *Assay*. Pipet 5 mL into a 50-mL volumetric flask, dilute with methanol to volume, mix, and pass through a membrane filter having a 0.5-µm or finer porosity.
Test solution—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of dimenhydrinate, to a 100-mL volumetric flask, dilute with methanol to volume, and mix to obtain a stock solution. Retain a portion of this stock solution for use in the *Assay*. Pipet 5 mL of this stock solution into a 50-mL volumetric flask, dilute with methanol to volume, mix, and pass through a membrane filter having a 0.5-µm or finer porosity.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector, a

USP Monographs

www.webofpharma.com

2-mm × 12.5-cm guard column that contains packing L2, and a 4.6-mm × 25-cm analytical column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph three replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of 8-chlorotheophylline ($C_7H_7ClN_4O_2$) in each mL of the Injection taken by the formula:

$$(214.61/469.96)(1000C/V)(r_U/r_S)$$

in which 214.61 and 469.96 are the molecular weights of 8-chlorotheophylline and dimenhydrinate, respectively; C is the concentration, in mg per mL, of USP Dimenhydrinate RS in the *Standard solution;* V is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively. An amount of 8-chlorotheophylline that is between 43.4% and 47.9% of the amount of dimenhydrinate obtained in the *Assay* is found.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Solution A, Solution B, Mobile phase, Internal standard solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Dimenhydrinate Tablets.*

Standard preparation—Use a portion of the Standard stock solution that was prepared for the *Standard solution* in the test for *Content of 8-chlorotheophylline.* Mix 5.0 mL of this Standard stock solution and 5.0 mL of *Internal standard solution,* and pass through a membrane filter having a 0.5-µm or finer porosity.

Assay preparation—Use a portion of the stock solution that was prepared for the *Test solution* in the test for *Content of 8-chlorotheophylline.* Mix 5.0 mL of this stock solution and 5.0 mL of *Internal standard solution,* and pass through a membrane filter having a 0.5-µm or finer porosity.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Dimenhydrinate Tablets.* Calculate the quantity, in mg, of dimenhydrinate ($C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$) in each mL of the Injection taken by the formula:

$$(200C/V)(R_U/R_S)$$

in which C is the concentration of USP Dimenhydrinate RS in the *Standard preparation;* V is the volume, in mL, of Injection taken; and the other terms are as defined therein.

Dimenhydrinate Oral Solution

» Dimenhydrinate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dimenhydrinate ($C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Dimenhydrinate RS

Identification—The relative retention times of the major peaks for 8-chlorotheophylline and diphenhydramine in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Content of 8-chlorotheophylline—

Ammonium bicarbonate solution, Diluent, Solution A, Solution B, Mobile phase, Internal standard solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Dimenhydrinate Tablets.*

Standard solution—Prepare as directed for *Standard preparation* in the *Assay* under *Dimenhydrinate Tablets.*

Test solution—Prepare as directed for *Assay preparation* in the *Assay.*

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg per mL, of 8-chlorotheophylline ($C_7H_7ClN_4O_2$) in the portion of Oral Solution taken by the formula:

$$(214.61/469.96)(0.05W)(R_U/R_S)$$

in which 214.61 and 469.96 are the molecular weights of 8-chlorotheophylline and dimenhydrinate, respectively; W is the weight, in mg, of USP Dimenhydrinate RS in the *Standard solution;* and R_U and R_S are peak area ratios of 8-chlorotheophylline to the internal standard obtained from the *Test solution* and the *Standard solution,* respectively. An amount of 8-chlorotheophylline that is between 43.4% and 47.9% of the amount of dimenhydrinate obtained in the *Assay* is found.

Alcohol Determination ⟨611⟩: between 4.0% and 6.0% of C_2H_5OH.

Assay—

Ammonium bicarbonate solution, Diluent, Solution A, Solution B, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Dimenhydrinate Tablets.*

Assay preparation—Pipet 5.0 mL of Oral Solution into a suitable container, add 5.0 mL of *Internal standard solution,* and mix. Transfer about 1 mL of this solution to a suitable container, add about 5 mL of *Diluent,* and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Dimenhydrinate Tablets.* Calculate the quantity, in mg per mL, of dimenhydrinate ($C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$) in the portion of the Oral Solution taken by the formula:

$$0.05W(R_U/R_S)$$

in which W is the weight, in mg, of USP Dimenhydrinate RS in the *Standard preparation;* and R_U and R_S are the peak area ratios of diphenhydramine to the internal standard obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dimenhydrinate Tablets

» Dimenhydrinate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dimenhydrinate ($C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$).

Packaging and storage—Preserve in well-closed containers.

www.webofpharma.com

USP Reference standards ⟨11⟩—
USP Dimenhydrinate RS

Identification—The relative retention times of the 8-chlorotheophylline and diphenhydramine peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 276 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Dimenhydrinate RS in the same *Medium*.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements, the following procedure being used where the test for *Content Uniformity* is required. Transfer 1 Tablet to a 50-mL volumetric flask, add about 5 mL of *Ammonium bicarbonate solution* obtained from the *Assay*, and shake gently to disperse, sonicating, if necessary. Add 20.0 mL of *Internal standard solution* obtained from the *Assay*, shake by mechanical means for 30 minutes, and centrifuge. To 1 mL of the clear supernatant add about 9 mL of *Diluent* obtained from the *Assay*, and mix. Continue as directed for *Procedure* in the *Assay*.

Content of 8-chlorotheophylline—
Ammonium bicarbonate solution, Diluent, Solution A, Solution B, Mobile phase, Internal standard solution, and *Chromatographic system*—Prepare as directed in the *Assay*.
Standard solution—Prepare as directed for *Standard preparation* in the *Assay*.
Test solution—Prepare as directed for *Assay preparation* in the *Assay*.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of 8-chlorotheophylline ($C_7H_7ClN_4O_2$) per Tablet taken by the formula:

$$(214.61/469.96)W(R_U/R_S)$$

in which 214.61 and 469.96 are the molecular weights of 8-chlorotheophylline and dimenhydrinate, respectively; W is the weight, in mg, of USP Dimenhydrinate RS in the *Standard solution;* and R_U and R_S are peak area ratios of 8-chlorotheophylline to the internal standard obtained from the *Test solution* and the *Standard solution*, respectively. An amount of 8-chlorotheophylline that is between 43.4% and 47.9% of the amount of dimenhydrinate obtained in the *Assay* is found.

Assay—
Ammonium bicarbonate solution—Dissolve 4 g of ammonium bicarbonate in 250 mL of water.
Diluent—Dissolve 4 g of ammonium bicarbonate in 200 mL of water. Add 50 mL of methanol, and mix.
Solution A—Dissolve 0.8 g of ammonium bicarbonate in 800 mL of water. Add 200 mL of methanol, filter, and degas.
Solution B—Dissolve 0.8 g of ammonium bicarbonate in 150 mL of water. Add 850 mL of methanol, filter, and degas.
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Prepare a solution in methanol containing 2.0 mg of 2-hydroxybenzyl alcohol per mL.
Standard preparation—Accurately weigh about 50 mg of USP Dimenhydrinate RS, add about 5 mL of *Ammonium bicarbonate solution* and 20.0 mL of *Internal standard solution*, and mix. To 1 mL of this solution add about 9 mL of *Diluent*, and mix.
Assay preparation—Transfer 5 Tablets into a 250-mL volumetric flask, add 25 mL of *Ammonium bicarbonate solution*, and shake gently to disperse, sonicating if necessary. Add 100.0 mL of *Internal standard solution*, shake vigorously for 30 minutes, and centrifuge. To 1 mL of the clear supernatant add about 9 mL of *Diluent*, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 229-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

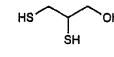
Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–7.0	100	0	isocratic
7.0–7.1	100→0	0→100	linear gradient
7.1–15	0	100	isocratic
15–15.1	0→100	100→0	linear gradient
15.1–22.0	100	0	isocratic

Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure:* the relative retention times are about 0.3 for 8-chlorotheophylline, 0.5 for the internal standard, and 1.0 for diphenhydramine; the resolution, R, between 8-chlorotheophylline and the internal standard is not less than 4.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of dimenhydrinate ($C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$) per Tablet taken by the formula:

$$W(R_U/R_S)$$

in which W is the weight, in mg, of USP Dimenhydrinate RS in the *Standard preparation;* and R_U and R_S are the peak area ratios of diphenhydramine to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dimercaprol



$C_3H_8OS_2$ 124.23
1-Propanol, 2,3-dimercapto.
2,3-Dimercapto-1-propanol [59-52-9].

USP Monographs

www.webofpharma.com

» Dimercaprol contains not less than 97.0 percent and not more than 100.5 percent of $C_3H_8OS_2$, and not more than 1.5 percent of 1,2,3-trimercaptopropane ($C_3H_8S_3$).

Packaging and storage—Preserve in tight containers, in a cold place.
Specific gravity ⟨841⟩: between 1.242 and 1.244.
Distilling range, *Method I* ⟨721⟩: between 66° and 68°, under a pressure of 0.2 mm of mercury.
Refractive index ⟨831⟩: between 1.567 and 1.573.

Limit of 1,2,3-trimercaptopropane and related impurities—
Adsorbent—Use a suitable chromatographic grade of 100-mesh silicic acid.
Standard buffer solution—Prepare 100 mL of pH 6.0 Phosphate Buffer (see *pH* ⟨791⟩), and dissolve in it 100 mg of sodium bisulfite.
Acid-washed solvent hexane—To 100 mL of solvent hexane contained in a separator add 10 mL of sulfuric acid, shake for not less than 12 hours, and allow the layers to separate. Transfer the acid-washed solvent to a distilling flask, and distill slowly, retaining only that portion distilling between 35° and 50°. Use only freshly distilled material.
Diisopropyl ether—Place 100 mL of diisopropyl ether in a distilling flask, and distil, retaining only that portion distilling between 68° and 69°. Use only freshly distilled material.
[**CAUTION**—Do not evaporate to the point of near-dryness, since diisopropyl ether tends to form explosive peroxides]
Solvent hexane-diisopropyl ether mixture (mobile solvent)—Mix 50 mL of *Diisopropyl ether* with 50 mL of *Acid-washed solvent hexane.*
Chromatographic tube—Insert a small plug of glass wool at the juncture of the tube and the stem of a 600- × 13-mm chromatographic tube.
Chromatographic column—Mix 20 g of *Adsorbent* with 20 mL of *Standard buffer solution.* Make into a slurry by mixing with 100 mL of chloroform. Transfer successive portions of the slurry into the *Chromatographic tube,* packing firmly and evenly with a close-fitting, ground-glass tamper after each addition. Keep a layer of liquid above the packed column to prevent the formation of air spaces. Wash the column free from chloroform with *Solvent hexane-diisopropyl ether mixture,* and allow the solvent to fall to the level of the *Adsorbent.*
Procedure—Place about 250 mg of Dimercaprol, accurately weighed and demonstrated to be free from hydrogen sulfide as directed in the *Assay,* in a 5-mL volumetric flask, add *Solvent hexane-diisopropyl ether mixture* to volume, and mix. Transfer 2.0 mL of the resulting solution to the prepared *Chromatographic column.* When the liquid has passed into the column, wash the wall of the tube with a 2-mL portion of *Solvent hexane-diisopropyl ether mixture,* and allow the washing to fall to the level of the *Adsorbent.* Fill the *Chromatographic tube* with solvent, and collect two successive fractions: (*A*) a 20-mL fraction containing all of the 1,2,3-trimercaptopropane, and (*B*) a 3-mL fraction that serves as a check on the separation. To each fraction add an equal volume of alcohol, and titrate with 0.1 N iodine VS until a permanent yellow color is produced. Perform a blank titration on 20 mL of the solvent mixture that has been passed through the column prior to introduction of the test specimen, and make any necessary correction. Fraction (*B*) does not decolorize 1 drop of 0.1 N iodine VS. Each mL of 0.1 N iodine added is equivalent to 4.676 mg of $C_3H_8S_3$. Not more than 1.5% of 1,2,3-trimercaptopropane ($C_3H_8S_3$) is found.
Assay—Test the Dimercaprol for the presence of hydrogen sulfide by examining the vapor above the assay specimen with moistened lead acetate test paper. If the paper darkens, bubble dry, oxygen-free nitrogen or carbon dioxide through the assay specimen until a fresh strip of test paper gives a negative test. Transfer about 2 mL of hydrogen sulfide-free Dimercaprol to a tared, glass-stoppered, 100-mL volumetric flask, weigh accurately, add methanol to volume, and mix. Pipet 10 mL of the solution into a 50-mL conical flask, and titrate with 0.1 N iodine VS until a permanent yellow color is produced. Perform a blank titration, and make any necessary correction. Calculate the percentage of $C_3H_8OS_2$ taken by the formula:

$$0.6211V/W - 1.328T$$

in which V is the volume, in mL, of 0.1 N iodine used, W is the weight, in g, of specimen in the aliquot taken, and T is the percentage of $C_3H_8S_3$ found in the determination of the *Limit of 1,2,3-trimercaptopropane and related impurities.*

Dimercaprol Injection

» Dimercaprol Injection is a sterile solution of Dimercaprol in a mixture of Benzyl Benzoate and vegetable oil. It contains, in each 100 g, not less than 9.0 g and not more than 11.0 g of dimercaprol ($C_3H_8OS_2$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 1 USP Endotoxin Unit per mg of dimercaprol.

Limit of 1,2,3-trimercaptopropane and related impurities—
Adsorbent, Standard buffer solution, Acid-washed solvent hexane, Diisopropyl ether, Solvent hexane-diisopropyl ether mixture, Chromatographic tube, and *Chromatographic column*—Prepare as directed in the test for *Limit of 1,2,3-trimercaptopropane and related impurities* under *Dimercaprol.*
Procedure—Place about 1 g of Injection, accurately weighed and demonstrated to be free from hydrogen sulfide as directed in the *Assay* under *Dimercaprol,* in a 5-mL beaker, add 2 mL of *Solvent hexane-diisopropyl ether mixture,* and mix. Transfer the resulting solution to the prepared *Chromatographic column.* When the liquid has passed into the column, wash the beaker with two 2-mL portions of *Solvent hexane-diisopropyl ether mixture,* and allow the washings to fall to the level of the *Adsorbent.* Proceed as directed for *Procedure* in the test for *Limit of 1,2,3-trimercaptopropane and related impurities* under *Dimercaprol.* The limit of 1,2,3-trimercaptopropane ($C_3H_8S_3$) is not more than 4.5%, by weight, of the content of dimercaprol.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that at times it may be turbid or contain small amounts of flocculent material.
Assay—Transfer about 2 mL of Injection to a tared conical flask, and weigh accurately. Add 100 mL of a mixture of 1 volume of chloroform and 3 volumes of methanol, agitate to dissolve the Injection, and titrate with 0.1 N iodine VS to the production of a permanent yellow color. Perform a blank determination, and make any necessary correction. Calculate the percentage of dimercaprol ($C_3H_8OS_2$) in the portion of Injection taken by the formula:

$$0.6211(V/W - v/w)$$

www.webofpharma.com

in which *V* is the volume, in mL, of 0.1 N iodine used; *W* is the weight, in g, of Injection taken; and *v* and *w* are the volume, in mL, of 0.1 N iodine and the weight, in g, of Injection, respectively, used in the test for *Limit of 1,2,3-trimercaptopropane and related impurities.*

Dimethyl Sulfoxide



C_2H_6OS 78.13
Methane, sulfinylbis-.
Methyl sulfoxide [67-68-5].

» Dimethyl Sulfoxide contains not less than 99.9 percent of C_2H_6OS, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Dimethyl Sulfoxide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197F*▲ (CN 1-May-2020), potassium bromide plates being used.

B: Add 1.5 mL cautiously and dropwise to 2.5 mL of hydriodic acid in a test tube cooled in ice. Filter the mixture rapidly, and collect the precipitate. Dry the precipitate in vacuum: a deep violet, crystalline solid is obtained, and it is soluble in chloroform, yielding a red solution.
Specific gravity ⟨841⟩: between 1.095 and 1.101.
Refractive index ⟨831⟩: between 1.4755 and 1.4775.
Acidity—Dissolve 50.0 g in 100 mL of water, and add phenolphthalein TS. If the solution remains colorless, titrate with 0.01 N sodium hydroxide until a pink color appears: not more than 5.0 mL of 0.01 N sodium hydroxide is consumed.
Water Determination, *Method I* ⟨921⟩: not more than 0.1%. [NOTE—Weigh and transfer the test specimen in an environment of low humidity to minimize absorption of atmospheric water.]
Ultraviolet absorbance—Maintain Dimethyl Sulfoxide in a water bath at a temperature of less than 20° [NOTE—Do not freeze.] , and purge with dry nitrogen for 30 minutes. Record the UV absorption spectrum between 270 and 350 nm in a 1-cm cell, using water as the blank: the spectrum is smooth with no absorption maxima; the absorbance at 275 nm is not more than 0.20, and the absorbance ratios, A_{285}/A_{275} and A_{295}/A_{275}, at the wavelengths indicated by the subscripts, are not more than 0.65 and 0.45, respectively.
Limit of nonvolatile residue—Evaporate about 100.0 g to dryness in a tared, preconditioned dish on a hot plate under a suitable fume hood. Evaporate gently so boiling does not occur. The weight of the residue does not exceed 0.01%.

Related compounds—
System suitability solution —Prepare a solution containing about 0.15 mg of dimethyl sulfone per mL and 0.1 mg of dibenzyl per mL in Dimethyl Sulfoxide.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and contains a 0.32-mm × 15-m column bonded with a 3-µm film of phase G2. Initially the column temperature is maintained at 100° for 15 minutes, then is increased at a rate of 10° per minute to a temperature of 170°, and maintained at 170° for 20 minutes. The injection port is maintained at a temperature of about 210°, and the detector block is maintained at a temperature of about 220°. Helium is used as the carrier gas, flowing at a rate of 1.7 mL per minute, and the split ratio is 33:1. [NOTE—The split ratio can be modified in order to optimize performance.] Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between dimethyl sulfone and dibenzyl is not less than 5.0; and the column efficiency, determined from the dimethyl sulfoxide peak, is not less than 1000 theoretical plates.
Procedure—Inject about 1 µL of Dimethyl Sulfoxide into the gas chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of total impurities in the portion of Dimethyl Sulfoxide taken by the formula:

$$100(r_U/r_S)$$

in which r_U is the sum of the areas of all the impurity peaks; and r_S is the sum of the areas of all the peaks: not more than 0.1% of total impurities is found.
Assay—Using the results from the test for *Limit of nonvolatile residue* and the test for *Related compounds,* calculate the percentage of C_2H_6OS in the portion of Dimethyl Sulfoxide taken by subtracting the percentage of nonvolatile residue and the percentage of total impurities found from 100.0%. [NOTE—A correction for percent water is not applied to the result.]

Dimethyl Sulfoxide Gel

» Dimethyl Sulfoxide Gel contains not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of C_2H_6OS.

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Dimethyl Sulfoxide RS
Identification—The retention time of the dimethyl sulfoxide peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Minimum fill ⟨755⟩**:** meets the requirements.
pH ⟨791⟩: between 4.7 and 6.7, determined on a mixture of 5 g of Gel and 5 mL of water.

Assay—
Internal standard solution—Transfer 1.0 mL of dimethylformamide to a 100-mL volumetric flask. Add about 75 mL of methanol, and shake for about 1 minute. Dilute with methanol to volume, and mix.
Standard preparation—Transfer about 2 g of USP Dimethyl Sulfoxide RS, accurately weighed, to a 100-mL volumetric flask, add 1.0 mL of dimethylformamide and 75 mL of methanol, and shake for about 1 minute. Dilute with methanol to volume, and mix. This solution contains about 20 mg of USP Dimethyl Sulfoxide RS per mL.

www.webofpharma.com

Assay preparation—Transfer an accurately weighed quantity of Gel, equivalent to 2 g of dimethyl sulfoxide, to a 100-mL volumetric flask. Add 1.0 mL of dimethylformamide and about 75 mL of methanol. Dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm × 1.8-m column packed with 15% liquid phase G39 on support S1A. The column is maintained at about 160° and the injection port and detector block at about 200°. Helium is used as the carrier gas, flowing at a rate of about 30 mL per minute. Chromatograph the *Internal standard solution*, and record the peak responses as directed for *Procedure:* examine the chromatogram to confirm that there are no peaks present that would interfere with the measurement of the dimethylformamide peaks and the dimethyl sulfoxide peaks in subsequent chromatograms. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.4 for dimethylformamide and 1.0 for dimethyl sulfoxide; the tailing factor is not more than 2.0; the resolution, *R*, between the dimethylformamide peak and the dimethyl sulfoxide peak is not less than 4; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for not less than 40 minutes, and measure the peak heights for the dimethylformamide and dimethyl sulfoxide peaks. Calculate the percentage (w/w) of C_2H_6OS in the portion of Gel taken by the formula:

$$10(C/W)(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Dimethyl Sulfoxide RS in the *Standard preparation; W* is the weight, in g, of Gel taken to prepare the *Assay preparation;* and R_U and R_S are the ratios of the dimethyl sulfoxide peak response to the dimethylformamide peak response in the chromatograms obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dimethyl Sulfoxide Irrigation

» Dimethyl Sulfoxide Irrigation is a sterile solution of Dimethyl Sulfoxide in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of C_2H_6OS.

Packaging and storage—Preserve in single-dose containers, and store at controlled room temperature, protected from strong light.
Labeling—Label it to indicate prominently that it is not intended for injection.
USP Reference standards ⟨11⟩—
USP Dimethyl Sulfoxide RS
Identification—A portion of the *Assay preparation*, chromatographed as directed in the *Assay*, exhibits a major peak for dimethyl sulfoxide, the retention time of which is identical with that exhibited by the *Standard preparation*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.5 Endotoxin Unit per mL.
Sterility Tests ⟨71⟩: meets the requirements.
pH ⟨791⟩: between 5.0 and 7.0, when diluted with water to obtain a solution containing 50 mg of dimethyl sulfoxide per mL.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Dimethyl Sulfoxide RS in acetone, and dilute quantitatively with acetone to obtain a solution having a known concentration of about 80 mg per mL.

Assay preparation—Transfer an accurately measured volume of Dimethyl Sulfoxide Irrigation, equivalent to about 2 g of dimethyl sulfoxide, to a 25-mL volumetric flask, dilute with acetone to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the test for *Dimethyl sulfone* under *Dimethyl Sulfoxide*. The relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

Procedure—Introduce equal volumes (about 1.0 µL) of the *Standard preparation* and the *Test preparation* into the gas chromatograph. Measure the peak responses, at corresponding retention times, so obtained, and calculate the quantity, in mg, of C_2H_6OS in each mL of Dimethyl Sulfoxide Irrigation taken by the formula:

$$25(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Dimethyl Sulfoxide RS in the *Standard preparation; V* is the volume, in mL, of Dimethyl Sulfoxide Irrigation taken; and r_U and r_S are the peak responses from the *Assay preparation* and the *Standard preparation*, respectively.

Dimethyl Sulfoxide Topical Solution

» Dimethyl Sulfoxide Topical Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of C_2H_6OS.

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Dimethyl Sulfoxide RS

Identification—

A: The chromatogram of the *Assay preparation*, obtained as directed in the *Assay*, exhibits a major peak for dimethyl sulfoxide, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

B: To 2 mL of Topical Solution in a test tube add 5 mL of cold hydriodic acid. Filter the mixture rapidly, and collect the precipitate. Dry the precipitate in vacuum: a deep violet, crystalline solid is obtained, and it is soluble in chloroform, yielding a red solution.

Change to read:

C: To 0.1 g of solid sodium hydroxide in a test tube add 10 mL of Topical Solution, and heat on a steam bath until the sodium hydroxide has dissolved. Add 10 mL of chloroform, and shake. Filter the chloroform layer through a filter funnel containing about 10 g of anhydrous sodium sulfate. Heat the filtrate on a steam bath to remove the chloroform. Determine the IR spectrum of the liquid residue as directed under ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy:*

www.webofpharma.com

197F▲ (CN 1-May-2020). The spectrum so obtained corresponds to that of a spectrum of USP Dimethyl Sulfoxide RS, similarly prepared.

Assay— [NOTE—Use glass equipment to prepare solutions.]

Internal standard solution—Transfer 3.0 mL of dimethylformamide to a 250-mL volumetric flask. Add about 200 mL of acetone, and shake for about 1 minute. Dilute with acetone to volume, and mix.

Standard preparation—Transfer about 2 g of USP Dimethyl Sulfoxide RS, accurately weighed, to a 250-mL volumetric flask, add 3.0 mL of dimethylformamide and 200 mL of acetone, and sonicate for about 1 minute. Dilute with acetone to volume, and mix to obtain a solution having a known concentration of about 8 mg of USP Dimethyl Sulfoxide RS per mL.

Assay preparation—Transfer an accurately measured volume of Topical Solution, equivalent to about 2 g of dimethyl sulfoxide, to a 250-mL volumetric flask, add 3.0 mL of dimethylformamide and about 200 mL of acetone, and sonicate for about 1 minute. Dilute with acetone to volume, and mix.

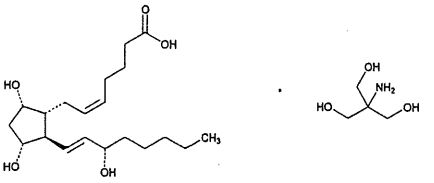
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm × 1.8-m column packed with 10% liquid phase G16 on support S1A. The column is maintained at about 170°, the injection port at about 200°, and the detector block at about 300°. The carrier gas is helium, flowing at a rate of about 20 mL per minute. Chromatograph two portions of the acetone used to prepare the *Standard preparation*, the *Assay preparation*, and the *Internal standard solution*, and record the peak responses as directed under *Procedure*. Discard the first chromatogram, and examine the second to confirm that there are no peaks present that would interfere with the measurement of the dimethylformamide peaks and the dimethyl sulfoxide peaks in subsequent chromatograms. Chromatograph the *Internal standard solution*, and record the peak responses as directed for *Procedure:* examine the chromatogram to confirm that there are no peaks present that would interfere with the measurement of the dimethylformamide peaks and the dimethyl sulfoxide peaks in subsequent chromatograms. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.2 for acetone, 0.5 for dimethylformamide, and 1.0 for dimethyl sulfoxide; the resolution, *R*, between the dimethylformamide peak and the dimethyl sulfoxide peak is not less than 2; the tailing factors for the dimethylformamide peak and the dimethyl sulfoxide peak are not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— [NOTE—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for not less than 20 minutes, and measure the responses for the dimethylformamide and dimethyl sulfoxide peaks. Calculate the percentage (v/v) of C_2H_6OS in the portion of Topical Solution taken by the formula:

$$(2.5/1.096)10(C/V)(R_U/R_S)$$

in which 1.096 is the specific gravity of dimethyl sulfoxide, *C* is the concentration, in mg per mL, of USP Dimethyl Sulfoxide RS in the *Standard preparation;* *V* is the volume, in mL, of Topical Solution taken to prepare the *Assay preparation;* and R_U and R_S are the ratios of the dimethyl sulfoxide peak response to the dimethylformamide peak response in the chromatograms obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dinoprost Tromethamine



$C_{20}H_{34}O_5 \cdot C_4H_{11}NO_3$ 475.62

Prosta-5,13-dien-1-oic acid, 9,11,15-trihydroxy-, (5*Z*,9α,11α,13*E*,15*S*)-, compd. with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1).

(*E*,*Z*)-(1*R*,2*R*,3*R*,5*S*)-7-[3,5-Dihydroxy-2-[(3*S*)-(3-hydroxy-1-octenyl)]cyclopentyl]-5-heptenoic acid compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1).

Prostaglandin $F_{2\alpha}$ tromethamine [38562-01-5].

» Dinoprost Tromethamine contains not less than 95.0 percent and not more than 105.0 percent of $C_{20}H_{34}O_5 \cdot C_4H_{11}NO_3$, calculated on the dried basis. [**CAUTION**—Great care should be taken to prevent inhaling particles of Dinoprost Tromethamine and exposing the skin to it.]

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Dinoprost Tromethamine RS

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M.*▲ (CN 1-May-2020)

Specific rotation ⟨781S⟩: between +19° and +26°.

Test solution: 20 mg per mL, in alcohol.

Loss on drying ⟨731⟩—Dry it in vacuum at room temperature and at a pressure not exceeding 5 mm of mercury for 16 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.5%.

Chromatographic purity— [NOTE—Prepare solutions immediately prior to use.]

Mobile phase—Proceed as directed in the *Assay*.

Standard stock solution—Prepare as directed for *Standard preparation* in the *Assay*.

Standard solution—Transfer 1.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Test solution—Prepare as directed for the *Assay preparation*.

Chromatographic system—Proceed as directed in the *Assay*. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between dinoprost tromethamine and any other adjacent peak is not less than 1.0.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Dinoprost Tromethamine taken by the formula:

$$2.5(C/W)F(r_i/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Dinoprost Tromethamine RS in the *Standard solution;* *W* is the weight, in mg, of Dinoprost Tromethamine taken to prepare the *Test solution;* *F* is the relative response factor and is equal to 0.25 for any peak at a relative retention time of about 0.30, 1.7 for

USP Monographs

www.webofpharma.com

any peak at a relative retention time of about 1.15, and 1.0 for any other peak; r_i is the peak response of each impurity obtained from the *Test solution;* and r_S is the peak response of dinoprost tromethamine obtained from the *Standard solution:* not more than 2.0% of any impurity having a relative retention time of about 0.94 is found; not more than 1.5% of any impurity having a relative retention time of about 0.84 is found; not more than 0.5% of any other impurity is found; and not more than 2.0% of all other impurities is found.

Assay— [NOTE—Prepare solutions immediately prior to use.]

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and phosphoric acid (750:250:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Dinoprost Tromethamine RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL.

Assay preparation—Transfer about 25.0 mg of Dinoprost Tromethamine, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 200-nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 6000 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{20}H_{34}O_5 \cdot C_4H_{11}NO_3$ in the portion of Dinoprost Tromethamine taken by the formula:

$$25C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dinoprost Tromethamine RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dinoprost Tromethamine Injection

» Dinoprost Tromethamine Injection is a sterile solution of Dinoprost Tromethamine in Water for Injection. It may contain a suitable preservative, such as benzyl alcohol. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dinoprost ($C_{20}H_{34}O_5$). [CAUTION—Extreme care should be exercised when handling dinoprost tromethamine as it is readily absorbed through the skin; accidental spillage on the skin should be washed off immediately with soap and water.]

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—
USP Dinoprost Tromethamine RS

Identification—The retention time of the derivatized dinoprost peak in the chromatogram of the *Assay preparation* corresponds to that of the derivatized dinoprost peak in the *Standard preparation,* both relative to the internal standard, obtained as directed in the *Assay.*

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 8 USP Endotoxin Units per 1.0 mg of dinoprost.

Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩**:** between 7.0 and 9.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Prepare a solution consisting of methylene chloride, 1,3-butanediol, and water (496: 3.5: 0.25).

Internal standard solution—Prepare a solution in *Mobile phase* containing about 0.75 mg of guaifenesin per mL.

Reagent preparations—

A—Prepare a solution containing about 10 mg of α-bromo-2′-acetonaphthone per mL of acetonitrile. Use a freshly prepared solution.

B—Prepare a solution containing 5 µL of diisopropylethylamine per mL of acetonitrile. Use a freshly prepared solution.

C —Prepare a citrate buffer solution by dissolving 10.5 g of citric acid monohydrate in about 75 mL of water and adding 5 N sodium hydroxide until a pH of 4.0 is obtained. Dilute with water to 100 mL, and mix.

Diluent—Use *Sterile Water for Injection* containing 0.945% of benzyl alcohol.

Standard preparation—Dissolve an accurately weighed quantity of USP Dinoprost Tromethamine RS in *Diluent* to obtain a solution having a known concentration of about 0.67 mg of dinoprost tromethamine per mL (*Standard stock preparation*). Transfer 1.0 mL of this solution to a suitable container, and add 1.0 mL of *Reagent C* and 20.0 mL of methylene chloride. Shake and centrifuge. Transfer 5.0 mL of the lower layer into a suitable container, and evaporate with the aid of nitrogen to dryness. Wash the inside of the container with 200 µL of *Reagent A.* Swirl to dissolve, add 100 µL of *Reagent B,* and mix. Allow the solution to stand for about 1 hour at room temperature, evaporate to dryness, add 4.0 mL of *Internal standard solution,* and mix to obtain a *Standard preparation* having a known concentration of about 0.0419 mg of USP Dinoprost Tromethamine RS per mL.

Assay preparation—Dilute and mix an accurately measured volume of Injection with *Diluent* to obtain a solution having a known concentration of about 0.5 mg of dinoprost per mL (*Assay stock preparation*). Proceed as directed for *Standard preparation,* beginning with "Transfer 1.0 mL of this solution."

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 30-cm column that contains packing L3. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure*: the resolution, *R,* between the dinoprost tromethamine and internal standard peaks is not less than 10, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject equal volumes (about 20 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the peak responses at equivalent retention times. The relative retention times are about 0.4, 0.5, 1.0, and 1.2 for the internal standard, the 15-*R* epimer, dinoprost tromethamine, and the 5,6-*trans* isomer, respectively. Calculate the quantity, in mg, of $C_{20}H_{34}O_5$ in each mL of the Injection taken by the formula:

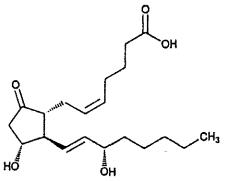
USP Monographs

www.webofpharma.com

$$(354.48/475.62)(D\ C)(R_U/R_S)$$

in which 354.48 and 475.62 are the molecular weights of dinoprost and dinoprost tromethamine, respectively; *D* is the dilution factor used in preparing the *Assay stock preparation;* *C* is the concentration, in mg per mL, of USP Dinoprost Tromethamine RS in the *Standard stock preparation;* and R_U and R_S are the ratios of the responses for the dinoprost tromethamine and internal standard peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dinoprostone



$C_{20}H_{32}O_5$ 352.47

Prosta-5,13-dien-1-oic acid, 11,15-dihydroxy-9-oxo-, (5*Z*, 11α,13*E*,15*S*)-;
(*E*,*Z*)-(1*R*,2*R*,3*R*)-7-[3-Hydroxy-2-[(3*S*)-(3-hydroxy-1-octenyl)]-5-oxocyclopentyl]-5-heptenoic acid;
Prostaglandin E_2 [363-24-6].

DEFINITION

Dinoprostone contains NLT 97.0% and NMT 103.0% of dinoprostone ($C_{20}H_{32}O_5$).

[NOTE—Prepare all solutions in all tests immediately before use.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.2% (v/v) of 5 N acetic acid in water
Mobile phase: Methanol and *Solution A* (29:21)
System suitability solution: 0.04 mg/mL each of USP Dinoprostone RS and USP Dinoprostone Related Compound C RS in *Mobile phase*
Standard solution: 2.5 mg/mL of USP Dinoprostone RS in *Mobile phase*
Sample solution: 2.5 mg/mL of Dinoprostone in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.8 between dinoprostone and dinoprostone related compound C, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dinoprostone ($C_{20}H_{32}O_5$) in the portion of Dinoprostone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dinoprostone RS in the *Standard solution* (mg/mL)
C_U = concentration of Dinoprostone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0%

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%
- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.
Standard stock solution: Prepare as directed for the *Standard solution* in the *Assay*.
Standard solution: Transfer 0.5 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.8 between dinoprostone and dinoprostone related compound C, *System suitability solution*
Column efficiency: NLT 6000 theoretical plates, *Standard solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Dinoprostone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of dinoprostone from the *Standard solution*
C_S = concentration of USP Dinoprostone RS in the *Standard solution* (mg/mL)
C_U = concentration of Dinoprostone in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

USP Monographs

www.webofpharma.com

Table 1

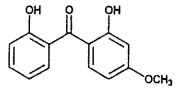
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
15-Oxo-dinoprostone	0.79	5	—*
15-Epi-dinoprostone	0.85	1.1	—*
8-Isodinoprostone	0.90	1.0	—*
Dinoprostone	1.00	—	—
Dinoprostone related compound C	1.15	1.0	2.0
(5*Z*,13*E*,15*S*)-15-Hydroxy-9-oxoprosta-5,10,13-triene-1-oic acid	1.80	5	1.0
(5*Z*,13*E*,15*S*)-15-Hydroxy-9-oxoprosta-5,8(12),13-trien-1-oic acid	1.90	1.43	1.0
Any individual unspecified impurity	—	1.0	0.10

* The sum of these three impurities is NMT 1.0%.

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 5 mg/mL in alcohol
  Acceptance criteria: −82.0° to −90.0°, at 20°
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dinoprostone RS
  USP Dinoprostone Related Compound C RS
  (*E*)-7-{(1*R*,2*R*,3*R*)-3-Hydroxy-2-[(*S*,*E*)-3-hydroxyoct-1-en-1-yl]-5-oxocyclopentyl}hept-5-enoic acid.
  $C_{20}H_{32}O_5$ 352.47

Dioxybenzone



$C_{14}H_{12}O_4$ 244.24
Methanone, (2-hydroxy-4-methoxyphenyl)(2-hydroxyphenyl)-;
2,2′-Dihydroxy-4-methoxybenzophenone [131-53-3].

DEFINITION
Dioxybenzone contains NLT 97.0% and NMT 103.0% of dioxybenzone ($C_{14}H_{12}O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Mobile phase: Methanol and water (60:40)
  Standard solution: 0.1 mg/mL of USP Dioxybenzone RS in *Mobile phase*. Sonicate if necessary to dissolve.
  Sample solution: 0.1 mg/mL of Dioxybenzone in *Mobile phase*. Sonicate if necessary to dissolve.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 286 nm
  Column: 4.6-mm × 7.5-cm; 3.5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 0.73%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of dioxybenzone ($C_{14}H_{12}O_4$) in the portion of Dioxybenzone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dioxybenzone RS in the *Standard solution* (mg/mL)
C_U = concentration of Dioxybenzone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

SPECIFIC TESTS
- **CONGEALING TEMPERATURE** ⟨651⟩**:** NLT 68.0°
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry a sample under vacuum at 40° for 2 h.
  Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dioxybenzone RS

Dioxybenzone and Oxybenzone Cream

DEFINITION
Dioxybenzone and Oxybenzone Cream is a mixture of approximately equal parts of Dioxybenzone and Oxybenzone in a suitable cream base. In each 100 g, it contains NLT 2.7 g and NMT 3.3 g each of dioxybenzone ($C_{14}H_{12}O_4$) and oxybenzone ($C_{14}H_{12}O_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Sample solutions: The solutions from the Cream used for measurement of absorbance in the *Assay*
  Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**
  Standard solution A: 12 µg/mL of USP Dioxybenzone RS in methanol

USP Monographs

www.webofpharma.com

Standard solution B: 12 µg/mL of USP Oxybenzone RS in methanol

Sample solution: Dissolve a portion of Cream, equivalent to 25 mg each of dioxybenzone and oxybenzone, in methanol in a 100-mL volumetric flask, and dilute with methanol to volume. Pipet 1 mL of this solution into a 15-mL conical test tube, evaporate on a water bath just to dryness using a gentle current of air, and dissolve the residue in 200 µL of methanol.

Analysis

Prepare sheets of chromatographic paper (Whatman No. 1 or equivalent), each measuring about 23 × 28.5 cm, as follows. Immerse the sheets in a 1-in-20 solution of light mineral oil in solvent hexane, withdraw them immediately, and allow to air-dry. On one sheet, mark a starting line about 2.5 cm from the long edge, and apply the entire *Sample solution* as a uniform streak along the starting line. Use a current of air or an air blower, if necessary, to maintain the width of the streak between 5 and 10 mm. Rinse the conical test tube that contained the *Sample solution* with 100 µL of methanol, and apply the rinse to the starting line. Similarly, repeat the rinsing and streaking with two additional portions of methanol, then allow the paper to air-dry for 5 min.

Staple together the short edges of the paper to form a cylinder, and place it in a 12-cm × 25-cm cylindrical chromatographic chamber containing 40 mL of a mobile solvent consisting of a mixture of equal volumes of acetone and water. Seal the chamber, and allow the chromatogram to develop for 2 h.

Remove the paper from the chamber, air-dry, remove the staples, and view the chromatogram under short-wavelength (254 nm) UV radiation. Mark the two bands representing the separated dioxybenzone and oxybenzone, respectively.

Determine the relative position of each benzone on the chromatogram by applying suitable aliquots of each *Standard solution* to another prepared chromatographic sheet and developing the chromatogram in a manner similar to that described for the *Sample solution*.

Cut the marked bands from the sheet, then, keeping the band segments separate, cut each into several pieces to facilitate extraction. Place the pieces from each band in separate glass-stoppered 50-mL conical flasks, add 20.0 mL of methanol to each flask, and shake gently for 30 min.

Blank: To provide the chromatographic blank, treat one of the prepared chromatographic sheets in the same manner as described above, but omit the application of the *Sample solution*. From the chromatographed paper, cut the areas corresponding to the bands produced by the benzones from the *Sample solution*, and in the same manner extract the blank bands for 30 min with 20.0 mL of methanol.

Instrumental conditions

Mode: UV

Cell: 1 cm

Analytical wavelength: Maximum absorbance at about 325 nm

Analysis

Samples: Resulting solutions from *Standard solution A*, *Standard solution B*, *Sample solution*, and *Blank*

Calculate the quantity, in mg, of dioxybenzone ($C_{14}H_{12}O_4$) in the portion of Cream taken:

$$\text{Result} = [(A_U - A_B)/A_S)] \times C \times 2$$

A_U = absorbance of the dioxybenzone solution from the *Sample solution*
A_B = absorbance of the *Blank*
A_S = absorbance of *Standard solution A*
C = concentration of USP Dioxybenzone RS in *Standard solution A* (µg/mL)

Calculate the quantity, in mg, of oxybenzone ($C_{14}H_{12}O_3$) in the portion of Cream taken:

$$\text{Result} = [(A_U - A_B)/A_S)] \times C \times 2$$

A_U = absorbance of the oxybenzone solution from the *Sample solution*
A_B = absorbance of the *Blank*
A_S = absorbance of *Standard solution B*
C = concentration of USP Oxybenzone RS in *Standard solution B* (µg/mL)

Acceptance criteria: In each 100 g, NLT 2.7 g and NMT 3.3 g each of dioxybenzone ($C_{14}H_{12}O_4$) and oxybenzone ($C_{14}H_{12}O_3$)

PERFORMANCE TESTS

• **Minimum Fill** ⟨755⟩**:** Meets the requirements

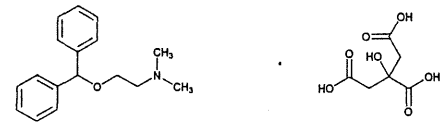
ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **USP Reference Standards** ⟨11⟩

USP Dioxybenzone RS
USP Oxybenzone RS

Diphenhydramine Citrate



$C_{17}H_{21}NO \cdot C_6H_8O_7$ 447.48

Ethanamine, 2-(diphenylmethoxy)-*N,N*-dimethyl-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1); 2-(Diphenylmethoxy)-*N,N*-dimethylethylamine citrate (1:1) [88637-37-0].

DEFINITION

Diphenhydramine Citrate contains NLT 98.0% and NMT 102.0% of diphenhydramine citrate ($C_{17}H_{21}NO \cdot C_6H_8O_7$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C. Identification Tests—General,** *Citrate* ⟨191⟩

ASSAY

• **Procedure**

Buffer: 5.4 g/L of monobasic potassium phosphate, adjusted with phosphoric acid to a pH of 3.0

Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	65	35

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Buffer (%)	Acetonitrile (%)
4	65	35
7	20	80
9	65	35
13	65	35

Diluent: Acetonitrile and *Buffer* (35:65)
System suitability solution: 0.15 mg/mL of USP Diphenhydramine Citrate RS and 0.1 mg/mL of USP Diphenhydramine Related Compound A RS in *Diluent*
Standard solution: 0.12 mg/mL of USP Diphenhydramine Citrate RS in *Diluent*
Sample solution: 0.12 mg/mL of Diphenhydramine Citrate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
[NOTE—The relative retention times for diphenhydramine related compound A and diphenhydramine are 0.9 and 1.0, respectively.]
Resolution: NLT 1.5 between diphenhydramine related compound A and diphenhydramine, *System suitability solution*
Tailing factor: NMT 1.8, *Standard solution*
Relative standard deviation: NMT 0.85% for six replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of diphenhydramine citrate ($C_{17}H_{21}NO \cdot C_6H_8O_7$) in the portion of Diphenhydramine Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for diphenhydramine from the *Sample solution*
r_S = peak response for diphenhydramine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Citrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Diphenhydramine Citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Sample: 8 g
Analysis: To the *Sample* add 5 mL of sulfuric acid, and char. After the substance is thoroughly charred, add 4 mL of nitric acid and a few drops of sulfuric acid, heat gently until fumes are no longer evolved, and ignite at 800 ± 25° until the carbon is consumed. Place in a muffle furnace at 550 ± 50° for about 1 h. Continue the ignition until constant weight is attained.
Acceptance criteria: NMT 0.1% remains.

• **ORGANIC IMPURITIES**
Buffer: 5.4 g/L of monobasic potassium phosphate, adjusted with phosphoric acid to a pH of 3.0
Mobile phase: Acetonitrile and *Buffer* (35:65)
System suitability solution: 0.15 mg/mL each of USP Diphenhydramine Related Compound A RS, benzhydrol, and USP Diphenhydramine Citrate RS in *Mobile phase*
Standard solution: 0.005 mg/mL of USP Diphenhydramine Citrate RS in *Mobile phase*
Sensitivity solution: 0.5 µg/mL of USP Diphenhydramine Citrate RS in *Mobile phase* from the *Standard solution*
Sample solution: 1.1 mg/mL of Diphenhydramine Citrate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 10 µL
Run time: 8 times the retention time of diphenhydramine
System suitability
[NOTE—See *Table 2* for the relative retention times.]
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between diphenhydramine related compound A and diphenhydramine
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Diphenhydramine Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of diphenhydramine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Citrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Diphenhydramine Citrate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.05% of the diphenhydramine peak.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citric acid[a]	0.3	—	—
Diphenhydramine related compound A[b]	0.9	1.6	0.5
Diphenhydramine	1.0	—	—
4-Methyldiphenhydramine[c]	1.4	1.4	0.3
4-Bromodiphenhydramine[d]	1.8	1.4	0.3
Benzhydrol[e]	3.8	2.4	0.3
Benzophenone[f]	7.7	1.4	0.3
Any other unspecified impurity	—	1.0	0.10

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	1.0

[a] Salt counter ion is included in the table for identification purposes only.
[b] 2-(Diphenylmethoxy)-*N*-methylethanamine.
[c] 2-[(*RS*)-(4-Methylphenyl)phenylmethoxy]-*N*,*N*-dimethylethanamine.
[d] 2-[(*RS*)-(4-Bromophenyl)phenylmethoxy]-*N*,*N*-dimethylethanamine.
[e] Diphenylmethanol.
[f] Diphenylmethanone.

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Sample: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Diphenhydramine Citrate RS
USP Diphenhydramine Related Compound A RS
2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.
$C_{16}H_{19}NO \cdot HCl$ 277.79

Diphenhydramine Citrate and Ibuprofen Tablets

DEFINITION
Diphenhydramine Citrate and Ibuprofen Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of diphenhydramine citrate ($C_{17}H_{21}NO \cdot C_6H_8O_7$) and ibuprofen ($C_{13}H_{18}O_2$).

IDENTIFICATION
• **A.** The retention times of the diphenhydramine and ibuprofen peaks from the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Buffer A: 6.9 g/L of monobasic sodium phosphate in water
Buffer B: 6.9 g/L of monobasic sodium phosphate in water. Adjust with triethylamine to a pH of 7.2.
Mobile phase: Acetonitrile, triethylamine, glacial acetic acid, and *Buffer A* (45: 0.2: 0.2: 55)
Diluent: Acetonitrile and *Buffer B* (3:2)
Standard solution: 1.1 mg/mL of USP Diphenhydramine Citrate RS and 5.7 mg/mL of USP Ibuprofen RS in *Diluent*. [Note—Sonicate as necessary.]
Sample solution: Transfer 10 Tablets into a suitable volumetric flask, add 350 mL of *Diluent*, and shake with a rotary shaker for 20 min. Sonicate for 20 min with intermediate shaking to obtain a solution containing about 1.1 mg/mL of diphenhydramine citrate and 5.7 mg/mL of ibuprofen. Centrifuge an aliquot at 4000 rpm for 10 min, and use the supernatant. [Note—Do not dilute to volume.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection size: 20 µL
Run time: 1.3 times the retention time of ibuprofen
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both diphenhydramine and ibuprofen
Relative standard deviation: NMT 2.0% for both diphenhydramine and ibuprofen
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of diphenhydramine citrate ($C_{17}H_{21}NO \cdot C_6H_8O_7$) and ibuprofen ($C_{13}H_{18}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*
r_S = peak response of the corresponding analyte from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% for both diphenhydramine citrate and ibuprofen

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: pH 6.5 phosphate buffer (Transfer 250 mL of 0.2 M monobasic potassium phosphate and 58 mL of 0.2 M sodium hydroxide to a 1000-mL volumetric flask, and dilute with water to volume.); 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 30 min
Buffer solution: 6.9 g/L of sodium dihydrogen phosphate monohydrate in water
Ibuprofen standard stock solution: 1.1 mg/mL of USP Ibuprofen RS in acetonitrile
Diphenhydramine citrate standard stock solution: 1.1 mg/mL of USP Diphenhydramine Citrate RS in water
Standard solution: Transfer 5 mL of the *Ibuprofen standard stock solution* and 1 mL of *Diphenhydramine citrate standard stock solution* to a 25-mL volumetric flask, and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Mobile phase: Acetonitrile, triethylamine, glacial acetic acid, and *Buffer solution* (45: 0.2: 0.2: 55)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm, 5-µm packing L1
Flow rate: 1.2 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both diphenhydramine and ibuprofen
Relative standard deviation: NMT 2.0% for both diphenhydramine and ibuprofen
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of diphenhydramine and ibuprofen dissolved:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of ibuprofen or diphenhydramine from the *Sample solution*
r_S = peak response of ibuprofen or diphenhydramine from the *Standard solution*
C_S = concentration of ibuprofen or diphenhydramine in the *Standard solution*
L = label claim for ibuprofen or diphenhydramine (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amounts of diphenhydramine and ibuprofen are dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer A: 1 mL of phosphoric acid in 1 L of water. Adjust with triethylamine to a pH of 3.2.

Buffer B: 1 mL of phosphoric acid and 1.0 g of monobasic potassium phosphate in 1 L of water. Adjust with triethylamine to a pH of 3.7.

Solution A: Acetonitrile and *Buffer A* (1:4)

Solution B: Acetonitrile and *Buffer B* (1:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	50	50
45	50	50
80	40	60
85	100	0
100	100	0

Standard solution: 0.004 mg/mL of USP Diphenhydramine Citrate RS and 0.02 mg/mL of USP Ibuprofen RS in *Solution B*

System suitability solution: 0.004 mg/mL of USP Diphenhydramine Related Compound A RS, 0.8 mg/mL of USP Diphenhydramine Citrate RS, and 4 mg/mL of USP Ibuprofen RS in *Solution B*

Sample solution: Transfer an amount of powder from ground Tablets (NLT 20) to a suitable volumetric flask. Add 70% of the flask volume of *Solution B*, sonicate for 20 min and dilute with *Solution B* to volume to obtain a solution containing about 0.8 mg/mL of diphenhydramine citrate and 4 mg/mL of ibuprofen. Centrifuge an aliquot at 4000 rpm for 10 min, and use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 0.8 between diphenhydramine and diphenhydramine related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for both diphenhydramine and ibuprofen, *Standard solution*

Relative standard deviation: NMT 5.0% for both diphenhydramine and ibuprofen, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the ibuprofen and diphenhydramine impurities using the relative retention times given in *Table 2*. Calculate the percentage of each diphenhydramine impurity and unspecified impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of diphenhydramine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Citrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine citrate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Calculate the percentage of the ibuprofen related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the ibuprofen related impurity from the *Sample solution*
r_S = peak response of ibuprofen from the *Standard solution*
C_S = concentration of USP Ibuprofen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ibuprofen in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Diphenhydramine related compound A[a]	0.95	1.3	0.26
Diphenhydramine	1.00	1.0	—
Unidentified diphenhydramine degradation product	1.32	1.0	0.2
Unidentified diphenhydramine degradation product	1.46	1.0	0.2
Unidentified ibuprofen degradation product	1.49	1.0	0.1
Methyl ibuprofen[b, c]	1.86	—	—
Unidentified diphenhydramine degradation product	1.96	1.0	0.2
Benzhydryl bromide[d]	2.49	2.4	0.26
Ibuprofen amide[b, e]	2.87	—	—
Isopropyl ibuprofen[b, f]	3.45	—	—
n-Propyl ibuprofen[b, g]	3.71	—	—
meta-Ibuprofen[b, h]	5.09	—	—
Ibuprofen	5.31	—	—
n-Butyl ibuprofen[b, i]	5.68	—	—

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other individual unspecified degradation product[i]	—	1.0	0.2
Total impurities[k]	—	—	1.0

[a] 2-(Diphenylmethoxy)-*N*-methylethanamine.
[b] Process impurity provided for information only; the content is not calculated and not reported.
[c] 2-*p*-Tolylpropanoic acid.
[d] (Bromomethylene)dibenzene.
[e] 2-(4-Isobutylphenyl) propanamide.
[f] 2-(4-Isopropylphenyl)propanoic acid.
[g] 2-(4-Propylphenyl)propanoic acid.
[h] 2-(3-Isobutylphenyl)propanoic acid.
[i] 2-(4-Butylphenyl)propanoic acid.
[j] Exclude peaks that elute before 4 min or after 80 min.
[k] Total impurities excludes ibuprofen related compound C.

• **Limit of Ibuprofen Related Compound C**
Buffer: 10 g/L of chloroacetic acid in water. Adjust with ammonium hydroxide to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (3:2)
Internal standard solution: 0.35 mg/mL of valerophenone in *Mobile phase*
Standard stock solution: 0.6 mg/mL of USP Ibuprofen Related Compound C RS in acetonitrile
Standard solution: 0.012 mg/mL of USP Ibuprofen Related Compound C RS in *Internal standard solution*; prepared by diluting 2 mL of *Standard stock solution* with *Internal standard solution* to 100 mL
Sample solution: Transfer an amount of powder equivalent to 1200 mg of ibuprofen from ground Tablets (NLT 20) to a suitable volumetric flask. Add 100 mL of *Internal standard solution*, and sonicate for 20 min to obtain a solution containing about 12 mg/mL of ibuprofen. Pass through a suitable filter, and use the filtrate. [Note—Do not dilute to volume.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection size: 5 µL
System suitability
Sample: *Standard solution*
[Note—The relative retention times for valerophenone and ibuprofen related compound C are 0.86 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.5 for both valerophenone and ibuprofen related compound C
Relative standard deviation: NMT 2.0%
Resolution: NLT 2.5 between the valerophenone and ibuprofen related compound C peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ibuprofen related compound C ($C_{12}H_{16}O$) in the portion of Tablets taken:

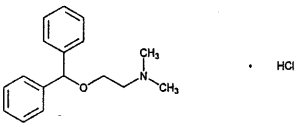
$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of ibuprofen related compound C to valerophenone from the *Sample solution*
R_S = peak area ratio of ibuprofen related compound C to valerophenone from the *Standard solution*
C_S = concentration of USP Ibuprofen Related Compound C RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1% of ibuprofen related compound C

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Diphenhydramine Citrate RS
USP Diphenhydramine Related Compound A RS
2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.
$C_{16}H_{19}NO \cdot HCl$ 277.79
USP Ibuprofen RS
USP Ibuprofen Related Compound C RS
4-Isobutylacetophenone.
$C_{12}H_{16}O$ 176.25

Diphenhydramine Hydrochloride



$C_{17}H_{21}NO \cdot HCl$ 291.82
Ethanamine, 2-(diphenylmethoxy)-*N*,*N*-dimethyl-, hydrochloride;
2-(Diphenylmethoxy)-*N*,*N*-dimethylethylamine hydrochloride [147-24-0].

DEFINITION
Diphenhydramine Hydrochloride contains NLT 98.0% and NMT 102.0% of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. Identification Tests—General,** *Chloride* ⟨191⟩

ASSAY
• **Procedure**
Buffer: 5.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH to 3.0.
Diluent: Acetonitrile and *Buffer* (35:65)
System suitability solution: 0.1 mg/mL each of USP Diphenhydramine Hydrochloride RS and USP Diphenhydramine Related Compound A RS in *Diluent*
Standard solution: 0.07 mg/mL of USP Diphenhydramine Hydrochloride RS in *Diluent*
Sample solution: 0.07 mg/mL of Diphenhydramine Hydrochloride in *Diluent*
Mobile phase: See *Table 1*.

www.webofpharma.com

Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	65	35
4	65	35
7	20	80
9	65	35
13	65	35

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
[NOTE—The relative retention times for diphenhydramine related compound A and diphenhydramine are 0.9 and 1.0, respectively.]
Resolution: NLT 1.5 between diphenhydramine related compound A and diphenhydramine, *System suitability solution*
Tailing factor: NMT 1.8, *Standard solution*
Relative standard deviation: NMT 0.85% for six replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Diphenhydramine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer: 5.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (35:65)
System suitability solution: 0.1 mg/mL each of USP Diphenhydramine Related Compound A RS, benzhydrol, and USP Diphenhydramine Hydrochloride RS in *Mobile phase*
Standard solution: 0.0035 mg/mL of USP Diphenhydramine Hydrochloride RS in *Mobile phase*
Sample solution: 0.7 mg/mL of Diphenhydramine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 10 µL
Run time: 7 times the retention time of diphenhydramine
System suitability
[NOTE—See *Table 2* for the relative retention times.]
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between diphenhydramine related compound A and diphenhydramine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Diphenhydramine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of diphenhydramine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Diphenhydramine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. [NOTE—Disregard peaks that are less than 0.05% of the diphenhydramine peak.]

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Diphenhydramine related compound A[a]	0.9	1.0	0.5
Diphenhydramine	1.0	—	—
4-Methyldiphenhydramine[b]	1.5	1.0	0.3
4-Bromodiphenhydramine[c]	1.8	1.0	0.3
Benzhydrol[d]	2.6	1.4	0.3
Benzophenone[e]	5.1	1.0	0.3
Any other unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 2-(Diphenylmethoxy)-*N*-methylethanamine.
[b] 2-[(*RS*)-(4-Methylphenyl)phenylmethoxy]-*N*,*N*-dimethylethanamine.
[c] 2-[(*RS*)-(4-Bromophenyl)phenylmethoxy]-*N*,*N*-dimethylethanamine.
[d] Diphenylmethanol.
[e] Diphenylmethanone.

SPECIFIC TESTS

- **ACIDITY OR ALKALINITY**

Sample solution: 50 mg/mL of Diphenhydramine Hydrochloride in carbon dioxide-free water
Analysis: To 10 mL of the *Sample solution*, add 0.15 mL of methyl red TS 2 and 0.25 mL of 0.01 N hydrochloric acid. The solution is pink. Titrate with 0.01 N sodium hydroxide.
Acceptance criteria: NMT 0.5 mL of 0.01 N sodium hydroxide is required to change the color of the solution to yellow.

- **LOSS ON DRYING** ⟨731⟩

Sample: Dry a sample at 105° for 3 h.
Acceptance criteria: NMT 0.5%

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Diphenhydramine Hydrochloride RS
  USP Diphenhydramine Related Compound A RS
  2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.
  $C_{16}H_{19}NO \cdot HCl$ 277.79

Diphenhydramine Hydrochloride Capsules

DEFINITION

Diphenhydramine Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$).

IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩**:** The contents of the Capsules meet the requirements.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 5.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Solution A: *Buffer*
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	65	35
4	65	35
7	20	80
9	65	35
13	65	35

Diluent: Acetonitrile and *Buffer* (35:65)
System suitability solution: 0.1 mg/mL each of USP Diphenhydramine Related Compound A RS and USP Diphenhydramine Hydrochloride RS in *Diluent*
Standard solution: Nominally 0.07 mg/mL of USP Diphenhydramine Hydrochloride RS in *Diluent*
Sample stock solution: Weigh and combine the contents of NLT 20 Capsules. Transfer an accurately weighed portion of the combined Capsule contents, equivalent to about 50 mg of diphenhydramine hydrochloride, to a 100-mL volumetric flask. Dissolve in and dilute with water to volume, and filter. Alternatively, dissolve NLT 20 Capsules in water at 50° and pipet the solution equivalent to about 50 mg of diphenhydramine hydrochloride to a 100-mL volumetric flask. Dissolve in and dilute with water to volume, and filter.
Sample solution: 0.07 mg/mL of diphenhydramine hydrochloride in *Diluent* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for diphenhydramine related compound A and diphenhydramine are about 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between diphenhydramine and diphenhydramine related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) in the portion of Capsule contents taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diphenhydramine from the *Sample solution*
r_S = peak response of diphenhydramine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
Procedure for a pooled sample
Medium: Water; 500 mL
Apparatus 1: 100 rpm
Time: 30 min
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
Injection volume: 50 µL
Standard solution: USP Diphenhydramine Hydrochloride RS in *Medium*, at a known concentration similar to that of the *Sample solution*
Sample solution: Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**
Buffer, Diluent, and **System suitability solution:** Prepare as directed in the *Assay*.
Mobile phase: Acetonitrile and *Buffer* (35:65)
Standard solution: 0.02 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.01 mg/mL of USP Diphenhydramine Related Compound A RS in *Diluent*
Sample solution: Nominally equivalent to 2 mg/mL of diphenhydramine hydrochloride in *Diluent* prepared as

www.webofpharma.com

follows. Remove the contents of NLT 20 Capsules as completely as possible, and weigh. Transfer a portion of the powder, nominally equivalent to 100 mg of diphenhydramine hydrochloride, to a 50-mL volumetric flask. Dilute with *Diluent* to volume. Sonicate the solution for 5 min.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 10 µL
Run time: 10 times the retention time of diphenhydramine

System suitability
Sample: *System suitability solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between diphenhydramine and diphenhydramine related compound A
Relative standard deviation: NMT 5.0% for diphenhydramine and diphenhydramine related compound A

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of diphenhydramine related compound A in the portion of Capsule contents taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diphenhydramine related compound A from the *Sample solution*
r_S = peak response of diphenhydramine related compound A from the *Standard solution*
C_S = concentration of USP Diphenhydramine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each degradation product in the portion of Capsule contents taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of diphenhydramine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Diphenhydramine related compound A	0.9	1.0	0.5
Diphenhydramine	1.0	—	—
Diphenhydramine *N*-oxide[a]	1.2	1.0	3.0
Benzhydrol[b]	4.7	1.5	2.0
Benzophenone[c]	9.3	0.8	0.4
Individual unspecified impurity	—	—	1.0

[a] 2-(Benzhydryloxy)-*N*,*N*-dimethylethan-1-amine oxide.
[b] Diphenylmethanol.
[c] Diphenylmethanone.

(Postponed indefinitely)

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Diphenhydramine Hydrochloride RS
  USP Diphenhydramine Related Compound A RS
  2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.
  $C_{16}H_{19}NO \cdot HCl$ 277.79

Diphenhydramine Hydrochloride Injection

DEFINITION
Diphenhydramine Hydrochloride Injection is a sterile solution of Diphenhydramine Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$).

IDENTIFICATION
- **A.**
  Sample solution: Dilute a volume of Injection equivalent to 50 mg of diphenhydramine hydrochloride with 0.03 N sulfuric acid to 25 mL.
  Analysis: Proceed as directed in *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Transfer the liquid to a separator".
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Buffer: 5.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
  Solution A: *Buffer*
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	65	35
4	65	35
7	20	80
9	65	35
13	65	35

www.webofpharma.com

Diluent: Acetonitrile and *Buffer* (35:65)
System suitability solution: 0.1 mg/mL each of USP Diphenhydramine Related Compound A RS and USP Diphenhydramine Hydrochloride RS in *Diluent*
Standard solution: 0.07 mg/mL of USP Diphenhydramine Hydrochloride RS in *Diluent*
Sample solution: Nominally equivalent to 0.07 mg/mL of diphenhydramine hydrochloride in *Diluent* prepared as follows. Transfer 5.0 mL of Injection, equivalent to 250 mg of diphenhydramine hydrochloride, to a 500-mL volumetric flask and dilute with water to volume. Transfer 7.0 mL of this solution to a 50-mL volumetric flask and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for diphenhydramine related compound A and diphenhydramine are about 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between diphenhydramine and diphenhydramine related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay* with a run time that is 10 times the retention time of diphenhydramine.
Mobile phase: Acetonitrile and *Buffer* (35:65)
Standard solution: 0.002 mg/mL of USP Diphenhydramine Hydrochloride RS in *Mobile phase*
Sample solution: Nominally equivalent to 2 mg/mL of diphenhydramine hydrochloride in water prepared as follows. Transfer a volume of Injection, equivalent to 500 mg of diphenhydramine hydrochloride, to a 250-mL volumetric flask and dilute with water to volume.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times. Inject a blank injection between the *System suitability solution* and *Standard solution*.]
Suitability requirements
Resolution: NLT 2.0 between diphenhydramine and diphenhydramine related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Diphenhydramine related compound A	0.9	1.1	0.4
Diphenhydramine	1.0	—	—
Benzhydrol[a]	4.3	1.5	0.4
Benzophenone[b]	8.2	0.8	0.2
Individual unspecified impurity	—	—	0.2
Total impurities	—	—	0.8

[a] Diphenylmethanol.
[b] Diphenylmethanone.

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.0–6.5
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 3.4 USP Endotoxin Units/mg of diphenhydramine hydrochloride
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Diphenhydramine Hydrochloride RS
USP Diphenhydramine Related Compound A RS
2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.
$C_{16}H_{19}NO \cdot HCl$ 277.79

www.webofpharma.com

Add the following:

▲Diphenhydramine Hydrochloride Oral Powder

DEFINITION

Diphenhydramine Hydrochloride Oral Powder contains NLT 90.0% and NMT 110.0% of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—The *System suitability solution*, *Standard solution*, and *Sample solution* should be passed through a polytetrafluoroethylene (PTFE) filter of 0.2-µm pore size.]

Buffer: 5.52 g/L of monobasic potassium hexafluorophosphate in water. Sonicate for 2–3 min, if necessary.

Solution A: *Buffer* and 0.05% phosphoric acid, prepared by adding 0.5 mL of phosphoric acid to 1 L of *Buffer*

Solution B: Acetonitrile, *Buffer*, and 0.05% phosphoric acid (700: 300: 0.5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
0.5	70	30
3.4	45	55
4.3	20	80
4.6	20	80
4.7	70	30
5.3	70	30

Diluent: Acetonitrile, water, and acetic acid (30:70:1)

System suitability solution: 0.0025 mg/mL each of USP Diphenhydramine Hydrochloride RS, USP Diphenhydramine Related Compound A RS, and USP Benzhydrol RS in *Diluent*

Standard solution: 0.25 mg/mL of USP Diphenhydramine Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.25 mg/mL of diphenhydramine hydrochloride in *Diluent* prepared as follows. Remove the contents of NLT 5 pouches as completely as possible, and weigh. Transfer a portion of the composite powder, equivalent to 50 mg of diphenhydramine hydrochloride, to a 200-mL volumetric flask. Dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.

Column: 2.1-mm × 5-cm; 1.7-µm packing L1

Column temperature: 40°

Flow rate: 0.9 mL/min

Injection volume: 2.5 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—For the relative retention times, see *Table 2*.]

System suitability

Resolution: NLT 2.0 between benzhydrol and diphenhydramine related compound A; NLT 2.0 between diphenhydramine related compound A and diphenhydramine, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) in the portion of Oral Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diphenhydramine from the *Sample solution*

r_S = peak response of diphenhydramine from the *Standard solution*

C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.5 µg/mL each of USP Diphenhydramine Hydrochloride RS and USP Benzophenone RS, 0.00125 mg/mL of USP Diphenhydramine Related Compound A RS, and 0.0025 mg/mL each of USP Benzhydrol RS and USP Diphenhydramine *N*-Oxide RS in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—For the relative retention times, see *Table 2*.]

Suitability requirements

Resolution: NLT 2.0 between benzhydrol and diphenhydramine related compound A; NLT 2.0 between diphenhydramine related compound A and diphenhydramine, *System suitability solution*

Relative standard deviation: NMT 5.0% for each peak, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of diphenhydramine related compound A, benzhydrol, benzophenone, or diphenhydramine *N*-oxide in the portion of Oral Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

www.webofpharma.com

r_U = peak response of diphenhydramine related compound A, benzhydrol, benzophenone, or diphenhydramine *N*-oxide from the *Sample solution*

r_S = peak response of diphenhydramine related compound A, benzhydrol, benzophenone, or diphenhydramine *N*-oxide from the *Standard solution*

C_S = concentration of USP Diphenhydramine Related Compound A RS, USP Benzhydrol RS, USP Benzophenone RS, or USP Diphenhydramine *N*-Oxide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Oral Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified degradation product from the *Sample solution*

r_S = peak response of diphenhydramine from the *Standard solution*

C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzhydrol	0.8	1.0
Diphenhydramine related compound A	0.9	0.5
Diphenhydramine	1.0	—
Diphenhydramine *N*-oxide	1.1	1.0
Benzophenone	1.2	0.2
Any unspecified degradation products	—	0.2
Total degradation products	—	3.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, unit-dose containers. Store at controlled room temperature
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Benzhydrol RS
Diphenylmethanol.
$C_{13}H_{12}O$ 184.23

USP Benzophenone RS
Diphenylmethanone.
$C_{13}H_{10}O$ 182.22

USP Diphenhydramine Hydrochloride RS

USP Diphenhydramine Related Compound A RS
2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.
$C_{16}H_{19}NO \cdot HCl$ 277.79

USP Diphenhydramine N-Oxide RS
2-(Benzhydryloxy)-*N*,*N*-dimethylethan-1-amine oxide hydrochloride.
$C_{17}H_{21}NO_2 \cdot HCl$ 307.82▲ (USP 1-May-2020)

Diphenhydramine Hydrochloride Oral Solution

DEFINITION

Diphenhydramine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$).

IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 11.24 g/L of sodium perchlorate monohydrate in water. Add 1 mL of trifluoroacetic acid to each L of solution prepared.
Solution B: Acetonitrile and trifluoroacetic acid (1000:1)
Solution C: *Solution A* and *Solution B* (82:18)
Solution D: *Solution A* and *Solution B* (50:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution C (%)	Solution D (%)
0	100	0
14.0	0	100
20.0	0	100
20.1	100	0
25.0	100	0

Diluent: Acetonitrile and water (18:82)
Standard stock solution: 1.0 mg/mL of USP Diphenhydramine Hydrochloride RS in *Diluent*
Standard solution: 0.25 mg/mL of USP Diphenhydramine Hydrochloride RS from the *Standard stock solution*
System suitability stock solution: 0.0125 mg/mL of USP Diphenhydramine Related Compound A RS prepared as follows. Transfer an appropriate amount of the Reference Standard to a volumetric flask. Add 5% of the flask volume of acetonitrile and dilute with *Diluent* to volume. Dilute this solution (1 in 10) with *Diluent*.
System suitability solution: 0.25 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.00025 mg/mL of USP Diphenhydramine Related Compound A RS in *Diluent* from the *Standard stock solution* and *System suitability stock solution*, respectively
Sample solution: Nominally 0.25 mg/mL of diphenhydramine hydrochloride from a suitable volume of Oral Solution in *Diluent*. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size, discarding the first few mL of filtrate. Use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

www.webofpharma.com

Detector: UV 220 nm. For *Identification A*, use a diode-array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between diphenhydramine and diphenhydramine related compound A, *System suitability solution*
Tailing factor: 0.5–2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diphenhydramine from the *Sample solution*
r_S = peak response of diphenhydramine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **ALCOHOL DETERMINATION** ⟨611⟩ (if present): 90.0%–110.0% of the labeled amount of ethanol (C_2H_5OH)

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase, Diluent, and **Sample solution:** Proceed as directed in the *Assay*.
Standard stock solution: 0.25 mg/mL of USP Diphenhydramine Hydrochloride RS in *Diluent*
Impurity stock solution: 0.25 mg/mL each of USP Diphenhydramine Related Compound A RS, USP Diphenhydramine Related Compound B RS, USP Diphenhydramine N-Oxide RS, USP Benzhydrol RS, and USP Benzophenone RS in *Diluent*
Standard solution: 0.0025 mg/mL each of USP Diphenhydramine Hydrochloride RS, USP Diphenhydramine Related Compound A RS, USP Diphenhydramine Related Compound B RS, USP Diphenhydramine N-Oxide RS, USP Benzhydrol RS, and USP Benzophenone RS in *Diluent* from the *Standard stock solution* and *Impurity stock solution*, respectively
System suitability stock solution: 0.0125 mg/mL each of USP Diphenhydramine Related Compound A RS and USP Diphenhydramine Related Compound B RS prepared as follows. Transfer an appropriate amount of each Reference Standard to a volumetric flask. Add 5% of the flask volume of acetonitrile and dilute with *Diluent* to volume. Dilute this solution (1 in 10) with *Diluent*.
System suitability solution: 0.00025 mg/mL each of USP Diphenhydramine Hydrochloride RS, USP Diphenhydramine Related Compound A RS, and USP Diphenhydramine Related Compound B RS in *Diluent* from the *Standard stock solution* and *System suitability stock solution*, respectively
Chromatographic system: Proceed as directed in the *Assay* except for the *Detector* parameters.
Detector: UV 272 nm. Switch to 220 nm at 8.5 min and 254 nm at 16.0 min.
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between diphenhydramine and diphenhydramine related compound A; NLT 1.5 between diphenhydramine related compound A and diphenhydramine related compound B, *System suitability solution*
Relative standard deviation: NMT 6.0% for each component, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each specified degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified degradation product from the *Sample solution*
r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of diphenhydramine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sodium benzoate[a]	0.50	—
Diphenhydramine	1.00	—
Diphenhydramine *N*-oxide	1.07	3.0
Benzhydrol	1.12	2.0
Benzophenone	1.45	0.4

www.webofpharma.com

Table 2 *(continued)*

Name		Relative Retention Time	Acceptance Criteria, NMT (%)
Unspecified degradation products	Diphenhydramine related compound B[b]	0.93	0.2 each
	Diphenhydramine related compound A	0.96	
	Any individual unspecified degradation product	—	
Total impurities		—	3.0

[a] Included in the table for identification only.
[b] Monitor for this degradation product in liquid formulations that contain glycerin.

(Postponed indefinitely)

PERFORMANCE TESTS
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 3.5–5.5
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the test for the absence of *Escherichia coli*. The total aerobic microbial count does not exceed 10^2 cfu/mL. The total yeasts and molds count does not exceed 10^1 cfu/mL.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Benzhydrol RS
Diphenylmethanol.
$C_{13}H_{12}O$ 184.23
(Postponed indefinitely)

USP Benzophenone RS
Diphenylmethanone.
$C_{13}H_{10}O$ 182.22
(Postponed indefinitely)

USP Diphenhydramine Hydrochloride RS

USP Diphenhydramine N-Oxide RS
2-(Benzhydryloxy)-*N*,*N*-dimethylethan-1-amine oxide hydrochloride.
$C_{17}H_{21}NO_2 \cdot HCl$ 307.82
(Postponed indefinitely)

USP Diphenhydramine Related Compound A RS
2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.
$C_{16}H_{19}NO \cdot HCl$ 277.79

USP Diphenhydramine Related Compound B RS
3-(Benzhydryloxy)propane-1,2-diol.
$C_{16}H_{18}O_3$ 258.32
(Postponed indefinitely)

Diphenhydramine Hydrochloride and Ibuprofen Capsules

DEFINITION
Diphenhydramine Hydrochloride and Ibuprofen Capsules contain NLT 95.0% and NMT 105.0% of the labeled amounts of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) and ibuprofen ($C_{13}H_{18}O_2$).

IDENTIFICATION
- **A.** The retention times of the diphenhydramine and ibuprofen peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the diphenhydramine and ibuprofen peaks of the *Sample solution* and those of the *Standard solution* exhibit maxima at the same wavelengths of 265 and 273 nm, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (38:62)
Standard solution: 0.05 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.4 mg/mL of USP Ibuprofen RS in *Mobile phase*
Sample stock solution: Nominally 0.25 mg/mL of diphenhydramine hydrochloride and 2.0 mg/mL of ibuprofen, prepared as follows. Transfer NLT 5 Capsules (including shells) to a suitable volumetric flask, add 4% of the final volume of water, and sonicate for 20 min. Dissolve and dilute with *Mobile phase* to volume. Pass a portion through a suitable filter of 0.45-µm pore size.
Sample solution: Nominally 0.05 mg/mL of diphenhydramine hydrochloride and 0.4 mg/mL of ibuprofen in *Mobile phase* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Assay: UV 220 nm
Identification ▲**B:**▲ (ERR 1-Jun-2019) Diode array, UV 200–400 nm
Column: 4.6-mm × 10-cm; 3-µm packing L11
Column temperature: 25°
Flow rate: 1.0 mL/min
Injection volume: 5 µL
Run time: NLT 4 times the retention time of diphenhydramine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both diphenhydramine and ibuprofen
Relative standard deviation: NMT 2.0% for both diphenhydramine and ibuprofen
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) and ibuprofen ($C_{13}H_{18}O_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diphenhydramine or ibuprofen from the *Sample solution*

www.webofpharma.com

r_S = peak response of diphenhydramine or ibuprofen from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS or USP Ibuprofen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride or ibuprofen in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩
Medium: Phosphate buffer, pH 7.2 (27.22 mg/mL of monobasic potassium phosphate in water and adjust with 100 mg/mL of sodium hydroxide to a pH of 7.2); 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 100 mg/mL of sodium hydroxide to a pH of 6.6.
Mobile phase: Methanol and *Buffer* (65:35)
Standard stock solution: 0.27 mg/mL of USP Diphenhydramine Hydrochloride RS and 2.2 mg/mL of USP Ibuprofen RS, prepared as follows. Transfer known amounts of USP Diphenhydramine Hydrochloride RS and USP Ibuprofen RS to a suitable volumetric flask. Add 5% of the final volume of methanol and sonicate to dissolve. Dilute with *Medium* to volume.
Standard solution: 0.027 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.22 mg/mL of USP Ibuprofen RS in *Medium* from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first few mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 5 µL
Run time: NLT 2.3 times the retention time of ibuprofen
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both diphenhydramine and ibuprofen
Relative standard deviation: NMT 2.0% for both diphenhydramine and ibuprofen
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) and ibuprofen ($C_{13}H_{18}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of diphenhydramine or ibuprofen from the *Sample solution*
r_S = peak response of diphenhydramine or ibuprofen from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS▲or▲ (ERR 1-Jun-2019) USP Ibuprofen RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim of diphenhydramine or ibuprofen (mg/Capsule)

Tolerances: NLT 75% (Q) of the labeled amounts of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) and ibuprofen ($C_{13}H_{18}O_2$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**
Buffer: Proceed as directed in the *Assay*.
Mobile phase: Acetonitrile and *Buffer* (32:68)
Diluent: Acetonitrile and *Buffer* (40:60)
System suitability solution: 0.0005 mg/mL of USP Diphenhydramine Related Compound A RS and 0.25 mg/mL of USP Diphenhydramine Hydrochloride RS in *Diluent*. Sonicate if necessary to dissolve.
Standard solution: 0.00125 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.01 mg/mL of USP Ibuprofen RS in *Diluent*. Sonicate to dissolve.
Sample solution: Nominally 0.25 mg/mL of diphenhydramine hydrochloride and 2 mg/mL of ibuprofen, prepared as follows. Transfer a suitable amount of Capsule contents from NLT 10 Capsules to a dry volumetric flask. Add about 60% of the final volume of *Diluent* and dissolve the contents completely by using a vortex. Dilute with *Diluent* to volume. Pass a portion through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 10-cm; 3-µm packing L11
Column temperature: 25°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Run time: NLT 20 times the retention time of diphenhydramine
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between diphenhydramine related compound A and diphenhydramine, *System suitability solution*
Relative standard deviation: NMT 5.0% for both diphenhydramine and ibuprofen, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of diphenhydramine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor of each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

www.webofpharma.com

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Diphenhydramine related compound A	0.9	1.0	0.2
Diphenhydramine	1.0	1.0	—
4-Methyldiphenhydramine[a]	1.3	1.0	0.2
4-Bromodiphenhydramine[b]	1.7	0.9	0.2
Benzhydrol[c]	2.3	1.6	0.2
Ibuprofen	4.1	—	—
Any other individual impurity	—	1.0	0.20
Total impurities	—	—	1.0

[a] *N,N*-Dimethyl-2-[phenyl(*p*-tolyl)methoxy]ethanamine.
[b] 2-[(4-Bromophenyl)(phenyl)methoxy]-*N,N*-dimethylethanamine.
[c] Diphenylmethanol.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: NMT 5×10^2 cfu/g for the total aerobic microbial count; NMT 10^2 cfu/g for total combined yeasts and molds count. It meets the requirements for absence of *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers and store at 20°–25°. Protect from light and excessive heat above 40°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Diphenhydramine Hydrochloride RS
  USP Diphenhydramine Related Compound A RS
  2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.
  $C_{16}H_{19}NO \cdot HCl$ 277.79
  USP Ibuprofen RS

Diphenhydramine and Phenylephrine Hydrochlorides Tablets

DEFINITION

Diphenhydramine and Phenylephrine Hydrochlorides Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) and phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$).

IDENTIFICATION

- **A.** The UV absorption spectra of the diphenhydramine and phenylephrine peaks of the *Sample solutions* and those of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.
- **B.** The retention times of the diphenhydramine and phenylephrine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

It is suggested to use plastic vials for analysis.

Buffer: 3.45 g/L of monobasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 3.5 ± 0.05 if necessary.
Mobile phase: Acetonitrile and *Buffer* (25:75)
Solution A: Dilute 10 mL of glacial acetic acid with 1000 mL of water.
Diluent: Methanol and *Solution A* (30:70)
Standard solution: 0.25 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.1 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.25 mg/mL of diphenhydramine hydrochloride and 0.1 mg/mL of phenylephrine hydrochloride prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask, add 50% final volume of *Solution A*, and stir for NLT 30 min. Add 30% final volume of methanol, and stir additionally for NLT 90 min. To ensure that particles do not collect above the solvent level, periodically rinse particulate into solution with *Solution A*. Allow the resulting solution to cool to room temperature, and dilute with *Solution A* to volume. Pass a portion through a suitable filter of 0.45-µm pore size. Discard the first 2–3 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm. For *Identification A*, use a diode-array detector in the range of 200–350 nm.
Column: 4.6-mm × 10-cm; 5-µm packing L9
Flow rate: 2.0 mL/min
Injection volume: 15 µL
Run time: NLT 3 times the retention time of phenylephrine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.5–3.0 for both phenylephrine and diphenhydramine
Relative standard deviation: NMT 2.0% for both phenylephrine and diphenhydramine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) and phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of diphenhydramine or phenylephrine from the *Sample solution*
r_S = peak response of diphenhydramine or phenylephrine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS or USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride or phenylephrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

It is suggested to use plastic vials for analysis.
Medium: Simulated gastric fluid without pepsin; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase: Proceed as directed in the *Assay*.

USP Monographs

www.webofpharma.com

Standard solution: (L_1/900) mg/mL of USP Diphenhydramine Hydrochloride RS and (L_2/900) mg/mL of USP Phenylephrine Hydrochloride RS in *Medium*, where L_1 is the label claim of diphenhydramine hydrochloride in mg/Tablet; and L_2 is the label claim of phenylephrine hydrochloride in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 10–20 µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 10-cm; 5-µm packing L9
Flow rate: 2.0 mL/min
Injection volume: 100 µL
Run time: NLT 3 times the retention time of phenylephrine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.5–3.0 for both phenylephrine and diphenhydramine
Relative standard deviation: NMT 2.0% for both phenylephrine and diphenhydramine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) and diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of diphenhydramine or phenylephrine from the *Sample solution*
r_S = peak response of diphenhydramine or phenylephrine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS or USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim of diphenhydramine hydrochloride (L_1) or phenylephrine hydrochloride (L_2) (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amounts of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) and phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

▲• **Organic Impurities**
Protect solutions containing diphenhydramine and phenylephrine from light. Use glass pipettes and silanized vials for analysis.
Solution A: Transfer 6 mL of 70% perchloric acid solution to a 100-mL volumetric flask, and dilute with water to volume.
Solution B: Transfer 8.31 g of potassium perchlorate to a suitable container. Add 10 mL of *Solution A* and 990 mL of water.
Solution C: Transfer 6 mL of 70% perchloric acid solution to a 100-mL volumetric flask, and dilute with acetonitrile to volume.
Solution D: Mix 10 mL of *Solution C* with 990 mL of acetonitrile.
Solution E: *Solution B* and *Solution D* (45:55)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution E (%)
0.0	99	1
17.0	99	1
25.0	53	47
33.0	53	47
50.0	5	95
55.0	5	95
55.1	99	1
63.0	99	1

Diluent: 1.26 g/L of ammonium formate in water. Adjust with formic acid to a pH of 4.0 ± 0.1.
Sensitivity solution: 0.0005 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.0002 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*
System suitability solution: 0.0002 mg/mL of USP Phenylephrine Related Compound F RS, 0.0005 mg/mL of USP Diphenhydramine Related Compound A RS, 0.15 mg/mL of USP Diphenhydramine Hydrochloride RS, and 0.2 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*
Standard solution: 0.005 mg/mL of USP Diphenhydramine Hydrochloride RS and 0.002 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.5 mg/mL of diphenhydramine hydrochloride and 0.2 mg/mL of phenylephrine hydrochloride prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask, using only PTFE stoppers. Add about 50% of final volume of *Diluent*, and shake on a flat bed shaker at low speed for NLT 1 h. Dilute with *Diluent* to volume. Centrifuge a portion until a clear supernatant is obtained, and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L60
Column temperature: 43.0°
Flow rate: 1.0 mL/min
Injection volume: 35 µL
System suitability
Samples: *Sensitivity solution*, *System suitability solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between phenylephrine related compound F and phenylephrine; NLT 2.0 between diphenhydramine related compound A and diphenhydramine, *System suitability solution*
Relative standard deviation: NMT 6.0% for phenylephrine and diphenhydramine, *Standard solution*
Signal-to-noise ratio: NLT 10 for phenylephrine and diphenhydramine, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Identify the diphenhydramine degradation products using the relative retention times in *Table 2*.
Calculate the percentage of each diphenhydramine related degradation product in the portion of Tablets taken:

USP Monographs

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual diphenhydramine related degradation product from the *Sample solution*
r_S = peak response of diphenhydramine from the *Standard solution*
C_S = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor of each individual impurity (see *Table 2*)

Identify the phenylephrine degradation products using the relative retention times in *Table 2*.
Calculate the percentage of each phenylephrine related degradation product and any other degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each phenylephrine related degradation product or any other degradation product from the *Sample solution*
r_S = peak response of phenylephrine from the *Standard solution*
C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of phenylephrine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor of each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peaks below 0.1%.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at 20°–25°.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Diphenhydramine Hydrochloride RS
  ▲USP Diphenhydramine Related Compound A RS
  2-(Diphenylmethoxy)-*N*-methylethanamine hydrochloride.
  $C_{16}H_{19}NO$ 241.33▲ (Postponed on 1-May-2016)
  USP Phenylephrine Hydrochloride RS
  ▲USP Phenylephrine Related Compound F RS
  2-Methyl-1,2,3,4-tetrahydroisoquinoline-4,8-diol.
  $C_{10}H_{13}NO_2$ 179.22▲ (Postponed on 1-May-2016)

Diphenhydramine and Pseudoephedrine Capsules

» Diphenhydramine and Pseudoephedrine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

Packaging and storage—Preserve in tight containers.
Labeling—Label Capsules to state both the contents of the active moieties and the contents of the salts used in formulating the article.
USP Reference standards ⟨11⟩—
USP Diphenhydramine Hydrochloride RS
USP Pseudoephedrine Hydrochloride RS

Identification—
A: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.
B: Developing solvent—Prepare a mixture of acetonitrile, methylene chloride, and *n*-propylamine (56:40:4).
Standard solution—Dissolve about 12.5 mg of USP Diphenhydramine Hydrochloride RS and 30 mg of USP Pseudoephedrine Hydrochloride RS in 5.0 mL of water in a 50-mL centrifuge tube.
Test solution—Transfer an amount of Capsule contents, equivalent to about 12.5 mg of diphenhydramine hydrochloride and about 30.0 mg of pseudoephedrine hydrochloride, to a 50-mL centrifuge tube. Add 5.0 mL of water, and shake.
Procedure—To each centrifuge tube add 5.0 mL of a saturated solution of sodium carbonate and 10 mL of methylene chloride. Insert the stoppers into the tubes and shake for about 1 minute. Centrifuge at about 2000 rpm for about 10 minutes until the layers are well separated. Draw off and discard the top layer. Add 5 g of anhydrous sodium sulfate to the remaining solution, insert the stopper tightly, and shake. Separately apply 30 µL (3 × 10 µL) each of the *Test solution* and the *Standard solution* to a suitable unactivated thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* ⟨621⟩) and allow the spots to dry. Develop the chromatograms in a paper lined chromatographic chamber equilibrated with the *Developing solvent* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, air-dry, place the plate in an iodine chamber, and allow to develop over several hours: the R_F values of the principal spots obtained from the *Test solution* correspond to those obtained from the *Standard solution*.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: water, 900 mL.
Apparatus 1: 100 rpm.
Time: 30 minutes.
Procedure—Inject a measured volume (about 50 µL) of a filtered portion of the solution under test into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Determine the quantities of $C_{17}H_{21}NO \cdot HCl$ and of $C_{10}H_{15}NO \cdot HCl$ dissolved by employing the procedures set forth in the *Assay*, making any necessary modifications.
Tolerances—Not less than 75% *(Q)* of the labeled amounts of $C_{17}H_{21}NO \cdot HCl$ and of $C_{10}H_{15}NO \cdot HCl$ are dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Related compounds—
Mobile phase—Prepare as directed in the *Assay*.
Diluting solution—Prepare a mixture of water, acetonitrile and methanol (64:26:10).
Standard solution—Transfer accurately weighed quantities of about 5 mg each of benzhydrol and benzophenone to a 500-mL volumetric flask, dissolve in *Diluting solution*, using

USP Monographs

www.webofpharma.com

Table 2 (Diphenhydramine and Phenylephrine Hydrochlorides Tablets)

Name		Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Isoquinoline 4,6-diol analog[a, b]		0.72	1.2	1.0
Phenylephrine		1.00	1.0	—
3-Hydroxybenzaldehyde[c, b]		2.37	5.3	0.6
Diphenhydramine		3.55	1.0	—
Diphenhydramine *N*-oxide[d, e]		3.71	0.92	1.2
Benzhydrol[f, e]		3.92	1.6	0.5
Benzophenone[e]		4.37	0.86	0.66
Unspecified degradation products quantified relative to phenylephrine	Phenylephrine carboxy analog (phenylephrine related compound G)[g, b]	0.80	1.2	0.3
	Phenylephrine related compound F[h, b]	0.94	1.0	
	Phenylephrone (phenylephrine related compound C)[i, b]	1.35	2.9	
	Phenylephrine isoquinolinone analog[j, b]	2.83	3.5	
	Individual phenylephrine related degradation product and any unspecified degradation product[b]	—	1.0	
Unspecified degradation products quantified relative to diphenhydramine	Diphenhydramine quarternary salt[k, e]	3.47	0.80	0.2
	Diphenhydramine related compound A[l, e]	3.51	1.1	
	Individual diphenhydramine related degradation product[e]	—	1.0	
Total degradation products quantitated relative to phenylephrine		—	—	2.5
Total degradation products quantitated relative to diphenhydramine		—	—	3.0

[a] 2-Methyl-1,2,3,4-tetrahydroisoquinoline-4,6-diol.
[b] Quantified relative to phenylephrine.
[c] 3-Hydroxybenzaldehyde.
[d] 2-(Benzhydryloxy)-*N*,*N*-dimethylethan-1-amine oxide.
[e] Quantified relative to diphenhydramine.
[f] Diphenylmethanol.
[g] (*R*)-*N*-[2-Hydroxy-2-(3-hydroxyphenyl)ethyl]-*N*-methylglycine.
[h] 2-Methyl-1,2,3,4-tetrahydroisoquinoline-4,8-diol.
[i] 1-(3-Hydroxyphenyl)-2-(methylamino)ethan-1-one.
[j] 1-(3-Hydroxybenzoyl)-2-methylisoquinolin-6(2*H*)-one.
[k] 2-{[2-(Benzhydryloxy)ethyl]dimethylammonio}acetate.
[l] 2-(Diphenylmethoxy)-*N*-methylethanamine.
▲ (Postponed on 1-May-2016)

heat and sonication if necessary, dilute with *Diluting solution* to volume, and mix. Dilute 5.0 mL of this solution quantitatively with *Diluting solution* to 25.0 mL, mix, and filter.

Test solution—Transfer 10 Capsules to a 500-mL volumetric flask, add about 350 mL of *Diluting solution*, sonicate in warm water at about 40° to effect dissolution, and cool. Dilute with *Diluting solution* to volume, and mix. Dilute 5.0 mL of this solution with *Diluting solution* to 25.0 mL, mix, and filter.

Resolution solution—Prepare a solution in *Diluting solution* containing about 2 µg each of benzhydrol and benzophenone, and 100 µg of USP Diphenhydramine Hydrochloride RS, per mL.

Chromatographic system—Proceed as directed for *Chromatographic system* under *Assay*. Chromatograph 50 µL of the *Resolution solution:* the resolution, *R*, between benz hydrol and diphenhydramine is not less than 1.3 and the resolution, *R*, between diphenhydramine and benzophenone is not less than 2.0.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution*, and record the areas of the benzhydrol and benzophenone peaks. Calculate

USP Monographs

www.webofpharma.com

the amounts of benzhydrol and benzophenone in the Capsules taken by the formula:

$$C(r_U/r_S)$$

in which *C* is the concentration of either benzhydrol or benzophenone, in µg per mL, in the *Standard solution,* and r_U and r_S are the areas of the corresponding analyte peaks obtained from the *Test solution* and the *Standard solution,* respectively: the sum of the amounts of benzhydrol and benzophenone does not exceed 2% (w/w) of the diphenhydramine hydrochloride.

Assay—

Aqueous solution—Dissolve 1.7 g of sodium 1-heptanesulfonate and 0.8 mL of triethylamine in about 800 mL of water, adjust with glacial acetic acid to a pH of 3.3 ± 0.05, dilute with water to 1 L, mix, and filter.

Mobile phase—Prepare a filtered and degassed mixture of *Aqueous solution,* acetonitrile, and methanol (64:26:10). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 25 mg of USP Diphenhydramine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask. Add 25*J* mg of USP Pseudoephedrine Hydrochloride RS, accurately weighed, *J* being the ratio of the labeled amount, in mg, of pseudoephedrine hydrochloride to the labeled amount, in mg, of diphenhydramine hydrochloride per capsule, dissolve in 0.5% glacial acetic acid, dilute with 0.5% glacial acetic acid to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.5% glacial acetic acid to volume, and mix.

Assay preparation—Remove as completely as possible, the contents of not less than 10 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 25 mg of diphenhydramine hydrochloride, to a 100-mL volumetric flask. Dissolve in 0.5% glacial acetic acid, dilute with 0.5% glacial acetic acid to volume, mix, and filter. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.5% glacial acetic acid to volume, and mix.

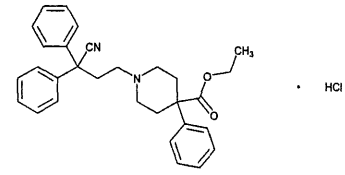
Chromatographic system—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the resolution, *R,* between the pseudoephedrine and diphenhydramine peaks is not less than 3.0. For each analyte peak, the tailing factor is not greater than 2.0, and the relative standard deviation for replicate injections is not greater than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for the two analytes. The relative retention times are about 1.0 for pseudoephedrine and 3.0 for diphenhydramine. Calculate the quantity, in mg, of diphenhydramine hydrochloride ($C_{17}H_{21}NO \cdot HCl$) in the portion of Capsules taken by formula:

$$C(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Diphenhydramine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Capsules taken by the same formula changing the terms to refer to pseudoephedrine hydrochloride.

Diphenoxylate Hydrochloride



$C_{30}H_{32}N_2O_2 \cdot HCl$ 489.05

4-Piperidinecarboxylic acid, 1-(3-cyano-3,3-diphenylpropyl)-4-phenyl-, ethyl ester, hydrochloride;

Ethyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenylpiperidine-4-carboxylate hydrochloride [3810-80-8].

DEFINITION

Diphenoxylate Hydrochloride contains NLT 98.0% and NMT 102.0% of diphenoxylate hydrochloride ($C_{30}H_{32}N_2O_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*: A saturated solution meets the requirements.

ASSAY

- **PROCEDURE**

Sample: 400 mg of Diphenoxylate Hydrochloride

Analysis: Dissolve the *Sample* in 40 mL of alcohol and add 5.0 mL of 0.01 N hydrochloric acid. Titrate with 0.1 N alcoholic sodium hydroxide VS (see *Titrimetry* ⟨541⟩), determining the endpoint potentiometrically. Read the volume of 0.1 N alcoholic sodium hydroxide added between the two points of inflection. Each mL of 0.1 N alcoholic sodium hydroxide is equivalent to 48.91 mg of diphenoxylate hydrochloride ($C_{30}H_{32}N_2O_2 \cdot HCl$).

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: Adjust 900 mL of water with phosphoric acid to a pH of 2.3, and dilute with water to 1000 mL.

Solution B: Acetonitrile

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
5	75	25
40	15	85

Diluent: Acetonitrile and *Solution A* (50:50)

System suitability solution: 1.0 mg/mL of USP Diphenoxylate Hydrochloride RS and 0.005 mg/mL of USP Diphenoxylate Related Compound A RS in *Diluent*. Sonicate for about 2 min to dissolve.

Standard solution: 1.0 µg/mL of USP Diphenoxylate Hydrochloride RS in *Diluent*

Sensitivity solution: 0.5 µg/mL of USP Diphenoxylate Hydrochloride RS in *Diluent* from *Standard solution*

www.webofpharma.com

Sample solution: 1.0 mg/mL of Diphenoxylate Hydrochloride in *Diluent*. Sonicate for about 2 min to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2.0 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 5.0 between diphenoxylate related compound A and diphenoxylate, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each individual impurity in the portion of Diphenoxylate Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of diphenoxylate from the *Standard solution*
C_S = concentration of USP Diphenoxylate Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Diphenoxylate Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Diphenoxylic acid (diphenoxylate related compound A)	0.8	0.50
Diphenoxylate	1.0	—
Any other individual impurity	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers, protected from light. Store at room temperature.
• **USP Reference Standards** ⟨11⟩
USP Diphenoxylate Hydrochloride RS
USP Diphenoxylate Related Compound A RS
Diphenoxylic acid;
1-(3-Cyano-3,3-diphenylpropyl)-4-phenylpiperidine-4-carboxylic acid.
$C_{28}H_{28}N_2O_2$ 424.53

Diphenoxylate Hydrochloride and Atropine Sulfate Oral Solution

» Diphenoxylate Hydrochloride and Atropine Sulfate Oral Solution contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of diphenoxylate hydrochloride ($C_{30}H_{32}N_2O_2 \cdot HCl$), and not less than 80.0 percent and not more than 120.0 percent of the labeled amount of atropine sulfate [$(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$].

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Atropine Sulfate RS
USP Diphenoxylate Hydrochloride RS
Identification—The retention times of two major peaks in the chromatogram of the *Assay preparation* correspond to the atropine and diphenoxylate peaks in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements with respect to diphenoxylate hydrochloride.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.
pH ⟨791⟩**:** between 3.0 and 4.3, determined in a dilution of the Oral Solution with an equal volume of water.
Alcohol Determination ⟨611⟩ **:** between 13.5% and 16.5% of C_2H_5OH.

Assay—
Solution A—Transfer 192 mg of sodium 1-pentanesulfonate monohydrate to a suitable container, add 200 mL of water, and sonicate to dissolve. Add 800 mL of water and 1.0 mL of phosphoric acid, and mix.
Solution B—Transfer 192 mg of sodium 1-pentane sulfonate monohydrate to a suitable container, add 200 mL of water, and sonicate to dissolve. Add 800 mL of acetonitrile, 1.0 mL of phosphoric acid, and mix.
Mobile phase—Prepare a filtered and degassed mixture of *Solution B* and *Solution A* (66:34). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Atropine stock preparation—Dissolve an accurately weighed quantity of USP Atropine Sulfate RS in dehydrated alcohol, and dilute quantitatively, and stepwise if necessary, with dehydrated alcohol to obtain a solution having a known concentration of about 0.04 mg per mL.
Standard preparation—Transfer about 20 mg of USP Diphenoxylate Hydrochloride RS to a 200-mL volumetric flask, add about 100 mL of dehydrated alcohol, and sonicate to dissolve. Accurately add 5.0 mL of *Atropine stock preparation* and 34 mL of water, and mix. Allow the solution to reach room temperature, and then dilute with dehydrated alcohol to volume. This solution contains about 0.1 mg of diphenoxylate hydrochloride and about 0.001 mg of atropine sulfate per mL.
Assay preparation—Transfer an accurately measured volume of the Oral Solution, equivalent to about 2.5 mg of diphenoxylate hydrochloride, based on the label claim, to a 25-mL volumetric flask, wash inside of the pipet with small portions of dehydrated alcohol, add the washings to the flask, dilute with dehydrated alcohol to volume, and mix. Pass a

USP Monographs

www.webofpharma.com

portion of the solution obtained through a 0.45-µm PTFE filter, discarding the first few mL, and use the clear filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L10. The flow rate is about 1.7 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.35 for atropine and 1.0 for diphenoxylate; the resolution, *R,* between atropine and diphenoxylate is not less than 5.0; the tailing factor is not more than 1.5 for atropine; and the relative standard deviation for replicate injections is not more than 2.0% for diphenoxylate and not more than 5.0% for atropine. [NOTE—If a significant tailing of the diphenoxylate peak is observed (greater than 2.5), it is recommended to maintain the column temperature at 25°, to stabilize the system.]

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of diphenoxylate hydrochloride ($C_{30}H_{32}N_2O_2 \cdot HCl$) in the portion of Oral Solution taken by the formula:

$$25C_D(r_U/r_S)$$

in which 25 is the volume, in mL, of the *Assay preparation;* C_D is the concentration, in mg per mL, of USP Diphenoxylate Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the diphenoxylate peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Calculate the quantity, in mg, of atropine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ in the portion of Oral Solution taken by the formula:

$$(694.83/676.83)(25)C_A(r_U/r_S)$$

in which 694.83 and 676.83 are the molecular weights of atropine sulfate monohydrate and anhydrous atropine sulfate, respectively; 25 is the volume, in mL, of the *Assay preparation;* C_A is the concentration, in mg per mL, of USP Atropine Sulfate RS in the *Standard preparation;* and r_U and r_S are the atropine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Diphenoxylate Hydrochloride and Atropine Sulfate Tablets

» Diphenoxylate Hydrochloride and Atropine Sulfate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of diphenoxylate hydrochloride ($C_{30}H_{32}N_2O_2 \cdot HCl$), and not less than 80.0 percent and not more than 120.0 percent of the labeled amount of atropine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Atropine Sulfate RS
USP Diphenoxylate Hydrochloride RS

Identification—The retention times of two major peaks in the chromatogram of the *Assay preparation* correspond to the atropine and diphenoxylate peaks in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: 0.2 M acetic acid; 500 mL.
Apparatus 1: 150 rpm.
Time: 45 minutes.

Determine the amount of $C_{30}H_{32}N_2O_2 \cdot HCl$ dissolved by employing the following method.

Mobile phase—Prepare a suitable degassed mixture of acetonitrile and 0.05 M monobasic potassium phosphate (65:35).

Standard solution—Dissolve an accurately weighed quantity of USP Diphenoxylate Hydrochloride RS in methanol to obtain a solution having a known concentration of about 250 µg per mL. Pipet 10 mL of this solution into a 500-mL volumetric flask, dilute with *Dissolution Medium* to volume, mix, and filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The flow rate is about 1.0 mL per minute. Chromatograph replicate injections of the *Standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5; and the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and of filtered portions of the solution under test into the chromatograph, record the chromatograms, measure the response for the major peak, and determine the amount of $C_{30}H_{32}N_2O_2 \cdot HCl$ dissolved.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{30}H_{32}N_2O_2 \cdot HCl$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements for *Content uniformity* with respect to diphenoxylate hydrochloride.

Assay—

Diluent—Use a mixture of acetonitrile and water (1:1).

Solution A—Transfer 192 mg of sodium 1-pentane sulfonate monohydrate to a suitable container, add 200 mL of water, and sonicate to dissolve. Add 800 mL of water and 1.0 mL of phosphoric acid, and mix.

Solution B—Transfer 192 mg of sodium 1-pentane sulfonate monohydrate to a suitable container, add 200 mL of water, and sonicate to dissolve. Add 800 mL of acetonitrile and 1.0 mL of phosphoric acid, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Solution B* and *Solution A* (66:34). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Atropine stock preparation—Dissolve an accurately weighed quantity of USP Atropine Sulfate RS in *Diluent,* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.04 mg per mL.

Standard preparation—Transfer about 20 mg of USP Diphenoxylate Hydrochloride RS to a 200-mL volumetric flask, add about 100 mL of *Diluent,* and sonicate to dissolve. Accurately add 5.0 mL of *Atropine stock preparation,* and mix. Allow the solution to reach room temperature, and then dilute with *Diluent* to volume. This solution contains about 0.1 mg of diphenoxylate hydrochloride and about 0.001 mg of atropine sulfate per mL.

Assay preparation—Transfer an accurately counted number of Tablets, equivalent to about 25 mg of diphenoxylate hydrochloride, based on the label claim, to a 250-mL volumetric flask, add approximately 100 mL of *Diluent,* and shake by mechanical means for at least 15 minutes or until the

www.webofpharma.com

Tablets are completely disintegrated. Sonicate for an additional 15 minutes, allow the solution to reach room temperature, dilute with *Diluent* to volume, and mix. Pass a portion of the solution obtained through a 0.45-µm PTFE filter, discarding the first few mL, and use the clear filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L10. The flow rate is about 1.7 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.35 for atropine and 1.0 for diphenoxylate; the resolution, *R,* between atropine and diphenoxylate is not less than 5.0; the tailing factor is not more than 1.5 for atropine; and the relative standard deviation for replicate injections is not more than 2.0% for diphenoxylate and not more than 5.0% for atropine. [NOTE—If a significant tailing of the diphenoxylate peak is observed (greater than 2.5), it is recommended to maintain the column temperature at 25°, to stabilize the system.]

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of diphenoxylate hydrochloride ($C_{30}H_{32}N_2O_2 \cdot HCl$) in the portion of Tablets taken by the formula:

$$250C_D(r_U/r_S)$$

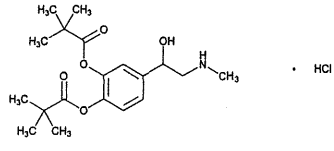
in which 250 is the volume, in mL, of the *Assay preparation;* C_D is the concentration, in mg per mL, of USP Diphenoxylate Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the diphenoxylate peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Calculate the quantity, in mg, of atropine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ in the portion of the Tablets taken by the formula:

$$(694.83/676.83)(250)C_A(r_U/r_S)$$

in which 694.83 and 676.83 are the molecular weights of atropine sulfate monohydrate and anhydrous atropine sulfate, respectively; 250 is the volume, in mL, of the *Assay preparation;* C_A is the concentration, in mg per mL, of USP Atropine Sulfate RS in the *Standard preparation;* and r_U and r_S are the atropine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dipivefrin Hydrochloride



$C_{19}H_{29}NO_5 \cdot HCl$ 387.90

Propanoic acid, 2,2-dimethyl-, 4-[1-hydroxy-2-(methylamino) ethyl]-1,2-phenylene ester, hydrochloride, (±)-; (±)-3,4-Dihydroxy-α-[(methylamino)methyl]benzyl alcohol 3,4-dipivalate hydrochloride [64019-93-8].

DEFINITION

Dipivefrin Hydrochloride contains NLT 98.5% and NMT 101.5% of dipivefrin hydrochloride ($C_{19}H_{29}NO_5 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
  Sample solution: 10 mg/mL of Dipivefrin Hydrochloride
  Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
  Mobile phase: Acetonitrile, 0.014 M sodium dodecyl sulfate, and glacial acetic acid (24:15:1)
  Standard solution: 5 mg/mL of USP Dipivefrin Hydrochloride RS in 0.0015 N hydrochloric acid
  Sample solution: 5 mg/mL of Dipivefrin Hydrochloride in 0.0015 N hydrochloric acid
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 25-cm; packing L1
  Flow rate: 2 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 0.6%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of dipivefrin hydrochloride ($C_{19}H_{29}NO_5 \cdot HCl$) in the portion of Dipivefrin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dipivefrin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Dipivefrin Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.3%
- **IRON** ⟨241⟩
  Standard iron solution: Use the *Standard Iron Solution* prepared as directed in the chapter.
  Hydroxylamine solution: 100 mg/mL of hydroxylamine hydrochloride in water
  Triazine solution: 1.25 mg/mL of 2,4,6-tri-(2-pyridyl)-*S*-triazine in methanol
  Standard solution: Into a 50-mL color-comparison tube pipet 1 mL of *Standard iron solution,* add 42.0 mL of water, and mix.
  Sample solution: Into a 50-mL color-comparison tube add 2.0 g of Dipivefrin Hydrochloride, 43.0 mL of water, and mix.
  Analysis: To each of the tubes containing the *Standard solution* and the *Sample solution* add 5.0 mL of *Hydroxylamine solution* and 2.0 mL of *Triazine solution,* and mix.

www.webofpharma.com

Acceptance criteria: NMT 5 ppm; the color of the solution from the *Sample solution* is not darker than that of the solution from the *Standard solution*.

• **LIMITS OF EPINEPHRINE AND ADRENALONE**

Protect the *Standard solution* and the *Sample solution* from light.

Solution A: 0.1% (v/v) Anhydrous formic acid in water

Solution B: Acetonitrile and methanol (60:40)

Mobile phase: See *Table 1*. Return to the original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
5	40	60
10	40	60

Diluent: 0.01 M hydrochloric acid

Standard solution: 0.02 mg/mL of USP Epinephrine Bitartrate RS and 0.01 mg/mL of USP Adrenalone Hydrochloride RS in *Diluent*.

Sample solution 10.0 mg/mL of Dipivefrin Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between epinephrine and adrenalone

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of epinephrine in the portion of Dipivefrin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of epinephrine from the *Sample solution*

r_S = peak response of epinephrine from the *Standard solution*

C_S = concentration of USP Epinephrine Bitartrate RS in the *Standard solution* (mg/mL)

C_U = concentration of Dipivefrin Hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of epinephrine, 183.20

M_{r2} = molecular weight of epinephrine bitartrate, 333.29

Calculate the percentage of adrenalone in the portion of Dipivefrin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of adrenalone from the *Sample solution*

r_S = peak response of adrenalone from the *Standard solution*

C_S = concentration of USP Adrenalone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Dipivefrin Hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of adrenalone, 181.19

M_{r2} = molecular weight of adrenalone hydrochloride, 217.65

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Epinephrine[a, b]	1.0	0.1
Adrenalone[c]	1.4	0.1

[a] 4-1[-Hydroxy-2-(methylamino)ethyl]benzene-1,2-diol.

[b] This impurity is a racemate of epinephrine (also known as racepinephrine or [±] adrenaline).

[c] 3′,4′-Dihydroxy-2-(methylamino)acetophenone.

• **ORGANIC IMPURITIES**

Solution A: Acetonitrile and methanol (60:40)

Solution B: 2.7 g/L of ammonium hydroxide, adjusted with 2 M acetic acid to a pH of 10.0

Mobile phase: *Solution A* and *Solution B* (55:45)

Diluent: *Solution A* and 0.01 M hydrochloric acid (55:45)

System suitability solution: 10 mg/mL of USP Dipivefrin Hydrochloride RS and 10 µg/mL of USP Dipivefrin Related Compound E RS in *Diluent*

Standard solution: 10 µg/mL each of USP Dipivefrin Hydrochloride RS and USP Dipivefrin Related Compound E RS in *Diluent*

Sample solution: 10 mg/mL of Dipivefrin Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 2.5 times the retention time of dipivefrin

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between dipivefrin and dipivefrin related compound E, *System suitability solution*

Relative standard deviation: NMT 5% for the dipivefrin peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dipivefrin related compound E in the portion of Dipivefrin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dipivefrin related compound E from the *Sample solution*

r_S = peak response of dipivefrin related compound E from the *Standard solution*

C_S = concentration of USP Dipivefrin Related Compound E RS in the *Standard solution* (mg/mL)

C_U = concentration of Dipivefrin Hydrochloride in the *Sample solution* (mg/mL)

www.webofpharma.com

Calculate the percentage of any other individual impurity in the portion of Dipivefrin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of dipivefrin from the *Standard solution*
C_S = concentration of USP Dipivefrin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Dipivefrin Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any impurity peak less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Pivaloyl racepinephrine[a] and 4-Pivaloyl racepinephrine[b]	0.4[c]	2.0	0.3
Dipivefrin	1.0	—	—
Dipivefrin related compound E[d]	1.3	—	0.1
N-Ethyl dipivefrin[e]	2.0	1.0	0.1
Unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] 2-Hydroxy-5-[1-hydroxy-2-(methylamino)ethyl]phenyl pivalate.
[b] 2-Hydroxy-4-[1-hydroxy-2-(methylamino)ethyl]phenyl pivalate.
[c] 5-Pivaloyl racepinephrine and 4-Pivaloyl racepinephrine coelutes.
[d] 4-[(Methylamino)acetyl]-1,2-phenylene dipivalate.
[e] 4-(2-[Ethyl(methyl)amino]-1-hydroxyethyl)-1,2-phenylene dipivalate.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry in a suitable vacuum drying tube over phosphorus pentoxide at 60° for 6 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Adrenalone Hydrochloride RS
3′,4′-Dihydroxy-2-(methylamino)acetophenone hydrochloride.
$C_9H_{11}NO_3 \cdot HCl$ 217.65
USP Dipivefrin Hydrochloride RS
USP Dipivefrin Related Compound E RS
4-[(Methylamino)acetyl]-1,2-phenylene dipivalate hydrochloride.
$C_{19}H_{27}NO_5 \cdot HCl$ 385.88
USP Epinephrine Bitartrate RS
$C_9H_{13}NO_3 \cdot C_4H_6O_6$ 333.29

Dipivefrin Hydrochloride Ophthalmic Solution

DEFINITION

Dipivefrin Hydrochloride Ophthalmic Solution is a sterile, aqueous solution of Dipivefrin Hydrochloride. It contains NLT 90.0% and NMT 115.0% of the labeled amount of dipivefrin hydrochloride ($C_{19}H_{29}NO_5 \cdot HCl$). It contains a suitable antimicrobial agent and may contain stabilizers, suitable buffers, and chelating agents.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Mobile phase: Acetonitrile, 0.014 M sodium dodecyl sulfate, and glacial acetic acid (24:15:1)
Standard solution: 1 mg/mL of USP Dipivefrin Hydrochloride RS in 0.0015 N hydrochloric acid
Sample solution: Transfer a volume of Ophthalmic Solution, nominally equivalent to 25 mg of dipivefrin hydrochloride, into a 25-mL volumetric flask, and dilute with 0.0015 N hydrochloric acid to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dipivefrin hydrochloride ($C_{19}H_{29}NO_5 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dipivefrin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dipivefrin hydrochloride in the *Sample solution*(mg/mL)

Acceptance criteria: 90.0%–115.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase: Acetonitrile, 0.014 M sodium dodecyl sulfate, and glacial acetic acid (24:15:1)
Diluent: 1.5 mM hydrochloric acid
System suitability solution: 1 mg/mL of USP Dipivefrin Hydrochloride RS and 0.01 mg/mL of USP Dipivefrin Related Compound E RS in *Diluent*
Standard solution: 0.004 mg/mL of USP Dipivefrin Hydrochloride RS and 0.01 mg/mL of USP Dipivefrin Related Compound E RS in *Diluent*
Sample solution: Nominally equivalent to 1 mg/mL of dipivefrin hydrochloride, from Ophthalmic Solution, diluted with *Diluent* if needed

USP Monographs

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*).
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between dipivefrin and dipivefrin related compound E, *System suitability solution*
Relative standard deviation: NMT 10% for the dipivefrin peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dipivefrin related compound E in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dipivefrin related compound E from the *Sample solution*
r_S = peak response of dipivefrin related compound E from the *Standard solution*
C_S = concentration of USP Dipivefrin Related Compound E RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dipivefrin hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of unspecified impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the unspecified impurity from the *Sample solution*
r_S = peak response of dipivefrin from the *Standard solution*
C_S = concentration of USP Dipivefrin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dipivefrin hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

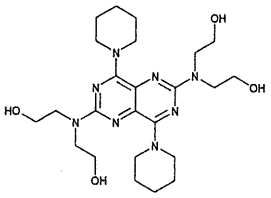
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dipivefrin related compound E[a]	1.2	1
Dipivefrin	1.0	—
Unspecified impurity	—	0.4

[a] 4-[(Methylamino)acetyl]-1,2-phenylene dipivalate.

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **pH** ⟨791⟩: 2.5–3.5

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Dipivefrin Hydrochloride RS
USP Dipivefrin Related Compound E RS
4-[(Methylamino)acetyl]-1,2-phenylene dipivalate hydrochloride.
$C_{19}H_{27}NO_5 \cdot HCl$ 385.88

Dipyridamole



$C_{24}H_{40}N_8O_4$ 504.63
Ethanol, 2,2′,2″,2‴-[(4,8-di-1-piperidinylpyrimido[5,4-*d*]pyrimidine-2,6-diyl)dinitrilo]tetrakis-;
2,2′,2″,2‴-[(4,8-Dipiperidinopyrimido[5,4-*d*]pyrimidine-2,6-diyl)dinitrilo]tetraethanol [58-32-2].

DEFINITION
Dipyridamole contains NLT 98.0% and NMT 102.0% of dipyridamole ($C_{24}H_{40}N_8O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Buffer: 0.77 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 4.0.
Mobile phase: Methanol and *Buffer* (700:300)
Standard solution: 0.2 mg/mL of USP Dipyridamole RS in methanol
Sample solution: 0.2 mg/mL of Dipyridamole in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 288 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
Run time: NLT 1.7 times the retention time of dipyridamole
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dipyridamole ($C_{24}H_{40}N_8O_4$) in the portion of Dipyridamole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dipyridamole from the *Sample solution*

USP Monographs

www.webofpharma.com

r_S = peak response of dipyridamole from the *Standard solution*
C_S = concentration of USP Dipyridamole RS in the *Standard solution* (mg/mL)
C_U = concentration of Dipyridamole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A: 10 mM ammonium formate. Adjust with formic acid to a pH of 5.0.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	80	20
2.0	80	20
17.0	5	95
19.0	5	95
21.0	80	20
25.0	80	20

Standard solution: 0.5 µg/mL each of USP Dipyridamole RS, USP Dipyridamole Related Compound A RS, USP Dipyridamole Related Compound B RS, USP Dipyridamole Related Compound C RS, USP Dipyridamole Related Compound D RS, USP Dipyridamole Related Compound E RS, and USP Dipyridamole Related Compound F RS in methanol
Sample solution: 500 µg/mL of Dipyridamole in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 295 nm
Column: 2.1-mm × 15-cm; 1.7-µm packing L1
Column temperature: 45°
Flow rate: 0.3 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between dipyridamole related compound D and dipyridamole
Relative standard deviation: NMT 2.8%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified impurity in the portion of Dipyridamole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each specified impurity from the *Sample solution*
r_S = peak response of corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of corresponding USP Reference Standard in the *Standard solution* (µg/mL)
C_U = concentration of Dipyridamole in the *Sample solution* (µg/mL)

Calculate the percentage of each unspecified impurity in the portion of Dipyridamole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of dipyridamole from the *Standard solution*
C_S = concentration of USP Dipyridamole RS in the *Standard solution* (µg/mL)
C_U = concentration of Dipyridamole in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dipyridamole related compound B	0.6	0.50
Dipyridamole related compound F	0.7	0.1
Dipyridamole related compound D	0.9	0.2
Dipyridamole	—	—
Dipyridamole related compound E	1.2	0.2
Dipyridamole related compound C	1.5	0.1
Dipyridamole related compound A	1.9	0.50
Any unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS

- **CHLORIDE**

Sample: 500 mg
Analysis: Dissolve the *Sample* in 5 mL of alcohol and 2 mL of 2 N nitric acid. Add 1 mL of silver nitrate TS.
Acceptance criteria: No turbidity or precipitate is produced.

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Dipyridamole RS
USP Dipyridamole Related Compound A RS
2,2′-{[4,6,8-Tri(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl]azanediyl}diethanol.
$C_{25}H_{40}N_8O_2$ 484.64
USP Dipyridamole Related Compound B RS
2,2′,2″,2‴,2⁗,2⁗′-{[8-(Piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,4,6-triyl]tris(azanetriyl)}hexaethanol.
$C_{23}H_{40}N_8O_6$ 524.61
USP Dipyridamole Related Compound C RS
2,2′-{[6-Chloro-4,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl]azanediyl}diethanol.
$C_{20}H_{30}ClN_7O_2$ 435.95
USP Dipyridamole Related Compound D RS
2,2′-({6-[(2-Hydroxyethyl)amino]-4,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl}azanediyl)diethanol.
$C_{22}H_{36}N_8O_3$ 460.57

USP Monographs

www.webofpharma.com

USP Dipyridamole Related Compound E RS
2,2′,2″,2‴-{[6,8-Di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,4-diyl]bis(azanetriyl)}tetraethanol.
$C_{24}H_{40}N_8O_4$ 504.63
USP Dipyridamole Related Compound F RS
2,2′,2″,2‴-({4-[(2-Hydroxyethyl)amino]-8-(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,6-diyl}bis(azanetriyl))tetraethanol.
$C_{21}H_{36}N_8O_5$ 480.56

Dipyridamole Injection

» Dipyridamole Injection is a sterile solution of Dipyridamole in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dipyridamole ($C_{24}H_{40}N_8O_4$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*. Protect from light, and avoid freezing.
USP Reference standards ⟨11⟩—
USP Dipyridamole RS

Identification—
A: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Test solution*—Use the Injection.
Standard solution: 5 mg per mL in a mixture of methanol, water, and 0.1 N hydrochloric acid (5:4:1).
Developing solvent system: a mixture of butyl alcohol, water, and glacial acetic acid (34:10:5).
Procedure—Proceed as directed in the chapter. Locate the yellow spots on the plate: the R_F value of the principal spot obtained from the *Test solution* corresponds to that of the principal spot obtained from the *Standard solution*. Spray the plate lightly with a spray reagent prepared as follows. Transfer 1 g of iodine and 3 g of potassium iodide to a 100-mL volumetric flask. Add 10 mL of alcohol to dissolve (heat gently). Add 20 mL of 2 N sulfuric acid, dilute with water to volume, and mix. Store in a dark place. Observe the plate, and locate the brown spots: the R_F value of the principal spot obtained from the *Test solution* corresponds to that of the principal spot obtained from the *Standard solution*.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 8.8 USP Endotoxin Units per mg of dipyridamole.
pH ⟨791⟩: between 2.2 and 3.2.
Chromatographic purity— [NOTE—Protect dipyridamole solutions from exposure to light.]
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*.
Test solution—Use the *Assay preparation* prepared as directed in the *Assay*.
Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Injection taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all of the peaks: not more than 2.0% of any individual impurity is found; and not more than 4.5% of total impurities is found.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—
[NOTE—Protect dipyridamole solutions from exposure to light.]
Acetate buffer—Dissolve a quantity of sodium acetate in water to obtain a concentration of about 6.8 mg per mL. Adjust with acetic acid to a pH of 5.1 ± 0.1.
Mobile phase—Prepare a filtered and degassed mixture of methanol and *Acetate buffer* (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Dipyridamole RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 25 mg of dipyridamole, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 276-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 2000 theoretical plates; the tailing factor is not greater than 1.7; and the relative standard deviation for replicate injections is not greater than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of dipyridamole ($C_{24}H_{40}N_8O_4$) in the portion of Injection taken by the formula:

$$25C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dipyridamole RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dipyridamole Compounded Oral Suspension

DEFINITION
Dipyridamole Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of dipyridamole ($C_{24}H_{40}N_8O_4$).
Prepare Dipyridamole Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Dipyridamole	1 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Place the required number of tablets into a suitable mortar and comminute to a fine powder, or add *Dipyridamole* powder to

USP Monographs

www.webofpharma.com

the mortar. Add 20 mL of *Vehicle*, and mix to a uniform paste. Add *Vehicle* in small portions, and mix well after each addition. Transfer, stepwise and quantitatively, to a graduated or calibrated bottle. Add *Vehicle* in portions to rinse the mortar, add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Solution A: 1 mg/mL of dibasic sodium phosphate. Adjust with dilute phosphoric acid (1 in 3) to a pH of 4.6.

Mobile phase: Methanol and *Solution A* (75:25). Pass through a membrane filter of a 0.5-µm pore size, and degas.

Standard solution: 100 µg/mL of USP Dipyridamole RS in *Mobile phase*

Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 288 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.3 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for dipyridamole is about 7.3 min.]

Suitability requirements

Relative standard deviation: NMT 2.3% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dipyridamole ($C_{24}H_{40}N_8O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Dipyridamole RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of dipyridamole in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **PH** ⟨791⟩: 3.8–4.8

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.

• **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature, or in a refrigerator

• **LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Dipyridamole RS

Dipyridamole Tablets

» Dipyridamole Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{24}H_{40}N_8O_4$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Dipyridamole RS

Identification—Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of dipyridamole, with 10 mL of 0.1 N hydrochloric acid, and filter, collecting the filtrate in a beaker. Add 0.1 N sodium hydroxide until the solution is basic and a precipitate forms. Heat the mixture on a steam bath for 1 minute, cool, and filter. Dry the residue at 105° for 1 hour: the residue so obtained responds to the *Identification* test under *Dipyridamole*.

Dissolution ⟨711⟩—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{24}H_{40}N_8O_4$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 282 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Dipyridamole RS in the same *Medium*.

Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{24}H_{40}N_8O_4$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—Transfer 1 Tablet to a 100-mL volumetric flask, add 50 mL of 1 N hydrochloric acid, heat in a steam bath for 5 minutes, and shake by mechanical means for 30 minutes. Cool to room temperature, dilute with 1 N hydrochloric acid to volume, and mix. Filter, discarding the first 25 mL of the filtrate. Dilute an accurately measured portion of the subsequent filtrate with 1 N hydrochloric acid to provide a solution containing about 10 µg of dipyridamole per mL. Concomitantly determine the absorbances of this solution and of a solution of USP Dipyridamole RS in the same medium having a known concentration of about 10 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 282 nm using 1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{24}H_{40}N_8O_4$ in the Tablet taken by the formula:

$$(TC/D)(A_U/A_S)$$

in which T is the labeled quantity, in mg, of dipyridamole in the Tablet; C is the concentration, in µg per mL, of USP Dipyridamole RS in the *Standard solution*; D is the concentration, in µg per mL, of dipyridamole in the solution from the Tablet based upon the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the solution from the Tablet and the *Standard solution*, respectively.

Assay—

Mobile phase—Dissolve 250 mg of dibasic sodium phosphate in 250 mL of water, and adjust with dilute phosphoric acid (1 in 3) to a pH of 4.6. Add 750 mL of methanol, mix, filter through a 0.5-µm membrane filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

USP Monographs

www.webofpharma.com

Standard preparation—Using an accurately weighed quantity of USP Dipyridamole RS, prepare a solution in *Mobile phase* having a known concentration of about 15 µg per mL.

Assay preparation—Transfer not less than 20 Tablets to a 1000-mL volumetric flask, add 100 mL of water, and sonicate for 15 minutes. Add about 750 mL of methanol, and shake by mechanical means for 30 minutes. Dilute with methanol to volume, mix, and centrifuge. Dilute an accurately measured volume (V_S mL) of the clear supernatant quantitatively with *Mobile phase* to obtain a solution (V_A mL) containing about 15 µg of dipyridamole per mL.

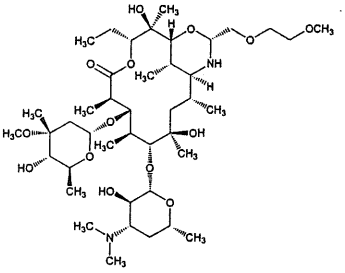
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 288-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 1000 theoretical plates, the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{24}H_{40}N_8O_4$ in the Tablets taken by the formula:

$$C(V_A/V_S)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Dipyridamole RS in the *Standard preparation;* V_A is the volume, in mL, of the *Assay preparation;* V_S is the volume, in mL, of supernatant taken for the *Assay preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dirithromycin



$C_{42}H_{78}N_2O_{14}$ 835.07

Erythromycin, 9-deoxo-11-deoxy-9,11-[imino[2-(2-methoxy ethoxy)ethylidene]oxy]-, 9*S*(*R*)-.

(9*S*)-9-Deoxo-11-deoxy-9,11-[imino[(1*R*)-2-(2-methoxy ethoxy)ethylidene]oxy]erythromycin [62013-04-1].

» Dirithromycin contains not less than 96.0 percent and not more than 102.0 percent of $C_{42}H_{78}N_2O_{14}$, consisting of the 16*R*- and 16*S*-epimers, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Dirithromycin RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Water Determination, *Method I* ⟨921⟩: not more than 1.0%.

Limit of dirithromycin 16 *S*-epimer—Using the chromatogram obtained in the test for *Chromatographic purity,* calculate the percentage of dirithromycin 16*S*-epimer in the portion of Dirithromycin taken by the formula:

$$1000(C/W)(r_E/r_S)$$

in which r_E is the response for dirithromycin 16*S*-epimer found in the chromatogram of the *Test solution;* and the other terms are as defined therein: not more than 1.5% of dirithromycin 16*S*-epimer is found.

Chromatographic purity—

Potassium phosphate buffer , *Mobile phase, System suitability solution, Solvent,* and *Chromatographic system*—Proceed as directed in the *Assay.*

Standard solution—Quantitatively dissolve an accurately weighed quantity of USP Dirithromycin RS in *Solvent* to obtain a solution having a known concentration of about 0.2 mg per mL.

Test solution—Transfer about 100 mg of Dirithromycin, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Solvent* to volume, and mix.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and record the chromatograms for a period of time that is not less than three times the retention time of dirithromycin (16*R*-epimer). Calculate the percentage of each impurity found in the portion of Dirithromycin taken by the formula:

$$1000(C/W)(r_i/r_S)$$

in which C is the concentration, in mg per mL, of USP Dirithromycin RS in the *Standard solution;* W is the quantity, in mg, of Dirithromycin taken to prepare the *Test solution;* r_i is the response for each impurity found in the chromatogram of the *Test solution;* and r_S is the response of the dirithromycin (16*R*-epimer) in the chromatogram of the *Standard solution:* not more than 1.5% of 9-(*S*)-erythromycylamine is found; not more than 1.0% of any other individual impurity is found; and not more than 4.0% of total impurities is found. [NOTE—Do not regard dirithromycin 16*S*-epimer as an impurity.]

Assay—

Potassium phosphate buffer—Dissolve 1.41 g of monobasic potassium phosphate and 6.91 g of dibasic potassium phosphate in 1 L of water, and pass through a filter having a porosity of 0.5 µm or finer.

Mobile phase—Prepare a degassed mixture of acetonitrile, *Potassium phosphate buffer,* and methanol (44:37:19).

System suitability solution—Dissolve an accurately weighed quantity of USP Dirithromycin RS in *Mobile phase* to obtain a solution having a concentration of about 2.5 mg per mL. Store this solution at room temperature for about 24 hours. [NOTE—The solution then contains an equilibrated mixture of dirithromycin (16*R*-epimer), dirithromycin 16*S*-epimer, and

www.webofpharma.com

9-(*S*)-erythromycylamine. The solution may be used for 1 month when stored at room temperature.]

Solvent—Prepare a mixture of acetonitrile and methanol (70:30).

Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Dirithromycin RS in *Solvent* to obtain a solution having a known concentration of about 2 mg per mL.

Assay preparation—Transfer about 20 mg of Dirithromycin, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Solvent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for 9-(*S*)-erythromycylamine, 1.0 for dirithromycin (16*R*-epimer), and 1.12 for dirithromycin 16*S*-epimer. The resolution, *R*, between dirithromycin (16*R*-epimer) and dirithromycin 16*S*-epimer is not less than 2.0, and between dirithromycin (16*R*-epimer) and 9-(*S*)-erythromycylamine is not less than 5.0, the tailing factor for the dirithromycin (16*R*-epimer) peak is not more than 2.0, and the relative standard deviation of the dirithromycin (16*R*-epimer) peak for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the percentage of $C_{42}H_{78}N_2O_{14}$ in the portion of Dirithromycin taken by the formula:

$$1000(C/W)(r_U/r_S) + P_E$$

in which *C* is the concentration, in mg per mL, of USP Dirithromycin RS in the *Standard preparation*; *W* is the quantity, in mg, of Dirithromycin taken to prepare the *Assay preparation*; r_U and r_S are the area responses for dirithromycin (16*R*-epimer) obtained from the *Assay preparation* and the *Standard preparation*, respectively; and P_E is the percentage of 16*S*-epimer as determined in the test for *Limit of dirithromycin 16S-epimer*.

Dirithromycin Delayed-Release Tablets

» Dirithromycin Delayed-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dirithromycin ($C_{42}H_{78}N_2O_{14}$), consisting of the 16*R*- and 16*S*-epimers.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Dirithromycin RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—Proceed as directed for *Procedure* for *Method B* under *Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms.*

ACID STAGE—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 10-mesh basket; 100 rpm.

Xanthydrol TS—[NOTE—Prepare this solution daily.] To about 150 mg of xanthydrol in a 100-mL volumetric flask, add 10 mL of glacial acetic acid, and swirl to dissolve. Dilute with hydrochloric acid to volume, and mix.

Standard solution—Quantitatively dissolve an accurately weighed quantity of USP Dirithromycin RS in 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 0.28 mg per mL.

Procedure—After 2 hours of operation, remove each Tablet, or the major portion thereof if the Tablet is not intact, from the individual vessel, and subject each Tablet to the test in the *Buffer stage*. Separately add 0.50 mL of acetic anhydride to 0.50 mL of the filtered solution under test and to 0.50 mL of the *Standard solution*, and mix. Add 5.0 mL of glacial acetic acid, allow to stand for 5 minutes, then add 0.50 mL of *Xanthydrol TS*, and allow 30 minutes for color development. Determine the amount of $C_{42}H_{78}N_2O_{14}$, including the 16*R*- and 16*S*-epimers, dissolved by employing UV absorption at the wavelength of maximum absorbance at about 540 nm.

BUFFER STAGE—

Medium: pH 6.8 phosphate buffer; 900 mL.

Procedure—Proceed as directed for *Acid stage* beginning with "Separately add 0.50 mL of acetic anhydride".

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{42}H_{78}N_2O_{14}$, including the 16*R*- and 16*S*-epimers, is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Water Determination, *Method I* ⟨921⟩: not more than 5.0%.

Chromatographic purity—

Potassium phosphate buffer, Mobile phase and *System suitability solution*—Proceed as directed in the *Assay* under *Dirithromycin*.

Phosphate buffer—Dissolve 4.35 g of dibasic potassium phosphate in 1 L of water, adjust with phosphoric acid to a pH of 8.0, and pass through a filter having a 0.5 µm or finer porosity.

Solvent 1—Prepare a mixture of acetonitrile and *Phosphate buffer* (60:40).

Solvent 2—Prepare a mixture of acetonitrile and *Phosphate buffer* (98:2). [NOTE—The mixture is cloudy.]

Standard solution—Quantitatively dissolve an accurately weighed quantity of USP Dirithromycin RS in *Solvent 1* to obtain a solution having a known concentration of about 0.2 mg per mL. [NOTE—Inject the *Standard solution* into the chromatograph immediately after preparation.]

Test solution—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 745 mg of dirithromycin, to a 50-mL volumetric flask, dissolve in and dilute with *Solvent 2* to volume, and mix. Centrifuge a portion of this solution, transfer 6.0 mL of the clear supernatant to a 10-mL volumetric flask, dilute with *Phosphate buffer* to volume, and mix. [NOTE—Inject the *Test solution* into the chromatograph immediately after preparation.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for 9-(*S*)-erythromycylamine, 1.0 for dirithromycin (16*R*-epimer), and 1.12 for dirithromycin 16*S*-epimer; the resolution, *R*, between the dirithromycin (16*R*-epimer) and dirithromycin 16*S*-epimer is not less than 2.0, and between dirithromycin (16*R*-epimer) and 9-(*S*)-erythromycylamine is

USP Monographs

www.webofpharma.com

not less than 5.0; and the tailing factor for the dirithromycin (16*R*-epimer) peak is not more than 2.0.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for a period of time that is not less than three times the retention time of dirithromycin (16*R*-epimer), and measure the peak areas. Calculate the percentage of each impurity found in the portion of Tablets taken by the formula:

$$50{,}000(C/6W)(L/T)(r_i/r_S)$$

in which C is the concentration, in mg per mL, of USP Dirithromycin RS in the *Standard solution*; W is the quantity, in mg, of Tablet powder taken to prepare the *Test solution*; L is the labeled amount, in mg, of dirithromycin in each Tablet; T is the average weight, in mg, of each Tablet; r_i is the peak response for each impurity obtained from the *Test solution*; and r_S is the peak response of dirithromycin (16*R*-epimer) obtained from the *Standard solution*: not more than 1.5% of 9-(*S*)-erythromycylamine is found; and not more than 5.0% of total impurities is found. [NOTE—Do not regard dirithromycin 16*S*-epimer as an impurity.]

Assay—

Potassium phosphate buffer, Mobile phase, System suitability solution, and *Solvent*—Proceed as directed in the *Assay* under *Dirithromycin.*

Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Dirithromycin RS in *Solvent* to obtain a solution having a known concentration of about 2.5 mg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of dirithromycin, to a 100-mL volumetric flask, dissolve in and dilute with *Solvent* to volume, and mix. Centrifuge a portion of this solution, and use the clear supernatant as the *Assay preparation.*

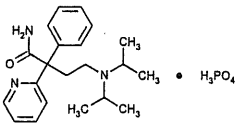
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for 9-(*S*)-erythromycylamine, 1.0 for dirithromycin (16*R*-epimer), and 1.12 for dirithromycin 16*S*-epimer; the resolution, *R,* between the dirithromycin (16*R*-epimer) and dirithromycin 16*S*-epimer is not less than 2.0, and between dirithromycin (16*R*-epimer) and 9-(*S*)-erythromycylamine is not less than 5.0; the tailing factor for the dirithromycin (16*R*-epimer) peak is not more than 2.0; and the relative standard deviation determined from the dirithromycin (16*R*-epimer) peak for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of dirithromycin ($C_{42}H_{78}N_2O_{14}$), which includes the 16*R*- and 16*S*-epimers, in the portion of Tablets taken by the formula:

$$40C(r_U + r_E)/r_S$$

in which C is the concentration, in mg per mL, of USP Dirithromycin RS in the *Standard preparation*; r_U and r_E are the peak responses for dirithromycin (16*R*-epimer) and dirithromycin 16*S*-epimer, respectively, obtained from the *Assay preparation*; and r_S is the peak response of dirithromycin (16*R*-epimer) obtained from the *Standard preparation.*

Disopyramide Phosphate



$C_{21}H_{29}N_3O \cdot H_3PO_4$ 437.47

2-Pyridineacetamide, α-[2-[bis(1-methylethyl)amino] ethyl]-α-phenyl-, (±)-, phosphate (1:1).

(±)-α-[2-(Diisopropylamino)ethyl]-α-phenyl-2-pyridineacetamide phosphate (1:1) [22059-60-5].

» Disopyramide Phosphate contains not less than 98.0 percent and not more than 102.0 percent of $C_{21}H_{29}N_3O \cdot H_3PO_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Disopyramide Phosphate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: A solution (1 in 200) meets the requirements of the tests for *Phosphate* ⟨191⟩.

pH ⟨791⟩: between 4.0 and 5.0 in a solution (1 in 20).

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

Chromatographic purity—

Standard solutions—Prepare solutions *A* and *B* of USP Disopyramide Phosphate RS in methanol having concentrations of about 50 and 100 µg per mL, respectively.

Test solution—Prepare a solution of Disopyramide Phosphate in methanol having a concentration of about 10 mg per mL.

Procedure—Separately apply 10-µL portions of *Standard solutions A* and *B* and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, dehydrated alcohol, and ammonium hydroxide (170:28:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow to air-dry, and spray with potassium bismuth iodide TS: the R_F value of the principal spot obtained from the *Test solution* corresponds to that obtained from *Standard solution B.* Estimate the levels of any additional spots observed in the chromatogram of the *Test solution* by comparison with the principal spots in the chromatograms of *Standard solutions A* and *B:* the sum of the intensities of any additional spots observed is not greater than that obtained from *Standard solution B* (equivalent to 1%).

Assay—Dissolve about 160 mg of Disopyramide Phosphate, accurately weighed, in 50 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make

www.webofpharma.com

any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 21.87 mg of $C_{21}H_{29}N_3O \cdot H_3PO_4$.

Disopyramide Phosphate Capsules

» Disopyramide Phosphate Capsules contain an amount of Disopyramide Phosphate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of disopyramide ($C_{21}H_{29}N_3O$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Disopyramide Phosphate RS
Identification—Transfer a portion of Capsule contents, equivalent to about 125 mg of disopyramide phosphate, to a 25-mL volumetric flask, add 20 mL of methanol, and shake by mechanical means for 20 minutes. Dilute with methanol to volume, mix, and filter through paper (Whatman No. 2 or equivalent), discarding the first 10 mL of the filtrate. Apply 10 µL each of the subsequent filtrate and of a solution of USP Disopyramide Phosphate RS in methanol containing 6.2 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, alcohol, and ammonium hydroxide (170:28:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Dissolution ⟨711⟩—
Medium: water; 1000 mL.
Apparatus 2: 50 rpm.
Time: 20 minutes.
Procedure—Filter 15 mL of the solution under test, and transfer 10.0 mL of the filtrate to a 25-mL volumetric flask. Dilute with 2 N sulfuric acid to volume, and mix. Determine the amount of disopyramide ($C_{21}H_{29}N_3O$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 268 nm of this solution, using water as the blank, in comparison with a Standard solution having a known concentration of USP Disopyramide Phosphate RS in the same medium.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{21}H_{29}N_3O$ is dissolved in 20 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Methanolic sulfuric acid—Cautiously add 5.4 mL of sulfuric acid to about 1800 mL of methanol with stirring, dilute with methanol to 2000 mL, and mix.
Procedure—Weigh the contents of not fewer than 20 Phosphate Capsules, and calculate the average weight per Capsule. Mix the combined contents of the Capsules, and transfer an accurately weighed portion, equivalent to about 125 mg of disopyramide phosphate, to a glass-stoppered, 125-mL flask. Add 50 mL of *Methanolic sulfuric acid*, and stir for 30 minutes. Filter through a medium-porosity, sintered-glass filter, and rinse thoroughly with *Methanolic sulfuric acid*. Transfer the combined filtrate and rinsings to a 100-mL volumetric flask, dilute with *Methanolic sulfuric acid* to volume, and mix. Dilute an accurately measured portion of this solution quantitatively and stepwise with the same solvent to obtain a solution having a concentration of about 40 µg per mL. Dissolve an accurately weighed portion of USP Disopyramide Phosphate RS in *Methanolic sulfuric acid*, and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about 40 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 268 nm, with a suitable spectrophotometer, using *Methanolic sulfuric acid* as the blank. Calculate the quantity, in mg, of $C_{21}H_{29}N_3O$ in the portion of Capsules taken by the formula:

$$3.125(339.48/437.47)C(A_U/A_S)$$

in which 339.48 and 437.47 are the molecular weights of disopyramide and disopyramide phosphate, respectively; C is the concentration, in µg per mL, of USP Disopyramide Phosphate RS in the Standard solution; and A_U and A_S are the absorbances of the solution from the Capsules and the Standard solution, respectively.

Disopyramide Phosphate Extended-Release Capsules

» Disopyramide Phosphate Extended-Release Capsules contain an amount of Disopyramide Phosphate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of disopyramide ($C_{21}H_{29}N_3O$).

Packaging and storage—Preserve in well-closed containers.
Labeling—The labeling indicates the *Dissolution Test* with which the product complies.
USP Reference standards ⟨11⟩—
USP Disopyramide Phosphate RS
Identification—Transfer a portion of Capsule contents, equivalent to about 195 mg of disopyramide phosphate, to a 25-mL volumetric flask, add 20 mL of methanol, and shake by mechanical means for 20 minutes. Dilute with methanol to volume, mix, and filter, discarding the first 10 mL of the filtrate. Apply 20 µL each of the subsequent filtrate and of a solution of USP Disopyramide Phosphate RS in methanol containing 7.7 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, absolute alcohol, and ammonium hydroxide (170:28:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Dissolution ⟨711⟩—
TEST 1—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1.*

USP Monographs

www.webofpharma.com

pH 2.5, 0.1 M Phosphate buffer—Dissolve 272 g of monobasic potassium phosphate in 20 L of water, and adjust with hydrochloric acid to a pH of 2.50 ± 0.04. [NOTE—Do not adjust back to pH 2.50 with base if too much acid is added. It is imperative that the ionic strength of the buffer be controlled.]

Medium: pH 2.5, 0.1 M Phosphate buffer; 1000 mL.

Apparatus 1: 100 rpm.

Times: 1 hour; 2 hours; 5 hours; 12 hours.

Procedure—Filter 10 mL of the solution under test at the required test points. Determine the amount of disopyramide ($C_{21}H_{29}N_3O$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 261 nm of this solution, suitably diluted with *Medium*, if necessary, using *Medium* as the blank, in comparison with a Standard solution having a known concentration of USP Disopyramide Phosphate RS dissolved in *Medium*.

Tolerances—The percentage of the labeled amount of disopyramide ($C_{21}H_{29}N_3O$) dissolved is within the range stated at each of the following times.

Time (hours)	Amount dissolved
1	between 5% and 25%
2	between 17% and 43%
5	between 50% and 80%
12	not less than 85%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.*

pH 2.5, *0.1 M Phosphate buffer,*and *Procedure*—Proceed as directed for *Test 1*.

Medium—Prepare as directed for *Test 1;* 900 mL.

Apparatus 2:100 rpm.

Times and Tolerances:

Time (hours)	Amount dissolved
1	between 5% and 30%
4	between 40% and 65%
8	between 60% and 90%
12	not less than 75%

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Disopyramide Phosphate RS in 0.1 N sulfuric acid, and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about 40 µg per mL.

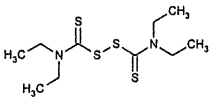
Assay preparation—Grind the contents of not fewer than 20 Capsules to a powder fine enough to pass through a 40-mesh screen. Transfer an accurately weighed portion of the powder, equivalent to about 650 mg of disopyramide phosphate, to a 500-mL volumetric flask. Add about 400 mL of 0.1 N sulfuric acid, and shake for 30 minutes. Dilute with 0.1 N sulfuric acid to volume, mix, and filter. Dilute an accurately measured portion of the filtrate quantitatively and stepwise with 0.1 N sulfuric acid to obtain a solution having a concentration of about 40 µg per mL.

Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 261 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid as the blank. Calculate the quantity, in mg, of $C_{21}H_{29}N_3O$ in the portion of Capsules taken by the formula:

$$16.25(339.48/437.47)C(A_U/A_S)$$

in which 339.48 and 437.47 are the molecular weights of disopyramide and disopyramide phosphate, respectively; C is the concentration, in µg per mL, of USP Disopyramide Phosphate RS in the *Standard preparation;* and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation,* respectively.

Disulfiram



$C_{10}H_{20}N_2S_4$ 296.54

Thioperoxydicarbonic diamide $[(H_2N)C(S)]_2S_2$, tetraethyl-; Bis(diethylthiocarbamoyl) disulfide [97-77-8].

DEFINITION

Disulfiram contains NLT 98.0% and NMT 102.0% of $C_{10}H_{20}N_2S_4$.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 45% potassium hydroxide solution to a pH of 7.0.

Mobile phase: Methanol and *Buffer* (7:3)

Standard stock solution: 1 mg/mL of USP Disulfiram RS in alcohol. [NOTE—Discard this solution after 5 days.]

Standard solution: 0.02 mg/mL of USP Disulfiram RS from the *Standard stock solution* diluted with *Mobile phase.* [NOTE—Prepare the *Standard solution* fresh daily.]

Sample stock solution: Transfer 50 mg of Disulfiram to a 50-mL volumetric flask, add 40 mL of alcohol, sonicate for 5 min to completely dissolve, cool, and dilute with alcohol to volume. [NOTE—Discard this solution after 5 days.]

Sample solution: 0.02 mg/mL of Disulfiram from the *Sample stock solution* diluted with *Mobile phase.* [NOTE—Prepare the *Sample solution* fresh daily.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 250 nm

Column: 4-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1800 theoretical plates

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of $C_{10}H_{20}N_2S_4$ in the portion of Disulfiram taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Disulfiram RS in the *Standard solution* (µg/mL)
C_U = concentration of Disulfiram in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

INORGANIC IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
- **SELENIUM** ⟨291⟩**:** 30 ppm
  [NOTE—Perform this test only if selenium is a known inorganic process impurity.]

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩**:** 69°–72°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Disulfiram RS

Disulfiram Tablets

DEFINITION

Disulfiram Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of disulfiram ($C_{10}H_{20}N_2S_4$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
  Standard: USP Disulfiram RS
  Sample: A portion of powdered Tablets
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 45% potassium hydroxide TS to a pH of 7.0.
  Mobile phase: Methanol and *Buffer* (70:30)
  Standard stock solution: 1 mg/mL of USP Disulfiram RS in alcohol. Use this solution within 5 days.
  Standard solution: 0.02 mg/mL of USP Disulfiram RS from the *Standard stock solution* diluted with *Mobile phase*. Use this solution within 1 day.
  Sample stock solution: Nominally 1 mg/mL of disulfiram from Tablets prepared as follows. Powder NLT 20 Tablets and transfer a suitable portion of the powder to an appropriate volumetric flask. Add 70% of the flask volume of alcohol and swirl, sonicate for about 5 min, and shake by mechanical means for 30 min or until dissolved. Dilute with alcohol to volume and pass through a suitable filter. Use the filtrate. Use this solution within 5 days.
  Sample solution: Nominally 0.02 mg/mL of disulfiram from *Sample stock solution* in *Mobile phase*. Use this solution within 1 day.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 250 nm
  Column: 3.9-mm × 15-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  Run time: NLT 1.5 times the retention time of disulfiram
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 1.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of disulfiram ($C_{10}H_{20}N_2S_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Disulfiram RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of disulfiram in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩
  Time: 15 min, the use of disks being omitted
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**
  Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 45% potassium hydroxide TS to a pH of 7.0.
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Methanol (%)
0	60	40
8	30	70
12.0	30	70
12.1	60	40
16.0	60	40

Diluent: Methanol and *Buffer* (50:50)
System suitability solution: 0.05 mg/mL of USP Disulfiram RS and 0.01 mg/mL of sulfiram in *Diluent*. Use this solution within 1 day.
Standard stock solution: 0.25 mg/mL of USP Disulfiram RS in methanol. Use this solution within 1 day.
Standard solution: 0.002 mg/mL of USP Disulfiram RS from *Standard stock solution* in *Diluent*. Use this solution within 1 day.
Sensitivity solution: 0.001 mg/mL of USP Disulfiram RS from *Standard solution* in *Diluent*. Use this solution within 1 day.

USP Monographs

www.webofpharma.com

Sample stock solution: Nominally 2.5 mg/mL of disulfiram from NLT 5 Tablets in methanol. Store at 4° and use it within 2 h.
Sample solution: Nominally 1 mg/mL of disulfiram from *Sample stock solution* in *Diluent.* Store at 4° and use it within 2 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 250 nm
Column: 3.9-mm × 15-cm; 5-µm, 300 Å packing L1
Autosampler temperature: 4°
Flow rate: 1 mL/min
Injection volume: 15 µL
System suitability
Samples: *System suitability solution, Standard solution,* and *Sensitivity solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 8.0 between sulfiram and disulfiram, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of disulfiram from the *Standard solution*
C_S = concentration of USP Disulfiram RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of disulfiram in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2.* The reporting threshold is 0.1%.

Table 2

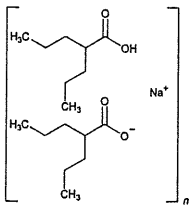
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Diethyldithiocarbamic acid	0.18	1.2	0.2
Tetraethylthiourea[a]	0.69	1.1	0.2
Sulfiram[b]	0.80	0.55	0.2
Disulfiram	1.0	—	—
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	1.0

[a] 1,1,3,3-Tetraethylthiourea.
[b] Diethylthiocarbamic thioanhydride.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Disulfiram RS

Divalproex Sodium



$(C_{16}H_{31}NaO_4)_n$ 310.41 (repeating unit molecular weight)
Pentanoic acid, 2-propyl-, sodium salt (2:1);
Sodium hydrogen bis(2-propylvalerate) oligomer.

DEFINITION

Divalproex Sodium contains NLT 98.0% and NMT 102.0% of available valproic acid ($C_8H_{16}O_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*
Sample: 100 mg of Divalproex Sodium
Analysis: Ignite the *Sample,* and dissolve the residue in 2 mL of water. Proceed as indicated in the chapter, starting with "Add 2 mL".
Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**
Buffer: 3.5 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 3.5. Dilute with water to 1 L.
Mobile phase: Acetonitrile and *Buffer* (50:50)
Impurity stock solution: 0.5 mg/mL of USP Valproic Acid Related Compound A RS in acetonitrile
Standard stock solution: 5.0 mg/mL of USP Valproic Acid RS in *Mobile phase*
System suitability solution: 0.5 mg/mL of valproic acid from the *Standard stock solution,* and 5 µg/mL of valproic acid related compound A from the *Impurity stock solution* in *Mobile phase*
Standard solution: 0.5 mg/mL of USP Valproic Acid RS from the *Standard stock solution* in *Mobile phase*
Sample solution: 0.5 mg/mL of Divalproex Sodium in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.8 times the retention time of valproic acid
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for valproic acid related compound A and valproic acid are 0.69 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 5.0 between valproic acid related compound A and valproic acid

USP Monographs

www.webofpharma.com

Tailing factor: NMT 1.5 for valproic acid
Relative standard deviation: NMT 2.0% for valproic acid

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of available valproic acid ($C_8H_{16}O_2$) in the portion of Divalproex Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/M) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight for divalproex sodium repeating unit, 310.41
M_{r2} = molecular weight for valproic acid, 144.21
M = number of moles of valproic acid per mole of divalproex sodium repeating unit, 2

Acceptance criteria: 98.0%–102.0%

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Divalproex Sodium RS
USP Valproic Acid RS
USP Valproic Acid Related Compound A RS
Diallyl acetic acid;
Also known as 2-Allylpent-4-enoic acid.
$C_8H_{12}O_2$ 140.18

Divalproex Sodium Delayed-Release Capsules

DEFINITION
Divalproex Sodium Delayed-Release Capsules contain an amount of divalproex sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of valproic acid ($C_8H_{16}O_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Diluent: Acetonitrile and water (1:1)
Standard: Prepare as directed in ⟨197F⟩ using USP Valproic Acid RS.
Sample: Dissolve the contents of 20 Capsules in 30 mL of *Diluent* in a 50-mL volumetric flask. Sonicate for 30 min to dissolve. Dilute with *Diluent* to volume. Centrifuge the solution at 3000 rpm for about 20 min. Pipet 20 mL of the supernatant into a separatory funnel. Extract with 50 mL of *n*-hexane. Collect the *n*-hexane layer and evaporate the solvent. Cast 1 mg of the liquid obtained after evaporation to sodium chloride (NaCl) windows.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 6.8 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (2:3)
Diluent: Acetonitrile and water (1:1)
Standard solution: Transfer a suitable amount of USP Valproic Acid RS to a suitable volumetric flask to obtain a solution having a final concentration of 2.5 mg/mL of valproic acid. Add 40% of the flask volume of *Diluent*. Sonicate for 5 min and add 20% of the flask volume of 0.1 N hydrochloric acid. Dilute with *Diluent* to volume.
Sample solution: Transfer an amount of contents (from NLT 20 Capsules) to a suitable volumetric flask to obtain a nominal concentration of 2.5 mg/mL of valproic acid. Dissolve in 20% of the flask volume of 0.1 N hydrochloric acid and sonicate for 5 min. Add 60% of the flask volume of *Diluent* and sonicate for an additional 25 min. Dilute with *Diluent* to volume. Centrifuge at 4000 rpm for 10 min and use the clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.8 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for valproic acid
Relative standard deviation: NMT 2.0% for valproic acid
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valproic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Phosphate buffer, pH 7.5 (6.8 g/L of monobasic potassium phosphate and 1.64 g/L of sodium hydroxide in water; adjusted with 0.08 N hydrochloric acid TS to a pH of 7.5); 500 mL, degassed
Apparatus 2: 50 rpm, with sinkers
Times: 2, 4, and 6 h
Buffer and **Mobile phase:** Prepare as directed in the *Assay*.
Standard stock solution: 1.6 mg/mL of USP Valproic Acid RS in *Mobile phase*
Standard solution: 0.26 mg/mL of valproic acid from the *Standard stock solution* and *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Replace the volume withdrawn with an equal volume of *Medium* previously heated at 37.0 ± 0.5°.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.8 mL/min
Injection volume: 40 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for valproic acid
Relative standard deviation: NMT 2.0% for valproic acid
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of valproic acid ($C_8H_{16}O_2$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of valproic acid in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	15–40
2	4	70–90
3	6	NLT 85

The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each time point conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Procedure A
Medium: 0.05 M phosphate buffer, pH 7.5 (6.8 g/L of monobasic potassium phosphate and 1.64 g/L of sodium hydroxide in water; adjusted with 2 N sodium hydroxide to a pH of 7.5); 500 mL
Apparatus 2: 50 rpm, contents of the Capsule
Time: 15 min
Standard solution A: 0.036 mg/mL of USP Valproic Acid RS in *Medium*. A volume of acetonitrile not exceeding 10% of the total volume may be used to dissolve the valproic acid.
Sample solution A: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Procedure B
Medium: 0.05 M phosphate buffer, pH 7.5 (6.8 g/L of monobasic potassium phosphate and 1.64 g/L of sodium hydroxide in water; adjusted with 2 N sodium hydroxide to a pH of 7.5); 900 mL
Apparatus 2: 50 rpm, with wire helix sinkers
Time: 4 h
Buffer A: 0.5 g/L of citric acid and 0.4 g/L of dibasic sodium phosphate in water
Buffer B: 6.8 g/L of monobasic potassium phosphate and 1.7 g/L of sodium hydroxide in water; adjusted with phosphoric acid to a pH of 7.4
Mobile phase: Acetonitrile, *Buffer A*, and *Buffer B* (30:35:35); adjusted with phosphoric acid to a pH of 3.0
Standard solution B: 0.13 mg/mL of USP Valproic Acid RS in *Medium*. A volume of acetonitrile not exceeding 10% of the total volume may be used to dissolve the valproic acid.
Sample solution B: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 3.9-mm × 15-cm; 4-µm packing L11
Flow rate: 1.2 mL/min
Injection volume: 200 µL for *Standard solution A* and *Sample solution A*; 50 µL for *Standard solution B* and *Sample solution B*
System suitability
Sample: *Standard solution B*
Suitability requirements
Tailing factor: NMT 2.0 for valproic acid
Relative standard deviation: NMT 2.0% for valproic acid
Analysis
Samples: *Standard solution A*, *Sample solution A*, *Standard solution B*, and *Sample solution B*
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each time point:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from *Sample solution A* or *Sample solution B*
r_S = peak response from *Standard solution A* or *Standard solution B*
C_S = concentration of *Standard solution A* or *Standard solution B* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*; 500 mL for *Sample solution A*, 900 mL for *Sample solution B*

Tolerances: NMT 20% ▲▲ (ERR 1-Aug-2018) of the labeled amount of valproic acid ($C_8H_{16}O_2$) is dissolved in 15 min (*Sample solution A*); NLT 80% (Q) of the labeled amount of valproic acid ($C_8H_{16}O_2$) is dissolved in 4 h (*Sample solution B*). The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at 4 h conforms to *Dissolution* ⟨711⟩, *Acceptance Table 1*.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
Medium
Acid stage medium: 0.08 N hydrochloric acid TS; 900 mL
Buffer stage medium: Phosphate buffer, pH 7.5 (6.8 g/L of monobasic potassium phosphate and 1.6 g/L of

USP Monographs

www.webofpharma.com

sodium hydroxide in water, prepared as follows. Transfer suitable quantities of monobasic potassium phosphate and sodium hydroxide to a suitable volumetric flask. Dissolve in 83% of the flask volume of water and adjust with 0.1 N hydrochloric acid, if necessary, to a pH of 7.5. Dilute the resulting solution with water to volume.); 900 mL

Times
Acid stage: 2 h
Buffer stage: 4 h
Apparatus 2: 50 rpm, with sinkers
Buffer: 0.25 g/L of citric acid, 0.2 g/L of anhydrous dibasic sodium phosphate, 3.4 g/L of monobasic potassium phosphate, and 0.85 g/L of sodium hydroxide in water
Mobile phase: Acetonitrile and *Buffer* (45:55); mixed, degassed, and adjusted with phosphoric acid to a pH of 2.5
Standard solution: 0.14 mg/mL of USP Valproic Acid RS prepared as follows. Transfer a portion of USP Valproic Acid RS to a suitable volumetric flask. Dissolve in methanol using 5.0% of the final volume. Dilute with *Buffer stage medium* to final volume and mix.
Sample solutions
Acid stage sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate.
Buffer stage sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 3.9-mm × 15-cm; 4-µm packing L11
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution*, *Acid stage sample solution*, and *Buffer stage sample solution*
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each time point:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Acid stage sample solution* or the *Buffer stage sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of the *Acid stage medium* or the *Buffer stage medium*, 900 mL

Tolerances: The requirements for the *Acid stage* and the *Buffer stage* must be met.
Acid stage: NMT 30% (Q) of the labeled amount of valproic acid ($C_8H_{16}O_2$) is dissolved in 2 h (*Acid stage sample solution*). The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at 2 h conforms to *Table 2*.

Table 2

Level	Number Tested	Criteria
A_1	6	No individual value exceeds Q dissolved.
A_2	6	Average of the 12 units ($A_1 + A_2$) is NMT Q dissolved; and no individual unit is greater than Q + 15% dissolved.
A_3	12	Average of the 24 units ($A_1 + A_2 + A_3$) is NMT Q dissolved; and no individual unit is greater than Q + 15% dissolved.

Buffer stage: NLT 80% (Q) of the labeled amount of valproic acid ($C_8H_{16}O_2$) is dissolved in 4 h (*Buffer stage sample solution*). The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at 4 h conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.
Medium: 0.05 M phosphate buffer, pH 7.5 (6.8 g/L of monobasic potassium phosphate in water; adjusted with 2 N sodium hydroxide to a pH of 7.5); 500 mL
Apparatus 2: 50 rpm
Times: 2, 4, and 8 h
Buffer A: 0.5 g/L of citric acid and 4 g/L of dibasic sodium phosphate in water
Buffer B: 6.8 g/L of monobasic potassium phosphate and 1.7 g/L of sodium hydroxide in water; adjusted with phosphoric acid to a pH of 7.4
Mobile phase: Acetonitrile, *Buffer A*, and *Buffer B* (30:35:35); adjusted with phosphoric acid to a pH of 3.0
Standard solution: 0.25 mg/mL of USP Valproic Acid RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 3.9-mm × 15-cm; 4-µm packing L11
Column temperature: 30°
Flow rate: 1.2 mL/min
Injection volume: 50 µL
Run time: NLT 1.5 times the retention time of valproic acid
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for valproic acid
Relative standard deviation: NMT 2.0% for valproic acid
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of valproic acid ($C_8H_{16}O_2$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

www.webofpharma.com

$$Result_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of valproic acid in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved (NLT %)
1	2	60
2	4	70
3	8	80

The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each time point conforms to *Dissolution* ⟨711⟩, *Acceptance Table 4*.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at controlled room temperature.
- **LABELING:** Divalproex Sodium Delayed-Release Capsules may be swallowed whole or may be administered by carefully opening the Capsule and sprinkling the entire contents on a small amount of soft food. This drug/food mixture should be swallowed immediately and not chewed. It should not be stored for future use. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Valproic Acid RS

Divalproex Sodium Delayed-Release Tablets

DEFINITION

Divalproex Sodium Delayed-Release Tablets contain an amount of Divalproex Sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of valproic acid ($C_8H_{16}O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.50 g/L of citric acid and 0.40 g/L of dibasic sodium phosphate in water
Mobile phase: Acetonitrile and *Solution A* (30:70). Adjust with phosphoric acid to a pH of 3.0 ± 0.1.
Standard solution: 0.5 mg/mL of USP Valproic Acid RS in *Mobile phase*
Sample stock solution: Nominally 10 mg/mL of valproic acid, prepared as follows. Transfer a number of whole Tablets to an appropriate volumetric flask. Add 60% of the flask volume of *Mobile phase*, and sonicate with frequent swirling for 30 min or until the Tablets completely disintegrate. Allow the solution to cool down to room temperature, and then dilute with *Mobile phase* to volume.
Sample solution: Nominally 0.5 mg/mL of valproic acid from *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm. For *Identification B*, use a diode array detector in the range of 190–400 nm.
Column: 3.9-mm × 15-cm; 4-µm packing L11
Flow rate: 0.9 mL/min
Injection volume: 15 µL
Run time: NLT 1.8 times the retention time of valproic acid
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) in the portion of Tablets taken:

$$Result = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of valproic acid from the *Sample solution*
r_S = peak area of valproic acid from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valproic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Acid stage
Acid stage medium: 0.08 N hydrochloric acid (prepared by adding 40 mL of hydrochloric acid to 5000 mL of water, adjusting with 2 N hydrochloric acid to a pH of 1.2, and diluting with water to 6000 mL); 900 mL
Apparatus 2: 50 rpm
Time: 1 h
At the end of 1 h, carefully transfer the Tablet to a dissolution vessel containing the *Buffer stage medium*. [NOTE—Do not perform an analysis of the *Acid stage medium*.]
Buffer stage
Buffer stage medium: pH 7.5 phosphate buffer (6.8 g/L of monobasic potassium phosphate and 1.6 g/L of sodium hydroxide in water, prepared as follows. Transfer suitable quantities of monobasic potassium phosphate and sodium hydroxide to an appropriate container. Dilute with water to 80% of the final volume, adjust with 0.08 N hydrochloric acid to a pH of 7.5, and dilute with water to the final volume); 900 mL
Apparatus 2: 50 rpm
Time: 1 h
Solution A: 0.5 g/L of citric acid and 0.4 g/L of dibasic sodium phosphate in water
Solution B: 6.8 g/L of monobasic potassium phosphate and 1.7 g/L of sodium hydroxide in water. Adjust with phosphoric acid to a pH of 7.4 ± 0.1.

USP Monographs

www.webofpharma.com

Mobile phase: Acetonitrile, *Solution A*, and *Solution B* (30:35:35). Adjust with phosphoric acid to a pH of 3.0 ± 0.1.
Standard solution: 0.12 mg/mL of USP Valproic Acid RS in *Buffer stage medium*. [NOTE—NMT 10.0% of the flask volume of acetonitrile may be used to dissolve the USP Valproic Acid RS.]
Sample solution: Nominally 0.12 mg/mL of valproic acid, prepared as follows. Pass a portion of the solution under test through a suitable filter. Dilute with *Buffer stage medium*, if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 3.9-mm × 15-cm; 4-µm packing L11
Flow rate: 1.2 mL/min
Injection volume: 50 µL
Run time: NLT 1.5 times the retention time of valproic acid
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (1/L) \times 100$$

r_U = peak area of valproic acid from the *Sample solution*
r_S = peak area of valproic acid from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
V = volume of *Buffer stage medium*, 900 mL
D = dilution factor of the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of valproic acid ($C_8H_{16}O_2$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Valproic Acid RS

Divalproex Sodium Extended-Release Tablets

DEFINITION
Divalproex Sodium Extended-Release Tablets contain an amount of divalproex sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of valproic acid ($C_8H_{16}O_2$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**
Buffer: 0.5 g/L of ▲anhydrous▲ (ERR 1-Mar-2019) citric acid and 0.4 g/L of ▲anhydrous▲ (ERR 1-Mar-2019) dibasic sodium phosphate in water
Mobile phase: Methanol and *Buffer* (55:45). Adjust with diluted sodium hydroxide or phosphoric acid to a pH of 5.0.
Diluent: *Buffer*, adjusted with phosphoric acid to a pH of 2.0
Standard stock solution: 2.5 mg/mL of USP Valproic Acid RS in methanol
Standard solution: 1.0 mg/mL of USP Valproic Acid RS from the *Standard stock solution* in *Diluent*
Sample stock solution: Nominally 2.5 mg/mL of valproic acid prepared as follows. Transfer an amount of powder (from NLT 20 Tablets) to a suitable volumetric flask. Dissolve in 50% of the flask volume of methanol by shaking for 1 h. Dilute with methanol to volume, pass through a suitable filter, and use the filtrate.
Sample solution: Nominally 1.0 mg/mL of valproic acid from the *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm. For *Identification B*, use a diode array detector in the range of 190–400 nm.
Column: 3.9-mm × 15-cm; 4-µm packing L11
Flow rate: 0.7 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of valproic acid
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for valproic acid
Relative standard deviation: NMT 2.0% for valproic acid
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of valproic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of valproic acid

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Test 1
Acid stage medium: 0.1 N hydrochloric acid; 500 mL
Buffer stage medium: 21.6 g/L of sodium dodecyl sulfate, 6.9 g/L of monobasic sodium phosphate, and 0.12 g/L of sodium hydroxide in water. Adjust with diluted sodium hydroxide or diluted phosphoric acid to a pH of 5.5; 900 mL
Apparatus 2: 100 rpm, with three-prong sinkers only for 250-mg Tablets, if necessary

USP Monographs

www.webofpharma.com

Times: 45 min in the *Acid stage medium*; 3, 9, 12, and 24 h in the *Buffer stage medium*
Procedure: After 45 min in the *Acid stage medium*, withdraw a sample from the solution, and immediately filter. Replace the *Acid stage medium* with the *Buffer stage medium*, and run the test for the times specified.
Buffer: 1.42 g/L of dibasic sodium phosphate in 0.008 M acetic acid TS. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Methanol and *Buffer* (65:35)
Standard stock solution: 2.5 mg/mL of USP Valproic Acid RS in methanol
Standard solution: 0.15 mg/mL of USP Valproic Acid RS from the *Standard stock solution* in the *Buffer stage medium*. [NOTE—Add 40% of the flask volume of methanol before diluting with *Buffer stage medium* to volume.]
Sample solution: Pass a portion of the solution under test through a suitable filter of 20-µm pore size. Use the *Sample solution* from the *Acid stage medium* as is. Dilute the *Sample solution* from the *Buffer stage medium* with methanol by a factor of 2.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 3.9-mm × 15-cm; 10-µm packing L11
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 80 µL
Run time: NLT 1.5 times the retention time of valproic acid
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution*, *Sample solution* from the *Acid stage medium*, and *Sample solution* from the *Buffer stage medium*
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved in the *Acid stage medium*:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
V = volume of the *Acid stage medium*, 500 mL
L = label claim (mg/Tablet)

Calculate the concentration (C_i) of valproic acid ($C_8H_{16}O_2$) in the sample withdrawn from the vessel at each time point *i* during the *Buffer stage*:

$$\text{Result}_i = (r_i/r_S) \times C_S \times D$$

r_i = peak response from the *Sample solution* at time point *i*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution* in the *Buffer stage medium*, 2

Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each time point *i* during the *Buffer stage*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of valproic acid in the *Sample solution* withdrawn at time point *i* (mg/mL)
V = volume of the *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point *i* during the *Buffer stage* (mL)

Tolerances
Acid stage: NMT 10% of the labeled amount of valproic acid ($C_8H_{16}O_2$) is dissolved.
Buffer stage: See *Table 1*.

Table 1

Time Point (*i*)	Time (h)	Amount Dissolved, Tablets labeled to contain 500 mg of valproic acid (%)	Amount Dissolved, Tablets labeled to contain 250 mg of valproic acid (%)
1	3	10–30	10–30
2	9	35–55	35–60
3	12	45–70	45–75
4	24	NLT 75	NLT 75

The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Acid stage medium: 0.1 N hydrochloric acid; 500 mL
Buffer stage concentrate: 15.53 g/L of monobasic sodium phosphate, 5.45 g/L of sodium hydroxide, and 48.7 g/L of sodium lauryl sulfate in water (final pH approximately 11); 400 mL
Buffer stage medium: Mix 400 mL of *Buffer stage concentrate* with 500 mL of *Acid stage medium* to a pH of 5.5 ± 0.05. [NOTE—If necessary, adjust the pH of *Buffer stage concentrate* with 1 N hydrochloric acid or 1 N sodium hydroxide to ensure that the final pH of the mixture of media is 5.5.] Retain this solution to dilute the solutions prepared later.
Apparatus 2: 100 rpm, with wire helix sinkers
Times: 45 min in the *Acid stage medium*; 3, 9, 12, and 21 h in the *Buffer stage medium*. The times in the *Buffer stage medium* include the time in the *Acid stage medium*.
Procedure: After 45 min in the *Acid stage medium*, stop and lift the paddles from the vessels. Do not perform an analysis of the *Acid stage medium*. Transfer 400 mL of *Buffer stage concentrate* to the vessels containing the *Acid stage medium*, and run the test for the times specified.
Buffer: 3.5 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Acetonitrile and *Buffer* (50:50)
Standard stock solution: 28 mg/mL of USP Valproic Acid RS in a suitable volumetric flask. Dissolve with 20% of the flask volume of 1 N sodium hydroxide, and dilute with water to volume. Dilute this solution with *Buffer stage medium* to obtain a final concentration of about 2.8 mg/mL.

www.webofpharma.com

Standard solutions: Prepare a series of dilutions in *Buffer stage medium* from the *Standard stock solution* at 0.028, 0.11, 0.22, 0.50, and 0.70 mg/mL.
Sample solution: Withdraw 10 mL of the solution under test, and pass through a suitable filter of 35-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: NLT 1.5 times the retention time of valproic acid
System suitability
Samples: 0.028, 0.11, 0.22, 0.50, and 0.70 mg/mL of the *Standard solutions*
Suitability requirements
Tailing factor: NMT 2.0, using the 0.50-mg/mL *Standard solution*
Correlation coefficient: NLT 0.999, using the five concentrations of the *Standard solution*
Relative standard deviation: NMT 2.0%, using the 0.50-mg/mL *Standard solution*
Analysis
Samples: *Sample solution*
From the standard curve, determine the concentration (C_i) of valproic acid ($C_8H_{16}O_2$) dissolved at each time point (i) using the response of each *Sample solution*.
Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each time point i during the *Buffer stage*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of valproic acid in the *Sample solution* withdrawn at time point i (mg/mL)
V = volume of the *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point i during the *Buffer stage* (mL)

Tolerances: The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at the times specified conform to *Table 2*.
Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Acid stage medium: 0.1 N hydrochloric acid; 250 mL (row 1)
Buffer stage medium: pH 6.8 buffer (6.8 g of monobasic potassium phosphate and 0.92 g of sodium hydroxide in 1 L of water. Adjust with phosphoric acid or sodium hydroxide to a pH of 6.8 ± 0.05); 250 mL (rows 2–4)
Apparatus 3: 30 dips/min, 20-mesh polypropylene screen on top and bottom; 30-s drip time
Times: 1 h in *Acid stage medium* (row 1); 2, 12, and 24 h in *Buffer stage medium* (rows 2–4). The times in the *Buffer stage medium* include the time in the *Acid stage medium*.
Buffer: 0.25 g/L of citric acid, 0.2 g/L of anhydrous dibasic sodium phosphate, 3.4 g/L of monobasic potassium phosphate, and 0.85 g/L of sodium hydroxide in water. Adjust with phosphoric acid to a pH of 3.0 ± 0.05.
Mobile phase: Acetonitrile and *Buffer* (30:70)
Acid stage standard stock solution: 1 mg/mL of USP Valproic Acid RS in *Acid stage medium*. Dissolve a suitable amount of USP Valproic Acid RS in a suitable volumetric flask in 10% of the flask volume of methanol to solubilize the valproic acid. Dilute with *Acid stage medium* to volume.
Buffer stage standard stock solution: 1 mg/mL of USP Valproic Acid RS in *Buffer stage medium*. Dissolve a suitable amount of USP Valproic Acid RS in a suitable volumetric flask in 10% of the flask volume of methanol to solubilize the valproic acid. Dilute with *Buffer stage medium* to volume.
Acid stage standard solution: (L/2500) mg/mL of USP Valproic Acid RS from *Acid stage standard stock solution* in *Acid stage medium*, where L is the Tablet label claim in mg
Buffer stage standard solution: (L/700) mg/mL of USP Valproic Acid RS from *Buffer stage standard stock solution* in *Buffer stage medium*, where L is the Tablet label claim in mg
Sample solutions: Centrifuge a portion of the solution under test. Use the supernatant. [NOTE—The use of a centrifuge speed of 3000 rpm for 20 min may be suitable.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 3.9-mm × 15-cm; 5-µm packing L11
Flow rate: 2 mL/min
Injection volume: 100 µL for Tablets labeled to contain 250 mg; 50 µL for Tablets labeled to contain 500 mg

Table 2 (Divalproex Sodium Extended-Release Tablets)

	Time Points (i)	1	2	3	4
	Times	**3 h**	**9 h**	**12 h**	**21 h**
L1	Individual Tablets	10%–27%	35%–70%	44%–92%	NLT 87%
L2	Average	10%–27%	35%–70%	44%–92%	NLT 87%
L2	Individual Tablets	0%–37%	25%–80%	34%–102%	NLT 77%
L3	Average	10%–27%	35%–70%	44%–92%	NLT 87%
L3	Individual Tablets	NMT 2 Tablets are outside the range of 0%–37%, and no individual Tablet is outside the range of 0%–47%.	NMT 2 Tablets are outside the range of 25%–80%, and no individual Tablet is outside the range of 15%–90%.	NMT 2 Tablets are outside the range of 34%–102%, and no individual Tablet is outside the range of 24%–112%.	NMT 2 Tablets release less than 77%, and no individual Tablet releases less than 67%.

www.webofpharma.com

Run time: NLT 1.5 times the retention time of valproic acid

System suitability

Samples: *Acid stage standard solution* and *Buffer stage standard solution*

Suitability requirements

Tailing factor: NMT 2.0 each for the *Acid stage standard solution* and the *Buffer stage standard solution*

Relative standard deviation: NMT 2.0% each for the *Acid stage standard solution* and the *Buffer stage standard solution*

Analysis

Samples: *Acid stage standard solution, Buffer stage standard solution,* and *Sample solutions*

Calculate the concentration (C_i) of valproic acid ($C_8H_{16}O_2$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_i/r_S) \times C_S$$

r_i = peak response from the *Sample solution* at time point i

r_S = peak response from the *Acid stage standard solution* or *Buffer stage standard solution*

C_S = concentration of USP Valproic Acid RS in the *Acid stage standard solution* or *Buffer stage standard solution* (mg/mL)

Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = (C_2 + C_1) \times V \times (1/L) \times 100$$

$$\text{Result}_3 = (C_3 + C_2 + C_1) \times V \times (1/L) \times 100$$

$$\text{Result}_4 = (C_4 + C_3 + C_2 + C_1) \times V \times (1/L) \times 100$$

C_i = concentration of valproic acid in the *Acid stage standard solution* or *Buffer stage standard solution* withdrawn at time point i (mg/mL)

V = volume of the *Acid stage medium* or *Buffer stage medium,* 250 mL

L = label claim (mg/Tablet)

Tolerances: See *Table 3*.

Table 3

Time Point (i)	Time (h)	Amount Dissolved, Tablets labeled to contain 500 mg of valproic acid (%)	Amount Dissolved, Tablets labeled to contain 250 mg of valproic acid (%)
1	1	NMT 10	NMT 10%
2	2	5–25	5–25
3	12	55–75	65–85
4	24	NLT 80	NLT 80

The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Acid stage medium: 0.1 N hydrochloric acid; 500 mL

Buffer stage stock medium: 19.0 g/L of tribasic sodium phosphate in water, adjusted with hydrochloric acid to a pH of 5.5

Buffer stage medium: 21.6 g/L of sodium lauryl sulfate in *Buffer stage stock medium*; 900 mL

Apparatus 2: 100 rpm, with sinkers for 250- and 500-mg Tablets

Times: 45 min in *Acid stage medium*; 3, 9, 12, and 18 h in *Buffer stage medium*. The times in the *Buffer stage medium* include the time in the *Acid stage medium*.

Buffer: 1.36 g/L of monobasic potassium phosphate and triethylamine (99.5: 0.5). Adjust with phosphoric acid to a pH of 2.75.

Solution A: 1.0 g/L of sodium lauryl sulfate in *Buffer*

Mobile phase: Acetonitrile and *Solution A* (50:50), degassed

Acid stage standard stock solution: 1 mg/mL of USP Valproic Acid RS prepared as follows. Transfer a suitable amount of USP Valproic Acid RS to a volumetric flask, and dissolve in 20% of the flask volume of acetonitrile to solubilize valproic acid. Dilute with *Acid stage medium* to volume.

Acid stage standard solution: (L/5000) mg/mL of valproic acid from *Acid stage standard stock solution* in *Acid stage medium*, where L is the Tablet label claim, in mg

Buffer stage standard solution: (L/900) mg/mL of USP Valproic Acid RS, prepared as follows. Transfer a suitable amount of USP Valproic Acid RS to a volumetric flask, and dissolve in (L/50)% of the flask volume of acetonitrile. Dilute with *Buffer stage medium* to volume. L is the Tablet label claim in mg.

Acid stage sample solution: Withdraw a 10.0-mL aliquot at each time point, and pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Buffer stage sample solution: Withdraw a 10.0-mL aliquot at each time point, and pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Replace the 10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Buffer stage medium*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

Run time: NLT 2.5 times the retention time of valproic acid

System suitability

Samples: *Acid stage standard solution* and *Buffer stage standard solution*

Suitability requirements

Tailing factor: NMT 2.0 each for the *Acid stage standard solution* and the *Buffer stage standard solution*

Relative standard deviation: NMT 2.0% each for the *Acid stage standard solution* and the *Buffer stage standard solution*

Analysis

Samples: *Acid stage standard solution, Buffer stage standard solution, Acid stage sample solution,* and *Buffer stage sample solutions*

Calculate the percentage of the labeled amount (Q_A) of valproic acid ($C_8H_{16}O_2$) dissolved in the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times C_S \times V_A \times (1/L) \times 100$$

r_U = peak response from the *Acid stage sample solution*

r_S = peak response from the *Acid stage standard solution*

C_S = concentration of USP Valproic Acid RS in the *Acid stage standard solution* (mg/mL)

www.webofpharma.com

V_A = volume of the *Acid stage medium*, 500 mL
L = label claim (mg/Tablet)

Calculate the concentration (C_i) of valproic acid ($C_8H_{16}O_2$) in the sample withdrawn from the vessel at each *Buffer stage* time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S \times 100$$

r_U = peak response from the *Buffer stage sample solution*
r_S = peak response from the *Buffer stage standard solution*
C_S = concentration of USP Valproic Acid RS in the *Buffer stage standard solution* (mg/mL)

Calculate the percentage of the labeled amount (Q_i) of valproic acid ($C_8H_{16}O_2$) dissolved at each *Buffer stage* time point *i*:

$$\text{Result}_1 = [C_1 \times V_B \times (1/L) \times 100] + Q_A$$

$$\text{Result}_2 = \{[(C_2 \times V_B) + (C_1 \times V_S)] \times (1/L) \times 100\} + Q_A$$

$$\text{Result}_3 = (\{(C_3 \times V_B) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100) + Q_A$$

$$\text{Result}_4 = (\{(C_4 \times V_B) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100) + Q_A$$

C_i = concentration of valproic acid in the *Buffer stage sample solution* withdrawn at time point *i* (mg/mL)
V_B = volume of the *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
Q_A = percentage of the labeled amount of valproic acid dissolved in the *Acid stage*
V_S = volume of the *Buffer stage sample solution* withdrawn from the vessel (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (*i*)	Time (h)	Amount Dissolved, Tablets labeled to contain 500 mg of valproic acid (%)	Amount Dissolved, Tablets labeled to contain 250 mg of valproic acid (%)
1	3	10–30	10–30
2	9	40–70	35–60
3	12	60–90	50–80
4	18	NLT 85	NLT 85

The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Acid stage medium: 0.1 N hydrochloric acid; 500 mL
Buffer stage stock medium: 7.8 g/L of monobasic sodium phosphate dihydrate in water, adjusted with 2 N sodium hydroxide solution to a pH of 5.5
Buffer stage medium: 21.6 g/L of sodium dodecyl sulfate in *Buffer stage stock medium*; 900 mL
Apparatus 2: 100 rpm, with three-prong sinkers
Times: 45 min in *Acid stage medium*; 3, 9, 12, and 24 h in *Buffer stage medium*. The times in the *Buffer stage medium* do not include the time in the *Acid stage medium*.
Procedure: After 45 min in *Acid stage medium*, discard the remainder of the *Acid stage medium* and add the *Buffer stage medium*.
Solution A: Dilute 5 mL of phosphoric acid with water to 25 mL.
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with *Solution A* to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (40:60), degassed
Standard stock solution: 1.4 mg/mL of USP Valproic Acid RS in *Mobile phase*
Buffer stage standard solution: (L/900) mg/mL of valproic acid from *Standard stock solution* in *Buffer stage medium*, where L is the Tablet label claim in mg
Buffer stage sample solution: Withdraw a 10.0-mL aliquot at each time point, and pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Replace the 10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Buffer stage medium*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: NLT 1.5 times the retention time of valproic acid
System suitability
Sample: *Buffer stage standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Buffer stage standard solution* and *Buffer stage sample solutions*
Calculate the concentration (C_i) of valproic acid ($C_8H_{16}O_2$) in the sample withdrawn from the vessel at each *Buffer stage* time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S$$

r_i = peak response from the *Buffer stage sample solution*
r_S = peak response from the *Buffer stage standard solution*
C_S = concentration of USP Valproic Acid RS in the *Buffer stage standard solution* (mg/mL)

Calculate the percentage of the labeled amount (Q_i) of valproic acid ($C_8H_{16}O_2$) dissolved at each *Buffer stage* time point *i*:

$$\text{Result}_1 = C_1 \times V_B \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V_B) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V_B) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V_B) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of valproic acid in the *Buffer stage sample solution* withdrawn at time point *i* (mg/mL)
V_B = volume of the *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Buffer stage sample solution* withdrawn from the vessel (mL)

www.webofpharma.com

Tolerances: See *Table 5*.

Table 5

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	3	10–30
2	9	40–60
3	12	45–85
4	24	NLT 85

The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium: pH 6.8 phosphate buffer (6.0 g/L of anhydrous monobasic sodium phosphate in water, adjusted with 240 g/L of sodium hydroxide in water to a pH of 6.8); 900 mL

Apparatus 2: 100 rpm

Times: 1, 4, 8, and 24 h in *Medium*

Buffer: 6.0 g/L of anhydrous monobasic sodium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (50:50). Adjust with phosphoric acid to a pH of 3.0.

Standard solution: (*L*/900) mg/mL of USP Valproic Acid RS, where *L* is the label claim in mg/Tablet, prepared as follows. Transfer USP Valproic Acid RS to an appropriate volumetric flask. Add 5% of the flask volume of methanol to dissolve the valproic acid. Dilute with *Medium* to volume.

Sample solutions: Withdraw an aliquot at each time point, and pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 100 µL

Run time: NLT 2.5 times the retention time of valproic acid

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the concentration (C_i) of valproic acid ($C_8H_{16}O_2$) in the sample withdrawn from the vessel at each time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S$$

r_i = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount (Q_i) of valproic acid ($C_8H_{16}O_2$) dissolved at each *Buffer stage* time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of valproic acid in the *Sample solution* withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the vessel (mL)

Tolerances: See *Table 6*.

Table 6

Time Point (*i*)	Time (h)	Amount Dissolved, Tablets labeled to contain 500 mg of valproic acid (%)	Amount Dissolved, Tablets labeled to contain 250 mg of valproic acid (%)
1	1	10–30	10–30
2	4	25–45	28–48
3	8	40–60	40–65
4	24	NLT 70	NLT 70

The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

Acid stage medium: 0.1 N hydrochloric acid; 500 mL

Buffer stage medium: pH 5.5 phosphate buffer with 75 mM sodium dodecyl sulfate (dissolve 78.0 g of monobasic sodium phosphate dihydrate in 10 L of water, adjust with 10 g/L of sodium hydroxide in water to a pH of 5.5, and add 216.3 g of sodium dodecyl sulfate); 900 mL

Apparatus 2: 100 rpm

Times: 45 min in *Acid stage medium*; 3, 9, 12, and 24 h in the *Buffer stage medium*. The times in the *Buffer stage medium* include the time in the *Acid stage medium*.

Procedure: After 45 min in the *Acid stage medium* and the collection of the *Acid stage sample solution*, discard the remainder of the *Acid stage medium* and add the *Buffer stage medium*.

Solution A: Dilute 10 mL of phosphoric acid with water to 100 mL.

Buffer: 3.5 g/L of monobasic sodium phosphate dihydrate in water, adjusted with *Solution A* to a pH of 3.5, and passed through a suitable filter

Mobile phase: Acetonitrile and *Buffer* (35:65)

Standard stock solution: 0.7 mg/mL of USP Valproic Acid RS prepared as follows. Transfer a suitable quantity of USP Valproic Acid RS to an appropriate volumetric flask and dissolve in 10% of the final flask volume of methanol. Sonication may be used to promote dissolution. Dilute with *Mobile phase* to volume.

Standard solution: 0.14 mg/mL of USP Valproic Acid RS from the *Standard stock solution* in *Mobile phase* passed through a suitable filter of 0.45-µm pore size

Acid stage sample solution: Withdraw a 10.0-mL aliquot at the time point, and pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Buffer stage sample stock solutions: Withdraw a 10.0-mL aliquot at each time point, and pass a portion of the solution under test through a suitable filter. Replace the

www.webofpharma.com

10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Buffer stage medium*.

Buffer stage sample solutions

For Tablets labeled to contain 500 mg of valproic acid: Dilute 5 mL of *Buffer stage sample stock solutions* with *Mobile phase* to 20 mL and pass through a suitable filter of 0.45-µm pore size.

For Tablets labeled to contain 250 mg of valproic acid: Dilute 5 mL of *Buffer stage sample stock solutions* with *Mobile phase* to 10 mL and pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 2.0 mL/min

Injection volume: 50 µL

Run time: NLT 2.5 times the retention time of valproic acid

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution*, *Acid stage sample solution*, and *Buffer stage sample solutions*

Calculate the percentage of the labeled amount (Q_A) of valproic acid ($C_8H_{16}O_2$) dissolved in the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times C_S \times V_A \times (1/L) \times 100$$

r_U = peak response from the *Acid stage sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
V_A = volume of the *Acid stage medium*, 500 mL
L = label claim (mg/Tablet)

Calculate the concentration (C_i) of valproic acid ($C_8H_{16}O_2$) in the sample withdrawn from the vessel at each *Buffer stage* time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S \times D$$

r_i = peak response from the *Buffer stage sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)
D = dilution factor between the *Buffer stage sample solution* and the *Buffer stage sample stock solution*

Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each *Buffer stage* time point *i*:

$$\text{Result}_1 = [C_1 \times V_B \times (1/L) \times 100] + Q_A$$

$$\text{Result}_2 = \{[(C_2 \times V_B) + (C_1 \times V_S)] \times (1/L) \times 100\} + Q_A$$

$$\text{Result}_3 = (\{(C_3 \times V_B) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100) + Q_A$$

$$\text{Result}_4 = (\{(C_4 \times V_B) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100) + Q_A$$

C_i = concentration of valproic acid in the *Buffer stage sample solution* withdrawn at time point *i* (mg/mL)
V_B = volume of the *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
Q_A = percentage of the labeled amount of valproic acid dissolved in the *Acid stage*
V_S = volume of the *Buffer stage sample solution* withdrawn at each time point and replaced with the *Buffer stage medium* (mL)

Tolerances: See *Table 7*.

Table 7

Time Point (*i*)	Time (h)	Amount Dissolved, Tablets labeled to contain 500 mg of valproic acid (%)	Amount Dissolved, Tablets labeled to contain 250 mg of valproic acid (%)
1	3	10–35	18–38
2	9	35–55	47–72
3	12	45–65	55–90
4	24	NLT 80	NLT 80

The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 8: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

Medium: pH 6.8 phosphate buffer with 2% sodium dodecyl sulfate (20.0 g/L of sodium dodecyl sulfate and 6.9 g/L of monobasic sodium phosphate dihydrate in water, adjusted with 10 g/L of sodium hydroxide in water to a pH of 6.8); 900mL

Apparatus 2: 50 rpm

Times: 2, 6, 12, and 24 h

Buffer A: 0.5 g/L of citric acid and 4 g/L of dibasic sodium phosphate in water

Buffer B: 6.8 g/L of monobasic potassium phosphate and 1.7 g/L of sodium hydroxide in water, adjusted with phosphoric acid to a pH of 7.4

Buffer C: *Buffer A* and *Buffer B* (50:50)

Mobile phase: Acetonitrile and *Buffer C* (30:70), adjusted with phosphoric acid to a pH of 3.0

Standard solution: (*L*/900) mg/mL of USP Valproic Acid RS prepared as follows. Transfer a suitable quantity of USP Valproic Acid RS to an appropriate volumetric flask and dissolve in 50% of the final volume of *Medium*. Sonication may be used to promote dissolution. Dilute with *Medium* to volume.

Sample solutions: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Replace with the same volume of *Medium*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 15-cm; 4-µm packing L11

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection volume: 50 µL

Run time: NLT 1.1 times the retention time of valproic acid

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solutions*
Calculate the concentration (C_i) of valproic acid ($C_8H_{16}O_2$) in the sample withdrawn from the vessel at each time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S$$

r_i = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of valproic acid in the *Sample solution* withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 8*.

Table 8

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	10–35
2	6	35–60
3	12	55–90
4	24	NLT 80

The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 1*.

Test 9: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.
Medium: pH 6.8 phosphate buffer with 75 mM sodium dodecyl sulfate [130 g/L of sodium dodecyl sulfate in water and pH 6.8 buffer (8.3 g/L of monobasic sodium phosphate in water, adjusted with 5 N hydrochloric acid or 5 N sodium hydroxide to a pH of 6.8 and then degassed) (17:83)]; 900 mL
Apparatus 2: 100 rpm, with spiral sinkers
Times: 2, 8, 12, and 24 h
Buffer: 6.8 g/L of monobasic potassium phosphate in water, adjusted with phosphoric acid to a pH of 2.2 and passed through a suitable filter
Mobile phase: Methanol, acetonitrile, and *Buffer* (50:10:40)
Standard solution: (L/900) mg/mL of USP Valproic Acid RS prepared as follows. Transfer a suitable quantity of USP Valproic Acid RS to an appropriate volumetric flask and dissolve in 10% of the final volume of acetonitrile. Dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 1.1 times the retention time of valproic acid
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of valproic acid ($C_8H_{16}O_2$) in the sample withdrawn from the vessel at each time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S$$

r_i = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Valproic Acid RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each time point *i*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of valproic acid in the *Sample solution* withdrawn at time point *i* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point *i* (mL)

Tolerances: See *Table 9*.

Table 9

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	2	15–40
2	8	40–70
3	12	50–85
4	24	NLT 70%

The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 10: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 10*.
Acid stage medium: 0.1 N hydrochloric acid, degassed; 500 mL
Buffer stage medium: pH 5.5 phosphate buffer with 75 mM sodium dodecyl sulfate (21.6 g/L of sodium dodecyl sulfate, 6.9 g/L of monobasic sodium phosphate, and 0.12

www.webofpharma.com

g/L of sodium hydroxide in water, adjusted with diluted phosphoric acid or diluted sodium hydroxide to a pH of 5.5); 900 mL
Apparatus 2: 100 rpm
Times: 45 min in *Acid stage medium*; 3, 9, and 15 h in *Buffer stage medium*. After 45 min in the *Acid stage medium*, discard the excess *Acid stage medium* and use the same Tablets in the *Buffer stage medium*. The time in the *Buffer stage medium* does not include the time in the *Acid stage medium*.
Buffer A: 0.5 g/L of citric acid and 0.4 g/L of anhydrous dibasic sodium phosphate in water
Buffer B: 6.8 g/L of monobasic potassium phosphate and 1.7 g/L of sodium hydroxide in water, adjusted with diluted phosphoric acid to a pH of 7.4
Mobile phase: Acetonitrile, *Buffer A*, and *Buffer B* (50:25:25). Adjust with diluted phosphoric acid to a pH of 3.0.
Acid stage standard solution: (*L*/5000) mg/mL of USP Valproic Acid RS in *Acid stage medium* where *L* is the label claim of valproic acid in mg/Tablet
Buffer stage standard solution: (*L*/900) mg/mL of USP Valproic Acid RS in *Buffer stage medium* where *L* is the label claim of valproic acid in mg/Tablet
Acid stage sample solution: Pass a portion of the solution under test through a suitable filter, discard the first 2 mL, and use the filtrate.
Buffer stage sample solution: Pass a portion of the solution under test through a suitable filter. Replace with the same volume of *Buffer stage medium*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.8 mL/min
Injection volume: 50 µL
Run time: NLT 2 times the retention time of valproic acid
System suitability
Samples: *Acid stage standard solution* and *Buffer stage standard solution*
Suitability requirements
Tailing factor: NMT 2.0, *Acid stage standard solution* and *Buffer stage standard solution*
Relative standard deviation: NMT 2.0%, *Acid stage standard solution* and *Buffer stage standard solution*
Analysis
Samples: *Acid stage standard solution*, *Buffer stage standard solution*, *Acid stage sample solution*, and *Buffer stage sample solution*
Calculate the percentage (Q_A) of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved in the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Acid stage sample solution*
r_S = peak response from the *Acid stage standard solution*
C_S = concentration of USP Valproic Acid RS in the *Acid stage standard solution* (mg/mL)
V = volume of *Acid stage medium*, 500 mL
L = label claim (mg/Tablet)

Calculate the concentration (C_i) of valproic acid ($C_8H_{16}O_2$) in the sample withdrawn from the vessel at each time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S$$

r_i = peak response from the *Buffer stage sample solution*
r_S = peak response from the *Buffer stage standard solution*
C_S = concentration of USP Valproic Acid RS in the *Buffer stage standard solution* (mg/mL)

Calculate the percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at each time point *i*:

$$\text{Result}_1 = [C_1 \times V \times (1/L) \times 100] + Q_A$$

$$\text{Result}_2 = \{[(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100\} + Q_A$$

$$\text{Result}_3 = (\{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100) + Q_A$$

C_i = concentration of valproic acid in the *Buffer stage sample solution* withdrawn at time point *i* (mg/mL)
V = volume of *Buffer stage medium*, 900 mL
L = label claim (mg/Tablet)
Q_A = percentage of the labeled amount of valproic acid dissolved in the *Acid stage*
V_S = volume of the *Buffer stage sample solution* withdrawn at each time point and replaced with the *Buffer stage medium* (mL)

Tolerances
Acid stage: NMT 10%
Buffer stage: See *Table 10*.

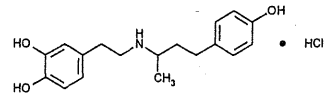
Table 10

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	3	15–40
2	9	40–70
3	15	NLT 85

The percentage of the labeled amount of valproic acid ($C_8H_{16}O_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Valproic Acid RS

Dobutamine Hydrochloride



$C_{18}H_{23}NO_3 \cdot HCl$ 337.84
1,2-Benzenediol, 4-[2-[[3-(4-hydroxyphenyl)-1-methylpropyl] amino]ethyl]-, hydrochloride, (±)-;
(±)-4-[2-[[3-(*p*-Hydroxyphenyl)-1-methylpropyl]amino] ethyl]-pyrocatechol hydrochloride [49745-95-1].

USP Monographs

www.webofpharma.com

DEFINITION

Dobutamine Hydrochloride contains NLT 98.0% and NMT 102.0% of dobutamine hydrochloride ($C_{18}H_{23}NO_3 \cdot HCl$), calculated on the anhydrous basis.

[**CAUTION**—Great care should be taken to prevent inhaling particles of dobutamine hydrochloride and exposing the skin to it. Protect the eyes.]

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*:▲ (CN 1-May-2020) 197K ▲or 197A▲ (USP 1-May-2020)

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

Change to read:

• ▲**C.**▲ (USP 1-May-2020) **IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*:Meets the requirements for dry chlorides

ASSAY

Change to read:

• **PROCEDURE:** Solutions containing dobutamine hydrochloride must be prepared fresh at the time of use and refrigerated until injected.

Solution A: Transfer 23 g of monobasic ammonium phosphate to a 2-L volumetric flask, and add 1900 mL of water. Adjust with phosphoric acid to a pH of 2.2, and dilute with water to volume.

Mobile phase: Acetonitrile and *Solution A* (20:80)

Standard solution: 0.5 mg/mL of USP Dobutamine Hydrochloride RS in water

▲▲ (USP 1-May-2020)

Sample solution: 0.5 mg/mL of Dobutamine Hydrochloride in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; ▲15–20-µm▲ (USP 1-May-2020) packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

▲▲ (USP 1-May-2020)

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT ▲0.73%▲ (USP 1-May-2020)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dobutamine hydrochloride ($C_{18}H_{23}NO_3 \cdot HCl$) in the portion of Dobutamine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dobutamine from the *Sample solution*

r_S = peak response of dobutamine from the *Standard solution*

C_S = concentration of USP Dobutamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Dobutamine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

Change to read:

• **ORGANIC IMPURITIES**

Solution A: 2.6 g/L of sodium 1-octanesulfonate in water. Pipet 3 mL of triethylamine into 1 L of this solution. Adjust the solution with phosphoric acid to a pH of 2.5.

Solution B: Methanol and acetonitrile (82:18)

Diluent: *Solution A* and *Solution B* (1:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	65	35
5	65	35
20	20	80
25	20	80
26	65	35
30	65	35

▲**Sensitivity solution:** 0.00125 mg/mL of USP Dobutamine Hydrochloride RS in *Diluent*▲ (USP 1-May-2020)

Standard solution: ▲0.025 mg/mL each of USP Dopamine Hydrochloride RS, USP Raspberry Ketone RS, and USP Dobutamine Related Compound C RS; and 0.005▲ (USP 1-May-2020) mg/mL of USP Dobutamine Hydrochloride RS in *Diluent*

Sample solution: 5 mg/mL of Dobutamine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; ▲5-µm▲ (USP 1-May-2020) packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: ▲*Sensitivity solution* and▲ (USP 1-May-2020) *Standard solution*

Suitability requirements

▲**Resolution:** NLT 1.5 between the dopamine and raspberry ketone peaks, *Standard solution*▲ (USP 1-May-2020)

Tailing factor: NMT 2.0 for the dobutamine peak, *Standard solution*

Relative standard deviation: NMT 2.0% for the dobutamine peak, *Standard solution*

▲**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*▲ (USP 1-May-2020)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Dobutamine Hydrochloride taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of the ▲corresponding impurity▲ (USP 1-May-2020) from the *Standard solution*
C_S = concentration of ▲the corresponding impurity Reference Standard▲ (USP 1-May-2020) in the *Standard solution* (mg/mL)
C_U = concentration of Dobutamine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of unspecified impurities:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of the ▲dobutamine▲ (USP 1-May-2020) from the *Standard solution*
C_S = concentration of USP Dobutamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Dobutamine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: ▲See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dopamine	0.33	0.5
Raspberry ketone	0.45	0.5
Dobutamine	1.00	—
Dobutamine related compound C	1.38	0.5
Any unspecified impurity	—	0.10
Total impurities	—	1.0▲ (USP 1-May-2020)

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 1.0%

Delete the following:

▲• **COLOR OF SOLUTION**
Sample solution: 500 mg of Dobutamine Hydrochloride to a 25-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, heating at 30°–35° to dissolve the sample, if necessary
Analysis: Immediately cool the *Sample solution* to room temperature and read the absorbance.
Spectrometric conditions
Mode: UV–Vis
Cell: 1 cm
Analytical wavelength: 480 nm
Blank: Water
Acceptance criteria: NMT 0.04▲ (USP 1-May-2020)

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dobutamine Hydrochloride RS
▲USP Dobutamine Related Compound C RS
N-(3,4-Dimethoxyphenethyl)-4-(4-methoxyphenyl) butan-2-amine.
$C_{21}H_{29}NO_3$ 343.46
USP Dopamine Hydrochloride RS
4-(2-Aminoethyl)benzene-1,2-diol hydrochloride.
$C_8H_{11}NO_2 \cdot HCl$ 189.64
USP Raspberry Ketone RS
4-(4-Hydroxyphenyl)butan-2-one.
$C_{10}H_{12}O_2$ 164.20▲ (USP 1-May-2020)

Dobutamine Injection

DEFINITION
Dobutamine Injection is a sterile solution of Dobutamine Hydrochloride in Water for Injection. It contains an amount of Dobutamine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of dobutamine ($C_{18}H_{23}NO_3$). It may contain one or more suitable antioxidants, chelating agents, or preservatives.

IDENTIFICATION
• **A.**
Standard solution: 10 mg/mL of USP Dobutamine Hydrochloride RS in methanol (freshly prepared)
Sample solution: Use the neat Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 10 µL
Developing solvent system: Ethyl acetate, *n*-propyl alcohol, glacial acetic acid, and water (100:40:5:15)
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry, and develop the chromatogram in the *Developing solvent system*, until the solvent front has moved about three-fourths of the length of the plate. Mark the solvent front, and allow it to evaporate at room temperature. Observe the plate under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY
• **PROCEDURE**
Ion-pair solution: Dissolve 3.38 g of sodium 1-octanesulfonate in 1 L of water, and pipet 3 mL of triethylamine into the solution. Adjust the solution with phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile, methanol, and *Ion-pair solution* (28:14:58)
[NOTE—The ratio of acetonitrile to methanol is critical to the elution order of the *System suitability solution* components.]
System suitability solution: 0.3 mg/mL of 4-(4-hydroxyphenyl)-2-butanone and 0.56 mg/mL of USP Dobutamine Hydrochloride RS in *Mobile phase*
Standard solution: 0.56 mg/mL (equivalent to 0.5 mg/mL of dobutamine) of USP Dobutamine Hydrochloride RS in *Mobile phase*

USP Monographs

www.webofpharma.com

Sample solution: Equivalent to a suitable volume of 0.5 mg/mL of dobutamine in *Mobile phase*, from Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm, base-deactivated packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for 4-(4-hydroxyphenyl)-2-butanone and dobutamine are about 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between 4-(4-hydroxyphenyl)-2-butanone and dobutamine
Tailing factor: NMT 1.5 for dobutamine
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dobutamine ($C_{18}H_{23}NO_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dobutamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dobutamine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dobutamine, 301.39
M_{r2} = molecular weight of dobutamine hydrochloride, 337.84

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 2.5–5.5
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩: Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 2.08 USP Endotoxin Units/mg of dobutamine.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
- **LABELING:** Label it to indicate that it is to be diluted with a suitable parenteral vehicle to appropriate strength before administration.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Dobutamine Hydrochloride RS

Dobutamine for Injection

DEFINITION
Dobutamine for Injection is a sterile mixture of Dobutamine Hydrochloride with suitable diluents. It contains an amount of dobutamine hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of dobutamine ($C_{18}H_{23}NO_3$).

[CAUTION—Great care should be taken to prevent inhaling particles of Dobutamine for Injection and exposing the skin to it. Protect the eyes.]

IDENTIFICATION
- **A.**
Standard solution: Freshly prepare 10 mg/mL of USP Dobutamine Hydrochloride RS in methanol.
Sample solution: 10 mg/mL of dobutamine hydrochloride in methanol, clarified by centrifugation
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 10 µL
Developing solvent system: Ethyl acetate, *n*-propyl alcohol, glacial acetic acid, and water (100:40:5:15)
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry, and develop the chromatogram in the *Developing solvent system*, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate at room temperature. Observe the plate under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY
- **PROCEDURE**
Ion-pair solution: Dissolve 3.38 g of sodium 1-octanesulfonate in 1000 mL of water, and pipet 3 mL of triethylamine into the solution. Adjust the solution with phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile, methanol, and *Ion-pair solution* (28:14:58)
[NOTE—The ratio of acetonitrile to methanol is critical to the elution order of the *System suitability solution* components.]
System suitability solution: 0.3 mg/mL of 4-(4-hydroxyphenyl)-2-butanone and 0.56 mg/mL of USP Dobutamine Hydrochloride RS in *Mobile phase*
Standard solution: 0.56 mg/mL (equivalent to 0.5 mg/mL of dobutamine) of USP Dobutamine Hydrochloride RS in *Mobile phase*
Sample solution: 0.5 mg/mL of dobutamine in *Mobile phase* prepared as follows. Inject 10 mL of *Mobile phase* into 1 vial of Dobutamine for Injection, taking care not to let pressure build up in the vial. Shake to dissolve the sample completely. Transfer the solution to a suitable volumetric flask, and dilute with *Mobile phase*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm, base-deactivated packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for 4-(4-hydroxyphenyl)-2-butanone and dobutamine are about 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between 4-(4-hydroxyphenyl)-2-butanone and dobutamine
Tailing factor: NMT 1.5 for dobutamine

www.webofpharma.com

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dobutamine ($C_{18}H_{23}NO_3$) in each container of Dobutamine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dobutamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dobutamine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dobutamine, 301.38
M_{r2} = molecular weight of dobutamine hydrochloride, 337.84

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: Dissolve contents of 1 vial in 10 mL of water.

Acceptance criteria: 2.5–5.5

• **Injections and Implanted Drug Products** ⟨1⟩: Meets the requirements

• **Particulate Matter in Injections** ⟨788⟩: Meets the requirements for small-volume injections

• **Bacterial Endotoxins Test** ⟨85⟩: NMT 5.56 USP Endotoxin Units/mg of dobutamine

• **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*. Do not use the constituted solution if it is brown or contains a precipitate.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, at controlled room temperature.

• **USP Reference Standards** ⟨11⟩

USP Dobutamine Hydrochloride RS

Dobutamine in Dextrose Injection

DEFINITION

Dobutamine in Dextrose Injection is a sterile solution of Dobutamine Hydrochloride and Dextrose in Water for Injection. It contains an amount of Dobutamine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of dobutamine ($C_{18}H_{23}NO_3$) and NLT 90.0% and NMT 110.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It may contain one or more suitable antioxidants or chelating agents.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay, Procedure 2: Dobutamine*.

• **B.**

Sample solution: Nominally 50 mg/mL of dextrose from Injection

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

ASSAY

• **Procedure 1: Dextrose**

Sample solution: Injection

Analysis

Sample: *Sample solution*

Determine the angular rotation of the *Sample solution* in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a) \times (l/\alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed rotation (°)
l = length of the polarimeter tube (dm)
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–110.0%

• **Procedure 2: Dobutamine**

Buffer: Transfer about 23 g of monobasic ammonium phosphate to a 2-L volumetric flask. Add 1900 mL of water. Adjust with phosphoric acid to a pH of 2.2, and dilute with water to volume.

Mobile phase: Acetonitrile and *Buffer* (20:80). Filter and degas.

System suitability solution: 0.5 mg/mL of USP Dobutamine Hydrochloride RS and 0.01 mg/mL of 5-hydroxymethylfurfural in water

Standard solution: 0.5 mg/mL of USP Dobutamine Hydrochloride RS in water. [Note—Prepare fresh daily, and refrigerate until injected.]

Sample solution: Nominally 0.446 mg/mL of dobutamine from Injection in water. [Note—Refrigerate until injected, and use within 8 h.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Relative retention time: 1.0 for dobutamine and NMT 0.62 for 5-hydroxymethylfurfural, *System suitability solution*

Retention time: NMT 5.3 min for dobutamine, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dobutamine ($C_{18}H_{23}NO_3$) in the portion of Injection taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of dobutamine from the *Sample solution*
r_S = peak response of dobutamine from the *Standard solution*
C_S = concentration of USP Dobutamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dobutamine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dobutamine, 301.39
M_{r2} = molecular weight of dobutamine hydrochloride, 337.84

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, System suitability solution, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay, Procedure 2: Dobutamine.*
Sample solution: Nominally 0.446 mg/mL of dobutamine from Injection in water
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity, excluding 5-hydroxymethylfurfural from all calculations, in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria
Any individual impurity: NMT 1.0%
Total impurities: NMT 2.0%

- **LIMIT OF 5-HYDROXYMETHYLFURFURAL**

Ion-exchange column: Fill an 8-mm chromatographic tube to a height of 40 mm with a 100- to 200-mesh, strongly acidic, styrene-divinylbenzene cation-exchange resin. Wash the column with 30 mL of water, and discard the eluate.
[NOTE—Prepare a new column for each *Sample solution* and *Blank*, and use each column only once.]
Sample solution: Transfer 2 mL of Injection to the *Ion-exchange column*, and collect the eluate in a 50-mL volumetric flask. Pass 25 mL of water through the column, and collect the eluate in the same volumetric flask. Dilute the eluate with water to volume. Remove the stopper from the flask, and allow the solution to stand for about 30 min in order to oxidize any bisulfite ions present.
Blank: Prepare as directed in the *Sample solution* by passing 27 mL of water through an *Ion-exchange column* and collecting the eluate in a 50-mL volumetric flask. Dilute with water to volume.
Instrumental conditions
Mode: UV
Analytical wavelength: 284 nm
Cell: 1 cm
Analysis
Samples: *Sample solution* and *Blank*
Determine the absorbance of the *Sample solution* after correcting for the *Blank*.
Acceptance criteria: NMT 0.25

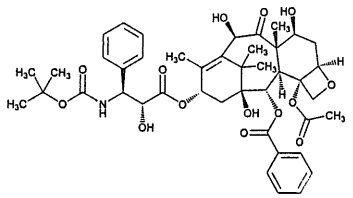
SPECIFIC TESTS

- **pH** ⟨791⟩: 2.5–5.5
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for large-volume injections
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 5.56 USP Endotoxin Units/mg of dobutamine
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type II glass, or of a suitable plastic material. Store at room temperature, avoid excessive heat, and protect from freezing.
- **LABELING:** The label states the total osmolar concentration in mOsmol/L.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Dobutamine Hydrochloride RS

Docetaxel



$C_{43}H_{53}NO_{14} \cdot 3H_2O$ 861.93
Anhydrous 807.88
Benzenepropanoic acid, β-[[(1,1-dimethylethoxy) carbonyl] amino]-α-hydroxy-, 12b-(acetyloxy)-12-(benzoyloxy)-2a, 3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,6,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-9-yl ester, [2a*R*-[2aα,4β, 4aβ,6β,9α(α*R**,β*S**),11α,12α,12aα,12bα]]-;
(2*R*,3*S*)-*N*-Carboxy-3-phenylisoserine, *N*-*tert*-butyl ester, 13-ester with 5β,20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate;
Trihydrate [148408-66-6].
Anhydrous [114977-28-5].

DEFINITION

Docetaxel contains NLT 97.5% and NMT 102.0% of docetaxel ($C_{43}H_{53}NO_{14}$), calculated on the anhydrous and solvent-free basis. [**CAUTION**—Docetaxel is cytotoxic. Great care should be taken to prevent inhaling particles of Docetaxel and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, 197M, or 197S▲ (CN 1-May-2020)
[NOTE—Use a solution containing 60 mg/mL of Docetaxel in methylene chloride for ⟨197S⟩.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

www.webofpharma.com

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	72	28
9.0	72	28
39.0	28	72
39.1	72	28
50	72	28

Diluent: Acetonitrile, water, and glacial acetic acid (100: 100: 0.1)
System suitability solution: 1 mg/mL of USP Docetaxel Identification RS in *Diluent*. [NOTE—USP Docetaxel Identification RS contains docetaxel and small amounts of 2-debenzoxyl 2-pentenoyl docetaxel, 6-oxodocetaxel, 4-epidocetaxel, and 4-epi-6-oxodocetaxel. See *Table 2*.]
Standard solution: 1.0 mg/mL of USP Docetaxel RS prepared as follows. Transfer a quantity of USP Docetaxel RS to a suitable volumetric flask, dissolve in alcohol equivalent to about 5% of the final volume, and dilute with *Diluent* to volume.
Sample solution: 1.0 mg/mL of Docetaxel prepared as follows. Transfer a quantity of Docetaxel to a suitable volumetric flask, dissolve in alcohol equivalent to about 5% of the final volume, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 232 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Temperatures
Autosampler: 10°
Column: 45°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.5 between 2-debenzoxyl 2-pentenoyl docetaxel and docetaxel, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of docetaxel ($C_{43}H_{53}NO_{14}$) in the portion of Docetaxel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Docetaxel RS in the *Standard solution* (mg/mL)
C_U = concentration of Docetaxel in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 1* is recommended if 10-deacetyl baccatin and 2-debenzoxyl 2-pentenoyl docetaxel are specified impurities. *Procedure 2* is recommended if O-BOC *N*-pyruvyl docetaxel is a specified impurity.]
System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.5 µg/mL of USP Docetaxel RS in *Diluent* from the *Standard solution*
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 3.5 between 2-debenzoxyl 2-pentenoyl docetaxel and docetaxel, *System suitability solution*
Signal-to-noise ratio: NLT 10 for the docetaxel peak, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Docetaxel taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*
F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
10-Deacetyl baccatin (if present)[a,b]	0.25	1.5	0.15
N-Formyl impurity (if present)[b,c]	0.57	1.1	0.15
2-Debenzoxyl 2-pentenoyl docetaxel	0.97	0.63	0.5
Docetaxel	1.00	—	—
6-Oxodocetaxel	1.08	1.0	0.3
4-Epidocetaxel	1.13	1.0	0.3
4-Epi-6-oxodocetaxel	1.18	1.0	0.2
6-Dichloroethoxycarbonyl docetaxel (if present)[b,d]	1.31	0.82	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] (2a*R*,4*S*,4a*S*,6*R*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5-one 12b-acetate, 12-benzoate.
[b] If possible from the manufacturing process.
[c] (2a*R*,4*S*,4a*S*,6*R*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-12b-Acetoxy-9-(((2*R*,3*S*)-3-formamido-2-hydroxy-3-phenylpropanoyl)oxy)-4,6,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1*H*-7,11-methanocyclodeca[3,4]benzo[1,2-*b*]oxet-12-yl benzoate.
[d] (2a*R*,4*S*,4a*S*,6*R*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-6-{[(2,2-Dichloroethoxy)carbonyl]oxy}-1,2a,3,4,4a,6,9,10,11,12,12a,12b-dodecahydro-4,9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5-one 12b-acetate, 12-benzoate, 9-ester with (2*R*,3*S*)-*N*-tert-butoxycarbonyl-3-phenylisoserine.

- **ORGANIC IMPURITIES, PROCEDURE 2**

Solution A: Water

www.webofpharma.com

Solution B: Acetonitrile
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	65	35
25	45	55
35	20	80
45	20	80
45.1	65	35
53	65	35

System suitability solution: 1 mg/mL of USP Docetaxel System Suitability Mixture RS in acetonitrile. [NOTE—USP Docetaxel System Suitability Mixture RS contains docetaxel and a small amount of 6-oxodocetaxel, O-BOC *N*-pyruvyl docetaxel, 4-epidocetaxel, and 4-epi-6-oxodocetaxel. See *Table 4*]
Standard solution: 5.0 µg/mL of USP Docetaxel RS in acetonitrile
Sample solution: 1.0 mg/mL of Docetaxel in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Temperatures
Column: 40°
Sample: 4°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between O-BOC *N*-pyruvyl docetaxel and 4-epidocetaxel
Tailing factor: 0.8–1.5 for the docetaxel peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Docetaxel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*
r_S = peak area of docetaxel from the *Standard solution*
C_S = concentration of USP Docetaxel RS in the *Standard solution* (mg/mL)
C_U = concentration of Docetaxel in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 4*)

Acceptance criteria: See *Table 4*. Disregard any peaks less than 0.05%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Docetaxel	1.0	—	—
6-Oxodocetaxel	1.19	1.1	0.15
O-BOC *N*-pyruvyl docetaxel[a]	1.24	0.80	0.15
4-Epidocetaxel	1.29	0.96	0.15
4-Epi-6-oxodocetaxel	1.42	1.2	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	2.0

[a] (2a*R*,4*R*,4a*S*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4, 9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2*R*,3*S*)-2-(*tert*-butoxycarbonyloxy)-3-(2-oxopropanamido)-3-phenylpropanoic acid.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial limit does not exceed 10^2 cfu/g. The total molds and yeasts count does not exceed 10 cfu/g.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.4 USP Endotoxin Units/mg.
- **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ic*: 5.0%–7.0%. If labeled as an anhydrous form: NMT 1.5%.
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL in methanol
Acceptance criteria: −39° to −41° (t = 20°), calculated on the anhydrous and solvent-free basis. If labeled as an anhydrous form: −35° to −45° (t = 20°), calculated on the as-is basis.

ADDITIONAL REQUIREMENTS

- **LABELING:** Where it is an anhydrous form, the label so indicates. If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at room temperature or between 2° and 8°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Docetaxel RS
USP Docetaxel Identification RS
It contains docetaxel and small amounts of the following:
2-Debenzoxyl 2-pentenoyl docetaxel: (2a*R*,4*S*,4a*S*,6*R*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5-one 12b-acetate, 12-[(*E*)-2-methylbut-2-enoate], 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine.
$C_{41}H_{55}NO_{14}$ 785.87
6-Oxodocetaxel: (2a*R*,4*S*,4a*S*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine.
$C_{43}H_{51}NO_{14}$ 805.86
4-Epidocetaxel: (2a*R*,4*R*,4a*S*,6*R*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5-one 12b-acetate, 12-benzoate, 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine.
$C_{43}H_{53}NO_{14}$ 807.88

www.webofpharma.com

4-Epi-6-oxodocetaxel: (2a*R*,4*R*,4a*S*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4, 9,11,12,12b-pentahydroxy-4a, 8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4] benz[1,2-*b*]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine.
$C_{43}H_{51}NO_{14}$ 805.86

USP Docetaxel System Suitability Mixture RS
Contains docetaxel and a small amount of 6-oxodocetaxel, O-BOC *N*-pyruvyl docetaxel, 4-epidocetaxel, and 4-epi-6-oxodocetaxel.

Docetaxel Injection

DEFINITION
Docetaxel Injection is a sterile solution of Docetaxel. It contains NLT 90.0% and NMT 110.0% of the labeled amount of docetaxel (anhydrous) ($C_{43}H_{53}NO_{14}$). It contains polysorbate 80 and/or other suitable solubilizing agents in the infusion vehicle. It may also contain dehydrated alcohol.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	72	28
9.0	72	28
39.0	28	72
39.1	0	100
49.0	0	100
49.1	72	28
60	72	28

Diluent: Acetonitrile, glacial acetic acid, and water (100:0.1:100)
System suitability solution: 1 mg/mL of USP Docetaxel Identification RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Docetaxel RS. Transfer USP Docetaxel RS into a suitable volumetric flask, and dissolve in alcohol equivalent to 5% of the final volume. Dilute with *Diluent* to volume.
Sample solution (for Injection labeled as one-vial formulation): Dilute a portion of the Injection with *Diluent* to obtain a solution containing 0.2 mg/mL of docetaxel (anhydrous).
Sample solution (for Injection labeled as two-vial formulation): Transfer the content of the vial containing the Injection concentrate to a suitable volumetric flask. Dissolve in an amount of alcohol equivalent to 5% of the final volume, and dilute with *Diluent* to volume to obtain a solution having a concentration of 0.2 mg/mL of docetaxel (anhydrous).
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 232 nm. For *Identification B*, use a diode array detector.
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Temperatures
Refrigerated autosampler: 10°
Column: 45°
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.5 between 2-debenzoxyl 2-pentenoyl docetaxel and docetaxel, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of docetaxel ($C_{43}H_{53}NO_{14}$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Docetaxel RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of docetaxel (anhydrous) in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
- **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Sensitivity solution: 0.2 µg/mL of USP Docetaxel RS in *Diluent* from the *Standard solution*
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 3.5 between 2-debenzoxyl 2-pentenoyl docetaxel and docetaxel, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Signal-to-noise ratio: NLT 10 for the docetaxel peak, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*
r_T = sum of all of the peak areas from the *Sample solution*
F = relative response factor for each individual impurity (see *Table 2*)

www.webofpharma.com

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.1% and any peak with a relative retention time less than 0.2 or greater than 1.3.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
10-Deacetyl baccatin[a]	0.27	1.5	0.30
2-Debenzoxyl 2-pentenoyl docetaxel[b]	0.97	—	—
Docetaxel	1.00	—	—
Crotonaldehyde analog[c]	1.05	1.0	1.3
6-Oxodocetaxel[d]	1.08	1.0	1.5
4-Epidocetaxel[e]	1.13	1.0	1.0
4-Epi-6-oxodocetaxel[f]	1.18	1.0	0.5
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	3.5

[a] (2a*R*,4*S*,4a*S*,6*R*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5-one 12b-acetate, 12-benzoate.

[b] (2a*R*,4*S*,4a*S*,6*R*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5-one 12b-acetate, 12-[(*E*)-2-methylbut-2-enoate], 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine. The alternative chemical name is 5β,20-Epoxy-1,7β,10β-trihydroxy-9-oxotax-11-ene-2α,4,13α-triyl 4-acetate 13-[(2*R*,3*S*)-3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate] 2-[(2*E*)-2-methylbut-2-enoate]. It is a process impurity and is listed in *Table 2* for identification only. It is controlled in the drug substance. It is not reported for the drug product and should not be included in the total impurities.

[c] (1*S*,2*S*,3*R*,9*S*,*E*)-3-[(*S*,*E*)-2-Acetoxy-1-hydroxy-5-oxopent-3-en-2-yl]-1,5,9-trihydroxy-4,8,11,11-tetramethyl-6-oxobicyclo[5.3.1]undeca-4,7-dien-2-yl benzoate, 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine.

[d] (2a*R*,4*S*,4a*S*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4, 9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine. The alternative chemical name is 5β,20-Epoxy-1,7β-dihydroxy-9,10-dioxotax-11-ene-2α,4,13α-triyl 4-acetate 2-benzoate 13-[(2*R*,3*S*)-3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate].

[e] (2a*R*,4*R*,4a*S*,6*R*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5-one 12b-acetate, 12-benzoate, 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine. The alternative chemical name is 5β,20-Epoxy-1,7α,10β-trihydroxy-9-oxotax-11-ene-2α,4,13α-triyl 4-acetate 2-benzoate 13-[(2*R*,3*S*)-3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate].

[f] (2a*R*,4*R*,4a*S*,9*S*,11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4, 9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2*R*,3*S*)-*N*-*tert*-butoxycarbonyl-3-phenylisoserine. The alternative chemical name is 5β,20-Epoxy-1,7α-dihydroxy-9,10-dioxotax-11-ene-2α,4,13α-triyl 4-acetate 2-benzoate 13-[(2*R*,3*S*)-3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate].

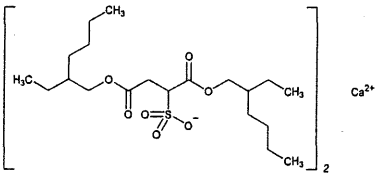
SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 1.94 USP Endotoxin Units/mg of docetaxel (anhydrous).
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store at controlled room temperature.
- **LABELING:** Label it to indicate whether it is a one-vial formulation or two-vial formulation (Injection concentrate and diluent), and also label it to indicate that it is to be diluted with a suitable parenteral vehicle before intravenous infusion.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Docetaxel RS
  USP Docetaxel Identification RS
  [NOTE—USP Docetaxel Identification RS contains docetaxel and small amounts of 2-debenzoxyl 2-pentenoyl docetaxel, 6-oxodocetaxel, 4-epidocetaxel, and 4-epi-6-oxodocetaxel.]

Docusate Calcium



$C_{40}H_{74}CaO_{14}S_2$ 883.22

Butanedioic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, calcium salt;
1,4-Bis(2-ethylhexyl) sulfosuccinate, calcium salt [128-49-4].

DEFINITION

Docusate Calcium contains NLT 91.0% and NMT 100.5% of docusate calcium ($C_{40}H_{74}CaO_{14}S_2$), calculated on the anhydrous basis.

IDENTIFICATION

- **A.**
  Sample: Place a small piece of Docusate Calcium on a salt plate, add 1 drop of acetone, and promptly cover with another salt plate. Rub the plates together to dissolve the specimen, slide the plates apart, and allow the acetone to evaporate.
  Acceptance criteria: The IR absorption spectrum of the film exhibits maxima only at the same wavelengths as that of a similar preparation of USP Docusate Calcium RS.
- **B.**
  Sample: 25 mg
  Analysis: Dissolve the *Sample* in 2 mL of acetone. Add 2 mL of water, and add 2 drops of sulfuric acid.
  Acceptance criteria: A white precipitate is formed.
- **C. THIN-LAYER CHROMATOGRAPHY**
  Standard solution: 10 mg/mL of USP Docusate Calcium RS in isopropyl alcohol
  Sample solution: 10 mg/mL of Docusate Calcium in isopropyl alcohol
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel
  Application volume: 10 µL. [NOTE—Apply with the aid of a stream of nitrogen.]
  Developing solvent system: Ethyl acetate, ammonium hydroxide, and alcohol (5:2:2)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove

www.webofpharma.com

the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Expose the plate to iodine vapors in a closed chamber for about 30 min, and locate the spots.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution.*

ASSAY

- **PROCEDURE**

Solution A: 2.500 g/L of tetra-*n*-butylammonium iodide in water

Solution B: A mixture of 100 g/L of anhydrous sodium sulfate and 10 g/L of sodium carbonate in water

Sample: 50 mg

Analysis: Dissolve the *Sample* in 50 mL of chloroform in a glass-stoppered, 250-mL conical flask. Add 50 mL of *Solution B* and 500 µL of bromophenol blue TS. Titrate with *Solution A* until 1 mL from the endpoint, and shake the stoppered flask vigorously for 2 min. Continue the titration in 2-drop increments, shaking vigorously for 10 s after each addition, and then allow the flask to stand for 10 s. Continue the titration until the chloroform layer just assumes a blue color. Each mL of *Solution A* is equivalent to 2.989 mg of docusate calcium ($C_{40}H_{74}CaO_{14}S_2$).

Acceptance criteria: 91.0%–100.5% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** 14.5%–16.5%, calculated on the anhydrous basis
- **LIMIT OF BIS(2-ETHYLHEXYL) MALEATE**

Mobile phase: Alcohol and water (78:22), filtered and degassed

Standard solution: 80 µg/mL of USP Bis(2-ethylhexyl) Maleate RS in alcohol

Sample solution: 20 mg/mL of Docusate Calcium in alcohol. [NOTE—If necessary, warm the mixture using the steam bath to achieve a complete dissolution.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 3-cm; 3.5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 3 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of bis(2-ethylhexyl) maleate in the portion of Docusate Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of bis(2-ethylhexyl) maleate from the *Sample solution*

r_S = peak response of bis(2-ethylhexyl) maleate from the *Standard solution*

C_S = concentration of USP Bis(2-ethylhexyl) Maleate RS in the *Standard solution* (mg/mL)

C_U = concentration of Docusate Calcium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.4%

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 2.0%
- **CLARITY OF SOLUTION**

Sample solution: 25 g in 94 mL of alcohol

Acceptance criteria: The *Sample solution* does not develop a haze within 24 h when maintained at a temperature of 25 ± 1°.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Bis(2-ethylhexyl) Maleate RS

$C_{20}H_{36}O_4$ 340.51

USP Docusate Calcium RS

Docusate Calcium Capsules

DEFINITION

Docusate Calcium Capsules contain NLT 85.0% and NMT 115.0% of the labeled amount of docusate calcium ($C_{40}H_{74}CaO_{14}S_2$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨191⟩

Sample solution: Ash the contents of 1 Capsule, dissolve the residue in 10 mL of dilute hydrochloric acid (1 in 12), and filter.

Acceptance criteria: The filtrate meets the requirements.

- **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 10 mg/mL of USP Docusate Calcium RS in isopropyl alcohol

Sample solution: Empty the contents of 1 Capsule into a conical flask, and add isopropyl alcohol to obtain a solution containing 10 mg/mL of docusate calcium.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 µL. [NOTE—Apply with the aid of a stream of nitrogen.]

Developing solvent system: Ethyl acetate, ammonium hydroxide, and alcohol (5:2:2)

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Expose the plate to iodine vapors in a closed chamber for about 30 min, and locate the spots.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution.*

ASSAY

- **PROCEDURE**

Basic fuchsin solution: Dissolve 100 mg of basic fuchsin, previously recrystallized from alcohol, in 3 mL of methanol in a 100-mL volumetric flask, and dilute with water to volume. Filter before use.

Standard stock solution: 5 mg/mL of USP Docusate Calcium RS in isopropyl alcohol

Standard solution: Transfer 5 mL of the *Standard stock solution* to a 500-mL volumetric flask containing 90 mL of a mixture of water and isopropanol (1:1) with swirling.

USP Monographs

www.webofpharma.com

Dilute with water to volume. The concentration of USP Docusate Calcium RS in this solution is 50 µg/mL.

Sample stock solution: To 10 Capsules in a 400-mL beaker add 300 mL of hot water. Heat on a steam bath, with occasional stirring, until the Capsules are completely disintegrated. Cool, and transfer with the aid of 100 mL of warm water to a 1000-mL volumetric flask. Rinse the beaker with 100 mL of isopropyl alcohol, add the rinsing to the volumetric flask, and shake to dissolve any previously undissolved particles. Dilute with a mixture of water and isopropyl alcohol (1:1) to volume.

Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Vis

Analytical wavelength: 545 nm

Cell: 1 cm

Blank: Chloroform

Analysis: Transfer 4.0 mL of the *Standard solution* to a 125-mL separator. To a second 125-mL separator transfer a volume of the *Sample solution*, equivalent to 200–240 µg of docusate calcium, and add a solution of isopropyl alcohol (1 in 100), if necessary, to bring the volume of the solution in the separator to 4.0 mL. To each separator transfer 20.0 mL of *pH 1.2 Hydrochloric Acid Buffer* (see *Reagents, Indicators, and Solutions—Solutions*) and 2.0 mL of *Basic fuchsin solution*. Add 20 mL of chloroform to each separator, and shake for 1 min. Allow the phases to separate, and transfer the chloroform extracts, through separate pledgets of absorbent cotton, into separate 100-mL volumetric flasks. Extract each of the solutions in the separators in the same manner with additional 20-mL portions of chloroform until no more color is visible in the extract, and pass each extract through the pledget of cotton used for the preceding extract into the flask containing the preceding extract. Dilute the contents of each flask to volume by passing chloroform through the cotton pledgets that had been used to filter the extracts, and mix.

Calculate the percentage of the labeled amount of docusate calcium ($C_{40}H_{74}CaO_{14}S_2$) in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of anhydrous USP Docusate Calcium RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of docusate calcium in the *Sample solution* (µg/mL)

Acceptance criteria: 85.0%–115.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 15 min

Analysis: Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each capsule shell to rupture.

Tolerances: The requirements are met if all of the Capsules tested rupture in NMT 15 min. If 1 or 2 of the Capsules rupture in more than 15 min but NMT 30 min, repeat the test on 12 additional Capsules. NMT 2 of the total of 18 Capsules tested rupture in more than 15 min but NMT 30 min.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements for *Content Uniformity* for solid-filled capsules and meet the requirements for *Weight Variation* for solution-filled capsules

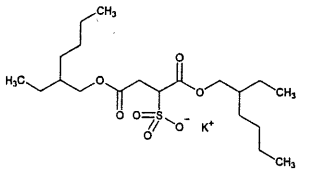
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature in a dry place.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Docusate Calcium RS

Docusate Potassium



$C_{20}H_{37}KO_7S$ 460.67

Butanedioic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, potassium salt;

Potassium 1,4-bis(2-ethylhexyl) sulfosuccinate [7491-09-0].

DEFINITION

Docusate Potassium contains NLT 95.0% and NMT 100.5% of docusate potassium ($C_{20}H_{37}KO_7S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

Sample: Place a small piece of Docusate Potassium on a salt plate, add 1 drop of acetone, and promptly cover with another salt plate. Rub the plates together to dissolve the specimen, slide the plates apart, and allow the acetone to evaporate.

Acceptance criteria: Meets the requirements

• **B.** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

ASSAY

• **PROCEDURE**

Solution A: 2.500 g/L of tetra-*n*-butylammonium iodide in water

Solution B: A solution containing 100 g/L of anhydrous sodium sulfate and 10 g/L of sodium carbonate in water

Sample: 100 mg

Analysis: Dissolve the *Sample* in 50 mL of chloroform in a glass-stoppered, 250-mL conical flask. Add 50 mL of *Solution B* and 500 µL of bromophenol blue TS. Titrate with *Solution A* until 1 mL from the endpoint, and shake the stoppered flask vigorously for 2 min. Continue the titration in 2-drop increments, shaking vigorously for 10 s after each addition, and then allow the flask to stand for 10 s. Continue the titration until the chloroform layer just assumes a blue color. Each mL of *Solution A* is equivalent to 3.118 mg of docusate potassium ($C_{20}H_{37}KO_7S$).

www.webofpharma.com

Acceptance criteria: 95.0%–100.5% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** 18.0%–20.0% on the dried basis

• **LIMIT OF BIS(2-ETHYLHEXYL) MALEATE**

Mobile phase: Alcohol and water (78:22), filtered and degassed

Standard solution: 80 µg/mL of USP Bis(2-ethylhexyl) Maleate RS in alcohol

Sample solution: 20 mg/mL of Docusate Potassium in alcohol. [NOTE—If necessary, warm the mixture by using the steam bath to achieve a complete dissolution.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 3-cm; 3.5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 3 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of bis(2-ethylhexyl) maleate in the portion of Docusate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of bis(2-ethylhexyl) maleate from the *Sample solution*

r_S = peak response of bis(2-ethylhexyl) maleate from the *Standard solution*

C_S = concentration of USP Bis(2-ethylhexyl) Maleate RS in the *Standard solution* (mg/mL)

C_U = concentration of Docusate Potassium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.4%

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry in a glass container at 105° for 4 h.

Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Bis(2-ethylhexyl) Maleate RS

$C_{20}H_{36}O_4$ 340.51

USP Docusate Potassium RS

Docusate Potassium Capsules

DEFINITION

Docusate Potassium Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of docusate potassium ($C_{20}H_{37}KO_7S$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Transfer 180 mL of water to a 1000-mL volumetric flask, and dilute with methanol to volume.

Tetrabutylammonium hydroxide solution: 250 mg/mL of tetrabutylammonium hydroxide in methanol

Mobile phase: Mix 180 mL of water, 6.0 mL of glacial acetic acid, and 8.0 mL of *Tetrabutylammonium hydroxide solution* in a 1000-mL volumetric flask, and dilute with methanol to volume. The water concentration may be varied to meet *System suitability* requirements and to provide a suitable elution time (about 5 min) for docusate potassium.

Standard solution: 4 mg/mL of USP Docusate Sodium RS, calculated on the anhydrous basis, in *Diluent*

Sample solution

Solid-filled capsules: Open and empty into a suitable container the contents of a counted number of Capsules, nominally equivalent to 1000 mg of docusate potassium. Place the Capsule shells in the container, and add 250.0 mL of *Diluent*. Shake by mechanical means for 20 min, and clarify a portion of the mixture by centrifuging. Pass a portion of the clear supernatant through a membrane filter of 0.5-µm or finer pore size.

Solution-filled capsules: Cut open a counted number of Capsules, nominally equivalent to 2.5 g of docusate potassium, and place the shells and contents in a suitable container. Add 25 mL of methanol, agitate for NLT 2 min, and decant the liquid into a 200-mL volumetric flask. Repeat the addition of methanol, agitation, and decanting NLT four times. Dilute with methanol to volume. Pipet 8 mL of this solution into a 25-mL volumetric flask, add 4.5 mL of water, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.8 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0% for five replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of docusate potassium ($C_{20}H_{37}KO_7S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of anhydrous USP Docusate Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of docusate potassium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of docusate potassium, 460.67

M_{r2} = molecular weight of docusate sodium, 444.56

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 15 min

Analysis: Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting

USP Monographs

www.webofpharma.com

rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

Tolerances: The requirements are met if all of the Capsules tested rupture in NMT 15 min. If 1 or 2 of the Capsules rupture in more than 15 min but NMT 30 min, repeat the test on 12 additional Capsules. NMT 2 of the total of 18 Capsules tested rupture in more than 15 min but NMT 30 min.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements for *Content Uniformity* for solid-filled capsules and meet the requirements for *Weight Variation* for solution-filled capsules

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩
USP Docusate Potassium RS
USP Docusate Sodium RS

Docusate Sodium

$C_{20}H_{37}NaO_7S$ 444.56

Butanedioic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt;

Sodium 1,4-bis(2-ethylhexyl) sulfosuccinate [577-11-7].

DEFINITION

Docusate Sodium contains NLT 99.0% and NMT 100.5% of $C_{20}H_{37}NaO_7S$, calculated on the anhydrous basis.

IDENTIFICATION

• **Procedure**

Sample: Place a small piece of Docusate Sodium on a salt plate, add one drop of acetone, and promptly cover with another salt plate. Rub the plates together to dissolve the specimen, slide the plates apart, and allow the acetone to evaporate.

Acceptance criteria: The IR absorption spectrum of the film exhibits maxima only at the same wavelengths as that of a similar preparation of USP Docusate Sodium RS.

ASSAY

• **Procedure**

Solution A: 2.5 g/L of tetra-*n*-butylammonium iodide in water

Solution B: A mixture of 100 g/L of anhydrous sodium sulfate and 10 g/L of sodium carbonate in water

Sample: 50 mg

Analysis: Dissolve the *Sample* in 50 mL of chloroform in a glass-stoppered, 250-mL conical flask. Add 50 mL of *Solution B* and 500 µL of bromophenol blue TS. Titrate with *Solution A* until 1 mL from the endpoint, and shake the stoppered flask vigorously for 2 min. Continue the titration in two-drop increments, shaking vigorously for 10 s after each addition, and then allow the flask to stand 10 s. Continue the titration until the chloroform layer just assumes a blue color. Each mL of *Solution A* is equivalent to 3.009 mg of $C_{20}H_{37}NaO_7S$.

Acceptance criteria: 99.0%–100.5% on the anhydrous basis

IMPURITIES

Inorganic Impurities

• **Residue on Ignition** ⟨281⟩: Between 15.5% and 16.5%, calculated on the anhydrous basis

Procedure: Transfer about 1 g, accurately weighed, to a tared crucible, ignite until thoroughly charred, and cool. Moisten the ash with 1 mL of sulfuric acid, and complete the ignition by heating at 800 ± 25° for 15-min periods to constant weight.

Organic Impurities

• **Procedure: Limit of Bis(2-Ethylhexyl) Maleate**

Mobile phase: Alcohol and water (78:22), filtered and degassed

Standard solution: 80 µg/mL of USP Bis(2-ethylhexyl) Maleate RS in alcohol

Sample solution: 20 mg/mL of Docusate Sodium in alcohol. [Note—If necessary, warm the mixture using the steam bath to achieve a complete dissolution.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 3-cm; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 3 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of bis(2-ethylhexyl) maleate in the portion of Docusate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of bis(2-ethylhexyl) maleate from the *Sample solution*
r_S = peak response of bis(2-ethylhexyl) maleate from the *Standard solution*
C_S = concentration of USP Bis(2-ethylhexyl) Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of Docusate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.4%

SPECIFIC TESTS

• **Water Determination,** *Method I* ⟨921⟩: NMT 2.0%

• **Clarity of Solution**
Sample solution: 25 g in 100 mL of alcohol
Acceptance criteria: The *Sample solution* does not develop a haze within 24 h.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards** ⟨11⟩
USP Bis(2-ethylhexyl) Maleate RS
$C_{20}H_{36}O_4$ 340.51
USP Docusate Sodium RS

Docusate Sodium Capsules

DEFINITION

Docusate Sodium Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of docusate sodium ($C_{20}H_{37}NaO_7S$).

www.webofpharma.com

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and 0.01 M tetrabutylammonium dihydrogen phosphate (66:34)

Diluent: Acetonitrile and water (50:50)

Standard solution: 0.1 mg/mL of USP Docusate Sodium RS in *Diluent*. Filter the solution, discarding the first 6 mL of the filtrate.

Sample stock solution: Transfer a number of Capsules, equivalent to 250 mg of docusate sodium, to a 250-mL volumetric flask, and add 50 mL of water. Heat the mixture with occasional swirling until the Capsule shells have ruptured and dissolved. [NOTE—Take special care to ensure that all of the Capsules have ruptured.] Remove from heat, and add 50 mL of acetonitrile. Allow this solution to cool to room temperature, and dilute with *Diluent* to volume.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, dilute with *Diluent* to volume, and filter, discarding the first 6 mL of filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; packing L1 that has been highly deactivated (carbon loading of 30%)

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of docusate sodium ($C_{20}H_{37}NaO_7S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of anhydrous docusate sodium in the *Standard solution*, as determined from the concentration of USP Docusate Sodium RS corrected for moisture by a titrimetric water determination (mg/mL)

C_U = nominal concentration of docusate sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 15 min

Analysis: Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

Tolerances: The requirements are met if all of the Capsules tested rupture in NMT 15 min. If 1 or 2 Capsules rupture in more than 15 min but NMT 30 min, repeat the test on 12 additional Capsules. NMT 2 of the total of 18 Capsules tested rupture in more than 15 min but NMT 30 min.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements for *Content Uniformity* for solid-filled capsules and meet the requirements for *Weight Variation* for solution-filled capsules

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Docusate Sodium RS

Docusate Sodium Solution

DEFINITION

Docusate Sodium Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of docusate sodium ($C_{20}H_{37}NaO_7S$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and 0.01 M tetrabutylammonium dihydrogen phosphate (66:34)

Diluent: Acetonitrile and water (50:50)

Standard solution: 0.1 mg/mL of USP Docusate Sodium RS in *Diluent*. Filter the solution, discarding the first 6 mL of the filtrate.

Sample stock solution: Transfer a volume of Solution, equivalent to 100 mg of docusate sodium, to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, dilute with *Diluent* to volume, and filter, discarding the first 6 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; packing L1 that has been highly deactivated (carbon loading of 30%)

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of docusate sodium ($C_{20}H_{37}NaO_7S$) in the portion of Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of anhydrous docusate sodium in the *Standard solution*, as determined from the concentration of USP Docusate Sodium RS corrected for moisture by a titrimetric water determination (mg/mL)

www.webofpharma.com

C_U = nominal concentration of docusate sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.5–6.9

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Docusate Sodium RS

Docusate Sodium Syrup

DEFINITION
Docusate Sodium Syrup contains NLT 90.0% and NMT 110.0% of the labeled amount of docusate sodium ($C_{20}H_{37}NaO_7S$).

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 2 mg/mL of USP Docusate Sodium RS in isopropyl alcohol

Sample solution: Dilute a volume of Syrup, equivalent to about 10 mg of docusate sodium, with isopropyl alcohol to obtain a preparation containing about 2 mg/mL, and mix. Use the upper layer of this preparation.

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 50 µL. [NOTE—Apply with the aid of a stream of nitrogen.]
Developing solvent system: Ethyl acetate, alcohol, water, and ammonium hydroxide (25:10:20:1). [NOTE—This is a two-phase system.]

Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry, and develop the chromatogram in the *Developing solvent system*, until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Expose the plate to iodine vapors in a closed chamber for about 30 min, and locate the spots.

Acceptance criteria: The *Sample solution* produces a spot at the same R_F value and of approximately the same size as that obtained from the *Standard solution*.

ASSAY
- **PROCEDURE**

Standard stock solution: 1.0 mg/mL of USP Docusate Sodium RS, first dissolved in alcohol using about 2.5% of the final volume, and then diluted with water to volume. This solution contains 1 mg/mL of USP Docusate Sodium RS.

Standard solution: 10 µg/mL in water from the *Standard stock solution*

Sample solution: Transfer an accurately measured volume of Syrup, equivalent to about 100 mg of docusate sodium, to a 1000-mL volumetric flask, allowing the pipet to drain for 15 min. Dilute with water to volume, and mix. Transfer 10.0 mL of the solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Vis
Analytical wavelength: 650 nm
Cell: 1 cm

Analysis
Samples: *Standard solution* and *Sample solution*
Transfer 20.0 mL each of the *Standard solution* and the *Sample solution* to two individual separators. Place 20 mL of water in a third separator (*Blank solution*). To each separator add 5 drops of hydrochloric acid, mix by swirling, add 1.0 mL of methylene blue solution (1 in 1000), and mix by swirling. To each separator add 20.0 mL of chloroform, and shake vigorously for 5 min. Wash each chloroform solution, in clean separators, with 20 mL of water, shaking vigorously for 60 s. Discard the washings, and filter each chloroform solution through a layer of 3 g of anhydrous granular sodium sulfate, supported on glass wool, into a 100-mL volumetric flask, washing each separator with two 10-mL portions of chloroform, and filtering the washings into each flask. Dilute each flask with chloroform to volume. Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*, using the *Blank solution* to set the instrument.

Calculate the percentage of the labeled amount of docusate sodium ($C_{20}H_{37}NaO_7S$) in the portion of Syrup taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Docusate Sodium RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of docusate sodium in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 5.5–6.5

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Docusate Sodium RS

Docusate Sodium Tablets

DEFINITION
Docusate Sodium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of docusate sodium ($C_{20}H_{37}NaO_7S$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Finely divide a suitable number of Tablets, extract with solvent hexane, filter, and evaporate the solvent hexane extract on a steam bath. Use the dry residue.
Acceptance criteria: Meet the requirements

www.webofpharma.com

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and 7 mM ammonium acetate (1:1)

Standard solution: Dissolve the USP Docusate Sodium RS in alcohol, and dilute with water to obtain a solution containing 1.0 mg/mL of USP Docusate Sodium RS.

Methylparaben solution: 0.15 mg/mL of methylparaben in water

System suitability solution: Mix 0.1 mL of *Methylparaben solution* and 10 mL of *Standard solution*.

Sample solution: Transfer 10 Tablets to a 1-L volumetric flask, add 200 mL of alcohol and 300 mL of water, and shake by mechanical means for NLT 90 min to completely disintegrate the Tablets. Dilute with water to volume, and filter, discarding the first 3 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 40 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for methylparaben and docusate are about 0.74 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between methylparaben and docusate, *System suitability solution*

Tailing factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 1.8%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of docusate sodium ($C_{20}H_{37}NaO_7S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Docusate Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of docusate sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩

Medium: Proceed as directed in the chapter, except substitute simulated gastric fluid TS for water in the test for *Uncoated Tablets*.

Time: 1 h

Acceptance criteria: Meet the requirements

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

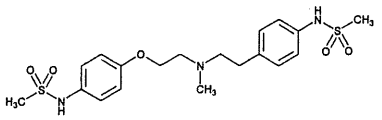
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Docusate Sodium RS

Dofetilide



$C_{19}H_{27}N_3O_5S_2$ 441.56

Methanesulfonamide, *N*-[4-[2-[methyl[2-[4-[(methylsulfonyl)amino]phenoxy]ethyl]amino]ethyl]phenyl]-; β-[(*p*-Methanesulfonamidophenethyl)methylamino]methane sulfono-*p*-phenetidide [115256-11-6].

DEFINITION

Dofetilide contains NLT 97.0% and NMT 103.0% of $C_{19}H_{27}N_3O_5S_2$, calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Potassium hydroxide solution: 0.56 g/mL of potassium hydroxide

Buffer: 1.36 g of monobasic potassium phosphate and 5 mg of ascorbic acid in 1 L of water. Adjust with *Potassium hydroxide solution* to a pH of 7.0.

Mobile phase: Acetonitrile and *Buffer* (1:3)

System suitability solution: 25 µg/mL of USP Dofetilide RS and 0.5 µg/mL of USP Dofetilide Related Compound A RS in *Mobile phase*

Standard solution: 25 µg/mL of USP Dofetilide RS in *Mobile phase*

Sample solution: 25 µg/mL of Dofetilide in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 3.9-mm × 15-cm; 4-µm packing L7

Temperature: 30°

Flow rate: 1 mL/min

Injection size: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 8.0 between dofetilide and dofetilide related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{19}H_{27}N_3O_5S_2$ in the portion of Dofetilide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dofetilide from the *Sample solution*

r_S = peak response of dofetilide from the *Standard solution*

C_S = concentration of dofetilide in the *Standard solution* (µg/mL)

www.webofpharma.com

C_U = concentration of Dofetilide in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0% on the anhydrous and solvent-free basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

• **Procedure**

Buffer: 0.78 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 5.0 ± 0.1.

Diluent: Acetonitrile and *Buffer* (3:22)

Solution A: *Buffer*

Solution B: Methanol

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	88	12
5	88	12
25	70	30
30	70	30

System suitability solution: 1.25 µg/mL each of USP Dofetilide RS and USP Dofetilide Related Compound A RS in *Diluent*

Standard solution: 1.25 µg/mL of USP Dofetilide RS in *Diluent*

Diluted standard solution: 0.125 µg/mL of USP Dofetilide RS in *Diluent* from the *Standard solution*

Sample solution: 0.25 mg/mL of Dofetilide in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Temperature: 30°

Flow rate: 1 mL/min

Injection size: 50 µL

System suitability

Samples: *System suitability solution* and *Diluted standard solution*

System suitability requirements

Resolution: NLT 5.0 between dofetilide and dofetilide related compound A, *System suitability solution*

Column efficiency: NLT 35,000 theoretical plates for the dofetilide peak, *System suitability solution*

Tailing factor: NMT 1.5 for the dofetilide peak, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Diluted standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Dofetilide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response of dofetilide from the *Standard solution*

C_S = concentration of USP Dofetilide RS in the *Standard solution* (mg/mL)

C_U = concentration of Dofetilide in the *Sample solution* (mg/mL)

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dofetilide related compound A[a]	0.9	1.04	0.5
Dofetilide	1.0	—	—
Any other individual unspecified impurity	—	1.00[b]	0.1
Total impurities	—	—	0.5

[a] *N*-[4-(2-(2-[4-(Methanesulfonamido)phenoxy]ethylamino)ethyl)phenyl] methanesulfonamide.

[b] Unless otherwise determined.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

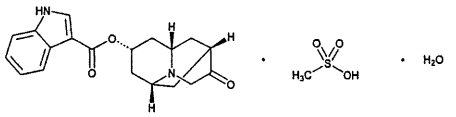
• **USP REFERENCE STANDARDS** ⟨11⟩

USP Dofetilide RS

USP Dofetilide Related Compound A RS

N-[4-(2-{2-[4-(Methanesulfonamido)phenoxy] ethylamino}ethyl)phenyl]methanesulfonamide. 427.54

Dolasetron Mesylate



$C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$ 438.49

1*H*-Indole-3-carboxylic acid, octahydro-3-oxo-2,6-methano-2*H*-quinolizin-8-yl ester, (2 α ,6 α ,8 α ,9a β)-, monomethanesulfonate monohydrate;

Indole-3-carboxylic acid, ester with (8*r*)-hexahydro-8-hydroxy-2,6-methano-2*H*-quinolizin-3(4*H*)-one, monomethanesulfonate monohydrate [878143-33-0].

Anhydrous [115956-13-3].

DEFINITION

Dolasetron Mesylate contains NLT 98.0% and NMT 102.0% of $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$, calculated on the as-is basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B. PROCEDURE**

Sample solution: 1 mg/mL

Analysis: Transfer 5–10 mg of 5,5′-methylenedisalicylic acid to a clean crucible, and heat in an oven at 150° for 5 min.

www.webofpharma.com

Remove from the oven, and add 10 drops of the *Sample solution*. Return to the oven, and evaporate to dryness.
Acceptance criteria: A red or pink color (presence of methanesulfonic acid) develops in the white residue.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, water, and 1 M ammonium formate (450:440:110), adding 0.19 mL of triethylamine to the acetonitrile portion
Standard solution: 0.04 mg/mL and 0.004 mg/mL respectively of USP Dolasetron Mesylate RS and indole-3-carboxylic acid in *Mobile phase*
Sample solution: 0.04 mg/mL of Dolasetron Mesylate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 4 between indole-3-carboxylic acid and dolasetron mesylate
Tailing factor: NMT 1.8
Relative standard deviation: NMT 1.5% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{19}H_{20}N_2O_3 \cdot CH_4O_3S \cdot H_2O$ in the Dolasetron Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dolasetron Mesylate RS in the *Standard solution* (mg/mL)
C_U = concentration of Dolasetron Mesylate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the as-is basis

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

0.01 M Dibasic ammonium phosphate solution: 1.32 g/L of dibasic ammonium phosphate. Adjust with 2.0 M phosphoric acid to a pH of 7.0.
Diluent: Acetonitrile and water (1:4)
Solution A: Acetonitrile and *0.01 M Dibasic ammonium phosphate solution* (53:1000), filtered and degassed
Solution B: Acetonitrile and *0.01 M Dibasic ammonium phosphate solution* (795:295), filtered and degassed
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
28	0	100
38	0	100
40	100	0
50	100	0

System suitability solution: 0.004 mg/mL and 0.03 mg/mL, respectively, of indole and USP Dolasetron Mesylate RS in *Diluent*
Standard solution A: 0.03 mg/mL of USP Dolasetron Mesylate RS in *Diluent*
Standard solution B: 6 mg/mL and 0.0072 mg/mL, respectively, of USP Dolasetron Mesylate RS and USP Dolasetron Mesylate Related Compound A RS in *Diluent*
Sample solution: 6 mg/mL of Dolasetron Mesylate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 1.5 mL/min
Injection size: 100 µL
System suitability
Suitability requirements
Resolution: NLT 1.5 between the first eluting peak, indole, and the second eluting peak, dolasetron mesylate from the *System suitability solution*. [NOTE—If the dolasetron mesylate peak is found to elute before the indole peak, condition the column as follows: fill up the column with *Solution A*, plug the column, and place the column in a convection oven at 105° for about 16 h. Retest the column.]
Relative standard deviation: NMT 5.0% for replicate injections of *Standard solution A*
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Calculate the percentage of dolasetron mesylate related compound A in the Dolasetron Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of dolasetron mesylate related compound A from the *Sample solution*
r_S = peak response of dolasetron mesylate related compound A from the *Standard solution B*
C_S = concentration of USP Dolasetron Mesylate Related Compound A RS in the *Standard solution B* (mg/mL)
C_U = concentration of Dolasetron Mesylate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dolasetron mesylate related compound A base, 181.2
M_{r2} = molecular weight of dolasetron mesylate related compound A hydrochloride, 217.8

Calculate the percentage of each impurity (other than dolasetron mesylate related compound A) in the portion of Dolasetron Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of dolasetron mesylate from the *Standard solution A*
C_S = concentration of USP Dolasetron Mesylate RS in the *Standard solution A* (mg/mL)
C_U = concentration of Dolasetron Mesylate in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: NMT 0.1%
Total impurities: NMT 0.3%
[NOTE—The reporting level for impurities is 0.05%.]

USP Monographs

www.webofpharma.com

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** Between 3.5% and 4.7%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dolasetron Mesylate RS
  USP Dolasetron Mesylate Related Compound A RS
  Hexahydro-8-hydroxy-2,6-methano-2*H*-quinolizin-3 (4*H*)-one, hydrochloride.

Dolasetron Mesylate Compounded Oral Solution

DEFINITION

Dolasetron Mesylate Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of dolasetron mesylate ($C_{19}H_{20}N_2O_3 \cdot CH_4O_3S$).

Prepare Dolasetron Mesylate Compounded Oral Solution 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Dolasetron Mesylate powder	1 g
Vehicle for Oral Solution (regular or sugar-free), *NF*, a sufficient quantity to make	100 mL

Add *Dolasetron Mesylate powder* and 15 mL of *Vehicle* to a mortar, and mix. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix.

ASSAY
- **PROCEDURE**

Solution A: 0.05 M ammonium acetate adjusted with diluted ammonium hydroxide to a pH of 7.5
Mobile phase: Acetonitrile and *Solution A* (24:76). Filter, and degas.
Diluent: Acetonitrile and water (24:76)
Standard stock solution: 500 µg/mL of USP Dolasetron Mesylate RS in *Diluent*
Standard solution: 10 µg/mL of USP Dolasetron Mesylate RS from *Standard stock solution* in *Mobile phase*
Sample solution: 10 µg/mL of dolasetron mesylate prepared from Oral Solution and *Diluent*. Shake each sample thoroughly by hand for 15 s, centrifuge at 1000 rpm for 2 min, and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 3-µm packing L10
Column temperature: 30°
Flow rate: 0.8 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for dolasetron mesylate is about 6.9 min.]
Suitability requirements
Relative standard deviation: NMT 1.4% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dolasetron mesylate ($C_{19}H_{20}N_2O_3 \cdot CH_4O_3S$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dolasetron Mesylate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dolasetron mesylate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 3.6–4.6

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature, or in a refrigerator
- **LABELING:** Label it to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dolasetron Mesylate RS

Dolasetron Mesylate Compounded Oral Suspension

DEFINITION

Dolasetron Mesylate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of dolasetron mesylate ($C_{19}H_{20}N_2O_3 \cdot CH_4O_3S$).

Prepare Dolasetron Mesylate Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Dolasetron Mesylate	1 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

If using tablets, place the required number in a suitable mortar, and comminute to a fine powder, or add *Dolasetron Mesylate* powder to the mortar. Add 20 mL of *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions, and mix well after each addition. Transfer, stepwise and quantitatively, to a calibrated bottle. Add the *Vehicle* in portions to rinse the mortar, add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY
- **PROCEDURE**

Solution A: 0.05 M ammonium acetate adjusted with diluted ammonium hydroxide to a pH of 7.5
Mobile phase: Acetonitrile and *Solution A* (24:76). Filter and degas.
Diluent: Acetonitrile and water (24:76)
Standard stock solution: 500 µg/mL of USP Dolasetron Mesylate RS in *Diluent*

www.webofpharma.com

Standard solution: 10 µg/mL of USP Dolasetron Mesylate RS prepared from the *Standard stock solution* in *Mobile phase*
Sample solution: 10 µg/mL of dolasetron mesylate prepared from Oral Suspension and *Diluent*. Shake each sample thoroughly by hand for 15 s, centrifuge at 1000 rpm for 2 min, and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 5-µm packing L10
Column temperature: 30°
Flow rate: 0.8 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for dolasetron mesylate is about 6.9 min.]
Suitability requirements
Relative standard deviation: NMT 1.4% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dolasetron mesylate ($C_{19}H_{20}N_2O_3 \cdot CH_4O_3S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

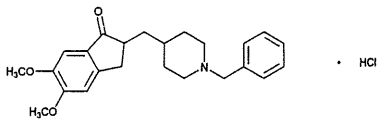
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Dolasetron Mesylate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of dolasetron mesylate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 3.6–4.6

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature, or in a refrigerator
• **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dolasetron Mesylate RS

Donepezil Hydrochloride



$C_{24}H_{29}NO_3 \cdot HCl$ 415.95
$C_{24}H_{29}NO_3 \cdot HCl \cdot H_2O$ 433.97
5,6-Dimethoxyindan-1-one, 2-[(1-benzyl-4-piperidyl) methyl]-, (±)-, hydrochloride;
(±)-2-[(1-Benzyl-4-piperidyl)methyl]-5,6-dimethoxy-1-indanone hydrochloride [120011-70-3].
Monohydrate [884740-09-4].

DEFINITION
Donepezil Hydrochloride contains NLT 98.0% and NMT 102.0% of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
[NOTE—If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the USP Donepezil Hydrochloride RS separately in dichloromethane, evaporate to dryness, and record new spectra using the residues.]
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩
Sample solution: 10 mg/mL
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Buffer: 3.9 g/L of sodium 1-decane sulfonate in water
Mobile phase: Acetonitrile and *Buffer* (35:65). Adjust with perchloric acid to a pH of 1.8.
System suitability solution: 0.4 mg/mL of USP Donepezil Hydrochloride RS and 0.016 mg/mL of USP Donepezil Related Compound A RS prepared as follows. Dissolve suitable quantities of USP Donepezil Hydrochloride RS and USP Donepezil Related Compound A RS using 40% of the flask volume of methanol, and dilute with water to volume.
Standard solution: 0.4 mg/mL of USP Donepezil Hydrochloride RS in *Mobile phase*
Sample solution: 0.4 mg/mL of Donepezil Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 271 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.4 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—Refer to *Table 1* under *Organic Impurities, Procedure 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between donepezil related compound A and donepezil, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) in the portion of Donepezil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of donepezil hydrochloride from the *Sample solution*

www.webofpharma.com

r_S = peak response of donepezil hydrochloride from the *Standard solution*
C_S = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Donepezil Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* is recommended if any of the impurities included in *Table 3* are potential related compounds.]

Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.8 µg/mL of USP Donepezil Hydrochloride RS in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—Refer to *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between donepezil related compound A and donepezil, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Donepezil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of donepezil hydrochloride from the *Standard solution*
C_S = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Donepezil Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desbenzyl donepezil[a]	0.33	0.2
Hydroxydonepezil[b]	0.54	0.2
Donepezil related compound A[c]	0.92	0.1
Donepezil hydrochloride	1.0	—
Any individual unspecified impurity	—	0.1
Total impurities	—	1.0

[a] 5,6-Dimethoxy-2-(piperidin-4-ylmethyl)indan-1-one.
[b] 2-[(1-Benzylpiperidin-4-yl)(hydroxy)methyl]-5,6-dimethoxyindan-1-one.
[c] (*E*)-2-[(1-Benzylpiperidin-4-yl)methylene]-5,6-dimethoxyindan-1-one.

- **ORGANIC IMPURITIES, PROCEDURE 2**

Solution A: Add 1 mL of phosphoric acid in 1 L of water. Adjust with triethylamine to a pH of 6.6 ± 0.1. Pass through a filter of 0.45-µm or finer pore size.

Solution B: Acetonitrile

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	75	25
10	40	60
40	40	60
41	75	25
50	75	25

[NOTE—The gradient was established on an HPLC system with a dwell volume of approximately 0.65 mL.]

Diluent: Acetonitrile and water (25:75)

System suitability solution: 1 mg/mL of USP Donepezil Hydrochloride RS and 0.002 mg/mL of USP Donepezil Related Compound A RS in *Diluent*

Sensitivity solution: 0.0015 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*

Standard solution: 0.01 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*. Sonication may be used to aid the dissolution.

Sample solution: 1.0 mg/mL of Donepezil Hydrochloride in *Diluent*. Sonication may be used to aid the dissolution.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 286 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between donepezil and donepezil related compound A, *System suitability solution*

Tailing factor: NMT 1.5 for donepezil, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Donepezil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of donepezil hydrochloride from the *Standard solution*
C_S = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Donepezil Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding impurity peak (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard peaks less than 0.03%.

www.webofpharma.com

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desbenzyl donepezil[a]	0.24	1.2	0.2
Donepezil alkene pyridine *N*-oxide[b]	0.32	2.3	0.15
Donepezil-*N*-oxide[c]	0.46	1.1	0.1
Donepezil pyridine analog (DPMI)[d]	0.52	1.4	0.15
3-Hydroxydonepezil[e]	0.59	1.0	0.15
Hydroxydonepezil[f]	0.68	0.86	0.2
Donepezil quaternary salt (donepezilbenzyl)[g]	0.77	0.74	0.15
Donepezil	1.0	—	—
Donepezil related compound A	1.08	3.4	0.1
Donepezil indene (dehydro-deoxy donepezil)[h]	1.63	2.2	0.15
Deoxydonepezil[i]	1.94	1.2	0.15
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	1.0

[a] 5,6-Dimethoxy-2-(piperidin-4-ylmethyl)indan-1-one.
[b] (*E*)-4-[(5,6-Dimethoxy-1-oxo-1,3-dihydro-2*H*-inden-2-ylidene)methyl] pyridine 1-oxide.
[c] 1-Benzyl-4-[(5,6-dimethoxy-1-oxo-2,3-dihydro-1*H*-inden-2-yl)methyl] piperidine 1-oxide.
[d] 5,6-Dimethoxy-2-(pyridin-4-ylmethyl)indan-1-one.
[e] 2-[(1-Benzylpiperidin-4-yl)methyl]-3-hydroxy-5,6-dimethoxy-1*H*-indan-one.
[f] 2-[(1-Benzylpiperidin-4-yl)(hydroxy)methyl]-5,6-dimethoxyindan-1-one.
[g] 1,1-Dibenzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidinium.
[h] 1-Benzyl-4-[(5,6-dimethoxyinden-2-yl)methyl]piperidine.
[i] 1-Benzyl-4-[(5,6-dimethoxyindan-2-yl)methyl]piperidine.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method Ia* ⟨921⟩
  Acceptance criteria
  Anhydrous form: NMT 0.4%
  Anhydrous form-I: NMT 7.0%
  Monohydrate form: NMT 7.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** Where it is the anhydrous form-I or the hydrated form, the label so indicates. If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Donepezil Hydrochloride RS
  USP Donepezil Related Compound A RS
  (*E*)-2-[(1-Benzylpiperidin-4-yl)methylene]-5,6-dimethoxyindan-1-one.
  $C_{24}H_{27}NO_3$ 377.48

Donepezil Hydrochloride Tablets

DEFINITION

Donepezil Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Wavelength range: 220–360 nm
  Sample solution: Crush a suitable number of Tablets, and transfer an amount of powder, equivalent to 10 mg of donepezil hydrochloride, to a 100-mL volumetric flask. Add 80 mL of ▲0.1 N hydrochloric acid VS,▲ (RB 1-Mar-2019) and sonicate for 5 min. Cool the solution to room temperature, and dilute with ▲0.1 N hydrochloric acid VS▲ (RB 1-Mar-2019) to volume. Transfer a portion of this solution to a centrifuge tube, and centrifuge for 15 min. Transfer 5 mL of the clear supernatant to a 25-mL volumetric flask, and dilute with ▲0.1 N hydrochloric acid VS▲ (RB 1-Mar-2019) to volume.
  Analysis: Using a 1-cm cell, record the UV spectrum of the *Sample solution*.
  Acceptance criteria: The solution exhibits absorption maxima at 230, 271, and 315 nm.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**
  Diluent: Methanol and ▲0.1 N hydrochloric acid VS▲ (RB 1-Mar-2019) (75:25)
  Mobile phase: Dissolve 2.5 g of sodium 1-decanesulfonate in 650 mL of water, and add 1.0 mL of perchloric acid and 350 mL of acetonitrile. If necessary, adjust with an additional 0.5 mL of perchloric acid to a pH of about 1.8.
  System suitability solution: 0.2 mg/mL of USP Donepezil Hydrochloride RS and 0.008 mg/mL of USP Donepezil Related Compound A RS. [NOTE—Dissolve in 40% of the flask volume of methanol, swirl, and dilute with water to volume.]
  Standard solution: 0.4 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*. [NOTE—Dissolve in 60% of the flask volume of *Diluent*, swirl, and dilute with *Diluent* to volume.]
  Sample solution: Nominally 0.4 mg/mL of donepezil hydrochloride prepared as follows. Dissolve a suitable number of Tablets in 75% of the flask volume of *Diluent*, and sonicate in an ultrasonic bath for 20 min. Swirl the mixture for 30 s, allow to cool to room temperature, and dilute with *Diluent* to volume. [NOTE—If necessary, add a magnetic stirring bar to the flask, and mix for 10 min on the magnetic stirrer, to aid in dissolution.] Allow a few min for the solids to settle. Pass through a suitable filter, discarding the first 2–3 mL of the filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 271 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Column temperature: 35°
  Flow rate: 1.4 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—The relative retention times for donepezil related compound A and donepezil are about 0.92 and 1.0, respectively.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 1.5 between donepezil related compound A and donepezil, *System suitability solution*
Tailing factor: NMT 1.5 for the donepezil peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of donepezil hydrochloride from the *Sample solution*
r_S = peak response of donepezil hydrochloride from the *Standard solution*
C_S = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of donepezil hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: ▲0.1 N hydrochloric acid VS;▲ (RB 1-Mar-2019) 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Analytical procedure: Determine the amount of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) dissolved, by using one of the following methods.
Chromatographic method
Diluent: Methanol and ▲0.1 N hydrochloric acid VS▲ (RB 1-Mar-2019) (75:25)
Mobile phase: Acetonitrile, water, and perchloric acid (35: 65: 0.1)
Standard stock solution A: 1.1 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*
Standard stock solution B: 0.11 mg/mL of USP Donepezil Hydrochloride RS from *Standard stock solution A* in *Medium*
Standard solution: (*L*/1000) mg/mL of USP Donepezil Hydrochloride RS from *Standard stock solution B* in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first few mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 271 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Column efficiency: NLT 5000 theoretical plates
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Spectrometric method
Standard stock solution: 0.11 mg/mL of USP Donepezil Hydrochloride RS in water
Standard solution: (*L*/900) mg/mL of USP Donepezil Hydrochloride RS from the *Standard stock solution* in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 230 nm
Blank: *Medium*
Calculate the percentage of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of donepezil hydrochloride is dissolved.
For Tablets which contain 23 mg of donepezil hydrochloride
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: pH 6.8 phosphate buffer; 900 mL
Apparatus 2: 50 rpm
Times: 1, 3, and 8 h
Buffer: 5.0 g/L of monobasic ammonium phosphate in water adjusted with phosphoric acid to a pH of 2.3
Mobile phase: Acetonitrile and *Buffer* (25:75)
Standard stock solution: 0.26 mg/mL of USP Donepezil Hydrochloride RS prepared as follows. Transfer a suitable quantity of USP Donepezil Hydrochloride RS to an appropriate volumetric flask. Add 70% of the flask volume of *Medium*. Sonicate to dissolve and dilute with *Medium* to volume.
Standard solution: (*L*/900) mg/mL of USP Donepezil Hydrochloride RS from *Standard stock solution* in *Medium*, where *L* is the label claim in mg/Tablet. Pass the solution through a suitable filter, discarding the first 3 mL of the filtrate.
Sample solution: Pass a portion of the solution under test through a suitable filter, discarding the first 3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35°

www.webofpharma.com

Flow rate: 1.5 mL/min
Injection volume: 50 µL
Run time: NLT 1.7 times the retention time of donepezil
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of donepezil from the *Sample solution*
r_S = peak response of donepezil from the *Standard solution*
C_S = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of donepezil hydrochloride in the portion of the sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	NMT 20
2	3	35–60
3	8	NLT 80

The percentages of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: pH 6.8 phosphate buffer; 900 mL
Apparatus 2: 50 rpm
Times: 1, 3, and 10 h
Standard stock solution: 0.25 mg/mL of USP Donepezil Hydrochloride RS prepared as follows. Transfer a suitable quantity of USP Donepezil Hydrochloride RS to an appropriate volumetric flask. Add 70% of the flask volume of water. Sonicate to dissolve and allow to cool to room temperature. Dilute with water to volume.
Standard solution: (*L*/900) mg/mL of USP Donepezil Hydrochloride RS from *Standard stock solution* in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 315 nm
Blank: *Medium*
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result}_i = (A_U/A_S) \times C_S$$

A_U = absorbance of donepezil from the *Sample solution*
A_S = absorbance of donepezil from the *Standard solution*
C_S = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of donepezil hydrochloride in the portion of the sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	10–30
2	3	33–53
3	10	NLT 80

The percentages of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.05 M sodium phosphate buffer, pH 6.8 [0.1 N hydrochloric acid VS and 76 g/L of tribasic sodium phosphate (25:75) adjusted with 2 N hydrochloric acid TS or 2 N sodium hydroxide TS to a pH of 6.8]; 900 mL, degassed
Apparatus 2: 50 rpm, with sinkers; see *Dissolution* ⟨711⟩, *Figure 2a*.
Times: 1, 3, and 8 h
Buffer: 1.36 g/L of monobasic potassium phosphate prepared as follows. To each 1 L of 1.36 g/L of

www.webofpharma.com

monobasic potassium phosphate in water, add 3 mL of triethylamine and adjust with phosphoric acid to a pH of 2.8.
Mobile phase: Methanol and *Buffer* (47:53)
Diluent: Methanol and water (50:50)
Standard stock solution: 0.53 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*
Standard solution: 0.027 mg/mL of USP Donepezil Hydrochloride RS from *Standard stock solution* in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Replace the portion removed with the same volume of *Medium*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 268 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.3 mL/min
Injection volume: 20 µL
Run time: NLT 1.7 times the retention time of donepezil
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of donepezil from the *Sample solution*
r_S = peak response of donepezil from the *Standard solution*
C_S = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of donepezil hydrochloride in the portion of the sample withdrawn at time point i (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	10–30
2	3	40–60
3	8	NLT 80

The percentages of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*. ▲ (RB 1-Mar-2019)

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES, PROCEDURE 1**
[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* is recommended if any of the impurities included in ▲*Table 6*▲ (RB 1-Mar-2019) are potential degradation products.]
Diluent, Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.0008 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for donepezil related compound A and donepezil are about 0.92 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between donepezil related compound A and donepezil, *System suitability solution*
Relative standard deviation: NMT 8.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Identify the impurities using the relative retention times given in *Table 3*.]
Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of donepezil hydrochloride from the *Standard solution*
C_S = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of donepezil hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see ▲*Table 4*)▲ (RB 1-Mar-2019)

Acceptance criteria: See ▲*Table 4*.

Table 4▲ (RB 1-Mar-2019)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desbenzyl donepezil[a]	0.33	1.0	0.5
Donepezil open ring[b]	0.70	0.6	0.5
Donepezil hydrochloride	1.0	—	—
Donepezil *N*-oxide[c]	1.2	1.0	0.5
Any individual unspecified degradation product	—	—	0.2

[a] 5,6-Dimethoxy-2-(piperidin-4-ylmethyl)indan-1-one.
[b] 2-(3-(1-Benzylpiperidin-4-yl)-2-oxopropyl)-4,5-dimethoxybenzoic acid.
[c] 2-[(1-Benzylpiperidin-4-yl)methyl]-5,6-dimethoxyindan-1-one *N*-oxide.

USP Monographs

www.webofpharma.com

Change to read:

- **ORGANIC IMPURITIES, PROCEDURE 2**

Diluent: Acetonitrile and water (25:75)
Solution A: Add 1 mL of phosphoric acid in 1 L of water. Adjust with triethylamine to a pH of 6.5. Pass through a filter of 0.45-µm or finer pore size.
Solution B: Acetonitrile
Mobile phase: See ▲*Table 5*.

Table 5▲ (RB 1-Mar-2019)

Time (min)	Solution A (%)	Solution B (%)
0	75	25
10	40	60
40	40	60
41	75	25
50	75	25

Standard solution: 0.01 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*. Sonication may be used to aid the dissolution.
Sample solution: Nominally 1.0 mg/mL of donepezil hydrochloride in *Diluent*. Sonication may be used to aid the dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 286 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%, for five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified impurity or any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of donepezil hydrochloride from the *Standard solution*
C_S = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of donepezil hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding impurity peak from ▲*Table 6*▲ (RB 1-Mar-2019)

Acceptance criteria: See ▲*Table 6*.

Table 6▲ (RB 1-Mar-2019)

Name	Relative Retention Time[a]	Relative Response Factor	Acceptance Criteria, NMT (%)
Desbenzyl donepezil[b]	0.23	1.5	0.15
Donepezil pyridine analog[c]	0.49	1.9	0.15
Donepezil quaternary salt[d]	0.68	0.74	0.15
Donepezil hydrochloride	1.0	1.0	—
Donepezil indene analog[e]	1.7	2.2	0.15
Deoxydonepezil[f]	2.1	1.3	0.15
Any individual degradation product	—	1.0	0.1
Total degradation products	—	—	1.0

[a] Relative retention times are based on 1-mL gradient delay volume.
[b] 5,6-Dimethoxy-2-(piperidin-4-ylmethyl)indan-1-one.
[c] 5,6-Dimethoxy-2-(pyridin-4-ylmethyl)indan-1-one; also known as DPMI.
[d] 1,1-Dibenzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidinium; also known as donepezil benzyl.
[e] 1-Benzyl-4-[(5,6-dimethoxyinden-2-yl)methyl]piperidine; also known as dehydrodeoxy donepezil.
[f] 1-Benzyl-4-[(5,6-dimethoxyindan-2-yl)methyl]piperidine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies. If a test for *Dissolution* other than *Test 1* is used, the labeling states the test with which the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Donepezil Hydrochloride RS
USP Donepezil Related Compound A RS
(*E*)-2-[(1-Benzylpiperidin-4-yl)methylene]-5,6-dimethoxyindan-1-one.
$C_{24}H_{27}NO_3$ 377.48

Donepezil Hydrochloride Orally Disintegrating Tablets

DEFINITION
Donepezil Hydrochloride Orally Disintegrating Tablets contains NLT 93.0% and NMT 107.0% of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: Crush a suitable number of Tablets, and transfer an amount of powder, equivalent to 10 mg of donepezil hydrochloride, to a 100-mL volumetric flask. Add 80 mL of 0.1 N hydrochloric acid, and sonicate for 5 min. Cool to room temperature, and dilute with 0.1 N hydrochloric acid to volume. Transfer a portion to a centrifuge tube, and centrifuge for 15 min. Transfer 5 mL of the clear supernatant to a 25-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.
Analysis
Wavelength range: 220–360 nm
Acceptance criteria: 230, 271, and 315 nm

www.webofpharma.com

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Diluent: Methanol and 0.1 N hydrochloric acid (3:1)

Mobile phase: Dissolve 2.5 g of sodium decanesulfonate in 650 mL of water, and add 1.0 mL of perchloric acid and 350 mL of acetonitrile. If necessary, adjust with an additional 0.5 mL of perchloric acid to a pH of about 1.8.

System suitability solution: 0.4 mg/mL of USP Donepezil Hydrochloride RS and 0.016 mg/mL of USP Donepezil Related Compound A RS, prepared by dissolving in 40% of the flask volume of methanol and diluting with water to volume.

Standard solution: 0.4 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*

Sample solution: 0.4 mg/mL of donepezil hydrochloride in *Diluent*, prepared by transferring a suitable number of Tablets to an appropriate volumetric flask containing 10 mL of 0.1 N hydrochloric acid. Shake to disintegrate the Tablets. Add 60% of the flask volume of *Diluent*, sonicate for 10 min, allow to cool to room temperature, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 271 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1.4 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times of donepezil related compound A and donepezil are about 0.92 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between donepezil related compound A and donepezil, *System suitability solution*

Tailing factor: NMT 1.5 for donepezil, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{24}H_{29}NO_3 \cdot HCl$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of donepezil hydrochloride from the *Sample solution*

r_S = peak response of donepezil hydrochloride from the *Standard solution*

C_S = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of donepezil hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩

Time: NMT 60 s

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Diluent: Methanol and 0.1 N hydrochloric acid (3:1)

Mobile phase: Acetonitrile, perchloric acid, and water (350:1:650)

Standard stock solution: 1.1 mg/mL of USP Donepezil Hydrochloride RS in *Diluent*. Dilute with *Medium* to obtain a concentration of 0.11 mg/mL.

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a concentration of L/1000 mg/mL, where L is the Tablet label claim in mg.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 271 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 5000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{24}H_{29}NO_3 \cdot HCl$ dissolved.

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of $C_{24}H_{29}NO_3 \cdot HCl$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.8 µg/mL of USP Donepezil Hydrochloride RS in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times of donepezil related compound A and donepezil are about 0.92 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between donepezil related compound A and donepezil, *System suitability solution*

Relative standard deviation: NMT 8.0%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*.

[NOTE—Identify the impurities, using the relative retention times given in *Impurity Table 1*.]

Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

www.webofpharma.com

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of donepezil hydrochloride from the *Standard solution*
C_S = concentration of USP Donepezil Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of donepezil hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor of each related compound, as listed in *Impurity Table 1*

Acceptance criteria
Individual impurities: See *Impurity Table 1*.

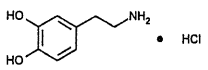
Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desbenzyl donepezil[a]	0.35	1.0	0.5
Donepezil open ring[b]	0.70	0.6	0.5
Donepezil hydrochloride	1.0	—	—
Donepezil *N*-oxide[c]	1.2	1.0	0.5
Individual unspecified degradation impurity	—	—	0.2
Total impurities	—	—	1.0

[a] 5,6-Dimethoxy-2-(piperidin-4-ylmethyl)indan-1-one.
[b] 2-(3-(1-Benzylpiperidin-4-yl)-2-oxopropyl)-4,5-dimethoxybenzoic acid.
[c] 2-[(1-Benzylpiperidin-4-yl)methyl]-5,6-dimethoxyindan-1-one *N*-oxide.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Donepezil Hydrochloride RS
  USP Donepezil Related Compound A RS
  (*E*)-2-[(1-Benzylpiperidin-4-yl)methylene]-5,6-dimethoxyindan-1-one.
  $C_{24}H_{27}NO_3$ 377.48

Dopamine Hydrochloride



$C_8H_{11}NO_2 \cdot HCl$ 189.64
1,2-Benzenediol, 4-(2-aminoethyl)-, hydrochloride.
4-(2-Aminoethyl)pyrocatechol hydrochloride [62-31-7].

» Dopamine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_8H_{11}NO_2 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers. Store at room temperature.
USP Reference standards ⟨11⟩—
USP Dopamine Hydrochloride RS
Clarity and color of solution—A solution of 400 mg in 10 mL of sodium bisulfite solution (1 in 1000) is clear and colorless or practically colorless.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 40 µg per mL.
Medium: sodium bisulfite in water (1 in 1000).
C: It responds to the tests for *Chloride* ⟨191⟩.
pH ⟨791⟩**:** between 3.0 and 5.5, in a solution (1 in 25).
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩**:** not more than 0.1%.
Sulfate ⟨221⟩—Dissolve 500 mg in 40 mL of water: any turbidity produced is not more than that produced in a solution containing 0.10 mL of 0.020 N sulfuric acid.
Readily carbonizable substances ⟨271⟩—Dissolve 100 mg in 5 mL of sulfuric acid : the solution has no more color than *Matching Fluid A*.

Chromatographic purity—
Standard solution and *Standard dilutions*—Prepare a solution of USP Dopamine Hydrochloride RS in methanol to contain 30 mg per mL *(Standard solution)*. Prepare a series of dilutions of the *Standard solution* in methanol to contain 0.6 mg, 0.3 mg, and 0.15 mg per mL *(Standard dilutions)*, corresponding to 2.0%, 1.0%, and 0.5% of impurities, respectively.
Test solution—Transfer 150 mg of Dopamine Hydrochloride to a 5-mL volumetric flask, dilute with methanol to volume, and mix.
Procedure—In a suitable chromatographic chamber arranged for thin-layer chromatography (see *Chromatography* ⟨621⟩), place a solvent system consisting of 13 volumes of chloroform, 9 volumes of methanol, and 4 volumes of dilute glacial acetic acid (3 in 10). Line the chamber with filter paper, and allow to equilibrate. Apply 10-µL portions of the *Standard solution*, the *Standard dilutions*, and the *Test solution* to a 20- × 20-cm chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram until the solvent front has moved about 15 cm. Remove the plate from the developing chamber, allow to dry at room temperature for several minutes, and spray evenly with a freshly prepared mixture containing equal volumes of ferric chloride solution (1 in 10) and potassium ferricyanide solution (1 in 20). [Dopamine and its related impurities appear as blue spots under visible light.] The *Test solution* exhibits its principal spot at an R_F value corresponding to that of the *Standard solution* and not more than three secondary spots. Estimate the concentration of any secondary spots exhibited by the *Test solution* by comparison with the *Standard dilutions:* the sum of the impurities is not greater than 1.0%.
Assay—Dissolve about 75 mg of Dopamine Hydrochloride, accurately weighed, in 5 mL of formic acid, add 25 mL of acetic anhydride, and mix. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 18.96 mg of $C_8H_{11}NO_2 \cdot HCl$.

Stimuli Article

www.webofpharma.com

Dopamine Hydrochloride Injection

» Dopamine Hydrochloride Injection is a sterile solution of Dopamine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_8H_{11}NO_2 \cdot HCl$. It may contain a suitable antioxidant.
[NOTE—Do not use the Injection if it is darker than slightly yellow or discolored in any other way.]

Packaging and storage—Preserve in single-dose containers of Type I glass.
Labeling—Label it to indicate that the Injection is to be diluted with a suitable parenteral vehicle prior to intravenous infusion.
USP Reference standards ⟨11⟩—
USP Dopamine Hydrochloride RS
Identification—Transfer a volume of Injection to a suitable container, and dilute if necessary, with dilute methanol (1 in 5) to obtain a test solution having a known concentration of 1.6 mg per mL. Prepare a Standard solution of USP Dopamine Hydrochloride RS in dilute methanol (1 in 5) having the same concentration as the test solution. The test solution so obtained responds to the *Thin-layer Chromatographic Identification Test* ⟨201⟩, a mixture of *n*-butyl alcohol, glacial acetic acid, and water (4:1:1) being used as the developing solvent and 5 µL each of the test solution and Standard solution being applied to the thin-layer chromatographic plate.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 16.67 USP Endotoxin Units per mg of dopamine hydrochloride.
pH ⟨791⟩: between 2.5 and 5.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of 0.005 M sodium 1-octanesulfonate in 1% glacial acetic acid and acetonitrile (87:13). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve a suitable quantity of USP Dopamine Hydrochloride RS, accurately weighed, in *Mobile phase* to obtain a solution having a concentration of about 1.6 mg per mL. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.16 mg of dopamine hydrochloride per mL.
System suitability solution—Prepare a solution of benzoic acid in methanol containing about 20 mg per mL. Dilute 1 volume of this solution with 3 volumes of the *Mobile phase* to obtain a solution having a final concentration of about 5 mg per mL. Transfer 10.0 mL of this solution and 10.0 mL of a Standard solution containing 1.6 mg of USP Dopamine Hydrochloride RS per mL to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 16 mg of dopamine hydrochloride, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column packed with packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed under *Procedure:* the resolution, *R*, between benzoic acid and dopamine hydrochloride is not less than 4.0. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the relative standard deviation for replicate injections is not more than 3.0%.
Procedure—Separately inject equal volumes (about 40 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_8H_{11}NO_2 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(100C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dopamine Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and r_U and r_S are the responses of dopamine hydrochloride obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dopamine Hydrochloride and Dextrose Injection

» Dopamine Hydrochloride and Dextrose Injection is a sterile solution of Dopamine Hydrochloride and Dextrose in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of dopamine hydrochloride ($C_8H_{11}NO_2 \cdot HCl$) and of dextrose ($C_6H_{12}O_6 \cdot H_2O$).
[NOTE—Do not use the Injection if it is darker than slightly yellow or discolored in any other way.]

Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.
USP Reference standards ⟨11⟩—
USP Dopamine Hydrochloride RS

Identification—
A: Add a few drops of a solution (1 in 20) to 5 mL of hot alkaline cupric tartrate TS. A copious red precipitate of cuprous oxide is formed.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for dopamine hydrochloride*.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 16.67 USP Endotoxin Units per mg of dopamine hydrochloride.
pH ⟨791⟩: between 2.5 and 4.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

www.webofpharma.com

Limit of 5-hydroxymethylfurfural and related substances—

Diluting solution—Prepare a solution containing approximately 0.022 N sodium hydroxide in water.

Cation-exchange column—Proceed as directed under *Column Partition Chromatography* (see *Chromatography* ⟨621⟩), using a chromatographic tube capable of providing a 0.8-cm × 4-cm bed volume (or about 2 mL) of 100- to 200-mesh, strongly acidic, styrene-divinylbenzene cation-exchange resin. Condition the column by washing with about 30 mL of water, discarding the eluate.

Procedure—Pass a volume of Injection containing about 100 mg of hydrous dextrose through the resin bed in the *Cation-exchange column*, allowing the specimen to flow down the wall of the column so as not to disturb the resin bed, and collect the eluate in a 50-mL volumetric flask. Wash the column with 25 mL of water, and collect the eluate in the same 50-mL volumetric flask. Dilute the eluate with *Diluting solution* to volume, and mix to obtain the test solution. In a similar manner, prepare a blank by passing 27 mL of water through a freshly conditioned *Cation-exchange column*, collecting the eluate in a 50-mL volumetric flask, diluting with *Diluting solution* to volume, and mixing. Determine the absorbance of the test solution against the blank in a 1-cm cell at 284 nm, with a suitable spectrophotometer: the absorbance is not more than 0.25.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

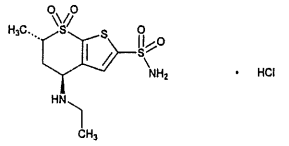
Assay for dopamine hydrochloride—Proceed with Injection as directed in the *Assay* under *Dopamine Hydrochloride Injection*.

Assay for dextrose—Transfer an accurately measured volume of Injection, containing 2 to 5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix. Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩). Calculate the percentage (g per 100 mL) of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively; *A* is 100 mm divided by the length of the polarimeter tube, in mm; and *R* is the observed rotation, in degrees.

Dorzolamide Hydrochloride



$C_{10}H_{16}N_2O_4S_3 \cdot HCl$ 360.90

4*H*-Thieno[2,3-*b*]thiopyran-2-sulfonamide, 4-(ethylamino)-5,6-dihydro-6-methyl-, 7,7-dioxide, monohydrochloride, (4*S*-*trans*)-;

(4*S*,6*S*)-4-(Ethylamino)-5,6-dihydro-6-methyl-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide, monohydrochloride [130693-82-2].

DEFINITION

Dorzolamide Hydrochloride contains NLT 99.0% and NMT 101.0% of dorzolamide hydrochloride ($C_{10}H_{16}N_2O_4S_3 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

ASSAY

- **PROCEDURE**

Buffer: 3.7 g/L of monobasic potassium phosphate in water
Solution A: Acetonitrile and *Buffer* (6.5: 94)
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
30	50	50
37	100	0
44	100	0

Standard solution: 0.6 mg/mL of USP Dorzolamide Hydrochloride RS in *Solution A*
Sample solution: 0.6 mg/mL of Dorzolamide Hydrochloride in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 6500 theoretical plates
Tailing factor: 0.6–1.2
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dorzolamide hydrochloride ($C_{10}H_{16}N_2O_4S_3 \cdot HCl$) in the portion of Dorzolamide Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the *Sample solution*
r_S = peak area of the *Standard solution*
C_S = concentration of USP Dorzolamide Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Dorzolamide Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 99.0%–101.0% on the anhydrous basis

USP Monographs

www.webofpharma.com

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%, an ignition temperature of 600° being used

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual impurity in the portion of Dorzolamide Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*

r_T = sum of all the peak areas from the *Sample solution*

Acceptance criteria

Any individual impurity: NMT 0.1%

Total impurities: NMT 0.5%

• **LIMIT OF DORZOLAMIDE HYDROCHLORIDE RELATED COMPOUND A**

Mobile phase: *tert*-Butyl methyl ether, chromatographic *n*-heptane, acetonitrile, and water (63: 35: 2: 0.2)

System suitability solution: Transfer 18 mg of USP Dorzolamide Hydrochloride RS and 2 mg of USP Dorzolamide Hydrochloride Related Compound A RS, each, to a 15-mL centrifuge tube. Dissolve in 4 mL of 0.5 N ammonium hydroxide, and add 4 mL of ethyl acetate. Separate the ethyl acetate layer, and transfer to a 15-mL centrifuge tube. Add 4 mL of ethyl acetate to the aqueous layer, and mix. Separate the ethyl acetate layer, and combine it with the first extract. Evaporate the combined organic layers to dryness on a water bath maintained at 50° under a stream of nitrogen. Dissolve the residue in 3 mL of acetonitrile, add 3 drops of (*S*)-(−)-α-methylbenzyl isocyanate (discard the reagent if it is colored), and allow to react for 5 min on a water bath maintained at 50°. Evaporate the mixture to dryness on a water bath maintained at 50° under a stream of nitrogen. Dissolve the residue in 10 mL of a mixture of *tert*-butyl methyl ether, glacial acetic acid, and acetonitrile (87:10:3).

Sample solution: Transfer 20 mg of Dorzolamide Hydrochloride to a 15-mL centrifuge tube, dissolve in 4 mL of 0.5 N ammonium hydroxide, and add 4 mL of ethyl acetate. Separate the ethyl acetate layer, and transfer to a 15-mL centrifuge tube. Add 4 mL of ethyl acetate to the aqueous layer, and mix. Separate the ethyl acetate layer, and combine it with the first extract. Evaporate the combined organic layers to dryness on a water bath maintained at 50° under a stream of nitrogen. Dissolve the residue in 3 mL of acetonitrile, add 3 drops of (*S*)-(−)-α-methylbenzyl isocyanate (discard the reagent if it is colored), and allow to react for 5 min on a water bath maintained at 50°. Evaporate the mixture to dryness on a water bath maintained at 50° under a stream of nitrogen. Dissolve the residue in 10 mL of a mixture of *tert*-butyl methyl ether, glacial acetic acid, and acetonitrile (87:10:3).

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L3

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for dorzolamide and dorzolamide hydrochloride related compound A are about 1.0 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 4.0 between dorzolamide and dorzolamide hydrochloride related compound A

Column efficiency: NLT 4000 theoretical plates for the dorzolamide hydrochloride peak

Tailing factor: NMT 1.4

Analysis

Samples: *System suitability solution* and *Sample solution*

Calculate the percentage of dorzolamide hydrochloride related compound A in the portion of Dorzolamide Hydrochloride taken:

$$\text{Result} = [r_i/(r_i + r_S)] \times 100$$

r_i = peak area of dorzolamide hydrochloride related compound A from the *Sample solution*

r_S = peak area of dorzolamide hydrochloride from the *Sample solution*

Acceptance criteria: NMT 0.5%

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩

Sample: 0.4 g

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light, and store at 15°–30°.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Dorzolamide Hydrochloride RS

USP Dorzolamide Hydrochloride Related Compound A RS

(4*R*,6*R*)-4-(Ethylamino)-5,6-dihydro-6-methyl-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide-7,7- dioxide, monohydrochloride.

$C_{10}H_{16}N_2O_4S_3 \cdot HCl$ 360.91

Dorzolamide Hydrochloride Ophthalmic Solution

DEFINITION

Dorzolamide Hydrochloride Ophthalmic Solution is a sterile, isotonic, buffered, slightly viscous, aqueous solution of Dorzolamide Hydrochloride. It contains an amount of Dorzolamide Hydrochloride ($C_{10}H_{16}N_2O_4S_3 \cdot HCl$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of dorzolamide ($C_{10}H_{16}N_2O_4S_3$). It may contain a suitable preservative.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 5.0 mg/mL of USP Dorzolamide Hydrochloride RS in methanol

Sample solution: Equivalent to 5.0 mg/mL of dorzolamide from Ophthalmic Solution in methanol

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture or equivalent

Application volume: 20 µL

Developing solvent system: Methylene chloride, methanol, and ammonium hydroxide (80:20:1)

Analysis: Spot the *Standard solution* and *Sample solution* approximately 2 cm from the bottom of the plate. Evaporate the solvent using a current of air. Saturate the

www.webofpharma.com

developing tank with the *Developing solvent system*, and equilibrate for approximately 1 h prior to use. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing tank, and allow the plate to air-dry in a fume hood. Examine the plate under short-wavelength UV light at 254 nm, or expose to iodine vapors.

Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Fill a 1-L volumetric flask approximately two-thirds full of water. Add 2.0 mL of phosphoric acid, and dilute with water to 900 mL. Adjust with triethylamine to a pH of 3.0, and dilute with water to volume.

Mobile phase: Acetonitrile and *Buffer* (5:95)

Standard solution: 0.11 mg/mL of USP Dorzolamide Hydrochloride RS and 0.5 µg/mL each of USP Dorzolamide Related Compound B RS and USP Dorzolamide Related Compound D RS in *Mobile phase*

Sample solution: Equivalent to 0.1 mg/mL of dorzolamide from Ophthalmic Solution in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 253 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between dorzolamide and dorzolamide related compound D; and NLT 3.0 between dorzolamide and dorzolamide related compound B

Tailing factor: NMT 1.8 for the dorzolamide peak

Relative standard deviation: NMT 2.0% for the dorzolamide peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dorzolamide ($C_{10}H_{16}N_2O_4S_3$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of dorzolamide from the *Sample solution*

r_S = peak response of dorzolamide from the *Standard solution*

C_S = concentration of USP Dorzolamide Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dorzolamide in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of dorzolamide, 324.44

M_{r2} = molecular weight of dorzolamide hydrochloride, 360.90

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Standard solution, Sample solution, and **System suitability:** Proceed as directed in the *Assay*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Proceed as directed in the *Assay*. In addition the run time is at least 1.4 times the retention time of dorzolamide.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dorzolamide related compound D in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of dorzolamide related compound D from the *Sample solution*

r_S = peak area of dorzolamide related compound D from the *Standard solution*

C_S = concentration of USP Dorzolamide Related Compound D RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dorzolamide in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of dorzolamide, 324.44

M_{r2} = molecular weight of dorzolamide related compound D, 332.85

Calculate the percentage of dorzolamide related compound B in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of dorzolamide related compound B from the *Sample solution*

r_S = peak area of dorzolamide related compound B from the *Standard solution*

C_S = concentration of USP Dorzolamide Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dorzolamide in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of dorzolamide, 324.44

M_{r2} = molecular weight of dorzolamide related compound B, 360.90

Calculate the percentage of each individual unspecified impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of dorzolamide from the *Standard solution*

C_S = concentration of USP Dorzolamide Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dorzolamide in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of dorzolamide, 324.44

M_{r2} = molecular weight of dorzolamide hydrochloride, 360.90

Acceptance criteria

Individual impurities: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dorzolamide related compound D[a]	0.87	0.5
Dorzolamide	1.00	—

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dorzolamide related compound B[b]	1.14	2.0
Total impurities[c]	—	3.0

[a] (4*S*,6*S*)-4-Amino-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide hydrochloride.
[b] (4*RS*,6*SR*)-4-(Ethylamino)-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide hydrochloride.
[c] The sum of dorzolamide related compound D, dorzolamide related compound B, and all unspecified impurities.

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **pH** ⟨791⟩: 5.4–5.9

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers protected from light, at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dorzolamide Hydrochloride RS
  USP Dorzolamide Related Compound B RS
  (4*R*,6*S*)-4-(Ethylamino)-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide hydrochloride.
  $C_{10}H_{16}N_2O_4S_3 \cdot HCl$ 360.90
  USP Dorzolamide Related Compound D RS
  (4*S*,6*S*)-4-Amino-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide hydrochloride.
  $C_8H_{12}N_2O_4S_3 \cdot HCl$ 332.85

Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution

DEFINITION
Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution is a sterile, isotonic, buffered, slightly viscous, aqueous solution of Dorzolamide Hydrochloride and Timolol Maleate. It contains an amount of dorzolamide hydrochloride ($C_{10}H_{16}N_2O_4S_3 \cdot HCl$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of dorzolamide ($C_{10}H_{16}N_2O_4S_3$), and also contains an amount of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of timolol ($C_{13}H_{24}N_4O_3S$). It may contain a suitable preservative.

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
  Standard solution A: 1.5 mg/mL of USP Timolol Maleate RS in *methanol*
  Standard solution B: 5.0 mg/mL of USP Dorzolamide Hydrochloride RS in *methanol*
  Sample solution: Nominally equivalent to 5.0 mg/mL of dorzolamide from Ophthalmic Solution in *methanol*
  Chromatographic system
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture or equivalent
  Application volume: 20 µL
  Developing solvent system: *Methylene chloride*, *methanol*, and *ammonium hydroxide* (80:20:1)
  Analysis: Spot *Standard solution A*, *Standard solution B*, and the *Sample solution* approximately 2 cm from the bottom of the plate. Evaporate the solvent using a current of air. Saturate the developing tank with the *Developing solvent system*, and equilibrate for approximately 1 h prior to use. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing tank, and allow the plate to air-dry in a fume hood. Examine the plate under short-wavelength UV light at 254 nm, or expose to iodine vapors.
  Acceptance criteria: The R_F values of the principal spots from the *Sample solution* correspond to the principal spots obtained from *Standard solution A* and *Standard solution B*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Procedure 1: Dorzolamide*.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Procedure 2: Timolol*.

ASSAY
- **PROCEDURE 1: DORZOLAMIDE**
  Solution A: *Acetonitrile*
  Solution B: 0.2% (v/v) *phosphoric acid* in *water*
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	5	95
15.0	5	95
15.1	95	5
20.0	95	5
20.1	5	95
30.0	5	95

Diluent: *Acetonitrile* and *Solution B* (5:95)
Standard solution: 0.11 mg/mL of USP Dorzolamide Hydrochloride RS and 0.5 µg/mL each of USP Dorzolamide Related Compound B RS and USP Dorzolamide Related Compound D RS in *Diluent*
Sample solution: Nominally equivalent to 0.1 mg/mL of dorzolamide from Ophthalmic Solution in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 253 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between dorzolamide and dorzolamide related compound D; NLT 3.0 between dorzolamide and dorzolamide related compound B
Relative standard deviation: NMT 2.0% for the dorzolamide peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dorzolamide ($C_{10}H_{16}N_2O_4S_3$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

www.webofpharma.com

r_U = peak response of dorzolamide from the *Sample solution*
r_S = peak response of dorzolamide from the *Standard solution*
C_S = concentration of USP Dorzolamide Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dorzolamide in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dorzolamide, 324.44
M_{r2} = molecular weight of dorzolamide hydrochloride, 360.90

Acceptance criteria: 90.0%–110.0%

Change to read:

- **PROCEDURE 2: TIMOLOL**

Buffer: Transfer 22 g of monobasic sodium phosphate sodium phosphate into a 2000-mL volumetric flask. Dissolve in 1995 mL of *water*, and adjust with *phosphoric acid* to a pH of 2.8. Dilute with *water* to volume.
Mobile phase: *Methanol* and *Buffer* (40:60)
System suitability solution: Transfer 88 mg of USP Timolol Maleate RS into a 50-mL volumetric flask. Transfer 8 mL of 0.1 M sodium hydroxide into the flask, mix, and heat at 70° for 15 h. Dilute with *Mobile phase* to volume, and mix well. Transfer 5 mL of this solution into a 25-mL volumetric flask, and add 28 mg of USP Dorzolamide Hydrochloride RS into the same flask. Dilute with *Mobile phase* to volume. [NOTE—The preparation generates timolol impurity G and timolol ▲related compound▲ 1S (USP41) B.]
Standard solution: 0.35 mg/mL of USP Timolol Maleate RS in *Mobile phase*
Sample solution: Nominally equivalent to 0.25 mg/mL of timolol from Ophthalmic Solution in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 295 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between timolol impurity G and timolol ▲related compound▲ 1S (USP41) B
Relative standard deviation: NMT 2.5% for timolol for five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of timolol ($C_{13}H_{24}N_4O_3S$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Timolol Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of timolol in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of timolol, 316.42
M_{r2} = molecular weight of timolol maleate, 432.49

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES: DORZOLAMIDE HYDROCHLORIDE**

Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay* for *Procedure 1: Dorzolamide*.
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between dorzolamide and dorzolamide related compound D; NLT 3.0 between dorzolamide and dorzolamide related compound B
Relative standard deviation: NMT 2.0% for dorzolamide
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of dorzolamide related compound D in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of dorzolamide related compound D from the *Sample solution*
r_S = peak area of dorzolamide related compound D from the *Standard solution*
C_S = concentration of ▲USP Dorzolamide Related Compound D RS▲ (ERR 1-Oct-2018) in the *Standard solution* (mg/mL)
C_U = nominal concentration of dorzolamide in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dorzolamide, 324.44
M_{r2} = molecular weight of dorzolamide related compound D, 332.85

Calculate the percentage of dorzolamide related compound B in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of dorzolamide related compound B from the *Sample solution*
r_S = peak area of dorzolamide related compound B from the *Standard solution*
C_S = concentration of ▲USP Dorzolamide Related Compound B RS▲ (ERR 1-Oct-2018) in the *Standard solution* (mg/mL)
C_U = nominal concentration of dorzolamide in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dorzolamide, 324.44
M_{r2} = molecular weight of dorzolamide related compound B, 360.90

Calculate the percentage of any individual unspecified impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of any individual unspecified impurity from the *Sample solution*
r_T = sum of all the peak areas from the *Sample solution*

Acceptance criteria: See *Table 2*. ▲The reporting threshold is 0.10%.▲ 1S (USP41)

www.webofpharma.com

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Maleic acid	0.33	Disregard
Dorzolamide related compound D ▲1S (USP41)	0.83	0.5
Dorzolamide	1.00	—
Dorzolamide related compound B ▲1S (USP41)	1.17	2.0
Any individual unspecified impurity	—	0.5
Total impurities ▲[a] ▲1S (USP41)	—	3.0

[a] It is the sum of all impurities in Table 2 and dorzolamide maleic acid adducts in *Table 5*.

Change to read:

• **Organic Impurities: Timolol Maleate**

Mobile phase, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay* for *Procedure 2: Timolol.*

Standard stock solution: Use the *Standard solution* as prepared in the *Assay* for *Procedure 2: Timolol.*

Standard solution: 3.5 µg/mL of USP Timolol Maleate RS from the *Standard stock solution* in *Mobile phase*

Chromatographic system: Proceed as directed in the *Assay* for *Procedure 2: Timolol* except for the *Run time.*

Run time: NLT 2 times the retention time of timolol

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between timolol impurity G and timolol ▲related compound▲ 1S (USP41) B

Relative standard deviation: NMT 2.5% for timolol for five replicate injections

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of timolol from the *Standard solution*

C_S = concentration of USP Timolol Maleate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of timolol in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of timolol, 316.42

M_{r2} = molecular weight of timolol maleate, 432.49

Acceptance criteria: See *Table 3*. ▲The reporting threshold is 0.10%.▲ 1S (USP41)

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dorzolamide and maleic acid	0.49	Disregard
Timolol impurity G[a]	0.58	0.5
Timolol ▲related compound▲ 1S (USP41) B[b]	0.70	1.0
Timolol	1.00	—
Timolol ▲related compound▲ 1S (USP41) D[c]	1.51	0.5
Any individual unspecified impurity	—	0.6
Total impurities	—	2.0

[a] 4-Morpholino-1,2,5-thiadiazol-3-ol 1-oxide.

[b] 3-(*tert*-Butylamino)-2-(4-morpholino-1,2,5-thiadiazol-3-yloxy)propan-1-ol.

[c] 4-Morpholino-1,2,5-thiadiazol-3-ol.

Add the following:

▲• **Limit of Dorzolamide Maleic Acid Adducts**

Solution A, Solution B, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* for *Procedure 1: Dorzolamide.*

Mobile phase: See *Table 4*. Return to original conditions and re-equilibrate the system for 6 min.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	5	95
15.0	5	95
15.1	13	87
20.0	13	87
25.0	95	5
29.0	95	5

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dorzolamide maleic acid adduct in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of dorzolamide maleic acid adduct from the *Sample solution*

r_S = peak area of dorzolamide from the *Standard solution*

C_S = concentration of USP Dorzolamide Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dorzolamide in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of dorzolamide, 324.44

M_{r2} = molecular weight of dorzolamide hydrochloride, 360.90

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dorzolamide	1.00	—

USP Monographs

www.webofpharma.com

Table 5 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dorzolamide maleic acid adducts[a, b]	1.71	0.5
	1.78	0.5

[a] The chromatographic system resolves the two epimers from each other.
[b] *N*-Ethyl-*N*-[(4*S*,6*S*)-6-methyl-7,7-dioxido-2-sulfamoyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-4-yl]aspartic acid. ▲1S (USP41)

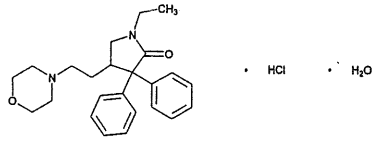
SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **pH** ⟨791⟩: 5.4–5.9 at 22°

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers protected from light, at controlled room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dorzolamide Hydrochloride RS
  USP Dorzolamide Related Compound B RS
  ▲(4*RS*,6*SR*)-4-(Ethylamino)-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide hydrochloride. ▲1S (USP41)
  $C_{10}H_{16}N_2O_4S_3 \cdot HCl$ 360.90
  USP Dorzolamide Related Compound D RS
  (4*S*,6*S*)-4-Amino-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide hydrochloride.
  $C_8H_{12}N_2O_4S_3 \cdot HCl$ 332.85
  USP Timolol Maleate RS

Doxapram Hydrochloride



$C_{24}H_{30}N_2O_2 \cdot HCl \cdot H_2O$ 432.99
$C_{24}H_{30}N_2O_2 \cdot HCl$ 414.97
2-Pyrrolidinone, 1-ethyl-4-[2-(4-morpholinyl)ethyl]-3,3-diphenyl-, monohydrochloride, monohydrate, (±)-;
(±)-1-Ethyl-4-(2-morpholinoethyl)-3,3-diphenyl-2-pyrrolidinone monohydrochloride monohydrate [7081-53-0].
Anhydrous [113-07-5].

DEFINITION
Doxapram Hydrochloride, dried at 105° for 2 h, contains NLT 98.0% and NMT 102.0% of doxapram hydrochloride ($C_{24}H_{30}N_2O_2 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*: Meets the requirements

ASSAY
- **PROCEDURE**

Solution A: To each L of water, add 0.1 mL of trifluoroacetic acid.
Solution B: To each L of acetonitrile, add 0.1 mL of trifluoroacetic acid.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20	50	50
25	50	50
25.1	90	10
30	90	10

Diluent: Acetonitrile and water (30:70)
Standard solution: 0.2 mg/mL of USP Doxapram Hydrochloride RS in *Diluent*
Sample solution: 0.2 mg/mL of Doxapram Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 5-cm; 2.5-µm packing L1
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of doxapram hydrochloride ($C_{24}H_{30}N_2O_2 \cdot HCl$) in the portion of Doxapram Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of doxapram from the *Sample solution*
r_S = peak response of doxapram from the *Standard solution*
C_S = concentration of USP Doxapram Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Doxapram Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%, previously dried at 105° for 2 h

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 2 mg/mL of USP Doxapram Hydrochloride RS and 0.04 mg/mL of USP Doxapram Related Compound B RS in *Diluent*

www.webofpharma.com

Standard solution: 0.004 mg/mL each of USP Doxapram Hydrochloride RS and USP Doxapram Related Compound B RS in *Diluent*
Sample solution: 2 mg/mL of Doxapram Hydrochloride in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between doxapram related compound B and doxapram, *System suitability solution*
Relative standard deviation: NMT 5.0% for doxapram related compound B and doxapram, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of doxapram related compound B in the portion of Doxapram Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of doxapram related compound B from the *Sample solution*
r_S = peak response of doxapram related compound B from the *Standard solution*
C_S = concentration of USP Doxapram Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Doxapram Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of doxapram chloroethyl analog or any individual unspecified impurity in the portion of Doxapram Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of doxapram chloroethyl analog or any individual unspecified impurity from the *Sample solution*
r_S = peak response of doxapram from the *Standard solution*
C_S = concentration of USP Doxapram Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Doxapram Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Doxapram related compound B	0.9	0.2
Doxapram	1.0	—
Doxapram chloroethyl analog[a]	3.9	0.2
Any individual unspecified impurity	—	0.2
Total impurities	—	1.0

[a] 4-(2-Chloroethyl)-1-ethyl-3,3-diphenylpyrrolidin-2-one.

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 10 mg/mL of Doxapram Hydrochloride in water
Acceptance criteria: 3.5–5.0
• **Loss on Drying** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: 3.0%–4.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Doxapram Hydrochloride RS
USP Doxapram Related Compound B RS
1-Ethyl-4-{2-[(2-hydroxyethyl)amino]ethyl}-3,3-diphenylpyrrolidin-2-one hydrochloride.
$C_{22}H_{29}ClN_2O_2$ 388.93

Doxapram Hydrochloride Injection

DEFINITION
Doxapram Hydrochloride Injection is a sterile solution of Doxapram Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of doxapram hydrochloride monohydrate ($C_{24}H_{30}N_2O_2 \cdot HCl \cdot H_2O$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Solution A: To each L of water, add 0.1 mL of trifluoroacetic acid.
Solution B: To each L of acetonitrile, add 0.1 mL of trifluoroacetic acid.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20	50	50
25	50	50
25.1	90	10
30	90	10

Diluent: Acetonitrile and water (30:70)
Standard solution: 0.2 mg/mL of USP Doxapram Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.2 mg/mL of doxapram hydrochloride monohydrate, equivalent to 0.19 mg/mL of doxapram hydrochloride from Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm. For *Identification* test *B*, use a diode array detector in the range of 190–300 nm.
Column: 4.6-mm × 5-cm; 2.5-µm packing L1
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*

USP Monographs

www.webofpharma.com

Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxapram hydrochloride monohydrate ($C_{24}H_{30}N_2O_2 \cdot HCl \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of doxapram from the *Sample solution*
r_S = peak response of doxapram from the *Standard solution*
C_S = concentration of USP Doxapram Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxapram hydrochloride monohydrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of doxapram hydrochloride monohydrate, 432.99
M_{r2} = molecular weight of anhydrous doxapram hydrochloride, 414.97

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 2 mg/mL of USP Doxapram Hydrochloride RS and 0.04 mg/mL of USP Doxapram Related Compound B RS in *Diluent*
Standard solution: 0.004 mg/mL of USP Doxapram Hydrochloride RS in *Diluent*
Sample solution: Nominally 2 mg/mL of doxapram hydrochloride monohydrate, equivalent to 1.9 mg/mL of doxapram hydrochloride from Injection in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between doxapram related compound B and doxapram, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of doxapram related compound B, doxapram chloroethyl analog, or any individual unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each corresponding impurity from the *Sample solution*
r_S = peak response of doxapram from the *Standard solution*
C_S = concentration of USP Doxapram Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxapram hydrochloride monohydrate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Doxapram related compound B	0.9	0.2
Doxapram	1.0	—
Doxapram chloroethyl analog[a]	3.9	0.2
Any individual unspecified degradation product	—	0.2
Total impurities	—	1.0

[a] 4-(2-Chloroethyl)-1-ethyl-3,3-diphenylpyrrolidin-2-one.

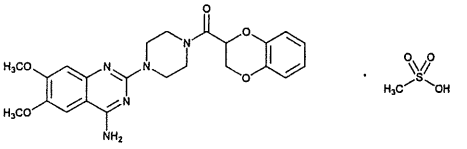
SPECIFIC TESTS

• **pH** ⟨791⟩: 3.5–5.0
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 3.3 USP Endotoxin Units/mg of doxapram hydrochloride.
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Doxapram Hydrochloride RS
USP Doxapram Related Compound B RS
1-Ethyl-4-{2-[(2-hydroxyethyl)amino]ethyl}-3,3-diphenylpyrrolidin-2-one hydrochloride.
$C_{22}H_{29}ClN_2O_2$ 388.93

Doxazosin Mesylate



$C_{23}H_{25}N_5O_5 \cdot CH_4O_3S$ 547.58
Piperazine, 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-[(2,3-dihydro-1,4-benzodioxin-2-yl)carbonyl]-, monomethanesulfonate;
1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-ylcarbonyl)piperazine monomethanesulfonate [77883-43-3].

DEFINITION

Doxazosin Mesylate contains NLT 98.0% and NMT 102.0% of doxazosin mesylate ($C_{23}H_{25}N_5O_5 \cdot CH_4O_3S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: 50 mg/mL (w/v) of phosphoric acid in water
Solution B: Acetonitrile
Solution C: Water
Solution D: Mix 100 mL of *Solution B* and 2 g of phosphoric acid.
Mobile phase: See *Table 1*.

Table 1[a]

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	20	10	70
10	20	22	58
35	20	50	30
40	20	50	30

[a] Between sample injections, the system is re-equilibrated for at least 7 min or until a stable baseline is obtained, representing the starting composition.

System suitability solution: 12 µg/mL of USP Doxazosin Related Compound A RS and 12 µg/mL of USP Doxazosin Related Compound B RS in a mixture of *Solution C* and *Solution D* (9:1). Initially add about 2.5 mL of *Solution D* and then add *Solution D* or *Solution C* to maintain a final composition of *Solution C* and *Solution D* in the ratio of 9:1. Sonicate briefly for complete dissolution.

Standard solution: 0.6 mg/mL of USP Doxazosin Mesylate RS in a mixture of *Solution C* and *Solution D* (9:1). Initially add about 2 mL of *Solution D* and then add *Solution D* or *Solution C* to maintain a final composition of *Solution C* and *Solution D* in the ratio of 9:1. Sonicate briefly for complete dissolution.

Sample solution: 0.6 mg/mL of Doxazosin Mesylate in a mixture of *Solution C* and *Solution D* (9:1). Initially add about 2 mL of *Solution D* and then add *Solution D* or *Solution C* to maintain a final composition of *Solution C* and *Solution D* in the ratio of 9:1. Sonicate briefly for complete dissolution.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4-mm × 25-cm; 5-µm packing L7
Column temperature: 35°
Flow rate: 0.8 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2 between doxazosin related compound A and doxazosin related compound B, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of doxazosin mesylate ($C_{23}H_{25}N_5O_5 \cdot CH_4O_3S$) in the portion of Doxazosin Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Doxazosin Mesylate RS in the *Standard solution* (mg/mL)
C_U = concentration of Doxazosin Mesylate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**

Solution A, Solution B, Solution C, Solution D, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.0015 mg/mL each of USP Doxazosin Mesylate RS, USP Doxazosin Related Compound A RS, USP Doxazosin Related Compound B RS, USP Doxazosin Related Compound C RS, USP Doxazosin Related Compound D RS, USP Doxazosin Related Compound E RS, USP Doxazosin Related Compound F RS, USP Terazosin Related Compound A RS, and USP Terazosin Related Compound C RS in a mixture of *Solution C* and *Solution D*. Initially dissolve USP Doxazosin Related Compounds A, B, C, D, E, and F RS and USP Terazosin Related Compounds A and C RS in approximately 2 mL of *Solution D* only and then add *Solution D* or *Solution C* to maintain a final composition of *Solution C* and *Solution D* in the ratio of 9:1. Sonicate briefly for complete dissolution.

Sample solution: 0.6 mg/mL of Doxazosin Mesylate in a mixture of *Solution C* and *Solution D* (9:1). Initially add about 2 mL of *Solution D* and then add *Solution D* or *Solution C* to maintain a final composition of *Solution C* and *Solution D* in the ratio of 9:1. Sonicate briefly for complete dissolution.

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2 between doxazosin related compound A and doxazosin related compound B, *System suitability solution*
Relative standard deviation: NMT 10% for all peaks, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Doxazosin Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r2}/M_{r1}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of each impurity or doxazosin mesylate (for calculating unspecified impurities) from the *Standard solution*
C_S = concentration of the corresponding USP Doxazosin Related Compound RS or USP Doxazosin Mesylate RS (for calculating unspecified impurities) in the *Standard solution* (mg/mL)
C_U = concentration of Doxazosin Mesylate in the *Sample solution* (mg/mL)
M_{r2} = molecular weight of the corresponding impurity in the sample (see *Table 2*)
M_{r1} = molecular weight of corresponding impurity Reference Standard (see *Table 2*)

Acceptance criteria: See *Table 2*.

www.webofpharma.com

Table 2

Name	Relative Retention Time	Molecular Weight of Reference Standard (M_{r1})	Molecular Weight of Corresponding Impurity in Sample (M_{r2})	Acceptance Criteria, NMT (%)
Terazosin related compound A[a, b]	0.20	362.25	481.55	0.3
Doxazosin related compound A[c, b]	0.44	248.28	344.39	0.25
Doxazosin related compound B[d]	0.48	222.20	222.20	0.25
Doxazosin related compound C[e, b]	0.56	239.66	335.77	0.25
Terazosin related compound C[f, b]	0.61	565.45	684.75	0.25
Doxazosin related compound D[g]	0.83	196.16	196.16	0.25
Doxazosin mesylate	1.00	—	—	—
Doxazosin related compound E[h]	1.45	259.09	259.09	0.25
Doxazosin related compound F[i]	1.55	410.42	410.42	0.25
Any unspecified impurity	—	—	—	0.10
Total impurities	—	—	—	1.0

[a] 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)piperazine, dihydrochloride.
[b] This impurity exists as a mesylate salt in the sample.
[c] *N*-1,4-Benzodioxane-2-carbonyl piperazine.
[d] 6,7-Dimethoxyquinazoline-2,4-dione.
[e] 2-Chloro-4-amino-6,7-dimethoxy quinazoline.
[f] 1,4-Bis(4-amino-6,7-dimethoxy-2-quinazolinyl)piperazine, dihydrochloride.
[g] 1,4-Benzodioxane-2-carboxylic acid.
[h] 2,4-Dichloro-6,7-dimethoxyquinazoline.
[i] *N,N'*-Bis(1,4-benzodioxane-2-carbonyl)piperazine.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
  Sample: 1.0 g
  Analysis: Dry the *Sample* under vacuum at 105° for 4 h.
  Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store below 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Doxazosin Mesylate RS
  USP Doxazosin Related Compound A RS
  N-1,4-Benzodioxane-2-carbonyl piperazine.
  $C_{13}H_{16}N_2O_3$ 248.28
  USP Doxazosin Related Compound B RS
  6,7-Dimethoxyquinazoline-2,4-dione.
  $C_{10}H_{10}N_2O_4$ 222.20
  USP Doxazosin Related Compound C RS
  2-Chloro-4-amino-6,7-dimethoxyquinazoline.
  $C_{10}H_{10}ClN_3O_2$ 239.66
  USP Doxazosin Related Compound D RS
  1,4-Benzodioxane-2-carboxylic acid.
  $C_9H_8O_5$ 196.16
  USP Doxazosin Related Compound E RS
  2,4-Dichloro-6,7-dimethoxyquinazoline.
  $C_{10}H_8Cl_2N_2O_2$ 259.09
  USP Doxazosin Related Compound F RS
  N,N'-Bis(1,4-benzodioxane-2-carbonyl)piperazine.
  $C_{22}H_{22}N_2O_6$ 410.42
  USP Terazosin Related Compound A RS
  1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)piperazine, dihydrochloride.
  $C_{14}H_{19}N_5O_2 \cdot 2HCl$ 362.25
  USP Terazosin Related Compound C RS
  1,4-Bis(4-amino-6,7-dimethoxy-2-quinazolinyl) piperazine, dihydrochloride.
  $C_{24}H_{28}N_8O_4 \cdot 2HCl$ 565.45

Doxazosin Tablets

DEFINITION

Doxazosin Tablets contain an amount of Doxazosin Mesylate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of doxazosin ($C_{23}H_{25}N_5O_5$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: Transfer 3.4 g of monobasic potassium phosphate into a 1-L flask, and add 800 mL of water and 4.0 mL of triethylamine to dissolve. Adjust with phosphoric acid to a pH of 4.5, and dilute with water to volume.
  Mobile phase: Methanol and *Buffer* (110:90)
  Diluent: Methanol and 0.1 N hydrochloric acid (90:10)
  Standard solution: 49 µg/mL of USP Doxazosin Mesylate RS in *Diluent*
  Sample stock solution: Transfer 10 Tablets, whole or ground, into a 250-mL volumetric flask, add 10 mL of water, and sonicate until the Tablets are disintegrated. Add 150 mL of *Diluent*, sonicate for 30 min, and dilute with *Diluent* to volume.
  Sample solution: Nominally 0.04 mg/mL of doxazosin in *Diluent* from *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 245 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 40°
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Capacity factor, *k'*: NLT 2.0
  Column efficiency: NLT 1000 theoretical plates
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of doxazosin ($C_{23}H_{25}N_5O_5$) in the portion of Tablets taken:

USP Monographs

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of doxazosin, 451.48
M_{r2} = molecular weight of doxazosin mesylate, 547.58

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: A known concentration of USP Doxazosin Mesylate RS in *Medium*
Sample solution: Pass a portion of solution under test through a suitable filter. Dilute with *Medium* as needed.
Instrumental conditions
Mode: UV
Analytical wavelength: 246 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of doxazosin mesylate ($C_{23}H_{25}N_5O_5 \cdot CH_4SO_3$) dissolved by comparing the UV absorption of the *Sample solution* and *Standard solution*.
Tolerances: NLT 70% (*Q*) of the labeled amount of doxazosin mesylate ($C_{23}H_{25}N_5O_5 \cdot CH_4SO_3$) is dissolved.

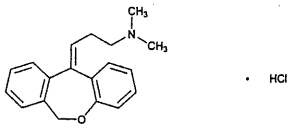
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Doxazosin Mesylate RS

Doxepin Hydrochloride



$C_{19}H_{21}NO \cdot HCl$ 315.84
1-Propanamine, 3-dibenz[*b*,*e*]oxepin-11(6*H*)ylidene-*N*,*N*-dimethyl-, hydrochloride;
N,*N*-Dimethyldibenz[*b*,*e*]oxepin-Δ^{11}(6*H*)-γ-propylamine hydrochloride [1229-29-4].
(*E*)-isomer [4698-39-9].
(*Z*)-isomer [25127-31-5].

DEFINITION

Doxepin Hydrochloride, an (*E*) and (*Z*) geometric isomer mixture, contains the equivalent of NLT 98.0% and NMT 102.0% of doxepin hydrochloride ($C_{19}H_{21}NO \cdot HCl$), calculated on the dried basis. It contains NLT 13.6% and NMT 18.1% of the (*Z*)-isomer, and NLT 81.4% and NMT 88.2% of the (*E*)-isomer.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds that of the *Standard solution*, as obtained in the *Assay*.

• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*
Diluent: Alcohol and water (50:50)
Sample solution: 10 mg/mL of Doxepin Hydrochloride in *Diluent*
Acceptance criteria: Meets the requirements of test *B*

ASSAY

• **PROCEDURE**
Mobile phase: Methanol and 0.2 M monobasic sodium phosphate buffer (3:7). Adjust with 2 N phosphoric acid to a pH of 2.5.
Standard solution: 0.1 mg/mL of USP Doxepin Hydrochloride RS in *Mobile phase*
Sample solution: 0.1 mg/mL of Doxepin Hydrochloride in *Mobile phase*. Sonication may be used to aid in dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 12.5-cm; packing L7
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between the (*E*)- and (*Z*)- isomers
Tailing factor: NMT 2.0 for both the (*E*)- and (*Z*)- isomers
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of doxepin hydrochloride ($C_{19}H_{21}NO \cdot HCl$) in the portion of Doxepin Hydrochloride taken:

$$\text{Result} = [(r_{U(Z)} + r_{U(E)})/(r_{S(Z)} + r_{S(E)}] \times (C_S/C_U) \times 100$$

$r_{U(Z)}$ = peak response of the (*Z*)-isomer from the *Sample solution*
$r_{U(E)}$ = peak response of the (*E*)-isomer from the *Sample solution*
$r_{S(Z)}$ = peak response of the (*Z*)-isomer from the *Standard solution*
$r_{S(E)}$ = peak response of the (*E*)-isomer from the *Standard solution*
C_S = concentration of USP Doxepin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Doxepin Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of the (*Z*)-isomer of doxepin hydrochloride ($C_{19}H_{21}NO \cdot HCl$) in the portion of Doxepin Hydrochloride taken:

$$\text{Result} = (r_{U(Z)}/r_{S(Z)}) \times (C_S/C_U) \times 100$$

$r_{U(Z)}$ = peak response of the (*Z*)-isomer from the *Sample solution*
$r_{S(Z)}$ = peak response of the (*Z*)-isomer from the *Standard solution*

USP Monographs

www.webofpharma.com

C_S = concentration of the (*Z*)-isomer in the *Standard solution* (mg/mL) based on the labeled percentage of (*Z*)-isomer in USP Doxepin Hydrochloride RS
C_U = concentration of Doxepin Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of the (*E*)-isomer of doxepin hydrochloride ($C_{19}H_{21}NO \cdot HCl$) in the portion of Doxepin Hydrochloride taken:

$$\text{Result} = (r_{U(E)}/r_{S(E)}) \times (C_S/C_U) \times 100$$

$r_{U(E)}$ = peak response of the (*E*)-isomer from the *Sample solution*
$r_{S(E)}$ = peak response of the (*E*)-isomer from the *Standard solution*
C_S = concentration of the (*E*)-isomer in the *Standard solution* (mg/mL) based on the labeled percentage of (*E*)-isomer in USP Doxepin Hydrochloride RS
C_U = concentration of Doxepin Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria
Doxepin hydrochloride: 98.0%–102.0% on the dried basis
(*Z*)-isomer of doxepin hydrochloride: 13.6%–18.1%
(*E*)-isomer of doxepin hydrochloride: 81.4%–88.2%

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%
- **ORGANIC IMPURITIES**

[NOTE—This procedure is not intended to resolve the (*E*)- and (*Z*)-isomers of doxepin hydrochloride. Minor variations in *Mobile phase* composition could result in a shoulder in the trailing edge of doxepin. In cases where there may be separation, both the (*E*)- and (*Z*)-isomers should be used in the appropriate calculation.]

Solution A: Water and phosphoric acid (10:1)
Buffer: 1.42 g/L of dibasic sodium phosphate. Adjust with *Solution A* to a pH of 7.7.
Mobile phase: Methanol, acetonitrile, and *Buffer* (50:20:30)
Diluent: *Mobile phase* and 2 N sodium hydroxide (1000:2)
Standard solution: 0.001 mg/mL each of USP Doxepin Hydrochloride RS, USP Doxepin Related Compound A RS, and USP Doxepin Related Compound B RS; and 0.002 mg/mL of USP Doxepin Related Compound C RS in *Diluent*. Sonication for about 1 min may be used to aid the initial dissolution of the compounds.
Sample solution: 1 mg/mL of Doxepin Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: 2.2 times the retention time of doxepin
System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for relative retention times. The doxepin related compound C peak will be the largest peak in the *Standard solution* chromatogram.]
Suitability requirements
Resolution: NLT 1.5 between doxepin related compound A and doxepin related compound C; NLT 1.5 between doxepin related compound C and doxepin related compound B
Signal-to-noise ratio: NLT 10 for all the peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each doxepin related compound in the portion of Doxepin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of doxepin related compound A, B, or C from the *Sample solution*
r_S = peak response of doxepin related compound A, B, or C from the *Standard solution*
C_S = concentration of USP Doxepin Related Compound A RS, USP Doxepin Related Compound B RS, or USP Doxepin Related Compound C RS in the *Standard solution* (mg/mL)
C_U = concentration of Doxepin Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Doxepin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified from the *Sample solution*
r_S = peak response of doxepin (sum of (*E*)- and (*Z*)-isomers) from the *Standard solution*
C_S = concentration of USP Doxepin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Doxepin Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peak with a relative retention time less than 0.25.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Doxepin related compound A	0.48	0.10
Doxepin related compound C	0.55	0.20
Doxepin related compound B	0.63	0.10
Doxepin	1.0	—
Any individual, unspecified impurity	—	0.10

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 60° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
USP Doxepin Hydrochloride RS

www.webofpharma.com

USP Doxepin Related Compound A RS
Dibenzo[*b*,*e*]oxepin-11(6*H*)-one.
$C_{14}H_{10}O_2$ 210.23

USP Doxepin Related Compound B RS
11(*RS*)-(3-(Dimethylamino)propyl)-6,11-dihydrodibenzo[*b*,*e*]oxepin-11-ol.
$C_{19}H_{23}NO_2$ 297.39

USP Doxepin Related Compound C RS
▲(*E*,*Z*)-3-(Dibenzo[*b*,*e*]oxepin-11(6*H*)-ylidene)-*N*-methylpropan-1-amine hydrochloride.▲ (ERR 1-Jun-2018)
$C_{18}H_{19}NO \cdot HCl$ 301.81

Doxepin Hydrochloride Capsules

DEFINITION

Doxepin Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of doxepin ($C_{19}H_{21}NO$).

IDENTIFICATION

• **A.** The retention times of the major peaks for (*E*)- and (*Z*)-isomers of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and 0.2 M monobasic sodium phosphate buffer (3:7). Adjust with 2 N phosphoric acid to a pH of 2.5.

Standard solution: 0.1 mg/mL of USP Doxepin Hydrochloride RS in *Mobile phase*

Sample stock solution: Nominally 0.5 mg/mL of doxepin hydrochloride from the contents of NLT 20 Capsules in *Mobile phase*, prepared as follows. Remove, as completely as possible, the contents of NLT 20 Capsules. Mix the combined contents, and transfer a suitable quantity of the powder, equivalent to 50 mg of doxepin hydrochloride, to a 100-mL volumetric flask. Add 70 mL of *Mobile phase*, and shake by mechanical means for 30 min. Dilute with *Mobile phase* to volume, and filter. Use the filtrate.

Sample solution: Nominally 0.1 mg/mL of doxepin hydrochloride from *Sample stock solution* in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 12.5-cm; packing L7
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between the (*E*)- and (*Z*)-isomers
Tailing factor: NMT 2.0 each for the (*E*)- and (*Z*)-isomers
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxepin ($C_{19}H_{21}NO$) in the portion of Capsules taken:

$$\text{Result} = [(r_{U(z)} + r_{U(E)})/(r_{S(z)} + r_{S(E)})] \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_{U(z)}$ = peak response of the (*Z*)-isomer from the *Sample solution*
$r_{U(E)}$ = peak response of the (*E*)-isomer from the *Sample solution*
$r_{S(z)}$ = peak response of the (*Z*)-isomer from the *Standard solution*
$r_{S(E)}$ = peak response of the (*E*)-isomer from the *Standard solution*
C_S = concentration of doxepin hydrochloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxepin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of doxepin, 279.38
M_{r2} = molecular weight of doxepin hydrochloride, 315.84

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 50 rpm
Time: 30 min
Standard solution: USP Doxepin Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to the same concentration as the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 292 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of doxepin ($C_{19}H_{21}NO$) dissolved using the absobances.
Tolerances: NLT 80% (*Q*) of the labeled amount of doxepin ($C_{19}H_{21}NO$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements
The following procedure is used where the test for *Content Uniformity* is required.
Procedure for Content Uniformity
Diluent: Methanol and 0.05 M monobasic sodium phosphate (50:50). Adjust with 2 N sodium hydroxide to a pH of 6.7.
Standard solution: 0.1 mg/mL of USP Doxepin Hydrochloride RS in *Diluent*. Filter, and use the resulting filtrate.
Sample solutions: Nominally 0.1 mg/mL of doxepin hydrochloride from 1 Capsule prepared as follows. Transfer the contents of 1 Capsule into an appropriate volumetric flask, add 80% of the final flask volume of *Diluent*, and shake the flask by mechanical means for about 30 min. Dilute with *Diluent* to volume. If necessary, transfer a suitable quantity of the resulting solution to another appropriate volumetric flask, and dilute with *Diluent* to volume. Prepare 10 *Sample solutions*.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 292 nm
Cell: 0.5 cm
Analysis
Samples: *Standard solution* and *Sample solutions*
Determine the amount of active ingredient in each unit of the *Sample solution* in comparison with the *Standard solution*.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩
Sample: Contents of 1 Capsule
Acceptance criteria: NMT 9.0%

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Doxepin Hydrochloride RS

Doxepin Hydrochloride Oral Solution

DEFINITION
Doxepin Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of doxepin ($C_{19}H_{21}NO$).

IDENTIFICATION
- **A.**

Mobile phase: Add 0.2 mL of diethylamine to a solution containing 250 mL of chloroform and 750 mL of acetonitrile in a vacuum flask. Before use, degas the contents of the flask by stirring vigorously with a magnetic stirrer, while applying vacuum, for 10 min.
Standard solution: 0.44 mg/mL of USP Doxepin Hydrochloride RS in *Mobile phase*
Sample solution: To 5.0 mL of the Oral Solution in a 60-mL separator add 1 mL of sodium hydroxide solution (1 in 25), 1 g of sodium chloride, and 5.0 mL of ethyl acetate. Shake the mixture vigorously for 1 min. Allow the phases to separate, transfer 1.0 mL of the clear upper phase to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 2-mm × 50-cm; packed with silica microspheres
Flow rate: 0.4 mL/min
Injection volume: 4 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The chromatogram of the *Sample solution* exhibits two peaks having retention times that are identical with those obtained with the *Standard solution*.

ASSAY
- **PROCEDURE**

Diluent: Dilute hydrochloric acid (1 in 120)
Standard stock solution: 1.1 mg/mL of USP Doxepin Hydrochloride RS (1.0 mg/mL of doxepin) in *Diluent*
Standard solution: Dilute 4.0 mL of *Standard stock solution* to 50 mL using *Diluent*. Transfer 15.0 mL of the resulting solution to a 125-mL separator, and extract with two 20-mL portions of ether. Dilute 10.0 mL of the extracted aqueous phase to 25.0 mL using *Diluent*.
Sample stock solution: Nominally 1 mg/mL of doxepin from Oral Solution in *Diluent*
Sample solution: Dilute 4.0 of *Sample stock solution* to 50 mL using *Diluent*. Transfer 15.0 mL of the resulting solution to a 125-mL separator, and extract with two 20-mL portions of ether. Dilute 10.0 mL of the extracted aqueous phase to 25.0 mL using *Diluent*.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 292 nm
Cell: 1 cm
Blank: Diluent
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxepin ($C_{19}H_{21}NO$) in the Oral Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Doxepin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxepin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of doxepin, 279.38
M_{r2} = molecular weight of doxepin hydrochloride, 315.84

Acceptance criteria: 90.0%–110%

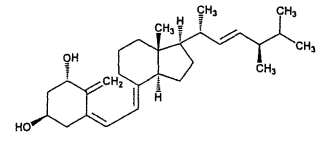
PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements for Oral Solution packaged in single-unit containers
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements for Oral Solution packaged in multiple-unit containers

SPECIFIC TESTS
- **pH** ⟨791⟩

Analysis: Allow the portion of Oral Solution under test to remain in contact with the electrodes for 15 min before the measurement.
Acceptance criteria: 4.0–7.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label the Oral Solution to indicate that each dose is to be diluted with water or other suitable fluid to approximately 120 mL just before administration.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Doxepin Hydrochloride RS

Doxercalciferol



$C_{28}H_{44}O_2$ 412.65
1α-Hydroxyvitamin D_2;
1α-Hydroxyergocalciferol;
9,10-Secoergosta-5,7,10(19),22-tetraene-1,3-diol, (1α,3β,5*Z*,7*E*,22*E*)-;
(5*Z*,7*E*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraene-1α,3β-diol [54573-75-0].

DEFINITION
Doxercalciferol contains NLT 98.0% and NMT 102.0% of doxercalciferol ($C_{28}H_{44}O_2$), calculated on the dried basis.
[**CAUTION**—Great care should be taken in handling Doxercalciferol, because it is a potentially cytotoxic agent.]

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Protect doxercalciferol solutions from light.]

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for 5 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	25	75
15	23	77
20	10	90
25	10	90

Standard solution: 1.0 mg/mL of USP Doxercalciferol RS in acetonitrile. Use sonication to dissolve if necessary.

System suitability solution: Heat a portion of the *Standard solution* at 40° for 1 h to obtain at least 0.1%–0.2% of pre-doxercalciferol.

Sample solution: 1.0 mg/mL of Doxercalciferol in acetonitrile. Use sonication to dissolve if necessary. [NOTE—Doxercalciferol solutions are stable for at least 9 h when stored at room temperature, and up to 7 days when stored at 5°.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Column temperature: 35°

Flow rate: 1.7 mL/min

Injection volume: 5 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for pre-doxercalciferol and doxercalciferol are 0.94 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between pre-doxercalciferol and doxercalciferol, *System suitability solution*

Tailing factor: 0.7–1.3, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of doxercalciferol ($C_{28}H_{44}O_2$) in the portion of Doxercalciferol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of doxercalciferol from the *Sample solution*

r_S = peak response of doxercalciferol from the *Standard solution*

C_S = concentration of USP Doxercalciferol RS in the *Standard solution* (mg/mL)

C_U = concentration of Doxercalciferol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **ORGANIC IMPURITIES**

[NOTE—Protect doxercalciferol solutions from light.]

Solution A, Solution B, and **Chromatographic system:** Proceed as directed in the *Assay*.

Mobile phase: See *Table 2*. Return to original conditions and re-equilibrate the system for 5 min.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	25	75
15	23	77
20	10	90
30	10	90
36	0	100
50	0	100

Standard stock solution: 4.0 mg/mL of USP Doxercalciferol RS. Dissolve first in ethyl acetate using about 20% of the final volume with sonication in an ice-water bath, if necessary, and dilute with acetonitrile to volume.

Standard solution: 0.004 mg/mL of USP Doxercalciferol RS in acetonitrile from the *Standard stock solution*

System suitability solution: Heat a portion of the *Standard stock solution* at 40° for 1 h or let it stand at room temperature for about 4–6 h, to obtain at least 0.1%–0.2% of pre-doxercalciferol

Sample solution: 4.0 mg/mL of Doxercalciferol. Dissolve first in ethyl acetate using about 20% of the final volume with sonication in an ice-water bath, if necessary, and dilute with acetonitrile to volume. [NOTE—The *Sample solution* should be prepared fresh before injection and injected within 5 min of completing its preparation.]

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between pre-doxercalciferol and doxercalciferol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Doxercalciferol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of doxercalciferol from the *Standard solution*

C_S = concentration of USP Doxercalciferol RS in the *Standard solution* (mg/mL)

C_U = concentration of Doxercalciferol in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard any peak observed in the blank.

www.webofpharma.com

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pre-doxercalciferol	0.94	0.15
trans-Doxercalciferol (if present)[a, b]	0.96	0.30
Doxercalciferol	1.0	—
β-Doxercalciferol (if present)[b, c]	1.07	0.50
Dihydrodoxercalciferol (if present)[b, d]	1.10	0.50
Any other individual impurity	—	0.50
Total impurities	—	1.0

[a] (5*E*,7*E*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraene-1α,3β-diol.
[b] If possible from manufacturing process.
[c] (5*Z*,7*E*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraene-1β,3β-diol.
[d] (5*Z*,7*E*)-9,10-Secoergosta-5,7,10(19)-triene-1α,3β-diol.

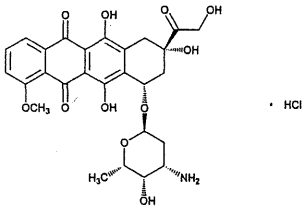
SPECIFIC TESTS

- **LOSS ON DRYING**
  (See *Thermal Analysis* ⟨891⟩.)
  Analysis: Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument, using 5–10 mg of Doxercalciferol. Heat the specimen under test at a rate of 5°/min in a stream of nitrogen at a flow rate of about 40 mL/min. Record the thermogram from ambient temperature to 150°.
  Acceptance criteria: NMT 0.50%
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 10 mg/mL in alcohol
  Acceptance criteria: +45° to +52°
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: [NOTE—This requirement applies only if the drug substance is intended for use in the manufacture of parenteral dosage forms.] The total aerobic microbial count is NMT 10^3 cfu/g, and the total combined yeasts and molds count is NMT 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at a lowered temperature not exceeding 8°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Doxercalciferol RS

Doxorubicin Hydrochloride



$C_{27}H_{29}NO_{11} \cdot HCl$ 579.98

5,12-Naphthacenedione, 10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxylacetyl)-1-methoxy-, hydrochloride (8*S*-*cis*)-;
(8*S*,10*S*)-10-[(3-Amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride [25316-40-9].

DEFINITION

Doxorubicin Hydrochloride contains NLT 98.0% and NMT 102.0% of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$), calculated on the anhydrous, solvent-free basis.
[**CAUTION**—Great care should be taken to prevent inhaling particles of doxorubicin hydrochloride and exposing the skin to it.]

IDENTIFICATION

- **A.** The retention time of the doxorubicin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

ASSAY

Change to read:

- **PROCEDURE**
  Solution A: 0.1% Trifluoroacetic acid prepared by transferring 1.0 mL of trifluoroacetic acid to 1 L of water
  Solution B: Acetonitrile, methanol, and trifluoroacetic acid (800:200:1)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
15	25	75
16	25	75
16.1	90	10
18	90	10

Diluent: *Solution A* and *Solution B* (50:50)
[NOTE—Protect solutions containing doxorubicin from light.]
System suitability solution: 0.1 mg/mL each of USP Doxorubicin Hydrochloride RS and USP Epirubicin Hydrochloride RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Doxorubicin Hydrochloride RS in *Diluent*
Sample solution: 0.1 mg/mL of Doxorubicin Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Temperatures
Column: 35°
Autosampler: 4°
Flow rate: 0.5 mL/min
Injection volume: 2 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for doxorubicin and epirubicin are 1.0 and 1.05, respectively.]
Suitability requirements
Resolution: NLT 1.5 between doxorubicin and epirubicin, *System suitability solution*
Tailing factor: 0.8–1.5, *Standard solution*

www.webofpharma.com

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$) in the portion of Doxorubicin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of doxorubicin from the *Sample solution*

r_S = peak response of doxorubicin from the *Standard solution*

C_S = concentration of USP Doxorubicin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Doxorubicin Hydrochloride in the *Sample solution* (mg/mL)

P = potency of doxorubicin ▲hydrochloride▲ (ERR 1-Jun-2019) in USP Doxorubicin Hydrochloride RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 98.0%–102.0% on the anhydrous, solvent-free basis

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

[NOTE—Protect solutions containing doxorubicin from light.]

Standard solution: 0.002 mg/mL each of USP Doxorubicin Hydrochloride RS, USP Doxorubicinone RS, USP Daunorubicin Hydrochloride RS, and USP Daunorubicinone RS in *Diluent*

Sample solution: 0.4 mg/mL of Doxorubicin Hydrochloride in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between doxorubicin and epirubicin, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of doxorubicinone in the portion of Doxorubicin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of doxorubicinone from the *Sample solution*

r_S = peak response of doxorubicinone from the *Standard solution*

C_S = concentration of USP Doxorubicinone RS in the *Standard solution* (mg/mL)

C_U = concentration of Doxorubicin Hydrochloride in the *Sample solution* (mg/mL)

P = potency of doxorubicinone in the USP Doxorubicinone RS (mg/mg)

Calculate the percentage of daunorubicinone in the portion of Doxorubicin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of daunorubicinone from the *Sample solution*

r_S = peak response of daunorubicinone from the *Standard solution*

C_S = concentration of USP Daunorubicinone RS in the *Standard solution* (mg/mL)

C_U = concentration of Doxorubicin Hydrochloride in the *Sample solution* (mg/mL)

P = potency of daunorubicinone in USP Daunorubicinone RS (mg/mg)

Calculate the percentage of daunorubicin in the portion of Doxorubicin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of daunorubicin from the *Sample solution*

r_S = peak response of daunorubicin from the *Standard solution*

C_S = concentration of USP Daunorubicin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Doxorubicin Hydrochloride in the *Sample solution* (mg/mL)

P = potency of daunorubicin in USP Daunorubicin Hydrochloride RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Calculate the percentage of any individual unspecified impurity in the portion of Doxorubicin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*

r_S = peak response of doxorubicin from the *Standard solution*

C_S = concentration of USP Doxorubicin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Doxorubicin Hydrochloride in the *Sample solution* (mg/mL)

P = potency of doxorubicin ▲hydrochloride▲ (ERR 1-Jun-2019) in USP Doxorubicin Hydrochloride RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Doxorubicin	1.0	—
Epirubicin[a]	1.05	—
Doxorubicinone[b]	1.08	0.5
Daunorubicin	1.23	0.5
Daunorubicinone[c]	1.35	0.5
Any individual unspecified impurity	—	0.5

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	2.0

[a] For resolution measurement only. Not to be reported; not to be included in total impurities.
[b] (8*S*,10*S*)-6,8,10,11-Tetrahydoxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[c] (8*S*,10*S*)-8-Acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.

• **LIMIT OF ACETONE AND ALCOHOL**
Internal standard solution: 1 mg/mL of dioxane in water
Standard solution: 0.2 mg/mL of USP Acetone RS, 0.3 mg/mL of dehydrated alcohol in *Internal standard solution*
Sample solution: 200 mg of Doxorubicin Hydrochloride in 3.0 mL (3.0 g) of *Internal standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 4-mm × 2-m; packed with 8%–10% liquid phase G16 and 2% potassium hydroxide on 100- to 120-mesh support S1A
Column temperature: 60°
Carrier gas: Helium
[NOTE—Adjust the column temperature and carrier gas flow rate so that dioxane elutes in about 6 min.]
Injection volume: 1 µL
Flow rate: Adjust the column temperature and carrier gas flow rate so that dioxane elutes in about 6 min.
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for acetone, alcohol, and dioxane are about 0.2, 0.5, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between adjacent peaks
Tailing factor: NMT 1.5 for the alcohol peak
Relative standard deviation: NMT 4.0% for the peak response ratios of acetone and alcohol to the internal standard
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage, by weight, of acetone and alcohol respectively, in the portion of Doxorubicin Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_A/C_D) \times (D_U/W_U) \times 100$$

R_U = peak response ratio of the analyte (acetone or alcohol) to dioxane from the *Sample solution*
R_S = peak response ratio of the analyte (acetone or alcohol) to dioxane from the *Standard solution*
C_A = concentration of the analyte (acetone or alcohol) in the *Standard solution* (mg/mL)
C_D = concentration of dioxane in the *Standard solution* (mg/mL)
D_U = weight of dioxane in the *Sample solution* (mg)
W_U = weight of Doxorubicin Hydrochloride in the *Sample solution* (mg)

Acceptance criteria
Acetone: NMT 0.5%
Total of acetone and alcohol: NMT 2.5%

SPECIFIC TESTS
• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements, except where it is labeled as amorphous, most particles do not exhibit birefringence and extinction positions
• **PH** ⟨791⟩
Sample solution: 5 mg/mL
Acceptance criteria: 4.0–5.5
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 4.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature except where it is labeled as amorphous, in which case it should be stored in the freezer.
• **LABELING:** The amorphous form is so labeled.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetone RS
USP Daunorubicin Hydrochloride RS
USP Daunorubicinone RS
(8*S*,10*S*)-8-Acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
$C_{21}H_{18}O_8$ 398.36
USP Doxorubicin Hydrochloride RS
USP Doxorubicinone RS
(8*S*,10*S*)-6,8,10,11-Tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
$C_{21}H_{18}O_9$ 414.36
USP Epirubicin Hydrochloride RS

Doxorubicin Hydrochloride Injection

DEFINITION
Doxorubicin Hydrochloride Injection is a sterile solution of Doxorubicin Hydrochloride in Sterile Water for Injection made isoosmotic with Sodium Chloride, Dextrose, or other suitable added substances. It contains NLT 90.0% and NMT 115.0% of the labeled amount of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the doxorubicin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the doxorubicin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
[NOTE—Protect solutions containing doxorubicin from light.]
Solution A: 0.1% trifluoroacetic acid TS
Solution B: Acetonitrile, methanol, and trifluoroacetic acid (800:200:1)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
15	25	75
16	25	75
16.1	90	10
18	90	10

USP Monographs

www.webofpharma.com

Diluent: *Solution A* and *Solution B* (50:50)
System suitability solution: 0.1 mg/mL each of USP Doxorubicin Hydrochloride RS and USP Epirubicin Hydrochloride RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Doxorubicin Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of doxorubicin hydrochloride in *Diluent* from Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification B*, use a diode array detector in the range of 190–400 nm.
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Temperatures
Autosampler: 4°
Column: 35°
Flow rate: 0.5 mL/min
Injection volume: 2 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between doxorubicin and epirubicin, *System suitability solution*
Tailing factor: 0.8–1.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of doxorubicin from the *Sample solution*
r_S = peak response of doxorubicin from the *Standard solution*
C_S = concentration of USP Doxorubicin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxorubicin hydrochloride in the *Sample solution* (mg/mL)
P = potency of doxorubicin in USP Doxorubicin Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

IMPURITIES

• **ORGANIC IMPURITIES**
[NOTE—Protect solutions containing doxorubicin from light.]
Mobile phase, Diluent, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.008 mg/mL each of USP Doxorubicin Hydrochloride RS and 0.012 mg/mL of USP Doxorubicinone RS in *Diluent*. [NOTE—It may be necessary to first dissolve in acetonitrile, using NMT 5% of the final volume, before diluting with *Diluent*.]
Sample solution: Nominally 0.4 mg/mL of doxorubicin hydrochloride in *Diluent* from Injection
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between doxorubicin and epirubicin, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of doxorubicinone in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of doxorubicinone from the *Sample solution*
r_S = peak response of doxorubicinone from the *Standard solution*
C_S = concentration of USP Doxorubicinone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxorubicin hydrochloride in the *Sample solution* (mg/mL)
P = potency of doxorubicinone in USP Doxorubicinone RS (mg/mg)

Calculate the percentage of any individual unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of doxorubicin from the *Standard solution*
C_S = concentration of USP Doxorubicin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxorubicin hydrochloride in the *Sample solution* (mg/mL)
P = potency of doxorubicin in USP Doxorubicin Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Doxorubicin	1.0	—
Epirubicin[a]	1.05	—
Doxorubicinone[b]	1.08	3.0
Daunorubicinone[c, d]	1.35	—
Any other individual degradation product	—	2.0
Total impurities	—	5.0

[a] For resolution measurement only. Not to be reported; not to be included in total impurities.
[b] (8*S*,10*S*)-6,8,10,11-Tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[c] (8*S*,10*S*)-8-Acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[d] The acceptance criteria of this impurity, if present, would fall under the acceptance criteria for "any other individual degradation product" and is included in the total impurities.

SPECIFIC TESTS

• **PH** ⟨791⟩: 2.5–4.5
• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: It meets the requirements when tested as directed, the entire contents of all the containers being collected aseptically.

www.webofpharma.com

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩
  Sample solution: 1.1 mg/mL of doxorubicin hydrochloride prepared from Injection in *Sterile Water for Injection*
  Acceptance criteria: NMT 2.2 USP Endotoxin Units/mg of doxorubicin hydrochloride
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Store in a refrigerator. Injection may be packaged in multiple-dose containers not exceeding 100 mL in volume.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Doxorubicin Hydrochloride RS
  USP Doxorubicinone RS
  (8*S*,10*S*)-6,8,10,11-Tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
  $C_{21}H_{18}O_9$ 414.36
  USP Epirubicin Hydrochloride RS

Doxorubicin Hydrochloride for Injection

DEFINITION

Doxorubicin Hydrochloride for Injection is a sterile mixture of Doxorubicin Hydrochloride and Lactose. It contains NLT 90.0% and NMT 115.0% of the labeled amount of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$).

[**CAUTION**—Great care should be taken to prevent inhaling particles of Doxorubicin Hydrochloride and exposing the skin to it.]

IDENTIFICATION

- **A.** The retention time of the doxorubicin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the doxorubicin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.1% Trifluoroacetic acid prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L
Solution B: Acetonitrile, methanol, and trifluoroacetic acid (800:200:1)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
15	25	75
16	25	75
16.1	90	10
18	90	10

Diluent: *Solution A* and *Solution B* (50:50)
[NOTE—Protect solutions containing doxorubicin from light.]
System suitability solution: 0.1 mg/mL each of USP Doxorubicin Hydrochloride RS and USP Epirubicin Hydrochloride RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Doxorubicin Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of doxorubicin hydrochloride in *Diluent* from Doxorubicin Hydrochloride for Injection prepared as follows. Add 5 mL of *Diluent* into the container of Doxorubicin Hydrochloride for Injection, and transfer the contents to a volumetric flask of appropriate size. Rinse the container with additional *Diluent* NLT 3 times. Dilute with *Diluent* to volume, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification* test *B*, use a photo-diode array detector in the range of 190–400 nm.
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Temperatures
Autosampler: 4°
Column: 35°
Flow rate: 0.5 mL/min
Injection volume: 2 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for doxorubicin and epirubicin are 1.0 and 1.05, respectively.]
Suitability requirements
Resolution: NLT 1.5 between doxorubicin and epirubicin, *System suitability solution*
Tailing factor: 0.8–1.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$) in the portion of Doxorubicin Hydrochloride for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of doxorubicin from the *Sample solution*
r_S = peak response of doxorubicin from the *Standard solution*
C_S = concentration of USP Doxorubicin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxorubicin hydrochloride in the *Sample solution* (mg/mL)
P = potency of doxorubicin in USP Doxorubicin Hydrochloride RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
[NOTE—Protect solutions containing doxorubicin from light.]
Standard solution: 0.002 mg/mL each of USP Doxorubicin Hydrochloride RS, USP Doxorubicinone RS, and USP Daunorubicinone RS in *Diluent*
Sample solution: Nominally 0.4 mg/mL of doxorubicin hydrochloride in *Diluent* from Doxorubicin Hydrochloride for Injection prepared as follows. Add 5 mL of *Diluent* into the container of Doxorubicin Hydrochloride for Injection, and transfer the contents to a volumetric flask of

USP Monographs

www.webofpharma.com

appropriate size. Rinse the container with additional *Diluent* NLT 3 times. Dilute with *Diluent* to volume, and mix.

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between doxorubicin and epirubicin, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of doxorubicinone in the portion of Doxorubicin Hydrochloride for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of doxorubicinone from the *Sample solution*

r_S = peak response of doxorubicinone from the *Standard solution*

C_S = concentration of USP Doxorubicinone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxorubicin hydrochloride in the *Sample solution* (mg/mL)

P = potency of doxorubicinone in USP Doxorubicinone RS (mg/mg)

Calculate the percentage of daunorubicinone in the portion of Doxorubicin Hydrochloride for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of daunorubicinone from the *Sample solution*

r_S = peak response of daunorubicinone from the *Standard solution*

C_S = concentration of USP Daunorubicinone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxorubicin hydrochloride in the *Sample solution* (mg/mL)

P = potency of daunorubicinone in USP Daunorubicinone RS (mg/mg)

Calculate the percentage of any individual unspecified degradation product in the portion of Doxorubicin Hydrochloride for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of doxorubicin from the *Standard solution*

C_S = concentration of USP Doxorubicin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxorubicin hydrochloride in the *Sample solution* (mg/mL)

P = potency of doxorubicin in USP Doxorubicin Hydrochloride RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Doxorubicin	1.0	—
Epirubicin[a]	1.05	—
Doxorubicinone[b]	1.08	0.5
Daunorubicinone[c]	1.35	0.5
Any individual unspecified degradation product	—	0.5
Total impurities	—	2.0

[a] For resolution measurement only. Not to be reported; not to be included in total impurities.

[b] (8*S*,10*S*)-6,8,10,11-Tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.

[c] (8*S*,10*S*)-8-Acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.

SPECIFIC TESTS

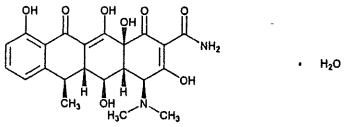
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*, the entire contents of all the containers being collected aseptically with the aid of 200 mL of *Fluid A* before filtering.
- **WATER DETERMINATION,** *Method I* ⟨921⟩
  Sample solution: Prepare as directed for a hygroscopic specimen.
  Acceptance criteria: NMT 4.0%
- **pH** ⟨791⟩
  Sample solution: Constitute as directed in the labeling using water as the diluent.
  Acceptance criteria: 4.5–6.5
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩
  Sample solution: 1.1 mg/mL of doxorubicin hydrochloride from Doxorubicin Hydrochloride for Injection
  Acceptance criteria: NMT 2.2 USP Endotoxin Units/mg of doxorubicin hydrochloride
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements
- **LABELING** ⟨7⟩, **LABELS AND LABELING FOR INJECTABLE PRODUCTS:** Meets the requirements.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, except that multiple-dose containers may provide for the withdrawal of NMT 100 mL when constituted as directed in the labeling. Store unreconstituted vials at controlled room temperature. Protect from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Daunorubicinone RS
  (8*S*,10*S*)-8-Acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
  $C_{21}H_{18}O_8$ 398.36
  USP Doxorubicin Hydrochloride RS
  USP Doxorubicinone RS
  (8*S*,10*S*)-6,8,10,11-Tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
  $C_{21}H_{18}O_9$ 414.36
  USP Epirubicin Hydrochloride RS

www.webofpharma.com

Doxycycline



$C_{22}H_{24}N_2O_8 \cdot H_2O$ 462.45

$C_{22}H_{24}N_2O_8$ 444.44

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, [4*S*-(4α,4aα,5α,5aα,6α,12aα)]-, monohydrate;
4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrate [17086-28-1].
Anhydrous [564-25-0].

DEFINITION

Doxycycline has a potency equivalent to NLT 880 µg/mg and NMT 980 µg/mg of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

• **A.**

Test solution: Equivalent to 1 mg/mL of doxycycline in methanol

Analysis: Proceed as directed in *Identification—Tetracyclines* ⟨193⟩, *Method II.*

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Transfer 2.72 g of monobasic potassium phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutylammonium hydrogen sulfate, and 0.40 g of edetate disodium to a 1000-mL volumetric flask. Add 850 mL of water, and stir to dissolve. Add 60 g of tertiary butyl alcohol with the aid of water, dilute with water to volume, and adjust with 1 N sodium hydroxide to a pH of 8.0 ± 0.1. Pass this solution through a filter of 0.5-µm or finer pore size, and degas before using. Decreasing the proportion of tertiary butyl alcohol results in a longer retention time of doxycycline and improved separation of doxycycline from the related compounds.

Diluent: 0.01 N hydrochloric acid

System suitability stock solution: 6 mg/mL of doxycycline in *Diluent*

System suitability solution: Transfer 5 mL of *System suitability stock solution* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in 0.01 N hydrochloric acid, and dilute with *Diluent* to volume. Pass a portion of this solution through a filter of 0.5-µm or finer pore size, and use the filtrate. This solution contains a mixture of 4-epidoxycycline, 6-epidoxycycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

Standard solution: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve. Protect the *Standard solution* from light.

Sample solution: 1.1 mg/mL of Doxycycline, prepared as follows. Transfer a suitable amount of Doxycycline to a suitable volumetric flask. Add 0.1 N hydrochloric acid, using 24% of the final volume, swirl to dissolve, and dilute with *Diluent* to volume. Pass through a filter of 0.5-µm or finer pore size. Protect the *Sample solution* from light.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; packing L21

Column temperature: 60 ± 1°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 1.7 times the retention time of doxycycline

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between the 4-epidoxycycline peak and the doxycycline peak, *System suitability solution*

Tailing factor: NMT 2.0 for doxycycline, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity of doxycycline ($C_{22}H_{24}N_2O_8$), in µg/mg, in the portion of Doxycycline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)

Acceptance criteria: 880–980 µg/mg

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay.*

Standard solution 1: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve. Protect *Standard solution 1* from light.

Standard solution 2: 0.024 mg/mL each of USP Doxycycline Hyclate RS and USP Methacycline Hydrochloride RS in *Diluent*. Protect *Standard solution 2* from light.

System suitability

Samples: *System suitability solution* and *Standard solution 1*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between 4-epidoxycycline and doxycycline, *System suitability solution*

Tailing factor: NMT 2.0 for doxycycline, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution 1*

Analysis

Samples: *Standard solution 2* and *Sample solution*

Calculate the percentage of methacycline in the portion of Doxycycline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methacycline from the *Sample solution*

r_S = peak response of methacycline from *Standard solution 2*

C_S = concentration of USP Methacycline Hydrochloride RS in *Standard solution 2* (mg/mL)

www.webofpharma.com

C_U = concentration of the *Sample solution* (mg/mL)

Calculate the percentage of each related compound other than methacycline in the portion of Doxycycline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of doxycycline from *Standard solution 2*
C_S = concentration of USP Doxycycline Hyclate RS in *Standard solution 2* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
4-Epidoxycycline[a]	0.4	0.5
Methacycline	0.6	2
6-Epidoxycycline	0.7	2
Doxycycline	1.0	—
Individual unspecified impurities[b]	—	0.5

[a] Main degradation product.
[b] Includes impurities that elute before methacycline and those that elute after doxycycline.

SPECIFIC TESTS
- **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
- **pH** ⟨791⟩
  Sample solution: An aqueous suspension containing 10 mg/mL
  Acceptance criteria: 5.0–6.5
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** 3.6%–4.6%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Doxycycline Hyclate RS
  USP Methacycline Hydrochloride RS

Doxycycline Capsules

DEFINITION
Doxycycline Capsules contain NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION
- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Protect solutions containing doxycycline from light.
  Solution A: Transfer 3.1 g of monobasic potassium phosphate, 0.5 g of edetate disodium, and 0.5 mL of triethylamine to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with 1 N sodium hydroxide to a pH of 8.5 ± 0.1.
  Solution B: Methanol
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid
Standard solution: 0.12 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.
Sample solution: Nominally 0.1 mg/mL of doxycycline in *Diluent*, prepared as follows. Transfer an adequate amount of doxycycline from the contents of NLT 20 Capsules to a suitable volumetric flask. Add 80% of the final volume of *Diluent*, sonicate for about 5 min, shake for about 15 min, and dilute with *Diluent* to volume. Centrifuge a portion of the solution for 10 min at 3000 rpm and use the supernatant for analysis.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm. For *Identification A*, a diode array detector may be used in the wavelength range of 200–400 nm.
Column: 2.1-mm × 5-cm; 1.7-µm packing L7. [NOTE—A 1.7-µm guard column with packing L7 was used during method validation.]
Column temperature: 60°
Flow rate: 0.6 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Test 1
  Medium: 0.01 N hydrochloric acid; 900 mL

www.webofpharma.com

Apparatus 2: 75 rpm
Time: 60 min
Standard solution: A known concentration of USP Doxycycline Hyclate RS in *Medium*
Sample solution: Filter a portion of the solution under test and dilute with *Medium*, if necessary.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Maximum absorbance at about 268 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times P \times F \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Tolerances: NLT 85% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

Test 2
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm, with sinkers
Time: 30 min
Standard solution: A known concentration of USP Doxycycline Hyclate RS in *Medium*
Sample solution: Filter a portion of the solution under test and dilute with *Medium*, if necessary.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 268 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times P \times F \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Tolerances: NLT 85% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
Protect solutions containing doxycycline from light.

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in *Diluent*
System suitability stock solution 2: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*
System suitability solution: Transfer 5 mL of *System suitability stock solution 2* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in *Diluent*, add 0.5 mL of *System suitability stock solution 1*, and dilute with *Diluent* to volume. Pass the solution through a suitable filter and use the filtrate. This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. [NOTE—The solution is stable up to 14 days when stored in a refrigerator at 2°–8°.]
Standard solution: 4.6 µg/mL of USP Doxycycline Hyclate RS in *Diluent*
Sample solution: Nominally 2.0 mg/mL of doxycycline in *Diluent*, prepared as follows. Accurately weigh and transfer a portion of the composite equivalent to 100.0 mg of doxycycline to a 50-mL volumetric flask. Add 80% of the final volume of *Diluent*, sonicate for about 5 min, shake for about 15 min, and dilute with *Diluent* to volume. Centrifuge a portion of the solution for 10 min at 3000 rpm and use the supernatant for analysis.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 2.0 between doxycycline related compound A and doxycycline, *System suitability solution*
Relative standard deviation: NMT 5.0% for the doxycycline peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of doxycycline from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methacycline[a, b]	0.64	—
4-Epidoxycycline[c]	0.79	▲0.5▲ (RB 1-May-2018)

Stop Emailing Me

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Doxycycline related compound A (6-epidoxycycline)[b, d]	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities	—	1.0

[a] (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide.
[b] Process impurities that are controlled in the drug substance are not to be reported. They are not to be included in total impurities. They are listed here for information only.
[c] (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.
[d] (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Doxycycline Hyclate RS
  USP Doxycycline Related Compound A RS
  [NOTE—May be available as a free base or a hydrochloride salt.]
  (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.
  $C_{22}H_{24}N_2O_8$ 444.43
  (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride.
  $C_{22}H_{24}N_2O_8 \cdot HCl$ 480.13
  USP Methacycline Hydrochloride RS

Doxycycline Extended-Release Capsules

DEFINITION

Doxycycline Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Transfer 2.72 g of monobasic potassium phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutylammonium hydrogen sulfate, and 0.40 g of edetate disodium to a 1000-mL volumetric flask. Add about 850 mL of water, and dissolve. Add 60 g of tertiary butyl alcohol with the aid of water, dilute with water to volume, and adjust to a pH of 8.0. Pass this solution through a suitable filter, and degas before using.

Buffer: pH 6.8 phosphate buffer (see *Buffer Solutions* in *Reagents, Indicators, and Solutions*)

System suitability stock solution: 7.2 mg/mL of USP Doxycycline Hyclate RS in 0.01 N hydrochloric acid prepared as follows. Transfer USP Doxycycline Hyclate RS to a suitable volumetric flask, and add 0.01 N hydrochloric acid to about 70% of the final volume. Sonicate as necessary to dissolve. Dilute with 0.01 N hydrochloric acid to volume, and mix. Protect this solution from light.

System suitability solution: 1.4 mg/mL of USP Doxycycline Hyclate RS from the *System suitability stock solution* in 0.01 N hydrochloric acid prepared as follows. Transfer NLT 5.0 mL of the *System suitability stock solution* to a suitable volumetric flask, and heat on a steam bath for 60 min. Evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in 0.01 N hydrochloric acid, dilute with 0.01 N hydrochloric acid to volume, and mix. Pass through a suitable filter. The filtrate contains a mixture of 4-epidoxycycline, 6-epidoxycycline, and doxycycline. Protect this solution from light.

Standard solution: 0.8 mg/mL of doxycycline from USP Doxycycline Hyclate RS in a mixture of *Buffer* and 0.1 N hydrochloric acid (1:9) prepared as follows. Transfer an appropriate amount of USP Doxycycline Hyclate RS to a volumetric flask, and add *Buffer* to 10% of the final volume. Sonicate to dissolve. Dilute with 0.1 N hydrochloric acid to volume, and mix. Protect this solution from light.

Sample solution: Nominally 0.8 mg/mL of doxycycline in a mixture of *Buffer* and 0.1 N hydrochloric acid (1:9) prepared as follows. Weigh, and transfer the contents of NLT 10 Capsules to a suitable volumetric flask, add *Buffer* to 10% of the final volume, and sonicate as necessary to dissolve. Dilute with 0.1 N hydrochloric acid to volume, and mix. Pass through a suitable filter. Protect this solution from light.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 25-cm; 7-µm packing L21
Column temperature: 60°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.7 times the retention time of the doxycycline peak

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between 4-epidoxycycline and doxycycline, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Tailing factor: NMT 2.0, *System suitability solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)

www.webofpharma.com

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Proceed as directed for *Apparatus 1* and *Apparatus 2, Delayed-Release Dosage Forms, Method A, Procedure.*

Acid stage

Acid stage medium: 0.1 N hydrochloric acid; 750 mL

Apparatus 2: 75 rpm

Time: 2 h

Blank: *Acid stage medium*

Standard solution: 0.04 mg/mL of doxycycline from USP Doxycycline Hyclate RS in the *Acid stage medium*. Sonicate as necessary to dissolve.

Capsule shell solution: Dissolve 10 empty Capsule shells in 1000 mL of the *Acid stage medium* by gently heating at 37°.

Acid stage capsule shell blank: 100 mL of *Capsule shell solution* and 650 mL of the *Acid stage medium*. Pass through a suitable filter.

Sample solution: Pass portions of the solution under test through a suitable filter.

Instrumental conditions

Mode: UV

Analytical wavelength: 345 nm

Cell: 0.2 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U - A_{CS})/A_S \times (C_S/L) \times V \times P \times F \times 100$$

A_U = absorbance of the *Sample solution*
A_{CS} = absorbance of the *Acid stage capsule shell blank*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of the *Acid stage medium*, 750 mL
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Tolerances: NLT 65% and NMT 90% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved. The percentage of the labeled amount of doxycycline dissolved at the time specified conforms to *Acceptance Table 2* in *Dissolution* ⟨711⟩.

Buffer stage

Conduct this stage of testing on the Capsules that were previously subjected to the acid stage testing by adding the *Buffer stage medium* to the same vessel and adjusting to a pH of 6.0 using either 2 N hydrochloric acid or 2 N sodium hydroxide.

Buffer stage medium: 28.4 g/L of dibasic sodium phosphate anhydrous and 6.4 g/L of sodium hydroxide; 200 mL

Buffer stage blank: 0.1 N hydrochloric acid and 0.2 M phosphate buffer (291:66). Adjust to a pH of 6.0 using either 2 N hydrochloric acid or 2 N sodium hydroxide.

Apparatus 2: 75 rpm

Time: 2 h. (The total time for this analysis is 4 h, where 2 h is from the *Acid stage* and 2 h is from the *Buffer stage*.)

Standard solution: 0.04 mg/mL of doxycycline from USP Doxycycline Hyclate RS in the *Buffer stage blank*. Sonicate as necessary to dissolve.

Buffer stage capsule shell blank: 100 mL of *Capsule shell solution* in 650 mL of *Acid stage medium* and 200 mL of *Buffer stage medium*. Adjust to a pH of 6.0 using either 2 N hydrochloric acid or 2 N sodium hydroxide, and pass through a suitable filter.

Sample solution: Pass portions of the solution under test through a suitable filter.

Blank: *Buffer stage*

Instrumental conditions

Mode: UV

Analytical wavelength: 345 nm

Cell: 0.2 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U - A_{CS})/A_S \times (C_S/L) \times V \times P \times F \times 100$$

A_U = absorbance of the *Sample solution*
A_{CS} = absorbance of the *Buffer stage capsule shell blank*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = total volume of dissolution medium (volume of medium from the *Acid stage* and the *Buffer stage*)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Tolerances: NLT 80% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved. The percentage of the labeled amount of doxycycline dissolved at the time specified conforms to *Acceptance Table 1* in *Dissolution* ⟨711⟩.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Buffer, System suitability solution, and **Sample solution:** Proceed as directed in the *Assay*. Store the *System suitability solution* and *Sample solution* under refrigerated conditions, and protect from light.

Standard stock solution: Use the *Standard solution* as directed in the *Assay*.

Standard solution 1: 0.016 mg/mL of doxycycline from the *Standard stock solution* in 0.1 N hydrochloric acid. Protect this solution from light, and store under refrigerated conditions.

Standard solution 2: 0.8 µg/mL of doxycycline from *Standard solution 1* in 0.1 N hydrochloric acid. Protect this solution from light, and store under refrigerated conditions.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 7-µm packing L21

Column temperature: 60°

Autosampler temperature: 4°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 3 times the retention time of the doxycycline peak

System suitability

Samples: *System suitability solution, Standard solution 1,* and *Standard solution 2*

www.webofpharma.com

Suitability requirements
Resolution: NLT 3.0 between 4-epidoxycycline and doxycycline, *System suitability solution*
Tailing factor: NMT 2.0 for doxycycline, *System suitability solution*
Relative standard deviation: NMT 5.0% for doxycycline, *Standard solution 1*
Signal-to-noise ratio: NLT 10 for doxycycline, *Standard solution 2*

Analysis
Samples: *Standard solution 2* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of doxycycline from *Standard solution 2*
C_S = concentration of USP Doxycycline Hyclate RS in *Standard solution 2* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F_1 = conversion factor, 0.001 mg/µg
F_2 = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-Epidoxycycline[a]	0.4	0.6	0.35
Methacycline[b]	0.6	—	—
Doxycycline related compound A (6-epi-doxycycline)[b]	0.7	—	—
Doxycycline	1.0	—	—
Other individual impurity	—	1.0	0.2
Total impurities	—	—	0.5

[a] (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrate.
[b] Process-related impurity, monitored in the drug substance, and not included in the total impurities.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Doxycycline Hyclate RS

Doxycycline for Injection

DEFINITION

Doxycycline for Injection contains an amount of Doxycycline Hyclate equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Protect solutions containing doxycycline from light.
Solution A: Transfer 3.1 g of monobasic potassium phosphate, 0.5 g of edetate disodium, and 0.5 mL of triethylamine to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with 1 N sodium hydroxide to a pH of 8.5 ± 0.1. Pass through a suitable filter of 0.22-µm pore size.
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid
Standard solution: 0.12 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.
Sample solution: Nominally 0.1 mg/mL of doxycycline prepared as follows. Dissolve the contents of one container of Doxycycline for Injection using 1% of the final volume of *Diluent* and transfer to a suitable volumetric flask. Dilute with *Diluent* to volume and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.
Column: 2.1-mm × 5-cm; 1.7-µm packing L7. [NOTE—A 1.7-µm guard column with packing L7 was used during method validation.]
Column temperature: 60°
Flow rate: 0.6 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Doxycycline for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

www.webofpharma.com

P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Protect solutions containing doxycycline from light.

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in *Diluent*

System suitability stock solution 2: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*

System suitability solution: Transfer 5 mL of *System suitability stock solution 2* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in *Diluent*, add 0.5 mL of *System suitability stock solution 1*, and dilute with *Diluent* to volume. Pass the solution through a suitable filter and use the filtrate. This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. [NOTE—The solution is stable up to 14 days when stored in a refrigerator.]

Standard solution: 2.3 µg/mL of USP Doxycycline Hyclate RS in *Diluent*

Sample solution: Nominally 2.0 mg/mL of doxycycline prepared as follows. Dissolve the contents of one container of Doxycycline for Injection using 20% of the final volume of *Diluent* and transfer to a suitable volumetric flask. Dilute with *Diluent* to volume and mix.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 2.0 between doxycycline related compound A and doxycycline, *System suitability solution*

Relative standard deviation: NMT 5.0% for the doxycycline peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Doxycycline for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of doxycycline from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methacycline[a, b]	0.64	—
4-Epidoxycycline[c]	0.79	2.2
Doxycycline related compound A (6-epidoxycycline)[b, d]	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.5
Total impurities	—	5.5

[a] (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide.
[b] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.
[c] (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.
[d] (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements. If the membrane filtration test is used, use *Fluid D* instead of *Fluid A*.

• **PH** ⟨791⟩

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 1.8–3.3

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry 100 mg in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

Acceptance criteria: NMT 2.0% for the article containing added substances; NMT 4.0% for the article containing no added substances

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Contains NMT 1.14 USP Endotoxin Units/mg of doxycycline

• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and Clarity of Solutions*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*, protected from light.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Doxycycline Hyclate RS

USP Doxycycline Related Compound A RS

[NOTE—May be available as a free base or a hydrochloride salt.]

(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

$C_{22}H_{24}N_2O_8$ 444.43

(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide, monohydrochloride.

$C_{22}H_{24}N_2O_8 \cdot HCl$ 480.13

USP Methacycline Hydrochloride RS

USP Monographs

www.webofpharma.com

Doxycycline for Oral Suspension

DEFINITION

Doxycycline for Oral Suspension contains the equivalent of NLT 90.0% and NMT 125.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) when constituted as directed. It contains one or more suitable buffers, colors, diluents, flavors, and preservatives.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197A▲** (CN 1-May-2020)

Standard: Transfer about 25 mg of USP Doxycycline Monohydrate RS to a suitable flask. Add 25 mL of acetonitrile and mix for approximately 10 min with a magnetic stir bar. Pass through suitable filter paper (Whatman No. 40 or equivalent), and remove the solvent by natural evaporation.

Sample: Place an amount of Doxycycline for Oral Suspension, nominally equivalent to about 25 mg of doxycycline, in a suitable flask. Add 25 mL of acetonitrile and mix for approximately 10 min with a magnetic stir bar. Pass through suitable filter paper (Whatman No. 40 or equivalent), and remove the solvent by natural evaporation.

Analysis: Examine the spectra of the *Standard* and the *Sample* in the range between 2000 and 650 cm^{-1}.

Acceptance criteria: The *Sample* exhibits bands at about 1644, 1577, 1518, 1452, 1396, 1167, 995, and 952 cm^{-1}, similar to the *Standard*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Protect solutions containing doxycycline from light.

Solution A: Transfer 3.1 g of monobasic potassium phosphate, 0.5 g of edetate disodium, and 0.5 mL of triethylamine to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with 1 N sodium hydroxide to a pH of 8.5 ± 0.2.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid

Standard solution: 0.3 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.

Sample solution: Nominally 0.25 mg/mL of doxycycline in *Diluent*, prepared as follows. Constitute Doxycycline for Oral Suspension as directed in the labeling. Transfer an accurately measured portion of the constituted suspension, freshly mixed and free from air bubbles, equivalent to about 25 mg of doxycycline, to a suitable volumetric flask. Add 50% of the final volume of *Diluent*, shake by mechanical means for about 15 min and dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 0.2-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 350 nm
Column: 2.1-mm × 5-cm; 1.7-µm packing L7. [NOTE—A 1.7-µm guard column with packing L7 was used during method validation.]
Column temperature: 60°
Flow rate: 0.6 mL/min
Injection volume: 5 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Doxycycline for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–125.0%

PERFORMANCE TESTS

- **DELIVERABLE VOLUME ⟨698⟩:** Meets the requirements
- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩**

For single-unit containers
Acceptance criteria: Meets the requirements

- **DISSOLUTION ⟨711⟩**

Protect solutions containing doxycycline from light.
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 25 rpm
Time: 10 min

Solution A: Transfer 1.36 g of monobasic potassium phosphate, 0.37 g of sodium hydroxide, 0.25 g of tetrabutylammonium hydrogen sulfate, and 0.2 g of edetate disodium to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Add 75 g of *tert*-butyl alcohol and dilute with water to volume. Adjust with 1 N sodium hydroxide or 5 N hydrochloric acid to a pH of 8.0 ± 0.05.

Solution B: Acetonitrile and water (80:20)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
13.0	100	0
15.0	20	80
21.0	20	80

Return to original conditions and equilibrate the system.

USP Monographs

www.webofpharma.com

Standard solution: 32 µg/mL of USP Doxycycline Hyclate RS in *Medium*

Sample solution: Reconstitute the suspension according to the label instructions for three separate containers. Transfer and combine the contents of the three containers into a separate suitable flask. Determine the density of the suspension. Using suitable syringes, measure portions of the reconstituted suspension containing nominally 25 mg of doxycycline. With the paddles lowered, gently empty the contents of each syringe into each vessel containing *Medium*. Start the paddle rotation. At the specified time, sample per ⟨711⟩ and pass a portion through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 355 nm

Column: 4.6-mm × 25-cm; 5-µm packing L21

Temperatures

Autosampler: 10°

Column: 60°

Flow rate: 1.5 mL/min

Injection volume: 100 µL

Run time: 30 min

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times (d/W) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of doxycycline in the *Standard solution* (mg/mL)

L = label claim of Doxycycline for Oral Suspension (mg/5 mL)

D = dilution factor, necessary only if the *Sample solution* requires dilution (mL/mL)

d = density of the *Sample solution* (g/mL)

W = weight of Doxycycline for Oral Suspension taken (g)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in *Diluent*

System suitability stock solution 2: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*

System suitability solution: Transfer 5 mL of *System suitability stock solution 2* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in *Diluent*, add 0.5 mL of *System suitability stock solution 1*, and dilute with *Diluent* to volume. Pass the solution through a suitable filter and use the filtrate. This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

Standard solution: 1.5 µg/mL of USP Doxycycline Hyclate RS in *Diluent*

Sample solution: Constitute Doxycycline for Oral Suspension as directed in the labeling. Nominally, a 0.25-mg/mL solution of doxycycline in *Diluent* is prepared as follows. Transfer an accurately measured portion of the constituted suspension, freshly mixed and free from air bubbles, equivalent to about 25 mg of doxycycline, to a 100-mL volumetric flask. Add 50 mL of *Diluent* and shake by mechanical means for about 15 min. Dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 0.2-µm pore size.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; and NLT 2.0 between doxycycline related compound A and doxycycline, *System suitability solution*

Relative standard deviation: NMT 5.0% for the doxycycline peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Doxycycline for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of doxycycline from the *Standard solution*

C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 3*. Disregard any impurity peak less than 0.1%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methacycline[a, b]	0.64	—
4-Epidoxycycline[c]	0.79	1.5
Doxycycline related compound A (6-epidoxycycline)[a, d]	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.20
Total impurities	—	2.5

[a] Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total impurities.

[b] (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide.

[c] (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

[d] (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

www.webofpharma.com

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: 5.0–6.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store dry powder at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Doxycycline Hyclate RS
USP Doxycycline Monohydrate RS
USP Doxycycline Related Compound A RS
[NOTE—May be available as a free base or a hydrochloride salt.]
(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.
$C_{22}H_{24}N_2O_8$ 444.43
(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide, monohydrochloride.
$C_{22}H_{24}N_2O_8 \cdot HCl$ 480.13
USP Methacycline Hydrochloride RS

Doxycycline Tablets

DEFINITION

Doxycycline Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Protect solutions containing doxycycline from light.
Solution A: Transfer 3.1 g of monobasic potassium phosphate, 0.5 g of edetate disodium, and 0.5 mL of triethylamine to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with 1 N sodium hydroxide to a pH of 8.5 ± 0.1.
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid
Standard solution: 0.12 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.
Sample solution: Nominally 0.1 mg/mL of doxycycline from NLT 20 Tablets prepared as follows. Transfer a suitable portion of finely powdered Tablets to a suitable volumetric flask. Add 50% of the final volume of *Diluent*, dissolve, dilute with *Diluent* to volume, and mix well. Centrifuge a portion of the solution and use the supernatant. [NOTE—The use of a centrifuge speed at 3,000 rpm for 10 min may be suitable.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm. For *Identification B*, a diode array detector may be used in the wavelength range of 200–400 nm.
Column: 2.1-mm × 5-cm; 1.7-µm packing L7. [NOTE—A 1.7-µm guard column with packing L7 was used during method validation.]
Column temperature: 60°
Flow rate: 0.6 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
▲Test 1▲ (RB 1-Apr-2018)
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 60 min
Standard solution: 0.01 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute a portion of the filtrate with *Medium* to a concentration that is similar to that of the *Standard solution*.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 268 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times P \times 100$$

www.webofpharma.com

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)

Tolerances: NLT 85% *(Q)* of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

▲**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Protect solutions containing doxycycline from light.
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm
Time: 15 min
Standard solution: 0.01 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute a portion of the filtrate with *Medium* to a concentration that is similar to that of the *Standard solution*.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 268 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times P \times F \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
D = dilution factor for the *Sample solution*, if applicable
V = volume of *Medium*, 900 mL
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Tolerances: NLT 80% *(Q)* of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved. ▲ (RB 1-Apr-2018)

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Protect solutions containing doxycycline from light.
Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in *Diluent*
System suitability stock solution 2: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*
System suitability solution: Transfer 5 mL of *System suitability stock solution 2* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in *Diluent*, add 0.5 mL of *System suitability stock solution 1*, and dilute with *Diluent* to volume. Pass the solution through a suitable filter and use the filtrate. This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. [Note—The solution is stable up to 14 days when stored in a refrigerator at 2°–8°.]
Standard solution: 7.0 µg/mL of USP Doxycycline Hyclate RS in *Diluent*
Sample solution: Nominally 2.0 mg/mL of doxycycline from NLT 20 Tablets prepared as follows. Transfer a suitable portion of finely powdered Tablets to a suitable volumetric flask. Add 50% of the final volume of *Diluent*, dissolve, dilute with *Diluent* to volume, and mix well. Centrifuge a portion of the solution and use the supernatant. [Note—The use of a centrifuge speed at 3,000 rpm for 10 min may be suitable.]
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 2.0 between doxycycline related compound A and doxycycline, *System suitability solution*
Relative standard deviation: NMT 5.0% for the doxycycline peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of doxycycline from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methacycline[a, b]	0.64	—
4-Epidoxycycline[c]	0.79	1.5
Doxycycline related compound A (6-epidoxycycline)[b, d]	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.3

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	2.5

[a] (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide.
[b] Process impurities that are controlled in the drug substance are not to be reported. They are not to be included in total impurities. They are listed here for information only.
[c] (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide. Main degradation product.
[d] (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

Add the following:

▲• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.▲ (RB 1-Apr-2018)

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Doxycycline Hyclate RS
  USP Doxycycline Related Compound A RS
  [NOTE—May be available as a free base or a hydrochloride salt.]
  (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.
  $C_{22}H_{24}N_2O_8$ 444.43
  (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide, monohydrochloride.
  $C_{22}H_{24}N_2O_8 \cdot HCl$ 480.13
  USP Methacycline Hydrochloride RS

Doxycycline Calcium Oral Suspension

DEFINITION

Doxycycline Calcium Oral Suspension is prepared from Doxycycline Hyclate and contains one or more suitable buffers, colors, diluents, flavors, and preservatives. It contains the equivalent of NLT 90.0% and NMT 125.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Protect solutions containing doxycycline from light.

Solution A: Transfer 3.1 g of monobasic potassium phosphate, 0.5 g of edetate disodium, and 0.5 mL of triethylamine to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with 1 N sodium hydroxide to a pH of 8.5 ± 0.1. Pass through a suitable filter of 0.22-µm pore size.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid

Standard solution: 0.12 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.

Sample solution: Nominally 0.1 mg/mL of doxycycline in *Diluent*, prepared as follows. Transfer an adequate amount of Oral Suspension, freshly mixed and free from air bubbles, to a suitable volumetric flask. Add 80% of the final volume of *Diluent*, sonicate for about 15 min, and dilute with *Diluent* to volume. Centrifuge a portion of the solution for 10 min at 3000 rpm and use the supernatant for analysis.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm. For *Identification A*, use a diode array detector in the range of 200-400 nm.
Column: 2.1-mm × 5-cm; 1.7-µm packing L7
[NOTE—A 1.7-µm guard column with packing L7 was used during method validation.]
Column temperature: 60°
Flow rate: 0.6 mL/min
Injection volume: 5 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–125.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  For single-unit containers
  Acceptance criteria: Meets the requirements
- **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

USP Monographs

www.webofpharma.com

IMPURITIES

• **ORGANIC IMPURITIES**

Protect solutions containing doxycycline from light.

Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in *Diluent*

System suitability stock solution 2: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*

System suitability solution: Transfer 5 mL of *System suitability stock solution 2* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in *Diluent*, add 0.5 mL of *System suitability stock solution 1*, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.20-µm pore size and use the filtrate. This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. [NOTE—The solution is stable up to 14 days when stored in a refrigerator.]

Standard solution: 2.3 µg/mL of USP Doxycycline Hyclate RS in *Diluent*

Sample solution: Nominally 2.0 mg/mL of doxycycline in *Diluent*, prepared as follows. Transfer an adequate amount of Oral Suspension, freshly mixed and free from air bubbles, to a suitable volumetric flask. Add 60% of the final volume of *Diluent*, sonicate for about 15 min, and dilute with *Diluent* to volume. Centrifuge a portion of the solution for 10 min at 3000 rpm and use the supernatant for analysis.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; and NLT 2.0 between doxycycline related compound A and doxycycline, *System suitability solution*

Relative standard deviation: NMT 5.0% for doxycycline, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of doxycycline from the *Standard solution*

C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methacycline[a, b]	0.64	—
4-Epidoxycycline[c]	0.79	0.5
Doxycycline related compound A (6-epidoxycycline)[b, d]	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.5

[a] (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide.

[b] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

[c] (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

[d] (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

SPECIFIC TESTS

• **pH** ⟨791⟩: 6.5–8.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Doxycycline Hyclate RS

USP Doxycycline Related Compound A RS

[NOTE—May be available as a free base or a hydrochloride salt.]

(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

$C_{22}H_{24}N_2O_8$ 444.43

(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride.

$C_{22}H_{24}N_2O_8 \cdot HCl$ 480.13

USP Methacycline Hydrochloride RS

Doxycycline Compounded Oral Suspension, Veterinary

DEFINITION

Change to read:

Doxycycline Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$). Prepare Oral Suspension, Veterinary 50 mg/mL ▲in a paraben solution▲ (USP 1-May-2020) as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩). For this preparation, a *Paraben solution* needs to be separately prepared.

Methylparaben	25 mg
Propylparaben	12.5 mg

www.webofpharma.com

Purified Water, a sufficient quantity to make	50 mL

Prepare a *Paraben solution* by dissolving the *Methylparaben* and *Propylparaben* in a sufficient amount of Purified Water to bring to final volume.

Doxycycline (as Doxycycline Hyclate) powder ▲▲ (USP 1-May-2020)	2.5 g of doxycycline (calculate)[a]
Calcium Hydroxide ▲▲ (USP 1-May-2020)	Calculate (see below)[b]
Glycerin	12.5 mL
Polysorbate 20 ▲▲ (USP 1-May-2020)	0.063 mL
Sodium Metabisulfite ▲▲ (USP 1-May-2020) (granular)	0.05 g
Carboxymethylcellulose Sodium ▲▲ (USP 1-May-2020) (medium viscosity)	0.25 g
Steviol Glycosides 95%	0.15 g
Paraben solution, a sufficient quantity to make	50 mL

[a] Calculate the amount of *Doxycycline Hyclate* powder required by dividing the weight of doxycycline required by the potency of the *Doxycycline Hyclate* powder obtained from the Certificate of Analysis. ▲ [NOTE—Unit conversion is needed in the calculation.]▲ (USP 1-May-2020)

[b] Calculate the amount of *Calcium Hydroxide* needed, in grams, by multiplying the amount of *Doxycycline Hyclate* needed, in grams, by 0.21.

Pour the calculated amount of *Doxycycline Hyclate* powder into a suitable mortar, and mix with about 12.5 mL of *Paraben solution* until all the solids are dissolved. Do not use more than 5 mL of *Paraben solution* for each gram of the calculated amount of *Doxycycline Hyclate*. Add the calculated amount of *Calcium Hydroxide* to the mortar, and mix with the pestle until the mixture thickens up and has a pasty consistency. [NOTE—Reaction may take a few minutes to occur. The pasty mixture may look dry and harden up which is an indication that the reaction is complete.]

In a separate beaker, mix *Glycerin* and *Polysorbate 20* with about 30 mL of the *Paraben solution*. Add *Sodium Metabisulfite* to the mixture of *Glycerin*, *Polysorbate 20*, and *Paraben solution* with mixing.

Add approximately two thirds of the separately prepared mixture of *Glycerin*, *Polysorbate 20*, *Sodium Metabisulfite*, and *Paraben solution* to the mortar in small increments with continuous mixing to make an even mixture free of lumps. Transfer the doxycycline mixture from the mortar to an appropriately sized beaker. Rinse the mortar three times, each time with one third of the remaining *Glycerin*, *Polysorbate 20*, *Sodium Metabisulfite*, and *Paraben solution*, and combine with the doxycycline mixture in the beaker. Add *Steviol Glycosides 95%* to the beaker. Disperse *Carboxymethylcellulose Sodium* through a 40-mesh sieve, and mix into the suspension until homogenous. Add sufficient *Paraben solution* to bring to final volume. Mix well.

▲Prepare Oral Suspension, Veterinary 100 mg/mL in a fixed oil base as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Doxycycline (as Doxycycline Hyclate) powder	10 g of doxycycline (calculate)[a]
Steviol Glycosides 95%	0.3 g
Acesulfame Potassium	0.3 g
Flavor (chicken, grilled, natural, oil miscible)[b]	3 mL
PCCA Fixed Oil Suspension Vehicle,[b] a sufficient quantity to make	100 mL

[a] Calculate the amount of *Doxycycline Hyclate* powder required by dividing the weight of doxycycline required by the potency of the *Doxycycline Hyclate* powder obtained from the Certificate of Analysis. [NOTE—Unit conversion is needed in the calculation.]

[b] PCCA, Houston, TX.

Place the calculated amount of *Doxycycline Hyclate* powder, *Steviol Glycosides 95%*, and *Acesulfame Potassium* into a suitable container and triturate to a fine powder. Add a sufficient amount of *PCCA Fixed Oil Suspension Vehicle* to form a smooth paste. Add *Flavor* and mix thoroughly. Add a sufficient amount of *PCCA Fixed Oil Suspension Vehicle* to make the contents pourable. Transfer the contents, stepwise and quantitatively, to a calibrated container using the *PCCA Fixed Oil Suspension Vehicle*. Add sufficient *PCCA Fixed Oil Suspension Vehicle* to bring to final volume, and mix well.▲ (USP 1-May-2020)

ASSAY

Change to read:

- **PROCEDURE ▲1: ORAL SUSPENSION IN PARABEN SOLUTION▲** (USP 1-May-2020)

Solution A: 20 mM monobasic sodium phosphate containing 0.74 g/L of sodium hydroxide. Adjust with phosphoric acid to a pH of 7.0.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Acetonitrile (%)	Solution A (%)
0	1	99
7	80	20
8	80	20
8.1	1	99
14	1	99

Diluent: Acetonitrile and 1 N hydrochloric acid (50:50)

Standard solution: 0.5 mg/mL of doxycycline prepared from USP Doxycycline Hyclate RS in *Diluent*

Sample solution: Shake each bottle of Oral Suspension, Veterinary thoroughly. Transfer 0.5 mL of Oral Suspension, Veterinary to a 50-mL volumetric flask, add approximately 10 mL of *Diluent*, and vortex for 30 s. Dilute with *Diluent* to volume, and mix well.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for doxycycline is about 4.7 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of doxycycline from the *Sample solution*
r_S = peak response of doxycycline from the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

Add the following:

▲• **PROCEDURE 2: ORAL SUSPENSION IN FIXED OIL**
Solution A: 1 mg/mL of trifluoroacetic acid in water
Solution B: 1 mg/mL of trifluoroacetic acid in acetonitrile
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
2.6	70	30
2.8	10	90
3.0	90	10

Standard solution: 0.125 mg/mL of doxycycline prepared from USP Doxycycline Hyclate RS in methanol
Sample solution: Transfer 0.5 mL of Oral Suspension, Veterinary to a centrifuge tube and add 39.5 mL of methanol. Vortex for 1 min, sonicate for 2 min, and vortex again for 1 min until the solution is mixed well. Centrifuge this solution for 10 min. Transfer 1 mL of the supernatant to a 10-mL volumetric flask and add sufficient methanol to bring to volume. Centrifuge again for 10 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 350 nm
Column: 2.1-mm × 50-mm; 1.7-µm packing L1
Autosampler temperature: 4°
Flow rate: 0.7 mL/min
Injection volume: 1.0 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for doxycycline is about 2.7 min.]
Suitability requirements
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of doxycycline from the *Sample solution*
r_S = peak response of doxycycline from the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% ▲(USP 1-May-2020)

SPECIFIC TESTS

Change to read:

• **pH** ⟨791⟩
▲**Oral Suspension in paraben solution:** ▲(USP 1-May-2020)
6.0–7.0

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. ▲Store the Oral Suspension, Veterinary in paraben solution in a refrigerator. Store the Oral Suspension, Veterinary in fixed oil at controlled room temperature, or in a refrigerator.▲ (USP 1-May-2020)
• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*. Label it to state that it is for veterinary use only.

Change to read:

• **BEYOND-USE DATE**
▲**Oral Suspension, Veterinary in paraben solution:** NMT 30 days after the date on which it was compounded when stored in a refrigerator
Oral Suspension, Veterinary in fixed oil: NMT 60 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator▲ (USP 1-May-2020)
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Doxycycline Hyclate RS

Doxycycline Hyclate

$(C_{22}H_{24}N_2O_8 \cdot HCl)_2 \cdot C_2H_6O \cdot H_2O$ 1025.87
2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride, compound with ethanol (2:1), monohydrate, [4*S*-(4α,4aα,5α,5aα,6α,12aα)]-;
4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride, compound with ethyl alcohol (2:1), monohydrate [24390-14-5].

DEFINITION
Doxycycline Hyclate has a potency equivalent to NLT 800 µg/mg and NMT 920 µg/mg of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

www.webofpharma.com

ASSAY

• **PROCEDURE**

Mobile phase: Transfer 2.72 g of monobasic potassium phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutylammonium hydrogen sulfate, and 0.40 g of edetate disodium to a 1000-mL volumetric flask. Add 850 mL of water, and stir to dissolve. Add 60 g of tertiary butyl alcohol with the aid of water, dilute with water to volume, and adjust with 1 N sodium hydroxide to a pH of 8.0 ± 0.1. Pass this solution through a filter of 0.5-µm or finer pore size, and degas before using. Decreasing the proportion of tertiary butyl alcohol results in a longer retention time of doxycycline and improved separation of doxycycline from the related compounds.

Diluent: 0.01 N hydrochloric acid

System suitability stock solution: 6 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Diluent*

System suitability solution: Transfer 5 mL of *System suitability stock solution* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in 0.01 N hydrochloric acid, and dilute with *Diluent* to volume. Pass a portion of this solution through a filter of 0.5-µm or finer pore size, and use the filtrate. This solution contains a mixture of 4-epidoxycycline, 6-epidoxycycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

Standard solution: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve. Protect the *Standard solution* from light.

Sample solution: 1.2 mg/mL of Doxycycline Hyclate in *Diluent*. Pass through a membrane filter of 0.5-µm or finer pore size. Protect the *Sample solution* from light.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; packing L21

Column temperature: 60 ± 1°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 1.7 times the retention time of doxycycline

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between the 4-epidoxycycline peak and the doxycycline peak, *System suitability solution*

Tailing factor: NMT 2.0 for doxycycline, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity of doxycycline ($C_{22}H_{24}N_2O_8$), in µg/mg, in the portion of Doxycycline Hyclate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)

Acceptance criteria: 800–920 µg/mg

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution 1: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve. Protect *Standard solution 1* from light.

Standard solution 2: 0.024 mg/mL each of USP Doxycycline Hyclate RS and USP Methacycline Hydrochloride RS in *Diluent*. Protect *Standard solution 2* from light.

System suitability

Samples: *System suitability solution* and *Standard solution 1*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between 4-epidoxycycline and doxycycline, *System suitability solution*

Tailing factor: NMT 2.0 for doxycycline, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution 1*

Analysis

Samples: *Standard solution 2* and *Sample solution*

Calculate the percentage of methacycline in the portion of Doxycycline Hyclate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methacycline from the *Sample solution*

r_S = peak response of methacycline from *Standard solution 2*

C_S = concentration of USP Methacycline Hydrochloride RS in *Standard solution 2* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Calculate the percentage of each related compound, other than methacycline, in the portion of Doxycycline Hyclate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of doxycycline from *Standard solution 2*

C_S = concentration of USP Doxycycline Hyclate RS in *Standard solution 2* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
4-Epidoxycycline[a]	0.4	0.5
Methacycline	0.6	2
6-Epidoxycycline	0.7	2
Doxycycline	1.0	—
Individual unspecified impurities[b]	—	0.5

[a] Main degradation product.

[b] Includes impurities that elute before methacycline and those that elute after doxycycline.

www.webofpharma.com

SPECIFIC TESTS
- **CRYSTALLINITY** ⟨695⟩: Meets the requirements
- **pH** ⟨791⟩
  Sample solution: Nominally 10 mg/mL of doxycycline from Doxycycline Hyclate
  Acceptance criteria: 2.0–3.0
- **WATER DETERMINATION,** *Method I* ⟨921⟩: 1.4%–2.8%
- **STERILITY TESTS** ⟨71⟩: Where the label states that Doxycycline Hyclate is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*, with *Fluid D* being used instead of *Fluid A*.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Doxycycline Hyclate is sterile or it must be subjected to further processing during the preparation of injectable dosage forms, it has NMT 1.14 USP Endotoxin Units/mg of doxycycline.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Doxycycline Hyclate RS
  USP Methacycline Hydrochloride RS

Doxycycline Hyclate Capsules

DEFINITION
Doxycycline Hyclate Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A**▲ (CN 1-May-2020)
  Standard solution: Transfer about 25 mg of USP Doxycycline Hyclate RS to a suitable flask. Add 25 mL of *acetonitrile* and mix for approximately 5 min with a magnetic stir bar. Pass the solution through a suitable filter, and remove the solvent by natural evaporation or by using a rotary evaporator under vacuum.
  Sample solution: Transfer the contents of NLT 10 Capsules, equivalent to 25 mg of doxycycline hyclate, to a suitable flask. Add 25 mL of *acetonitrile* and mix for approximately 5 min with a magnetic stir bar. Pass the solution through a suitable filter, and remove the solvent by natural evaporation or by using a rotary evaporator under vacuum.
  Analysis: Examine the spectra of the *Standard solution* and the *Sample solution* in the range between 2000 and 650 cm^{-1}.
  Acceptance criteria: The *Sample solution* exhibits bands at about 1663, 1611, 1576, 1453, 1213, 1037, 1002, 935, and 659 cm^{-1}, similar to the *Standard solution*.

ASSAY
- **PROCEDURE**
  Protect solutions containing doxycycline from light.

Solution A: Transfer 3.1 g of monobasic potassium phosphate, 0.5 g of edetate disodium, and 0.5 mL of triethylamine to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with *1 N sodium hydroxide VS* to a pH of 8.5 ± 0.2.
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid
System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in *Diluent*
System suitability stock solution 2: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*
System suitability solution: Transfer 5 mL of *System suitability stock solution 2* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in *Diluent*, add 0.5 mL of *System suitability stock solution 1*, and dilute with *Diluent* to volume. Pass the solution through a suitable filter and use the filtrate. This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.
Standard solution: 0.3 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.
Sample solution: Nominally 0.25 mg/mL of doxycycline in *Diluent*, prepared as follows. Empty as completely as possible the contents of NLT 20 Capsules. Mix the combined contents and transfer a suitable portion of the powder to a suitable volumetric flask. Add 75% of the final volume of *Diluent*, sonicate for about 5 min, shake for about 15 min, and dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 0.2-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 350 nm
Column: 2.1-mm × 5-cm; 1.7-µm packing L7. [NOTE—A 1.7-µm guard column with packing L7 was used during method validation.]
Column temperature: 60°
Flow rate: 0.6 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 1.5 between doxycycline related compound A and doxycycline, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 75 rpm, the distance between the blade and the inside bottom of the vessel being maintained at 4.5 ± 0.5 cm during the test
Time: 30 min
Standard solution: USP Doxycycline Hyclate RS in *Medium*
Sample solution: Dilute with *Medium* to a concentration that is similar to the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: 276 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1.5 µg/mL of USP Doxycycline Hyclate RS in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 1.5 between doxycycline related compound A and doxycycline, *System suitability solution*
Relative standard deviation: NMT 5.0% for the doxycycline peak, *Standard solution*

Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of doxycycline from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.2%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methacycline[a, b]	0.64	—
4-Epidoxycycline[c]	0.79	1.0
Doxycycline related compound A (6-epidoxycycline)[a, d]	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.5
Total impurities	—	2.0

[a] Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total impurities.
[b] (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide.
[c] (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.
[d] (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Doxycycline Hyclate RS
USP Doxycycline Related Compound A RS
[Note—May be available as a free base or a hydrochloride salt.]
(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.
$C_{22}H_{24}N_2O_8$ 444.43
(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide, monohydrochloride.
$C_{22}H_{24}N_2O_8 \cdot HCl$ 480.13
USP Methacycline Hydrochloride RS

www.webofpharma.com

Doxycycline Hyclate Delayed-Release Capsules

DEFINITION

Doxycycline Hyclate Delayed-Release Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

• **A.**

Test solution: Nominally 1 mg/mL of doxycycline in methanol from finely powdered Capsule contents. Pass through a filter, and use the filtrate.

Analysis: Proceed as directed in *Identification—Tetracyclines* ⟨193⟩, *Method II.*

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Transfer 2.72 g of monobasic potassium phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutylammonium hydrogen sulfate, and 0.40 g of edetate disodium to a 1000-mL volumetric flask. Add 850 mL of water, and stir to dissolve. Add 60 g of tertiary butyl alcohol with the aid of water, dilute with water to volume, and adjust with 1 N sodium hydroxide to a pH of 8.0 ± 0.1. Pass this solution through a filter of 0.5-µm or finer pore size, and degas before using. Decreasing the proportion of tertiary butyl alcohol results in a longer retention time of doxycycline and improved separation of doxycycline from the related compounds.

Diluent: 0.01 N hydrochloric acid

System suitability stock solution: 6 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Diluent*

System suitability solution: Transfer 5 mL of *System suitability stock solution* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in 0.01 N hydrochloric acid, and dilute with *Diluent* to volume. Pass a portion of this solution through a filter of 0.5-µm or finer pore size, and use the filtrate. This solution contains a mixture of 4-epidoxycycline, 6-epidoxycycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

Standard solution: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve. Protect the *Standard solution* from light.

Sample solution: Nominally 1 mg/mL of doxycycline in *Diluent*, prepared as follows. Remove as completely as possible the contents of NLT 20 Capsules. Mix the combined contents, and transfer a suitable portion of the powder to a suitable volumetric flask. Add 75% of the final volume of *Diluent*, sonicate for 5 min, shake for 15 min, and dilute with *Diluent* to volume. Pass through a membrane filter of 0.5-µm or finer pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; packing L21

Column temperature: 60 ± 1°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 1.7 times the retention time of doxycycline

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 4-epidoxycycline (the main degradation product), 6-epidoxycycline, and doxycycline are about 0.4, 0.7, and 1.0, respectively, *System suitability solution.*]

Suitability requirements

Resolution: NLT 3.0 between the 4-epidoxycycline peak and the doxycycline peak, *System suitability solution*

Tailing factor: NMT 2.0 for doxycycline, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩**:** Proceed as directed for *Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B, Procedure.*

Acid stage

Conduct the test by transferring the contents of each Capsule to the individual basket units of the apparatus.

Medium: 0.06 N hydrochloric acid; 900 mL

Apparatus 1: 50 rpm

Time: 20 min

Detector: UV 345 nm

Diluent: 0.1 N hydrochloric acid

Standard solution: 10 µg/mL of USP Doxycycline Hyclate RS in *Diluent*

Sample solution: Dilute filtered portions of the solution under test with *Diluent* to a concentration that is similar to the *Standard solution.*

Tolerances

Level 1 (6 Capsules tested): No individual value exceeds 50% dissolved.

Level 2 (6 Capsules tested): NMT 2 individual values of 12 tested are greater than 50% dissolved.

Buffer stage

Conduct this stage of testing on separate specimens, selecting Capsules that were not previously subjected to *Acid stage* testing and transferring the contents of each Capsule to the individual basket units of the apparatus.

Medium: pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 1000 mL

Apparatus 1: 50 rpm

Time: 30 min

Detector: UV 345 nm

Diluent: 0.1 N hydrochloric acid

Standard solution: 10 µg/mL of USP Doxycycline Hyclate RS in *Diluent*

Sample solution: Dilute filtered portions of the solution under test with *Diluent* to a concentration that is similar to the *Standard solution.*

www.webofpharma.com

Tolerances: NLT 85% (*Q*) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 5.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** The label indicates that the contents of the Capsules are enteric coated.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Doxycycline Hyclate RS

Doxycycline Hyclate Tablets

DEFINITION

Doxycycline Hyclate Tablets contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A**▲ (CN 1-May-2020)

Standard solution: Transfer about 25 mg of USP Doxycycline Hyclate RS to a suitable flask. Add 25 mL of *acetonitrile* and mix for approximately 5 min with a magnetic stir bar. Pass the solution through a suitable filter, and remove the solvent by natural evaporation or by using a rotary evaporator under vacuum.

Sample solution: Transfer powdered Tablets (NLT 25), equivalent to 25 mg of doxycycline hyclate, to a suitable flask. Add 25 mL of *acetonitrile* and mix for approximately 5 min with a magnetic stir bar. Pass the solution through a suitable filter, and remove the solvent by natural evaporation or by using a rotary evaporator under vacuum.

Analysis: Examine the spectra of the *Standard solution* and the *Sample solution* in the range between 2000 and 650 cm^{-1}.

Acceptance criteria: The *Sample solution* exhibits bands at about 1663, 1611, 1576, 1453, 1213, 1037, 1002, 935, and 659 cm^{-1}, similar to the *Standard solution*.

ASSAY

- **PROCEDURE**

Protect solutions containing doxycycline from light.

Solution A: Transfer 3.1 g of monobasic potassium phosphate, 0.5 g of edetate disodium, and 0.5 mL of triethylamine to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with 1 N sodium hydroxide to a pH of 8.5 ± 0.2.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid

System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in *Diluent*

System suitability stock solution 2: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*

System suitability solution: Transfer 5 mL of *System suitability stock solution 2* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in *Diluent*, add 0.5 mL of *System suitability stock solution 1*, and dilute with *Diluent* to volume. Pass the solution through a suitable filter and use the filtrate. This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days.

Standard solution: 0.3 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.

Sample solution: Nominally 0.25 mg/mL of doxycycline in *Diluent*, prepared as follows. Transfer a suitable portion of NLT 20 finely powdered Tablets to a suitable volumetric flask. Add 50% of the final volume of *Diluent*, sonicate for about 5 min, shake for about 15 min, and dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 0.2-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 350 nm

Column: 2.1-mm × 5-cm; 1.7-µm packing L7. [NOTE—A 1.7-µm guard column with packing L7 was used during method validation.]

Column temperature: 60°

Flow rate: 0.6 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solution* [NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 1.5 between doxycycline related compound A and doxycycline, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)

www.webofpharma.com

C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Protect solutions containing doxycycline from light.
Test 1
Medium: Water; 900 mL
Apparatus 2: 75 rpm, the distance between the blade and the inside bottom of the vessel being maintained at 4.5 ± 0.5 cm during the test
Time: 90 min
Standard solution: USP Doxycycline Hyclate RS in *Medium*
Sample solution: Dilute with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 276 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm, the distance between the blade and the inside bottom of the vessel being maintained at 4.5 ± 0.5 cm during the test
Time: 30 min
Standard solution: 22 µg/mL of doxycycline from USP Doxycycline Hyclate RS, in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Blank: *Medium*
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 276 nm
Cell: 0.5 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of doxycycline is dissolved.
▲**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Protect solutions containing doxycycline from light.
Medium: Water; 900 mL
Apparatus 2: 75 rpm
Time: 30 min
Standard solution: 0.016 mg/mL of doxycycline from USP Doxycycline Hyclate RS, in *Medium*. Sonicate, if necessary, in a cool water bath.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and dilute with *Medium*, to a concentration that is similar to the *Standard solution*.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 276 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved. ▲ (RB 1-Nov-2018)
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1.5 µg/mL of USP Doxycycline Hyclate RS in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 1.5 between doxycycline related compound A and doxycycline, *System suitability solution*
Relative standard deviation: NMT 5.0% for the doxycycline peak, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

USP Monographs

www.webofpharma.com

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of doxycycline from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.2%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methacycline[a, b]	0.64	—
4-Epidoxycycline[c]	0.79	1.5
Doxycycline related compound A (6-epidoxycycline)[a, d]	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.5
Total impurities	—	2.0

[a] Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total impurities.
[b] (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide.
[c] (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.
[d] (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Doxycycline Hyclate RS
  USP Doxycycline Related Compound A RS
  [NOTE—May be available as a free base or a hydrochloride salt.]
  (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.
  $C_{22}H_{24}N_2O_8$ 444.43
  (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide, monohydrochloride.
  $C_{22}H_{24}N_2O_8 \cdot HCl$ 480.13
  USP Methacycline Hydrochloride RS

Doxycycline Hyclate Delayed-Release Tablets

DEFINITION

Doxycycline Hyclate Delayed-Release Tablets contain an amount of Doxycycline Hyclate equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

- **PROCEDURE**

▲Protect solutions containing doxycycline from light.

Solution A: Transfer 3.1 g of monobasic potassium phosphate, 0.5 g of edetate disodium, and 0.5 mL of triethylamine to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with 1 N sodium hydroxide to a pH of 8.5 ± 0.1. Pass through a suitable filter of 0.22-µm pore size.
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid
Standard solution: 0.12 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.
Sample solution: Nominally 0.1 mg/mL of doxycycline from NLT 20 Tablets prepared as follows. Transfer a suitable portion of finely powdered Tablets to a suitable volumetric flask. Add 80% of the final volume of *Diluent*, sonicate for about 15 min, shake for about 15 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.2-µm pore size and use the filtrate for analysis.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 2.1-mm × 5-cm; 1.7-µm packing L7. [NOTE—A 1.7-µm guard column with packing L7 was used during method validation.]
Column temperature: 60°
Flow rate: 0.6 mL/min
Injection volume: 5 µL

USP Monographs

www.webofpharma.com

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg ▲(USP 1-May-2019)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
▲Protect solutions containing doxycycline from light.▲ (USP 1-May-2019)
Test 1: Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure.*
Acid stage
Medium: 0.06 N hydrochloric acid; 900 mL, degassed with helium
Apparatus 1: 50 rpm
Time: 20 min
Standard solution: 0.128 mg/mL of USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS. [NOTE—Sonicate if necessary to dissolve.]
Sample solution: Pass portions of the solution under test through a suitable PVDF filter of 0.45-µm pore size.
Detector: UV 346 nm
Cell: 0.1-cm quartz
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances
Level 1 (6 Tablets tested): No individual value is more than 30% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved in 20 min.
Level 2 (6 Tablets tested): NMT 2 individual values of the 12 tested are greater than 30% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in 20 min.
Buffer stage
Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.
Medium: pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Solutions, Buffer Solutions*); 900 mL, degassed
Apparatus 1: 50 rpm
Time: 30 min
Standard solution: 0.128 mg/mL of USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS. [NOTE—Sonicate if necessary to dissolve.]
Sample solution: Pass portions of the solution under test through a suitable PVDF filter of 0.45-µm pore size.
Analysis: Determine the percentage of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved by the procedure described for the *Acid stage*.
Tolerances: NLT 85% (Q) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*. Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure.*
Acid stage
Medium, Apparatus 1, Time, Blank, and **Analysis:** Proceed as directed for *Acid stage* in *Test 1*.
Standard solution: (L/900) mg/mL of USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS. Sonicate if necessary to dissolve.
Sample solution: Pass portions of the solution under test through a suitable filter.
Detector: UV 345 nm
Cell: See ▲*Table 2*.▲ (USP 1-May-2019)

Table ▲2▲ (USP 1-May-2019)

Tablet Strength (mg/Tablet)	Cell Size (cm)
75	0.5
100	0.5
150	0.2

Tolerances
Level 1 (6 Tablets tested): No individual value is more than 50% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved in 20 min.
Level 2 (6 Tablets tested): NMT 2 individual values of the 12 tested are greater than 50% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in 20 min.
Buffer stage
Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.
Medium: pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Solutions, Buffer Solutions*); 1000 mL, degassed
Apparatus 1 and **Analysis:** Proceed as directed for *Buffer stage* in *Test 1*.
Time: 45 min

USP Monographs

www.webofpharma.com

Standard solution: (*L*/1000) mg/mL of USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS. Sonicate if necessary to dissolve.

Sample solution: Pass portions of the solution under test through a suitable filter.

Detector and **Cell:** Proceed as directed for *Acid stage* in *Test 2*.

Tolerances: NLT 70% (*Q*) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*. Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure.*

Acid stage

Apparatus 1 and **Time:** Proceed as directed for *Acid stage* in *Test 1*.

Medium: 0.06 N hydrochloric acid; 900 mL

Standard solution: Prepare the solutions from USP Doxycycline Hyclate RS in *Medium* as directed in ▲*Table 3.*▲ (USP 1-May-2019) Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.

Table ▲3▲ (USP 1-May-2019)

Tablet Strength (mg/Tablet)	Concentration of Doxycycline (mg/mL)
75	0.1
100	0.1
150	0.17

Sample solution: Pass portions of the solution under test through a suitable filter.

Detector: UV 345 nm

Cell: 0.2 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: See ▲*Table 4.*▲ (USP 1-May-2019)

Table ▲4▲ (USP 1-May-2019)

Level	Number of Tablets Tested	Tolerances	
		Tablets Labeled to Contain 75 or 100 mg of Doxycycline	Tablets Labeled to Contain 150 mg of Doxycycline
A_1	6	No individual value exceeds 50% at 20 min.	No individual value exceeds 30% at 20 min.

▲4▲ (USP 1-May-2019) *(continued)*

Level	Number of Tablets Tested	Tolerances	
		Tablets Labeled to Contain 75 or 100 mg of Doxycycline	Tablets Labeled to Contain 150 mg of Doxycycline
A_2	6	Average of 12 units ($A_1 + A_2$) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.	Average of 12 units ($A_1 + A_2$) is NMT 30% at 20 min, and no individual unit is greater than 45% dissolved.
A_3	12	Average of 24 units ($A_1 + A_2 + A_3$) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.	Average of 24 units ($A_1 + A_2 + A_3$) is NMT 30% at 20 min, and no individual unit is greater than 45% dissolved.

Buffer stage

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.

Medium: pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Solutions, Buffer Solutions*); 1000 mL

Apparatus 1: 50 rpm

Time: 60 min

Standard solution: Prepare the solutions from USP Doxycycline Hyclate RS in *Medium* as directed in ▲*Table 5.*▲ (USP 1-May-2019) Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.

Table ▲5▲ (USP 1-May-2019)

Tablet Strength (mg/Tablet)	Concentration of Doxycycline (mg/mL)
75	0.1
100	0.1
150	0.15

Sample solution: Pass portions of the solution under test through a suitable filter.

Detector: UV 345 nm

Cell: 0.2 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 1000 mL

Tolerances: See ▲*Table 6.*▲ (USP 1-May-2019)

USP Monographs

www.webofpharma.com

Table ▲6▲ (USP 1-May-2019)

Tablets Labeled to Contain 75 or 100 mg of Doxycycline	Tablets Labeled to Contain 150 mg of Doxycycline
NLT 80% (*Q*) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.	NLT 70% (*Q*) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*. Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure.*

Acid stage

Medium: 0.06 N hydrochloric acid; 900 mL, degassed
Apparatus 1: 50 rpm
Time: 20 min
Standard solution: 0.1 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.
Sample solution: Pass portions of the solution under test through a suitable filter.
Detector: UV 345 nm
Cell: 0.2-cm quartz
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances
Level 1 (6 Tablets tested): No individual value is more than 30% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved in 20 min.
Level 2 (6 Tablets tested): NMT 2 individual values of the 12 tested are greater than 30% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) ▲dissolved▲ (USP 1-May-2019) in 20 min.

Buffer stage

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.
Medium: pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Solutions, Buffer Solutions*); 1000 mL, degassed
Apparatus 1: 50 rpm
Time: 30 min
Standard solution: 0.1 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Medium*
Sample solution: Pass portions of the solution under test through a suitable filter. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 1000 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) is dissolved.

Test 5: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 5*. Proceed as directed for *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B Procedure.*

Acid stage

Medium: 0.06 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 20 min
Standard solution: 0.06 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.
Sample solution: Pass portions of the solution under test through a suitable filter.
Detector: UV 345 nm
Cell: 1.0 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of doxycycline in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: See ▲*Table 7*.▲ (USP 1-May-2019)

Table ▲7▲ (USP 1-May-2019)

Level	Number of Tablets Tested	Tolerances
A_1	6	No individual value exceeds 50% at 20 min.
A_2	6	Average of 12 units ($A_1 + A_2$) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.
A_3	12	Average of 24 units ($A_1 + A_2 + A_3$) is NMT 50% at 20 min, and no individual unit is greater than 65% dissolved.

Buffer stage

Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.
Medium: pH 5.5 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Solutions, Buffer Solutions*); 900 mL

USP Monographs

www.webofpharma.com

Apparatus 1: 100 rpm
Time: 30 min
Standard solution: 0.06 mg/mL of doxycycline from USP Doxycycline Hyclate RS in *Medium*. Calculate the concentration, C_S, in mg/mL, of doxycycline using the designated potency, in µg/mg, of doxycycline in USP Doxycycline Hyclate RS.
Sample solution: Pass portions of the solution under test through a suitable filter.
Blank: *Medium*

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
▲Protect solutions containing doxycycline from light.
Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride RS in *Diluent*
System suitability stock solution 2: 1.2 mg/mL of USP Doxycycline Hyclate RS in *Diluent*
System suitability solution: Transfer 5 mL of *System suitability stock solution 2* to a 25-mL volumetric flask, heat on a steam bath for 60 min, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in *Diluent*, add 0.5 mL of *System suitability stock solution 1*, and dilute with *Diluent* to volume. Pass the solution through a suitable filter and use the filtrate. This solution contains a mixture of 4-epidoxycycline, doxycycline related compound A, methacycline, and doxycycline. [NOTE—The solution is stable up to 14 days when stored in a refrigerator.]
Sensitivity solution: 2 µg/mL of USP Doxycycline Hyclate RS in *Diluent*
Standard solution: 4.6 µg/mL of USP Doxycycline Hyclate RS in *Diluent*
Sample solution: Nominally 2.0 mg/mL of doxycycline from NLT 20 Tablets prepared as follows. Transfer a suitable portion of finely powdered Tablets to a suitable volumetric flask. Add 80% of the final volume of *Diluent*, sonicate for about 15 min, shake for about 15 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.2-µm pore size and use the filtrate for analysis.
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between methacycline and 4-epidoxycycline; NLT 1.5 between 4-epidoxycycline and doxycycline related compound A; NLT 2.0 between doxycycline related compound A and doxycycline, *System suitability solution*
Relative standard deviation: NMT 5.0% for the doxycycline peak, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of doxycycline from the *Standard solution*
C_S = concentration of USP Doxycycline Hyclate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxycycline in the *Sample solution* (mg/mL)
P = potency of doxycycline in USP Doxycycline Hyclate RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 8*. The reporting threshold is 0.1%.

Table 8

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxytetracycline[a, b]	0.39	—
Methacycline[b, c]	0.64	—
4-Epidoxycycline[d]	0.79	1.0
Doxycycline related compound A (6-epidoxycycline)[b, e]	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.2

[a] (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.
[b] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.
[c] (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide.
[d] (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide. Main degradation product.
[e] (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide. ▲(USP 1-May-2019)

ADDITIONAL REQUIREMENTS

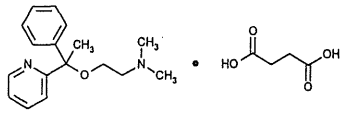
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Doxycycline Hyclate RS
USP Doxycycline Related Compound A RS
▲[NOTE—May be available as a free base or a hydrochloride salt.]▲ (USP 1-May-2019)
6-Epidoxycycline, or ▲(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.▲ (USP 1-May-2019)
$C_{22}H_{24}N_2O_8$ 444.43
▲(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide, monohydrochloride.
$C_{22}H_{24}N_2O_8 \cdot HCl$ 480.13▲ (USP 1-May-2019)
USP Methacycline Hydrochloride RS
▲▲ (USP 1-May-2019)

www.webofpharma.com

Doxylamine Succinate



$C_{17}H_{22}N_2O \cdot C_4H_6O_4$ 388.46

Ethanamine, *N*,*N*-dimethyl-2-[1-phenyl-1-(2-pyridinyl) ethoxy]-, butanedioate (1:1);
2-[α-[2-(Dimethylamino)ethoxy]-α-methylbenzyl]pyridine succinate (1:1);
N,*N*-Dimethyl-2-[1-phenyl-1-(pyridin-2-yl)ethoxy]ethan-1-amine succinate [562-10-7].

DEFINITION

Change to read:

Doxylamine Succinate contains NLT 98.0% and ▲NMT 102.0%▲ (USP 1-May-2019) of doxylamine succinate ($C_{17}H_{22}N_2O \cdot C_4H_6O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Analytical wavelength: 262 nm
Sample solution: 20 µg/mL of Doxylamine Succinate in 0.1 N hydrochloric acid
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%

Delete the following:

▲• **B. IDENTIFICATION—ORGANIC NITROGENOUS BASES** ⟨181⟩: It meets the requirements.▲ (USP 1-May-2019)

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

• **C.**
Sample solution: Dissolve 500 mg of Doxylamine Succinate in 5 mL of water, and add a slight excess of 6 N ammonium hydroxide. Extract the liberated doxylamine with several portions of ether, discard the ether extracts, and evaporate the aqueous solution on a steam bath to dryness. Add 2 mL of 3 N hydrochloric acid, and again evaporate on the steam bath to dryness. Cool, add about 10 mL of ether, allow to stand for a few minutes, and decant the clear supernatant. Evaporate the ether solution to dryness, and dry the residue at 105° for 30 min.
Acceptance criteria: The succinic acid melts between 184° and 188°, as indicated for Class I under *Melting Range or Temperature* ⟨741⟩, *Procedures*.

ASSAY

Change to read:

• **PROCEDURE**
▲Solution A: 50 mM ammonium acetate in water; adjusted with glacial acetic acid to a pH of 4.0
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
2	90	10
15	40	60
20	40	60
21	90	10
25	90	10

Diluent: *Solution A* and *Solution B* (90:10)
Standard solution: 0.1 mg/mL of USP Doxylamine Succinate RS in *Diluent*
Sample solution: 0.1 mg/mL of Doxylamine Succinate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 262 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of doxylamine succinate ($C_{17}H_{22}N_2O \cdot C_4H_6O_4$) in the portion of Doxylamine Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of doxylamine from the *Sample solution*
r_S = peak response of doxylamine from the *Standard solution*
C_S = concentration of USP Doxylamine Succinate RS in the *Standard solution* (mg/mL)
C_U = concentration of Doxylamine Succinate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis▲ (USP 1-May-2019)

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Delete the following:

▲• **VOLATILE RELATED COMPOUNDS**
Sample solution: Dissolve 650 mg in 20 mL of 0.1 N hydrochloric acid in a separator. Render the solution alkaline with 2.5 N sodium hydroxide, and immediately extract with four 25-mL portions of ether, filtering each extract through an ether-saturated pledget of cotton. Evaporate the combined ether extracts on a water bath with the aid of a current of air to dryness at a temperature not exceeding 50°, and dissolve the residue in 5 mL of alcohol.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

USP Monographs

www.webofpharma.com

Mode: GC
Detector: Flame ionization
Column: 4-mm × 2-m glass column containing 5% packing G16 and 5% packing G12 on 60- to 80-mesh S1A
Temperatures
Column: 212°
Injection port: 250°
Detector block: 250°
Carrier gas: Dry helium
Injection volume: 1 µL
Acceptance criteria: NMT 2.0%, the total relative area of all extraneous peaks (except that of the solvent peak); and NMT 1.0%, the relative area of any individual extraneous peak ▲ (USP 1-May-2019)

Add the following:

▲• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.001 mg/mL each of USP Doxylamine Succinate RS and USP Carbinoxamine Related Compound C RS in *Diluent*
Sample solution: 1.0 mg/mL of Doxylamine Succinate in *Diluent*
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2 between doxylamine and carbinoxamine related compound C
Relative standard deviation: NMT 5.0% for the doxylamine and carbinoxamine related compound C peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of carbinoxamine related compound C in the portion of Doxylamine Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of carbinoxamine related compound C from the *Sample solution*
r_S = peak response of carbinoxamine related compound C from the *Standard solution*
C_S = concentration of USP Carbinoxamine Related Compound C RS in the *Standard solution* (mg/mL)
C_U = concentration of Doxylamine Succinate in the *Sample solution* (mg/mL)

Calculate the percentage of each specified and any unspecified impurity in the portion of Doxylamine Succinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each specified and any unspecified impurity from the *Sample solution*
r_S = peak response of doxylamine from the *Standard solution*
C_S = concentration of USP Doxylamine Succinate RS in the *Standard solution* (mg/mL)
C_U = concentration of Doxylamine Succinate in the *Sample solution* (mg/mL)
F = relative response factor of each specified and any unspecified impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Doxylamine pyridinyl *N*-oxide[a]	0.64	2.1	0.10
Doxylamine dioxide[b]	0.70	1.8	0.10
Doxylamine pyridine-4-yl isomer[c]	0.81	1.0	0.15
Carbinoxamine related compound C	0.95	—	0.5
Doxylamine	1.0	—	—
Doxylamine ethylamine *N*-oxide[d]	1.06	1.0	0.10
Doxylamine alcohol[e]	1.30	1.8	0.15
2-Benzoylpyridine[f]	1.44	5.8	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 2-[1-(2-(Dimethylamino)ethoxy)-1-phenylethyl]pyridine 1-oxide.
[b] *N,N*-Dimethyl-2-(1-(1-oxidopyridin-2-yl)-1-phenylethoxy)ethan-1-amine oxide.
[c] *N,N*-Dimethyl-2-[1-phenyl-1-(pyridin-4-yl)ethoxy]ethan-1-amine.
[d] *N,N*-Dimethyl-2-[1-phenyl-1-(pyridin-2-yl)ethoxy]ethan-1-amine oxide.
[e] 1-Phenyl-1-(pyridin-2-yl)ethan-1-ol.
[f] Phenyl(pyridin-2-yl)methanone. ▲ (USP 1-May-2019)

SPECIFIC TESTS

Delete the following:

▲• **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩: 103°–108°, the range between the beginning and end of melting does not exceed 3°. ▲ (USP 1-May-2019)
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum over phosphorus pentoxide for 5 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲USP Carbinoxamine Related Compound C RS
N,N-Dimethyl-2-[phenyl(pyridin-2-yl)methoxy]ethan-1-amine;
Desmethyl doxylamine.
$C_{16}H_{20}N_2O$ 256.35 ▲ (USP 1-May-2019)
USP Doxylamine Succinate RS

www.webofpharma.com

Doxylamine Succinate Oral Solution

» Doxylamine Succinate Oral Solution contains not less than 92.0 percent and not more than 108.0 percent of the labeled amount of doxylamine succinate ($C_{17}H_{22}N_2O \cdot C_4H_6O_4$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Doxylamine Succinate RS
Identification—Use a volume of Oral Solution equivalent to about 50 mg of doxylamine succinate, and proceed as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Transfer the liquid to a separator." The Oral Solution meets the requirements of the test.
Assay—Proceed with Oral Solution as directed under *Salts of Organic Nitrogenous Bases* ⟨501⟩, determining the absorbance at the wavelength of maximum absorbance at about 262 nm. Calculate the quantity, in mg, of doxylamine succinate ($C_{17}H_{22}N_2O \cdot C_4H_6O_4$) in each mL of the Oral Solution taken by the formula:

$$(0.05C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Doxylamine Succinate RS in the *Standard Preparation;* and V is the volume, in mL, of Oral Solution taken.

Doxylamine Succinate Tablets

DEFINITION
Doxylamine Succinate Tablets contain NLT 92.0% and NMT 108.0% of the labeled amount of doxylamine succinate ($C_{17}H_{22}N_2O \cdot C_4H_6O_4$).

IDENTIFICATION

Delete the following:

▲• **A.** Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.▲ (USP 1-May-2019)

Add the following:

▲• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*▲ (USP 1-May-2019)

Add the following:

▲• **B.** The UV-Vis absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution,* as obtained in the *Assay.*▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**
▲**Solution A:** 50 mM ammonium acetate in water; adjusted with glacial acetic acid to a pH of 4.0
Solution B: Acetonitrile
Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
2	90	10
15	40	60
20	40	60
21	90	10
25	90	10

Diluent: *Solution A* and *Solution B* (90:10)
Standard solution: 0.1 mg/mL of USP Doxylamine Succinate RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of doxylamine succinate in *Diluent* prepared as follows. Transfer an appropriate quantity of doxylamine succinate from NLT 10 finely powdered Tablets to a suitable volumetric flask and dilute with *Diluent* to volume. [NOTE—Sonication or shaking may be necessary. Centrifuge or pass a portion of this solution through a suitable filter.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 262 nm. For *Identification B,* use a diode array detector in the range of 230–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of doxylamine succinate ($C_{17}H_{22}N_2O \cdot C_4H_6O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of doxylamine from the *Sample solution*
r_S = peak response of doxylamine from the *Standard solution*
C_S = concentration of USP Doxylamine Succinate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxylamine succinate in the *Sample solution* (mg/mL)▲ (USP 1-May-2019)

Acceptance criteria: 92.0%–108.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Instrumental conditions
▲(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)▲ (USP 1-May-2019)
Mode: UV
Analytical wavelength: 262 nm

www.webofpharma.com

Standard solution: USP Doxylamine Succinate RS in *Medium*
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of doxylamine succinate ($C_{17}H_{22}N_2O \cdot C_4H_6O_4$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amount of doxylamine succinate ($C_{17}H_{22}N_2O \cdot C_4H_6O_4$) is dissolved.

Change to read:

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements
▲▲ (USP 1-May-2019)

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability solution: 0.001 mg/mL each of USP Doxylamine Succinate RS and USP Carbinoxamine Related Compound C RS in *Diluent*
Standard solution: 0.001 mg/mL of USP Doxylamine Succinate RS in *Diluent*
Sample solution: Nominally 1.0 mg/mL of doxylamine succinate in *Diluent* prepared as follows. Finely powder NLT 10 Tablets and transfer a portion of the powder to an appropriate volumetric flask. Dilute with *Diluent* to volume. [NOTE—Sonication or shaking may be necessary. Centrifuge or pass a portion of this solution through a suitable filter.]
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between doxylamine and carbinoxamine related compound C, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each specified and unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each specified and unspecified degradation product from the *Sample solution*
r_S = peak response of doxylamine from the *Standard solution*
C_S = concentration of USP Doxylamine Succinate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of doxylamine succinate in the *Sample solution* (mg/mL)
F = relative response factor of each specified and any unspecified degradation product (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Doxylamine pyridinyl *N*-oxide[a]	0.64	2.1	0.2
Doxylamine dioxide[b]	0.70	1.8	0.2
Doxylamine pyridine-4-yl isomer[c, d]	0.81	—	—
Carbinoxamine related compound C[d]	0.95	—	—
Doxylamine	1.0	—	—
Doxylamine ethylamine *N*-oxide[e]	1.06	1.0	0.2
Doxylamine alcohol[f]	1.30	1.8	0.2
2-Benzoylpyridine[d, g]	1.44	—	—
Any unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	2.0

[a] 2-[1-(2-(Dimethylamino)ethoxy)-1-phenylethyl]pyridine 1-oxide.
[b] *N,N*-Dimethyl-2-(1-(1-oxidopyridin-2-yl)-1-phenylethoxy)ethan-1-amine oxide.
[c] *N,N*-Dimethyl-2-[1-phenyl-1-(pyridin-4-yl)ethoxy]ethan-1-amine.
[d] Process related impurity. Do not include in calculation of total degradation products.
[e] *N,N*-Dimethyl-2-[1-phenyl-1-(pyridin-2-yl)ethoxy]ethan-1-amine oxide.
[f] 1-Phenyl-1-(pyridin-2-yl)ethan-1-ol.
[g] Phenyl(pyridin-2-yl)methanone. ▲ (USP 1-May-2019)

ADDITIONAL REQUIREMENTS

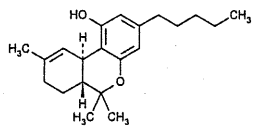
Change to read:

• **PACKAGING AND STORAGE:** ▲Store at controlled room temperature in tight, ▲ (USP 1-May-2019) light-resistant containers.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲USP Carbinoxamine Related Compound C RS
N,N-Dimethyl-2-[phenyl(pyridin-2-yl)methoxy]ethan-1-amine;
Desmethyl doxylamine.
$C_{16}H_{20}N_2O$ 256.35 ▲ (USP 1-May-2019)
USP Doxylamine Succinate RS

Dronabinol



$C_{21}H_{30}O_2$ 314.46
6*H*-Dibenzo[*b,d*]pyran-1-ol, 6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-, (6a*R-trans*)-.
(6a*R*,10a*R*)-6a,7,8,10a-Tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b,d*]-pyran-1-ol [1972-08-3].

www.webofpharma.com

» Dronabinol is Δ^9-tetrahydrocannabinol. It contains not less than 95.0 percent of $C_{21}H_{30}O_2$.

Packaging and storage—Preserve in tight, light-resistant glass containers in inert atmosphere. Store as per labeling instructions.

USP Reference standards ⟨11⟩—
USP Δ^9-Tetrahydrocannabinol RS
USP exo-Tetrahydrocannabinol RS
(6a*R*, 10a*R*)-6,6-Dimethyl-9-methylene-3-pentyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-1-ol
$C_{21}H_{30}O_2$ 314.46

Identification—
A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
B: Visualizing agent—Transfer about 100 mg of Fast Blue B salt to a suitable flask containing about 100 mL of methanol, stir for about 5 minutes, and allow to settle. Decant the clear liquid into the sprayer reservoir. [NOTE—Prepare fresh daily.]
Identification solution—Use the *Standard preparation,* prepared as directed in the *Assay.*
Test solution—Use the *Assay preparation.*
Procedure—Apply separately 10 µL each of the *Identification solution* and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the plate in a chromatographic chamber that has been equilibrated (for about 2 minutes) with vapors from a solvent mixture of *n*-hexane and methylene chloride (1:1) until the solvent front has moved about 10 cm. Remove the plate from the developing chamber, quickly mark the solvent front, and allow the plate to dry at room temperature for about 5 minutes. Spray the plate with the *Visualizing agent* until it is uniformly damp (not saturated). Heat the plate at about 80° until the spots are developed: the color and R_F value of the spots from the *Test solution* correspond to those obtained from the *Identification solution.*

Related compounds— [NOTE—Minimize exposure to air and light for the *Standard solution* and the *Test solution.* Analyze all samples within 24 hours.]
Mobile phase, System suitability solution, and *Standard preparation*—Proceed as directed in the *Assay.*
Standard solution—Dilute an accurately measured volume of the *Standard preparation* quantitatively, and stepwise if necessary, with dehydrated alcohol to obtain a solution having a known concentration of about 0.004 mg per mL.
Sensitivity solution—Quantitatively dilute an accurately measured volume of the *Standard solution* with dehydrated alcohol to obtain a solution having a concentration of about 0.2 µg per mL.
Test solution—Use the *Assay preparation.*
Chromatographic system—Proceed as directed in the *Assay.* In addition, chromatograph the *Sensitivity solution,* and calculate the signal-to-noise ratio, *S/N,* by the formula:

$$(2H)/h$$

in which H is the measured height of the peak, and h is the amplitude of the average measured baseline noise; the signal-to-noise ratio is not less than 10.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for all of the peaks.
Calculate the percentage of each impurity in the portion of Dronabinol taken by the formula:

$$100(1/F)(CV/W)(r_U/r_S)$$

in which F is the relative response factor for each impurity (see *Table 1*); C is the concentration, in mg per mL, of Δ^9-tetrahydrocannabinol in the *Standard solution; V* is the volume, in mL, of the *Test solution; W* is the weight, in mg, of Dronabinol taken to prepare the *Test solution;* r_U is the peak area response of each impurity in the *Test solution;* and r_S is the peak area response of Δ^9-tetrahydrocannabinol in the *Standard solution.* In addition to not exceeding the limits in *Table 1* not more than 5.0% of total impurities is found.

Table 1

Name	Relative Retention Time	Relative Response Factor	Limit (%)
Cannabinol	0.78	2.7	1.5
Δ^9-Tetrahydrocannabinol	1.00	1.0	—
Exo-tetrahydrocannabinol[1]	1.07	0.92	0.5
Δ^8-Tetrahydrocannabinol	1.18	0.90	2.0
Any other individual impurity	—	1.0	1.0

[1] (6a*R*, 10a*R*)-6,6-Dimethyl-9-methylene-3-pentyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-1-ol.

Assay— [NOTE—Minimize exposure to air and light for the *Standard preparation* and the *Assay preparation.* Analyze all samples within 24 hours.]
Mobile phase—Prepare a filtered and degassed mixture of methanol, water, tetrahydrofuran, and acetonitrile (45:25:20:10), making adjustments, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Transfer accurately measured volumes of USP Δ^9-Tetrahydrocannabinol RS and USP Exo-tetrahydrocannabinol RS to a suitable volumetric flask, and dilute with dehydrated alcohol to prepare a solution that contains about 200 µg of Δ^9-tetrahydrocannabinol and about 10 µg of *exo*-tetrahydrocannabinol per mL.
Standard preparation—Quantitatively dilute an accurately measured volume of USP Δ^9-Tetrahydrocannabinol RS with dehydrated alcohol to obtain a solution having a known concentration of about 0.2 mg per mL.
Assay preparation—Transfer about 20 mg of Dronabinol, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with dehydrated alcohol to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 228-nm detector and a 4.6-mm × 15-cm analytical column that contains 4-µm packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at 20°. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between Δ^9-tetrahydrocannabinol and *exo*-tetrahydrocannabinol is not less than 1.5; and the tailing factor of Δ^9-tetrahydrocannabinol is not more than 2.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all of the peaks. Calculate the quantity, in mg, of $C_{21}H_{30}O_2$ in the portion of Dronabinol taken by the formula:

$$CV(r_U/r_S)$$

USP Monographs

www.webofpharma.com

in which C is the concentration, in mg per mL, of Δ^9-tetrahydrocannabinol in the *Standard preparation; V* is the volume, in mL, of the *Assay preparation;* and r_U and r_S are the Δ^9-tetrahydrocannabinol peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dronabinol Capsules

DEFINITION

Dronabinol Capsules contain dronabinol in Sesame Oil. Dronabinol Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of dronabinol ($C_{21}H_{30}O_2$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, tetrahydrofuran, and water (71:5:24)

System suitability stock solution: 1.0 mg/mL of Δ^8-tetrahydrocannabinol in methanol

System suitability solution: 0.5 mg/mL of USP Δ^9-Tetrahydrocannabinol RS and 0.5 mg/mL of Δ^8-tetrahydrocannabinol. Mix equal volumes of USP Δ^9-Tetrahydrocannabinol RS and *System suitability stock solution.*

Standard solution: 0.2 mg/mL of USP Δ^9-Tetrahydrocannabinol RS in dehydrated alcohol

Sample solution: Equivalent to 0.2 mg/mL of dronabinol, from Capsule contents (NLT 20) in dehydrated alcohol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 228 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Guard column: 4.6-mm × 30-mm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for Δ^9-tetrahydrocannabinol and Δ^8-tetrahydrocannabinol are about 1.0 and 1.14, respectively.]

Suitability requirements

Resolution: NLT 2.0 between dronabinol and Δ^8-tetrahydrocannabinol, *System suitability solution*

Tailing factor: NMT 2.0 of Δ^9-tetrahydrocannabinol, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{21}H_{30}O_2$ in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dronabinol from the *Sample solution*

r_S = peak response of dronabinol from the *Standard solution*

C_S = concentration of USP Δ^9-Tetrahydrocannabinol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dronabinol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 15 min

Analysis: Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

Tolerances: The requirements are met if all of the Capsules tested rupture in NMT 15 min. If 1 or 2 of the Capsules rupture in NLT 15 but NMT 30 min, repeat the test on 12 additional Capsules. NMT 2 of the total of 18 Capsules tested rupture in NLT 15 min but NMT 30 min.

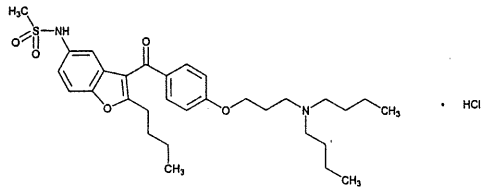
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, in a cool place.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Δ^9-Tetrahydrocannabinol RS

Dronedarone Hydrochloride



$C_{31}H_{44}N_2O_5S \cdot HCl$ 593.2

N-{2-Butyl-3-[4-(3-dibutylaminopropoxy)benzoyl] benzofuran-5-yl}methanesulfonamide, hydrochloride;

Methanesulfonamide, *N*-[2-butyl-3-[4-[3-(dibutylamino) propoxy]benzoyl]-5-benzofuranyl]-, monohydrochloride [141625-93-6].

DEFINITION

Dronedarone Hydrochloride contains NLT 98.0% and NMT 101.5% of dronedarone hydrochloride, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020): Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

Sample solution: Dissolve 100 mg of Dronedarone Hydrochloride in 8 mL of methanol and dilute with water to 10.0 mL. Use 2.0 mL for the analysis.

Acceptance criteria: Meets the requirements

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: Combine 2.0 mL of triethylamine with 1 L of water and adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (50:50)

System suitability stock solution: 1 mg/mL each of USP Dronedarone Hydrochloride RS and USP Dronedarone Related Compound A RS in methanol

System suitability solution: 0.01 mg/mL each of USP Dronedarone Hydrochloride RS and USP Dronedarone Related Compound A RS in *Mobile phase* from *System suitability stock solution*

Standard solution: 0.1 mg/mL of USP Dronedarone Hydrochloride RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Dronedarone Hydrochloride in *Mobile phase*

Chromatographic system

Mode: LC

Detector: UV 288 nm

Column: 4.6-mm × 25-cm; 5-µm packing L10

Flow rate: 0.8 mL/min

Injection volume: 20 µL

Run time: NLT 2.15 times the retention time of dronedarone

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for dronedarone related compound A and dronedarone are 0.71 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8 between dronedarone and dronedarone related compound A, *System suitability solution*

Tailing factor: 0.8–2.2 for dronedarone, *System suitability solution*

Relative standard deviation: NMT 0.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dronedarone hydrochloride ($C_{31}H_{44}N_2O_5S \cdot HCl$) in the portion of Dronedarone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dronedarone from the *Sample solution*

r_S = peak response of dronedarone from the *Standard solution*

C_S = concentration of USP Dronedarone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Dronedarone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the anhydrous and solvent-free basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **ORGANIC IMPURITIES**

Solution A: Combine 2 mL of triethylamine with 950 mL of water. Adjust with phosphoric acid to a pH of 4.0 and then dilute with water to 1 L.

Solution B: Acetonitrile

Mobile phase: See *Table 1*. [NOTE—Collect data for 58 min.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
15	60	40
25	60	40
40	50	50
45	40	60
58	40	60
60	70	30
70	70	30

Diluent: Acetonitrile and *Solution A*, 50:50

Standard stock solution: 0.2 mg/mL of USP Dronedarone Hydrochloride RS and 0.4 mg/mL of USP Dronedarone Related Compound A RS, respectively, in methanol

Standard solution: 2 µg/mL of USP Dronedarone Hydrochloride RS and 4 µg/mL of USP Dronedarone Related Compound A RS, respectively, in *Diluent* from the *Standard stock solution*

Sensitivity solution: 1 µg/mL of USP Dronedarone Hydrochloride RS and 2 µg/mL of USP Dronedarone Related Compound A RS in *Diluent* from the *Standard solution*

Sample solution: 2 mg/mL of Dronedarone Hydrochloride in *Diluent*. Sonicate for 5 min to dissolve completely. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

Mode: LC

Detector: UV 246 nm

Column: 4.6-mm × 25-cm; 5-µm packing L10

Flow rate: 0.8 mL/min

Injection volume: 25 µL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[NOTE—The relative retention times for dronedarone related compound A and dronedarone are 0.58 and 1.00, respectively.]

Suitability requirements

Resolution: NLT 25 between dronedarone and dronedarone related compound A, *Standard solution*

Signal-to-noise ratio: NLT 10 for dronedarone and NLT 20 for dronedarone related compound A, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dronedarone related compound A in the portion of Dronedarone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dronedarone related compound A from the *Sample solution*

r_S = peak response of dronedarone related compound A from the *Standard solution*

C_S = concentration of USP Dronedarone Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Dronedarone Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Dronedarone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak response of any unspecified impurity from the *Sample solution*
r_S = peak response of dronedarone from the *Standard solution*
C_S = concentration of USP Dronedarone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Dronedarone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: Disregard peaks below 0.05%.
Dronedarone related compound A: NMT 0.15%
Any unspecified impurity: NMT 0.10%
Total impurities: NMT 0.3%

SPECIFIC TESTS
- **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Protect from light and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Dronedarone Hydrochloride RS
  USP Dronedarone Related Compound A RS
  N-(2-Butyl-3-{4-[3-(butylamino)propoxy]benzoyl} benzofuran-5-yl)methanesulfonamide.
  $C_{27}H_{36}N_2O_5S$ 500.65

Dronedarone Tablets

DEFINITION
Dronedarone Tablets contain an amount of dronedarone hydrochloride equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of dronedarone free base ($C_{31}H_{44}N_2O_5S$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the dronedarone peak in the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: Combine 2.0 mL of triethylamine with 1 L of water and adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (50:50)
System suitability stock solution: 0.2 mg/mL each of USP Dronedarone Hydrochloride RS and USP Dronedarone Related Compound A RS in methanol
System suitability solution: 0.01 mg/mL each of USP Dronedarone Hydrochloride RS and USP Dronedarone Related Compound A RS in *Mobile phase* from the *System suitability stock solution*
Standard stock solution: 2.13 mg/mL of USP Dronedarone Hydrochloride RS in methanol
Standard solution: 0.11 mg/mL of USP Dronedarone Hydrochloride RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: Nominally equivalent to 4 mg/mL of dronedarone in methanol prepared as follows. Dissolve and dilute in methanol to volume, an amount equivalent to 400 mg of dronedarone from NLT 20 finely powdered Tablets, taken in a 100-mL volumetric flask. Sonicate for about 5 min and allow to settle at room temperature.
Sample solution: Nominally equivalent to 0.1 mg/mL of dronedarone in *Mobile phase* from the *Sample stock solution*. Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Assay: UV 288 nm
Identification test B: UV diode array
Column: 4.6-mm × 25-cm; 5-µm packing L10
Flow rate: 0.8 mL/min
Injection volume: 20 µL
Run time: NLT 2.15 times the retention time of dronedarone
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for dronedarone related compound A and dronedarone are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 8 between dronedarone and dronedarone related compound A
Tailing factor: 0.8–2.1 for dronedarone
Relative standard deviation: NMT 1.5%, for dronedarone
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dronedarone free base ($C_{31}H_{44}N_2O_5S$) in the portion of Tablets taken:

$$(r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of dronedarone from the *Sample solution*
r_S = peak response of dronedarone from the *Standard solution*
C_S = concentration of USP Dronedarone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dronedarone in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dronedarone free base, 556.76
M_{r2} = molecular weight of dronedarone hydrochloride, 593.22

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Medium: 13.61 g/L of monobasic potassium phosphate in water. Adjust with 0.1 M hydrochloric acid or 0.1 M sodium hydroxide as needed to a pH of 4.5; 1000 mL
Apparatus 2: 75 rpm, with sinker ring
Times: 30 and 90 min
Standard solution: 0.43 mg/mL of USP Dronedarone Hydrochloride RS prepared as follows. Dissolve a suitable amount of USP Dronedarone Hydrochloride RS in 2% of the total volume of methanol and dilute with *Medium* to volume.
Sample solution: Pass a portion of sample under test through a suitable filter.
Instrumental conditions
Analytical wavelength: UV 288 nm
Cell: 1 mm

www.webofpharma.com

Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of dronedarone dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of USP Dronedarone Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Tablet)
M_{r1} = molecular weight of dronedarone free base, 556.76
M_{r2} = molecular weight of dronedarone hydrochloride, 593.22

Tolerances
30 min: 20.0%–60.0% ▲▲ (ERR 1-Jun-2019) of the labeled amount of dronedarone free base ($C_{31}H_{44}N_2O_5S$) is dissolved.
90 min: NLT 80% (*Q*) of the labeled amount of dronedarone free base ($C_{31}H_{44}N_2O_5S$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer, Mobile phase, and **System suitability stock solution:** Proceed as directed in the *Assay*.
System suitability solution: 0.01 mg/mL each of USP Dronedarone Hydrochloride RS and USP Dronedarone Related Compound A RS prepared as follows. To a suitable amount of *System suitability stock solution*, add 20% of the total volume of methanol and dilute with *Mobile phase* to volume.
Standard stock solution: 0.4 mg/mL of USP Dronedarone Hydrochloride RS in methanol
Standard solution: 0.002 mg/mL of USP Dronedarone Hydrochloride RS prepared as follows. To a suitable amount of *Standard stock solution*, add 25% of the total volume of methanol and dilute with *Mobile phase* to volume.
Sensitivity solution: 0.0005 mg/mL of USP Dronedarone Hydrochloride RS prepared as follows. To a suitable amount of the *Standard solution*, add 20% of the total volume of methanol and dilute with *Mobile phase* to volume.
Sample stock solution: Nominally equivalent to 4 mg/mL of dronedarone in methanol prepared as follows. Dissolve and dilute in methanol to volume, an amount equivalent to 400 mg of dronedarone from NLT 20 finely powdered Tablets, taken in a 100-mL volumetric flask. Sonicate for about 5 min and allow to settle at room temperature.
Sample solution: Nominally equivalent to 1 mg/mL of dronedarone in *Mobile phase* from *Sample stock solution*. Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 246 nm
Column: 4.6-mm × 25-cm; 5-µm packing L10
Flow rate: 0.8 mL/min
Injection volume: 20 µL
Run time: NLT 3.6 times the retention time of dronedarone
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
[NOTE—The relative retention times for dronedarone related compound A and dronedarone are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 8 between dronedarone and dronedarone related compound A, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$(r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

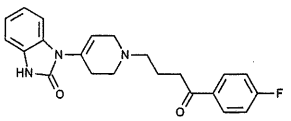
r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of dronedarone from the *Standard solution*
C_S = concentration of USP Dronedarone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of dronedarone in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of dronedarone free base, 556.76
M_{r2} = molecular weight of dronedarone hydrochloride, 593.22

Acceptance criteria: Disregard peaks less than 0.05%.
Any unspecified impurity: NMT 0.20%
Total impurities: NMT 0.4%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dronedarone Hydrochloride RS
USP Dronedarone Related Compound A RS
N-(2-Butyl-3-{4-[3-(butylamino)propoxy]benzoyl}benzofuran-5-yl)methanesulfonamide.
$C_{27}H_{36}N_2O_5S$ 500.65

Droperidol



$C_{22}H_{22}FN_3O_2$ 379.43
2*H*-Benzimidazol-2-one, 1-[1-[4-(4-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydro-4-pyridinyl]-1,3-dihydro-.
1-1-[3-(*p*-Fluorobenzoyl)propyl]-1,2,[3,6-tetrahydro-4-pyridyl]-2-benzimidazolinone [548-73-2].

» Droperidol, dried in vacuum at 70° for 4 hours, contains not less than 98.0 percent and not more than 102.0 percent of $C_{22}H_{22}FN_3O_2$.

Packaging and storage—Preserve in tight, light-resistant containers, under nitrogen, in a cool place.

USP Monographs

www.webofpharma.com

USP Reference standards ⟨11⟩—
USP Droperidol RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 15 µg per mL.
Medium: 0.1 N hydrochloric acid in isopropyl alcohol (1 in 10).
Loss on drying ⟨731⟩—Dry it in vacuum at 70° for 4 hours: it loses not more than 5.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.
Limit of 4,4′-bis[1,2,3,6-tetrahydro-4-(2-oxo-1-benzimidazolinyl)-1-pyridyl]butyrophenone— Dissolve about 30.0 mg of the sample in 70 mL of isopropyl alcohol in a 100-mL volumetric flask. Add 10.0 mL of 0.1 N hydrochloric acid, dilute with isopropyl alcohol to volume, and mix. The absorptivity of the solution in 1-cm cells at the wavelength of maximum absorbance at about 330 nm, with a suitable spectrophotometer, using a 1 in 10 solution of 0.1 N hydrochloric acid in isopropyl alcohol as the blank, is not more than 0.7 (equivalent to limit of 1.5%).
Assay—Dissolve about 240 mg of Droperidol, previously dried and accurately weighed, in 50 mL of glacial acetic acid, add *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 37.94 mg of $C_{22}H_{22}FN_3O_2$.

Droperidol Injection

» Droperidol Injection is a sterile solution of Droperidol in Water for Injection, prepared with the aid of Lactic Acid. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{22}H_{22}FN_3O_2$, as the lactate.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Droperidol RS

Identification—
A: Dissolve about 25 mg of USP Droperidol RS in 10 mL of water containing 0.3 mL of 50% acetic acid in a separator to obtain a solution containing about 2.5 mg per mL. Transfer a volume of Injection, equivalent to about 25 mg of droperidol, to a second separator. Separately add 2 mL of ammonia TS to each separator, and mix. Extract each solution with two 10-mL portions of chloroform, collecting the chloroform extracts from the solutions in separate 50-mL beakers. Apply separately 20 µL of the test solution and the Standard solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a chamber with an unequilibrated solvent system consisting of a mixture of ethyl acetate, chloroform, methanol, and acetate buffer (0.2 M sodium acetate adjusted with 50% acetic acid to a pH of 4.7) (54:23:18:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with dinitrophenylhydrazine TS, then examine under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 35.7 USP Endotoxin Units per mg of droperidol.
pH ⟨791⟩: between 3.0 and 3.8.

Chromatographic purity—
Mobile phase, System suitability preparation, Standard stock preparation, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay*.
Test preparation—Use the *Assay preparation*.
Procedure—Inject a volume (about 100 µL) of the *Test preparation* into the chromatograph, record the chromatogram of twice the retention time of droperidol, and measure the peak responses. Calculate the percentage of each impurity in the portion of Droperidol taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity, and r_s is the sum of the responses of all the peaks: the sum of all impurities is not more than 2.0%.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Borate buffer—Dissolve 31 g of boric acid in about 800 mL of water. Slowly add sodium hydroxide solution (1 in 5) in small quantities until all of the boric acid is dissolved and the pH is constant at 7.0. Quantitatively transfer the solution to a glass-stoppered, 1000-mL volumetric flask, dilute with water to volume, and mix.
Mobile phase—Prepare a filtered and degassed mixture of methanol, water, and *Borate buffer* (700:280:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard stock preparation—Dissolve an accurately weighed quantity of USP Droperidol RS in methanol to obtain a solution having a known concentration of about 1 mg per mL.
Standard preparation—Transfer 5.0 mL of *Standard stock preparation* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
System suitability preparation—Prepare a solution of 4′-fluoroacetophenone in methanol containing about 1 mg per mL. Transfer 5.0 mL of this solution and 5.0 mL of *Standard stock preparation* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of droperidol, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 10-µm packing L1. The flow rate is about 1.0 mL per minute.
Chromatograph the *System suitability preparation* and the *Standard preparation*, and record the peak responses as

www.webofpharma.com

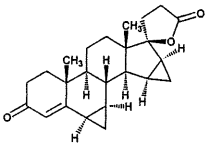
directed for *Procedure:* the resolution, *R*, between 4′-fluoroacetophenone and droperidol is not less than 5.0; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of droperidol ($C_{22}H_{22}FN_3O_2$) in each mL of the Injection taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Droperidol RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Drospirenone



$C_{24}H_{30}O_3$ 366.49

(6*R*,7*R*,8*R*,9*S*,10*R*,13*S*,14*S*,15*S*,16*S*,17*S*)-1,3′,4′,6,6a,7,8,9,10, 11,12,13,14,15,15a,16-Hexadecahydro-10,13-dimethylspiro-[17*H*-dicyclopropa[6,7:15,16]cyclopenta[*a*]phenanthrene-17,2′(5′*H*)-furan]-3,5′(2*H*)-dione;
17-Hydroxy-6β,7β:15β,16β-dimethylene-3-oxo-17 α-pregn-4-ene-21-carboxylic acid, γ-lactone [67392-87-4].

DEFINITION

Drospirenone contains NLT 98.0% and NMT 102.0% of $C_{24}H_{30}O_3$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	63	37
2.0	63	37
16.0	52	48
23.0	52	48
31.0	20	80
39.0	20	80
39.1	63	37
49.0	63	37

Diluent: Acetonitrile and water (1:1)
System suitability solution: 60 µg/mL of USP Drospirenone RS and 60 µg/mL of USP Drospirenone Related Compound A RS in *Diluent*
Standard solution: 0.6 mg/mL of USP Drospirenone RS in *Diluent*
Sample solution: 0.6 mg/mL of Drospirenone in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 245 nm
Column: 4.6-mm × 25-cm; 3-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between drospirenone and drospirenone related compound A, *System suitability solution*
Tailing factor: Between 0.8 and 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of drospirenone ($C_{24}H_{30}O_3$) in the portion of Drospirenone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Drospirenone RS in the *Standard solution* (mg/mL)
C_U = concentration of drospirenone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

INORGANIC IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

- **Procedure 1: Limit of 1,2-Dimethoxyethane and Diisopropyl Ether** (if present)

Standard solution: 0.1 mg/mL of 1,2-dimethoxyethane and 0.05 mg/mL of diisopropyl ether in dimethylformamide
Sample solution: 50 mg/mL of Drospirenone in dimethylformamide
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 30-m; 1.4-µm coating of phase G43
Temperature
Injector: 160°
Detector: 250°
Column: See *Table 2*.

USP Monographs

www.webofpharma.com

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	10
40	5	70	0
70	30	220	0

Carrier gas: Helium
Flow rate: 32 ± 8 cm/s. [NOTE—For pressure- controlled systems, a column pressure of about 130 kPa is necessary.]
Injector type: Headspace
Sample volume: 2.0 mL/vial
Vial treatment: Maintain at 80° for 60 min before injection.
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for diisopropyl ether and 1,2-dimethoxyethane are about 0.6 and 1.0, respectively.]
Suitability requirements
Relative standard deviation: NMT 4.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 1,2-dimethoxyethane and diisopropyl ether in the portion of Drospirenone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 1,2-dimethoxyethane or diisopropyl ether from the *Sample solution*
r_S = peak response of 1,2-dimethoxyethane or diisopropyl ether from the *Standard solution*
C_S = concentration of 1,2-dimethoxyethane or diisopropyl ether in the *Standard solution* (mg/mL)
C_U = concentration of Drospirenone in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: NMT 0.2% of 1,2-dimethoxyethane and NMT 0.1% of diisopropyl ether

• **Procedure 2**
Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	63	37
2.0	63	37
16.0	52	48
23.0	52	48
31.0	20	80
39.0	20	80
39.1	63	37
49.0	63	37

Diluent: Acetonitrile and water (1:1)
System suitability solution: 60 µg/mL of USP Drospirenone RS and 60 µg/mL of USP Drospirenone Related Compound A RS in *Diluent*
Standard solution: 0.6 µg/mL of USP Drospirenone RS in *Diluent*
Sample solution: 0.6 mg/mL of Drospirenone in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 195 nm and 245 nm
Column: 4.6-mm × 25-cm; 3-µm packing L1
Column temperature: 35 ± 5°
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between drospirenone and drospirenone related compound A, *System suitability solution*
Tailing factor: Between 0.8 and 1.5, *Standard solution*
Relative standard deviation: NMT 15.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Drospirenone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of drospirenone from the *Standard solution*
C_S = concentration of USP Drospirenone RS in the *Standard solution* (µg/mL)
C_U = concentration of Drospirenone in the *Sample solution* (µg/mL)
F = relative response factor for each individual impurity (see *Table 4*)

[NOTE—The percentage of hydroxydrospirenone is calculated at 195 nm.]
Acceptance criteria
[NOTE—Disregard any peaks that are less than 0.05% of the drospirenone peak.]
Individual impurities: See *Table 4*.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
7-Hydroxymethyl drospirenone at 245 nm[a]	0.43	1.9	0.1
5-Hydroxydrospirenone at 195 nm[b]	0.57	0.57	0.1
17-Keto drospirenone at 245 nm[c]	0.77	1.2	0.1
Drospirenone at 245 and 195 nm	1.00	—	—
Drospirenone 6-ene at 245 nm[d]	1.04	0.30	0.1
Drospirenone related compound A at 245 nm[e]	1.11	1.0	0.1
6,7-Epidrospirenone at 245 nm[f]	1.14	1.3	0.1
6,7-Desmethylene drospirenone at 245 nm[g]	1.18	2.2	0.1

www.webofpharma.com

Table 4 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
15-Methyl drospirenone at 245 nm[h]	1.34	0.99	0.1
7-Chloromethyl drospirenone at 245 nm[i]	1.38	1.6	0.1
7-Chloromethyl 17-epidrospirenone at 245 nm[j]	1.51	1.9	0.1
7-Hydroxymethyl 3,5(6)-diene drospirenone at 245 nm[k]	1.55	1.4	0.1
Any unspecified impurity at 245 nm	—	1.0	0.1
Total impurities	—	—	0.4

[a] 17-Hydroxy-7β-hydroxymethyl-15β,16β-methylene-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.
[b] 5β,17-Dihydroxy-6β,7β:15β,16β-dimethylene-3-oxo-17α-pregnan-21-carboxylic acid, γ-lactone.
[c] 6β,7β:15β,16β-Dimethyleneandrost-4-ene-3,17-dione.
[d] 17-Hydroxy-15β,16β-methylene-3-oxo-17α-pregn-4,6-diene-21-carboxylic acid, γ-lactone.
[e] 17-Hydroxy-6β,7β:15β,16β-dimethylene-3-oxo-17β-pregn-4-ene-21-carboxylic acid, γ-lactone; 17-epidrospirenone.
[f] 17-Hydroxy-6α,7α:15β,16β-dimethylene-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.
[g] 17-Hydroxy-15β,16β-methylene-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.
[h] 17-Hydroxy-15β-methyl-6β,7β-methylene-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.
[i] 17-Hydroxy-7β-chloromethyl-15β,16β-methylene-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone.
[j] 17-Hydroxy-7β-chloromethyl-15β,16β-methylene-3-oxo-17β-pregn-4-ene-21-carboxylic acid, γ-lactone.
[k] 17-Hydroxy-7β-hydroxymethyl-15β,16β-methylene-17α-pregn-3,5(6)-diene-21-carboxylic acid, γ-lactone.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL in methanol
  Acceptance criteria: −187° to −193° at 20° on the anhydrous and solvent-free basis
- **MELTING RANGE OR TEMPERATURE,** *Class 1* ⟨741⟩**:** 198°–203°. [NOTE—Dry over silica gel for NLT 24 h before testing.]
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Drospirenone RS
  USP Drospirenone Related Compound A RS
  17-Hydroxy-6β,7β:15β,16β-dimethylene-3-oxo-17β-pregn-4-ene-21-carboxylic acid, γ-lactone.
  $C_{24}H_{30}O_3$ 366.49

Drospirenone and Ethinyl Estradiol Tablets

DEFINITION

Drospirenone and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of drospirenone ($C_{24}H_{30}O_3$) and NLT 90.0% and NMT 110.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

- **A.** The retention times of the drospirenone and ethinyl estradiol peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 132 g of dibasic ammonium phosphate in 0.8 L of water, adjust with phosphoric acid to a pH of 6.8, and dilute to 1 L.
Solution B: *Solution A* and water (1:24)
Mobile phase: Acetonitrile and *Solution B* (1:1). Adjust with phosphoric acid to a pH of 6.8.
Standard solution: (*L*/25) mg/mL of USP Drospirenone RS and USP Ethinyl Estradiol RS in *Mobile phase*, where *L* is the Tablet label claim, in mg/Tablet, of each compound
Sample solution: Transfer 10 Tablets to a 250-mL volumetric flask, add 230 mL of *Mobile phase*, and sonicate with intermittent shaking for NLT 10 min, or until the Tablets are completely dispersed. Equilibrate to room temperature. Dilute with *Mobile phase* to volume, and centrifuge the sample until a clear supernatant is obtained. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector 1: UV 270 nm for drospirenone
Detector 2: Fluorescence, excitation wavelength at 285 nm, emission wavelength at 315 nm for ethinyl estradiol. [NOTE—*Detector 1* and *Detector 2* are connected in series.]
Column: 4.0-mm × 12.5-cm; 3-µm packing L1
Column temperature: 25 ± 3°
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: Between 0.8 and 1.8 for both drospirenone and ethinyl estradiol
Relative standard deviation: NMT 2.0% for both drospirenone and ethinyl estradiol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of drospirenone ($C_{24}H_{30}O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of drospirenone from the *Sample solution*
r_S = peak response of drospirenone from the *Standard solution*
C_S = concentration of USP Drospirenone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of drospirenone in the *Sample solution* (mg/mL)

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ethinyl estradiol from the *Sample solution*
r_S = peak response of ethinyl estradiol from the *Standard solution*
C_S = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (mg/mL)

USP Monographs

www.webofpharma.com

C_U = nominal concentration of ethinyl estradiol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of ethinyl estradiol; 90.0%–110.0% of drospirenone

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1: For drug products labeled to contain 3 mg of drospirenone and 0.03 mg of ethinyl estradiol, or 3 mg of drospirenone and 0.02 mg of ethinyl estradiol
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: (*L*/900) mg/mL of USP Drospirenone RS and USP Ethinyl Estradiol RS in *Medium*, where *L* is the Tablet label claim of each compound. A volume of methanol not exceeding 2% of the final total volume of solution may be used to aid in dissolving these compounds.
Sample solution: Pass a portion of the solution under test through a suitable cellulose filter of 0.45-µm pore size, discarding the first 10 mL.
Mobile phase: Acetonitrile and water (40:60)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm (for drospirenone), in series with a fluorescence detector (for ethinyl estradiol), with excitation at 210 nm and detection at 315 nm, or with excitation at 281 nm and detection at 305 nm
Column: 4.6-mm × 6-cm; 3-µm packing L1
Column temperature: 22°
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 for both drospirenone and ethinyl estradiol
Tailing factor: Between 0.8 and 1.5 for both drospirenone and ethinyl estradiol
Relative standard deviation: NMT 3% for both drospirenone and ethinyl estradiol
Samples: *Standard solution* and *Sample solution*
[NOTE—In *Medium*, drospirenone is partially converted into 17-epidrospirenone, which has a relative retention time of approximately 1.2 relative to drospirenone. The amount of drospirenone dissolved is calculated from the sum of drospirenone and 17-epidrospirenone.]
Calculate the percentage of the labeled amounts of drospirenone ($C_{24}H_{30}O_3$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (*Q*) of the labeled amount of drospirenone and NLT 75% (*Q*) of the labeled amount of ethinyl estradiol is dissolved.

Test 2: For drug products labeled to contain 3 mg of drospirenone and 0.02 mg of ethinyl estradiol. If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: (*L*/900) mg/mL of USP Drospirenone RS and USP Ethinyl Estradiol RS in *Medium*, where *L* is the Tablet label claim of each compound
Sample solution: Centrifuge a portion of the solution under test at 3500 rpm for 15 min, and use the supernatant.
Mobile phase: Acetonitrile, methanol, and water (40:5:55)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm (for drospirenone), in series with a fluorescence detector (for ethinyl estradiol), with excitation at 280 nm and detection at 310 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Temperatures
Column: 30°
Autosampler: 5°
Flow rate: 1 mL/min
Injection volume: 200 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2 for both drospirenone and ethinyl estradiol
Relative standard deviation: NMT 3% for both drospirenone and ethinyl estradiol
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of drospirenone ($C_{24}H_{30}O_3$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of drospirenone and NLT 85% (*Q*) of the labeled amount of ethinyl estradiol is dissolved.

Test 3: For drug products labeled to contain 3 mg of drospirenone and 0.03 mg of ethinyl estradiol, or 3 mg of drospirenone and 0.02 mg of ethinyl estradiol. If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Mobile phase: Acetonitrile and water (50:50)
Standard stock solution A: 2.8 µg/mL of USP Ethinyl Estradiol RS in acetonitrile. Sonicate as needed.
Standard stock solution B: 168 µg/mL of USP Drospirenone RS in acetonitrile. Sonicate as needed.
Standard solution: (*L*/900) mg/mL of USP Drospirenone RS and USP Ethinyl Estradiol RS in *Medium* from *Standard stock solution A* and *Standard stock solution B*, where *L* is the Tablet label claim of each compound.
Sample solution: Pass a portion of the solution under test through a suitable glass filter of 1-µm pore size, discarding the first 5 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

www.webofpharma.com

Detector: UV 265 nm (for drospirenone), in series with a fluorescence detector (for ethinyl estradiol), with excitation at 285 nm and detection at 310 nm
Column: 4.6-mm × 15-cm; 5-µm packing L11
Temperatures
Column: 30°
Autosampler: 10°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for both drospirenone and ethinyl estradiol
Relative standard deviation: NMT 2.0% for both drospirenone and ethinyl estradiol
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of drospirenone ($C_{24}H_{30}O_3$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of drospirenone and NLT 75% (*Q*) of the labeled amount of ethinyl estradiol is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**
Solution A: Acetonitrile, methanol, and water (26:19:55)
Solution B: Acetonitrile, methanol, and water (76:19:5)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Flow (mL/min)	Solution A (%)	Solution B (%)
0	0.5	90	10
40	0.5	90	10
53	0.5	0	100
59	1.0	0	100
60	0.5	90	10
70	0.5	90	10

System suitability stock solution: (L_1 × 18/100) µg/mL of USP Drospirenone RS in *Solution A*, where L_1 is the label claim (µg/Tablet) of drospirenone
System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* into a 10-mL volumetric flask, add 1.0 mL of 0.1 N hydrochloric acid, and heat for 30 min in a 40° water bath. Immediately add 1 mL of 0.1 N sodium hydroxide (NaOH) and allow to reach room temperature. Dilute with *Solution A* to volume to obtain a solution containing drospirenone and 17-epidrospirenone. [Note—Sodium hydroxide (NaOH) must be added immediately after heating for the reaction to proceed properly. The drospirenone to 17-epidrospirenone ratio must be between 3:1 and 7:1.]
Standard solution: (L_1 × 15/1000) µg/mL of USP Drospirenone RS, (L_2 × 30/1000) µg/mL of USP Ethinyl Estradiol RS, and (L_2 × 30/1000) µg/mL of USP Ethinyl Estradiol Related Compound B RS in *Solution A*, where L_1 and L_2 are the label claim (µg/Tablet) of drospirenone and ethinyl estradiol, respectively
Sensitivity solution: (L_1 × 15/10,000) µg/mL of USP Drospirenone RS, (L_2 × 30/10,000) µg/mL of USP Ethinyl Estradiol RS, and (L_2 × 30/10,000) µg/mL of USP Ethinyl Estradiol Related Compound B RS in *Solution A*, where L_1 and L_2 are the label claim (µg/Tablet) of drospirenone and ethinyl estradiol, respectively, prepared from the *Standard solution*
Sample solution: Transfer 15 Tablets to a 10-mL glass-stoppered test tube, and add 5.0 mL of *Solution A*. Shake vigorously, sonicate for NLT 5 min, and place in an ice bath for NLT 10 min. Centrifuge the sample at least until an almost clear supernatant is obtained. Filter the supernatant, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector 1: UV 222 nm
Detector 2: Fluorescence, excitation wavelength at 215 nm, emission wavelength at 315 nm. Monitor the signal at 344 nm for ethinyl estradiol related compound B (typically between 37 and 42 min). [Note—*Detector 1* and *Detector 2* are connected in series. Use the response at 344 nm to quantify ethinyl estradiol related compound B.]
Column: 3.0-mm × 30-cm; 3-µm packing L1 followed, in series, by a 4.6-mm × 10-cm; chromolith packing L1
Column temperature: 40°
Flow rate: See *Table 1*.
Injection volume: 20 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Tailing factor: Between 0.8 and 1.5 for both drospirenone and ethinyl estradiol, *Standard solution*
Resolution: NLT 2.0 between drospirenone and 17-epidrospirenone, *System suitability solution*
Relative standard deviation: NMT 5.0% for both drospirenone and ethinyl estradiol, *Standard solution*
Signal-to-noise ratio: NLT 10 for drospirenone and ethinyl estradiol related compound B and NLT 7.0 for ethinyl estradiol, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Identify the ethinyl estradiol degradation products using the relative retention times given in *Table 2*.
Calculate the percentage of each ethinyl estradiol degradation product and unspecified degradation products in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each ethinyl estradiol degradation product from the *Sample solution*
r_S = peak response of ethinyl estradiol from the *Standard solution*
C_S = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of ethinyl estradiol in the *Sample solution* (µg/mL)

USP Monographs

www.webofpharma.com

F = relative response factor for each degradation product (see *Table 2*)

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ethinyl estradiol related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ethinyl estradiol related compound B from the *Sample solution*
r_S = peak response of ethinyl estradiol related compound B from the *Standard solution*
C_S = concentration of USP Ethinyl Estradiol Related Compound B RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of ethinyl estradiol in the *Sample solution* (µg/mL)

Samples: *Standard solution* and *Sample solution*
Identify the drospirenone degradation products using the relative retention times given in *Table 3*.
Calculate the percentage of each drospirenone degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each drospirenone degradation product from the *Sample solution*
r_S = peak response of drospirenone from the *Standard solution*
C_S = concentration of USP Drospirenone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of drospirenone in the *Sample solution* (µg/mL)
F = relative response factor for each degradation product (see *Table 3*)

Acceptance criteria [NOTE—Report only degradation products greater than 0.1%.]
Individual degradation products: See *Table 2* for ethinyl estradiol and *Table 3* for drospirenone.
Total degradation products: NMT 3.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Drospirenone RS
  USP Ethinyl Estradiol RS
  USP Ethinyl Estradiol Related Compound B RS
  19-Nor-17α-pregna-1,3,5(10),9(11)-tetraen-20-yne-3,17-diol monohydrate.
  $C_{20}H_{22}O_2 \cdot H_2O$ 312.40

Duloxetine Delayed-Release Capsules

DEFINITION
Duloxetine Delayed-Release Capsules contain an amount of Duloxetine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of duloxetine ($C_{18}H_{19}NOS$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F▲** (CN 1-May-2020)
  Buffer: 6.9 g/L of *monobasic sodium phosphate* in *water* adjusted with *5 N sodium hydroxide* to a pH of 7.5
  Spectral range: 1650 cm^{-1} to 900 cm^{-1}
  Standard: 1 mg/mL of USP Duloxetine Hydrochloride RS in *methylene chloride*. Shake the contents, and sonicate for 1 min. Transfer 15 mL of filtrate into a separatory funnel, and add 15 mL of *Buffer*. Collect the organic layer, and evaporate to dryness. Redissolve the residue with a few drops of *methylene chloride*, and transfer to a potassium bromide or sodium chloride plate. Allow it to dry.

Table 2 (Drospirenone and Ethinyl Estradiol Tablets)

Name	Detection Mode	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)[a]	Acceptance Criteria, NMT (%)[b]
6α-Hydroxy ethinyl estradiol[c]	Fl (215 nm/315 nm)[g]	0.25	0.73	0.8	0.8
6β-Hydroxy ethinyl estradiol[d]	Fl (215 nm/315 nm)	0.27	0.64	0.8	0.8
6-Keto ethinyl estradiol[e]	UV 222 nm	0.41	2.3	1.5	▲0.8▲ (RB 1-Apr-2019)
Ethinyl estradiol related compound B[f]	Fl (215 nm/344 nm)	0.88	—	1.0	1.0
Ethinyl estradiol	Fl (215 nm/315 nm)	1.0	—	—	—
Any unspecified degradation product	Fl (215 nm/315 nm) and UV 222[h]	—	1.0	▲0.4▲ (RB 1-Apr-2019)	0.5
Total degradation product	—	—	—	3.0	2.5

[a] Limits for drug products labeled to contain 3 mg of drospirenone and 0.03 mg of ethinyl estradiol.
[b] Limits for drug products labeled to contain 3 mg of drospirenone and 0.02 mg of ethinyl estradiol.
[c] 19-Nor-6α,17α-pregna-1,3,5(10)-trien-20-yne-3,6,17-triol.
[d] 19-Nor-6β,17α-pregna-1,3,5(10)-trien-20-yne-3,6,17-triol.
[e] 19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol-6-one.
[f] Δ9,11-Ethinyl estradiol. 19-Nor-17α-pregna-1,3,5(10),9(11)-tetraen-20-yne-3,17-diol.
[g] Fl = Fluorescence.
[h] Determine unknown impurities using both modes of detection. Report the values from the detection mode that yield higher impurity levels.

www.webofpharma.com

Table 3 (Drospirenone and Ethinyl Estradiol Tablets)

Name	Detection Mode (λ nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)[a]	Acceptance Criteria, NMT (%)[b]
Drospirenone	UV 222	0.75	—	—	—
17-Epidrospirenone[c]	UV 222	0.83	1.0	0.3	0.3
Ethinyl estradiol	UV 222	1.0	—	—	—
Any unspecified degradation product	UV 222	—	1.0	0.3	0.5
Total degradation product	—	—	—	0.5	1.0

[a] Limits for drug products labeled to contain 3 mg of drospirenone and 0.03 mg of ethinyl estradiol.
[b] Limits for drug products labeled to contain 3 mg of drospirenone and 0.02 mg of ethinyl estradiol.
[c] 17-Hydroxy-6β,7β:15β,16β-dimethylene-3-oxo-17β-pregn-4-ene-21-carboxylic acid, γ-lactone.

Sample: 1 mg/mL of duloxetine, from the contents of NLT 10 Capsules in *methylene chloride*. Proceed as directed in the *Standard*.
Acceptance criteria: Meet the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**
Protect solutions of duloxetine from light.
Buffer A: 3.4 g/L of ▲monobasic potassium phosphate▲ (ERR 1-Jun-2019) in *water*. To 1 L of this solution add 15 mL of *triethylamine*, and adjust with *phosphoric acid* to a pH of 5.5.
Buffer B: 0.2 g/L of ▲monobasic ammonium phosphate▲ (ERR 1-Jun-2019) and 4.5 g/L of *dibasic potassium phosphate* in *water*. Adjust with *phosphoric acid* to a pH of 8.0.
Mobile phase: *Methanol*, *tetrahydrofuran*, and *Buffer A* (323:90:587)
Diluent: *Methanol* and *Buffer B* (50:50)
System suitability solution: 0.1 mg/mL of USP Duloxetine Hydrochloride RS, 0.05 mg/mL of α-naphthol, 0.01 mg/mL of USP Duloxetine Related Compound F RS, and 0.025 mg/mL of USP Duloxetine Related Compound H RS in *Diluent*. [NOTE—Add 1 mL of *methanol* before diluting to volume to assist with dissolving contents. Duloxetine related compound H is used for peak identification purposes in this solution.]
Standard solution: 0.1 mg/mL of USP Duloxetine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.1 mg/mL of duloxetine from the contents of NLT 5 Capsules, in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
[NOTE—It is recommended to preheat the *Mobile phase* to 45°.]
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 7.5-cm; 3- or 3.5-µm packing L7
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: 6 times the retention time of duloxetine
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* in *Organic Impurities* for relative retention times.]
Suitability requirements
Resolution: NLT 1.6 between duloxetine and duloxetine related compound F; NLT 2 between α-naphthol and duloxetine related compound H, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of duloxetine ($C_{18}H_{19}NOS$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Duloxetine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of duloxetine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of duloxetine free base, 297.42
M_{r2} = molecular weight of duloxetine hydrochloride, 333.88

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

DISSOLUTION ⟨711⟩
Test 1
Acid stage
Acid stage medium: 0.1 N hydrochloric acid VS; 1000 mL
Apparatus 1: 100 rpm
Time: 2 h
Buffer stage
Buffer stage medium: *pH 6.8 phosphate buffer*; 1000 mL
Apparatus 1: 100 rpm
Time: 60 min for Capsules containing 20% w/w pellets; 90 min for Capsules containing 32% w/w pellets
Buffer A and **Mobile phase:** Proceed as directed in the *Assay*.
Standard stock solution: 0.28 mg/mL of USP Duloxetine Hydrochloride RS, equivalent to 0.25 mg/mL of duloxetine, in *Buffer stage medium*. Use a small amount of methanol, not exceeding 2% of the final volume, to dissolve duloxetine.
Acid stage standard solution: 0.0023 mg/mL of USP Duloxetine Hydrochloride RS, equivalent to 0.002

USP Monographs

www.webofpharma.com

mg/mL of duloxetine, from the *Standard stock solution* diluted with *Buffer stage medium*

Buffer stage standard solution: 0.023 mg/mL of USP Duloxetine Hydrochloride RS, equivalent to 0.02 mg/mL of duloxetine, from the *Standard stock solution* diluted with *Buffer stage medium*

Sample solution: After 2 h in the *Acid stage medium*, pass a portion of the solution under test through a suitable filter. Transfer the basket containing the pellets to the vessel containing the *Buffer stage medium*. After the appropriate time in the *Buffer stage medium*, pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 7.5-cm; 3- or 3.5-µm packing L7

Column temperature: 45°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Acid stage standard solution*

[NOTE—The relative retention times for duloxetine and α-naphthol are 1.0 and 1.4, respectively.]

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Acid stage standard solution*, *Buffer stage standard solution*, and *Sample solution*

Calculate the concentration of duloxetine in the *Acid stage medium* (C_1):

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response of duloxetine from the *Sample solution*

r_S = peak response of duloxetine from the *Acid stage standard solution*

C_S = concentration of USP Duloxetine Hydrochloride RS in the *Acid stage standard solution* (mg/mL)

M_{r1} = molecular weight of duloxetine free base, 297.42

M_{r2} = molecular weight of duloxetine hydrochloride, 333.88

Calculate the equivalent concentration of duloxetine from α-naphthol in the *Acid stage medium* (C_2):

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times (M_{r1}/M_{r3})$$

r_U = peak response of α-naphthol from the *Sample solution*

r_S = peak response of duloxetine from the *Acid stage standard solution*

C_S = concentration of USP Duloxetine Hydrochloride RS in the *Acid stage standard solution* (mg/mL)

M_{r1} = molecular weight of duloxetine free base, 297.42

M_{r2} = molecular weight of duloxetine hydrochloride, 333.88

M_{r3} = molecular weight of α-naphthol, 144.17

Calculate the percentage of the labeled amount of duloxetine dissolved in the *Acid stage medium* (Q_A):

$$\text{Result} = (C_1 + C_2) \times V \times (1/L) \times 100$$

C_1 = concentration of duloxetine in the *Acid stage medium* (mg/mL)

C_2 = equivalent concentration of duloxetine from α-naphthol in the *Acid stage medium* (mg/mL)

V = volume of *Medium*, 1000 mL

L = label claim of duloxetine (mg/Capsule)

Calculate the percentage of the labeled amount of duloxetine dissolved in the *Buffer stage medium*:

$$\text{Result} = [(r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100] + Q_A$$

r_U = peak response of duloxetine from the *Sample solution*

r_S = peak response of duloxetine from the *Buffer stage standard solution*

C_S = concentration of USP Duloxetine Hydrochloride RS in the *Buffer stage standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of *Medium*, 1000 mL

M_{r1} = molecular weight of duloxetine free base, 297.42

M_{r2} = molecular weight of duloxetine hydrochloride, 333.88

Q_A = percentage of the labeled amount of duloxetine dissolved in the *Acid stage medium*

Tolerances

Acid stage: No individual unit releases more than 10% of the labeled amount of duloxetine in 2 h.

Buffer stage

For Capsules containing 20% w/w pellets: NLT 75% (*Q*) of the labeled amount of duloxetine is dissolved in 60 min.

For Capsules labeled to contain 32% w/w pellets: NLT 75% (*Q*) of the labeled amount of duloxetine is dissolved in 90 min.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Protect solutions of duloxetine from light.

Acid stage

Acid stage medium: 0.1 N hydrochloric acid VS; 750 mL

Apparatus 2: 100 rpm

Time: 2 h in *Acid stage medium*

Buffer stage

Buffer stage medium: *pH 6.8 phosphate buffer* (after 2 h, add 250 mL of 76 g/L of tribasic sodium phosphate, previously heated to 37 ± 0.5°, to the *Acid stage medium*); 1000 mL

Apparatus 2: 100 rpm

Time: 3 h in *Buffer stage medium*. The time in *Buffer stage medium* includes the time in *Acid stage medium*.

Solution A: A mixture of *triethylamine* and *water* prepared as follows. Add 15 mL of *triethylamine* to 1 L of *water* and adjust with *phosphoric acid* to a pH of 2.5 ± 0.05.

Mobile phase: *Acetonitrile* and *Solution A* (40:60)

Diluent: 0.1 N hydrochloric acid VS and 76 g/L of tribasic sodium phosphate (75:25)

Standard stock solution: 0.46 mg/mL of USP Duloxetine Hydrochloride RS, equivalent to 0.4 mg/mL of duloxetine, prepared as follows. Transfer a suitable amount of USP Duloxetine Hydrochloride RS to an appropriate volumetric flask and dissolve in 50% of the final flask volume of *Mobile phase*. Dilute with *Mobile phase* to volume.

Standard solution: 0.046 mg/mL of USP Duloxetine Hydrochloride RS, equivalent to 0.04 mg/mL of duloxetine, from the *Standard stock solution* in *Diluent*

Acid stage sample solution and **Buffer stage sample solution:** Pass a portion of the solution under test

USP Monographs

www.webofpharma.com

through a suitable filter, and use the filtrate. [NOTE—A cannula-style filter with a 20-µm pore size may be suitable.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 290 nm
Column: 4.6-mm × 15.0-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1.3 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution, Acid stage sample solution,* and *Buffer stage sample solution*
Calculate the percentage of the labeled amount of duloxetine ($C_{18}H_{19}NOS$) dissolved in *Acid stage medium*:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_U = peak response of duloxetine from the *Acid stage sample solution*
r_S = peak response of duloxetine from the *Standard solution*
C_S = concentration of USP Duloxetine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of duloxetine free base, 297.42
M_{r2} = molecular weight of duloxetine hydrochloride, 333.88
V = volume of *Acid stage medium*, 750 mL
L = label claim of duloxetine (mg/Capsule)

Calculate the percentage of the labeled amount of duloxetine ($C_{18}H_{19}NOS$) dissolved in *Buffer stage medium*:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_U = peak response of duloxetine from the *Buffer stage sample solution*
r_S = peak response of duloxetine from the *Standard solution*
C_S = concentration of USP Duloxetine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of duloxetine free base, 297.42
M_{r2} = molecular weight of duloxetine hydrochloride, 333.88
V = volume of *Buffer stage medium*, 1000 mL
L = label claim of duloxetine (mg/Capsule)

Tolerances
Acid stage: For each individual value, NMT 10% of the labeled amount of duloxetine ($C_{18}H_{19}NOS$). The percentage of the labeled amount of duloxetine dissolved at the time specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 3*.
Buffer stage: NLT 80% (*Q*) of the labeled amount of duloxetine ($C_{18}H_{19}NOS$). The percentage of the labeled amount of duloxetine dissolved at the time specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 4*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Protect solutions of duloxetine from light.

Acid stage
Acid stage medium: 0.1 N hydrochloric acid VS with *pepsin* (mix 8.5 mL of hydrochloric acid and 3.2 g of *pepsin* in 1 L of *water*); 1000 mL
Apparatus 1: 100 rpm
Time: 2 h in *Acid stage medium*

Buffer stage
Buffer stage medium: *pH 6.8 phosphate buffer* (6.8 g/L of monobasic potassium phosphate and 0.9 g/L of sodium hydroxide in *water*, adjusted with *phosphoric acid* or 1 N sodium hydroxide VS to a pH of 6.80); 1000 mL
Apparatus 1: 100 rpm
Time: 3 h in *Buffer stage medium*. The time in *Buffer stage medium* includes the time in *Acid stage medium*.

Procedure: After 2 h in the *Acid stage medium,* withdraw a sample from the solution and immediately filter. Remove and rinse each basket with NMT 25 mL of ▲0.1 N hydrochloric acid VS.▲ (ERR 1-Jun-2019) Then transfer the baskets to the *Buffer stage medium*.

Solution A: 2.7 g/L of monobasic potassium phosphate in *water*. Add 2.0 mL of *triethylamine* per liter of solution and adjust with *phosphoric acid* to a pH of 6.0.

Mobile phase: *Acetonitrile* and *Solution A* (38:62)

Standard stock solution: 1.1 mg/mL of USP Duloxetine Hydrochloride RS, equivalent to 1.0 mg/mL of duloxetine, prepared as follows. Transfer a suitable amount of USP Duloxetine Hydrochloride RS to an appropriate volumetric flask and dissolve in 60% of the flask volume of *water*. Sonication may be used to promote dissolution. Dilute with *water* to volume.

System suitability stock solution: 0.011 mg/mL of USP Duloxetine Hydrochloride RS from the *Standard stock solution* in 0.1 N hydrochloric acid VS. Store this solution in a 37° water bath for 30 min.

System suitability solution: 0.002 mg/mL of USP Duloxetine Hydrochloride RS from the *System suitability stock solution* in *Mobile phase*. Pass the resulting solution through a suitable filter and use the filtrate.

Acid stage standard stock solution: 0.11 mg/mL of USP Duloxetine Hydrochloride RS, equivalent to 0.10 mg/mL of duloxetine, from the *Standard stock solution* in *water*

Acid stage standard solution: (*L*/50,000) mg/mL of duloxetine from the *Standard stock solution* in *Mobile phase,* where *L* is the label claim, in mg/Capsule

Buffer stage standard solution: (*L*/1,000) mg/mL of duloxetine from the *Standard stock solution* in *Buffer stage medium,* where *L* is the label claim, in mg/Capsule

Acid stage sample solution: Transfer 2 mL of the solution under test to a suitable container and dilute with *Mobile phase* to 10 mL. Pass a portion of the resulting solution through a suitable filter, discard NLT 3 mL, and use the filtrate.

Buffer stage sample solution: Pass a portion of the solution under test through a suitable filter, discard NLT 1 mL, and use the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15.0-cm; 3.5-µm packing L1
Column temperature: 50°
Flow rate: 1.1 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of duloxetine

System suitability
Samples: *System suitability solution* and *Buffer stage standard solution*

USP Monographs

www.webofpharma.com

[NOTE—The relative retention times for duloxetine and α-naphthol are 1.0 and 1.7, respectively.]

Suitability requirements

Resolution: NLT 5 between duloxetine and α-naphthol, *System suitability solution*

Tailing factor: NMT 2.0, *Buffer stage standard solution*

Relative standard deviation: NMT 2.0%, *Buffer stage standard solution*

Analysis

Samples: *Acid stage standard solution, Buffer stage standard solution, Acid stage sample solution,* and *Buffer stage sample solution*

Calculate the concentration of duloxetine ($C_{18}H_{19}NOS$) dissolved in the *Acid stage medium* (C_1):

$$\text{Result} = (r_U/r_S) \times C_S \times D \times (M_{r1}/M_{r2})$$

r_U = peak response of duloxetine from the *Acid stage sample solution*

r_S = peak response of duloxetine from the *Acid stage standard solution*

C_S = concentration of USP Duloxetine Hydrochloride RS in the *Acid stage standard solution* (mg/mL)

D = dilution factor of the *Acid stage sample solution,* 5

M_{r1} = molecular weight of duloxetine free base, 297.42

M_{r2} = molecular weight of duloxetine hydrochloride, 333.88

Calculate the equivalent concentration of duloxetine from α-naphthol in the *Acid stage medium* (C_2):

$$\text{Result} = (r_U/r_S) \times C_S \times D \times (1/F) \times (M_{r1}/M_{r2})$$

r_U = peak response of α-naphthol from the *Acid stage sample solution*

r_S = peak response of duloxetine from the *Acid stage standard solution*

C_S = concentration of USP Duloxetine Hydrochloride RS in the *Acid stage standard solution* (mg/mL)

D = dilution factor of the *Acid stage sample solution,* 5

F = relative response factor of α-naphthol, 1.7

M_{r1} = molecular weight of duloxetine free base, 297.42

M_{r2} = molecular weight of duloxetine hydrochloride, 333.88

Calculate the equivalent concentration of duloxetine from all of the unspecified degradation products in the *Acid stage medium* (C_3):

$$\text{Result} = (r_i/r_S) \times C_S \times D \times (M_{r1}/M_{r2})$$

r_i = sum of the peak responses from all of the unspecified degradation products in the *Acid stage sample solution*

r_S = peak response of duloxetine from the *Acid stage standard solution*

C_S = concentration of USP Duloxetine Hydrochloride RS in the *Acid stage standard solution* (mg/mL)

D = dilution factor of the *Acid stage sample solution,* 5

M_{r1} = molecular weight of duloxetine free base, 297.42

M_{r2} = molecular weight of duloxetine hydrochloride, 333.88

Calculate the percentage of the labeled amount of duloxetine dissolved in the *Acid stage medium* (Q_A):

$$\text{Result} = (\Sigma C_i) \times V \times (1/L) \times 100$$

C_i = concentration or equivalent concentration of duloxetine in the *Acid stage medium* (mg/mL)

V = volume of *Acid stage medium,* 1000 mL

L = label claim of duloxetine (mg/Capsule)

Calculate the percentage of the labeled amount of duloxetine dissolved in both the *Acid stage medium* and the *Buffer stage medium*:

$$\text{Result} = [(r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100] + Q_A$$

r_U = peak response of duloxetine from the *Buffer stage sample solution*

r_S = peak response of duloxetine from the *Buffer stage standard solution*

C_S = concentration of USP Duloxetine Hydrochloride RS in the *Buffer stage standard solution* (mg/mL)

M_{r1} = molecular weight of duloxetine free base, 297.42

M_{r2} = molecular weight of duloxetine hydrochloride, 333.88

V = volume of *Buffer stage medium,* 1000 mL

L = label claim (mg/Capsule)

Q_A = percentage of the labeled amount of duloxetine dissolved in the *Acid stage medium*

Tolerances

Acid stage: For each individual value, NMT 10% of the labeled amount of duloxetine ($C_{18}H_{19}NOS$). The percentage of the labeled amount of duloxetine dissolved at the time specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 3.*

Buffer stage: NLT 75% (Q) of the labeled amount of duloxetine ($C_{18}H_{19}NOS$). The percentage of the labeled amount of duloxetine dissolved at the time specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 4.*

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4.*

Protect solutions of duloxetine from light.

Acid stage

Acid stage medium: 0.1 N hydrochloric acid VS; 1000 mL

Apparatus 1: 100 rpm

Time: 2 h in *Acid stage medium*

Buffer stage

Buffer stage medium: *pH 6.8 phosphate buffer* (6.8 g/L of monobasic potassium phosphate and 0.9 g/L of sodium hydroxide in *water,* adjusted with *phosphoric acid* or 1 N sodium hydroxide VS to a pH of 6.80); 1000 mL

Apparatus 1: 100 rpm

Time: 3 h in *Buffer stage medium.* The time in *Buffer stage medium* includes the time in *Acid stage medium.*

Procedure: After 2 h in the *Acid stage medium,* withdraw a sample from the solution under test and immediately filter. Remove the *Acid stage medium* and add the *Buffer stage medium.*

Solution A: *Acetonitrile* and *water* (20:80). To each liter add 1.0 mL of *phosphoric acid.*

Solution B: *Acetonitrile*

Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	35	65
11	100	0

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
15	100	0

Acid stage standard stock solution: 0.011 mg/mL of USP Duloxetine Hydrochloride RS, equivalent to 0.010 mg/mL of duloxetine, in *methanol*. Use this solution within 10 h.

Acid stage standard solution: (*L*/20,000) mg/mL of duloxetine from the *Standard stock solution* in solution prepared as follows, where *L* is the label claim, in mg/Capsule. Transfer a suitable volume of *Acid stage standard stock solution* to an appropriate volumetric flask. Add 45% of the flask volume of 0.1 N hydrochloric acid VS and dilute with 0.1 N sodium hydroxide VS to volume. Use this solution within 10 h.

Buffer stage standard stock solution: 0.67 mg/mL of USP Duloxetine Hydrochloride RS, equivalent to 0.6 mg/mL of duloxetine, in *acetonitrile*

Buffer stage standard solution: (*L*/1,000) mg/mL of duloxetine from *Buffer stage standard stock solution* in *Buffer stage medium*

Acid stage sample stock solution: Pass a portion of the solution under test through a suitable filter, discard NLT 1 mL, and use the filtrate. Use this solution within 4 h.

Acid stage sample solution: Dilute 5.0 mL of the *Acid stage sample stock solution* with 0.1 N sodium hydroxide VS to 10 mL. Use this solution within 4 h.

Buffer stage sample solution: Pass a portion of the solution under test through a suitable filter, discard NLT 2 mL, and use the filtrate. Further dilute with *Buffer stage medium*, if needed.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 10.0-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Acid stage standard solution*

[NOTE—The relative retention times for duloxetine 4-naphthyl isomer, duloxetine, and α-naphthol are 0.8, 1.0, and 1.5, respectively.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 291 nm

Blank: *Buffer stage medium*

System suitability

Sample: *Buffer stage standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Acid stage standard solution, Buffer stage standard solution, Acid stage sample solution,* and *Buffer stage sample solution*

Calculate the concentration of duloxetine ($C_{18}H_{19}NOS$) dissolved in the *Acid stage medium* (C_1):

$$\text{Result} = (r_U/r_S) \times C_S \times D \times (M_{r1}/M_{r2})$$

r_U = peak response of duloxetine from the *Acid stage sample solution*

r_S = peak response of duloxetine from the *Acid stage standard solution*

C_S = concentration of USP Duloxetine Hydrochloride RS in the *Acid stage standard solution* (mg/mL)

D = dilution factor of the *Acid stage sample solution*, 2

M_{r1} = molecular weight of duloxetine free base, 297.42

M_{r2} = molecular weight of duloxetine hydrochloride, 333.88

Calculate the equivalent concentration of duloxetine from α-naphthol in the *Acid stage medium* (C_2):

$$\text{Result} = (r_U/r_S) \times C_S \times D \times (1/F) \times (M_{r1}/M_{r2})$$

r_U = peak response of α-naphthol from the *Acid stage sample solution*

r_S = peak response of duloxetine from the *Acid stage standard solution*

C_S = concentration of USP Duloxetine Hydrochloride RS in the *Acid stage standard solution* (mg/mL)

D = dilution factor of the *Acid stage sample solution*, 2

F = relative response factor of α-naphthol, 2.0

M_{r1} = molecular weight of duloxetine free base, 297.42

M_{r2} = molecular weight of α-naphthol, 144.17

Calculate the equivalent concentration of duloxetine from duloxetine 4-naphthyl isomer (4-[3-(Methylamino)-1-(thiophen-2-yl)propyl] naphthalen-1-ol) in the *Acid stage medium* (C_3):

$$\text{Result} = (r_U/r_S) \times C_2 \times D \times (1/F) \times (M_{r1}/M_{r2})$$

r_U = peak response of duloxetine 4-naphthyl isomer from the *Acid stage sample solution*

r_S = peak response of duloxetine from the *Acid stage standard solution*

C_2 = equivalent concentration of duloxetine from α-naphthol in the *Acid stage medium* (mg/mL)

D = dilution factor of the *Acid stage sample solution*, 2

F = relative response factor of duloxetine 4-naphthyl isomer, 0.70

M_{r1} = molecular weight of duloxetine free base, 297.42

M_{r2} = molecular weight of duloxetine 4-naphthyl isomer, 297.42

Calculate the percentage of the labeled amount of duloxetine dissolved in *Acid stage medium* (Q_A):

$$\text{Result} = \Sigma(C_i) \times V \times (1/L) \times 100$$

C_i = concentration or equivalent concentration of duloxetine in the *Acid stage medium* (mg/mL) associated with duloxetine, α-naphthol, and duloxetine 4-naphthyl isomer

V = volume of *Acid stage medium*, 1000 mL

L = label claim of duloxetine (mg/Capsule)

Calculate the percentage of the labeled amount of duloxetine dissolved in the *Buffer stage medium*:

$$\text{Result} = (A_U/A_S) \times [C_S \times (M_{r1}/M_{r2})] \times V \times D \times (1/L) \times 100$$

A_U = absorbance of duloxetine from the *Buffer stage sample solution*

A_S = absorbance of duloxetine from the *Buffer stage standard solution*

C_S = concentration of USP Duloxetine Hydrochloride RS in the *Buffer stage standard solution* (mg/mL)

M_{r1} = molecular weight of duloxetine free base, 297.42

USP Monographs

www.webofpharma.com

M_{r2} = molecular weight of duloxetine hydrochloride, 333.88
V = volume of *Buffer stage medium*, 1000 mL
D = dilution factor of the *Buffer stage sample solution*, if needed
L = label claim (mg/Capsule)

Tolerances

Acid stage: NMT 10% of the labeled amount of duloxetine ($C_{18}H_{19}NOS$). The percentage of the labeled amount of duloxetine dissolved at the time specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 3*.

Buffer stage: NLT 75% (Q) of the labeled amount of duloxetine ($C_{18}H_{19}NOS$). The percentage of the labeled amount of duloxetine dissolved at the time specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 4*.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Protect solutions of duloxetine from light.

Buffer A, Buffer B, Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Duloxetine	1.0	—
Duloxetine related compound F[a]	1.1	—
α-Naphthol[b]	1.5	0.2
Duloxetine related compound H[c]	2.2	0.2
Any individual unspecified degradation product	—	0.2
Total impurities	—	0.4

[a] This is a process impurity that is included ▲(ERR 1-Jun-2019)▲ for identification purposes only. It is controlled in the drug substance and is not to be reported or included in total impurities.
[b] Naphthalen-1-ol.
[c] May not be present in all formulations.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **LABELING:** The labeling states with which *Dissolution* test the article complies, if other than *Test 1*.

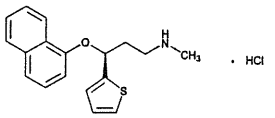
• **USP REFERENCE STANDARDS** ⟨11⟩

USP Duloxetine Hydrochloride RS

USP Duloxetine Related Compound F RS
(*S*)-*N*-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-3-yl) propan-1-amine hydrochloride.
$C_{18}H_{19}NOS \cdot HCl$ 333.88

USP Duloxetine Related Compound H RS
(*S*)-4-{Methyl[3-(naphthalen-1-yloxy)-3-(thiophen-2-yl) propyl]amino}-4-oxobutanoic acid.
$C_{22}H_{23}NO_4S$ 397.49

Duloxetine Hydrochloride



$C_{18}H_{19}NOS \cdot HCl$ 333.88

2-Thiophenepropanamine, *N*-methyl-γ-(1-naphthalenyloxy)-, hydrochloride, (*S*)-;
(+)-(*S*)-*N*-Methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride [136434-34-9].

DEFINITION

Duloxetine Hydrochloride contains NLT 97.0% and NMT 102.0% of duloxetine hydrochloride ($C_{18}H_{19}NOS \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the duloxetine *S*-isomer from the *System suitability solution* in the test for *Limit of Duloxetine Related Compound A*.

• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

Sample solution: 5 mg/mL in methanol

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Protect solutions of duloxetine from light.

Buffer: 2.9 g/L of phosphoric acid in water. Adjust with sodium hydroxide solution to a pH of 2.5. To each L of this solution add 10.3 g of sodium 1-hexanesulfonate monohydrate, and dissolve.

Mobile phase: Acetonitrile, *n*-propanol, and *Buffer* (13:17:70)

Diluent: Acetonitrile and water (25:75)

System suitability solution: 0.2 mg/mL of USP Duloxetine Hydrochloride RS (contains duloxetine related compound F) in *Mobile phase*. Heat the solution to at least 40° for a minimum of 1 h. [NOTE—The resulting solution contains duloxetine alcohol, duloxetine 4-naphthyl isomer, α-naphthol, duloxetine β-naphthol-1-yl isomer, and duloxetine related compound F.]

Standard solution: 0.1 mg/mL of USP Duloxetine Hydrochloride RS in *Diluent*

Sample solution: 0.1 mg/mL of Duloxetine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

www.webofpharma.com

Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Column temperature: 40 ± 3°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of duloxetine
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between duloxetine and duloxetine related compound F
Tailing factor: NMT 1.5 for duloxetine
Relative standard deviation: NMT 1.0% for duloxetine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of duloxetine hydrochloride ($C_{18}H_{19}NOS \cdot HCl$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Duloxetine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Duloxetine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

• **ORGANIC IMPURITIES**

Protect solutions of duloxetine from light.
Buffer, Mobile phase, Diluent, and **System suitability solution:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.2 µg/mL of USP Duloxetine Hydrochloride RS in *Diluent*
Sample solution: 0.2 mg/mL of Duloxetine Hydrochloride in *Diluent*
Chromatographic system: Proceed as directed in the *Assay*, except for *Run time*.
Run time: 2.4 times the retention time of duloxetine
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between duloxetine 4-naphthyl isomer and α-naphthol; NLT 1.5 between duloxetine and duloxetine related compound F, *System suitability solution*
Tailing factor: NMT 1.5 for duloxetine, *System suitability solution*
Relative standard deviation: NMT 1.0% for duloxetine, *System suitability solution*
Signal-to-noise ratio: NLT 20 for the duloxetine peak, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of any individual impurity in the portion of Duloxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Duloxetine alcohol[a]	0.15	0.36	0.1
Duloxetine 4-napthyl isomer[b]	0.43	1.0	0.1
α-Napthol[c]	0.48	1.8	0.1
Duloxetine β-naphthol-1-yl isomer[d]	0.74	1.0	0.1
Duloxetine	1.0	—	—
Duloxetine related compound F[e]	1.1	1.0	0.5
Fluoronaphthalene[f]	1.4	0.51	0.1
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.6

[a] 3-(Methylamino)-1-(thiophen-2-yl)propan-1-ol.
[b] 4-[3-(Methylamino)-1-(thiophen-2-yl)propyl]naphthalen-1-ol.
[c] Naphthalen-1-ol.
[d] 2-[3-(Methylamino)-1-(thiophen-2-yl)propyl]naphthalen-1-ol.
[e] (*S*)-*N*-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-3-yl)propan-1-amine.
[f] 1-Fluoronaphthalene.

• **LIMIT OF DULOXETINE RELATED COMPOUND A**

Mobile phase: Hexane and isopropyl alcohol (83:17). To 1 L of this mixture add 2 mL of diethylamine.
System suitability solution: 0.1 mg/mL each of USP Duloxetine Hydrochloride RS and USP Duloxetine Related Compound A RS in *Mobile phase*. Sonication may be used to aid in dissolution.
Sensitivity solution: 0.1 µg/mL of USP Duloxetine Hydrochloride RS in *Mobile phase*
Sample solution: 0.1 mg/mL of Duloxetine Hydrochloride in *Mobile phase*. Sonication may be used to aid in dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L40
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 2 times the retention time of duloxetine
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
[NOTE—The relative retention times for duloxetine and duloxetine related compound A are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 3.5 between duloxetine and duloxetine related compound A, *System suitability solution*
Tailing factor: 0.8–1.5 each for duloxetine and duloxetine related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0% for duloxetine, *System suitability solution*
Signal-to-noise ratio: NLT 3, *Sensitivity solution*
Analysis
Sample: *Sample solution*

www.webofpharma.com

Calculate the percentage of duloxetine related compound A in the portion of Duloxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of duloxetine related compound A from the *Sample solution*

r_T = sum of the peak responses of duloxetine and duloxetine related compound A from the *Sample solution*

Acceptance criteria: NMT 0.5%

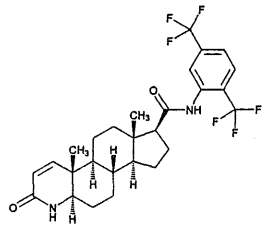
SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Protect from light. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Duloxetine Hydrochloride RS
  USP Duloxetine Related Compound A RS
  (*R*)-*N*-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl) propan-1-amine hydrochloride.
  $C_{18}H_{19}NOS \cdot HCl$ 333.88

Dutasteride



$C_{27}H_{30}F_6N_2O_2$ 528.53

(5α,17β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1-ene-17-carboxamide;
α,α,α,α′,α′,α′-Hexafluoro-3-oxo-4-aza-5α-androst-1-ene-17β-carboxy-2′,5′-xylidide [164656-23-9].

DEFINITION

Dutasteride contains NLT 97.0% and NMT 102.0% of dutasteride ($C_{27}H_{30}F_6N_2O_2$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Diluent: Acetonitrile and water (60:40)
  Mobile phase: Acetonitrile, water, and trifluoroacetic acid (52: 48: 0.025)
  System suitability solution: 0.5 mg/mL of USP Dutasteride Resolution Mixture RS in *Diluent*. Sonicate to dissolve.
  Standard solution: 0.5 mg/mL of USP Dutasteride RS in *Diluent*. Sonicate to dissolve.
  Sample solution: 0.5 mg/mL of Dutasteride in *Diluent*. Sonicate to dissolve.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 220 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 35°
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  Run time: 1.5 times the retention time of dutasteride
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  [NOTE—See *Table 3* for the relative retention times.]
  Suitability requirements
  Resolution: NLT 1.5 between dutasteride 17α-epimer and dutasteride, *System suitability solution*
  Relative standard deviation: NMT 1.5%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of dutasteride ($C_{27}H_{30}F_6N_2O_2$) in the portion of Dutasteride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Dutasteride RS in the *Standard solution* (mg/mL)

C_U = concentration of Dutasteride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF PLATINUM**
  [NOTE—Perform this test only if platinum is a known inorganic impurity of the manufacturing process.]
  Diluent: Hydrochloric acid and dimethyl sulfoxide (2:98). Prepare in a plastic volumetric flask.
  Standard stock solution: 10 µg/mL of platinum in *Diluent*. Prepare by diluting (1:100) a 1000-µg/mL commercially available platinum standard.
  Standard solution 1: 1.0 µg/mL of platinum in *Diluent* from the *Standard stock solution*
  Standard solution 2: 0.1 µg/mL of platinum in *Diluent* from *Standard solution 1*
  Sample solution: 0.01 g/mL of Dutasteride in *Diluent*. Sonicate to dissolve.
  Instrumental conditions
  (See *Plasma Spectrochemistry* ⟨730⟩.)
  Mode: ICP–OES
  Analytical wavelength: 306.471 nm
  Spectrophotometric system: Use an inductively coupled plasma–optical emission spectrophotometric system, and construct a calibration curve using the response from the *Diluent*, *Standard solution 1*, and *Standard solution 2*.
  System suitability
  Samples: *Diluent*, *Standard solution 1*, and *Standard solution 2*
  Suitability requirements
  Limit of quantitation: 3 µg/g for platinum
  Calculate the limit of quantitation from the *Diluent*:

$$\text{Result} = 10 \times (SD/C_S)$$

www.webofpharma.com

SD = standard deviation of platinum from *Diluent* (µg/mL)
C_S = nominal concentration of dutasteride in the *Sample solution* (g/mL)

Correlation coefficient: NLT 0.99 from the *Diluent*, *Standard solution 1*, and *Standard solution 2*

Analysis
Samples: *Diluent*, *Standard solution 1*, *Standard solution 2*, and *Sample solution*
Plot the responses of the *Diluent*, *Standard solution 1*, and *Standard solution 2* versus their content (0, 0.1, and 1.0 µg/mL) of platinum. Determine the concentration, in µg/mL, of platinum in the *Sample solution* from the calibration curve.
Calculate the concentration, in µg/g, of platinum in the portion of Dutasteride taken:

$$\text{Result} = C_S/C_U$$

C_S = concentration of platinum in the *Sample solution* (µg/mL)
C_U = concentration of Dutasteride in the *Sample solution* (g/mL)

Acceptance criteria: NMT 5 µg/g

• **LIMIT OF RESIDUAL SOLVENTS**
Standard stock solution: 5 mg/mL each of acetonitrile, ethyl acetate, pyridine, toluene, dioxane, and *n*-heptane in dimethyl sulfoxide
Standard solution: 10 µg/mL each of acetonitrile, ethyl acetate, pyridine, toluene, dioxane, and *n*-heptane in dimethyl sulfoxide from the *Standard stock solution*
Sample solution: 10 mg/mL of Dutasteride in dimethyl sulfoxide
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m; capillary coated with 5-µm film of phase G1
Temperatures
Injection port: 180°
Detector: 260°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	—	50	3
50	10	200	2

Carrier gas: Helium
Flow rate: Head pressure at 12 psi
Split flow: 10 mL/min
Septum purge: 2 mL/min
Injector type: Headspace
Sample volume: 2 mL
Temperatures
Sample: 85°
Needle: 100°
Transfer line: 110°
Times
Equilibration: 1 min
Thermostating: 15 min
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between *n*-heptane and dioxane peaks
Relative standard deviation: NMT 5% for each solvent
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each solvent in the portion of Dutasteride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each solvent from the *Sample solution*
r_S = peak response of each solvent from the *Standard solution*
C_S = concentration of each solvent in the *Standard solution* (mg/mL)
C_U = concentration of Dutasteride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acetonitrile	0.30	0.3
Ethyl acetate	0.60	0.2
Dioxane	0.83	0.1
n-Heptane	0.85	0.5
Pyridine	0.92	0.2
Toluene	1.0	0.2

• **ORGANIC IMPURITIES, PROCEDURE 1**
Diluent, Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between dutasteride 17α-epimer and dutasteride
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Dutasteride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area for each impurity from the *Sample solution*
r_T = sum of all the peak areas from the *Sample solution*
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dutasteride acid[a]	0.10	1.0	0.2
Dutasteride dimethylamide[b]	0.11	1.4	0.2

www.webofpharma.com

Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dutasteride methyl ester[c]	0.28	1.0	0.15
Dutasteride ethyl ester[d]	0.39	1.0	0.2
Dutasteride 17α-5-ene[e]	0.90	1.0	0.2
Dutasteride 17α-epimer	0.93	1.0	0.3
Dutasteride	1.00	—	—
Chlorodutasteride[f]	1.15	0.33	0.4
Dutasteride 5-ene[g]	1.20	1.0	0.3
Any other individual impurity	—	—	0.1

[a] (5α,17β)-3-Oxo-4-azaandrost-1-ene-17-carboxylic acid.
[b] (5α,17β)-*N*,*N*-Dimethyl-3-oxo-4-azaandrost-1-ene-17-carboxamide.
[c] Methyl (5α,17β)-3-oxo-4-azaandrost-1-ene-17-carboxylate.
[d] Ethyl (5α,17β)-3-oxo-4-azaandrost-1-ene-17-carboxylate.
[e] (17α)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1,5(6)-diene-17-carboxamide.
[f] (1α,5α,17β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-1-chloro-3-oxo-4-azaandrostane-17-carboxamide.
[g] (17β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1,5(6)-diene-17-carboxamide.

• **Organic Impurities, Procedure 2**
Diluent, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.
Mobile phase: Acetonitrile and water (80:20)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L11
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 5 times the retention time of dutasteride
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between dutasteride α-dimer and dutasteride β-dimer peaks
Analysis
Sample: *Sample solution*
Integrate the dutasteride peak and all drug-related peaks eluting after the dutasteride peak.
Calculate the percentage of each impurity in the portion of Dutasteride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_T = sum of all the peak areas from the *Sample solution*
F = relative response factor (see *Table 4*)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dutasteride	1.0	—	—
Dihydrodutasteride[a]	1.19	1.0	0.15

Table 4 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dutasteride α-dimer	3.7	1.0	0.3
Dutasteride β-dimer	4.3	1.0	0.5
Any other individual impurity	—	1.0	0.1
Total impurities[b]	—	—	2.0

[a] (5α,17β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrostane-17-carboxamide.
[b] Sum of impurities from *Table 3* and *Table 4*.

SPECIFIC TESTS
• **Water Determination** ⟨921⟩, *Method I, Method Ic*
Sample: 100 mg
Analysis: The *Sample* is heated in a tube at 180° for 4 min in a stream of dry inert gas.
Acceptance criteria
For the anhydrous form: NMT 0.50%
For the hydrate form: NMT 2.0%. For the hydrate form, *Water Determination* ⟨921⟩, *Method I, Method Ia* may also be used.
• **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL in chloroform and alcohol (98:2)
Acceptance criteria: +15.0° to +25.0°

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers, and store below 30°.
• **Labeling:** Where it is the hydrate form, the label so indicates.
• **USP Reference Standards** ⟨11⟩
USP Dutasteride RS
USP Dutasteride Resolution Mixture RS
The mixture contains Dutasteride and the following impurities (other impurities may also be present):
Dutasteride 17α-epimer: (5α,17α)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1-ene-17-carboxamide.
$C_{27}H_{30}F_6N_2O_2$ 528.53
Dutasteride α-dimer: {[(5α,17β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1-ene-17-carboxamide-]4-yl}{[(5α,17α)-3-oxo-4-azaandrost-1-ene]-17-yl}methanone.
$C_{46}H_{55}F_6N_3O_4$ 827.94
Dutasteride β-dimer: {[(5α,17β)-*N*-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaandrost-1-ene-17-carboxamide-]4-yl}{[(5α,17β)-3-oxo-4-azaandrost-1-ene]-17-yl}methanone.
$C_{46}H_{55}F_6N_3O_4$ 827.94

Absorbable Dusting Powder

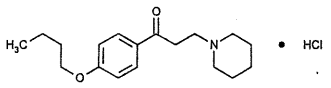
» Absorbable Dusting Powder is an absorbable powder prepared by processing cornstarch and intended for use as a lubricant for surgical gloves. It contains not more than 2.0 percent of magnesium oxide.

USP Monographs

www.webofpharma.com

Packaging and storage—Preserve in well-closed containers. It may be preserved in sealed paper packets.
Identification—A 1 in 10 suspension is colored purplish blue to deep blue by iodine TS.
Stability to autoclaving—Transfer about 2 g to a suitable paper packet, and seal or close the packet with a double fold. Wrap the paper packet in muslin, transfer to an autoclave, heat to 121° for 30 minutes, and cool: the powder is not caked, and any lumps are easily crushed between the fingers.
Sedimentation—Boil 100 mL of a 1 in 10 suspension in water for 20 minutes. Cool, transfer to a 100-mL graduated cylinder, dilute with water to volume, and allow to stand undisturbed for 24 hours: the volume occupied by the settled Powder is between 45 mL and 75 mL.
pH ⟨791⟩: between 10.0 and 10.8, in a 1 in 10 suspension.
Loss on drying ⟨731⟩—Dry about 2 g, accurately weighed, at 105° to constant weight: it loses not more than 12% of its weight.
Residue on ignition ⟨281⟩—Heat about 1 g, accurately weighed, in a covered platinum crucible until most of the carbon is burned away, but do not ignite the sample. Remove the cover, and ignite to constant weight: not more than 3.0% of residue remains.
Assay for magnesium oxide—Weigh accurately about 2.5 g, and transfer to a beaker. Add 25 mL of water and 2 mL of 3 N hydrochloric acid, and stir the mixture for 5 minutes. Add 5 mL of hydroxylamine hydrochloride solution (1 in 10), 15 mL of ammonia-ammonium chloride TS, 5 mL of potassium cyanide solution (1 in 10), and 5 drops of eriochrome black TS, mix, and titrate with 0.05 M edetate disodium VS until the solution becomes distinctly blue in color. Each mL of 0.05 M edetate disodium is equivalent to 2.015 mg of MgO.

Dyclonine Hydrochloride



$C_{18}H_{27}NO_2 \cdot HCl$ 325.87
1-Propanone, 1-(4-butoxyphenyl)-3-(1-piperidinyl)-hydro chloride.
4′-Butoxy-3-piperidinopropiophenone hydrochloride [536-43-6].

» Dyclonine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{18}H_{27}NO_2 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Dyclonine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

C: Add 2 mL of silver nitrate TS to 10 mL of Dyclonine Hydrochloride solution (1 in 100): a white precipitate is formed. Add 2 mL of nitric acid, centrifuge, and discard the supernatant. Wash the precipitate twice by adding 10 mL of 2 N nitric acid, centrifuging, and discarding the supernatant: the precipitate so obtained is soluble in 6 N ammonium hydroxide.
Melting range ⟨741⟩: between 173° and 178°.
pH ⟨791⟩: between 4.0 and 7.0, in a solution (1 in 100).
Loss on drying ⟨731⟩—Dry it at 105° for 1 hour: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.

Assay—
Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Dyclonine Hydrochloride Gel.*
Assay preparation—Transfer about 50 mg of Dyclonine Hydrochloride, accurately weighed, to a 500-mL volumetric flask, dissolve in 0.001 N phosphoric acid, dilute with 0.001 N phosphoric acid to volume, and mix.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{27}NO_2 \cdot HCl$ in the portion of Dyclonine Hydrochloride taken by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dyclonine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dyclonine Hydrochloride Gel

» Dyclonine Hydrochloride Gel contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{18}H_{27}NO_2 \cdot HCl$. It may contain suitable stabilizers and antimicrobial agents.

Packaging and storage—Preserve in collapsible, opaque plastic tubes or in tight, light-resistant glass containers. [NOTE—Do not use aluminum or tin tubes.]
USP Reference standards ⟨11⟩—
USP Dyclonine Hydrochloride RS
Identification—Shake a portion of Gel, equivalent to about 400 mg of dyclonine hydrochloride, with 25 mL of chloroform, and allow the layers to separate. Remove the chloroform layer, evaporate on a steam bath to dryness, and dry the residue at 105° for 1 hour: the dyclonine hydrochloride so obtained responds to the *Identification*— tests under *Dyclonine Hydrochloride.*
pH ⟨791⟩: between 2.0 and 4.0.

Assay—
Mobile phase—Dissolve 0.20 g of monobasic potassium phosphate and 0.45 mL of *n*-heptylamine in about 350 mL of water. Adjust with phosphoric acid to a pH of 3.0, dilute with water to 400 mL, add 600 mL of acetonitrile, and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Dyclonine Hydrochloride RS in 0.001 N

USP Monographs

www.webofpharma.com

phosphoric acid to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer an accurately measured portion of Gel, equivalent to about 5.0 mg of dyclonine hydrochloride, to a 50-mL volumetric flask. Add 10 mL of 0.001 N phosphoric acid, and sonicate to dissolve the gel. Dilute with 0.001 N phosphoric acid to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 25-cm column that contains 5-µm diameter packing L13. The flow rate is about 1.2 mL per minute. Adjust the flow rate, if necessary, so that the retention time of dyclonine hydrochloride is not less than 5 minutes. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0 and the relative standard deviation is not more than 3.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of dyclonine hydrochloride ($C_{18}H_{27}NO_2 \cdot HCl$) in the portion of Gel taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dyclonine Hydrochloride RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dyclonine Hydrochloride Topical Solution

» Dyclonine Hydrochloride Topical Solution is a sterile, aqueous solution of Dyclonine Hydrochloride. It contains not less than 92.0 percent and not more than 108.0 percent of the labeled amount of $C_{18}H_{27}NO_2 \cdot HCl$. It may contain suitable stabilizers and antimicrobial agents.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Dyclonine Hydrochloride RS

Identification—To a volume of Topical Solution, equivalent to about 300 mg of dyclonine hydrochloride, add 15 mL of chloroform, shake, and allow the layers to separate. Remove a portion of the chloroform solution, evaporate on a steam bath to dryness, and dry the residue at 105° for 1 hour: the dyclonine hydrochloride so obtained responds to the *Identification*— tests under *Dyclonine Hydrochloride*.

Sterility Tests ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 3.0 and 5.0.

Assay—

Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay*— under *Dyclonine Hydrochloride Gel.*

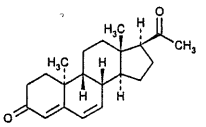
Assay preparation—Transfer an accurately measured volume of Topical Solution, equivalent to about 50 mg of dyclonine hydrochloride, to a 500-mL volumetric flask. Dilute with water to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{27}NO_2 \cdot HCl$ in each mL of the Topical Solution taken by the formula:

$$500(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dyclonine Hydrochloride RS in the *Standard preparation*, V is the volume, in mL, of the Topical Solution taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dydrogesterone



$C_{21}H_{28}O_2$ 312.45

Pregna-4,6-diene-3,20-dione, (9β,10α)-.

9β,10α-Pregna-4,6-diene-3,20-dione [152-62-5].

» Dydrogesterone contains not less than 98.0 percent and not more than 102.0 percent of $C_{21}H_{28}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Dydrogesterone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 6 µg per mL.
Medium: methanol.
Absorptivities at 285 nm, calculated on the dried basis, do not differ by more than 2.5%.

Melting range ⟨741⟩: between 167° and 171°.

Specific rotation ⟨781S⟩: between −442° and −462°.
Test solution: 10 mg per mL, in trichloroethane.

Loss on drying ⟨731⟩—Dry it in vacuum at 50° for 1 hour: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—

Mobile phase , System suitability preparation, and *Chromatographic system*—Prepare as directed in the *Assay.*

Test solution—Prepare a solution of Dydrogesterone in *Mobile phase* having a concentration of about 0.1 mg per mL.

Procedure—Inject about 20 µL of the *Test solution* into the chromatograph, record the chromatograms for not less than 20 minutes, and measure the peak area responses. The sum of

www.webofpharma.com

the areas of the secondary peaks, is not more than 2.0% of the total peak area.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water, alcohol, and acetonitrile (530:260:210). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Dydrogesterone RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer about 100 mg of Dydrogesterone, accurately weighed, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 10.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

System suitability preparation—Transfer 10 mg of dydrogesterone to a 100-mL volumetric flask, add 30 mL of alcohol, and mix to dissolve the solid. Add 1 mL of 0.2 N sodium hydroxide, and heat the mixture at 85° for 10 minutes. Cool to room temperature, neutralize with 1 mL of 0.2 N hydrochloric acid, add 20 mL of acetonitrile, dilute with water to volume, and mix. This solution contains dydrogesterone and 17 α-dydrogesterone.

Chromatographic system—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains 3-µm packing L1 and is maintained at 40°. The flow rate is about 1 mL per minute. Chromatograph 20 µL of the *System suitability preparation*, and record the peak responses as directed under *Procedure:* the resolution between the dydrogesterone and 17 α-dydrogesterone is not less than 5, and the relative standard deviation of dydrogesterone peak responses from replicate injections is not more than 1.5%. The relative retention times are about 1.0 for dydrogesterone and about 1.3 for 17 α-dydrogesterone.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{28}O_2$ in the portion of Dydrogesterone taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dydrogesterone RS in the *Standard preparation*, and r_U and r_S are the peak responses for dydrogesterone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Dydrogesterone Tablets

» Dydrogesterone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{28}O_2$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Dydrogesterone RS

Identification—Extract a quantity of the powdered Tablets, containing about 60 mg of Dydrogesterone, with 20 mL of methanol, filter, and evaporate to dryness: the residue so obtained responds to *Identification*— test *A:* under *Dydrogesterone.*

Dissolution ⟨711⟩—

Medium: 0.3% sodium lauryl sulfate; 500 mL.

Apparatus 2: 100 rpm.

Time: 60 minutes.

Procedure—Determine the amount of $C_{21}H_{28}O_2$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 295 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Dydrogesterone RS in the same medium.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{21}H_{28}O_2$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase, Standard preparation, System suitability preparation, and *Chromatographic system*—Proceed as directed in the *Assay*— under *Dydrogesterone.*

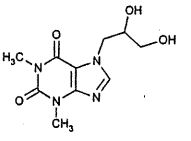
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer a portion of the powder, equivalent to about 20 mg of Dydrogesterone, to a 200-mL volumetric flask, add about 100 mL of *Mobile phase*, and sonicate for 10 minutes. Cool to room temperature, dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and determine the peak responses by area measurement. Calculate the quantity, in mg, of $C_{21}H_{28}O_2$ in the portion of Tablets taken by the formula:

$$200C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Dydrogesterone RS in the *Standard preparation*, and r_U and r_S are the Dydrogesterone peak area responses from the *Assay preparation* and the *Standard preparation*, respectively.

Dyphylline



$C_{10}H_{14}N_4O_4$ 254.24

1*H*-Purine-2,6-dione, 7-(2,3-dihydroxypropyl)-3,7-dihydro-1,3-dimethyl-, (±)-.

(±)-7-(2,3-Dihydroxypropyl)theophylline [479-18-5].

» Dyphylline contains not less than 98.0 percent and not more than 102.0 percent of $C_{10}H_{14}N_4O_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Dyphylline RS

www.webofpharma.com

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: water.
Melting range ⟨741⟩: between 160° and 164°.
pH ⟨791⟩: between 5.0 and 7.5, in a solution (1 in 100).
Loss on drying ⟨731⟩—Dry about 1 g, accurately weighed, at 105° for 3 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.15%.
Chloride ⟨221⟩: A 1.0-g portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.035%).
Sulfate ⟨221⟩: A 1.0-g portion shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.010%).
Limit of theophylline—Dissolve 2.0 g in 10 mL of hot water. Cool, add 10 drops of bromothymol blue TS and 5.0 mL of silver nitrate TS, and mix. Not more than 0.50 mL of 0.10 N sodium hydroxide is required to change the color of the solution to blue (0.5%).
Related compounds—Dissolve about 200 mg in a mixture of 30 volumes of methanol and 20 volumes of water in a 10-mL volumetric flask, dilute with the same mixture to volume, and mix *(Test solution)*. Dilute 1 mL of the *Test solution* with methanol to 100 mL (*Diluted test solution*). Separately apply 10 µL of each solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of binder-free chromatographic silica gel mixture. Develop the plate in a suitable chamber containing a mixture of chloroform, dehydrated alcohol, and ammonium hydroxide (90:10:1) until the solvent front has moved about 15 cm. Remove the plate, air-dry at room temperature, and examine under short-wavelength UV light: no spot in the chromatogram of the *Test solution* other than the principal spot is larger or more intense than the principal spot from the *Diluted test solution* (1.0%).
Assay—Dissolve about 200 mg of Dyphylline, accurately weighed, in 2 mL of formic acid, and carefully add, with stirring, 50 mL of acetic anhydride. Add 0.2 mL of Sudan IV TS, and titrate with 0.1 N perchloric acid VS to a deep violet endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 25.42 mg of $C_{10}H_{14}N_4O_4$.

Dyphylline Injection

» Dyphylline Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{10}H_{14}N_4O_4$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. To avoid precipitation, store at a temperature of not below 15°, but avoid excessive heat.
USP Reference standards ⟨11⟩—
USP Dyphylline RS

Labeling—Label it to indicate that the Injection is not to be used if crystals have separated.

Identification—
A: Dilute about 5 mL with 20 mL of water, and add 1.0 mL of 2 N sodium hydroxide and 2 drops of potassium permanganate TS: a green color is produced.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay*.
C: Transfer a portion of Injection, equivalent to about 100 mg of dyphylline, to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 2 mL to a 100-mL volumetric flask, dilute with water to volume, and mix: the UV absorption spectrum of the solution exhibits maxima and minima at the same wavelengths as that of a solution of USP Dyphylline RS containing 10 µg per mL, concomitantly measured.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.7 USP Endotoxin Unit per mg of dyphylline.
pH ⟨791⟩: between 5.0 and 8.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Dyphylline Tablets*.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of dyphylline, to a 500-mL volumetric flask, add water to volume, and mix.
Procedure—Proceed as directed in the *Assay*— under *Dyphylline Tablets*. Calculate the quantity, in mg, of $C_{10}H_{14}N_4O_4$ in each mL of the Injection taken by the formula:

$$(0.5C/V)(r_U/r_S)$$

in which V is the volume, in mL, of Injection taken, and the other terms are as defined therein.

Dyphylline Oral Solution

» Dyphylline Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dyphylline ($C_{10}H_{14}N_4O_4$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Dyphylline RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Alcohol content—
Standard preparation—Pipet 5 mL of dehydrated alcohol and 5 mL of acetone into a 200-mL volumetric flask containing 50 mL of water, add water to volume, and mix. Pipet 10 mL of this solution into a 200-mL volumetric flask, add water to volume, and mix.
Test preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 5 mL of alcohol, to a 200-mL volumetric flask containing about 30 mL of water. Pipet 5 mL of acetone into the flask, add water to volume, and mix. Pipet 10 mL of this solution into a 200-mL volumetric flask, add water to volume, and mix.

www.webofpharma.com

Chromatographic system—The gas chromatograph is equipped with a flame-ionization detector and contains a 75-cm × 4-mm column packed with 20% phase G20 on support S1AB, conditioned as directed (see *Chromatography* ⟨621⟩). The column is maintained at a temperature of about 85°, and the injection port and detector block are maintained at about 175° and 225°, respectively. Nitrogen is used as the carrier gas at a flow rate of about 18 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* is not less than 2.0; the tailing factor of the alcohol peak is not greater than 1.5; and the relative standard deviation for replicate injections is not more than 2.0% in the ratio of the peak of alcohol to the peak of acetone.

Procedure—Separately inject equal volumes (about 4 µL) of the *Test preparation* and the *Standard preparation,* in duplicate, into the gas chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of alcohol in the specimen taken by the formula:

$$(500/V)(R_U/R_S)$$

in which V is the volume, in mL, of Oral Solution taken, and R_U and R_S are the peak response ratios of alcohol to that of acetone obtained from the *Assay preparation* and the *Standard preparation,* respectively: the alcohol content, obtained as the average of the calculated results, is between 90.0% and 110.0% of the labeled amount of C_2H_5OH.

Assay—

Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay* under *Dyphylline Tablets.*

Standard preparation—Dissolve an accurately weighed quantity of USP Dyphylline RS in *Mobile phase* to obtain a solution having a known concentration of about 500 µg per mL.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 100 mg of dyphylline, to a 200-mL volumetric flask, add *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 10 µL), of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of dyphylline ($C_{10}H_{14}N_4O_4$) in the volume of Oral Solution taken by the formula:

$$0.2C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Dyphylline RS in the *Standard preparation;* and r_U and r_S are the dyphylline peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Dyphylline Tablets

» Dyphylline Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{10}H_{14}N_4O_4$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Dyphylline RS

Identification—

A: To a solution of 5 Tablets in 20 mL of water add 1 mL of 2 N sodium hydroxide and 2 drops of potassium permanganate TS: a green color is produced.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation,* as obtained in the *Assay.*

C: Weigh a portion of finely powdered Tablets, equivalent to about 100 mg of dyphylline, and transfer to a 200-mL volumetric flask. Add about 100 mL of water, shake by mechanical means for about 15 minutes, dilute with water to volume, and mix. Filter, and transfer 2 mL of the filtrate to a 100-mL volumetric flask, dilute with water to volume, and mix: the UV absorption spectrum of the solution exhibits maxima and minima at the same wavelengths as that of a solution of USP Dyphylline RS containing 10 µg per mL, concomitantly measured.

Dissolution ⟨711⟩—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{10}H_{14}N_4O_4$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 273 nm of filtered portions of the solution under test, suitably diluted with water, in comparison with a Standard solution having a known concentration of USP Dyphylline RS in the same medium.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{10}H_{14}N_4O_4$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase—Dissolve 1.4 g of monobasic potassium phosphate in 1350 mL of water in a 2-liter volumetric flask, add methanol to volume, and mix. Filter through a 0.5-µm porosity membrane filter. [NOTE—The composition may be varied to meet system suitability requirements.]

Standard preparation—Dissolve an accurately weighed quantity of USP Dyphylline RS in water to obtain a solution having a known concentration of about 200 µg per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of dyphylline, to a 500-mL volumetric flask, add 200 mL of water, insert the stopper, and shake by mechanical means for 10 minutes. Add water to volume, and mix. Just prior to injection, filter about 5 mL through a 1.2-µm porosity membrane filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph five replicate injections of the *Standard preparation,* and record the peak responses as directed under *Procedure:* the relative standard deviation is not more than 2.0%. The tailing factor is not more than 2.0.

Procedure—Inject separately equal volumes, accurately measured (about 10 µL), of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of $C_{10}H_{14}N_4O_4$ in the portion of Tablets taken by the formula:

$$0.5C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Dyphylline RS in the *Standard preparation,* and r_U and r_S are the dyphylline peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

USP Monographs

www.webofpharma.com

Dyphylline and Guaifenesin Oral Solution

DEFINITION

Dyphylline and Guaifenesin Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amounts of dyphylline ($C_{10}H_{14}N_4O_4$) and guaifenesin ($C_{10}H_{14}O_4$).

IDENTIFICATION

- **A.** The UV spectra of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.01 M monobasic potassium phosphate

Mobile phase: Methanol and *Buffer* (21:79)

System suitability solution: 0.1 mg/mL each of USP Guaifenesin RS and USP Dyphylline RS and 0.01 mg/mL of guaiacol in *Mobile phase*

Standard solution: 0.1 mg/mL of USP Guaifenesin RS and 0.1*J* mg/mL of USP Dyphylline RS in *Mobile phase*, *J* being the ratio of the labeled amount of dyphylline to that of guaifenesin

Sample solution: Nominally 0.1 mg/mL of guaifenesin, prepared as follows. Transfer 100 mg of guaifenesin from a volume of Oral Solution to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm. For *Identification* test *A*, use a diode array detector in the range of 200–400 nm.

Columns

Guard: Packing L1

Analytical: 4.6-mm × 15-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for dyphylline, guaiacol, and guaifenesin are about 0.25, 0.7, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.8 between the guaiacol and guaifenesin peaks; NLT 9.0 between the guaiacol and dyphylline peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% for both dyphylline and guaifenesin, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of the labeled amounts of dyphylline ($C_{10}H_{14}N_4O_4$) and guaifenesin ($C_{10}H_{14}O_4$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of dyphylline or guaifenesin from the *Sample solution*

r_S = peak response of dyphylline or guaifenesin from the *Standard solution*

C_S = concentration of USP Dyphylline RS or USP Guaifenesin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of dyphylline or guaifenesin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of dyphylline ($C_{10}H_{14}N_4O_4$) and 90.0%–110.0% of the labeled amount of guaifenesin ($C_{10}H_{14}O_4$)

OTHER COMPONENTS

- **ALCOHOL DETERMINATION** *Method IIb* ⟨611⟩: 90.0%–110.0% of the labeled amount of alcohol (C_2H_5OH)

SPECIFIC TESTS

- **pH** ⟨791⟩: 5.0–7.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Alcohol Determination—Acetonitrile RS
USP Alcohol Determination—Alcohol RS
USP Dyphylline RS
USP Guaifenesin RS

Dyphylline and Guaifenesin Tablets

» Dyphylline and Guaifenesin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of dyphylline ($C_{10}H_{14}N_4O_4$) and guaifenesin ($C_{10}H_{14}O_4$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Dyphylline RS
USP Guaifenesin RS

Identification—

A: Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of dyphylline, with 20 mL of water, and transfer to a test tube. Add 2.0 mL of 2 N sodium hydroxide and 2 drops of potassium permanganate TS: a green color is produced.

B: Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of guaifenesin, with 10 mL of chloroform, filter, and on a watch glass evaporate 1 mL of the filtrate, on a steam bath, to dryness. To the residue add 1 drop of formaldehyde TS and a few drops of sulfuric acid: a deep cherry-red to purple color is produced.

C: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amounts of dyphylline ($C_{10}H_{14}N_4O_4$) and guaifenesin ($C_{10}H_{14}O_4$) dissolved, using the procedure set forth in the *Assay*, making any necessary modifications.

Tolerances—Not less than 75% *(Q)* of the labeled amounts of $C_{10}H_{14}N_4O_4$ and $C_{10}H_{14}O_4$ are dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase, Resolution solution, and *Standard preparation*—Prepare as directed in the *Assay* under *Dyphylline and Guaifenesin Oral Solution.*

www.webofpharma.com

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of guaifenesin, to a 100-mL volumetric flask, add about 80 mL of *Mobile phase*, sonicate for 30 minutes, and shake by mechanical means for an additional 30 minutes. Dilute with *Mobile phase* to volume, mix, and pass through a membrane filter having a 0.45-µm or finer porosity, discarding the first 10 mL of the filtrate. Transfer 5.0 mL of the clear filtrate to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

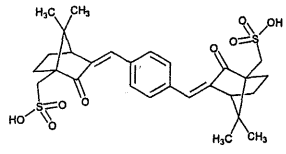
Chromatographic system—Prepare as directed in the *Assay* under *Dyphylline and Guaifenesin Oral Solution*, except omit the use of the guard column.

Procedure—Proceed as directed in the *Assay* under *Dyphylline and Guaifenesin Oral Solution*. Calculate the quantities, in mg, of dyphylline ($C_{10}H_{14}N_4O_4$) and guaifenesin ($C_{10}H_{14}O_4$) in the portion of Tablets taken by the formula:

$$1000C(r_U/r_S)$$

in which the terms are as defined therein.

Ecamsule Solution



$C_{28}H_{34}O_8S_2$ 562.69

Bicyclo[2.2.1]heptane-1-methanesulfonic acid, 3,3′-(1,4-phenylenedimethylidyne)bis[7,7-dimethyl-2-oxo]-. (±)-(3*E*,3′*E*)-3,3′-(*p*-Phenylenedimethylidyne)bis[2-oxo-10-bornanesulfonic acid] [92761-26-7].

» Ecamsule Solution is an aqueous solution of $C_{28}H_{34}O_8S_2$. It contains not less than 30.0 percent and not more than 34.0 percent, by weight, of ecamsule ($C_{28}H_{34}O_8S_2$).

Packaging and storage—Preserve in tight containers. Protect from light, and store at room temperature.

USP Reference standards ⟨11⟩—

USP Ecamsule Solution RS

USP Ecamsule Related Compound A RS
1,4-Phenylenedimethanol.
$C_8H_{10}O_2$ 138.16

USP Ecamsule Related Compound B RS
4-(Hydroxymethyl)benzoic acid.
$C_8H_8O_3$ 152.15

USP Ecamsule Related Compound C RS
Terephthalic acid.
$C_8H_6O_4$ 166.13

USP Ecamsule Related Compound D RS
((1*SR*,4*RS*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)methanesulfonic acid.
$C_{10}H_{16}O_4S$ 232.30

USP Ecamsule Related Compound E RS
Sodium ((1*SR*,4*SR*,*E*)-3-(4-(hydroxymethyl) benzylidene)-7,7-dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)methanesulfonate.
$C_{18}H_{21}NaO_5S$ 372.41

USP Ecamsule Related Compound F RS
4-((*E*)-((1*SR*,4*SR*)-7,7-Dimethyl-3-oxo-4-(sulfomethyl) bicyclo[2.2.1]heptan-2-ylidene)methyl)benzoic acid.
$C_{18}H_{20}O_6S$ 364.41

USP Ecamsule Related Compound G RS
4-((*E*)-((1*SR*,4*SR*)-7,7-Dimethyl-3-oxo-4-(sulfomethyl) bicyclo[2.2.1]heptan-2-ylidene)methyl)benzaldehyde, sodium salt.
$C_{18}H_{19}NaO_5S$ 370.40

USP Ecamsule Triethanolamine RS
Bicyclo[2.2.1]heptane-1-methanesulfonic acid, 3,3′-(1,4-phenylenedimethylidyne)bis[7,7-dimethyl-2-oxo]-, ditriethanolamine salt (1:2).
$C_{28}H_{34}O_8S_2 \cdot (C_6H_{15}NO_3)_2$ 861.07

Labeling—The label states that this article is not intended for direct administration to humans or animals.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197A*▲ (CN 1-May-2020)—Place a drop of Ecamsule Solution on a diamond sampling surface and dry it with a stream of warm air. The IR absorption spectrum conforms to that of USP Ecamsule Solution RS, similarly obtained.

Change to read:

B:▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—*Solution*—Transfer 0.25 g of Ecamsule Solution to a 100-mL volumetric flask, and dilute with water to volume. Further dilute 2 mL of this solution with water to 100 mL.

The *Solution* exhibits absorption maximum between 342 and 346 nm.

Limit of chloride—Dissolve about 10 g of Ecamsule Solution, accurately weighed, in 70 mL of water. Titrate this solution with 0.01 N silver nitrate, determine the endpoint potentiometrically (see *Titrimetry* ⟨541⟩), and calculate the percentage of chloride in the portion of $C_{28}H_{34}O_8S_2$ taken by the formula:

$$100(35.5)(VN/W)(100/A)$$

in which 35.5 is the atomic weight, in g per mole, of chloride; V is the volume, in mL, of silver nitrate used for titration; N is the concentration, in normality, of silver nitrate; W is the weight, in mg, of Ecamsule Solution taken for determination; and A is the assay, in percent, of Ecamsule Solution: not more than 0.3% of chloride is found.

Limit of sodium—

Diluent—Transfer 5 mL of nitric acid in a 1000-mL volumetric flask containing about 500 mL of water, and dilute with water to volume.

Test solution—Transfer about 1 g of Ecamsule Solution, accurately weighed, to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Standard solutions—Dilute quantitatively, and stepwise if necessary, a commercially available sodium atomic absorption standard solution containing 1000 µg of sodium per mL with *Diluent* to obtain solutions having known concentrations of 1, 5, 10, and 20 µg per mL, respectively.

Procedure (see *Atomic Absorption Spectroscopy* ⟨852⟩)—Concomitantly determine the absorbances of the *Standard solutions* and the *Test solution* at the sodium emission line of 330 nm or 589 nm with a suitable atomic absorption spectrophotometer equipped with a sodium lamp and an air–acetylene flame, using *Diluent* as the blank. Determine the

USP Monographs

www.webofpharma.com

concentration of sodium, in µg per mL, in the *Test solution* using the calibration graph. Calculate the percentage of sodium in the portion of $C_{28}H_{34}O_8S_2$ taken by the formula:

$$100 \times 10^{-6}(CV/W)(100/A)$$

in which C is the concentration, in µg per mL, of sodium in the *Test solution,* the multiplier of 10^{-6} is for conversion of µg per mL to g per mL; V is the volume, in mL, of *Test solution;* W is the weight, in g, of Ecamsule Solution taken for determination; and A is the assay, in percent, of Ecamsule Solution: not more than 0.3% of sodium is found.

Related compounds—

Test for related compounds A to F—

Solvent A—Prepare a mixture of acetonitrile and 85% phosphoric acid (1000:1).

Solvent B—Prepare a mixture of water and 85% phosphoric acid (1000:1).

Standard solution—Dissolve an accurately weighed quantity of USP Ecamsule Related Compound A RS, USP Ecamsule Related Compound B RS, USP Ecamsule Related Compound C RS, USP Ecamsule Related Compound D RS, USP Ecamsule Related Compound E RS, and USP Ecamsule Related Compound F RS in water, sonicating if necessary, to obtain a solution having known concentrations as found in *Table 1.*

Test solution—Transfer about 100 mg, accurately weighed, of Ecamsule Solution to a 50-mL volumetric flask, and dilute with water to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with either a programmable variable wavelength detector or two separate detectors capable of monitoring at 200 nm and 300 nm and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–25	20	80	isocratic
25–27	20→80	80→20	linear gradient
27–47	80	20	isocratic
47–50	80→20	20→80	linear gradient
50–65	20	80	equilibration

Chromatograph the *Standard solution,* and record the peak areas as directed for *Procedure:* the relative standard deviations of the ecamsule related compound peaks for replicate injections are not more than 10.0%; and the resolution, R, between all adjacent peak pairs of ecamsule related compounds is not less than 1.5 measured at 200 nm.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, allow the chromatogram to run for about 20 minutes for the *Standard solution* and 60 minutes for the *Test solution,* record the chromatograms at 200 nm from 0 to 8 minutes and at 300 nm after 8 minutes, and measure the peak areas. Calculate the percentage of ecamsule related compounds A, B, C, D, and F in the portion of $C_{28}H_{34}O_8S_2$ taken by the formula:

$$100[100C_S/(C_U \times A)](r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of the ecamsule related compound in the *Standard solution;* C_U is the concentration, in mg per mL, of the *Test solution;* A is the assay, in percent, obtained from the *Assay;* and r_U and r_S are the peak areas of the ecamsule related compound obtained from the *Test solution* and *Standard solution,* respectively. Calculate the percentage of ecamsule related compound E in the portion of $C_{28}H_{34}O_8S_2$ taken by the formula:

$$100(350.43/372.41)[100C_S/(C_U \times A)](r_U/r_S)$$

in which 350.43 and 372.41 are the molecular weights of ecamsule related compound E (free acid) and USP Ecamsule Related Compound E RS (sodium salt), respectively; C_S is the concentration, in mg per mL, of ecamsule related compound E in the *Standard solution;* C_U is the concentration, in mg per mL, of the *Test solution;* A is the assay, in percent, obtained from the *Assay;* and r_U and r_S are the peak areas of the ecamsule related compound E obtained from the *Test solution* and *Standard solution,* respectively. The limits are given in *Table 1.*

Test for related compound G, Ecamsule exo-2-hydroxyecamsule, Ecamsule endo-2-hydroxyecamsule, and unspecified impurities—

Mobile phase—Proceed as directed in the *Assay.*

Standard solution 1—Dissolve an accurately weighed quantity of USP Ecamsule Related Compound G RS in water to obtain a solution having a known concentration of about 0.005 mg per mL.

Standard solution 2—Use the *Standard preparation,* as described in the *Assay.*

Test solution—Use the *Assay preparation,* prepared as directed in the *Assay.*

Chromatographic system—Prepared as directed in the *Assay.* Use the liquid chromatograph equipped with a 310-nm detector in addition to using a 343-nm detector.

[NOTE—Ecamsule related compound G is detected at 310 nm; and Ecamsule exo-2-hydroxyecamsule, Ecamsule endo-

Table 1 (Ecamsule Solution)

Name	Concentration (mg/mL) in the *Standard solution*	Detection wavelength (nm)	RRT[1]	Limit (%)
Ecamsule related compound A	0.001	200	0.42	0.2
Ecamsule related compound B	0.001	200	0.54	0.2
Ecamsule related compound C	0.001	200	0.70	0.2
Ecamsule related compound D	0.008	200	1.00	1.3
Ecamsule related compound E (free acid)	0.004	300	2.52	0.7
Ecamsule related compound F	0.004	300	3.26	0.7

[1] Ecamsule elutes after 27 minutes and is a broad peak in the *Test for related compounds A to F.* The relative retention times of related compounds are measured with respect to ecamsule related compound D.

www.webofpharma.com

2-hydroxyecamsule, and unspecified impurities are detected at 343 nm.]

Procedure—Separately inject equal volumes (about 20 µL) of *Standard solution 1*, *Standard solution 2*, and the *Test solution* into a chromatograph, record the chromatograms for not less than 6 times the retention time of ecamsule trans-trans isomer, and measure the peak areas. Calculate the percentage of ecamsule related compound G in the portion of $C_{28}H_{34}O_8S_2$ taken by the formula:

$$100(348.41/370.40)[100C_S/(C_U \times A)](r_U/r_S)$$

in which 348.41 and 370.40 are the molecular weights of ecamsule related compound G and USP Ecamsule Related Compound G RS, respectively; C_S is the concentration, in mg per mL, of USP Ecamsule Related Compound G RS in *Standard solution 1*; C_U is the concentration, in mg per mL, of the *Test solution*; *A* is the assay, in percent, obtained from the *Assay*; and r_U and r_S are the peak areas of ecamsule related compound G obtained from the *Test solution* and *Standard solution 1*, respectively. Calculate the percentage of Ecamsule exo-2-hydroxyecamsule and Ecamsule endo-2-hydroxyecamsule in the portion of $C_{28}H_{34}O_8S_2$ taken by the formula:

$$100(1/F)[100C_S/(C_U \times A)](r_i/r_S)$$

in which *F* is the relative response factor for each impurity obtained from *Table 2*; C_S is the concentration, in mg per mL, of USP Ecamsule Triethanolamine RS in *Standard solution 2*; C_U is the concentration, in mg per mL, of the *Test solution*; *A* is the assay, in percent, obtained from the *Assay*; r_i is the peak area for each impurity obtained from the *Test solution*; and r_S is the sum of peak areas corresponding to the trans-trans and cis-trans isomers obtained from the *Standard solution*. Calculate the percentage of any unspecified impurity in the portion of $C_{28}H_{34}O_8S_2$ taken by the formula:

$$100(r_i/r_S)$$

in which r_i is the peak area for each unspecified impurity obtained from the *Test solution*; and r_S is the sum of all peak areas obtained from the *Test solution*. The limits are given in *Table 2*.

Table 2

Name	RRT[3]	*F*	Limit (%)
Ecamsule related compound G	0.9	—	0.2
Ecamsule exo-2-hydroxyecamsule[1]	1.4	0.6	0.2
Ecamsule endo-2-hydroxyecamsule[2]	1.6	0.6	0.3
Any single unspecified impurity	—	1.0	0.5

[1] [(1*SR*,2*R*,4*SR*,*E*)-3-(4-{(*E*)-[(1*SR*,4*SR*)-7,7-dimethyl-3-oxo-4-(sulfomethyl) bicyclo[2.2.1]heptan-2-ylidene]methyl}benzylidene)-2-hydroxy-7,7-dimethylbicyclo[2.2.1]heptan-1-yl]methanesulfonic acid [$C_{28}H_{36}O_8S_2$, 564.71].

[2] [(1*SR*,2*S*,4*SR*,*E*)-3-(4-{(*E*)-[(1*SR*,4*SR*)-7,7-dimethyl-3-oxo-4-(sulfomethyl) bicyclo[2.2.1]heptan-2-ylidene]methyl}benzylidene)-2-hydroxy-7,7-dimethylbicyclo[2.2.1]heptan-1-yl]methanesulfonic acid [$C_{28}H_{36}O_8S_2$, 564.71].

[3] The relative retention times are measured with respect to ecamsule trans-trans isomer.

Total impurities—Calculate the sum of the related compounds and unspecified impurities from the *Test for related compounds A to F* and the *Test for related compound G, Ecamsule exo-2-hydroxyecamsule, Ecamsule endo-2-hydroxyecamsule, and unspecified impurities:* not more than 5.0% of total impurities is found.

Assay— [NOTE—Prepare solutions immediately before use, and protect them from light in low-actinic glassware.]

1% Triethylamine solution—Prepare a mixture of water and triethylamine (100:1), and adjust with phosphoric acid to a pH of 7.

Mobile phase—Prepare a mixture of *1% Triethylamine solution* and methanol (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Ecamsule Triethanolamine RS in *Mobile phase*, sonicating if necessary, to obtain a solution having a known concentration of about 0.12 mg per mL of ecamsule triethanolamine.

Assay preparation—Transfer about 500 mg, accurately weighed, of Ecamsule Solution to a 100-mL volumetric flask, and dilute with water to volume. Transfer 5.0 mL of this solution into a 100-mL volumetric flask, and dilute with water to volume.

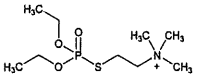
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 343-nm detector and a 4.0-mm × 125-mm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for ecamsule trans-trans isomer and 2.9 for the ecamsule cis-trans isomer; the relative standard deviation of the sum of the ecamsule trans-trans and cis-trans peak areas for replicate injections is not more than 2.0%; and the number of theoretical plates of the peak corresponding to the trans-trans isomer is not less than 1430.

Procedure—Inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of $C_{28}H_{34}O_8S_2$ in the portion of Ecamsule Solution taken by the formula:

$$100(562.69/861.07)(V/W)C(r_U/r_S)$$

in which 562.69 and 861.07 are the molecular weights of ecamsule and ecamsule triethanolamine, respectively; *V* is the volume, in mL, of the *Assay preparation*; *W* is the weight, in mg, of Ecamsule Solution used for the *Assay preparation*; *C* is the concentration, in mg per mL, of ecamsule triethanolamine in the *Standard preparation*; and r_U and r_S are the sum of peak areas corresponding to trans-trans and cis-trans isomers obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Echothiophate Iodide



$C_9H_{23}INO_3PS$ 383.23

Ethanaminium, 2-[(diethoxyphosphinyl)thio]-*N*,*N*,*N*-trimethyl-, iodide;
(2-Mercaptoethyl)trimethylammonium iodide *S*-ester with *O*,*O*-diethyl phosphorothioate [513-10-0].

DEFINITION

Echothiophate Iodide contains NLT 95.0% and NMT 100.5% of echothiophate iodide ($C_9H_{23}INO_3PS$), calculated on the dried basis.

www.webofpharma.com

IDENTIFICATION

• **A.**

Sample solution: 50 mg/mL

Analysis: To 2 mL of the *Sample solution* in a test tube add 1 mL of nitric acid. Transfer 1 drop of this mixture to another test tube, add 1 mL of solvent hexane, and shake.

Acceptance criteria: (1) A brown precipitate of iodine is formed after addition of nitric acid. (2) The solvent hexane acquires a pink color.

• **B. IDENTIFICATION TESTS—GENERAL,** *Phosphate* ⟨191⟩

Analysis: Heat the remainder of the brown reaction mixture from *Identification* test *A* over a flame until a colorless solution remains (about 3 min). Cool, and dilute with water to 10 mL.

Acceptance criteria: A 2-mL portion of this solution meets the requirements.

• **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩**:** A 2-mL portion of the solution obtained in *Identification* test *B* meets the requirements.

ASSAY

• **PROCEDURE**

In the preparation of all reagents, and throughout this procedure, wherever water is specified, use only water that has been distilled, boiled for 10 min, and cooled while protected from the atmosphere.

Solution A: Transfer 5.44 g of anhydrous dibasic sodium phosphate to a 100-mL volumetric flask, and add a volume of 1 N sodium hydroxide VS that contains 36.5 mEq of sodium hydroxide. Add 40 mL of water, shake to dissolve the sodium phosphate, and dilute with water to volume.

Solution B: Dilute 0.1 N iodine with water to 0.004 N, and standardize the solution on the day of use as follows. Weigh 150 mg of arsenic trioxide, and dissolve in 20 mL of 1 N sodium hydroxide, by warming if necessary, in a 500-mL volumetric flask. Dilute with 40 mL of water, add 2 drops of methyl orange TS, then add 3 N hydrochloric acid until the yellow color is changed to pink. Add 2 g of sodium bicarbonate, then add water to volume. Transfer 5.0 mL of this solution to a titration vessel, and add 50 mL of *Solution A* (see *Reagents, Indicators, and Solutions—Solutions*). Titrate with 0.004 N iodine, determining the endpoint potentiometrically, using platinum and silver–silver chloride electrodes. Calculate the normality. Each 197.8 µg of arsenic trioxide is equivalent to 1 mL of 0.004 N iodine.

Sample solution: 1.25 mg/mL of Echothiophate Iodide in water

Analysis: Transfer 10.0 mL of the *Sample solution* to a titration vessel containing 30 mL of water. Add 10.0 mL of *Solution A,* cover, and allow to stand for 20 min at 25 ± 3°. Add 2 mL of glacial acetic acid rapidly and with mixing. Titrate with *Solution B,* determining the endpoint potentiometrically, using platinum and silver–silver chloride electrodes. Correct for the amount of free thiol sulfur by repeating the procedure but adding the glacial acetic acid first, then *Solution A,* and titrating immediately. Subtract the volume of *Solution B* used in the second titration from that used in the first. Each mL of 0.004 N iodine is equivalent to 1.533 mg of echothiophate iodide ($C_9H_{23}INO_3PS$).

Acceptance criteria: 95.0%–100.5% on the dried basis

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum over phosphorus pentoxide at 50° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, preferably at a temperature below 0°.

Echothiophate Iodide for Ophthalmic Solution

DEFINITION

Echothiophate Iodide for Ophthalmic Solution is sterile Echothiophate Iodide. It may contain Mannitol or other suitable diluent. It contains NLT 95.0% and NMT 115.0% of the labeled amount of echothiophate iodide ($C_9H_{23}INO_3PS$).

IDENTIFICATION

• **A.**

Sample solution: Nominally equivalent to 1.2 mg/mL of echothiophate iodide in water from Echothiophate Iodide for Ophthalmic Solution

Analysis: To 1 mL of the *Sample solution* add 0.2 mL of 2 N hydrochloric acid and 0.2 mL of 30% hydrogen peroxide. Add a few drops of solvent hexane, and shake.

Acceptance criteria: (1) The color turns brown after the addition of 30% hydrogen peroxide. (2) The solvent hexane acquires a pink color.

• **B.**

Analysis: To 5 mL of the *Sample solution* from *Identification* test *A* add 0.5 mL of sodium hydroxide solution (1 in 2), heat at 50° for 2 min, cool to room temperature, and add 1 mL of sodium nitroferricyanide TS.

Acceptance criteria: A deep-red color is produced.

ASSAY

• **PROCEDURE**

In the preparation of all reagents, and throughout this procedure, wherever water is specified, use only water that has been distilled, boiled for 10 min, and cooled while protected from the atmosphere.

Solution A: Transfer 5.44 g of anhydrous dibasic sodium phosphate to a 100-mL volumetric flask, and add a volume of 1 N sodium hydroxide VS that contains 36.5 mEq of sodium hydroxide. Add 40 mL of water, shake to dissolve the sodium phosphate, and dilute with water to volume.

Solution B: Dilute 0.1 N iodine with water to 0.004 N, and standardize the solution on the day of use as follows. Weigh 150 mg of arsenic trioxide, and dissolve in 20 mL of 1 N sodium hydroxide, by warming if necessary, in a 500-mL volumetric flask. Dilute with 40 mL of water, add 2 drops of methyl orange TS, then add 3 N hydrochloric acid until the yellow color is changed to pink. Add 2 g of sodium bicarbonate, then add water to volume. Transfer 5.0 mL of this solution to a titration vessel, and add 50 mL of *Solution A* (see *Reagents, Indicators, and Solutions—Solutions*). Titrate with 0.004 N iodine, determining the endpoint potentiometrically, using platinum and silver–silver chloride electrodes. Calculate the normality. Each 197.8 µg of arsenic trioxide is equivalent to 1 mL of 0.004 N iodine.

Sample solution: Dissolve the contents of a counted number of vials of Echothiophate Iodide for Ophthalmic Solution, equivalent to 30 mg of echothiophate iodide, by adding 5 mL of water to each vial. Combine the solutions, and mix. Dilute a portion of the mixture, equivalent to 12 mg of echothiophate iodide, with water to 40 mL.

Analysis: To the *Sample solution* add 10.0 mL of *Solution A,* cover, and allow to stand for 20 min at 25 ± 3°. Add 2 mL of glacial acetic acid rapidly and with mixing. Titrate with *Solution B,* determining the endpoint potentiometrically, using platinum and silver–silver chloride electrodes. Correct

www.webofpharma.com

for the amount of free thiol sulfur by repeating the procedure but adding the glacial acetic acid first, then *Solution A*, and titrating immediately. Subtract the volume of *Solution B* used in the second titration from that used in the first. Each mL of 0.004 N iodine is equivalent to 1.533 mg of echothiophate iodide ($C_9H_{23}INO_3PS$).

Acceptance criteria: 95.0%–115.0%

SPECIFIC TESTS

- **COMPLETENESS OF SOLUTION** ⟨641⟩: The contents of one container dissolve in 10 mL of carbon dioxide-free water to yield a clear solution.
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **WATER**

Dry all glassware used in the following procedure at 105° for 4 h, and store in a desiccator or dry box. Perform as many operations as possible in a dry box.

Solution A: Wash 150 g of 8- to 12-mesh type 3A molecular sieve with several portions of dehydrated alcohol to remove the fine particles. Place the washed molecular sieve in a shallow borosilicate glass tray, heat in an oven at 350° for 2 h, and cool in a dry box. Transfer the dry molecular sieve to a 1-L conical flask, add 700 mL of dehydrated alcohol, insert the stopper, and allow to stand for NLT 48 h before using.

Internal standard solution: Place 0.17 mL of methanol in a 100-mL volumetric flask, and add *Solution A* to volume. Prepare this solution fresh daily.

Standard solutions: Into three 25-mL volumetric flasks, each containing 15 mL of *Internal standard solution*, transfer 5, 40, and 75 µL of water, respectively. Dilute with *Internal standard solution* to volume. Prepare these solutions fresh daily.

Sample solution: Carefully remove the protective retainer and cap from five vials of Echothiophate Iodide for Ophthalmic Solution without removing the elastomeric septum closure. Discard the separated parts, and weigh each closed vial and contents. Inject through the septum of each vial 400 µL of *Internal standard solution*, using a suitable gas-tight syringe, and allow to stand for 1 h, swirling occasionally to dissolve the residue. After 1 h, remove 300 µL of solution from each vial by using a gas-tight syringe; transfer to a dry small-volume, sample-collecting vial equipped with a sampling valve system;[1] and mix the combined solutions.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Thermal conductivity

Column: 2-mm × 1.8-m silylated glass; packed with 80- to 100-mesh surface-silanized packing S3

Temperatures

Column: 115°

Injection port: 200°

Detector: 225°

Carrier gas: Dry helium

Flow rate: 45 mL/min

Injection volume: 3–4 µL

System suitability

Samples: *Standard solutions*, chromatograph a sufficient number of injections

Suitability requirements

Resolution: NLT 2.0 between the water and methanol peaks

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solutions* and *Sample solution*

Measure the responses for the first (water) and second (methanol) major peaks obtained for each. Plot the ratios of the peak responses of water to methanol versus the concentration, in mg/mL, of water in each *Standard solution*. If the plot is not linear, discard it, and repeat the chromatography on additional portions of the *Standard solutions*. Similarly, inject a portion of the *Sample solution*, record the chromatogram, and measure the responses for the two major peaks. By comparison with the linear standard plot, determine the concentration, in mg/mL, of water in the *Sample solution* as that corresponding to the ratio of the peak responses of water to methanol from the *Sample solution*. Remove the elastomeric septum closure from each test vial, discard the contents, and rinse each vial and closure with several portions of methanol. Dry the vials and the closures in a stream of dry nitrogen, and subtract this weight from that of the closed vials and contents obtained as directed in the *Sample solution*.

Calculate the percentage of water content in the portion of Echothiophate Iodide for Ophthalmic Solution taken:

$$\text{Result} = [(C/V)/W] \times 100$$

C = concentration of water in the *Sample solution* (mg/mL)

V = volume of *Internal standard solution*, 0.4 mL

W = average weight of Echothiophate Iodide for Ophthalmic Solution in the vials (mg)

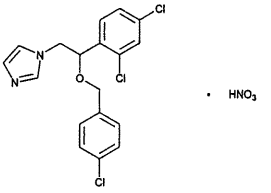
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, preferably of Type I glass, at controlled room temperature.

[1] Suitable sample-collecting vials and sampling valve systems are available as Catalog Nos. 13098 and 13099 3-mL and 5-mL vials and Catalog No. 10135 valve from Pierce Chemical Co., P.O. Box 117, Rockford, IL 61105.

Econazole Nitrate



$C_{18}H_{15}Cl_3N_2O \cdot HNO_3$ 444.70

1*H*-Imidazole, 1-[2-[(4-chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-, mononitrate, (±)-;
(±)-1-[2,4-Dichloro-β-[(*p*-chlorobenzyl)oxy]phenethyl]-imidazole mononitrate [24169-02-6].

DEFINITION

Econazole Nitrate contains NLT 98.0% and NMT 102.0% of econazole nitrate ($C_{18}H_{15}Cl_3N_2O \cdot HNO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

• **C.**
Sample solution: 10 mg with 5 mL of water
Analysis: Shake the *Sample solution*, and cool the resulting suspension in ice. Keeping the suspension cool, add 0.4 mL of potassium chloride solution (1 in 10), 0.1 mL of diphenylamine TS, and, dropwise with shaking, 5 mL of sulfuric acid.
Acceptance criteria: An intense blue color develops.

ASSAY

• **PROCEDURE**
Solution A: 0.77 g/L of ammonium acetate in water
Solution B: Methanol and *Solution A* (20:80)
Solution C: Acetonitrile and methanol (60:40)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	60	40
25	10	90
27	10	90
27.1	60	40
30	60	40

Diluent: Methanol and water (40:60)
Standard solution: 0.4 mg/mL of USP Econazole Nitrate RS in *Diluent*. [NOTE—Sonication may be needed to dissolve the Standard.]
Sample solution: 0.4 mg/mL of Econazole Nitrate in *Diluent*. [NOTE—Sonication may be needed to dissolve the sample.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of econazole nitrate ($C_{18}H_{15}Cl_3N_2O \cdot HNO_3$) in the portion of Econazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of econazole from the *Sample solution*
r_S = peak response of econazole from the *Standard solution*
C_S = concentration of USP Econazole Nitrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Econazole Nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **ORGANIC IMPURITIES**
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.02 mg/mL each of USP Econazole Related Compound A RS, USP Econazole Related Compound B RS, and USP Econazole Related Compound C RS, and 0.01 mg/mL of USP Econazole Nitrate RS in methanol
Sample solution: 10 mg/mL of Econazole Nitrate in methanol
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between econazole related compound C and econazole
Relative standard deviation: NMT 3% for econazole related compound A, econazole related compound B, econazole related compound C, and econazole
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of econazole related compound A, econazole related compound B, or econazole related compound C in the portion of Econazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of econazole related compound A, econazole related compound B, or econazole related compound C from the *Sample solution*
r_S = peak response of econazole related compound A, econazole related compound B, or econazole related compound C from the *Standard solution*
C_S = concentration of USP Econazole Related Compound A RS, USP Econazole Related Compound B RS, or USP Econazole Related Compound C RS in the *Standard solution* (mg/mL)
C_U = concentration of Econazole Nitrate in the *Sample solution* (mg/mL)

Calculate the percentage of any other unspecified impurities in the portion of Econazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of econazole from the *Standard solution*
C_S = concentration of USP Econazole Nitrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Econazole Nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Econazole related compound A	0.2	0.375
Econazole related compound B	0.6	0.375
Econazole related compound C	0.8	0.375
Econazole	1.0	—

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

SPECIFIC TESTS

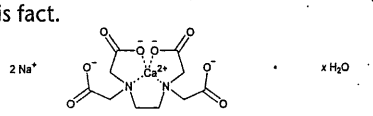
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Econazole Nitrate RS
USP Econazole Related Compound A RS
Deschlorobenzyl econazole;
1-(2,4-Dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol.
$C_{11}H_{10}Cl_2N_2O$ 257.12
USP Econazole Related Compound B RS
Econazole amine;
2-[(4-Chlorobenzyl)oxy]-2-(2,4-dichlorophenyl) ethanamine.
$C_{15}H_{14}Cl_3NO$ 330.64
USP Econazole Related Compound C RS
Econazole quarternary salt;
1-(4-Chlorobenzyl)-3-{2-[(4-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazol-3-ium nitrate (salt).
$C_{25}H_{21}Cl_{14}N_3O_4$ 569.26

Edetate Calcium Disodium

Portions of this monograph that are national *USP* text, and are not part of the harmonized text, are marked with symbol (• •) to specify this fact.



$C_{10}H_{12}CaN_2Na_2O_8 \cdot xH_2O$ 374.27
Calciate (2-), [[*N*,*N*′-1,2-ethanediylbis[*N*-(carboxymethyl) glycinato]](4-)-*N*,*N*′,*O*,*O*′,O^N,$O^{N'}$]-, disodium, hydrate, (*OC*-6-21)-;
Disodium[(ethylenedinitrilo)tetraacetato]calciate(2-) hydrate [23411-34-9].
Anhydrous [62-33-9].

DEFINITION

Edetate Calcium Disodium contains NLT 98.0% and NMT 102.0% of edetate calcium disodium ($C_{10}H_{12}CaN_2Na_2O_8$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **•A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020) •
• **B. PROCEDURE**
Sample: 2 g
Analysis: Dissolve the *Sample* in 10 mL of water, add 6 mL of lead (II) nitrate solution (33 in 1000), shake, and add 3 mL of potassium iodide TS: no yellow precipitate is formed. Make this solution alkaline by the addition of diluted ammonia solution (7 in 50), and add 3 mL of ammonium oxalate TS.
Acceptance criteria: A white precipitate is formed.
• **C. PROCEDURE**
Sample: 0.5 g
Analysis: Dissolve 0.5 g in 10 mL of water, and add 10 mL of potassium pyroantimonate TS.
Acceptance criteria: A white, crystalline precipitate is formed. The formation of the precipitate is accelerated by rubbing the inside wall of the test tube with a glass rod.

ASSAY

• **PROCEDURE**
Sample: 500 mg of Edetate Calcium Disodium
Analysis: Transfer the *Sample* into a 200-mL volumetric flask. Dissolve in and dilute with water to volume, and mix. Pipet 20 mL of this solution to 80 mL of water, and adjust with diluted nitric acid to a pH of 2–3. Add two drops of xylenol orange TS, and titrate with 0.01 M bismuth nitrate VS until the color of the solution changes from yellow to red. Each mL of 0.01 M bismuth nitrate VS is equivalent to 3.743 mg of edetate calcium disodium ($C_{10}H_{12}CaN_2Na_2O_8$).
Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample solution: To 0.70 g of Edetate Calcium Disodium add 20 mL of water and 30 mL of diluted nitric acid. Allow to stand for 30 min, and filter. To 10 mL of the filtrate add water to make 50 mL.
Control solution: Prepare with 0.40 mL of 0.01 M hydrochloric acid VS.
Analysis: Proceed as directed in the chapter.
Acceptance criteria: NMT 0.10%

ORGANIC IMPURITIES

• **Procedure 1: Disodium Edetate**
Sample: 1.00 g of Edetate Calcium Disodium
Analysis: Dissolve the *Sample* in 50 mL of water. Add 5 mL of ammonia–ammonium chloride buffer TS, and 40 mg of eriochrome black T–sodium chloride indicator. Titrate with 0.01 M magnesium chloride VS until the color of the solution changes from blue to red-violet.
Acceptance criteria: NMT 3.0 mL of 0.01 M magnesium chloride VS is consumed (NMT 1.0%).
• **•Procedure 2: Limit of Nitrilotriacetic Acid**
Mobile phase: Add 10 mL of 1.0 M tetrabutylammonium hydroxide in methanol to 200 mL of water, and adjust with 1 M phosphoric acid to a pH of 7.5 ± 0.1. Transfer the solution to a 1000-mL volumetric flask, add 90 mL of methanol, dilute with water to volume, pass through a filter of 0.5-µm or finer pore size, and degas.
Cupric nitrate solution: 10 mg/mL of cupric nitrate [$Cu(NO_3)_2$] in water
Standard stock solution: Transfer 100 mg of nitrilotriacetic acid to a 10-mL volumetric flask, add 0.5 mL of ammonium hydroxide, and mix. Dilute with water to volume, and mix.
Standard solution: Transfer 1.0 g of Edetate Calcium Disodium to a 100-mL volumetric flask, add 100 µL of *Standard stock solution*, dilute with *Cupric nitrate solution* to volume, and mix. Sonicate, if necessary, to achieve complete solution.
System suitability solution: Transfer 10 mg of Edetate Calcium Disodium to a 100-mL volumetric flask, add 100 µL of *Standard stock solution*, dilute with *Cupric nitrate solution* to volume, and mix. Sonicate, if necessary, to dissolve.

USP Monographs

www.webofpharma.com

Sample solution: 10 mg/mL of Edetate Calcium Disodium in *Cupric nitrate solution*. Sonicate, if necessary, to achieve complete solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; packing L7
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for nitrilotriacetic acid, copper, and edetate are about 0.35, 0.65, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3 between nitrilotriacetic acid and copper, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the responses for the major peaks.
Acceptance criteria: The peak response of nitrilotriacetic acid from the *Sample solution* does not exceed the difference between the nitrilotriacetic acid peak responses obtained from the *Standard solution* and the *Sample solution* (0.1%).

SPECIFIC TESTS
- **pH** ⟨791⟩
Sample solution: A solution (1 in 5)
Acceptance criteria: 6.5–8.0
- **WATER DETERMINATION,** *Method I* ⟨921⟩
Sample: 0.2 g
Acceptance criteria: 5.0%–13.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. No storage requirements specified.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Edetate Calcium Disodium RS

Edetate Calcium Disodium Injection

» Edetate Calcium Disodium Injection is a sterile solution of Edetate Calcium Disodium in Water for Injection. It contains, in each mL, not less than 180 mg and not more than 220 mg of $C_{10}H_{12}CaN_2Na_2O_8$.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Edetate Calcium Disodium RS

Identification—
A: Transfer a volume of Injection, equivalent to about 1 g of edetate calcium disodium, to an evaporating dish, and evaporate on a steam bath to dryness: the residue responds to *Identification* test *A* under *Edetate Calcium Disodium*.
B: It responds to *Identification* test *B* under *Edetate Calcium Disodium*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.01 USP Endotoxin Unit per mg of edetate calcium disodium.
pH ⟨791⟩: between 6.5 and 8.0.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Dilute an accurately measured volume of Injection, equivalent to about 1 g of edetate calcium disodium with water to about 75 mL. Add 25 mL of 1 N acetic acid and 1 mL of diphenylcarbazone TS, mix, and titrate slowly with 0.1 M mercuric nitrate VS to the appearance of the first purplish color. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M mercuric nitrate is equivalent to 37.43 mg of $C_{10}H_{12}CaN_2Na_2O_8$.

Edetate Disodium

$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ 372.24
$C_{10}H_{14}N_2Na_2O_8$ 336.21
Glycine, *N*,*N*′-1,2-ethanediylbis[*N*-(carboxymethyl)-, disodium salt, dihydrate;
Disodium (ethylenedinitrilo)tetraacetate dihydrate [6381-92-6].
Anhydrous [139-33-3].

DEFINITION
Edetate Disodium contains NLT 99.0% and NMT 101.0% of edetate disodium ($C_{10}H_{14}N_2Na_2O_8$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.**
Sample: 50 mg
Analysis: To 5 mL of water in a test tube add 2 drops of ammonium thiocyanate TS and 2 drops of ferric chloride TS. To the deep red solution add the *Sample*.
Acceptance criteria: The red color is discharged, leaving a yellowish solution.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sodium*: It meets the requirements of test *A*.

ASSAY
- **PROCEDURE**
Sample solution: Dissolve 5 g of Edetate Disodium in about 100 mL of water contained in a 250-mL volumetric flask. Add water to volume.
Analysis: Place 200 mg of chelometric standard calcium carbonate, previously dried at 110° for 2 h and cooled in a desiccator, into a 400-mL beaker. Add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and without removing the latter, add 2 mL of 3 N hydrochloric acid from a pipet. Swirl the contents of the beaker, and dissolve the calcium carbonate. With water, wash down the sides of the beaker, the outer surface of the pipet, and the watch glass, and dilute with water to 100 mL. While stirring the solution, preferably with a magnetic stirrer, add 30 mL of the *Sample solution* from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 0.30 g of hydroxy naphthol blue, and continue the titration with the *Sample solution* to a blue endpoint.
Calculate the weight of edetate disodium ($C_{10}H_{14}N_2Na_2O_8$) in the portion of Edetate Disodium taken:

$$\text{Result} = (V_T/V_U) \times W \times (M_{r1}/M_{r2})$$

www.webofpharma.com

V_T = total volume of the *Sample solution* (mL)
V_U = volume of the *Sample solution* consumed in the titration (mL)
W = weight of calcium carbonate (mg)
M_{r1} = molecular weight of edetate disodium, 336.21
M_{r2} = molecular weight of calcium carbonate, 100.09

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

• **CALCIUM**

Sample solution: 1 g of Edetate Disodium in 20 mL of water

Analysis: To the *Sample solution* add 2 drops of methyl red TS, and neutralize with 6 N ammonium hydroxide. Add 3 N hydrochloric acid dropwise until the solution is just acidic, and then add 1 mL of ammonium oxalate TS.

Acceptance criteria: No precipitate is formed.

• **LIMIT OF NITRILOTRIACETIC ACID**

Mobile phase: Add 10 mL of 1.0 M tetrabutylammonium hydroxide in methanol to 200 mL of water, and adjust with 1 M phosphoric acid to a pH of 7.5 ± 0.1. Transfer the solution so obtained to a 1000-mL volumetric flask. Add 90 mL of methanol, and dilute with water to volume. Pass through a filter of 0.5-µm or finer pore size, and degas.

Cupric nitrate solution: 10 mg/mL of cupric nitrate [$Cu(NO_3)_2$]

Standard stock solution: Transfer 100 mg of nitrilotriacetic acid to a 10-mL volumetric flask. Add 0.5 mL of ammonium hydroxide, mix, and dilute with water to volume.

Standard solution: Transfer 1.0 g of Edetate Disodium to a 100-mL volumetric flask. Add 100 µL of *Standard stock solution*, and dilute with *Cupric nitrate solution* to volume. If necessary, sonicate to dissolve.

System suitability solution: Transfer 10 mg of Edetate Disodium to a 100-mL volumetric flask. Add 100 µL of *Standard stock solution*, and dilute with *Cupric nitrate solution* to volume. If necessary, sonicate to dissolve.

Sample solution: 10 mg/mL of Edetate Disodium in *Cupric nitrate solution*. If necessary, sonicate to dissolve.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; packing L7
Flow rate: 2 mL/min
Injection volume: 50 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for nitrilotriacetic acid, copper, and edetate are about 0.35, 0.65, and 1.0, respectively.]

Suitability requirements
Resolution: NLT 3 between nitrilotriacetic acid and copper, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*

Acceptance criteria: NMT 0.1%; the response of the nitrilotriacetic acid peak of the *Sample solution* does not exceed the difference between the nitrilotriacetic acid peak responses from the *Standard solution* and the *Sample solution*.

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: 50 mg/mL

Acceptance criteria: 4.0–6.0

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 150° for 6 h.

Acceptance criteria: 8.7%–11.4%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Edetate Disodium RS

Edetate Disodium Injection

» Edetate Disodium Injection is a sterile solution of Edetate Disodium in Water for Injection, which, as a result of pH adjustment, contains varying amounts of the disodium and trisodium salts. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{10}H_{14}N_2Na_2O_8$.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.

USP Reference standards ⟨11⟩—
USP Edetate Disodium RS

Identification—Transfer a volume of Injection, equivalent to about 1 g of edetate disodium, to an evaporating dish, adjust with 3 N hydrochloric acid to a pH of 5.0, and evaporate on a steam bath to dryness: the residue responds to *Identification* tests *A* and *C* under *Edetate Disodium*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.2 USP Endotoxin Unit per mg of edetate disodium.

pH ⟨791⟩**:** between 6.5 and 7.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

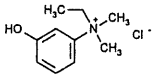
Assay preparation—Dilute an accurately measured volume of Injection, equivalent to about 2 g of edetate disodium, with water to volume in a 100-mL volumetric flask, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Edetate Disodium*. Calculate the weight, in mg, of $C_{10}H_{14}N_2Na_2O_8$ in the volume of Injection taken by the formula:

$$(336.21/100.09)W(V_T/V)$$

in which 336.21 and 100.09 are the molecular weights of edetate disodium and calcium carbonate, respectively; W is the weight, in mg, of calcium carbonate; V_T is the volume, in mL, of the *Assay preparation*; and V is the volume, in mL, of the *Assay preparation* consumed in the titration.

Edrophonium Chloride



$C_{10}H_{16}ClNO$ 201.69
Benzenaminium, *N*-ethyl-3-hydroxy-*N*,*N*-dimethyl-, chloride.
Ethyl(*m*-hydroxyphenyl)dimethylammonium chloride [116-38-1].

USP Monographs

www.webofpharma.com

» Edrophonium Chloride contains not less than 98.0 percent and not more than 100.5 percent of $C_{10}H_{16}ClNO$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Edrophonium Chloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 50 µg per mL.
Medium: 0.1 N hydrochloric acid.
Absorptivities at 273 nm, calculated on the dried basis, do not differ by more than 2.0%.

C: To 10 mL of a solution (1 in 10) add 1 drop of ferric chloride TS: a violet-blue color is produced.

D: It responds to the tests for *Chloride* ⟨191⟩.
Melting range ⟨741⟩: between 165° and 170°, with decomposition.
pH ⟨791⟩: between 4.0 and 5.0, in a solution (1 in 10).
Loss on drying ⟨731⟩—Dry it in a suitable vacuum drying tube, using phosphorus pentoxide as the desiccant, for 3 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Limit of dimethylaminophenol—Dissolve about 500 mg, accurately weighed, in 5 mL of water in a separator. Add 5 mL of pH 8.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), and shake. Extract with five 20-mL portions of chloroform, pooling the extracts in a 100-mL volumetric flask, and add chloroform to volume. The absorbance of this solution, determined in a 1-cm cell at 252 nm, with a suitable spectrophotometer, is not more than that of a 1 in 200,000 solution of dimethylaminophenol in chloroform, similarly measured (0.1%).
Assay—Transfer about 175 mg of Edrophonium Chloride, accurately weighed, to a suitable flask, and dissolve in about 20 mL of glacial acetic acid. Add 5 mL of mercuric acetate TS, then add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 20.17 mg of $C_{10}H_{16}ClNO$.

Edrophonium Chloride Injection

» Edrophonium Chloride Injection is a sterile solution of Edrophonium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{10}H_{16}ClNO$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
Labeling—Label the Injection in multiple-dose containers to indicate an expiration date of not later than 3 years after the date of manufacture, and label the Injection in single-dose containers to indicate an expiration date of not later than 4 years after the date of manufacture.
USP Reference standards ⟨11⟩—
USP Edrophonium Chloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—Test solution versus standard solution prepared as directed in the *Assay*.

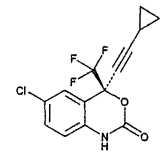
B: Place in a small separator a volume of Injection, equivalent to about 30 mg of edrophonium chloride, add 15 mL of pH 9.6 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) and 5 mL of a 1 in 1000 solution of thymol blue in pH 9.6 alkaline borate buffer, and mix. Add 10 mL of chloroform, shake thoroughly, and allow to settle: a yellow color is produced in the chloroform layer.

C: It responds to the tests for *Chloride* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 8.33 USP Endotoxin Units per mg of edrophonium chloride.
pH ⟨791⟩: between 5.0 and 5.8.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Pipet a volume of Injection, equivalent to about 50 mg of edrophonium chloride, into a glass-stoppered, 50-mL centrifuge tube. Add 5 mL of pH 8.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) and 5 g of sodium chloride. Wash the solution with four 20-mL portions of a mixture of equal volumes of solvent hexane and ether. Transfer the aqueous phase to a 100-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Transfer a 5-mL aliquot of this solution to a 50-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix test solution. Dissolve an accurately weighed quantity of USP Edrophonium Chloride RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the acid to obtain a Standard solution having a known concentration of about 50 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{10}H_{16}ClNO$ in each mL of the Injection taken by the formula:

$$(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Edrophonium Chloride RS in the Standard solution; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the test solution and the Standard solution, respectively.

Efavirenz



$C_{14}H_9ClF_3NO_2$ 315.67

www.webofpharma.com

1*H*-Benzo[*d*][1,3]oxazin-2(4*H*)-one, 6-chloro-4-cyclopropylethynyl-4-trifluoromethyl;
(*S*)-6-Chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one [154598-52-4].

DEFINITION

Efavirenz contains NLT 98.0% and NMT 102.0% of $C_{14}H_9ClF_3NO_2$, calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
Sample: Dry at 105° for 30 min, and cool in dessicator.

Change to read:

• **B. ▲Spectroscopic Identification Tests ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: 197U▲** (CN 1-May-2020)
Solvent: Methanol
Standard solution: 10 µg/mL of USP Efavirenz RS in *Solvent*
Sample solution: 10 µg/mL of Efavirenz in *Solvent*
Acceptance criteria: Meets the requirements

ASSAY

• **Procedure**
[Note—Protect solutions of efavirenz from light. Use of polypropylene HPLC vials is recomended to avoid possible degradation from certain types of glass vials.]
Diluent: Acetonitrile and water (1:1)
Solution A: Methanol, trifluoroacetic acid, and water (1: 0.005: 9). [Note—Use only freshly-opened trifluoroacetic acid, ≤6 months.]
Solution B: Methanol, trifluoroacetic acid, and water (9: 0.005: 1). [Note—Use only freshly-opened trifluoroacetic acid, ≤6 months.]
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	60	40
16	50	50
23	35	65
28	30	70
29	20	80
31	20	80
32	60	40
40	60	40

Standard solution: 250 µg/mL of USP Efavirenz RS and 1.0 µg/mL of USP Efavirenz Related Compound B RS in *Diluent*. [Note—Dissolve in about 65% of the flask volume in *Diluent* and shake for 30 min or until dissolved before diluting with *Diluent* to volume.]
Sample solution: 250 µg/mL of Efavirenz in *Diluent*. [Note—Dissolve in about 65% of the flask volume of *Diluent*, and shake for 30 min or until dissolved before diluting with *Diluent* to volume.]
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 15-cm; 5-µm packing L10
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 35 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between efavirenz related compound B and efavirenz
Relative standard deviation: NMT 1.0% for efavirenz
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_9ClF_3NO_2$ in the portion of Efavirenz taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for efavirenz from the *Sample solution*
r_S = peak response for efavirenz from the *Standard solution*
C_S = concentration of USP Efavirenz RS in the *Standard solution* (mg/mL)
C_U = concentration of efavirenz in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous, solvent-free basis

IMPURITIES

Inorganic Impurities

• **Residue on Ignition ⟨281⟩:** NMT 0.2%, use a platinum crucible

Organic Impurities

• **Procedure 1**
Diluent, Solution A, Solution B, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability solution: Use the *Standard solution* prepared as directed in the *Assay*.
Standard solution: 1.25 µg/mL of USP Efavirenz RS in *Diluent*, prepared from the *System suitability solution*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between efavirenz related compound B and efavirenz, *System suitability solution*
Relative standard deviation: NMT 5.0% for efavirenz, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Efavirenz taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for any individual impurity from the *Sample solution*
r_S = peak response for efavirenz from the *Standard solution*
C_S = concentration of USP Efavirenz RS in the *Standard solution* (mg/mL)
C_U = concentration of Efavirenz in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
[Note—Disregard any peak less than 0.05%.]
Total impurities: NMT 1.0%

USP Monographs

www.webofpharma.com

Impurity Table 1 (Efavirenz)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Efavirenz aminoalcohol[a]	0.48	0.26	0.15
Efavirenz ethene analog[b] (efavirenz related compound B)	0.93	0.91	0.40
Efavirenz pent-3-ene-1-yne (*trans*)[c]	1.16	1.0	0.10[q]
Efavirenz pent-3-ene-1-yne (*cis*)[d]	1.16	1.0	0.10[q]
Efavirenz penteneyne[e]	1.16	1.0	0.10[q]
Efavirenz pentyne analog[f]	1.2	1.0	0.15
Methyl efavirenz[g]	1.28	1.0	0.10
Efavirenz amino alcohol methyl carbamate[h]	1.33	0.83	0.10
N-Benzylefavirenz[i]	1.8	0.71	0.25
Efavirenz benzoylaminoalcohol[j]	1.9	0.56	0.15
Quinoline analog[k]	1.45	2.0	0.10
Efavirenz amino alcohol ethyl carbamate[l]	1.53	0.83	0.10
Unidentified impurity[m]	1.60	1.0	0.10
Efavirenz amino alcohol bis(ethoxycarbonyl)[n]	1.63	0.34	0.10
Unidentified impurity[o]	2.1	1.0	0.10
Cyclobutenylindole analog[p]	2.18	0.48	0.10
Any other unknown individual impurity	—	1.0	0.10

[a] (*S*)-2-(2-Amino-5-chlorophenyl)-4-cyclopropyl-1,1,1-trifluorobut-3-yn-2-ol.
[b] (*S,E*)-6-Chloro-4-(2-cyclopropylvinyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[c] (*S,E*)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[d] (*S,Z*)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[e] (*S*)-6-Chloro-4-(3-methylbut-3-en-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[f] (*S*)-6-Chloro-4-(pent-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[g] (*S*)-6-Chloro-4-{[(2*RS*,2*RS*)-2-methylcyclopropyl]ethynyl}-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[h] (*S*)-Methyl 4-chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl)phenylcarbamate.
[i] (*S*)-6-Chloro-4-(cyclopropylethynyl)-1-(4-methoxybenzyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[j] (*S*)-*N*-(4-Chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl)phenyl)-4-methoxybenzamide.
[k] 6-Chloro-2-cyclopropyl-4-(trifluoromethyl)quinoline.
[l] (*S*)-Ethyl 4-chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl)phenylcarbamate.
[m] Relative retention time of 1.60.
[n] (*S*)-Ethyl 4-chloro-2-[4-cyclopropyl-2-(ethoxycarbonyloxy)-1,1,1-trifluorobut-3-yn-2-yl]phenylcarbamate.
[o] Relative retention time of 2.1.
[p] Ethyl 5-chloro-2-cyclobutenyl-3-(trifluoromethyl)-1*H*-indole-1-carboxylate.
[q] [NOTE—If results exceed 0.10%, perform *Procedure 2* to separate the three coeluting impurities and ensure that each impurity meets the limit.]

- **Procedure 2**

[NOTE—Perform *Procedure 2* in addition to *Procedure 1* if the result for the total of the three impurities at a relative retention time of 1.16 in *Procedure 1* exceeds 0.10%.]

Diluent: Acetonitrile, trifluoroacetic acid, and water (55: 0.05: 45)

Solution A: Acetonitrile, trifluoroacetic acid, and water (4: 0.005: 6). [NOTE—Use only freshly-opened trifluoroacetic acid, ≤6 months.]

Solution B: Acetonitrile, trifluoroacetic acid, and water (8: 0.005: 2). [NOTE—Use only freshly-opened trifluoroacetic acid, ≤6 months.]

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	0	100
45	0	100
45.1	100	0
50	100	0

Standard solution: 1.25 µg/mL of USP Efavirenz RS in *Diluent*
Sample solution: 250 µg/mL of Efavirenz in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*

USP Monographs

www.webofpharma.com

Suitability requirements
Tailing factor: NMT 1.5 for efavirenz
Relative standard deviation: NMT 5.0% for efavirenz
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each of the three specified impurities in the portion of Efavirenz taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each of the three specified impurities from the *Sample solution*
r_S = peak response for efavirenz from the *Standard solution*
C_S = concentration of USP Efavirenz RS in the *Standard solution* (µg/mL)
C_U = concentration of Efavirenz in the *Sample solution* (µg/mL)
F = relative response factor (see *Impurity Table 2*)

Acceptance criteria
Individual impurities: See *Impurity Table 2*.
[NOTE—Disregard any peak less than 0.05%.]

SPECIFIC TESTS
• **COMPLETENESS OF SOLUTION** ⟨641⟩**:** Meets the requirements
Solvent: Methanol
Sample solution: 50 mg/mL of Efavirenz in *Solvent*
• **WATER DETERMINATION,** *Method Ic* ⟨921⟩
Sample: 100 mg/mL of Efavirenz in methanol
Acceptance criteria: NMT 0.5%
• **ENANTIOMERIC PURITY**
Mobile phase: Hexane and absolute alcohol (97:3)
Retention time solution: 1 mg/mL of USP Efavirenz RS in *Mobile phase*
Standard solution: 10 µg/mL of USP Efavirenz Racemic RS in *Mobile phase*
Sample solution: 1 mg/mL of Efavirenz in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm column; 5-µm packing L10 connected in-line and before 4.6-mm × 25-cm column; 10-µm packing L40
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution* and *Retention time solution*
Suitability requirements
Efavirenz identification: Identify the (*S*)-efavirenz enantiomer peak, *Retention time solution*
Resolution: NLT 3.0 between (*R*)-efavirenz enantiomer and (*S*)-efavirenz enantiomer, *Standard solution*
Relative standard deviation: NMT 5.0% for (*R*)-efavirenz enantiomer
Analysis
Samples: *Retention time solution*, *Standard solution*, and *Sample solution*
[NOTE—Verify the identification of the efavirenz peak based on the chromatogram of the *Retention time solution*. The relative retention times for (*R*)-efavirenz enantiomer and (*S*)-efavirenz enantiomer are 0.88 and 1.00, respectively.]
Calculate the percentage of (*R*)-efavirenz enantiomer in the portion of Efavirenz taken:

$$\text{Result} = 100 \times [r_R/(r_R + r_S)]$$

r_R = peak response of (*R*)-efavirenz enantiomer from the *Sample solution*
r_S = peak response of (*S*)-efavirenz enantiomer from the *Sample solution*

Acceptance criteria: NMT 0.5% of (*R*)-efavirenz enantiomer

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Efavirenz RS
USP Efavirenz Related Compound B RS
(*S,E*)-6-Chloro-4-(2-cyclopropylvinyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
$C_{14}H_{11}ClF_3NO_2$ 317.69
USP Efavirenz Racemic RS

Efavirenz Capsules

DEFINITION
Efavirenz Capsules contain NLT 92.0% and NMT 108.0% of the labeled amount of efavirenz ($C_{14}H_9ClF_3NO_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample solution: Dissolve the contents of 1 Capsule in about 5 mL of acetonitrile by mixing on a vortex mixer. Allow to settle, remove about 3 mL of the solution, and centrifuge for about 5 min. Transfer 1–2 mL of supernatant to a clean suitable container, and evaporate to dryness

Impurity Table 2 (Efavirenz)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Efavirenz	1.0	1.0	—
Efavirenz pent-3-ene-1-yne (*trans*)[a]	1.10	1.1	0.10
Efavirenz pent-3-ene-1-yne (*cis*)[b]	1.13	1.1	0.10
Efavirenz penteneyne[c]	1.14	1.0	0.10

[a] (*S,E*)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[b] (*S,Z*)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[c] (*S*)-6-Chloro-4-(3-methylbut-3-en-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.

USP Monographs

www.webofpharma.com

under nitrogen. Mix 0.5–1 mg of the powder with 200 mg of potassium bromide.

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Solvent: Acetonitrile
Standard solution: 10 µg/mL in *Solvent*
Sample solution: Dissolve the contents of 1 Capsule in about 40 mL of *Solvent* by shaking for about 30 min. Pass through a suitable nylon or PVDF membrane filter, discarding the first 2 mL of filtrate, and dilute a portion with acetonitrile to a concentration of 10 µg/mL of efavirenz.
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as does the *Standard solution*.

ASSAY

• **PROCEDURE**
Diluent: Acetonitrile and water (1:1)
Solution A: Methanol, trifluoroacetic acid, and water (1: 0.005: 9). [NOTE—Use only freshly-opened trifluoroacetic acid, ≤6 months.]
Solution B: Methanol, trifluoroacetic acid, and water (9: 0.005: 1). [NOTE—Use only freshly-opened trifluoroacetic acid, ≤6 months.]
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	60	40
16	50	50
23	35	65
28	30	70
29	20	80
31	20	80
32	60	40
40	60	40

Standard solution 1: 0.2 mg/mL of USP Efavirenz Related Compound B RS in *Diluent*
Standard solution 2: 5 mg/mL of USP Efavirenz RS in acetonitrile. [NOTE—Sonicate to dissolve before diluting to final volume.]
Standard solution: 250 µg/mL of USP Efavirenz RS and 1 µg/mL of USP Efavirenz Related Compound B RS in *Diluent* prepared from *Standard solution 2* and *Standard solution 1*, respectively. [NOTE—Store protected from light. For the HPLC analysis, it is recommended to use polypropylene vials, because degradation has been noted with certain brands made of glass.]
Sample stock solution: Transfer the contents of NLT 10 Capsules to a suitable container, and extract the contents in acetonitrile by mixing for about 30 min to obtain a quantitative solution equivalent to about 5 mg/mL of efavirenz. [NOTE—Store protected from light.]
Sample solution: Filter a portion of the *Sample stock solution*, and dilute the filtrate with *Diluent* to obtain a solution of about 250 µg/mL of efavirenz. [NOTE—Store protected from light. For the HPLC analysis, it is recommended to use polypropylene vials, because degradation has been noted with certain brands made of glass.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 15-cm; packing L10
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 35 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between efavirenz related compound B and efavirenz
Relative standard deviation: NMT 2.0% for efavirenz
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of efavirenz ($C_{14}H_9ClF_3NO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of efavirenz from the *Sample solution*
r_S = peak response of efavirenz from the *Standard solution*
C_S = concentration of USP Efavirenz RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of efavirenz in the *Sample solution* (mg/mL)

Acceptance criteria: 92.0%–108.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 1.0% (w/v) sodium lauryl sulfate in water; 900 mL. [NOTE—Do not deaerate.]
Apparatus 2: 50 rpm, with helix sinker
Time: 45 min
Standard solution: (L/900) mg/mL of USP Efavirenz RS in *Medium*, where L is the Capsule label claim in mg. A small volume of methanol, NMT 10% of the final volume, could be used to solubilize efavirenz. Dilute this solution with *Medium* to obtain a final concentration of about 0.01 mg/mL for Capsules labeled to contain 50 mg, or about 0.02 mg/mL for Capsules labeled to contain 100 mg or 200 mg.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and dilute with *Medium* to obtain a theoretical concentration similar to the *Standard solution*, assuming complete dissolution of the Capsule label claim.
Analytical wavelength: UV 247 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of efavirenz dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of efavirenz is dissolved.

www.webofpharma.com

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

Procedure for content uniformity

Standard solution: 10 µg/mL of USP Efavirenz RS in acetonitrile

Sample solution: Transfer the contents of 1 Capsule into a suitable container, and dissolve in 40.0 mL of acetonitrile. Shake for about 30 min and pass through a suitable nylon or PVDF membrane filter. Dilute a portion of the filtrate to an efavirenz concentration of about 10 µg/mL.

Spectrometric conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV absorption spectroscopy

Analytical wavelength: UV 246 nm

Cell: 1 cm

Blank: Acetonitrile

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of efavirenz ($C_{14}H_9ClF_3NO_2$) in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U = absorbance of efavirenz from the *Sample solution*

A_S = absorbance of efavirenz from the *Standard solution*

C_S = concentration of USP Efavirenz RS in the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of the *Sample solution*

D = dilution factor of the *Sample solution*

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Diluent, Solution A, Solution B, Sample solution, and **Chromatographic system:** Prepare as directed in the *Assay*.

System suitability solution: Use the *Standard solution* prepared as directed in the *Assay*.

Standard solution: 1.25 µg/mL of USP Efavirenz RS and 0.005 µg/mL of USP Efavirenz Related Compound B RS in *Diluent* from the *System suitability solution*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between efavirenz related compound B and efavirenz, *System suitability solution*

Relative standard deviation: NMT 5.0% for efavirenz, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any individual impurity (degradation product) from the *Sample solution*

r_S = peak response of efavirenz from the *Standard solution*

C_S = concentration of USP Efavirenz RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of efavirenz in the *Sample solution* (mg/mL)

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*. [NOTE—Disregard any peak less than 0.05%.]

Total impurities: NMT 0.50%. [NOTE—Include only the degradation products in the calculation of the total impurities.]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Efavirenz aminoalcohol (degradation product)[a]	0.48	0.26	0.25
Efavirenz ethene analog[b]	0.93	—	*
Efavirenz pent-3-ene-1-yne (*cis*)[c]	1.16	—	*
Efavirenz pent-3-ene-1-yne (*trans*)[d]	1.16	—	*
Efavirenz penteneyne[e]	1.16	—	*
Efavirenz pentyne analog[f]	1.2	—	*
Methylefavirenz[g]	1.28	—	*
Efavirenz aminoalcohol methyl carbamate[h]	1.33	—	*
N-Benzylefavirenz[i]	1.8	—	*
Efavirenz benzoylaminoalcohol[j]	1.9	—	*
Quinoline analog (degradation product)[k]	1.45	2.0	0.20
Efavirenz aminoalcohol ethyl carbamate[l]	1.53	—	*
Unidentified impurity	1.60	—	*
Efavirenz aminoalcohol bis(ethoxycarbonyl)[m]	1.63	—	*
Unidentified impurity	2.1	—	*
Cyclobutenylindole analog[n]	2.18	—	*
Any other individual degradation product	—	1.0	0.20

* For information purposes only. These are process impurities monitored in the drug substance and are not included in the total impurities.

[a] (*S*)-2-(2-Amino-5-chlorophenyl)-4-cyclopropyl-1,1,1-trifluorobut-3-yn-2-ol.

[b] (*S,E*)-6-Chloro-4-(2-cyclopropylvinyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.

[c] (*S,E*)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.

[d] (*S,Z*)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.

[e] (*S*)-6-Chloro-4-(3-methylbut-3-en-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.

[f] (*S*)-6-Chloro-4-(pent-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.

[g] (*S*)-6-Chloro-4-[[(2*RS*,2*RS*)-2-methylcyclopropyl]ethynyl]-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.

[h] (*S*)-Methyl 4-chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl) phenylcarbamate.

[i] (*S*)-6-Chloro-4-(cyclopropylethynyl)-1-(4-methoxybenzyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.

[j] (*S*)-*N*-[4-Chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl) phenyl]-4-methoxybenzamide.

[k] 6-Chloro-2-cyclopropyl-4-(trifluoromethyl)quinoline.

[l] (*S*)-Ethyl 4-chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl) phenylcarbamate.

[m] (*S*)-Ethyl 4-chloro-2-[4-cyclopropyl-2-(ethoxycarbonyloxy)-1,1,1-trifluorobut-3-yn-2-yl]phenylcarbamate.

[n] Ethyl 5-chloro-2-cyclobutenyl-3-(trifluoromethyl)-1*H*-indole-1-carboxylate.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store in well-closed containers at controlled room temperature.

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Efavirenz RS
USP Efavirenz Related Compound B RS
(*S,E*)-6-Chloro-4-(2-cyclopropylvinyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
$C_{14}H_{11}ClF_3NO_2$ 317.69

Efavirenz Tablets

DEFINITION
Efavirenz Tablets contain NLT 92.0% and NMT 108.0% of the labeled amount of efavirenz ($C_{14}H_9ClF_3NO_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample solution: Completely remove the coating film from a Tablet using a mortar and pestle to crack the Tablet, grind the contents into fine powder, and place the powder into a suitable container. Dissolve the contents in about 5 mL of acetonitrile by mixing on a vortex mixer. Allow to settle, remove about 3 mL of the solution, and centrifuge for about 5 min using a suitable container. Transfer 1–2 mL of supernatant to a clean suitable container, and evaporate to dryness under nitrogen. Mix 0.5–1 mg of the powder with 200 mg of potassium bromide.
Acceptance criteria: Meet the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Diluent: Acetonitrile and water (500:500)
Solution A: Methanol, trifluoroacetic acid, and water (100:0.5:900)
Solution B: Methanol, trifluoroacetic acid, and water (900:0.5:100)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
16	50	50
23	35	65
28	30	70
29	20	80
31	20	80
32	60	40
40	60	40

Standard stock solution 1: 0.2 mg/mL of USP Efavirenz Related Compound B RS in *Diluent*
Standard stock solution 2: 5 mg/mL of USP Efavirenz RS in *Diluent*. Sonicate to dissolve before diluting to final volume.
Standard solution: 250 µg/mL of USP Efavirenz RS and 1 µg/mL of USP Efavirenz Related Compound B RS in *Diluent* prepared from *Standard solution stock 2* and *Standard stock solution 1*, respectively. Store protected from light. For the HPLC analysis, it is recommended to use polypropylene vials, because degradation may occur with glass containers.
Sample stock solution: Nominally, 12 mg/mL of efavirenz in *Diluent* prepared as follows. Transfer NLT 10 Tablets to a suitable container, and extract the contents in *Diluent* by mixing for about 90 min. Store protected from light.
Sample solution: Nominally, 240 µg/mL of efavirenz in *Diluent* prepared as follows. Filter a portion of the *Sample stock solution*, and dilute the filtrate with *Diluent*. Store protected from light. For the HPLC analysis, it is recommended to use polypropylene vials, because degradation may occur with glass containers.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 15-cm; 5-µm packing L10
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 35 µL
System suitability
Sample: *Standard solution*
[NOTE—The typical retention times for efavirenz related compound B and efavirenz are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.2 between efavirenz related compound B and efavirenz
Relative standard deviation: NMT 2.0% for efavirenz
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of efavirenz ($C_{14}H_9ClF_3NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of efavirenz from the *Sample solution*
r_S = peak response of efavirenz from the *Standard solution*
C_S = concentration of USP Efavirenz RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of efavirenz in the *Sample solution* (mg/mL)

Acceptance criteria: 92.0%–108.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 2.0% (w/v) sodium lauryl sulfate in water; 1000 mL. Do not deaerate.
Apparatus 2: 50 rpm, with helix sinker. In addition, paddle and shaft must be composed of stainless steel and not coated with Teflon or other material. Also, all sampling devices and dissolution vessels must be washed with methanol or ethanol followed by a water wash.
Time: 30 min
Standard solution: (*L*/1000) mg/mL of USP Efavirenz RS in *Medium*, where *L* is the Tablet label claim in mg. A small volume of methanol, NMT 1% of the final volume, could be used to solubilize efavirenz. Dilute this solution with *Medium* to obtain a final concentration of about 0.012 mg/mL.
Sample solution: Pass a portion of the solution under test through a suitable polyethylene filter, and dilute with *Medium* to obtain a concentration similar to the *Standard solution*, assuming complete dissolution of the Tablet label claim.
Instrumental conditions
Analytical wavelength: UV 247 nm

www.webofpharma.com

Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of efavirenz ($C_{14}H_9ClF_3NO_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
D = dilution for the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of efavirenz ($C_{14}H_9ClF_3NO_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩
Procedure for content uniformity
Diluent: Acetonitrile and water (50:50)
Standard solution: 12 µg/mL of USP Efavirenz RS in *Diluent*
Sample solution: Transfer NLT 10 Tablets into separate and suitable containers, and dissolve in 250 mL of *Diluent*. Stir for about 90 min, and centrifuge a portion of each solution for 10 min. Pass about 10 mL through a suitable nylon or PVDF membrane filter. Immediately dilute a portion of the filtrate to an efavirenz concentration of about 12 µg/mL.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: UV 246 nm
Cell: 1 cm
Blank: *Diluent*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of efavirenz ($C_{14}H_9ClF_3NO_2$) in each Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U = absorbance of efavirenz from the *Sample solution*
A_S = absorbance of efavirenz from the *Standard solution*
C_S = concentration of USP Efavirenz RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of the *Sample solution* (mL)
D = dilution factor of the *Sample solution*

Acceptance criteria: Meet the requirements

IMPURITIES

• **Organic Impurities**
Diluent, Solution A, Solution B, Sample solution, and **Chromatographic system:** Prepare as directed in the *Assay*.
System suitability solution: Use the *Standard solution* prepared as directed in the *Assay*.
Standard solution: 1.25 µg/mL of USP Efavirenz RS and 0.005 µg/mL of USP Efavirenz Related Compound B RS in *Diluent* from the *System suitability solution*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between efavirenz related compound B and efavirenz, *System suitability solution*
Relative standard deviation: NMT 5.0% for efavirenz, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual degradation product in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any individual impurity (degradation product) from the *Sample solution*
r_S = peak response of efavirenz from the *Standard solution*
C_S = concentration of USP Efavirenz RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of efavirenz in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Efavirenz aminoalcohol (degradation product)[a]	0.48	0.26	0.25
Efavirenz ethene analog (efavirenz related compound B)[b, c]	0.93	—	—
Efavirenz	1.0	—	—
Efavirenz pent-3-ene-1-yne (*cis*)[c, d]			
Efavirenz pent-3-ene-1-yne (*trans*)[c, e]		—	—
Efavirenz penteneyne[c, f]	1.16		
Efavirenz pentyne analog[c, g]	1.2	—	—
Methylefavirenz[c, h]	1.28	—	—
Efavirenz aminoalcohol methyl carbamate[c, i]	1.33	—	—
Quinoline analog (degradation product)[j]	1.45	2.0	0.20
Efavirenz aminoalcohol ethyl carbamate[c, k]	1.53	—	—
Unidentified impurity[c]	1.60	—	—
Efavirenz aminoalcohol bis(ethoxycarbonyl)[c, l]	1.63	—	—
N-Benzylefavirenz[c, m]	1.8	—	—
Efavirenz benzoylaminoalcohol[c, n]	1.9	—	—
Unidentified impurity[c]	2.1	—	—
Cyclobutenylindole analog[c, o]	2.18	—	—
Any other individual degradation product	—	1.0	0.20

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total[c, p]	—	—	0.50

[a] (*S*)-2-(2-Amino-5-chlorophenyl)-4-cyclopropyl-1,1,1-trifluorobut-3-yn-2-ol.
[b] (*S,E*)-6-Chloro-4-(2-cyclopropylvinyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[c] For information purposes only. These are process impurities monitored in the drug substance and are not included in the total impurities. Include only the degradation products in the calculation of the total impurities.
[d] (*S,E*)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[e] (*S,Z*)-6-Chloro-4-(pent-3-en-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[f] (*S*)-6-Chloro-4-(3-methylbut-3-en-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[g] (*S*)-6-Chloro-4-(pent-1-ynyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[h] (*S*)-6-Chloro-4-{[(2*RS*,2*RS*)-2-methylcyclopropyl]ethynyl}-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[i] (*S*)-Methyl 4-chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl) phenylcarbamate.
[j] 6-Chloro-2-cyclopropyl-4-(trifluoromethyl)quinoline.
[k] (*S*)-Ethyl 4-chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl) phenylcarbamate.
[l] (*S*)-Ethyl 4-chloro-2-[4-cyclopropyl-2-(ethoxycarbonyloxy)-1,1,1-trifluorobut-3-yn-2-yl]phenylcarbamate.
[m] (*S*)-6-Chloro-4-(cyclopropylethynyl)-1-(4-methoxybenzyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
[n] (*S*)-*N*-[4-Chloro-2-(4-cyclopropyl-1,1,1-trifluoro-2-hydroxybut-3-yn-2-yl) phenyl]-4-methoxybenzamide.
[o] Ethyl 5-chloro-2-cyclobutenyl-3-(trifluoromethyl)-1*H*-indole-1-carboxylate.
[p] Disregard any peak less than 0.05%.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in well-closed containers at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Efavirenz RS
  USP Efavirenz Related Compound B RS
  (*S,E*)-6-Chloro-4-(2-cyclopropylvinyl)-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.
  $C_{14}H_{11}ClF_3NO_2$ 317.69

Multiple Electrolytes Injection Type 1

DEFINITION

Multiple Electrolytes Injection Type 1 is a sterile solution of suitable salts in Water for Injection to provide sodium, potassium, magnesium, and chloride ions. In addition, the salts provide ions of acetate, acetate and gluconate, or acetate, gluconate, and phosphate. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), chloride (Cl), acetate ($C_2H_3O_2$), gluconate ($C_6H_{11}O_7$), and phosphate (PO_4). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Magnesium* and *Chloride*: Meets the requirements
- **B. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.
- **C. POTASSIUM:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]
- **D.** The retention time of the acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the *Assay* for *Acetate*.
- **E.** Where gluconate is purported to be present, the retention time of the gluconate peak of the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the *Assay* for *Gluconate*.
- **F.** Where phosphate is purported to be present, proceed as follows.
  Sample solution: Add 5 mL of Injection and 1 mL of ammonium molybdate TS to a test tube and mix.
  Acceptance criteria: A yellow precipitate, which is soluble in 6 N ammonium hydroxide, is formed.

ASSAY

- **ACETATE**
  Mobile phase: 0.05 N sulfuric acid
  Standard solution: 1.2 mg/mL of sodium acetate trihydrate (0.0088 mEq/mL of acetate) in water
  Sample solution: Nominally 0.0088 mEq/mL of acetate from a volume of Injection in water
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Columns
  Guard: 4.6-mm × 3-cm; packing L17
  Analytical: 7.8-mm × 30-cm; packing L17
  Column temperature: 60°
  Flow rate: 0.8 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of acetate ($C_2H_3O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetate from the *Sample solution*
r_S = peak response of acetate from the *Standard solution*
C_S = concentration of acetate in the *Standard solution* (mEq/mL)
C_U = nominal concentration of acetate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

- **CHLORIDE**
  Sample solution: Transfer a volume of Injection equivalent to 55 mg of chloride (1.55 mEq) to a suitable conical flask, and add water, if necessary, to bring the volume to 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.
  Titrimetric system
  Mode: Direct titration
  Titrant: 0.1 N silver nitrate VS
  Endpoint detection: Visual
  Analysis
  Sample: *Sample solution*
  Titrate, with shaking, with *Titrant* to a pink endpoint.
  Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

www.webofpharma.com

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample solution* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 35.45 mg/mEq
W = nominal amount of chloride in the *Sample solution* (mg)

Acceptance criteria: 90.0%–110.0%

• **GLUCONATE** (if present)

Mobile phase: 0.05 N sulfuric acid

Standard solution: 1 mg/mL of USP Potassium Gluconate RS (0.0043 mEq/mL of gluconate) in water

Sample solution: Nominally 0.004 mEq/mL of gluconate from a volume of Injection in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Columns

Guard: 4.6-mm × 3-cm; packing L17

Analytical: 7.8-mm × 30-cm; packing L17

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of gluconate ($C_6H_{11}O_7$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of gluconate from the *Sample solution*
r_S = peak response of gluconate from the *Standard solution*
C_S = concentration of USP Potassium Gluconate RS in the *Standard solution* (mEq/mL)
C_U = nominal concentration of gluconate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **MAGNESIUM**

[NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Solution B: Mix 678 mL of hydrochloric acid with water to make 3000 mL.

Standard stock solution A: 1.00 mg/mL of magnesium (Mg) prepared as follows. Transfer 1.00 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water. Slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with *Solution B* to volume.

Standard stock solution B: 100 µg/mL of magnesium (Mg) prepared as follows. Transfer 10.0 mL of *Standard stock solution A* to a 100-mL volumetric flask, and dilute with *Solution B* to volume.

Standard solutions: 10.0, 15.0, and 20.0 µg/mL of magnesium (Mg) prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution B*. Dilute the contents of each flask with *Solution B* to volume.

Sample solution: Nominally 20.0 µg/mL of magnesium from Injection prepared as follows. Transfer a volume of Injection, equivalent to 20 mg of magnesium (Mg) to a 1000-mL volumetric flask containing 50.0 mL of *Solution A*. Dilute with *Solution B* to volume.

Blank: 5.0 mL of *Solution A* diluted with *Solution B* to 100.0 mL

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Magnesium emission line at 285.2 nm

Lamp: Magnesium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of magnesium (Mg), and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (C) in µg/mL, of magnesium (Mg) in the *Sample solution*.

Calculate the percentage of the labeled amount of magnesium (Mg) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of magnesium (Mg) in the *Sample solution* (µg/mL), interpolated from the graph obtained
C_U = nominal concentration of magnesium (Mg) in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

• **PHOSPHATE** (if present)

Solution A: 50 g/L of ammonium molybdate prepared as follows. Transfer a suitable amount of ammonium molybdate to an appropriate volumetric flask. Add 60% of the final flask volume of water, and swirl to dissolve. Add 15% of the final flask volume of sulfuric acid, and swirl. Allow to cool, and dilute with water to volume.

Solution B: Dissolve 0.5 g of hydroquinone in 100 mL of water, and add 1 drop of sulfuric acid. Prepare this solution fresh daily.

Solution C: Dissolve 1 g of sodium sulfite in water to make 5 mL. Prepare this solution fresh daily.

Standard solution: 0.11 mg/mL of monobasic potassium phosphate (0.0008 mEq/mL of phosphate) in water

Sample solution: Nominally 0.0008 mEq/mL of phosphate from a volume of Injection in water

Blank: Water

Instrumental conditions

Mode: Vis

Analytical wavelength: 640 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use water to zero the instrument. Transfer 2.0 mL each of the *Standard solution*, *Sample solution*, and the *Blank* to separate test tubes. To each test tube add 1.0 mL of *Solution A*, mix, and allow to stand for 3 min. Add 1.0 mL of *Solution B*, and mix. Add 1.0 mL of *Solution C*, mix, and allow to stand for 30 min.

Calculate the percentage of the labeled amount of phosphate (PO_4) in the portion of Injection taken:

USP Monographs

www.webofpharma.com

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*, corrected for any absorbance of the solution from the *Blank*
A_S = absorbance of the *Standard solution*, corrected for any absorbance of the solution from the *Blank*
C_S = concentration of phosphate (PO_4) in the *Standard solution* (mEq/mL)
C_U = nominal concentration of phosphate (PO_4) in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **POTASSIUM AND SODIUM**

Internal standard solution: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Add a suitable nonionic surfactant, and then dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride (1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Standard stock solution: 0.0391*J* mg/mL of potassium (K) from *Potassium stock solution* and 0.02299*J′* mg/mL of sodium (Na) from *Sodium stock solution* prepared as follows. Transfer 0.1*J* mL of *Potassium stock solution* and 0.1*J′* mL of *Sodium stock solution* to a 100-mL volumetric flask, where *J* and *J′* are the labeled amounts, in mEq/L, of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Dilute 5.0 mL of the *Standard stock solution* with *Internal standard solution* to 500.0 mL.

Sample solution: Dilute 5.0 mL of Injection with *Internal standard solution* to 500.0 mL.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: Maximum at 766 nm

Sodium: Maximum at 589 nm

Lithium: Maximum at 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of potassium to lithium from the *Sample solution*
R_S = emission reading ratio of potassium to lithium from the *Standard solution*
C_S = concentration of potassium (K) in the *Standard solution* (mg/mL)
C_U = nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[NOTE—Each mg of potassium (K) is equivalent to 0.02558 mEq of potassium (K).]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of sodium to lithium from the *Sample solution*
R_S = emission reading ratio of sodium to lithium from the *Standard solution*
C_S = concentration of sodium (Na) in the *Standard solution* (mg/mL)
C_U = nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[NOTE—Each mg of sodium (Na) is equivalent to 0.04350 mEq of sodium (Na).]

Acceptance criteria

Potassium: 90.0%–110.0%

Sodium: 90.0%–110.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.5 USP Endotoxin Units/mL

• **pH** ⟨791⟩**:** 4.0–8.0

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers of Type I or Type II glass are preferable.

• **LABELING:** The label states the content of each electrolyte in terms of milliequivalents (mEq) in a given volume. The label states the total osmolar concentration in mOsmol/L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Potassium Gluconate RS

Multiple Electrolytes Injection Type 2

DEFINITION

Multiple Electrolytes Injection Type 2 is a sterile solution of suitable salts in Water for Injection to provide sodium, potassium, calcium, magnesium, and chloride ions. In addition, the salts provide ions of either acetate and citrate, or acetate and lactate. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), chloride (Cl), acetate ($C_2H_3O_2$), citrate ($C_6H_5O_7$), and lactate ($C_3H_5O_3$). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Magnesium* and *Chloride***:** Meets the requirements

• **B. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.

• **C. POTASSIUM:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

• **D. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Calcium***:** Meets the requirements of test *A*

• **E.** The retention time of the acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the *Assay* for *Acetate*.

www.webofpharma.com

• **F.** Where citrate is purported to be present, the retention time of the citrate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Citrate*.

• **G.** Where lactate is purported to be present, the retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Lactate*.

ASSAY

• **ACETATE**

Mobile phase: 0.05 N sulfuric acid

Standard solution: 1.2 mg/mL of sodium acetate trihydrate (0.0088 mEq/mL of acetate) in water

Sample solution: Nominally 0.0088 mEq/mL of acetate from a volume of Injection in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Columns

Guard: 4.6-mm × 3-cm; packing L17

Analytical: 7.8-mm × 30-cm; packing L17

Column temperature: 60°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetate ($C_2H_3O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetate from the *Sample solution*

r_S = peak response of acetate from the *Standard solution*

C_S = concentration of acetate in the *Standard solution* (mEq/mL)

C_U = nominal concentration of acetate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **CALCIUM**

[NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Solution B: Mix 678 mL of hydrochloric acid with water to make 3000 mL.

Standard stock solution: 1000 µg/mL of calcium prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of *Solution B*, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 10.0, 15.0, and 20.0 µg/mL of calcium prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 1.0, 1.5, and 2.0 mL, respectively, of *Standard stock solution*. Dilute the contents of each flask with *Solution B* to volume.

Sample solution: Nominally 20.0 µg/mL of calcium from Injection prepared as follows. Transfer a volume of Injection, equivalent to 20 mg of calcium, to a 1000-mL volumetric flask containing 50.0 mL of *Solution A*. Dilute with *Solution B* to volume.

Blank: 5.0 mL of *Solution A* diluted with *Solution B* to 100.0 mL

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of calcium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (C), in µg/mL, of calcium in the *Sample solution*.

Calculate the percentage of the labeled amount of calcium (Ca) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of calcium in the *Sample solution* (µg/mL), interpolated from the graph obtained

C_U = nominal concentration of calcium in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

• **CHLORIDE**

Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride (1.55 mEq), to a suitable conical flask, and add water, if necessary, to bring the volume to 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample solution* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 35.45 mg/mEq

W = nominal amount of chloride in the *Sample solution* (mg)

Acceptance criteria: 90.0%–110.0%

• **CITRATE** (if present)

Mobile phase and **Chromatographic system:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Standard solution: 0.3 mEq/L of citrate ($C_6H_5O_7$) from USP Citric Acid RS in freshly prepared 1 mM sodium hydroxide

Sample solution: Nominally 0.3 mEq/L of citrate in 1 mM sodium hydroxide from a volume of Injection diluted with freshly prepared sodium hydroxide

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in ⟨345⟩.

www.webofpharma.com

Calculate the percentage of the labeled amount of citrate ($C_6H_5O_7$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of citrate from the *Sample solution*
r_S = peak response of citrate from the *Standard solution*
C_S = concentration of citrate in the *Standard solution* (mEq/L)
C_U = nominal concentration of citrate in the *Sample solution* (mEq/L)

Acceptance criteria: 90.0%–110.0%

• **LACTATE** (if present)

Mobile phase: Formic acid, dicyclohexylamine, and water (1:1:998)

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water

Standard solution: 2 mg/mL of USP Sodium Lactate RS (0.018 mEq/mL of lactate) in water

Sample solution

For Injections containing >20 mEq/L of lactate: Nominally 0.02 mEq/mL of lactate from Injection in water

For Injections containing ≤20 mEq/L of lactate: Use the undiluted Injection.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between acetate and lactate, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lactate ($C_3H_5O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lactate from the *Sample solution*
r_S = peak response of lactate from the *Standard solution*
C_S = concentration of lactate in the *Standard solution* (mEq/mL)
C_U = nominal concentration of lactate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **MAGNESIUM**

[NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Solution B: Mix 678 mL of hydrochloric acid with water to make 3000 mL.

Standard stock solution A: 1.00 mg/mL of magnesium (Mg) prepared as follows. Transfer 1.00 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water. Slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with *Solution B* to volume.

Standard stock solution B: 100 µg/mL of magnesium (Mg) prepared as follows. Transfer 10.0 mL of *Standard stock solution A* to a 100-mL volumetric flask, and dilute with *Solution B* to volume.

Standard solutions: 10.0, 15.0, and 20.0 µg/mL of magnesium (Mg) prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution B*. Dilute the contents of each flask with *Solution B* to volume.

Sample solution: Nominally 20.0 µg/mL of magnesium from Injection prepared as follows. Transfer a volume of Injection, equivalent to 20 mg of magnesium (Mg) to a 1000-mL volumetric flask containing 50.0 mL of *Solution A*. Dilute with *Solution B* to volume.

Blank: 5.0 mL of *Solution A* diluted with *Solution B* to 100.0 mL

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Magnesium emission line at 285.2 nm

Lamp: Magnesium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of magnesium (Mg), and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (C) in µg/mL, of magnesium (Mg) in the *Sample solution*.

Calculate the percentage of the labeled amount of magnesium (Mg) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of magnesium (Mg) in the *Sample solution* (µg/mL), interpolated from the graph obtained
C_U = nominal concentration of magnesium (Mg) in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

• **POTASSIUM AND SODIUM**

Internal standard solution: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Add a suitable nonionic surfactant, and then dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride (1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Standard stock solution: 0.0391J mg/mL of potassium (K) from *Potassium stock solution* and 0.02299J' mg/mL of sodium (Na) from *Sodium stock solution* prepared as follows. Transfer 0.1J mL of *Potassium stock solution* and 0.1J' mL of *Sodium stock solution* to a 100-mL volumetric flask, where J

www.webofpharma.com

and *J′* are the labeled amounts, in mEq/L, of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Dilute 5.0 mL of *Standard stock solution* with *Internal standard solution* to 500.0 mL.

Sample solution: Dilute 5.0 mL of Injection with *Internal standard solution* to 500.0 mL.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: Maximum at 766 nm

Sodium: Maximum at 589 nm

Lithium: Maximum at 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of potassium to lithium from the *Sample solution*

R_S = emission reading ratio of potassium to lithium from the *Standard solution*

C_S = concentration of potassium (K) in the *Standard solution* (mg/mL)

C_U = nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[NOTE—Each mg of potassium (K) is equivalent to 0.02558 mEq of potassium (K).]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of sodium to lithium from the *Sample solution*

R_S = emission reading ratio of sodium to lithium from the *Standard solution*

C_S = concentration of sodium (Na) in the *Standard solution* (mg/mL)

C_U = nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[NOTE—Each mg of sodium (Na) is equivalent to 0.04350 mEq of sodium (Na).]

Acceptance criteria

Potassium: 90.0%–110.0%

Sodium: 90.0%–110.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Units/mL
- **pH** ⟨791⟩: 4.0–8.0
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers of Type I or Type II glass are preferable.
- **LABELING:** The label states the content of each electrolyte in terms of milliequivalents (mEq) in a given volume. The label states the total osmolar concentration in mOsmol/L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Citric Acid RS
  USP Sodium Lactate RS

Multiple Electrolytes and Dextrose Injection Type 1

DEFINITION

Multiple Electrolytes and Dextrose Injection Type 1 is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, potassium, magnesium, and chloride ions. In addition, the salts provide ions of acetate, or acetate and gluconate, or acetate and phosphate, or phosphate and lactate, or phosphate and sulfate. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), acetate ($C_2H_3O_2$), gluconate ($C_6H_{11}O_7$), phosphate (PO_4), lactate ($C_3H_5O_3$), and sulfate (SO_4); NLT 90.0% and NMT 120.0% of the labeled amount of chloride (Cl); and NLT 90.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

IDENTIFICATION

- **A.**

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Magnesium* and *Chloride*: Meets the requirements
- **C. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.
- **D. POTASSIUM:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]
- **E.** Where acetate is purported to be present, the retention time of the acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Acetate*.
- **F.** Where gluconate is purported to be present, the retention time of the gluconate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Gluconate*.
- **G.** Where phosphate is purported to be present, proceed as follows.

Sample solution: Add 5 mL of Injection and 1 mL of ammonium molybdate TS to a test tube and mix.

Acceptance criteria: A yellow precipitate, which is soluble in 6 N ammonium hydroxide, is formed.

- **H.** Where lactate is purported to be present, the retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Lactate*.
- **I. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sulfate*: Where sulfate is purported to be present, it meets the requirements of test *A*.

USP Monographs

www.webofpharma.com

ASSAY

• **ACETATE** (if present)

Mobile phase: 0.05 N sulfuric acid

Standard solution: 1.2 mg/mL of sodium acetate trihydrate (0.0088 mEq/mL of acetate) in water

Sample solution: Nominally 0.0088 mEq/mL of acetate from a volume of Injection in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Columns

Guard: 4.6-mm × 3-cm; packing L17

Analytical: 7.8-mm × 30-cm; packing L17

Column temperature: 60°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetate ($C_2H_3O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetate from the *Sample solution*

r_S = peak response of acetate from the *Standard solution*

C_S = concentration of acetate in the *Standard solution* (mEq/mL)

C_U = nominal concentration of acetate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **CHLORIDE**

Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride (1.55 mEq), to a suitable conical flask, and add water, if necessary, to bring the volume to 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample solution* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 35.45 mg/mEq

W = nominal amount of chloride in the *Sample solution* (mg)

Acceptance criteria: 90.0%–120.0%

• **DEXTROSE**

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis

Sample: *Sample solution*

Determine the angular rotation in a polarimeter tube (see *Optical Rotation* ⟨781⟩).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times \alpha)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

α = observed angular rotation of the *Sample solution* (°)

l = length of the polarimeter tube (dm)

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–105.0%

• **GLUCONATE** (if present)

Mobile phase: Dissolve 7.0 g of calcium acetate in 1900 mL of water, and adjust with glacial acetic acid to a pH of 4.5 ± 0.1. Dilute with water to 2000 mL, mix, pass through a filter of 0.5-µm pore size or finer, and degas. Maintain the *Mobile phase* at 70 ± 2° at all times.

System suitability solution: 5 mg/mL each of USP Sodium Lactate RS and USP Potassium Gluconate RS in water

Standard solution: 4.6 mg/mL of USP Potassium Gluconate RS (0.02 mEq/mL of gluconate) in water

Sample solution: Nominally 0.023 mEq/mL of gluconate from a volume of Injection in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 7.8-mm × 30-cm; packing L19 in the calcium form. Condition the column with *Mobile phase* for 60 min.

Column temperature: 70 ± 2°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between lactate and gluconate, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of gluconate ($C_6H_{11}O_7$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of gluconate from the *Sample solution*

r_S = peak response of gluconate from the *Standard solution*

C_S = concentration of gluconate in the *Standard solution* (mEq/mL)

C_U = nominal concentration of gluconate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **LACTATE** (if present)

Mobile phase: Formic acid, dicyclohexylamine, and water (1:1:998)

www.webofpharma.com

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water
Standard solution: 2 mg/mL of USP Sodium Lactate RS (0.018 mEq/mL of lactate) in water
Sample solution
For Injections containing >20 mEq/L of lactate: Nominally 0.02 mEq/mL of lactate from Injection in water
For Injections containing ≤20 mEq/L of lactate: Use the undiluted Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2 between acetate and lactate, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lactate ($C_3H_5O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lactate from the *Sample solution*
r_S = peak response of lactate from the *Standard solution*
C_S = concentration of lactate in the *Standard solution* (mEq/mL)
C_U = nominal concentration of lactate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **MAGNESIUM**
[NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]
Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.
Solution B: Mix 678 mL of hydrochloric acid with water to make 3000 mL.
Standard stock solution A: 1.00 mg/mL of magnesium (Mg) prepared as follows. Transfer 1.00 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water. Slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with *Solution B* to volume.
Standard stock solution B: 100 µg/mL of magnesium (Mg) prepared as follows. Transfer 10.0 mL of *Standard stock solution A* to a 100-mL volumetric flask, and dilute with *Solution B* to volume.
Standard solutions: 10.0, 15.0, and 20.0 µg/mL of magnesium (Mg) prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution B*. Dilute the contents of each flask with *Solution B* to volume.
Sample solution: Transfer a volume of Injection, equivalent to 20 mg of magnesium (Mg) to a 1000-mL volumetric flask containing 50.0 mL of *Solution A*. Dilute with *Solution B* to volume.
Blank: 5.0 mL of *Solution A* diluted with *Solution B* to 100.0 mL
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Magnesium emission line at 285.2 nm
Lamp: Magnesium hollow-cathode
Flame: Air–acetylene
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of magnesium (Mg), and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (C) in µg/mL, of magnesium (Mg) in the *Sample solution*.
Calculate the percentage of the labeled amount of magnesium (Mg) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of magnesium (Mg) in the *Sample solution* (µg/mL), interpolated from the graph obtained
C_U = nominal concentration of magnesium (Mg) in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

• **PHOSPHATE** (if present)
Solution A: 50 g/L of ammonium molybdate prepared as follows. Transfer a suitable amount of ammonium molybdate to an appropriate volumetric flask. Add 60% of the final flask volume of water, and swirl to dissolve. Add 15% of the flask volume of sulfuric acid, and swirl. Allow to cool, and dilute with water to volume.
Solution B: Dissolve 0.5 g of hydroquinone in 100 mL of water, and add 1 drop of sulfuric acid. Prepare this solution fresh daily.
Solution C: Dissolve 1 g of sodium sulfite in water to make 5 mL. Prepare this solution fresh daily.
Standard solution: 0.11 mg/mL of monobasic potassium phosphate (0.0008 mEq/mL of phosphate) in water
Sample solution: Nominally 0.0008 mEq/mL of phosphate from a volume of Injection in water
Blank: Water
Instrumental conditions
Mode: Vis
Analytical wavelength: 640 nm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Use water to zero the instrument. Transfer 2.0 mL each of the *Standard solution*, *Sample solution*, and the *Blank* to separate test tubes. To each test tube add 1.0 mL of *Solution A*, mix, and allow to stand for 3 min. Add 1.0 mL of *Solution B*, and mix. Add 1.0 mL of *Solution C*, mix, and allow to stand for 30 min.
Calculate the percentage of the labeled amount of phosphate (PO_4) in the portion of the Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*, corrected for any absorbance of the solution from the *Blank*
A_S = absorbance of the *Standard solution*, corrected for any absorbance of the solution from the *Blank*

www.webofpharma.com

C_S = concentration of phosphate (PO_4) in the *Standard solution* (mEq/mL)
C_U = nominal concentration of phosphate (PO_4) in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

- **POTASSIUM AND SODIUM**

Internal standard solution: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Add a suitable nonionic surfactant, and then dilute with water to volume.
Potassium stock solution: 74.56 mg/mL of potassium chloride (1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.
Sodium stock solution: 58.44 mg/mL of sodium chloride (1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.
Standard stock solution: 0.0391*J* mg/mL of potassium (K) from *Potassium stock solution* and 0.02299*J′* mg/mL of sodium (Na) from *Sodium stock solution* prepared as follows. Transfer 0.1*J* mL of *Potassium stock solution* and 0.1*J′* mL of *Sodium stock solution* to a 100-mL volumetric flask, where *J* and *J′* are the labeled amounts, in mEq/L, of potassium and sodium, respectively, in the Injection. Dilute with water to volume.
Standard solution: Dilute 5.0 mL of *Standard stock solution* with *Internal standard solution* to 500.0 mL.
Sample solution: Dilute 5.0 mL of Injection with *Internal standard solution* to 500.0 mL.
Instrumental conditions
Mode: Flame photometer
Analytical wavelengths
Potassium: Maximum at 766 nm
Sodium: Maximum at 589 nm
Lithium: Maximum at 671 nm
Blank: *Internal standard solution*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and *Sample solution*.
Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of potassium to lithium from the *Sample solution*
R_S = emission reading ratio of potassium to lithium from the *Standard solution*
C_S = concentration of potassium (K) in the *Standard solution* (mg/mL)
C_U = nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[NOTE—Each mg of potassium (K) is equivalent to 0.02558 mEq of potassium (K).]
Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of sodium to lithium from the *Sample solution*
R_S = emission reading ratio of sodium to lithium from the *Standard solution*
C_S = concentration of sodium (Na) in the *Standard solution* (mg/mL)
C_U = nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[NOTE—Each mg of sodium (Na) is equivalent to 0.04350 mEq of sodium (Na).]
Acceptance criteria
Potassium: 90.0%–110.0%
Sodium: 90.0%–110.0%

- **SULFATE** (if present)

Sample solution: Equivalent to 120 mg (1.25 mEq) of sulfate from a volume of injection. Dilute, if necessary, to 200 mL, and add 1 mL of hydrochloric acid.
Analysis: Heat the *Sample solution* to boiling, and gradually add in small portions and while constantly stirring, an excess of hot barium chloride TS (about 8 mL). Heat the mixture on a steam bath for 1 h, collect the precipitate of barium sulfate on a tared filtering crucible, wash until free from chloride, dry, ignite, and weigh.
Calculate the percentage of the labeled amount of sulfate (SO_4) in the portion of Injection taken:

$$\text{Result} = \{[W \times (M_{r1}/M_{r2}) \times (1/M_{r1})]/(L \times V)\} \times 100$$

W = weight of barium sulfate after ignition (mg)
M_{r1} = molecular weight of sulfate, 96.06
M_{r2} = molecular weight of barium sulfate, 233.43
L = label claim of sulfate in the Injection (mEq/mL)
V = volume of Injection in the *Sample solution* (mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Units/mL
- **PH** ⟨791⟩: 4.0–6.5
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **LABELING:** The label states the content of each electrolyte in terms of milliequivalents (mEq) in a given volume. The label states the total osmolar concentration in mOsmol/L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Potassium Gluconate RS
USP Sodium Lactate RS

Multiple Electrolytes and Dextrose Injection Type 2

DEFINITION

Multiple Electrolytes and Dextrose Injection Type 2 is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, potassium, magnesium, calcium, and chloride ions. In addition, the salts provide ions of acetate, or acetate and citrate, or acetate and lactate, or gluconate and sulfate. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), acetate ($C_2H_3O_2$), citrate ($C_6H_5O_7$), lactate ($C_3H_5O_3$), gluconate ($C_6H_{11}O_7$), and sulfate (SO_4); NLT 90.0% and NMT 120.0% of the labeled amount of chloride

www.webofpharma.com

(Cl); and NLT 90.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

IDENTIFICATION

• **A.**

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Magnesium* and *Chloride* **:** Meets the requirements

• **C. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.

• **D. POTASSIUM:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

• **E. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Calcium*: Meets the requirements of test *A*

• **F.** Where acetate is purported to be present, the retention time of the acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Acetate*.

• **G.** Where citrate is purported to be present, the retention time of the citrate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Citrate*.

• **H.** Where lactate is purported to be present, the retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Lactate*.

• **I.** Where gluconate is purported to be present, the retention time of the gluconate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Gluconate*.

• **J. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Sulfate*: Where sulfate is purported to be present, it meets the requirements of test *A*.

ASSAY

• **ACETATE** (if present)

Mobile phase: 0.05 N sulfuric acid

Standard solution: 1.2 mg/mL of sodium acetate trihydrate (0.0088 mEq/mL of acetate) in water

Sample solution: Nominally 0.0088 mEq/mL of acetate from a volume of Injection in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Columns

Guard: 4.6-mm × 3-cm; packing L17

Analytical: 7.8-mm × 30-cm; packing L17

Column temperature: 60°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetate ($C_2H_3O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetate from the *Sample solution*

r_S = peak response of acetate from the *Standard solution*

C_S = concentration of acetate in the *Standard solution* (mEq/mL)

C_U = nominal concentration of acetate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **CALCIUM**

[NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Solution B: Mix 678 mL of hydrochloric acid with water to make 3000 mL.

Standard stock solution: 1000 µg/mL of calcium prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of *Solution B*, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 10.0, 15.0, and 20.0 µg/mL of calcium prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 1.0, 1.5, and 2.0 mL, respectively, of *Standard stock solution*. Dilute the contents of each flask with *Solution B* to volume.

Sample solution: Transfer a volume of Injection, equivalent to 20 mg of calcium, to a 1000-mL volumetric flask containing 50.0 mL of *Solution A*. Dilute with *Solution B* to volume.

Blank: 5.0 mL of *Solution A* diluted with *Solution B* to 100.0 mL

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of calcium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (C), in µg/mL, of calcium in the *Sample solution*.

Calculate the percentage of the labeled amount of calcium (Ca) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of calcium in the *Sample solution* (µg/mL), interpolated from the graph obtained

C_U = nominal concentration of calcium in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

USP Monographs

www.webofpharma.com

• **CHLORIDE**

Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride (1.55 mEq), to a suitable conical flask, and add water, if necessary, to bring the volume to 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = Titrant volume consumed by the *Sample solution* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 35.45 mg/mEq
W = nominal amount of chloride in the *Sample solution* (mg)

Acceptance criteria: 90.0%–120.0%

• **DEXTROSE**

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis

Sample: *Sample solution*

Determine the angular rotation in a polarimeter tube (see *Optical Rotation* ⟨781⟩.)

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* (°)
l = length of the polarimeter tube (dm)
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–105.0%

• **CITRATE** (if present)

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay for Citric Acid/Citrate and Phosphate* ⟨345⟩.

Standard solution: 0.3 mEq/L of citrate ($C_6H_5O_7$) from USP Citric Acid RS in freshly prepared 1 mM sodium hydroxide

Sample solution: Nominally 0.3 mEq/L of citrate in 1 mM sodium hydroxide from a volume of Injection diluted with freshly prepared sodium hydroxide

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in ⟨345⟩.

Calculate the percentage of the labeled amount of citrate ($C_6H_5O_7$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of citrate from the *Sample solution*
r_S = peak response of citrate from the *Standard solution*
C_S = concentration of citrate in the *Standard solution* (mEq/L)
C_U = nominal concentration of citrate in the *Sample solution* (mEq/L)

Acceptance criteria: 90.0%–110.0%

• **GLUCONATE** (if present)

Mobile phase: Dissolve 7.0 g of calcium acetate in 1900 mL of water, and adjust with glacial acetic acid to a pH of 4.5 ± 0.1. Dilute with water to 2000 mL, mix, pass through a filter of 0.5-µm pore size or finer, and degas. Maintain the *Mobile phase* at 70 ± 2° at all times.

System suitability solution: 5 mg/mL each of USP Sodium Lactate RS and USP Potassium Gluconate RS in water

Standard solution: 4.6 mg/mL of USP Potassium Gluconate RS (0.02 mEq/mL of gluconate) in water

Sample solution: Nominally 0.023 mEq/mL of gluconate from a volume of Injection in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 7.8-mm × 30-cm; packing L19 in the calcium form. Condition the column with *Mobile phase* for 60 min.

Column temperature: 70 ± 2°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between lactate and gluconate, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of gluconate ($C_6H_{11}O_7$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of gluconate from the *Sample solution*
r_S = peak response of gluconate from the *Standard solution*
C_S = concentration of gluconate in the *Standard solution* (mEq/mL)
C_U = nominal concentration of gluconate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **LACTATE** (if present)

Mobile phase: Formic acid, dicyclohexylamine, and water (1:1:998)

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water

Standard solution: 2 mg/mL of USP Sodium Lactate RS (0.018 mEq/mL of lactate) in water

Sample solution

For Injections containing >20 mEq/L of lactate: Nominally 0.02 mEq/mL of lactate from Injection in water

For Injections containing ≤20 mEq/L of lactate: Use the undiluted Injection.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

www.webofpharma.com

Column: 4.6-mm × 10-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2 between acetate and lactate, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lactate ($C_3H_5O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lactate from the *Sample solution*
r_S = peak response of lactate from the *Standard solution*
C_S = concentration of lactate in the *Standard solution* (mEq/mL)
C_U = nominal concentration of lactate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **MAGNESIUM**

[NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Solution A: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.
Solution B: Mix 678 mL of hydrochloric acid with water to make 3000 mL.
Standard stock solution A: 1.00 mg/mL of magnesium (Mg) prepared as follows. Transfer 1.00 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water. Slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with *Solution B* to volume.
Standard stock solution B: 100 µg/mL of magnesium (Mg) prepared as follows. Transfer 10.0 mL of *Standard stock solution A* to a 100-mL volumetric flask, and dilute with *Solution B* to volume.
Standard solutions: 10.0, 15.0, and 20.0 µg/mL of magnesium (Mg) prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Solution A*, add 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution B*. Dilute the contents of each flask with *Solution B* to volume.
Sample solution: Transfer a volume of Injection, equivalent to 20 mg of magnesium (Mg) to a 1000-mL volumetric flask containing 50.0 mL of *Solution A*. Dilute with *Solution B* to volume.
Blank: 5.0 mL of *Solution A* diluted with *Solution B* to 100.0 mL

Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Magnesium emission line at 285.2 nm
Lamp: Magnesium hollow-cathode
Flame: Air–acetylene

Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of magnesium (Mg), and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (C) in µg/mL, of magnesium (Mg) in the *Sample solution*.
Calculate the percentage of the labeled amount of magnesium (Mg) in each portion of Injection taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of magnesium (Mg) in the *Sample solution* (µg/mL), interpolated from the graph obtained
C_U = nominal concentration of magnesium (Mg) in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

• **POTASSIUM AND SODIUM**

Internal standard solution: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Add a suitable nonionic surfactant, and then dilute with water to volume.
Potassium stock solution: 74.56 mg/mL of potassium chloride (1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.
Sodium stock solution: 58.44 mg/mL of sodium chloride (1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.
Standard stock solution: 0.0391J mg/mL of potassium (K) from *Potassium stock solution* and 0.02299J' mg/mL of sodium (Na) from *Sodium stock solution* prepared as follows. Transfer 0.1J mL of *Potassium stock solution* and 0.1J' mL of *Sodium stock solution* to a 100-mL volumetric flask, where J and J' are the labeled amounts, in mEq/L, of potassium and sodium, respectively, in the Injection. Dilute with water to volume.
Standard solution: Dilute 5.0 mL of *Standard stock solution* with *Internal standard solution* to 500.0 mL.
Sample solution: Dilute 5.0 mL of Injection with *Internal standard solution* to 500.0 mL.

Instrumental conditions
Mode: Flame photometer
Analytical wavelengths
Potassium: Maximum at 766 nm
Sodium: Maximum at 589 nm
Lithium: Maximum at 671 nm
Blank: *Internal standard solution*

Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and *Sample solution*.
Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of potassium to lithium from the *Sample solution*
R_S = emission reading ratio of potassium to lithium from the *Standard solution*
C_S = concentration of potassium (K) in the *Standard solution* (mg/mL)
C_U = nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

www.webofpharma.com

[NOTE—Each mg of potassium (K) is equivalent to 0.02558 mEq of potassium (K).]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of sodium to lithium from the *Sample solution*
R_S = emission reading ratio of sodium to lithium from the *Standard solution*
C_S = concentration of sodium (Na) in the *Standard solution* (mg/mL)
C_U = nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[NOTE—Each mg of sodium (Na) is equivalent to 0.04350 mEq of sodium (Na).]

Acceptance criteria
Potassium: 90.0%–110.0%
Sodium: 90.0%–110.0%

- **SULFATE** (if present)

Sample solution: Equivalent to 120 mg (1.25 mEq) of sulfate, from a volume of Injection. Dilute, if necessary, to 200 mL, and add 1 mL of hydrochloric acid.

Analysis
Sample: *Sample solution*

Heat the *Sample solution* to boiling, and gradually add, in small portions and while constantly stirring, an excess of hot barium chloride TS (about 8 mL). Heat the mixture on a steam bath for 1 h, collect the precipitate of barium sulfate on a tared filtering crucible, wash until free from chloride, dry, ignite, and weigh.

Calculate the percentage of the labeled amount of sulfate (SO_4) in the portion of Injection taken:

$$\text{Result} = \{[W \times (M_{r1}/M_{r2}) \times (1/M_{r1})]/(L \times V)\} \times 100$$

W = weight of barium sulfate after ignition (mg)
M_{r1} = molecular weight of sulfate, 96.06
M_{r2} = molecular weight of barium sulfate, 233.43

L = label claim of sulfate in the Injection (mEq/mL)
V = volume of Injection in the *Sample solution* (mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 0.5 USP Endotoxin Units/mL.
- **PH** ⟨791⟩: 4.0–6.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **LABELING:** The label states the content of each electrolyte in terms of milliequivalents (mEq) in a given volume. The label states the total osmolar concentration in mOsmol/L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Citric Acid RS
USP Potassium Gluconate RS
USP Sodium Lactate RS

Multiple Electrolytes and Dextrose Injection Type 3

DEFINITION

Multiple Electrolytes and Dextrose Injection Type 3 is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, potassium, and chloride ions. In addition, the salts provide ions of ammonium, or acetate and phosphate, or phosphate and lactate. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of sodium (Na), potassium (K), ammonium (NH_4), acetate ($C_2H_3O_2$), phosphate (PO_4), and lactate ($C_3H_5O_3$); NLT 90.0% and NMT 120.0% of the labeled amount of chloride (Cl); and NLT 90.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It may contain Hydrochloric Acid or Sodium Hydroxide used to adjust the pH. It contains no antimicrobial agents.

IDENTIFICATION

- **A.**

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride* and *Ammonium*: Meets the requirements
- **C. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.
- **D. POTASSIUM:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]
- **E.** Where acetate is purported to be present, the retention time of the acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Acetate*.
- **F.** Where phosphate is purported to be present proceed as follows.

Sample solution: Add 5 mL of Injection and 1 mL of ammonium molybdate TS to a test tube and mix.

Acceptance criteria: A yellow precipitate, which is soluble in 6 N ammonium hydroxide, is formed.

- **G.** Where lactate is purported to be present, the retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Lactate*.

ASSAY

- **ACETATE** (if present)

Mobile phase: 0.05 N sulfuric acid
Standard solution: 1.2 mg/mL of sodium acetate trihydrate (0.0088 mEq/mL of acetate) in water
Sample solution: Nominally 0.0088 mEq/mL of acetate from a volume of Injection in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Columns
Guard: 4.6-mm × 3-cm; packing L17
Analytical: 7.8-mm × 30-cm; packing L17
Column temperature: 60°
Flow rate: 0.8 mL/min

www.webofpharma.com

Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetate ($C_2H_3O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetate from the *Sample solution*
r_S = peak response of acetate from the *Standard solution*
C_S = concentration of acetate in the *Standard solution* (mEq/mL)
C_U = nominal concentration of acetate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **AMMONIUM** (if present)
Sample solution: Transfer a volume of Injection, equivalent to 63 mg (3.5 mEq) of ammonium, to a 500-mL Kjeldahl flask. Dilute with water to 200 mL, and add 50 mL of sodium hydroxide solution (2 in 5).
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N sulfuric acid VS
Endpoint detection: Visual
Analysis
Sample: *Sample solution*
Immediately connect the flask containing the *Sample solution* by means of a distillation trap to a well-cooled condenser, the delivery tube of which dips into 40 mL of boric acid solution (1 in 25) contained in a suitable receiver. Heat to boiling, and distill about 200 mL. Cool the liquid in the receiver, if necessary, then add methyl red TS, and titrate with *Titrant*. Perform a blank determination, and make any necessary correction.
Calculate the percentage of the labeled amount of ammonium (NH_4) in the portion of Injection taken:

$$\text{Result} = (V_S - V_B) \times N \times (F/W) \times 100$$

V_S = *Titrant* volume consumed by the *Sample solution* (mL)
V_B = *Titrant* volume consumed by the blank (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 18.04 mg/mEq
W = nominal amount of ammonium in the *Sample solution* (mg)

Acceptance criteria: 90.0%–110.0%

• **CHLORIDE**
Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride (1.55 mEq), to a suitable conical flask and add water, if necessary, to bring the volume to about 10 mL. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS
Endpoint detection: Visual
Analysis
Sample: *Sample solution*
Titrate, with shaking, with *Titrant* to a pink endpoint.
Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample solution* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 35.45 mg/mEq
W = nominal amount of chloride in the *Sample solution* (mg)

Acceptance criteria: 90.0%–120.0%

• **DEXTROSE**
Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.
Analysis
Sample: *Sample solution*
Determine the angular rotation in a polarimeter tube (see *Optical Rotation* ⟨781⟩).
Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* (°)
l = length of the polarimeter tube (dm)
α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)
M_{r1} = molecular weight of dextrose monohydrate, 198.17
M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–105.0%

• **LACTATE** (if present)
Mobile phase: Formic acid, dicyclohexylamine, and water (1:1:998)
System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water
Standard solution: 2 mg/mL of USP Sodium Lactate RS (0.018 mEq/mL of lactate) in water
Sample solution
For Injections containing >20 mEq/L of lactate: Nominally 0.02 mEq/mL of lactate from Injection in water
For Injections containing ≤20 mEq/L of lactate: Use the undiluted Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2 between acetate and lactate, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lactate ($C_3H_5O_3$) in the portion of Injection taken:

USP Monographs

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lactate from the *Sample solution*
r_S = peak response of lactate from the *Standard solution*
C_S = concentration of lactate in the *Standard solution* (mEq/mL)
C_U = nominal concentration of lactate in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **PHOSPHATE** (if present)

Solution A: 50 g/L of ammonium molybdate prepared as follows. Transfer a suitable amount of ammonium molybdate to an appropriate volumetric flask. Add 60% of the final flask volume of water, and swirl to dissolve. Add 15% of the flask volume of sulfuric acid, and swirl. Allow to cool, and dilute with water to volume.

Solution B: Dissolve 0.5 g of hydroquinone in 100 mL of water, and add 1 drop of sulfuric acid. Prepare this solution fresh daily.

Solution C: Dissolve 1 g of sodium sulfite in water to make 5 mL. Prepare this solution fresh daily.

Standard solution: 0.11 mg/mL of monobasic potassium phosphate (0.0008 mEq/mL of phosphate) in water

Sample solution: Nominally 0.0008 mEq/mL of phosphate from a volume of Injection

Blank: Water

Instrumental conditions

Mode: Vis

Analytical wavelength: 640 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use water to zero the instrument. Transfer 2 mL each of the *Standard solution*, the *Sample solution*, and the *Blank* to separate test tubes. To each test tube add 1 mL of *Solution A*, and allow to stand for 3 min. Add 1 mL of *Solution B*. Add 1 mL of *Solution C*, and allow to stand for 30 min.

Calculate the percentage of the labeled amount of phosphate (PO_4) in the portion of the Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*, corrected for any absorbance of the solution from the *Blank*
A_S = absorbance of the *Standard solution*, corrected for any absorbance of the solution from the *Blank*
C_S = concentration of phosphate in the *Standard solution* (mEq/mL)
C_U = nominal concentration of phosphate (PO_4) in the *Sample solution* (mEq/mL)

Acceptance criteria: 90.0%–110.0%

• **POTASSIUM AND SODIUM**

Internal standard solution: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, then dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride (1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Standard stock solution: 0.0391*J* mg/mL of potassium (K) from *Potassium stock solution* and 0.02299*J′* mg/mL of sodium (Na) from *Sodium stock solution* prepared as follows. Transfer 0.1*J* mL of *Potassium stock solution* and 0.1*J′* mL of *Sodium stock solution* to a 100-mL volumetric flask, where *J* and *J′* are the labeled amounts, in mEq/L, of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Dilute 5.0 mL of *Standard stock solution* with *Internal standard solution* to 500.0 mL.

Sample solution: Dilute 5.0 mL of Injection with *Internal standard solution* to 500.0 mL.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: Maximum at 766 nm

Sodium: Maximum at 589 nm

Lithium: Maximum at 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of potassium to lithium from the *Sample solution*
R_S = emission reading ratio of potassium to lithium from the *Standard solution*
C_S = concentration of potassium (K) in the *Standard solution* (mg/mL)
C_U = nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[NOTE—Each mg of potassium (K) is equivalent to 0.02558 mEq of potassium (K).]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of sodium to lithium from the *Sample solution*
R_S = emission reading ratio of sodium to lithium from the *Standard solution*
C_S = concentration of sodium (Na) in the *Standard solution* (mg/mL)
C_U = nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[NOTE—Each mg of sodium (Na) is equivalent to 0.04350 mEq of sodium (Na).]

Acceptance criteria

Potassium: 90.0%–110.0%

Sodium: 90.0%–110.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Units/mL

• **pH** ⟨791⟩: 4.0–6.5

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

• **LABELING:** The label states the content of each electrolyte in terms of milliequivalents (mEq) in a given volume. The label

www.webofpharma.com

states the total osmolar concentration in mOsmol/L. When the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Sodium Lactate RS

Trace Elements Injection

» Trace Elements Injection is a sterile solution in Water for Injection of two or more of the following: Zinc Chloride or Zinc Sulfate, Cupric Chloride or Cupric Sulfate, Chromic Chloride, Manganese Chloride or Manganese Sulfate, Selenious Acid, Sodium Iodide, and Ammonium Molybdate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of zinc (Zn), copper (Cu), chromium (Cr), manganese (Mn), selenium (Se), iodine (I), and molybdenum (Mo).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.
Labeling—Label the Injection to specify that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration. The label shows by an appropriate number juxtaposed to the official name, the number of trace elements contained in the Injection according to the following: zinc and copper (2), and then cumulatively, chromium (3), manganese (4), selenium (5), iodine (6), and molybdenum (7). Other combinations are indicated separately by citing the number of trace elements contained in each followed by an asterisk that is repeated with the list of labeled ingredients. Label the Injection for its contents of zinc chloride ($ZnCl_2$), zinc sulfate ($ZnSO_4 \cdot 7H_2O$), cupric chloride ($CuCl_2$), cupric sulfate ($CuSO_4$), chromic chloride ($CrCl_3$), manganese chloride ($MnCl_2$), manganese sulfate ($MnSO_4$), selenious acid (H_2SeO_3), sodium iodide (NaI), and ammonium molybdate [$(NH_4)Mo_7 \cdot 4H_2O$], and for elemental zinc (Zn), copper (Cu), chromium (Cr), manganese (Mn), selenium (Se), iodine (I), and molybdenum (Mo), as appropriate in relation to the ingredients claimed to be present.

Identification—
A: The *Assay preparation* prepared as directed in the *Assay for zinc*, the *Assay for copper*, the *Assay for chromium*, the *Assay for manganese*, the *Assay for selenium*, and the *Assay for molybdenum*, as appropriate, based on the elements claimed in the labeling to be present, exhibit maximum absorption at the relevant wavelengths specified when tested as directed for the *Procedures* in the respective *Assays*.
B: If iodine is claimed in the labeling to be present, the retention time of the iodine peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay for iodine*.
Pyrogen—When diluted with Sodium Chloride Injection so that each mL contains not more than 20 µg of zinc, 20 µg of copper, 0.1 µg of chromium, 4 µg of manganese, 4 µg of selenium, 5 µg of iodine, or 5 µg of molybdenum, it meets the requirements of the *Pyrogen Test* ⟨151⟩.
pH ⟨791⟩**:** between 1.5 and 3.5.
Particulate Matter in Injections ⟨788⟩**:** meets the requirements.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for zinc—
Standard preparations—Prepare as directed in the *Assay* under *Zinc Chloride Injection*.
Assay preparation—Using the Injection, proceed as directed for *Assay preparation* in the *Assay* under *Zinc Chloride Injection*.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Zinc Chloride Injection*. Calculate the quantity, in µg, of zinc (Zn) in each mL of the Injection taken by the formula:

$$5C/V$$

in which *C* is the concentration, in µg per mL, of zinc in the *Assay preparation*, and *V* is the volume, in mL, of Injection taken.

Assay for copper—
Sodium chloride solution, Copper stock solution, and Standard preparations—Prepare as directed in the *Assay preparation* under *Cupric Chloride Injection*
Assay preparation—Using the Injection, proceed as directed for the *Assay preparation* in the *Assay preparation* under *Cupric Chloride Injection*
Procedure—Proceed as directed for *Procedure* in the *Assay preparation* under *Cupric Chloride Injection* Calculate the quantity, in mg, of copper (Cu) in each mL of the Injection taken by the formula:

$$2C/3V$$

in which *C* is the concentration, in µg per mL, of copper in the *Assay preparation*, and *V* is the volume, in mL, of Injection taken.

Assay for chromium *(if present)*—
INJECTIONS CONTAINING 3 µg *OR MORE OF* Cr *PER* mL—*Sodium chloride solution, Chromium stock solution, Standard preparations, Assay preparation,* and *Procedure*—Proceed as directed in the *Assay* under *Chromic Chloride Injection* Calculate the quantity, in µg, of chromium (Cr) in each mL of the Injection taken by the formula:

$$25C/V$$

in which *C* is the concentration, in µg per mL, of chromium in the *Assay preparation*, and *V* is the volume, in mL, of Injection taken.
INJECTIONS CONTAINING LESS THAN 3 µg *OF* Cr *PER* mL—*Sodium chloride solution* and *Chromium stock solution*—Proceed as directed in the *Assay* under *Chromic Chloride Injection*
Standard preparations—Prepare as directed in the *Assay* under *Chromic Chloride Injection* If the Injection is not labeled as containing sodium chloride, omit the addition of the *Sodium chloride solution*.
Assay preparation—Use the undiluted Injection.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Chromic Chloride Injection*. Calculate the quantity, in µg, of chromium (Cr) in each mL of the Injection taken by the formula:

$$25C/V$$

in which *C* is the concentration, in µg per mL, of chromium in the *Assay preparation*, and *V* is the volume, in mL, of Injection taken.

www.webofpharma.com

Assay for manganese *(if present)*—
Sodium chloride solution, Manganese stock solution, and *Standard preparations*—Prepare as directed in the *Assay* under *Manganese Chloride Injection.*
Assay preparation—Using the Injection, proceed as directed for the *Assay preparation* in the *Assay* under *Manganese Chloride Injection.*
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Manganese Chloride Injection.* Calculate the quantity, in µg, of manganese (Mn) in each mL of the Injection taken by the formula:

$$0.5C/V$$

in which C is the concentration, in µg per mL, of manganese in the *Assay preparation,* and V is the volume, in mL, of Injection taken.

Assay for selenium *(if present)*—
Selenium stock solution and *Standard preparations*—Prepare as directed in the *Assay* under *Selenious Acid Injection.*
Assay preparation—Using the Injection, proceed as directed for *Assay preparation* in the *Assay* under *Selenious Acid Injection.*
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Selenious Acid Injection.* Calculate the quantity, in mg, of selenium (Se) in each mL of the Injection taken by the formula:

$$LC/D$$

in which L is the labeled quantity, in mg per mL, of selenium in the Injection taken, C is the concentration, in µg per mL, in the *Assay preparation,* and D is the concentration, in µg of selenium per mL, of the *Assay preparation* on the basis of the labeled quantity in the Injection and the extent of dilution.

Assay for iodine *(if present)*—
Mobile phase—Prepare a suitable mixture of 0.05 M dibasic sodium phosphate, 0.0025 M cetyltrimethylammonium chloride (prepared by diluting 3.20 g of a 25% solution of cetyltrimethylammonium chloride with water to 1000 mL), and acetonitrile (45:25:25). Filter this mixture through a suitable filter of 0.5 µm or finer porosity, adjust with phosphoric acid to a pH of 6.8 ± 0.1, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparations—Transfer about 52 mg of potassium iodide, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with water to volume, and mix. This solution contains about 5.2 µg of potassium iodide per mL (equivalent to about 4 µg of iodine per mL).
Assay preparation—Dilute an accurately measured volume of Injection quantitatively with water to obtain a solution containing about 4 µg of iodine per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 226-nm detector, a precolumn containing packing L3, and a 4.6 × 25-cm column that contains packing L1. The flow rate is about 2.5 mL per minute. [NOTE—Equilibrate the column with *Mobile phase* recirculating at a rate of about 1 mL per minute for at least 12 hours before use.] Chromatograph the *Standard preparation,* and record the peak responses as directed under *Procedure:* the tailing factor for the iodine peak is not more than 2, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the iodine peaks. Calculate the quantity, in µg, of iodine in each mL of the Injection taken by the formula:

$$(126.9/166.0)(CL/D)$$

in which 126.9 is the atomic weight of iodine, 166.0 is the molecular weight of potassium iodide, C is the concentration, in µg per mL, of potassium iodide, calculated on the dried basis, in the *Standard preparation,* L is the labeled concentration, in µg per mL, of iodine in the Injection, and D is the concentration, in µg per mL, of iodine in the *Assay preparation* based on the labeled concentration and the extent of dilution.

Assay for molybdenum *(if present)*—
Ammonium hydroxide diluent, Sodium sulfate solution, Molybdenum stock solution, and *Standard preparations*—Prepare as directed in the *Assay* under *Ammonium Molybdate Injection*
Assay preparation—Using the Injection, proceed as directed for the *Assay preparation* in the *Assay* under *Ammonium Molybdate Injection*
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Ammonium Molybdate Injection* Calculate the quantity, in µg, of molybdenum (Mo) in each mL of the Injection taken by the formula:

$$25C/V$$

in which C is the concentration, in µg per mL, of molybdenum in the *Assay preparation,* and V is the volume, in mL, of Injection taken.

Elm

DEFINITION
Elm is the dried inner bark of *Ulmus rubra* Muhl. (*Ulmus fulva* Michx.) (Fam. Ulmaceae).

IDENTIFICATION
• **A. MUCILAGINOUS SUBSTANCE**
Sample: 1 g of finely powdered Elm
Analysis: Macerate the *Sample* with 40 mL of cold water for 1 h.
Acceptance criteria: The resulting mixture is of a thick mucilaginous consistency and yellowish brown in color.
• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**
Standard solution: 0.025% rutin in methanol
Sample solution: Extract 1 g of powdered Elm with 10 mL of 60% methanol on a water bath for 15 min. Cool, filter, and concentrate the filtrate to 2.5 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, typically 20 cm long (TLC plates)
Application volume: 20 µL
Developing solvent system: Ethyl acetate, anhydrous formic acid, glacial acetic acid, and water (100:11:11:27)
Spray reagent: 1% solution of 2-aminoethyl diphenylborinate ester in methanol, followed by a 5% solution of polyethylene glycol 4000 in alcohol
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatograms in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, and allow to air-dry. Spray the

www.webofpharma.com

plate with *Spray reagent*, and examine the plate under UV light at 366 nm.

Acceptance criteria: The R_F values of the principal spots relative to rutin are 1.05 (blue) and 0.8 (orange).

SPECIFIC TESTS

- **BOTANIC CHARACTERISTICS**

Macroscopic

Unground Elm: Unground Elm occurs as broad, flat, oblong pieces 1–4 mm in thickness. The outer surface is yellow-orange with some brown outer bark or cork layers attached; the inner surface, which is pale yellow, is marked faintly with striated phloem lines. The fracture is fibrous with projections of five bast bundles.

Powdered Elm:

Weak yellowish orange with a distinctive fenugreek-like odor

Microscopic

Powdered Elm: Bast fibers are numerous, very long, usually broken, up to 25 µm in diameter, thick-walled, unlignified, or with only a thin outer sheath of the wall lignified; have calcium oxalate prisms 10–35 µm in length; have starch grains that are spheroidal, or polygonal, usually 3–15 µm in diameter, occasionally up to 25 µm in length; and have numerous mucilage fragments, frequently lamellated. Cork cells are few or absent.

- **OUTER BARK:** Contains NMT 2% of adhering outer bark
- **ARTICLES OF BOTANICAL ORIGIN,** *Foreign Organic Matter* ⟨561⟩**:** NMT 2%
- **LOSS ON DRYING** ⟨731⟩**:** Dry 2 g at 105° to constant weight: it loses NMT 12% of its weight.
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨561⟩**:** NMT 10% on the dried basis
- **ARTICLES OF BOTANICAL ORIGIN,** *Acid-Insoluble Ash* ⟨561⟩**:** NMT 0.65% on the dried basis

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store in a cool, dry place.

Emedastine Ophthalmic Solution

» Emedastine Ophthalmic Solution is a sterile, aqueous solution containing an amount of Emedastine Difumarate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of emedastine ($C_{17}H_{26}N_4O$).

Packaging and storage—Preserve in tight, light-resistant containers, in a refrigerator or at controlled room temperature.

USP Reference standards ⟨11⟩—

USP Emedastine Difumarate RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩**:** between 5.0 and 8.0.

Assay—

Buffer solution—Dissolve 13.8 g of monobasic sodium phosphate and 10 mL of triethylamine in 800 mL of water. Adjust with phosphoric acid to a pH of 5.7, dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (83:17). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Emedastine Difumarate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.057 mg of emedastine per mL.

System suitability solution—Add 50 µL of 30 percent hydrogen peroxide to 2 mL of *Standard preparation,* and heat at 100° for 30 minutes. Add another 2 mL of *Standard preparation,* mix, and use immediately.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution into a suitable volumetric flask to obtain a solution having a known concentration of about 0.057 mg of emedastine per mL.

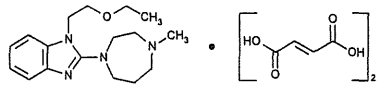
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 15-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for emedastine and 1.2 for emedastine *N*-oxide; the resolution, *R,* between emedastine and emedastine *N*-oxide is not less than 1.5; the column efficiency determined from the emedastine peak is not less than 1000 theoretical plates; and the tailing factor is not more than 2.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for emedastine. Calculate the quantity, in mg, of emedastine ($C_{17}H_{26}N_4O$) in each mL of the Ophthalmic Solution taken by the formula:

$$(302.42/534.57)C(V_1/V_2)(r_U/r_S)$$

in which 302.42 and 534.57 are the molecular weights of emedastine and emedastine difumarate, respectively; C is the concentration, in mg per mL, of USP Emedastine Difumarate RS in the *Standard preparation;* V_1 is the volume, in mL, of the volumetric flask used to prepare the *Assay preparation;* V_2 is the volume, in mL, of Ophthalmic Solution taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Emedastine Difumarate



$C_{17}H_{26}N_4O \cdot 2C_4H_4O_4$ 534.56

1*H*-Benzimidazole, 1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1*H*-1,4-diazepin-1-yl)-, (*E*)-2-butenedioate (1:2).

1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1*H*-1,4-diazepin-1-yl)benzimidazole fumarate (1:2) [87233-62-3].

» Emedastine Difumarate contains not less than 98.5 percent and not more than 101.0 percent of $C_{17}H_{26}N_4O \cdot 2C_4H_4O_4$, calculated on the dried basis.

USP Monographs

www.webofpharma.com

Packaging and storage—Preserve in tight, light-resistant containers, at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Emedastine Difumarate RS

Identification—

Change to read:

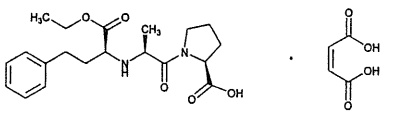
A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—
Test specimen—Dry for 3 hours at 105°.
B: The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that of emedastine in the chromatogram of the *System suitability solution*, as obtained from the *Chromatographic purity* test.
C: Dissolve about 23 mg of Emedastine Difumarate in 25 mL of water. Add 1 mL of a solution prepared by mixing 20 mL of cupric sulfate solution (1 in 5) and 8 mL of pyridine: a precipitate is formed in the blue solution within one minute.
pH ⟨791⟩: between 3.0 and 4.5, in a solution (2 in 1000).
Loss on drying ⟨731⟩—Dry at 105° for 3 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Buffer solution—Dissolve 3.9 g of monobasic sodium phosphate and 2.5 g of sodium dodecyl sulfate in 1.0 L of water. Adjust with phosphoric acid to a pH of 2.4.
Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Prepare a solution in *Mobile phase* containing 0.10 mg of USP Emedastine Difumarate RS and 0.04 mg of 4-methylbenzophenone per mL.
Standard solution—Dissolve an accurately weighed quantity of USP Emedastine Difumarate RS in *Mobile phase*, and dilute, stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.003 mg per mL.
Test solution—Prepare a solution of Emedastine Difumarate in *Mobile phase* containing about 1.0 mg per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute.
Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.2 for fumaric acid, 1.0 for emedastine, and 2.0 for 4-methylbenzophenone; the resolution, *R*, is not less than 2.0; the column efficiency determined from the emedastine peak is not less than 1500 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Mobile phase*, *Standard solution*, and *Test solution* into the chromatograph, and record the chromatograms, allowing the elution to continue for a period of not less than twice the retention time of emedastine. Measure the areas for all of the peaks, disregarding the fumaric acid peak and peaks corresponding to those obtained from the *Mobile phase*. Calculate the percentage of each impurity in the portion of Emedastine Difumarate taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity obtained from the *Test solution;* and r_s is the peak response for emedastine in the *Standard solution:* not more than 0.3% of any individual impurity is found; and not more than 1.0% of total impurities is found.
Assay—Dissolve about 200 mg of Emedastine Difumarate, accurately weighed, in 50.0 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using suitable electrodes (see *Titrimetry* ⟨541⟩). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 26.73 mg of $C_{17}H_{26}N_4O \cdot 2C_4H_4O_4$.

Enalapril Maleate



$C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ 492.52
L-Proline, 1-[*N*-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-, (*S*)-, (*Z*)-2-butenedioate (1:1);
1-[*N*-[(*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]-L-proline, 1′-ethyl ester, maleate (1:1) [76095-16-4].

DEFINITION
Enalapril Maleate contains NLT 98.0% and NMT 102.0% of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: ⟨197M⟩ or ⟨197A⟩▲ (USP 1-Dec-2019)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Buffer 1: 2.8 g of sodium phosphate, monobasic in 900 mL of water in a 1000-mL volumetric flask. Adjust with a 9 M sodium hydroxide solution to a pH of ▲▲ (USP 1-Dec-2019) 6.8, and dilute with water to volume.
Buffer 2: 2.8 g of sodium phosphate, monobasic in 900 mL of water in a 1000-mL volumetric flask. Adjust with phosphoric acid to a pH of ▲▲ (USP 1-Dec-2019) 2.5, and dilute with water to volume.
Solution A: Acetonitrile and *Buffer 1* (5:95)
Solution B: Acetonitrile and *Buffer 1* (66:34)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	40	60
25	40	60
26	95	5
30	95	5

USP Monographs

www.webofpharma.com

Diluent: Acetonitrile and *Buffer 2* (5:95)
Standard solution: 0.3 mg/mL of USP Enalapril Maleate RS in *Diluent*
▲ (USP 1-Dec-2019)
Sample solution: 0.3 mg/mL of Enalapril Maleate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.1-mm × 15-cm; ▲5-µm▲ (USP 1-Dec-2019) packing L21
Column temperature: 70°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*▲ (USP 1-Dec-2019)
Suitability requirements
▲Tailing factor: NMT 2.0▲ (USP 1-Dec-2019)
Relative standard deviation: NMT ▲0.73%▲ (USP 1-Dec-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) in the portion of Enalapril Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of enalapril from the *Sample solution*
r_S = peak response of enalapril from the *Standard solution*
C_S = concentration of USP Enalapril Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of Enalapril Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

Change to read:

• **ORGANIC IMPURITIES**
▲Buffer: 0.14 g/L of potassium phosphate, monobasic in water. Adjust with phosphoric acid to a pH of 2.0.
Solution A: Acetonitrile and *Buffer* (10:90)
Solution B: Acetonitrile and *Buffer* (80:20)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
21	80	20
30	15	85
40	100	0
50	100	0

Diluent: Acetonitrile and *Buffer* (20:80)
System suitability solution: 1 mg/mL of USP Enalapril Maleate RS and 3 µg/mL of USP Moexipril Related Compound F RS in *Diluent*. Sonicate to dissolve as needed.
Standard solution: 0.01 mg/mL of USP Enalapril Maleate RS in *Diluent*
Sensitivity solution: 0.5 µg/mL of USP Enalapril Maleate RS in *Diluent* from the *Standard solution*
Sample solution: 1 mg/mL of Enalapril Maleate in *Diluent*. Sonicate to dissolve as needed.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Temperatures
Autosampler: 20°
Column: 70°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[NOTE—See *Table 3* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.0 between moexipril related compound F and enalapril, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Enalapril Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of enalapril from the *Standard solution*
C_S = concentration of USP Enalapril Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of Enalapril Maleate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. The reporting threshold is 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Maleic acid[a]	0.13	—	—
Enalaprilat[b]	0.55	1.41	0.3
Moexipril related compound F	0.93	0.85	0.3
Enalapril	1.00	—	—
Enalapril cyclohexyl analog[c]	1.33	0.45	0.3
Enalapril related compound D[d]	1.48	1.27	0.3
Any unspecified impurity	—	1.00	0.10

www.webofpharma.com

Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	2.0

[a] This peak is due to the counterion and is not to be reported or included in the total impurities.
[b] 1-[*N*-[(*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]-L-proline.
[c] 1-(*N*-[(*S*)-1-Ethoxycarbonyl-3-cyclohexylpropyl]-L-alanyl}-L-proline.
[d] (*S*)-Ethyl 2-[(3*S*,8a*S*)-3-methyl-1,4-dioxohexahydropyrrolo[1,2-α]pyrazin-2(1*H*)-yl]-4-phenylbutanoate. ▲ (USP 1-Dec-2019)

SPECIFIC TESTS
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures*, *Specific Rotation*
  Sample solution: 10 mg/mL in methanol
  Acceptance criteria: −41.0° to −43.5°
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 2 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Enalapril Maleate RS
  ▲USP Moexipril Related Compound F RS
  (*S*)-2-[(*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-ylamino] propanoic acid;
  Also known as *N*-[(*S*)-1-Ethoxycarbonyl-3-phenylpropyl]-L-alanine.
  $C_{15}H_{21}NO_4$ 279.33 ▲ (USP 1-Dec-2019)

Enalapril Maleate Compounded Oral Suspension

DEFINITION
Enalapril Maleate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled content of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$).
Prepare Enalapril Maleate Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Enalapril Maleate tablets[a] equivalent to	100 mg of enalapril maleate
Vehicle: a 1:1 mixture of Ora-Sweet[b] (regular or sugar-free) and Ora-Plus,[b] a sufficient quantity to make	100 mL

[a] Vasotec 20-mg tablets, Merck Sharp & Dohme, West Point, PA.
[b] Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Enalapril Maleate tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make an enalapril maleate liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY
- **PROCEDURE**
  Buffer solution: 1 M monobasic potassium phosphate, adjusted with phosphoric acid to a pH of 4.0
  Mobile phase: Acetonitrile, *Buffer solution*, and water (30:2:68). Filter and degas.
  Standard solution: 0.1 mg/mL of USP Enalapril Maleate RS in *Mobile phase*
  Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of the Oral Suspension into a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 0.1 mg/mL of enalapril maleate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 215 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L7
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The retention time for enalapril maleate is about 8.8 min.]
  Suitability requirements
  Relative standard deviation: NMT 2.1% for the replicate injections
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Enalapril Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.2–5.3

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded, when stored in a refrigerator or at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Enalapril Maleate RS

Enalapril Maleate Compounded Oral Suspension, Veterinary

DEFINITION
Enalapril Maleate Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled content of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$).

www.webofpharma.com

Prepare Enalapril Maleate Compounded Oral Suspension, Veterinary 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Enalapril maleate	1 g
Ora-Blend[a], a sufficient quantity to make	100 mL

[a] Perrigo, Minneapolis, MN.

Pour the *Enalapril maleate* powder into a suitable container. Wet the powder with a small amount of *Ora-Blend*, and triturate to make a smooth paste. Add the *Ora-Blend* to make the mortar contents pourable. Transfer the contents of the mortar stepwise and quantitatively to a calibrated container using the *Ora-Blend*. Add sufficient *Ora-Blend* to bring the preparation to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: Dissolve 2.8 g of monobasic sodium phosphate in 1000 mL of water. Adjust with 1 N sodium hydroxide to a pH of 6.8.

Solution B: Acetonitrile and *Solution A* (5:95)

Solution C: Acetonitrile and *Solution A* (66:34)

Solution D: Dissolve 2.8 g of monobasic sodium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.5.

Diluent: Acetonitrile and *Solution D* (5:95)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	95	5
20	40	60
25	40	60
26	95	5
30	95	5

Standard solution: 1 mg/mL of USP Enalapril Maleate RS in *Diluent*

Sample solution: Shake thoroughly each bottle of Oral Suspension. Transfer 1.0 mL of the Oral Suspension into a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix well to dissolve. Pass through a PVDF filter of 0.45-µm pore size, discarding the first 3 mL to waste.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-µm packing L21

Column temperature: 60°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for enalapril maleate is about 14.2 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of enalapril maleate from the *Sample solution*

r_S = peak response of enalapril maleate from the *Standard solution*

C_S = concentration of enalapril maleate in the *Standard solution* (mg/mL)

C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 2.6–3.6

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.

• **LABELING:** Label it to indicate that it is to be shaken vigorously immediately before use, and to state the *Beyond-Use Date*. Label it to state that it is for veterinary use only.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8°; NMT 60 days when stored at controlled room temperature

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Enalapril Maleate RS

Enalapril Maleate Tablets

DEFINITION

Enalapril Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 1.38 g of monobasic sodium phosphate in 800 mL of water, adjust with phosphoric acid to a pH of 2.2, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (250:750)

Enalapril diketopiperazine solution: Place 20 mg of USP Enalapril Maleate RS in a 100-mL beaker to form a mound on the bottom of the beaker. Place the beaker on a hot plate at one-half the maximum hot plate temperature setting to melt the solid. When melting is observed (after 5–10 min of heating), immediately remove the beaker from the hot plate, and allow it to cool. Avoid overheating beyond the melting initially observed to prevent heat-induced degradation, which would give rise to a brown color. To the cooled residue in the beaker add 50 mL of acetonitrile, and sonicate for a few min to dissolve the residue. The solution typically contains between 0.2 and 0.4 mg/mL of enalapril diketopiperazine.

Enalaprilat stock solution: 0.4 mg/mL of USP Enalaprilat RS in water

USP Monographs

www.webofpharma.com

Standard solution: 0.2 mg/mL of USP Enalapril Maleate RS and 0.002 mg/mL of USP Enalaprilat RS in *Buffer* prepared as follows. To a suitable amount of USP Enalapril Maleate RS in a suitable volumetric flask add an appropriate amount of *Enalaprilat stock solution* to the flask, and add 50% of the total volume of *Buffer* to dissolve. Sonicate if necessary, then dilute with *Buffer* to volume.

System suitability solution: Dilute 0.5 mL of *Enalapril diketopiperazine solution* with *Standard solution* to a final volume of 25 mL.

Sample solution: Nominally 0.2 mg/mL of enalapril maleate in *Buffer* prepared as follows. Transfer NLT 10 Tablets to a volumetric flask of capacity such that when filled to volume it will produce a 0.2-mg/mL solution. Add a volume of *Buffer* that is about one-half the nominal volume of the flask, sonicate for 15 min, and shake by mechanical means for 30 min. Dilute with *Buffer* to volume, shake well, and sonicate for another 15 min. Pass the solution through a suitable filter of 0.45-µm pore size, and discard the first portion of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 50°
Flow rate: 2 mL/min
Injection volume: 50 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for maleic acid, enalaprilat, enalapril, and enalapril diketopiperazine are about 0.3, 0.5, 1.0, and 1.5, respectively, *System suitability solution*. A peak response for a heat-induced degradation product of enalapril diketopiperazine (if present with a relative retention time of about 1.2) is NMT 15% of the response for enalapril diketopiperazine.]
Suitability requirements
Resolution: NLT 2.0 between maleic acid and enalaprilat; NLT 2.0 between enalaprilat and enalapril; NLT 2.0 between enalapril and enalapril diketopiperazine, *System suitability solution*
Column efficiency: NLT 1000 theoretical plates for enalaprilat; NLT 300 theoretical plates for enalapril; NLT 2500 theoretical plates for enalapril diketopiperazine, *System suitability solution*
Tailing factor: NMT 2.0 for enalapril, *System suitability solution*
Relative standard deviation
Enalapril peak: NMT 2.0%, *Standard solution*
Enalaprilat peak: Responses agree within 5%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Enalapril Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: 0.1 mg/mL of USP Enalapril Maleate RS in *Medium*. Sonicate if necessary. Dilute in *Medium* per *Table 1*.

Table 1

Tablet Strength (mg)	Volume of Standard solution (mL)	Volumetric Flask Size (mL)
2.5	5	200
5	10	200
10	10	100
20	10	50
40	10	25

Sample solution: Pass a portion of solution under test through a suitable filter. Dilute as needed with *Medium* to a concentration that is similar to that of the *Standard solution*.
Analysis: Determine the amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) dissolved as directed in *Uniformity of Dosage Units* ⟨905⟩.
Tolerances: NLT 80% (*Q*) of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Buffer and **Mobile phase:** Prepare as directed in the *Assay*.
Standard solution: 0.1 mg/mL of USP Enalapril Maleate RS in *Buffer*
Sample solution: 0.1 mg/mL of enalapril maleate from 1 Tablet in *Buffer*. Add a volume of *Buffer* that is one-half the nominal volume of the flask, sonicate for 15 min, and shake by mechanical means for 30 min. Dilute with *Buffer* to volume, shake well, and sonicate for an additional 15 min. Pass through a suitable filter of 0.45-µm pore size, and discard the first portion of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 50°
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 300 theoretical plates
Tailing factor: NMT 2.0
Capacity factor, *k'*: NLT 1.5
Relative standard deviation: NMT 2.0%
[NOTE—The enalapril peak tailing factor may be minimized by controlling the column temperature between 45° and 50° and by raising the pH of the aqueous component of the *Mobile phase* from 2.2 to 2.6; the capacity factor may be increased by decreasing the amount of acetonitrile in the *Mobile phase*.]
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Enalapril Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, Enalapril diketopiperazine solution, Standard solution, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Diluted standard solution: Dilute 1.0 mL of *Standard solution* with *Buffer* to 100 mL.

Analysis

Samples: *Buffer*, *Standard solution*, *Sample solution*, and *Diluted standard solution*

Measure the responses for all of the peaks in the *Sample solution* greater than 0.1% of the response of the enalapril peak that are not observed in the *Buffer*.

Calculate the percentage of anhydrous enalaprilat (as enalapril maleate) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Enalaprilat RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of enalapril maleate, 492.52
M_{r2} = molecular weight of anhydrous enalaprilat, 348.39

Calculate the percentage of enalapril diketopiperazine (as enalapril maleate) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of enalapril diketopiperazine from the *Sample solution*
r_S = peak response of enalapril from the *Diluted standard solution*
C_S = concentration of USP Enalapril Maleate RS in the *Diluted standard solution* (mg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)
F = relative response factor of enalapril diketopiperazine, 1.25
M_{r1} = molecular weight of enalapril maleate, 492.52
M_{r2} = molecular weight of enalapril diketopiperazine, 358.44

Calculate the percentage of the sum of all other individual impurities in the portion of Tablets taken:

$$\text{Result} = (r_T/r_S) \times (C_S/C_U) \times 100$$

r_T = sum of the responses of all other individual impurities other than maleic acid, enalapril, enalaprilat, and enalapril diketopiperazine from the *Sample solution*
r_S = peak response of enalapril maleate from the *Diluted standard solution*
C_S = concentration of USP Enalapril Maleate RS in the *Diluted standard solution* (mg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 5.0% for the sum of all impurities including those from enalaprilat and enalapril diketopiperazine

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Enalaprilat RS
USP Enalapril Maleate RS

Enalapril Maleate and Hydrochlorothiazide Tablets

DEFINITION

Enalapril Maleate and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Enalapril Maleate*.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Hydrochlorothiazide*.

ASSAY

• **ENALAPRIL MALEATE**

Buffer: 136 mg/L of monobasic potassium phosphate in water. Initially add water to 80% of the total volume, adjust with phosphoric acid to a pH of 2.0, and dilute with water to volume. Adjust the pH before makeup to final volume.

Mobile phase: Acetonitrile and *Buffer* (400:600)

Standard solution: 0.2 mg/mL of USP Enalapril Maleate RS prepared as follows. Dissolve a suitable amount of USP Enalapril Maleate RS in about 25% of the total volume with methanol, and dilute to volume with *Buffer*.

Sample solution: Transfer a portion of the powder from NLT 20 Tablets, equivalent to 40 mg of enalapril maleate, to a 200-mL volumetric flask. Add 50 mL of *Buffer*, and sonicate for 15 min. Add 50 mL of methanol, sonicate for an additional 15 min, dilute with *Buffer* to volume, and filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 65°
Flow rate: 1.5 mL/min
Injection volume: 50 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 700 theoretical plates
Tailing factor: NMT 3.5

www.webofpharma.com

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of enalapril from the *Sample solution*

r_S = peak response of enalapril from the *Standard solution*

C_S = concentration of USP Enalapril Maleate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

• **HYDROCHLOROTHIAZIDE**

Buffer: Prepare as directed in the *Assay* for *Enalapril Maleate*.

Mobile phase: Acetonitrile and *Buffer* (100:900)

Standard solution: 0.1 mg/mL of USP Hydrochlorothiazide RS prepared as follows. Dissolve a suitable quantity of USP Hydrochlorothiazide RS in 25% of the total volume of methanol, and dilute with *Buffer* to volume.

Sample solution: Transfer a portion of the powder from NLT 20 Tablets, equivalent to 20 mg of hydrochlorothiazide, to a 200-mL volumetric flask. Add 50 mL of *Buffer*, and sonicate for 15 min. Add 50 mL of methanol, sonicate for an additional 15 min, dilute with *Buffer* to volume, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 310 nm

Column: 4.6-mm × 20-cm; 10-µm packing L7

Column temperature: 30°

Flow rate: 2.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 2.0

Capacity factor, k′: NLT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydrochlorothiazide from the *Sample solution*

r_S = peak response of hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Enalapril maleate

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Enalapril Maleate RS in *Medium*

Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.

Analysis: Determine the percentage of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) dissolved, using the test for *Uniformity of Dosage Units*, *Procedure for content uniformity of enalapril maleate*.

Hydrochlorothiazide

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Detector: UV 320 and 360 nm

Cell size: 1 cm

Standard solution: USP Hydrochlorothiazide RS dissolved in 20 mL of methanol and diluted with *Medium*

Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U = difference in absorbances ($A_{320} - A_{360}$) at the wavelengths indicated for the *Sample solution*

A_S = difference in absorbances ($A_{320} - A_{360}$) at the wavelengths indicated for the *Standard solution*

C_S = concentration of hydrochlorothiazide in the *Standard solution* (mg/mL)

L = label claim of hydrochlorothiazide (mg/Tablet)

V = volume of *Medium*, 900 mL

D = dilution factor of the *Sample solution*

Tolerances: NLT 80% (Q) of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) and NLT 60% ($Q$) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity of enalapril maleate

Buffer: 136 g/L of monobasic potassium phosphate in water. Initially add water to 80% of the total volume, adjust with phosphoric acid to a pH of 4.0, and then dilute to volume with water.

Solution A: *Buffer* and water (10:490)

Mobile phase: Acetonitrile, water, and *Buffer* (150:340:10)

Standard solution: 100 µg/mL of USP Enalapril Maleate RS in *Solution A*

Sample solution: Transfer 1 finely powdered Tablet to a 50-mL volumetric flask, add 30 mL of *Solution A*, and sonicate for 15 min. Shake by mechanical means for 30 min, dilute with *Solution A* to volume, sonicate for 30 min, mix, and filter, discarding the first portion of the filtrate. Dilute a portion of the filtrate with *Solution A* to obtain a solution containing about 100 µg/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 20-cm; 10-µm packing L7

Column temperature: 80°

Flow rate: 2 mL/min

Injection volume: 50 µL

www.webofpharma.com

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 2.0
Capacity factor, k′: NLT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of enalapril from the *Sample solution*
r_S = peak response of enalapril from the *Standard solution*
C_S = concentration of USP Enalapril Maleate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

Procedure for content uniformity of hydrochlorothiazide
Buffer and **Solution A:** Prepare as directed in *Procedure for content uniformity of enalapril maleate.*
Standard stock solution: 0.25 mg/mL of USP Hydrochlorothiazide RS prepared as follows. Dissolve a suitable amount of USP Hydrochlorothiazide RS in 10% of the total volume of methanol, and dilute with *Solution A* to volume.
Standard solution: 50 µg/mL of USP Hydrochlorothiazide RS in *Solution A* from *Standard stock solution*
Sample stock solution: Transfer 1 Tablet to a volumetric flask of a suitable size such that, when the hydrochlorothiazide is dissolved from the Tablet, a solution having a concentration of about 250 µg/mL is obtained. Add a volume of *Solution A* equal to about half the capacity of the flask, and sonicate with occasional shaking for 15 min. Shake by mechanical means for 30 min, dilute with *Solution A* to volume, and sonicate for 30 more min. Filter, and discard the first portion of the filtrate.
Sample solution: Transfer 5.0 mL of the clear filtrate to a 25-mL volumetric flask, and dilute with *Solution A* to volume.
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: 320 and 360 nm
Cell: 1 cm
Blank: *Solution A*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = difference in absorbances ($A_{320} - A_{360}$) at the wavelengths indicated for the *Sample solution*
A_S = difference in absorbances ($A_{320} - A_{360}$) at the wavelengths indicated for the *Standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer, Mobile phase, and **Sample solution:** Proceed as directed in the *Assay* for *Enalapril Maleate.*
Enalaprilat stock solution: 0.4 mg/mL of USP Enalaprilat RS in water
Enalapril diketopiperazine solution: Carefully place 20 mg of USP Enalapril Maleate RS in a 100-mL beaker to form a mound on the bottom of the beaker. Place the beaker on a hot plate at about one-half the maximum hot plate temperature setting. Heat for about 5–10 min until the solid is melted. Immediately remove the beaker from the hot plate, and allow to cool. To the cooled residue in the beaker add 50 mL of acetonitrile, and sonicate for a few min to dissolve. The solution typically contains between 0.2 and 0.4 mg/mL of enalapril diketopiperazine. [NOTE—Avoid overheating to prevent heat-induced degradation, which gives rise to a brown color.]
Standard solution: Transfer about 40 mg of USP Enalapril Maleate RS, accurately weighed, to a 200-mL volumetric flask, and dissolve with about 50 mL of methanol. Pipet 1 mL each of *Enalaprilat stock solution* and *Enalapril diketopiperazine solution* into the volumetric flask, and dilute with *Buffer* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 65°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for enalaprilat, enalapril diketopiperazine, and enalapril are 0.3, 0.4, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.3 between enalapril and any peak
Column efficiency: NLT 700 theoretical plates for enalapril, NLT 1500 for enalaprilat, and NLT 1500 for enalapril diketopiperazine
Tailing factor: NMT 3.5
Relative standard deviation: NMT 5.0% for enalaprilat and enalapril diketopiperazine, and NMT 2.0% for enalapril
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of enalaprilat in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of enalaprilat from the *Sample solution*
r_S = peak response of enalaprilat from the *Standard solution*
C_S = concentration of enalaprilat in the *Standard solution* (mg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Calculate the percentage of enalapril diketopiperazine in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

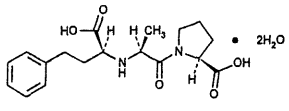
r_U = peak response of enalapril diketopiperazine from the *Sample solution*
r_S = peak response of enalapril diketopiperazine from the *Standard solution*
C_S = concentration of enalapril diketopiperazine in the *Standard solution* (mg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 5.0% of total impurities

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Enalapril Maleate RS
  USP Enalaprilat RS
  L-Proline, 1-[*N*-(1-carboxy-3-phenylpropyl)-L-alanyl]-, dihydrate (*S*)-.
  $C_{18}H_{24}N_2O_5 \cdot 2H_2O$
  USP Hydrochlorothiazide RS

Enalaprilat



$C_{18}H_{24}N_2O_5 \cdot 2H_2O$ 384.42
L-Proline, 1-[*N*-(1-carboxy-3-phenylpropyl)-L-alanyl]-, dihydrate, (*S*)-.
1-[*N*-[(*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]-L-proline dihydrate [84680-54-6].

» Enalaprilat contains not less than 98.0 percent and not more than 101.0 percent of $C_{18}H_{24}N_2O_5$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Enalaprilat RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020)— [NOTE—If the spectrum is not comparable to that of the Reference Standard, expose the specimen and Reference Standard to an environment of 98% relative humidity (use a chamber conditioned with a saturated solution of calcium sulfate) for 1 to 3 days to equilibrate them. Prepare dispersions from the equilibrated specimen and Reference Standard, and record the spectra.]

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

Specific rotation ⟨781S⟩: between −53.0° and −56.0°.
Test solution: 10 mg per mL, in methanol.

Water Determination, *Method I* ⟨921⟩: between 7.0% and 11.0%.

Residue on ignition ⟨281⟩: not more than 0.2%.

Assay—

pH 3 buffer—Dissolve 1.36 g of monobasic potassium phosphate in 950 mL of water, adjust with phosphoric acid to a pH of 3.0 ± 0.1, dilute with water to 1000 mL, and mix.

Solvent mixture—Prepare a mixture of acetonitrile, methanol, and *pH 3 buffer* (2:2:1). Adjust with phosphoric acid to a pH of 3.0 ± 0.1, and mix.

Diluent—Prepare a mixture of *pH 3 buffer* and *Solvent mixture* (92:8), and filter.

Mobile phase—Prepare a filtered and degassed mixture of *pH 3 buffer* and *Solvent mixture* (85:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Enalaprilat RS in *Diluent* to obtain a solution having a known concentration of about 0.3 mg per mL. [NOTE—Use this solution within 24 hours.]

Assay preparation—Transfer about 30 mg of Enalaprilat, accurately weighed, to a 100-mL volumetric flask, dissolve in *Diluent*, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 15-cm column that contains 4-µm packing L1 and is maintained at 70°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 500 theoretical plates; the tailing factor for the analyte peak is not more than 1.7; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{24}N_2O_5$ in the portion of Enalaprilat taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Enalaprilat RS in the *Standard preparation;* and r_U and r_S are the enalaprilat peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Enalaprilat Injection

» Enalaprilat Injection is a sterile solution of enalaprilat in a suitable vehicle for injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of enalaprilat ($C_{18}H_{24}N_2O_5$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, and store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Benzyl Alcohol RS
USP Enalaprilat RS
USP Enalapril Maleate RS

Identification—

A: The retention time of the enalaprilat peak in the chromatogram of the *Assay preparation* corresponds to that of the corresponding peak in the chromatogram of the *Standard preparation,* obtained as directed in the *Assay*.

USP Monographs

www.webofpharma.com

Bacterial Endotoxins Test ⟨85⟩—It contains no more than 280 USP Endotoxin Units per mg of enalaprilat.
Sterility Tests ⟨71⟩: meets the requirements.
pH ⟨791⟩: between 6.5 and 7.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Osmolality and Osmolarity ⟨785⟩, *Osmolality*: between 276 to 305 mOsm per kg.

Related compounds—
Diluent—Prepare a mixture of water and acetonitrile (84:16).
Buffer solution—Dilute 3.4 mL of phosphoric acid with water to 1 L. Adjust with potassium hydroxide to a pH of 2.5.
Solution A—Use a mixture of *Buffer solution* and acetonitrile (84:16).
Solution B—Use acetonitrile.
Mobile phase—Use variable mixtures of *Solution A* and *Solution B*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Stock solution A—Dissolve 20 mg of USP Enalaprilat RS in a 100-mL volumetric flask with approximately 80 mL of *Diluent*. Heat at 80° for 24 hours to generate enalaprilat related compound A. Cool the solution to room temperature, and dilute with *Diluent* to volume.
Stock solution B—Dissolve 10 mg of USP Enalapril Maleate RS in a 200-mL volumetric flask, and dilute with *Diluent* to volume.
Stock solution C—Dissolve accurately weighed quantities of benzyl alcohol, benzaldehyde, and benzoic acid in *Diluent*. Dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of 0.1 mg per mL of each of the three substances.
System suitability solution—Pipet 4.0 mL of *Stock solution A*, 5 mL of *Stock solution B*, and 5 mL of *Stock solution C* into a 25-mL volumetric flask, and dilute with *Diluent* to volume to obtain a solution containing about 0.0032 mg per mL of enalaprilat related impurity A, 0.01 mg per mL of enalapril, and 0.02 mg per mL of each of benzyl alcohol, benzaldehyde, and benzoic acid, respectively.
Test solution—Transfer an accurately measured volume of Injection, equivalent to about 12.5 mg of enalaprilat, to a 25-mL volumetric flask. Dilute with *Diluent* to volume, and mix.
Diluted test solution—Dilute the *Test solution* (1 in 100) with *Diluent* to obtain a solution having a known concentration of 0.005 mg per mL (corresponds to 1% of the *Test solution*).
Chromatographic system (see *Chromatography* ⟨621⟩)—The chromatograph is equipped with 215-nm detector and a 4.6-mm × 15-cm, 5-µm column that contains packing L1. The column temperature is maintained at 60°. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows:

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–5	97.0	3.0	isocratic
5–20	97→77.5	3→22.5	linear gradient
20–25	77.5→10	22.5→90	linear gradient
25–25.01	10→97	90→3.0	step gradient
25.01–30	97	3.0	re-equilibrium

Chromatograph the *System suitability solution* and the *Diluted test solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between enalapril maleate and enalaprilat related impurity A is not less than 1.2; the capacity factor for enalaprilat is not less than 1.5; and the relative standard deviation of the enalaprilat peak in replicate injections of the *Diluted test solution* is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Diluted test solution* and the *Test solution* into the chromatograph, record the chromatogram, and measure the peak response for enalaprilat in the *Diluted test solution* and all of the peak responses from the *Test solution* that do not correspond to enalaprilat, benzyl alcohol, benzoic acid, benzaldehyde, and benzyl alcohol related compounds (see *Table 1* for RRT). Calculate the percentage of specified and unspecified impurities using the formula:

$$(r_i/r_S)$$

in which r_i is the peak response for each specified impurity in the *Test solution;* and r_S is the peak response for enalaprilat in the *Diluted test solution:* the impurities meet the specified limits in *Table 1*.
Benzyl alcohol content *(if present)*—
Buffer solution, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay*.
Standard solution—Dissolve an accurately weighed quantity of USP Benzyl Alcohol RS in *Mobile phase* to obtain a solution having a known concentration of 0.72 mg per mL.
Test solution—Use the *Assay preparation,* prepared as directed in the *Assay*.
Procedure—Proceed as directed in the *Assay*. Calculate the percentage of benzyl alcohol, based on the label claim, in the volume of Injection taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Benzyl Alcohol RS in the *Standard solution;* C_U is the concentration, in mg per mL, of benzyl alcohol in the *Test solution;* and r_U and r_S are the benzyl alcohol peak responses obtained from the *Test solution* and the *Standard solution,* respectively: between 75.0% and 120.0% of the labeled amount is found.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Buffer solution—Prepare a solution of 0.05 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (84 : 16). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability preparation—Use the *Standard preparation.*
Standard preparation—Dissolve an accurately weighed quantity of USP Enalaprilat RS and USP Benzyl Alcohol RS in *Buffer solution,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL of enalaprilat and 0.72 mg per mL of benzyl alcohol.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of enalaprilat, to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm and 258-nm detector (use 215 nm as the initial wavelength, and switch to 258 nm after the elution of enalaprilat and before the elution of benzyl alcohol) and a 4.6-mm × 15-cm, 5-µm column that contains packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 60°.
Chromatograph the *System suitability preparation,* and record

USP Monographs

www.webofpharma.com

Table 1 (Enalaprilat Injection)

Component	Relative Retention Time (minutes)	Limit (w/w, %)
Enalaprilat heat degradation product	0.6 vs enalaprilat	0.5
Enalaprilat	1 vs enalaprilat	—
Benzyl alcohol	1 vs benzyl alcohol	—
Benzyl alcohol related unknown impurity 1	1.2 vs benzyl alcohol	—
Benzoic acid	1.4 vs benzyl alcohol	—
Benzyl alcohol related unknown impurity 2	1.7 vs benzyl alcohol	—
Benzaldehyde	2.1 vs benzyl alcohol	—
Enalapril maleate	4.7 vs enalaprilat	0.25
Enalaprilat related impurity A	5.1 vs enalaprilat	1.0
Any other unspecified individual impurity	—	0.10
Total impurities	—	2.0

the peak responses as directed for *Procedure:* the resolution, *R*, between benzyl alcohol and enalaprilat is not less than 3.0; the tailing factor for benzyl alcohol and the enalaprilat peaks is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the quantity, in percentage of the label claim, of enalaprilat ($C_{18}H_{24}N_2O_5$) in the portion of Injection taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Enalaprilat RS in the *Standard preparation;* C_U is the concentration, in mg per mL, of enalaprilat in the *Assay preparation,* based on the label claim; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Endotoxin Indicator for Depyrogenation

DEFINITION

An Endotoxin Indicator is an article (challenge vial of endotoxin or a carrier spiked with endotoxin) designed for use in depyrogenation studies. The endotoxin (a purified lipopolysaccharide) is validated for use in or on an Endotoxin Indicator. The carrier is made from a material appropriate for the intended depyrogenation processes to which it will be subjected. The endotoxin on a carrier is added at a concentration sufficient to allow recovery of a minimum of 1000 USP Endotoxin Units/carrier. The Endotoxin Indicator would allow for accurate indication of at least a 3-log reduction in USP Endotoxin Units during depyrogenation process challenges.

IDENTIFICATION

- **CHARACTERISTICS**

The endotoxin (lipopolysaccharide) has equivalent characteristics to those of the USP Endotoxin RS.

PERFORMANCE TESTS

- **CARRIER:** The carrier should be the same as or chemically similar to the surface or material used for measuring depyrogenation, e.g., glass or stainless steel. If not similar, then the carrier and endotoxin combination must be at least as resistant to the depyrogenation process as the surface or material being measured. The carrier must be depyrogenated, or the inherent endotoxin level of the carrier should be determined before the addition of the endotoxin to the carrier.
- **ENDOXIN RECOVERY TESTS**

Analysis: Proceed as directed for the relevant technique under *Bacterial Endotoxins Test* ⟨85⟩.

Acceptance criteria: The determined endotoxin concentration is within a factor of 2 of the labeled endotoxin concentration.

SPECIFIC TESTS

- **PURITY**

Absence of assay enhancing substances

Analysis: Proceed as directed in the test for interfering factors in *Bacterial Endotoxins Test* ⟨85⟩.

Acceptance criteria: The Endotoxin Indicator should contain no substances (e.g., glucans) that can result in enhancement of endotoxin spike recovery.

Absence of depyrogenation enhancing/inhibiting substances: No substance (e.g., lactose, albumin, polyethylene glycol) should be present as filler for the endotoxin, as this can result in enhanced or inhibited depyrogenation effects.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in the original package under conditions recommended on the label, and protect from light, toxic substances, excessive heat, and moisture. The packaging and container materials do not adversely affect the performance of the article, when used as directed on the labeling.
- **EXPIRATION DATE:** The expiration date is determined on the basis of stability studies from the date of manufacture, which is the date on which the first determination of Endotoxin Units was made.
- **LABELING:** The label states that the article is or may be used as an Endotoxin Indicator. It indicates the concentration in USP Endotoxin Units/indicator and the recommended storage conditions. The labeling states the source of the endotoxin (e.g. species and strain number) and includes instructions for preparation and safe disposal of the indicator. If the carrier is labeled, the label must be either

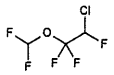
www.webofpharma.com

easily removable or heat resistant, with no substances that could interfere with the assay. For Endotoxin Indicator vials, the label must include instructions for removal of the stopper from vials before use. If the stopper is heat resistant and designed to be left in the vial, the maximum processing temperature is stated.

• **DISPOSAL:** For destruction or discard, follow instructions recommended by the manufacturer, or depyrogenate at 250° for NLT 120 min.

• **USP REFERENCE STANDARDS**
USP Endotoxin RS

Enflurane



$C_3H_2ClF_5O$ 184.49

Ethane, 2-chloro-1-(difluoromethoxy)-1,1,2-trifluoro-, (±)-; (±)-2-Chloro-1,1,2-trifluoroethyl difluoromethyl ether [13838-16-9].

DEFINITION

Enflurane contains NLT 99.9% and NMT 100.0% of enflurane ($C_3H_2ClF_5O$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Sample solution: Enflurane

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Thermal conductivity
Column: 4-mm × 3-m stainless steel; packed with 20% liquid phase G4 on 60- to 80-mesh S1A
Temperatures
Injection port: 200°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	6	125	0

Carrier gas: Dry helium
Flow rate: 60 mL/min
Injection volume: NMT 30 µL

Analysis
Sample: *Sample solution*
Calculate the percentage of purity:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of Enflurane
r_T = sum of all the peak areas

Acceptance criteria: 99.9%–100.0%

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample: Shake 25 mL with 25 mL of water for 5 min, and allow the liquids to separate completely. Draw off the water layer, and add to it 1 drop of nitric acid and 5 drops of silver nitrate TS.
Acceptance criteria: Any turbidity produced is not greater than that produced in a solution containing 0.35 mL of 0.020 N hydrochloric acid.

• **LIMIT OF FLUORIDE**
[NOTE—Use plasticware throughout this test.]
Buffer: Dissolve 110 g of sodium chloride and 1 g of sodium citrate in 700 mL of water in a 2000-mL volumetric flask. Cautiously add 150 g of sodium hydroxide, and dissolve with shaking. Cool to room temperature, and, while stirring, cautiously add 450 mL of glacial acetic acid to the cooled solution. Cool, add 600 mL of isopropyl alcohol, and dilute with water to volume; the pH of this solution is 5.0–5.5.
Standard stock solution: 1 mg/mL of fluoride ion, prepared as follows. Transfer 221 mg of sodium fluoride, previously dried at 150° for 4 h, to a 100-mL volumetric flask. Add 20 mL of water, and mix to dissolve. Add 1.0 mL of sodium hydroxide solution (1 in 2500), and dilute with water to volume. [NOTE—Store in a tightly closed plastic container.]
Standard solution A: 100 mL of a solution containing 1 µg/mL of fluoride ion in *Buffer*, from *Standard stock solution*
Standard solution B: 100 mL of a solution containing 3 µg/mL of fluoride ion in *Buffer*, from *Standard stock solution*
Standard solution C: 100 mL of a solution containing 5 µg/mL of fluoride ion in *Buffer*, from *Standard stock solution*
Standard solution D: 100 mL of a solution containing 10 µg/mL of fluoride ion in *Buffer*, from *Standard stock solution*
Sample stock solution: Enflurane and water (1:1). Shake the *Sample stock solution* for 5 min, allow the liquids to separate completely, and use the water layer.
Sample solution: *Sample stock solution* and *Buffer* (1:1). Use volumetric glassware.
Electrode system: Use a pH meter capable of a minimum reproducibility of ±0.2 mV, equipped with a glass-sleeved, calomel-fluoride, specific-ion electrode system (see *pH* ⟨791⟩).
Analysis
Samples: *Standard solutions* and *Sample solution*
Transfer the solution to a 150-mL beaker, add a polytef-coated stirring bar, and immerse the electrodes in the solution. Stir with a magnetic stirrer having an insulated top until equilibrium is obtained (1–2 min), and record the potential, in mv. [NOTE—Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the specific-ion electrode.]
Plot the logarithm of the fluoride-ion concentrations, in µg/mL, of the *Standard solutions* versus the potential, in mV. From the measured potential of the *Sample solution* and the standard curve, determine the concentration, in µg/mL, of fluoride ions in the *Sample solution*.
Acceptance criteria: NMT 10 µg/mL

• **LIMIT OF NONVOLATILE RESIDUE**
Analysis: Allow 10.0 mL to evaporate at room temperature in a tared evaporating dish, dry the residue at 50° for 2 h, and weigh.
Acceptance criteria: NMT 2 mg of residue remains.

SPECIFIC TESTS

• **SPECIFIC GRAVITY** ⟨841⟩: 1.516–1.519
• **REFRACTIVE INDEX** ⟨831⟩: 1.3020–1.3038 at 20°
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.14%
• **ACIDITY OR ALKALINITY**
Sample: Shake 20 mL of Enflurane with 20 mL of carbon dioxide-free water for 3 min, and allow the layers to

USP Monographs

www.webofpharma.com

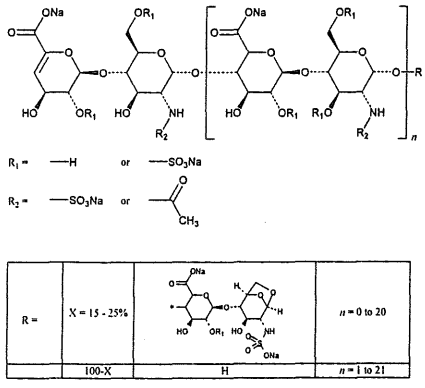
separate. Draw off the water layer, add bromocresol purple TS as the indicator, and titrate with 0.010 N sodium hydroxide or 0.010 N hydrochloric acid.

Acceptance criteria: NMT 0.10 mL of 0.010 N sodium hydroxide or NMT 0.60 mL of 0.010 N hydrochloric acid is required for neutralization.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Enflurane RS

Enoxaparin Sodium



[9041-08-1].

DEFINITION

Enoxaparin Sodium is the sodium salt of a depolymerized heparin. It is obtained by alkaline depolymerization of heparin benzyl ester. The starting material, heparin, is obtained exclusively from porcine intestinal mucosa. Heparin source material used in the manufacture of Enoxaparin Sodium complies with the compendial requirements stated in the *Heparin Sodium* monograph. Enoxaparin Sodium consists of a complex set of oligosaccharides that have not yet been completely characterized. The majority of the components have a 4-enopyranose uronate structure at the nonreducing end of their chain. About 20% of the materials contain a 1,6-anhydro derivative on the reducing end of the chain, the range being between 15% and 25%. The weight-average molecular weight of Enoxaparin Sodium is 4500 Da, the range being between 3800 and 5000 Da; about 16% have a molecular weight of less than 2000 Da, the range being between 12.0% and 20.0%; about 74% have a molecular weight between 2000 and 8000 Da, the range being between 68.0% and 82.0%. NMT 18.0% have a molecular weight higher than 8000 Da. When prepared as a solution, the solution is analyzed for clarity and degree of color using a validated method. The degree of sulfation is NLT 1.8 per disaccharide unit. It has a potency of NLT 90 and NMT 125 Anti-Factor Xa International Units (IU)/mg, and NLT 20.0 and NMT 35.0 Anti-Factor IIa IU/mg, calculated on the dried basis. The ratio of Anti-Factor Xa activity to Anti-Factor IIa activity is between 3.3 and 5.3.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Medium: 0.01 N hydrochloric acid
  Sample solution: 500 µg/mL
  Acceptance criteria: The spectra exhibit maxima at 231 ± 2 nm.
- **B. ^{13}C NMR SPECTRUM**
  (See *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩.)
  Standard solution: Dissolve 200 mg of USP Enoxaparin Sodium RS in a mixture of 0.2 mL of deuterium oxide and 0.8 mL of water. Add 0.05 mL of deuterated methanol to serve as an internal reference.
  Sample solution: Dissolve 200 mg of Enoxaparin Sodium in a mixture of 0.2 mL of deuterium oxide and 0.8 mL of water. Add 0.05 mL of deuterated methanol.
  Analysis: Transfer the *Standard solution* and the *Sample solution* to NMR tubes of 5-mm diameter. Using a pulsed (Fourier transform) NMR spectrometer operating at NLT 75 MHz for ^{13}C, record the ^{13}C NMR spectra of the *Standard solution* and the *Sample solution* at 40°.
  Acceptance criteria: The spectra are similar.
- **C.** The ratio of the numerical value of the Anti-Factor Xa activity, in Anti-Factor Xa IU/mg, to the numerical value of the Anti-Factor IIa activity, in Anti-Factor IIa IU/mg, as determined by the *Assay* (*Anti-Factor Xa Activity*) and *Impurities* (*Anti-Factor IIa Activity*), respectively, is NLT 3.3 and NMT 5.3.
- **D. MOLECULAR WEIGHT DISTRIBUTION AND WEIGHT-AVERAGE MOLECULAR WEIGHT**
  Mobile phase: Prepare a 0.5 M lithium nitrate solution. Pass through a membrane filter of 0.45-µm or smaller pore size, and degas with helium.
  Standard solution: 10 mg/mL of USP Enoxaparin Sodium RS in *Mobile phase*
  Sample solution: 10 mg/mL of Enoxaparin Sodium in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: Size exclusion LC
  Detector: Differential refractive index
  Column
  Guard: 6-mm × 40-mm; packing L59
  Analytical: Two 7.8-mm × 300-mm columns in series; packing L59
  Temperature: Room temperature
  Flow rate: 0.6 mL/min maintained constant to ± 1.0%
  Analysis: Reconstitute 1 vial each of USP Enoxaparin Sodium Molecular Weight Calibrant A RS and USP Enoxaparin Sodium Molecular Weight Calibrant B RS in 1 mL of *Mobile phase*. Separately inject 20 µL of USP Enoxaparin Sodium Molecular Weight Calibrant A RS and USP Enoxaparin Sodium Molecular Weight Calibrant B RS, record the chromatograms, and measure the retention times. Inject in duplicate 20 µL each of the *Standard solution* and the *Sample solution*, and record the chromatograms for a length of time to ensure complete elution, including salt and solvent peaks. Calculate the total peak areas under each of the *Standard solution* and *Sample solution* chromatograms, excluding salt and solvent peaks at the end.
  Calibration curve: Plot the retention times on the *x*-axis against the peak molecular weights on the *y*-axis for the peaks from USP Enoxaparin Sodium Molecular Weight Calibrant A RS and USP Enoxaparin Sodium Molecular Weight Calibrant B RS, and fit the data to a third-order

www.webofpharma.com

polynomial, using suitable gel permeation chromatography (GPC) software.

Calculations: Compute the data, using the same GPC software; determine the weight-average molecular weight, M_w, for each of the duplicate chromatograms of the *Standard solution* and the *Sample solution*; and take the average for each solution. Correct the mean value of M_w to the nearest 50. The *Chromatographic system* is suitable if M_w for USP Enoxaparin Sodium RS is within 150 Da of the labeled M_w value. The M_w for the *Sample solution* is between 3800 and 5000 Da. Using the same software, determine for each of the duplicate *Sample solution* chromatograms the percentage of Enoxaparin Sodium chains with molecular weights lower than 2000 Da, M_{2000}, the percentage of Enoxaparin Sodium chains with molecular weights in the range 2000–8000 Da, $M_{2000\text{–}8000}$, and the percentage of Enoxaparin Sodium chains with molecular weights greater than 8000 Da, M_{8000}. Average the duplicate values, and express to the nearest 0.5%.

Acceptance criteria: M_{2000} is between 12.0% and 20.0%, $M_{2000\text{–}8000}$ is between 68.0% and 82.0%, and M_{8000} is NMT 18.0%.

• **E. Identification Tests—General,** *Sodium* ⟨191⟩**:** Meets the requirements

ASSAY

• **Anti-Factor Xa Activity**

Acetic acid solution: Glacial acetic acid and water (42:58)

pH 7.4 polyethylene glycol 6000 buffer: Dissolve 6.08 g of tris(hydroxymethyl)aminomethane and 8.77 g of sodium chloride in 500 mL of water. Add 1.0 g of polyethylene glycol 6000, adjust with hydrochloric acid to a pH of 7.4, and dilute with water to 1000 mL.

pH 7.4 buffer: Dissolve 6.08 g of tris(hydroxymethyl) aminomethane and 8.77 g of sodium chloride in 500 mL of water. Adjust with hydrochloric acid to a pH of 7.4, and dilute with water to 1000 mL.

pH 8.4 buffer: Dissolve 3.03 g of tris(hydroxymethyl) aminomethane, 5.12 g of sodium chloride, and 1.40 g of edetate sodium in 250 mL of water. Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 500 mL.

Human antithrombin III solution: Reconstitute a vial of antithrombin III (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution containing 5 Antithrombin III Units/mL. Dilute this solution with *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution having a concentration of 1.0 Antithrombin III Unit/mL.

Factor Xa solution: Reconstitute a weighed quantity of bovine factor Xa (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution that gives an increase in absorbance value at 405 nm of NMT 0.20 absorbance units/min when assayed as described below but using as an appropriate volume, *V*, the volume in µL of *pH 7.4 buffer* instead of *V* µL of the enoxaparin solution.

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for factor Xa in water to obtain a concentration of about 3 mM. Dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 0.5 mM.

Standard solutions: Reconstitute the entire contents of an ampul of USP Enoxaparin Sodium for Bioassays RS with water, and dilute with *pH 7.4 buffer* to obtain four dilutions in the concentration range between 0.025 and 0.2 Anti-Factor Xa IU/mL.

Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of Enoxaparin Sodium similar to those obtained for the *Standard solutions*.

Analysis

Samples: *Standard solutions, Sample solutions, Human antithrombin III solution, pH 7.4 buffer, Factor Xa solution, Chromogenic substrate solution,* and *Acetic acid solution*

Label 18 suitable tubes: B1 and B2 for blanks; T1, T2, T3, and T4 each in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each in duplicate for the dilutions of the *Standard solutions*. [Note—Treat the tubes in the order B1, S1, S2, S3, S4, T1, T2, T3, T4, T1, T2, T3, T4, S1, S2, S3, S4, B2.] To each tube add the same volume, *V* (20–50 µL), of *Human antithrombin III solution* and an equal volume, *V*, of either the blank *(pH 7.4 buffer)* or an appropriate dilution of the *Sample solutions* or the *Standard solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for 1.0 min. Add to each tube 2*V* (40–100 µL) of *Factor Xa solution*, and incubate for 1.0 min. Add a 5*V* (100–250 µL) volume of *Chromogenic substrate solution*. Stop the reaction after 4.0 min with a 5*V* (100–250 µL) volume of *Acetic acid solution*. Measure the absorbance of each solution at 405 nm, using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) against blank B1. The reading of blank B2 relative to blank B1 is NMT ± 0.05 absorbance unit.

Calculations: For each series, calculate the regression of the absorbance against log concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the potency of the Enoxaparin Sodium in IU of Anti-Factor Xa activity/mL, using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated. Express the Anti-Factor Xa activity of Enoxaparin Sodium/mg.

Acceptance criteria: The potency is NLT 90 and NMT 125 Anti-Factor Xa IU/mg on the dried basis.

OTHER COMPONENTS

• **Benzyl Alcohol Content**

Mobile phase: Acetonitrile, methanol, and water (3:1:16)

Standard solution: 0.1 mg/mL of USP Benzyl Alcohol RS in water

Sample solution: Weigh 0.5 g of Enoxaparin Sodium into a 10-mL volumetric flask, and dissolve in 5.0 mL of 1 N sodium hydroxide. Allow to stand at room temperature for about 1 h. Add 1.0 mL of glacial acetic acid, dilute with water to volume, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 256 nm

Column: 4.6-mm × 15-cm stainless steel; packing L7

Flow rate: 1.0 mL/min, maintained constant to ±10%

Injection volume: 20 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of benzyl alcohol in the portion of Enoxaparin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of benzyl alcohol from the *Sample solution*

r_S = peak area of benzyl alcohol from the *Standard solution*

C_S = concentration of benzyl alcohol in the *Standard solution* (mg/mL)

C_U = concentration of Enoxaparin Sodium in the *Sample solution* (mg/mL)

www.webofpharma.com

Acceptance criteria: NMT 0.1%

• **NITROGEN DETERMINATION,** *Method II* ⟨461⟩**:** 1.8%–2.5% on the dried basis

• **SODIUM CONTENT**

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Cesium chloride solution: 1.27 mg/mL of cesium chloride in 0.1 N hydrochloric acid

Standard solution A: 0.0025% of sodium chloride in *Cesium chloride solution*

Standard solution B: 0.0050% of sodium chloride in *Cesium chloride solution*

Standard solution C: 0.0075% of sodium chloride in *Cesium chloride solution*

Sample solution: Transfer 50.0 mg of Enoxaparin Sodium to a 100-mL volumetric flask, and dissolve in and dilute with *Cesium chloride solution* to volume.

Analysis

Samples: *Standard solution A, Standard solution B, Standard solution C, Cesium chloride solution,* and *Sample solution*

Concomitantly determine the absorbances of the *Cesium chloride solution* (blank), *Sample solution,* and *Standard solutions* at 330.3 nm, using a sodium hollow-cathode lamp and an air–acetylene flame. Using the absorbances of *Standard solutions A–C,* determine the sodium content in the *Sample solution* after an appropriate blank correction.

Acceptance criteria: 11.3%–13.5% on the dried basis

IMPURITIES

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 6.2–7.7 for a 10.0% solution in water

• **LOSS ON DRYING** ⟨731⟩**:** Dry 1 g in a vacuum at 70° for 6 h: it loses NMT 10.0% of its weight.

• **SPECIFIC ABSORBANCE**

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Sample solution: 0.5 mg/mL of Enoxaparin Sodium in 0.01 N hydrochloric acid

Analysis: Obtain the UV spectra of the *Standard solution* and the *Sample solution* between 200 nm and 300 nm against a 0.01 N hydrochloric acid blank.

Calculate the specific absorbance at the wavelength of maximum absorbance at 231 ± 2 nm, with reference to the dried substance:

$$\text{Result} = A \times 100 \times 1000/[M \times l \times (100 - E)]$$

A = absorbance at the wavelength of maximum absorbance

M = weight of Enoxaparin Sodium in the *Sample solution* (mg)

l = path length (typically 1 cm)

E = loss on drying (%)

Acceptance criteria: 14.0–20.0 on the dried basis

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.01 USP Endotoxin Unit/IU of Anti-Factor Xa activity.

• **ANTI-FACTOR IIa ACTIVITY**

Acetic acid solution, pH 7.4 polyethylene glycol 6000 buffer, pH 7.4 buffer, pH 8.4 buffer, and **Human antithrombin III solution:** Proceed as directed in the *Assay* for *Anti-Factor Xa Activity,* except that the concentration of the *Human antithrombin III solution* is 0.5 Antithrombin III Unit/mL.

Thrombin human solution: Reconstitute thrombin human (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water, and dilute in *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution having a concentration of 5 Thrombin Units/mL.

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic substrate for an amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for thrombin in water to obtain a concentration of about 3 mM. Immediately before use, dilute with *pH 8.4 buffer* to 0.5 mM.

Standard solutions: Reconstitute the entire contents of an ampul of USP Enoxaparin Sodium for Bioassays RS with water, and dilute with *pH 7.4 buffer* to obtain four dilutions having concentrations in the range between 0.015 and 0.075 IU of Anti-Factor IIa activity/mL.

Sample solutions: Proceed as directed under *Standard solutions* to obtain concentrations of Enoxaparin Sodium similar to those obtained for the *Standard solutions.*

Analysis: Proceed as directed in the *Assay* for *Anti-Factor Xa Activity,* except to use *Thrombin human solution* instead of *Factor Xa solution* and to use *Human antithrombin III solution* as described above.

Calculations: For each series, calculate the regression of the absorbance against log concentrations of the *Sample solutions* and of the *Standard solutions,* and calculate the potency of the Enoxaparin Sodium in IU of Anti-Factor IIa activity/mg, using statistical methods for parallel-line assays. The four independent dilution estimates are then combined to obtain the final weighted mean. Then calculate the confidence limits. Express the Anti-Factor IIa activity of Enoxaparin Sodium/mg.

Acceptance criteria: It has a potency of NLT 20.0 and NMT 35.0 Anti-Factor IIa IU/mg on the dried basis.

• **MOLAR RATIO OF SULFATE TO CARBOXYLATE**

Mobile phase: Carbon dioxide-free water

Sample solution: 5 mg/mL of Enoxaparin Sodium in carbon dioxide-free water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: Ion

Column: Two columns; one 1.5-cm × 2.5-cm column, packed with an anion-exchange resin L64 packing; and one 1.5-cm × 7.5-cm column, packed with a cation-exchange resin L65 packing. The outlet of the anion-exchange column is connected to the inlet of the cation-exchange column.

Flow rate: 1 mL/min

Analysis

Sample: *Sample solution*

[NOTE—Regenerate the anion-exchange column and the cation-exchange column with 1 N sodium hydroxide and 1 N hydrochloric acid, respectively, between two injections.]

Inject the *Sample solution* into the anion-exchange column, and collect the eluate from the cation-exchange column in a beaker at the outlet until the ion detector reading returns to the baseline value. Quantitatively transfer the eluate to a titration vessel containing a magnetic stirring bar, and dilute with carbon dioxide-free water to about 60 mL. Position the titration vessel on a magnetic stirrer, and immerse the electrodes. Note the initial conductivity reading, and titrate with approximately 0.1 N sodium hydroxide added in 100-µL portions. [NOTE—Prepare the sodium hydroxide solution in carbon dioxide-free water.] Record the buret reading and the conductivity meter reading after each addition of the sodium hydroxide solution.

Calculations: Plot the conductivity measurements on the *y*-axis against the volumes of sodium hydroxide added on the *x*-axis. The graph will have three linear sections—an initial downward slope, a middle slight rise, and a final rise. For each of these sections draw the best-fit straight lines,

www.webofpharma.com

using linear regression analysis. At the points where the first and second straight lines intersect and where the second and third lines intersect, draw perpendiculars to the *x*-axis to determine the volumes of sodium hydroxide taken up by the sample at those points. The point where the first and second lines intersect corresponds to the volume of sodium hydroxide taken up by the sulfate groups (V_S). The point where the second and third lines intersect corresponds to the volume of sodium hydroxide consumed by the sulfate and the carboxylate groups together (V_T).
Calculate the molar ratio of sulfate to carboxylate:

$$\text{Result} = V_S/(V_T - V_S)$$

Acceptance criteria: The molar ratio of sulfate to carboxylate is NLT 1.8.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store below 40°, preferably at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzyl Alcohol RS
  USP Enoxaparin Sodium RS
  USP Enoxaparin Sodium Molecular Weight Calibrant A RS
  USP Enoxaparin Sodium Molecular Weight Calibrant B RS
  USP Enoxaparin Sodium for Bioassays RS

Enoxaparin Sodium Injection

DEFINITION

Enoxaparin Sodium Injection is a sterile solution of Enoxaparin Sodium in Water for Injection. Its appearance is analyzed for clarity and degree of color, using a validated method. Its potency value is NLT 90% and NMT 110% of the potency stated on the label in terms of International Anti-Factor Xa Units (IU). It may contain, in multiple-dose containers, a suitable antimicrobial preservative, such as benzyl alcohol.

IDENTIFICATION

- **A.**

Analysis: Transfer the total contents of a single-dose container or 0.4 mL from a multiple-dose container to a glass test tube, add 2 mL of water and 1 mL of 2% (w/v) protamine sulfate solution, and mix.

Acceptance criteria: A creamy white precipitate is formed.

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Medium: 0.01 N hydrochloric acid

Standard solution: 500 µg/mL

Sample solution: Transfer the total content of a single-dose container or 0.4 mL from a multiple-dose container to a 100-mL volumetric flask. Dilute with *Medium* to volume.

Acceptance criteria: The spectra exhibit maxima at 231 ± 2 nm.

- **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩**:** Meets the requirements

ASSAY

- **ANTI-FACTOR Xa ACTIVITY**

Acetic acid solution: Glacial acetic acid and water (42:58)

pH 7.4 polyethylene glycol 6000 buffer: Dissolve 6.08 g of tris(hydroxymethyl)aminomethane and 8.77 g of sodium chloride in 500 mL of water. Add 1.0 g of polyethylene glycol 6000, adjust with hydrochloric acid to a pH of 7.4, and dilute with water to 1000 mL.

pH 7.4 buffer: Dissolve 6.08 g of tris(hydroxymethyl) aminomethane and 8.77 g of sodium chloride in 500 mL of water. Adjust with hydrochloric acid to a pH of 7.4, and dilute with water to 1000 mL.

pH 8.4 buffer: Dissolve 3.03 g of tris(hydroxymethyl) aminomethane, 5.12 g of sodium chloride, and 1.40 g of edetate sodium in 250 mL of water. Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 500 mL.

Human antithrombin III solution: Reconstitute a vial of antithrombin III (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution containing 5 Antithrombin III Units/mL. Dilute this solution with *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution having a concentration of 1.0 Antithrombin III Unit/mL.

Factor Xa solution: Reconstitute a weighed quantity of bovine factor Xa (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution that gives an increase in absorbance value at 405 nm of NMT 0.20 absorbance units/min when assayed as described below but using as an appropriate volume, *V*, the volume in µL of *pH 7.4 buffer* instead of *V* µL of the enoxaparin solution.

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic substrate for an amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for Factor Xa in water to obtain a concentration of about 3 mM. Dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 0.5 mM.

Standard solutions: Reconstitute the entire contents of an ampul of USP Enoxaparin Sodium for Bioassays RS with water, and dilute with *pH 7.4 buffer* to obtain four dilutions in the concentration range between 0.025 and 0.2 Anti-Factor Xa IU/mL.

Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of Injection similar to those obtained for the *Standard solutions*.

Analysis

Samples: *Standard solutions*, *Sample solutions*, *Human antithrombin III solution*, *pH 7.4 buffer*, *Factor Xa solution*, *Chromogenic substrate solution*, and *Acetic acid solution*

Label 18 suitable tubes: B1 and B2 for blanks; T1, T2, T3, and T4 each in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each in duplicate for the dilutions of the *Standard solutions*. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, T1, T2, T3, T4, T1, T2, T3, T4, S1, S2, S3, S4, B2.] To each tube add the same volume, *V* (20–50 µL), of *Human antithrombin III solution* and an equal volume, *V*, of either the blank *(pH 7.4 buffer)* or an appropriate dilution of the *Sample solutions* or the *Standard solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for 1.0 min. Add to each tube 2*V* (40–100 µL) of *Factor Xa solution*, and incubate for 1.0 min. Add a 5*V* (100–250 µL) volume of *Chromogenic substrate solution*. Stop the reaction after 4.0 min with a 5*V* (100–250 µL) volume of *Acetic acid solution*. Measure the absorbance of each solution at 405 nm, using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) against blank B1. The reading of blank B2 relative to blank B1 is NMT ±0.05 absorbance unit.

Calculations: For each series, calculate the regression of the absorbance against log concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the potency of the enoxaparin sodium in the Injection in IU of Anti-Factor Xa activity/mL, using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated.

www.webofpharma.com

Acceptance criteria: The potency is NLT 90% and NMT 110% of the potency stated on the label in terms of International Anti-Factor Xa Units (IU).

• **ANTI-FACTOR Xa TO ANTI-FACTOR IIa RATIO:** The ratio of the numerical value of the Anti-Factor Xa activity in Anti-Factor Xa IU/mL to the numerical value of the Anti-Factor IIa activity in Anti-Factor IIa IU/mL, as determined by *Anti-Factor Xa Activity* and *Anti-Factor IIa Activity*, respectively, is NLT 3.3 and NMT 5.3.

OTHER COMPONENTS

• **BENZYL ALCOHOL CONTENT (IF PRESENT)**

Mobile phase: Acetonitrile, methanol, and water (3:1:16)

Standard solution: 1.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*

Sample solution: Transfer exactly 5.0 mL of the Injection to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 256 nm

Column: 4.6-mm × 15-cm stainless steel; packing L7[1]

Flow rate: 1.0 mL/min, maintained constant to ±10%

Injection volume: 20 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage (w/v) of benzyl alcohol in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times C$$

r_U = peak area of benzyl alcohol from the *Sample solution*

r_S = peak area of benzyl alcohol from the *Standard solution*

C = concentration of benzyl alcohol in the *Standard solution* (mg/mL)

Acceptance criteria: 1.35%–1.65%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 5.5–7.5

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains less than 0.01 USP Endotoxin Unit/unit of Anti-Factor Xa activity in Anti-factor Xa IU.

• **ANTI-FACTOR IIa ACTIVITY**

Acetic acid solution, pH 7.4 polyethylene glycol 6000 buffer, pH 7.4 buffer, pH 8.4 buffer, and **Human antithrombin III solution:** Proceed as directed in the *Assay* for *Anti-Factor Xa Activity*, except that the concentration of the *Human antithrombin III solution* is 0.5 Antithrombin III Unit/mL.

Thrombin human solution: Reconstitute thrombin human (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water, and dilute in *pH 7.4 polyethylene glycol 6000 buffer* to obtain a solution having a concentration of 5 Thrombin Units/mL.

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic substrate for an amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for thrombin in water to obtain a concentration of about 3 mM. Immediately before use, dilute with *pH 8.4 buffer* to 0.5 mM.

Standard solutions: Reconstitute the entire contents of an ampul of USP Enoxaparin Sodium for Bioassays RS with water, and dilute with *pH 7.4 buffer* to obtain four dilutions having concentrations in the range between 0.015 and 0.075 IU of Anti-Factor IIa activity/mL.

Sample solutions: Proceed as directed under *Standard solutions* to obtain concentrations of Injection similar to those obtained for the *Standard solutions*.

Analysis: Proceed as directed in the *Assay* for *Anti-Factor Xa Activity*, except to use *Thrombin human solution* instead of *Factor Xa solution* and to use *Human antithrombin III solution* as described above.

Calculations: For each series, calculate the regression of the absorbance against log concentrations of the *Sample solutions* and of the *Standard solutions*, and calculate the potency of the enoxaparin sodium in the Injection in IU of Anti-Factor IIa activity/mL, using statistical methods for parallel-line assays. The four independent dilution estimates are then combined to obtain the final weighted mean. Then calculate the confidence limits.

Acceptance criteria: The Anti-Factor IIa activity IU (or IU/mL) is NLT 20.0% and NMT 35.0% of the potency stated on the label in terms of International Anti-Factor Xa Units (IU or IU/mL).

• **FREE SULFATE CONTENT**

Mobile phase: 3.0 mM sodium carbonate solution

System suitability solution: 3 µg/mL of sulfate anion and 5 µg/mL of oxalate anion

Standard sulfate stock solution: Prepare a solution of sodium sulfate in *Mobile phase* in a suitable sulfate-free container such that the concentration of sulfate is accurately known at about 1 mg/mL. Transfer 5 g of the solution to a similar container, and add *Mobile phase* to obtain 25 g of solution.

Standard solution A: 0.1 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*

Standard solution B: 0.5 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*

Standard solution C: 1 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*

Standard solution D: 2 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*

Standard solution E: 4 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*

Standard solution F: 5 µg/g of sulfate from *Standard sulfate stock solution* in *Mobile phase*

Sample solution: Transfer a known quantity, *m*, of Enoxaparin Sodium Injection, accurately weighed, to a suitable previously tared sulfate-free vial. Add *Mobile phase* to obtain a solution having a known concentration of about 10 mg/g.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System suitability*.)

Mode: Ion chromatography

Detector: Conductivity

Column

Guard: 4-mm × 5-cm; packing L61

Analytical: 4-mm × 25-cm; packing L61

[NOTE—Use a micromembrane anion autosuppressor[2] or a suitable chemical suppression system.]

Flow rate: 2.0 mL/min

Injection size: 25 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1 between the sulfate and oxalate peaks

Analysis

Samples: *Standard solutions A–F* and *Sample solution*

[1] Available as Lichrospher 100 RP 18, pore size 100 Å, particle size 5 µm, or equivalent.

[2] Available as Anion Self-Regenerating Suppressor (ASRS) from Dionex Inc, or equivalent.

www.webofpharma.com

Plot the standard curve of sulfate peak height as a function of sulfate concentration (in µg/g) in *Standard solutions A–F*. From the sulfate peak height determine the concentration of sulfate, *C*, in µg/g, in the *Sample solution*, using the standard curve.

Calculate the percentage of free sulfate content (w/w) in the portion of Injection taken:

$$\text{Result} = [(C \times M_S)/10m)]$$

M_S = total mass of the *Sample solution* (g)
m = mass of Injection taken to prepare the *Sample solution* (mg)

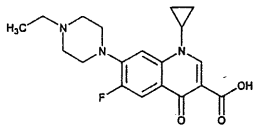
Acceptance criteria: The percentage of free sulfate is NMT 0.12% (w/w).

- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers in Type I glass. Store between 20° and 25°, excursions permitted between 15° and 30°.
- **LABELING:** Label it to indicate the amount (mg) of Enoxaparin Sodium in the total volume of contents. The label states also that the Enoxaparin Sodium starting material is porcine derived.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzyl Alcohol RS
  USP Enoxaparin Sodium RS
  USP Enoxaparin Sodium for Bioassays RS

Enrofloxacin



$C_{19}H_{22}FN_3O_3$ 359.39

3-Quinolinecarboxylic acid, 1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-;
1-Cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid [93106-60-6].

» Enrofloxacin contains not less than 98.5 percent and not more than 101.5 percent of $C_{19}H_{22}FN_3O_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, tight, light-resistant containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩**—**
USP Enrofloxacin RS
USP Fluoroquinolonic Acid RS
USP *N*-Ethylpiperazine RS
USP Enrofloxacin Related Compound Mixture RS
Contains a mixture of USP Enrofloxacin RS, desfluoro-enrofloxacin, and USP Ciprofloxacin RS.
*Desfluoro-enrofloxacin:*1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-1,4-dihydro-4-oxo-3-quinoline-carboxylic acid.

Change to read:

Clarity of solution—

Hydrazine sulfate solution—Transfer 1.0 g of hydrazine sulfate to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Allow to stand for 4 to 6 hours.

Hexamethylenetetramine solution—Transfer 2.5 g of hexamethylenetetramine to a 100-mL volumetric flask, add 25.0 mL of water, insert a glass stopper, and mix to dissolve.

Primary opalescent suspension— [NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well-mixed before use.] Transfer 25.0 mL of the *Hydrazine sulfate solution* to the *Hexamethylenetetramine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 hours.

Opalescence standard— [NOTE—This suspension should not be used beyond 24 hours after preparation.] Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, dilute with water to volume, and mix.

Reference suspension—Transfer 10.0 mL of the *Opalescence standard* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Test solution—To 1.0 g of Enrofloxacin add about 0.25 g of potassium hydroxide and 7 mL of water. Sonicate to dissolve, and dilute with water to 10.0 mL.

Procedure—Transfer a sufficient portion of the *Test solution,* the *Reference suspension,* and water to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 to 25 mm to obtain a depth of 40 mm. Compare the *Test solution,* the *Reference suspension,* and water in diffused daylight 5 minutes after preparation of the *Reference suspension,* viewing vertically against a black background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)). [NOTE—The diffusion of light must be such that the *Reference suspension* can be readily distinguished from water.] The *Test solution* shows the same clarity as that of water or its opalescence is not more pronounced than that of the *Reference suspension.*

Change to read:

Color of solution—

Standard stock solution—Combine 9.6 mL of ferric chloride CS, 0.2 mL of cobaltous chloride CS, and 0.2 mL of cupric sulfate CS, and mix.

Standard solution— [NOTE—Prepare the *Standard solution* immediately before use.] Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with dilute hydrochloric acid (10 g per 1000 mL).

Test solution—To 1.0 g of Enrofloxacin add about 0.25 g of potassium hydroxide and 7 mL of water. Sonicate to dissolve, and dilute with water to 10.0 mL.

Procedure—Transfer a sufficient portion of the *Test solution,* the *Standard solution,* and water to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm to obtain a depth of 40 mm. Compare the *Test solution,* the *Standard solution,* and water in diffused daylight, viewing vertically against a white background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)). [NOTE—The diffusion of light must be such that the *Reference suspension* can be readily distinguished from water.] The *Test solution* has the appearance of water or is not more intensely colored than the *Standard solution.*

www.webofpharma.com

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Test solution:* Prepare a solution containing 10 mg of Enrofloxacin per mL of 0.05 M hydrochloric acid.
Application volume: 5 µL.
Developing solvent solution: methylene chloride, methanol, 25% ammonia solution, and acetonitrile (2: 2: 1: 0.5).
Procedure—Proceed as directed in the chapter except apply 5-µL portions of the *Test solution* and the *Standard solution* to the starting line of the chromatographic plate. Place the plate in an atmosphere of ammonia for approximately 15 minutes. Develop the chromatogram in an unsaturated chamber with the *Developing solvent solution.*

Loss on drying ⟨731⟩—Dry a 2-g sample, accurately weighed, 5 to 7 hours under vacuum at 120° to constant weight: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%, based on a test specimen of about 2 g.

Change to read:

Chloride ⟨221⟩—Add 58 mL of water to 0.5 g of Enrofloxacin, shake for 5 minutes, and pass through a chloride-free filter paper, add 2 mL of 2 M acetic acid, and mix. Transfer 15.0 mL of the filtrate to a 50-mL color-comparison tube *(Test solution).* To a second matched 50-mL color-comparison tube transfer 10.0 mL of a *Standard solution* of sodium chloride having a concentration of 8.2 µg per mL, corresponding to 5 µg of chloride per mL, add 5.0 mL of water, and mix. To each tube add 1 mL of 2 N nitric acid, mix, add 1 mL of silver nitrate TS, and mix. Allow the solutions to stand for 5 minutes, protected from light. Examine the tubes vertically against a black background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)). Any opalescence in the *Test solution* is not more intense than that in the *Standard solution* (0.04%).

Change to read:

Sulfate ⟨221⟩—Dissolve 0.5 g of Enrofloxacin in 5.0 mL of 2 N acetic acid and 15.0 mL of water (*Test solution*). To each of two 50-mL matched color-comparison tubes transfer 1.50 mL of a *Standard solution* of potassium sulfate in 30% alcohol having a concentration of 18.1 µg per mL, equivalent to 10 µg of sulfate per mL. To each tube add, successively and with continuous shaking, 1.0 mL of barium chloride solution (1 in 4), and allow to stand for 1 minute. To one of the tubes transfer 15.0 mL of the *Standard solution* and 0.5 mL of 30% acetic acid, and mix. To the second tube add 15.0 mL of the *Test solution* and 0.5 mL of 30% acetic acid, and mix. Allow solutions to stand for 5 minutes. Examine the tubes vertically against a black background (see ▲*Visual Comparison* ⟨630⟩▲ (CN 1-May-2019)). Any opalescence in the *Test solution* is not more intense than that in the *Standard solution* (0.04%).

Limit of *N*-ethylpiperazine—

Internal standard solution—Dissolve an accurately weighed quantity of *n*-decane in chloroform, and dilute quantitatively, and stepwise if necessary, with chloroform to obtain a solution having a known concentration of about 0.1 mg per mL.

Standard stock solution—Dissolve an accurately weighed quantity of USP *N*-Ethylpiperazine RS in chloroform, and dilute quantitatively, and stepwise if necessary, with chloroform to obtain a solution having a known concentration of about 9.0 mg per mL.

Standard solution—To 2.0 mL of the *Internal standard solution* add 20 µL of the *Standard stock solution,* and mix.

Test solution—To 200 mg of Enrofloxacin, accurately weighed, add 2.0 mL of the *Internal standard solution,* and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a split injector system and contains a 0.32-mm × 50-m column with 100% liquid phase G1 with a film thickness of about 5.0 µm. The carrier gas is hydrogen (helium may be used), flowing at a rate of about 2.9 mL per minute. The auxiliary gas is nitrogen flowing at a rate of about 30 mL per minute. The chromatograph is programmed as follows. Initially the temperature of the column is equilibrated at 80°, then the temperature is increased at a rate of 10° per minute to 240°, and maintained at 240° for 15 minutes. The split injector (25:1 split ratio) temperature is maintained at 200°, and the detector is maintained at 250°. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.90 for *N*-ethylpiperazine and 1.0 for *n*-decane.

Procedure—Inject a volume (about 1.0 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Calculate the percentage of the impurity in the portion of Enrofloxacin taken by the formula:

$$100(C_S/C_U)(R_U/R_S)$$

in which C_S is the concentration of the impurity, in mg per mL, in the *Standard solution;* C_U is the concentration, in mg per mL, of Enrofloxacin in the *Test solution;* R_U is the peak response ratio of the impurity peak to the internal standard peak obtained from the *Test solution;* and R_S is the peak response ratio of the impurity peak to the internal standard peak obtained from the *Standard solution.* Not more than 0.1% of the impurity is found.

Related compounds—

TEST 1 (FOR FLUOROQUINOLONIC ACID)—
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.
Test solution—Prepare as directed for the *Test solution* in *Identification* test *B.*
Diluent—Transfer 0.1 mL of 6 M ammonium to a 100-mL volumetric flask, mix, and dilute with water to volume.
Standard stock solution—Dissolve and mix an accurately weighed quantity of USP Fluoroquinolonic Acid RS with *Diluent* to prepare a solution containing about 0.10 mg per mL, and mix.
Standard solution 1 (0.1%)—Transfer 1.0 mL of the *Standard stock solution* to a 10-mL volumetric flask, dilute with water to volume, and mix.
Standard solution 2 (0.2%)—Transfer 2.0 mL of the *Standard stock solution* to a 10-mL volumetric flask, dilute with water to volume, and mix.
Application volume: 5 µL.
Developing solvent system— [NOTE—Carefully follow the mixing order stated below.] Shake butyl acetate, *n*-butanol, water, and glacial acetic acid (50:9:15:25), and allow to settle. Use the upper layer as the mobile phase and discard the lower layer.
Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. Apply separately the *Test solution, Standard solution 1,* and *Standard solution 2* to the thin-layer plate, and chromatograph using the *Developing solvent system.* Dry the developed chromatogram in the air under a fume hood for 30 to 60 minutes, then view under short-wavelength UV light. Determine the quantity of fluoroquinolonic acid by comparing

www.webofpharma.com

the size and intensity of the spots from the *Test solution* to the *Standard solutions*. The intensity of any spot from the *Test solution* at about the same retardation factor, R_F, as that of the *Standard solutions* is not greater than the intensity of the *Standard solution 2 (0.2%)* spot.

TEST 2 (FOR CIPROFLOXACIN, DES-FLUORO COMPOUND AND OTHER UNSPECIFIED IMPURITIES)—

Phosphoric acid buffer—Prepare 25 mM phosphoric acid, and adjust with triethylamine to a pH of 3.0.

Mobile phase—Prepare a solution containing *Phosphoric acid buffer* and acetonitrile (87:13).

Control solution—Dissolve about 5 mg of USP Enrofloxacin Related Compound Mixture RS, accurately weighed, in *Mobile phase* in a 5-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Test solution 1—Dissolve about 50 mg of Enrofloxacin, accurately weighed, in *Mobile phase* in a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Test solution 2—Transfer 1.0 mL of *Test solution 1* into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 1.0 mL of this solution into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm × 25-cm stainless steel column that contains 5-µm packing L1. The column temperature is maintained at 40°, and the flow rate is about 1.5 mL per minute. Chromatograph the *Control solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.58 for the des-fluoro compound, 0.74 for ciprofloxacin, and 1.0 for enrofloxacin. The resolution, *R,* between the des-fluoro compound and ciprofloxacin is not less than 1.5.

Procedure—Inject a volume (about 25 µL) of *Test solution 1, Test solution 2,* and the *Control solution* into the chromatograph, record the chromatogram, and measure the peak responses. Identify the ciprofloxacin and the des-fluoro compound peaks in *Test solution 2* by comparing their retention times with those from the *Control solution*. Calculate the percentage of each related compound in the portion of Enrofloxacin taken by the formula:

$$100C(r_i/r_s)$$

in which C is the concentration of Enrofloxacin in *Test solution 2* as a percentage of *Test solution 1 (0.2%)*; r_i is the individual peak response of each related compound obtained from *Test solution 1;* and r_s is the individual peak area of enrofloxacin obtained from *Test solution 2:* not more than 0.1% of des-fluoro compound, not more than 0.3% of ciprofloxacin, not more than 0.1% of any unspecified impurity, and not more than 0.5% of total impurities are found.

Assay—Transfer about 250 mg of Enrofloxacin, accurately weighed, to a 125-mL flask, dissolve in 100 mL of anhydrous acetic acid, and titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M perchloric acid is equivalent to 35.94 mg of $C_{19}H_{22}FN_3O_3$.

Enrofloxacin Compounded Oral Suspension, Veterinary

DEFINITION

Enrofloxacin Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of enrofloxacin ($C_{19}H_{22}FN_3O_3$).

Prepare Enrofloxacin Compounded Oral Suspension, Veterinary 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Enrofloxacin powder	2 g
Vehicle: a 1:1 mixture of hypromellose 1% solution and Syrpalta,[a] a sufficient quantity to make	100 mL

[a] Humco, Texarkana, TX.

Prepare a hypromellose 1% solution by heating 25 mL of Purified Water to boiling in a calibrated beaker, and slowly adding 0.5 g of hypromellose with stirring. When the hypromellose is completely dispersed, take the beaker off heat, and add cold Purified Water with continuous stirring until gelled. Add cold Purified Water to bring to a final volume of 50 mL with stirring. Continue mixing until a clear, homogenous solution results. Place the solution in a refrigerator overnight to allow for complete hydration. Prepare the *Vehicle* by mixing equal volumes of the previously prepared hypromellose 1% solution and the *Syrpalta.*

Place the *Enrofloxacin powder* into a suitable mortar. Wet the powder with a small amount of *Vehicle,* and triturate to make a smooth paste. Add sufficient *Vehicle* to make the mortar contents pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated container using the remainder of the *Vehicle.* Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and 50 mM phosphoric acid (15:85). Filter, and degas.

System suitability solution: Dissolve about 5 mg of USP Enrofloxacin Related Compound Mixture RS in a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Standard solution: 0.1 mg/mL of enrofloxacin prepared from USP Enrofloxacin RS in *Mobile phase*

Sample solution: Shake thoroughly each bottle of Oral Suspension, Veterinary. Transfer 1 mL of Oral Suspension, Veterinary into a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix well. Pass through a PVDF filter of 0.45-µm pore size, discarding the first 3 mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV-Vis 277 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for ciprofloxacin and enrofloxacin are 0.76 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between enrofloxacin and ciprofloxacin, *System suitability solution*

USP Monographs

www.webofpharma.com

Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of enrofloxacin ($C_{19}H_{22}FN_3O_3$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

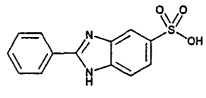
r_U = peak response of enrofloxacin from the *Sample solution*
r_S = peak response of enrofloxacin from the *Standard solution*
C_S = concentration of USP Enrofloxacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of enrofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩: 6.0–7.0

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Package in tight, light-resistant containers. Store in a refrigerator (2°–8°) or at controlled room temperature.
• **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored in a refrigerator (2°–8°) or at controlled room temperature
• **Labeling:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*. Label it to state that it is for veterinary use only.
• **USP Reference Standards** ⟨11⟩
USP Enrofloxacin RS
USP Enrofloxacin Related Compound Mixture RS

Ensulizole



$C_{13}H_{10}N_2O_3S$ 274.30
1*H*-Benzimidazole-5-sulfonic acid-2-phenyl-;
2-Phenylbenzimidazole-5-sulfonic acid [27503-81-7].

DEFINITION
Ensulizole contains NLT 98.0% and NMT 102.0% of $C_{13}H_{10}N_2O_3S$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 5 µg/mL
[Note—Transfer 100 mg of Ensulizole to a 100-mL volumetric flask, dissolve in 4 mL of 1 N sodium hydroxide, and dilute with water to volume. Transfer 0.5 mL of the solution to a 100-mL volumetric flask, and dilute with water to volume.]
Acceptance criteria: The absorptivities, calculated on the dried basis, at the wavelength of maximum absorbance at about 302 nm, do not differ by more than 3.0%.

ASSAY
• **Procedure**
Sample solution: 48 mg/mL of Ensulizole in 0.5 N sodium hydroxide
Titrant: 0.5 N hydrochloric acid VS
Analysis: To the *Sample solution*, add phenolphthalein TS, and titrate the excess with *Titrant*. Perform a blank determination, and make any necessary corrections (see *Titrimetry* ⟨541⟩). Each mL of 0.5 N sodium hydroxide is equivalent to 137.15 mg of $C_{13}H_{10}N_2O_3S$.
Calculate the percentage of $C_{13}H_{10}N_2O_3S$ in the portion of Ensulizole taken:

$$\text{Result} = [(N \times (V_B - V_A))/(W \times (1 - \text{LOD}))] \times M_{r1} \times 100$$

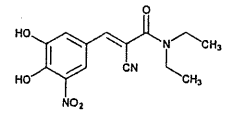
N = actual normality of the *Titrant*
V_B = volume of *Titrant* used for the blank (mL)
V_A = volume of *Titrant* used for the *Sample solution* (mL)
W = weight of Ensulizole taken for the *Sample solution* (mg)
LOD = percentage of loss on drying expressed as a decimal fraction, as determined in the test for *Loss on Drying*
M_{r1} = molecular weight of ensulizole (274.30)

Acceptance criteria: 98.0%–102.0% on the dried basis

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩: Dry a sample at 105° for 4 h: it loses NMT 2.0% of its weight.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers in a cool place.
• **USP Reference Standards** ⟨11⟩
USP Ensulizole RS

Entacapone



$C_{14}H_{15}N_3O_5$ 305.29
(*E*)-α-Cyano-*N*,*N*-diethyl-3,4-dihydroxy-5-nitrocinnamamide;
2-Propenamide, 2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-*N*,*N*-diethyl-,(*E*)- [130929-57-6].

DEFINITION
Entacapone contains NLT 98.0% and NMT 102.0% of $C_{14}H_{15}N_3O_5$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

USP Monographs

www.webofpharma.com

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 2.34 g/L of monobasic sodium phosphate dihydrate. Adjust with phosphoric acid to a pH of 2.1.
Diluent: Methanol and tetrahydrofuran (7:3)
Mobile phase: Methanol, tetrahydrofuran, and *Buffer* (22:1:27)
System suitability solution: 1 µg/mL each of USP Entacapone Related Compound A RS and USP Entacapone RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Entacapone RS in *Diluent*
Sample solution: 0.1 mg/mL of Entacapone in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for entacapone related compound A and entacapone are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between entacapone related compound A and entacapone, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{14}H_{15}N_3O_5$ in the portion of Entacapone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Entacapone RS in the *Standard solution* (mg/mL)
C_U = concentration of entacapone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

• **Procedure**

Buffer, Diluent, and **Mobile phase:** Proceed as directed in the *Assay*.
Standard solution: Use the *System suitability solution* prepared as directed in the *Assay*.
Sample solution: 1.0 mg/mL of Entacapone in *Diluent*
Chromatographic system: Prepare as directed in the *Assay*.
Run time: 2 times the retention time of the entacapone peak
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between entacapone related compound A and entacapone
Relative standard deviation: NMT 10.0% based on the entacapone peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Entacapone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of entacapone from the *Standard solution*
C_S = concentration of USP Entacapone RS in the *Standard solution* (mg/mL)
C_U = concentration of Entacapone in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.2%
[NOTE—Do not include entacapone related compound A in the calculation of total impurities.]

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Entacapone related compound A[a]	0.8	0.1
Entacapone	1.0	—
Any other unknown individual impurity	—	0.10
Total impurities	—	0.2

[a] (Z)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-*N*,*N*-diethylacrylamide.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry 1.0 g of the sample in vacuum at a pressure not exceeding 49 mm of mercury at 65° to constant weight.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Entacapone RS
USP Entacapone Related Compound A RS
(*Z*)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-*N*,*N*-diethylacrylamide.
$C_{14}H_{15}N_3O_5$ 305.29

Entacapone Tablets

DEFINITION

Entacapone Tablets contain an amount of entacapone equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of entacapone ($C_{14}H_{15}N_3O_5$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)**:** The sample shows a medium band at about 2216 cm^{-1} and strong bands at

www.webofpharma.com

about 1628, 1604, 1544, 1512, 1440, 1376, 1348, 1296, 1280, and 1208 cm^{-1} similar to the reference preparation.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Protect solutions from light.

Buffer: 2.1 g/L of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.1.

Diluent: Methanol and tetrahydrofuran (70:30)

Mobile phase: Methanol, tetrahydrofuran, and *Buffer* (44:2:54)

Standard solution: 0.5 mg/mL of USP Entacapone RS in *Diluent*

Sample solution: Nominally 0.5 mg/mL of entacapone prepared as follows. Finely powder NLT 20 Tablets, and transfer a suitable portion of the powder to an appropriate volumetric flask. Add NLT 30% of the final flask volume of tetrahydrofuran, and sonicate for 3 min. Add NLT 30% of the final flask volume of methanol, and shake for 5 min. Dilute with methanol to volume. Centrifuge a portion of this solution, and use the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 300 nm

Column: 4.6-mm × 25-cm; 5-µm packing L11

Flow rate: 1 mL/min

Run time: 1.5 times the retention time of the entacapone peak

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of entacapone ($C_{14}H_{15}N_3O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Entacapone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of entacapone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: pH 5.5 phosphate buffer (6.8 g/L of monobasic potassium phosphate in water, adjusted with 1 M sodium hydroxide to a pH of 5.5); 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard stock solution: 0.22 mg/mL of USP Entacapone RS, prepared as follows. Transfer a suitable quantity of USP Entacapone RS to an appropriate volumetric flask, and dissolve in 2% of the flask volume of tetrahydrofuran. Dilute with *Medium* to volume. Protect this solution from light.

Standard solution: 0.022 mg/mL of USP Entacapone RS from the *Standard stock solution* in *Medium*. Protect this solution from light.

Sample solution: Pass a portion of the solution through a suitable filter of 20-µm pore size. Dilute with *Medium*, if necessary. Protect this solution from light.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 313 nm

Path length: 1 cm

Blank: Tetrahydrofuran and *Medium* (0.2: 99.8)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of entacapone ($C_{14}H_{15}N_3O_5$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of entacapone ($C_{14}H_{15}N_3O_5$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: pH 5.5 phosphate buffer (6.8 g/L of monobasic potassium phosphate in water, adjusted with 5 M sodium hydroxide to a pH of 5.5); 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard stock solution: 0.45 mg/mL of USP Entacapone RS prepared as follows. Transfer a suitable quantity of USP Entacapone RS to an appropriate volumetric flask, and dissolve in 5% of the flask volume of methanol. Dilute with *Medium* to volume. Use this solution within 6.5 h.

Standard solution: 0.018 mg/mL of USP Entacapone RS from the *Standard stock solution* in *Medium*. Use this solution within 6.5 h.

Sample solution: Pass a portion of the solution under test through a suitable filter. Transfer 2 mL of the filtrate to a 25-mL volumetric flask, and dilute with *Medium* to volume. Pass the resulting solution through a suitable filter of 0.45-µm pore size. Use this solution within 6.5 h.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 313 nm

Path length: 1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of entacapone ($C_{14}H_{15}N_3O_5$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

D = dilution factor for the *Sample solution*, 12.5

Tolerances: NLT 80% (Q) of the labeled amount of entacapone ($C_{14}H_{15}N_3O_5$) is dissolved.

www.webofpharma.com

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: pH 5.5 phosphate buffer (6.8 g/L of monobasic potassium phosphate and 0.05 g/L of sodium hydroxide in water, adjusted with 5 M sodium hydroxide to a pH of 5.5); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard stock solution: 0.45 mg/mL of USP Entacapone RS prepared as follows. Transfer a suitable quantity of USP Entacapone RS to an appropriate volumetric flask, and dissolve in 30% of the flask volume of methanol. Sonicate to dissolve, and allow the solution to cool to room temperature. Dilute with methanol to volume. Use this solution within 6 h.
Standard solution: 0.009 mg/mL of USP Entacapone RS from the *Standard stock solution* in *Medium*. Use this solution within 6 h.
Sample stock solution: Pass a portion of the solution through a suitable filter of 0.45-µm pore size. Use this solution within 6 h.
Sample solution: Transfer 2.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Medium* to volume. Use this solution within 6 h.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 378 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of entacapone ($C_{14}H_{15}N_3O_5$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*, 25

Tolerances: NLT 70% (Q) of the labeled amount of entacapone ($C_{14}H_{15}N_3O_5$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Protect solutions from light.
Buffer, Diluent, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.03 mg/mL each of USP Entacapone RS and USP Entacapone Related Compound A RS in *Diluent*
Standard solution: 0.003 mg/mL of USP Entacapone RS in *Diluent*
Sample solution: Nominally 3 mg/mL of entacapone prepared as follows. Finely powder NLT 20 Tablets, and transfer a suitable portion of the powder to an appropriate volumetric flask. Add NLT 30% of the final flask volume of tetrahydrofuran, and sonicate for 3 min. Add NLT 30% of the final flask volume of methanol, and shake for 5 min. Dilute with methanol to volume. Centrifuge a portion of this solution, and use the supernatant within 7 h.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between entacapone related compound A and entacapone, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of entacapone from the *Standard solution*
C_S = concentration of USP Entacapone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of entacapone in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

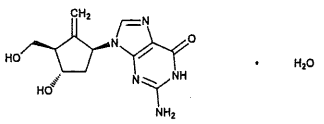
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Entacapone related compound A	0.8	0.2
Entacapone	1.0	—
Any individual unspecified degradation product	—	0.1
Total impurities[a]	—	0.2

[a] Do not include entacapone related compound A in the calculation of total impurities.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in light-resistant containers. Store at controlled room temperature.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Entacapone RS
USP Entacapone Related Compound A RS
(*Z*)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-*N*,*N*-diethylacrylamide.
$C_{14}H_{15}N_3O_5$ 305.29

Entecavir



$C_{12}H_{15}N_5O_3 \cdot H_2O$ 295.29
6*H*-Purin-6-one, 2-amino-1,9-dihydro-9-[(1*S*,3*R*,4*S*)-4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-, monohydrate;
9-[(1*S*,3*R*,4*S*)-4-Hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]guanine monohydrate [209216-23-9].
Anhydrous 277.28

www.webofpharma.com

DEFINITION

Entecavir is a monohydrate and contains NLT 98% and NMT 102% of entecavir ($C_{12}H_{15}N_5O_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Acetonitrile and water (3:97)
Solution B: Acetonitrile
Mobile phase: See *Table 1*. [NOTE—The gradient elution times are established on an HPLC system with a dwell volume of approximately 1.0 mL.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
8	100	0
50	77	23
75	17	83
90	100	0
100	100	0

System suitability stock solution: 1.0 mg/mL of USP Entecavir System Suitability Mixture RS in methanol
System suitability solution: 0.2 mg/mL of USP Entecavir System Suitability Mixture RS in *Solution A* from *System suitability stock solution*
Standard stock solution: 1.0 mg/mL of USP Entecavir Monohydrate RS in methanol. Sonicate as needed.
Standard solution: 0.2 mg/mL of USP Entecavir Monohydrate RS in *Solution A* from the *Standard stock solution*
Sample stock solution: 1.0 mg/mL of Entecavir in methanol. Sonicate as needed.
Sample solution: 0.2 mg/mL of Entecavir in *Solution A* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times of the components in the *System suitability solution*.]
Suitability requirements
Resolution: NLT 3.5 between entecavir 1-epimer and entecavir; NLT 2.0 between entecavir and 8-hydroxy entecavir, *System suitability solution*
Tailing factor: 0.8–1.5 for entecavir, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of entecavir ($C_{12}H_{15}N_5O_3$) in the portion of Entecavir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of entecavir from the *Sample solution*
r_S = peak response of entecavir from the *Standard solution*
C_S = concentration of USP Entecavir Monohydrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Entecavir in the *Sample solution* (mg/mL)

Acceptance criteria: 98%–102% on the anhydrous basis

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, System suitability stock solution, System suitability solution, Sample stock solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: Use the *Standard solution* from the *Assay*.
Standard solution: 0.2 µg/mL of USP Entecavir Monohydrate RS in *Solution A* from the *Standard stock solution*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times of the components in the *System suitability solution*.]
Suitability requirements
Resolution: NLT 3.5 between entecavir 1-epimer and entecavir; NLT 2.0 between entecavir and 8-hydroxy entecavir, *System suitability solution*
Tailing factor: 0.8–1.5 for entecavir, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Entecavir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of entecavir from the *Standard solution*
C_S = concentration of USP Entecavir Monohydrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Entecavir in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Furoentecavir[a]	0.73	1.0	0.1
Entecavir 1-epimer[b]	0.93	1.0	0.1
Entecavir 3-epimer[c]	0.96	1.0	0.1

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Entecavir	1.0	—	—
8-Hydroxy entecavir[d]	1.03	0.67	0.1
Entecavir 4-epimer[e]	1.08	1.0	0.1
8-Methoxy entecavir[f]	1.27	0.67	0.1
4-Dimethylsilyl entecavir[g]	1.84	1.0	0.1
Entecavir related compound A	3.41	—	—[h]
Any unspecified impurity	—	1.0	0.1
Total impurities[i]	—	—	0.3

[a] 9-[(3a*S*,4*S*,6*S*,6a*R*)-3a,6-Dihydroxyhexahydro-1*H*-cyclopenta[*c*]furan-4-yl] guanine.
[b] 9-[(1*R*,3*R*,4*S*)-4-Hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl] guanine.
[c] 9-[(1*S*,3*S*,4*S*)-4-Hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl] guanine.
[d] 8-Hydroxy-9-[(1*S*,3*R*,4*S*)-4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]guanine.
[e] 9-[(1*S*,3*R*,4*R*)-4-Hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl] guanine.
[f] 8-Methoxy-9-[(1*S*,3*R*,4*S*)-4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]guanine.
[g] 9-[(1*S*,3*R*,4*S*)-4-Hydroxydimethylsilyl-3-(hydroxymethyl)-2-methylenecyclopentyl]guanine.
[h] For information only; quantitated in the test for *Limit of Entecavir Related Compound A*.
[i] Includes the sum of all the impurities found in the tests for *Limit of Entecavir Related Compound A* and *Organic Impurities*.

• **Limit of Entecavir Related Compound A**
Solution A: 0.1% (v/v) trifluoroacetic acid in water
Solution B: 0.1% (v/v) trifluoroacetic acid in acetonitrile
Mobile phase: See *Table 3*. [Note—The gradient elution times are established on an HPLC system with a dwell volume of approximately 1.0 mL.]

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	65	35
8	53	47
8.1	65	35
11	65	35

Standard solution: 2 µg/mL of USP Entecavir Related Compound A RS in methanol
Sample solution: 1.0 mg/mL of Entecavir in methanol. Sonicate as needed.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 5-cm; 5-µm packing L1
Temperatures
Autosampler: 4°
Column: 30°
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.8–1.5
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of entecavir related compound A in the portion of Entecavir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of entecavir related compound A from the *Sample solution*
r_S = peak response of entecavir related compound A from the *Standard solution*
C_S = concentration of USP Entecavir Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Entecavir in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS
• **Water Determination** ⟨921⟩, *Method I, Method Ic*: 5.5%–7.0%
• *Optical Rotation* ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL of Entecavir in a mixture of dimethylformamide and methanol (50:50)
Acceptance criteria: +24° to +30°

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers, protected from light. Store at room temperature.
• *USP Reference Standards* ⟨11⟩
USP Entecavir Monohydrate RS
USP Entecavir Related Compound A RS
3-Benzyl-4-silyl entecavir;
9-[(1*S*,3*R*,4*S*)-4-Dimethylphenylsilyl-3-(benzyloxymethyl)-2-methylenecyclopentyl]guanine.
$C_{27}H_{31}N_5O_2Si$ 485.65
USP Entecavir System Suitability Mixture RS
The mixture contains entecavir monohydrate and the following impurities (other impurities may also be present):
Entecavir 1-epimer.
8-Hydroxy entecavir.
8-Methoxy entecavir.

Add the following:

▲Entecavir Oral Solution

DEFINITION
Entecavir Oral Solution contains NLT 90.0% and NMT 105.0% of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Solution A: 19.1 g/L of sodium tetraborate, decahydrate in water. Add 35 mL of methanol per liter of the solution. Pass through a suitable filter of 0.45-µm pore size.

www.webofpharma.com

Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
12	100	0
30	68.5	31.5
31	50	50
34	50	50
35	100	0
43	100	0

Diluent: Methanol and water (20:80)
System suitability solution: 0.05 mg/mL of USP Entecavir Monohydrate RS and 1.5 mg/mL of methylparaben prepared as follows. Transfer an amount of USP Entecavir Monohydrate RS and methylparaben to a suitable volumetric flask and add about 5% of the flask volume of methanol. Sonicate to dissolve. Dilute with *Diluent* to volume.
Standard stock solution: 0.2 mg/mL of USP Entecavir Monohydrate RS prepared as follows. Transfer an amount of USP Entecavir Monohydrate RS to a suitable volumetric flask and add about 4% of the flask volume of methanol. Sonicate to dissolve. Dilute with *Diluent* to volume.
Standard solution: 0.05 mg/mL of USP Entecavir Monohydrate RS in *Diluent* from *Standard stock solution*
Sample solution: Nominally 0.05 mg/mL of entecavir from a measured volume of Oral Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for entecavir and methylparaben are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between entecavir and methylparaben, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of entecavir from the *Sample solution*
r_S = peak response of entecavir from the *Standard solution*
C_S = concentration of USP Entecavir Monohydrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of entecavir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–105.0%

PERFORMANCE TESTS
• **DELIVERABLE VOLUME** ⟨698⟩
For multiple-unit containers: Meets the requirements

IMPURITIES
• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Standard stock solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Entecavir	1.0	—
Epimer-1[a]	1.5	1.0
Epimer-2[a]	1.6	1.0
Individual unspecified impurity	—	0.5
Total impurities	—	2.2

[a] 2-({9-[(1*S*,3*R*,4*S*)-4-Hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl}amino)propanoic acid. Configuration of epimers may be interchangeable.

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/mL. The total yeasts and molds count is NMT 10^1 cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Entecavir Monohydrate RS▲ 2S (USP41)

Entecavir Tablets

DEFINITION
Entecavir Tablets contain NLT 90.0% and NMT 105.0% of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$).

www.webofpharma.com

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Acetonitrile, trifluoroacetic acid, and water (1:0.1:99)

Solution B: Acetonitrile, trifluoroacetic acid, and water (30:0.1:70)

Mobile phase: See *Table 1*. [NOTE—The gradient elution times are established on an HPLC system with a dwell volume of approximately 1.1 mL.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3.5	100	0
21	69	31
24	51	49
27	0	100
28	100	0
35	100	0

Diluent: 0.01 N hydrochloric acid

Standard stock solution: 0.2 mg/mL of USP Entecavir Monohydrate RS prepared as follows. Transfer a suitable quantity of USP Entecavir Monohydrate RS into an appropriate volumetric flask. Dissolve in NMT 20% of the flask volume of methanol, and sonicate if necessary. Dilute with *Diluent* to volume.

Standard solution: 10 µg/mL of USP Entecavir Monohydrate RS in *Diluent* from *Standard stock solution*

Sample solution: Nominally 10 µg/mL of entecavir prepared as follows. Transfer NLT 5 Tablets into an appropriate volumetric flask. Add 80% of the flask volume of *Diluent*, and sonicate for 30 min. Cool to room temperature. Dilute with *Diluent* to volume, and centrifuge for 10 min. Pass the supernatant through a suitable filter, and use the filtrate for analysis.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Temperatures

Column: 30°

Autosampler: 4°

Flow rate: 1 mL/min

Injection volume: 75 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Entecavir Monohydrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of entecavir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–105.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: Simulated intestinal fluid TS without enzyme; 1000 mL

Apparatus 2: 50 rpm

Time: 30 min

Mobile phase: Acetonitrile and water (8:92)

Standard stock solution: 0.1 mg/mL of USP Entecavir Monohydrate RS in *Medium* prepared as follows. Transfer a suitable amount of USP Entecavir Monohydrate RS to a suitable flask and add *Medium* to about 66% of the flask volume. Sonicate until dissolved. Dilute with *Medium* to volume.

Standard solution: Dilute an appropriate volume from the *Standard stock solution* in *Medium* to obtain a similar concentration as the *Sample solution*. Prepare fresh on the day of use.

Sample solution: Pass the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 100 µL

Run time: NLT 2 times the retention time of entecavir

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of entecavir from the *Sample solution*

r_S = peak response of entecavir from the *Standard solution*

C_S = concentration of USP Entecavir Monohydrate RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 1000 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: pH 6.8 phosphate buffer; 1000 mL

Apparatus 2: 50 rpm

Time: 15 min

Buffer: 2 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 5.0. Pass through a suitable filter.

Mobile phase: Acetonitrile and *Buffer* (7:93)

Standard stock solution: 0.6 mg/mL of USP Entecavir Monohydrate RS in methanol prepared as follows. Transfer a suitable amount of USP Entecavir Monohydrate

www.webofpharma.com

RS to a suitable volumetric flask and add methanol to about 20% of the flask volume. Sonicate until dissolved. Dilute with methanol to volume.
Standard solution: Dilute an appropriate volume from the *Standard stock solution* in *Medium* to obtain a similar concentration as the *Sample solution*.
Sample solution: Pass the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 100 µL
Run time: NLT 2 times the retention time of entecavir
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of entecavir from the *Sample solution*
r_S = peak response of entecavir from the *Standard solution*
C_S = concentration of USP Entecavir Monohydrate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of entecavir ($C_{12}H_{15}N_5O_3$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES
• **Organic Impurities**
Solution A, Solution B, Mobile phase, Diluent, Standard stock solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

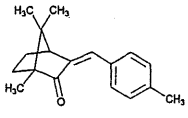
r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: Disregard any peak less than 0.10%.
Individual impurities: NMT 0.5%
Total impurities: NMT 2.0%

SPECIFIC TESTS
• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count is NMT 10^3 cfu/g. The total yeasts and molds count is NMT 10^2 cfu/g. It meets the requirements of the tests for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.
• **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP Reference Standards** ⟨11⟩
USP Entecavir Monohydrate RS

Enzacamene



$C_{18}H_{22}O$ 254.37
Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-3-[(4-methylphenyl)methylene]-;
(±)-3-(*p*-Methylbenzylidene)camphor [36861-47-9].

DEFINITION
Enzacamene contains NLT 98.0% and NMT 102.0% of enzacamene ($C_{18}H_{22}O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 10 µg/mL in methanol
Acceptance criteria: Meets the requirements

ASSAY
• **Procedure**
Standard solution: 250 µg/mL each of camphor and USP Methyl Benzylidene Camphor RS in dichloromethane
Sample solution: 250 µg/mL of Enzacamene in dichloromethane
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m fused-silica capillary; coated with a 0.25-µm layer of phase G1
Temperatures
Injection port: 295°
Detector: 305°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	—	100	5
100	10	230	10
230	10	300	—

USP Monographs

www.webofpharma.com

The column temperature is maintained at 300° until the end of the procedure.
Carrier gas: Helium
Flow rate: 1.2 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.0 for the enzacamene peak
Relative standard deviation: NMT 2.0% for the enzacamene peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of enzacamene ($C_{18}H_{22}O$) in the portion of Enzacamene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response of enzacamene from the *Standard solution*
C_S = concentration of USP Methyl Benzylidene Camphor RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **PROCEDURE**
Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Enzacamene taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of enzacamene from the *Standard solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Camphor[a]	0.2	0.02
Enzacamene	1.0	—
Any individual unspecified impurity	—	0.1
Total impurities	—	0.5

[a] Bicyclo[2.2.1]heptane-2-one, 1,7,7-trimethyl.

SPECIFIC TESTS

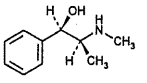
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry it under vacuum at 50° to constant weight.
Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Methyl Benzylidene Camphor RS
3-(4-Methylbenzylidene)-camphor.
$C_{18}H_{22}O$ 254.37
[NOTE—It is the same as Enzacamene.]

Ephedrine



$C_{10}H_{15}NO$ 165.23
Benzenemethanol, α-[1-(methylamino)ethyl]-, [*R*-(*R**,*S**)]-.
(–)-Ephedrine [299-42-3].
Hemihydrate 174.24
[50906-05-3].

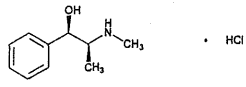
» Ephedrine is anhydrous or contains not more than one-half molecule of water of hydration. It contains not less than 98.5 percent and not more than 100.5 percent of $C_{10}H_{15}NO$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers, in a cold place.
Labeling—Label it to indicate whether it is hydrous or anhydrous. Where the quantity of Ephedrine is indicated in the labeling of any preparation containing Ephedrine, this shall be understood to be in terms of anhydrous Ephedrine.
USP Reference standards ⟨11⟩—
USP Ephedrine Sulfate RS
Identification—Accurately weigh about 100 mg, and add by buret the exact volume of 0.1 N sulfuric acid, determined in the *Assay*, to neutralize it. Dilute with water in a volumetric flask to 25 mL. Mix 2 mL with 10 mL of alcohol, and evaporate on a steam bath with the aid of a current of air to dryness: the residue so obtained responds to *Identification* test *A* under *Ephedrine Sulfate*.
Specific rotation ⟨781S⟩: between –40.3° and –43.3°.
Test solution: 25 mg per mL, in 0.6 N hydrochloric acid.
Water Determination, *Method Ib* ⟨921⟩ : between 4.5% and 5.5%, for hydrated Ephedrine; not more than 0.5% for anhydrous Ephedrine.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chloride ⟨221⟩—A solution of 500 mg shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.030%).
Sulfate—Dissolve 100 mg in 40 mL of water, and add 1 mL of 3 N hydrochloric acid and 1 mL of barium chloride TS: no turbidity develops within 10 minutes.

Ordinary impurities ⟨466⟩—
Test solution: methanol.
Standard solution: methanol.
Eluant: a mixture of isopropyl alcohol, ammonium hydroxide, and chloroform (80:15:5).
Visualization: 1, followed by 4.
Assay—Dissolve about 500 mg of Ephedrine, accurately weighed, in 10 mL of neutralized alcohol, and add 5 drops of methyl red TS and 40.0 mL of 0.1 N hydrochloric acid VS. Titrate the excess acid with 0.1 N sodium hydroxide VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 0.1 N hydrochloric acid is equivalent to 16.52 mg of $C_{10}H_{15}NO$.

www.webofpharma.com

Ephedrine Hydrochloride



$C_{10}H_{15}NO \cdot HCl$ 201.69

Benzenemethanol, α-[1-(methylamino)ethyl]-, hydrochloride, [*R*-(*R**,*S**)]-;
(-)-Ephedrine hydrochloride [50-98-6].

DEFINITION

Change to read:

Ephedrine Hydrochloride contains NLT 98.0% and NMT ▲102.0%▲ (USP 1-Aug-2019) of ephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL ⟨191⟩, *Chemical Identification Tests*, *Chloride*:** Meets the requirements

Add the following:

▲• **C.** The retention time of the ephedrine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

- **PROCEDURE**

▲Buffer: 11.6 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 4.0.
Mobile phase: Methanol and *Buffer* (6:94)
Diluent: Methanol and water (6:94)
System suitability solution: 0.1 mg/mL each of USP Ephedrine Hydrochloride RS and USP Pseudoephedrine Hydrochloride RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Ephedrine Hydrochloride RS in *Diluent*
Sample solution: 0.2 mg/mL of Ephedrine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 257 nm
Column: 4.6-mm × 15-cm; 3-µm packing L11
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of ephedrine
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for ephedrine and pseudoephedrine are 1.0 and 1.1, respectively.]
Suitability requirements
Resolution: NLT 2.0 between ephedrine and pseudoephedrine, *System suitability solution*
Tailing factor: NMT 2.0 for ephedrine, *Standard solution*
Relative standard deviation: NMT 0.73% for ephedrine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Ephedrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ephedrine from the *Sample solution*
r_S = peak response of ephedrine from the *Standard solution*
C_S = concentration of USP Ephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Ephedrine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis▲ (USP 1-Aug-2019)

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%
- **CHLORIDE AND SULFATE ⟨221⟩, *Sulfate***

Sample solution: 1.25 mg/mL of Ephedrine Hydrochloride in water
Analysis: Add 1 mL of 3 N hydrochloric acid and 1 mL of barium chloride TS to 40 mL of the *Sample solution*.
Acceptance criteria: No turbidity develops within 10 min.

Change to read:

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*, except for the *Run time*.
Run time: ▲NLT▲ (USP 1-Aug-2019) 2.5 times the retention time of ephedrine
System suitability solution: 0.1 mg/mL each of USP Ephedrine Hydrochloride RS and USP Pseudoephedrine Hydrochloride RS in *Mobile phase*
Sensitivity solution: 3.8 µg/mL of USP Ephedrine Hydrochloride RS in *Mobile phase*
Standard solution: 30 µg/mL of USP Ephedrine Hydrochloride RS in *Mobile phase*
Sample solution: 7.5 mg/mL of Ephedrine Hydrochloride in *Mobile phase*
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between ephedrine and pseudoephedrine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲α-acetylbenzyl alcohol or any unspecified▲ (USP 1-Aug-2019) impurity in the portion of Ephedrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of ▲α-acetylbenzyl alcohol or any unspecified▲ (USP 1-Aug-2019) impurity from the *Sample solution*
r_S = peak response of ephedrine from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Ephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Ephedrine Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. ▲The reporting threshold is 0.05%.▲ (USP 1-Aug-2019)

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ephedrine	1.0	—	—
Pseudoephedrine[a]	1.1	—	—
α-Acetylbenzyl alcohol[b]	1.4	2.5	0.2
Any unspecified impurity	—	1.0	0.1
Total impurities[c]	—	—	0.5

[a] Included for identification only. It is not to be reported and not to be included in the total impurities.
[b] (-)-(1*R*)-1-Hydroxy-1-phenylpropan-2-one.
[c] Excludes α-acetylbenzyl alcohol.

SPECIFIC TESTS
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 50 mg/mL of Ephedrine Hydrochloride in water
  Acceptance criteria: −33.0° to −35.5°
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 0.5%
- **ACIDITY OR ALKALINITY**
  Sample solution: 50 mg/mL of Ephedrine Hydrochloride in water
  Analysis: To 20 mL of *Sample solution* add 1 drop of methyl red TS.
  Acceptance criteria: If the solution is yellow, it is changed to red by NMT 0.10 mL of 0.020 N sulfuric acid. If the solution is pink, it is changed to yellow by NMT 0.20 mL of 0.020 N sodium hydroxide.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ephedrine Hydrochloride RS
  USP Pseudoephedrine Hydrochloride RS

Ephedrine Sulfate

$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ 428.54
Benzenemethanol, α-[1-(methylamino)ethyl]-, [*R*-(*R**,*S**)]-, sulfate (2:1) (salt).
(−)-Ephedrine sulfate (2:1) (salt) [134-72-5].

» Ephedrine Sulfate contains not less than 98.0 percent and not more than 101.0 percent of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Ephedrine Sulfate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: A solution of it responds to the tests for *Sulfate* ⟨191⟩.
Specific rotation ⟨781S⟩: between −30.5° and −32.5°.
Test solution: 50 mg per mL, in water.
Acidity or alkalinity—Dissolve 1.0 g in 20 mL of water, and add 1 drop of methyl red TS. If the solution is yellow, it is changed to red by not more than 0.10 mL of 0.020 N sulfuric acid. If the solution is pink, it is changed to yellow by not more than 0.20 mL of 0.020 N sodium hydroxide.
Loss on drying ⟨731⟩—Dry about 500 mg, accurately weighed, at 105° for 3 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chloride ⟨221⟩—A 200-mg portion shows no more chloride than corresponds to 0.40 mL of 0.020 N hydrochloric acid (0.14%).

Ordinary impurities ⟨466⟩—
Test solution: alcohol.
Standard solution: alcohol.
Eluant: a mixture of isopropyl alcohol, ammonium hydroxide, and chloroform (80:15:5).
Visualization: 1, followed by 4.
Assay—Transfer about 300 mg of Ephedrine Sulfate, accurately weighed, to a separator, and dissolve in about 10 mL of water. Saturate the solution with sodium chloride (about 3 g), add 5 mL of 1 N sodium hydroxide, and extract with four 25-mL portions of chloroform. Wash the combined chloroform extracts by shaking with 10 mL of a saturated solution of sodium chloride, and filter through chloroform-saturated purified cotton into a beaker. Extract the wash solution with 10 mL of chloroform, and add to the main chloroform extract. Add methyl red TS, and titrate with 0.1 N perchloric acid in dioxane VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 21.43 mg of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$.

Ephedrine Sulfate Capsules

» Ephedrine Sulfate Capsules contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Ephedrine Sulfate RS
Identification—Macerate the contents of a sufficient number of Capsules, equivalent to about 200 mg of ephedrine sulfate, with 15 mL of warm alcohol for 20 minutes, filter, and evaporate the filtrate on a steam bath to dryness: the residue so obtained responds to the *Identification* tests under *Ephedrine Sulfate*.

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 1: 100 rpm.

USP Monographs

www.webofpharma.com

Time: 30 minutes.

Procedure—Dilute filtered portions of the solutions under test with water to a concentration of about 25 µg per mL. Transfer 5.0-mL portions to suitable tubes. Add 1 mL of a saturated sodium carbonate solution and 2 mL of sodium metaperiodate solution (2 in 100) to each, mix, and allow to stand for 10 minutes. Add 20.0 mL of hexanes, shake for 30 seconds, and allow the phases to separate. Measure the absorbances of the hexanes extract in 1-cm cells at the wavelength of maximum absorbance, at about 242 nm, with a suitable spectrophotometer, using hexanes as the blank. Determine the amount of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ dissolved by comparison with a similarly treated Standard solution having a known concentration of USP Ephedrine Sulfate RS in water. Remove the contents of 1 Capsule as completely as possible, with the aid of a current of air, dissolve the empty capsule shell in the *Medium*, determine the absorbance at the same dilution and in the same manner as for the Capsules, and make any necessary corrections.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Standard preparation—Weigh accurately about 25 mg of USP Ephedrine Sulfate RS, transfer to a 50-mL volumetric flask with the aid of 10 mL of water, add methanol to volume, and mix. Dilute 5.0 mL of this solution with water to 100.0 mL.

Assay preparation—Weigh accurately the contents of not less than 20 Capsules, and mix. Transfer an accurately weighed portion of the mixture, equivalent to about 25 mg of ephedrine sulfate, to a glass-stoppered conical flask, and add by pipet 50 mL of a 1 in 5 mixture of water in methanol. Shake by mechanical means for 10 minutes, and filter. Dilute 5.0 mL of the filtrate with water to 100.0 mL.

Procedure—Transfer 5-mL portions of the *Assay preparation* and the *Standard preparation* to separate glass-stoppered, 50-mL centrifuge tubes. Add 1 mL of saturated sodium carbonate solution and 2 mL of sodium metaperiodate solution (1 in 50) to each tube, mix, and allow to stand for 10 minutes. Pipet 20 mL of *n*-hexane into each tube, shake for 30 seconds, and allow the phases to separate. Concomitantly determine the absorbances of the *n*-hexane extracts in 1-cm cells at the wavelength of maximum absorbance at about 242 nm, with a suitable spectrophotometer, using *n*-hexane as the blank. Calculate the quantity, in mg, of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ in the portion of Capsule contents taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Ephedrine Sulfate RS in the *Standard preparation*, and A_U and A_S are the absorbances of the hexane extracts of the *Assay preparation* and the *Standard preparation*, respectively.

Ephedrine Sulfate Injection

DEFINITION

Ephedrine Sulfate Injection is a sterile solution of Ephedrine Sulfate in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of ephedrine sulfate $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Mix 1 mL of Injection with 5 mL of alcohol, and evaporate on a steam bath with the aid of a current of air to dryness.

Acceptance criteria: The residue obtained from the *Sample* meets the requirements.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: To 1 L of water add 4 mL of triethylamine. Adjust with phosphoric acid to a pH of 4.0.

Mobile phase: Acetonitrile and *Solution A* (1:99)

System suitability solution: 0.1 mg/mL of USP Ephedrine Sulfate RS and 0.01 mg/mL of USP Pseudoephedrine Sulfate RS in water

Standard solution: 0.1 mg/mL of USP Ephedrine Sulfate RS in water

Sample solution: Nominally 0.1 mg/mL of ephedrine sulfate in water prepared as follows. Transfer a portion of Injection to a suitable volumetric flask. Dilute with water to volume, and mix well.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 206 nm

Column: 2.1-mm × 10-cm; 2.6-µm packing L11

Column temperature: 30°

Flow rate: 0.6 mL/min

Injection volume: 10 µL

Run time: NLT 3 times the retention time of ephedrine

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between ephedrine and pseudoephedrine, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ephedrine sulfate $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$ in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ephedrine from the *Sample solution*

r_S = peak response of ephedrine from the *Standard solution*

C_S = concentration of USP Ephedrine Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ephedrine sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

USP Monographs

www.webofpharma.com

Standard solution: 1.0 µg/mL of USP Ephedrine Sulfate RS in water
Sample solution: Nominally 500 µg/mL of ephedrine sulfate in water prepared as follows. Transfer a portion of Injection to a suitable volumetric flask. Dilute with water to volume, and mix well.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between ephedrine and pseudoephedrine, *System suitability solution*
Relative standard deviation: NMT 2.0% for ephedrine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of ephedrine from the *Standard solution*
C_S = concentration of USP Ephedrine Sulfate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of ephedrine in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ephedrine	1.0	—
Pseudoephedrine[a]	1.2	—
Any individual impurity	—	0.2
Total impurities	—	2.0

[a] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities of the drug product.

SPECIFIC TESTS
- **PH** ⟨791⟩: 4.5–7.0
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 1.7 USP Endotoxin Units/mg of ephedrine sulfate

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose, light-resistant containers, preferably of Type I glass. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Ephedrine Sulfate RS
USP Pseudoephedrine Sulfate RS
$(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ 428.54

Ephedrine Sulfate Nasal Solution

» Ephedrine Sulfate Nasal Solution contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Ephedrine Sulfate RS
Identification—It responds to the *Identification* tests under *Ephedrine Sulfate Injection*.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Assay—
Standard preparation—Weigh accurately about 26 mg of USP Ephedrine Sulfate RS, transfer to a 50-mL volumetric flask with the aid of 10 mL of water, add methanol to volume, and mix. Pipet 5 mL of the resulting solution into a 100-mL volumetric flask, dilute with water to volume, and mix.
Assay preparation—Transfer an accurately measured volume of Nasal Solution, equivalent to about 26 mg of ephedrine sulfate, to a 50-mL volumetric flask, dilute with a 1 in 5 mixture of water in methanol to volume, and mix. Pipet 5 mL of the resulting solution into a 100-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Transfer 5-mL portions of the *Assay preparation* and the *Standard preparation* to separate glass-stoppered, 50-mL centrifuge tubes. Add 1 mL of saturated sodium carbonate solution and 2 mL of sodium metaperiodate solution (1 in 50) to each tube, mix, and allow to stand for 10 minutes. Pipet 20 mL of *n*-hexane into each tube, shake for 30 seconds, and allow the phases to separate. Concomitantly determine the absorbances of the *n*-hexane extracts in 1-cm cells at the wavelength of maximum absorbance at about 242 nm, with a suitable spectrophotometer, using *n*-hexane as the blank. Calculate the quantity, in mg, of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ in each mL of the Nasal Solution taken by the formula:

$$(C/V)(A_U/A_S)$$

in which V is the volume, in mL, of Nasal Solution taken, C is the concentration, in µg per mL, of USP Ephedrine Sulfate RS in the *Standard preparation*, and A_U and A_S are the absorbances of the hexane extracts of the *Assay preparation* and the *Standard preparation*, respectively.

Ephedrine Sulfate Oral Solution

» Ephedrine Sulfate Oral Solution contains, in each 100 mL, not less than 360 mg and not more than 440 mg of ephedrine sulfate $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
USP Reference standards ⟨11⟩—
USP Ephedrine Sulfate RS
Identification, *Angular rotation* ⟨781A⟩—Use the 0.1 N sulfuric acid extract of the chloroform solution obtained as

USP Monographs

www.webofpharma.com

directed for *Assay preparation:* the angular rotation is levorotatory.

Alcohol Determination ⟨611⟩ **:** between 2.0% and 4.0% of C_2H_5OH.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Ephedrine Sulfate RS in 0.1 N sulfuric acid to obtain a solution having a known concentration of about 20 µg per mL.

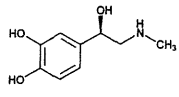
Assay preparation—Transfer 5 mL of Oral Solution to a separator, add 1 mL of 1 N sulfuric acid, and extract with 10 mL of chloroform. Discard the extract, and add 5 mL of potassium carbonate solution (1 in 5). After gas evolution has ceased, extract the solution with three 10-mL portions of chloroform, and combine the extracts in a second separator. Extract the chloroform solution with 50.0 mL of 0.1 N sulfuric acid. Filter the acid layer through paper, and dilute 5.0 mL of it with 0.1 N sulfuric acid to 100.0 mL.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Ephedrine Sulfate Capsules*. Calculate the quantity, in mg, of ephedrine sulfate $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$ in the portion of Oral Solution taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Ephedrine Sulfate RS in the *Standard preparation;* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Epinephrine



$C_9H_{13}NO_3$ 183.20

1,2-Benzenediol, 4-[1-hydroxy-2-(methylamino)ethyl]-, (*R*)-; (−)-3,4-Dihydroxy-α-[(methylamino)methyl]benzyl alcohol [51-43-4].

DEFINITION

Epinephrine contains NLT 98.0% and NMT 102.0% of epinephrine ($C_9H_{13}NO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Protect the *Standard solution* and the *Sample solution* from light.]

Buffer: 5.0 g/L of potassium dihydrogen phosphate and 2.6 g/L of sodium octanesulfonate in water. Adjust with phosphoric acid to a pH of 2.8. Pass through a suitable filter of 0.45-µm pore size.

Solution A: Acetonitrile and *Buffer* (5:95)

Solution B: Acetonitrile and *Buffer* (45:55)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	50	50
21	50	50
23	95	5
30	95	5

Standard solution: 0.5 mg/mL of USP Epinephrine RS prepared as follows. To a suitable amount of USP Epinephrine RS in a suitable volumetric flask, add about 10% of the total volume of 0.1 N hydrochloric acid and dilute with *Solution A* to volume.

Sample solution: 0.5 mg/mL of Epinephrine prepared as follows. To a suitable amount of Epinephrine in a suitable volumetric flask, add about 10% of the total volume of 0.1 N hydrochloric acid and dilute with *Solution A* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Temperatures

Autosampler: 4°

Column: 50°

Flow rate: 1.2 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of epinephrine ($C_9H_{13}NO_3$) in the portion of Epinephrine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of epinephrine from the *Sample solution*

r_S = peak response of epinephrine from the *Standard solution*

C_S = concentration of USP Epinephrine RS in the *Standard solution* (mg/mL)

C_U = concentration of Epinephrine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩

Sample: 1 g

Acceptance criteria: NMT 0.1%

- **ENANTIOMERIC PURITY**

Buffer: 1.2 g/L of sodium dihydrogen phosphate in water. Adjust with 0.1 N sodium hydroxide to a pH of 5.8. Add 18.6 mg/L of edetate disodium. Pass through a suitable filter of 0.45-µm pore size.

Mobile phase: Isopropanol and *Buffer* (5:95)

System suitability solution: 0.03 mg/mL of USP Racepinephrine Hydrochloride RS in *Mobile phase*

Sensitivity solution: 0.005 mg/mL of USP Epinephrine RS prepared as follows. To a suitable amount of USP Epinephrine RS in a suitable volumetric flask, add about 2%

www.webofpharma.com

of the total volume of 0.1 N hydrochloric acid and dilute with *Mobile phase* to volume.

Sample solution: 1 mg/mL of Epinephrine in *Mobile phase* prepared as follows. Transfer an appropriate amount of Epinephrine to a suitable volumetric flask and dissolve in 2% of the total volume of 1 N hydrochloric acid. Dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 10-cm; 5-µm packing L108
Column temperature: 25°
Flow rate: 0.9 mL/min
Injection volume: 10 µL
Run time: 15 min

System suitability
Samples: *System suitability solution* and *Sensitivity solution*
[NOTE—The relative retention times for L-epinephrine and D-epinephrine are 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 1.5 between L-epinephrine and D-epinephrine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Sample: *Sample solution*
Calculate the percentage of D-epinephrine ($C_9H_{13}NO_3$) in the portion of Epinephrine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of D-epinephrine from the *Sample solution*
r_T = sum of the peak responses of L-epinephrine and D-epinephrine from the *Sample solution*

Acceptance criteria: NMT 3.0%

• **ORGANIC IMPURITIES**
[NOTE—Protect the *Standard solution* and the *Sample solution* from light.]
Solution A, Solution B, and **Mobile phase:** Prepare as directed in the *Assay*.
System suitability solution: 80 µg/mL each of USP Epinephrine RS and USP Norepinephrine Bitartrate RS prepared as follows. Transfer an accurately weighed amount of USP Epinephrine RS and USP Norepinephrine Bitartrate RS to a suitable volumetric flask. Add 10% of the total volume of 0.1 N hydrochloric acid and dissolve. Dilute with *Solution A* to volume.
Standard solution: 0.8 µg/mL of USP Epinephrine RS, 3.2 µg/mL of USP Norepinephrine Bitartrate RS, and 1.9 µg/mL of USP Adrenalone Hydrochloride RS prepared as follows. Transfer an accurately weighed amount of USP Epinephrine RS, USP Norepinephrine Bitartrate RS, and USP Adrenalone Hydrochloride RS to a suitable volumetric flask. Add 10% of the total volume of 0.1 N hydrochloric acid and dissolve. Dilute with *Solution A* to volume.
Sample solution: 0.8 mg/mL of Epinephrine prepared as follows. Transfer an accurately weighed amount of Epinephrine to a suitable volumetric flask. Add 10% of the total volume of 0.1 N hydrochloric acid and dissolve. Dilute with *Solution A* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Temperatures
Autosampler: 4°
Column: 50°
Flow rate: 1.2 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between epinephrine and norepinephrine, *System suitability solution*
Relative standard deviation: NMT 2.0% for epinephrine, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of norepinephrine in the portion of Epinephrine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of norepinephrine from the *Sample solution*
r_S = peak response of norepinephrine from the *Standard solution*
C_S = concentration of USP Norepinephrine Bitartrate RS in the *Standard solution* (µg/mL)
C_U = concentration of Epinephrine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of norepinephrine, 169.18
M_{r2} = molecular weight of norepinephrine bitartrate monohydrate, 337.28

Calculate the percentage of adrenalone in the portion of Epinephrine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of adrenalone from the *Sample solution*
r_S = peak response of adrenalone from the *Standard solution*
C_S = concentration of USP Adrenalone Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Epinephrine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of adrenalone, 181.19
M_{r2} = molecular weight of adrenalone hydrochloride, 217.65

Calculate the percentage of each specified and any individual, unspecified impurity in the portion of Epinephrine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each specified and any individual, unspecified impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Epinephrine RS in the *Standard solution* (µg/mL)
C_U = concentration of Epinephrine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

www.webofpharma.com

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Epinephrine sulfonate[a]	0.19	1.0	0.2
Norepinephrine[b]	0.81	—	0.2
Epinephrine	1.0	—	—
Oxedrine[c]	1.26	0.5	0.10
Adrenalone[d]	1.34	—	0.2
Epinine[e]	1.57	1.0	0.10
Epinephrine methoxy analog[f]	1.85	1.0	0.10
N-Benzyl epinephrine[g]	3.28	1.4	0.1
N-Benzyl adrenalone[h]	3.66	1.7	0.1
Any individual, unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] (*R*)-1-(3,4-Dihydroxyphenyl)-2-(methylamino)ethanesulfonic acid.
[b] (*R*)-4-(2-Amino-1-hydroxyethyl) benzene-1,2-diol.
[c] 1-(4-Hydroxyphenyl)-2-(methylamino)ethanol.
[d] 3′,4′-Dihydroxy-2-(methylamino)acetophenone.
[e] 4-[2-(Methylamino)ethyl]benzene-1,2-diol.
[f] 4-[1-Methoxy-2-(methylamino)ethyl]benzene-1,2-diol.
[g] (*R*)-4-{2-[Benzyl(methyl)amino]-1-hydroxyethyl}benzene-1,2-diol.
[h] 2-[Benzyl(methyl)amino]-1-(3,4-dihydroxyphenyl)ethan-1-one.

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩
  Analysis: Dry under vacuum over silica gel for 18 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. [Note—For shelf-life stability purposes, packaging under vacuum may be necessary.]
- **USP Reference Standards** ⟨11⟩
  USP Adrenalone Hydrochloride RS
  3′,4′-Dihydroxy-2-(methylamino)acetophenone hydrochloride.
  $C_9H_{11}NO_3 \cdot HCl$ 217.65
  USP Epinephrine RS
  USP Norepinephrine Bitartrate RS
  (*R*)-4-(2-Amino-1-hydroxyethyl)benzene-1,2-diol bitartrate monohydrate.
  $C_8H_{11}NO_3 \cdot C_4H_6O_6 \cdot H_2O$ 337.28
  USP Racepinephrine Hydrochloride RS

Delete the following:

▲Epinephrine Inhalation Aerosol

» Epinephrine Inhalation Aerosol is a solution of Epinephrine in propellants and Alcohol prepared with the aid of mineral acid in a pressurized container. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of epinephrine ($C_9H_{13}NO_3$).

Packaging and storage—Preserve in small, nonreactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

USP Reference standards ⟨11⟩ —
USP Epinephrine Bitartrate RS

Identification—Place 10 mL of water in a small beaker, and deliver 2 sprays from the Inhalation Aerosol under the surface of the water, actuating the valve by pressing the tip against the bottom of the beaker. To 5 mL of the solution add 1 drop of dilute sulfuric acid (1 in 200), add 0.5 mL of 0.1 N iodine, allow to stand for 5 minutes, and add 1 mL of 0.1 N sodium thiosulfate: a red-brown color is produced.

Delivered dose uniformity over the entire contents: meets the requirements for *Metered-Dose Inhalers* under *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* ⟨601⟩.

PROCEDURE FOR DOSE UNIFORMITY—

Ferro-citrate solution and *Buffer solution*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Standard preparation—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in a freshly prepared sodium bisulfite solution (1 in 500), and dilute quantitatively and stepwise with the same sodium bisulfite solution as necessary to obtain a solution having a known concentration of about 18 µg per mL.

Test preparation—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of a freshly prepared sodium bisulfite solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform, insert the stopper, shake vigorously for 1 minute, and centrifuge for 5 minutes. Use the clear supernatant as directed in the *Procedure*.

Procedure—Into three separate flasks, transfer the *Test preparation*, 20.0 mL of the *Standard preparation*, and 20.0 mL of water to provide the blank. To each flask add 100 µL of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation*, at the wavelength of maximum absorbance at about 530 nm, against the blank. Calculate the quantity, in µg, of $C_9H_{13}NO_3$ contained in the minimum dose taken by the formula:

$$(183.20/333.29)(20CN)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*; N is the number of sprays discharged to obtain the minimum recommended dose; 183.20 and 333.29 are the molecular weights of epinephrine and epinephrine bitartrate, respectively; and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

Assay—Weigh the Inhalation Aerosol, chill to a temperature below −30°, remove the valve by suitable means, and allow the Inhalation Aerosol to warm slowly to room temperature to expel the more volatile propellant fractions. Transfer the residues in the aerosol container and valve to a 125-mL separator with the aid of six 5-mL portions of dilute sulfuric acid (1 in 1000), and extract the solution with three 25-mL portions of chloroform. Proceed as directed in the *Assay* under *Epinephrine Nasal Solution*, beginning with "Rinse the stopper

www.webofpharma.com

and mouth of the separator," but use 10.0 mL instead of 5.0 mL of chloroform in the determination of the specific rotation. Dry the empty aerosol container and valve, weigh them, and determine the net weight of the Inhalation Aerosol. Calculate the quantity, in mg, of $C_9H_{13}NO_3$ in the Inhalation Aerosol taken by the formula:

$$(183.20/309.32)(W)(0.5 + 0.5R/93)$$

in which 183.20 and 309.32 are the molecular weights of epinephrine and triacetylepinephrine, respectively, and W is the weight, in mg, and R is the specific rotation (in degrees, without regard to the sign), of the isolated triacetylepinephrine. ▲ 2S (USP41)

Epinephrine Injection

» Epinephrine Injection is a sterile solution of Epinephrine in Water for Injection prepared with the aid of Hydrochloric Acid or other suitable buffers. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of epinephrine ($C_9H_{13}NO_3$).

Packaging and storage—Preserve in single-dose or multiple-dose, light-resistant containers, preferably of Type I glass.
Labeling—The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.
USP Reference standards ⟨11⟩—
USP Epinephrine Bitartrate RS

Color and clarity—
Standard solution—Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Visually examine a portion of the Injection *(Test solution)* in a suitable clear glass test tube against a white background: it is not pinkish and it contains no precipitate. If any yellow color is observed in the *Test solution*, concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm: the absorbance of the *Test solution* does not exceed that of the *Standard solution*.

Identification—
A: It responds to the *Identification* test under *Epinephrine Nasal Solution*.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 357.0 USP Endotoxin Units per mg of epinephrine.
pH ⟨791⟩**:** between 2.2 and 5.0.
Total acidity—Transfer 5.0 mL of Injection to a flask, add 10 mL of water, and titrate with 0.01 N sodium hydroxide VS to a pH of 7.40. Perform a blank determination, and make any necessary correction. Not more than 25.0 mL of 0.01 N sodium hydroxide is required.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase—To 1 L of 0.05 M monobasic sodium phosphate add about 519 mg of sodium 1-octanesulfonate and about 45 mg of edetate disodium, and mix. Adjust by the dropwise addition of phosphoric acid, if necessary, to a pH of 3.8. Mix 85 volumes of this solution with 15 volumes of methanol. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg of epinephrine per mL.
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 1 mg of epinephrine, to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
System suitability preparation—Dissolve 10 mg of dopamine hydrochloride in 100 mL of the *Standard preparation*, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for epinephrine and 2.0 for dopamine hydrochloride; the resolution, *R,* between epinephrine and dopamine hydrochloride is not less than 3.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of epinephrine ($C_9H_{13}NO_3$) in each mL of the Injection taken by the formula:

$$(183.20/333.29)(10)(C/V)(r_U/r_S)$$

in which 183.20 and 333.29 are the molecular weights of epinephrine and epinephrine bitartrate, respectively; C is the concentration, in mg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Epinephrine Inhalation Solution

» Epinephrine Inhalation Solution is a sterile solution of Epinephrine in Purified Water prepared with the aid of Hydrochloric Acid. It contains, in each 100 mL, not less than 0.9 g and not more than 1.15 g of $C_9H_{13}NO_3$.

Packaging and storage—Preserve in small, well-filled, tight, light-resistant containers.
Labeling—The label indicates that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.
Color and clarity—Using the Inhalation Solution as the *Test solution*, proceed as directed for *Color and clarity* under *Epinephrine Injection*.
Identification—It meets the requirements for the *Identification* test under *Epinephrine Nasal Solution*.
Sterility Tests ⟨71⟩: meets the requirements.

USP Monographs

www.webofpharma.com

Assay—Pipet 10 mL of Inhalation Solution into a 125-mL separator, and extract the solution with two 10-mL portions of chloroform. Proceed as directed in the *Assay* under *Epinephrine Nasal Solution*, beginning with "Rinse the stopper and mouth of the separator," but use for the acetylation 1.05 g of sodium bicarbonate and 0.50 mL of acetic anhydride, and extract the acetylated product with six 15-mL portions of chloroform instead of the 25-mL portions specified therein, and use 15.0 mL of chloroform instead of 5.0 mL in the determination of the specific rotation.

Epinephrine Nasal Solution

» Epinephrine Nasal Solution is a solution of Epinephrine in Purified Water prepared with the aid of Hydrochloric Acid. It contains, in each 100 mL, not less than 90 mg and not more than 115 mg of $C_9H_{13}NO_3$.

Packaging and storage—Preserve in small, well-filled, tight, light-resistant containers.
Labeling—The label indicates that the Nasal Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.
Color and clarity—Using the Nasal Solution as the *Test solution*, proceed as directed for *Color and clarity* under *Epinephrine Injection*.
Identification—To 5 mL of pH 4.0 acid phthalate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) add 0.5 mL of Nasal Solution and 1.0 mL of 0.1 N iodine. Mix, and allow to stand for 5 minutes. Add 2 mL of sodium thiosulfate solution (1 in 40): a deep red color is produced.
Assay—Pipet 30 mL of Nasal Solution into a 125-mL separator, add 25 mL of chloroform, shake vigorously for 1 minute, allow the liquids to separate, and discard the chloroform. Wash twice more with chloroform, separating and discarding the lower layer as completely as possible each time. Rinse the stopper and mouth of the separator with a few drops of water. Add 0.2 mL of starch TS, then while swirling the separator add iodine and potassium iodide TS 1 dropwise until the blue color formed persists, and immediately add just sufficient 0.1 N sodium thiosulfate to discharge the blue color. [NOTE—Proceed with the assay from this point without delay.]

Add to the liquid in the separator 2.10 g of sodium bicarbonate, preventing it from coming in contact with the mouth of the separator, and swirl until most of the bicarbonate has dissolved. By means of a 1-mL syringe that is not fitted with a needle, rapidly inject 1.0 mL of acetic anhydride directly into the contents of the separator. Immediately insert the stopper in the separator, and shake vigorously until the evolution of carbon dioxide has ceased (7 to 10 minutes), releasing the pressure as necessary through the stopcock. Allow to stand for 5 minutes, and extract the solution with six 25-mL portions of chloroform, filtering each extract through a small pledget of cotton, previously washed with chloroform, into a beaker.

Evaporate the combined chloroform extracts on a steam bath in a current of air to about 3 mL, transfer the residue by means of small portions of chloroform to a tared 50-mL beaker, and heat again to evaporate the solvent completely. Heat further at 105° for 30 minutes, cool in a desiccator, and weigh the residue of triacetylepinephrine. Add 5.0 mL of chloroform, cover the beaker, gently swirl the contents until the residue has completely dissolved, and determine the specific rotation, *R*, using a 200-mm semimicro polarimeter tube.

Calculate the quantity, in mg, of $C_9H_{13}NO_3$ in the volume of Nasal Solution taken by the formula:

$$(183.20/309.32)(W)(0.5 + 0.5R/93)$$

in which 183.20 and 309.32 are the molecular weights of epinephrine and triacetylepinephrine, respectively; and *W* is the weight, in mg, and *R* is the specific rotation (in degrees, without regard to the sign), of the isolated triacetylepinephrine.

Epinephrine Ophthalmic Solution

» Epinephrine Ophthalmic Solution is a sterile, aqueous solution of Epinephrine prepared with the aid of Hydrochloric Acid. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_9H_{13}NO_3$. It contains a suitable antibacterial agent and may contain an anti-oxidant, suitable buffers, and chelating and tonicity-adjusting agents.

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—The label indicates that the Ophthalmic Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.
USP Reference standards ⟨11⟩—
USP Epinephrine Bitartrate RS
Color and clarity—Using the Ophthalmic Solution as the *Test solution*, proceed as directed for *Color and clarity*— under *Epinephrine Injection*.

Identification—
A: The UV absorption spectrum of the *Assay preparation* prepared as directed in the *Assay* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Epinephrine Bitartrate RS.
B: A solution (1 in 2) is levorotatory.
Sterility Tests ⟨71⟩: meets the requirements.
pH ⟨791⟩: between 2.2 and 4.5.

Assay—
pH 5.8 Buffer—Mix 1 volume of 1 M dibasic potassium phosphate with 9 volumes of 1 M monobasic potassium phosphate. Adjust by the addition of small volumes of either solution to a pH of 5.80 ± 0.05.
Standard preparation—Dissolve a suitable quantity of USP Epinephrine Bitartrate RS, accurately weighed, in 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 40 µg of epinephrine per mL.
Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 20 mg of epinephrine, to a 250-mL beaker containing 2.0 mL of *pH 5.8 Buffer*. Add 9 g of chromatographic siliceous earth, and mix. Carefully transfer the mixture to a 45- × 2.2-cm chromatographic tube containing a pledget of glass wool at the bottom, and tap the column gently to effect packing. Dry-wash the beaker with about 1 g of chromatographic siliceous earth, add to the column, and plug the top with a pledget of glass wool. Wash the column with 100 mL of water-washed ether, and discard the eluant. Add 10.0 mL of 0.1 N hydrochloric acid to a 125-mL separator, and place the

www.webofpharma.com

separator under the column. To about 100 mL of water-washed ether add 1 mL of bis(2-ethylhexyl) phosphoric acid, and elute the column with this solution, collecting the eluate in the separator. Extract the epinephrine into the aqueous acid layer, and carefully transfer the aqueous layer to a 500-mL volumetric flask. Shake the ether layer with two 50-mL portions of 0.1 N hydrochloric acid, add the acidic aqueous extracts to the volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 280 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_9H_{13}NO_3$ in each mL of the Ophthalmic Solution taken by the formula:

$$0.5(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of epinephrine in the *Standard preparation*; V is the volume, in mL, of Ophthalmic Solution taken; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Epinephrine Bitartrate

$C_9H_{13}NO_3 \cdot C_4H_6O_6$ 333.29

1,2-Benzenediol, 4-[1-hydroxy-2-(methylamino)ethyl]-, (*R*)-, [*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1) (salt).

(–)-3,4-Dihydroxy-α-[(methylamino)methyl]benzyl alcohol (+)-tartrate (1:1) salt [51-42-3].

» Epinephrine Bitartrate contains not less than 97.0 percent and not more than 102.0 percent of $C_9H_{13}NO_3 \cdot C_4H_6O_6$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Epinephrine Bitartrate RS
USP Norepinephrine Bitartrate RS

Identification—Dissolve about 500 mg in 20 mL of water containing about 100 mg of sodium bisulfite. Add 6 N ammonium hydroxide until the solution has a distinct odor of ammonia, and allow to stand in a refrigerator for 1 hour. Filter the precipitate, wash it with three 2-mL portions of cold water, then with 5 mL of cold alcohol, and finally with 5 mL of cold ether, and dry in vacuum over silica gel for 3 hours. The epinephrine so obtained responds to the *IDENTIFICATION* test under *Epinephrine*, and its specific rotation (see *Optical Rotation* ⟨781⟩), determined by dissolving 200 mg, accurately weighed, in sufficient dilute hydrochloric acid (1 in 20) to make 10.0 mL, is between –50° and –53.5°.

Melting range ⟨741⟩: between 147° and 152°, with decomposition.

Loss on drying ⟨731⟩—Dry it in vacuum over silica gel for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: negligible, from 100 mg.

Limit of adrenalone—Its absorptivity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) at 310 nm, determined in a solution in dilute hydrochloric acid (1 in 200) containing 4 mg per mL, is not more than 0.2.

Limit of norepinephrine bitartrate—
Epinephrine standard solution—Dilute with methanol an accurately measured volume of an aqueous solution of USP Epinephrine Bitartrate RS containing about 200 mg per mL to obtain a solution having a known concentration of about 20 mg per mL.

Norepinephrine standard solution—Dilute with methanol an accurately measured volume of an aqueous solution of USP Norepinephrine Bitartrate RS containing 8.0 mg per mL to obtain a solution having a known concentration of 0.80 mg per mL.

Test solution—Dissolve 200 mg of Epinephrine Bitartrate in 1.0 mL of water, dilute with methanol to 10.0 mL, and mix.

Procedure—Apply 5-µL portions of *Epinephrine standard solution*, *Norepinephrine standard solution*, and *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in an unsaturated tank using a solvent system consisting of *n*-butanol, water, and formic acid (7:2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in warm circulating air. Spray with Folin-Ciocalteu Phenol TS, followed by sodium carbonate solution (1 to 10): the R_F value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Epinephrine standard solution*. Any spot obtained from the *Test solution* is not larger nor more intense than the spot with the same R_F value obtained from *Norepinephrine standard solution*, corresponding to not more than 4.0% of norepinephrine bitartrate.

Assay—Dissolve about 500 mg of Epinephrine Bitartrate, accurately weighed, in 20 mL of glacial acetic acid, warming slightly if necessary to effect solution. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 33.33 mg of $C_9H_{13}NO_3 \cdot C_4H_6O_6$.

Delete the following:

▲Epinephrine Bitartrate Inhalation Aerosol

» Epinephrine Bitartrate Inhalation Aerosol is a suspension of microfine Epinephrine Bitartrate in propellants in a pressurized container. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of epinephrine bitartrate ($C_9H_{13}NO_3 \cdot C_4H_6O_6$).

Packaging and storage—Preserve in small, nonreactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

USP Reference standards ⟨11⟩—
USP Epinephrine Bitartrate RS

Identification—
A: Place 10 mL of water in a small beaker, and deliver 3 sprays from the Aerosol under the surface of the water, actuating the valve by pressing the tip against the bottom of the beaker. Filter, and to 5 mL of the filtrate add 1 drop of dilute hydrochloric acid (1 in 120). Add 0.5 mL of 0.1 N iodine,

USP Monographs

www.webofpharma.com

allow to stand for 5 minutes, and add 1 mL of 0.1 N sodium thiosulfate: a red-brown color is produced.

B: Actuate the valve of the Aerosol by pressing the tip against a station of a white porcelain spot plate. Cover the spot with 2 or 3 drops of a mixture of 3 volumes of pyridine and 1 volume of acetic anhydride: an emerald-green color is produced.

Delivered dose uniformity over the entire contents: meets the requirements for *Metered-Dose Inhalers* under *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* ⟨601⟩.

PROCEDURE FOR DOSE UNIFORMITY—

Ferro-citrate solution and *Buffer solution*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Standard preparation—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in a freshly prepared sodium bisulfite solution (1 in 500), and dilute quantitatively and stepwise with the same sodium bisulfite solution as necessary to obtain a solution having a known concentration of about 15 µg per mL.

Test preparation—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of a freshly prepared sodium bisulfite solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform, insert the stopper, shake vigorously for 1 minute and centrifuge for 5 minutes. Use the clear supernatant as directed in the *Procedure*.

Procedure—Into three separate flasks, transfer the *Test preparation*, 20.0 mL of the *Standard preparation*, and 20.0 mL of water to provide the blank. To each flask add 100 µL of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation*, at the wavelength of maximum absorbance at about 530 nm, against the blank. Calculate the quantity, in µg of $C_9H_{13}NO_3 \cdot C_4H_6O_6$ contained in the minimum dose taken by the formula:

$$(20CN)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*; N is the number of sprays discharged to obtain the minimum recommended dose; and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

Particle size—Proceed with Epinephrine Bitartrate Inhalation Aerosol as directed in the test for *Particle size* under *Isoproterenol Sulfate Inhalation Aerosol*. It meets the limits of the test.

Assay—

Ferro-citrate solution and *Buffer solution*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Standard preparation—Prepare as directed under *Delivered dose uniformity over the entire contents*.

Assay preparation— [NOTE—A suitable specimen beaker is one having a small indentation formed on its inside bottom surface having dimensions adequate to accept the aerosol valve stem during actuation, thereby preventing particle entrapment and side-of-stem leakage during the delivery of the specimen.] Place 20 mL of chloroform in a suitable 100-mL beaker. Prime the valve of Epinephrine Bitartrate Inhalation Aerosol by alternately shaking and firing it 10 times through its oral inhalation actuator. Accurately weigh the Aerosol, shake it, and immediately deliver a single spray under the surface of the chloroform, actuating the valve by pressing the tip into the indentation in the bottom of the beaker. Raise the Aerosol above the surface of the chloroform, and shake it gently preparatory to delivering another spray similarly under the surface of the chloroform. Deliver a total of 3 sprays in this manner. Rinse the valve stem and ferrule with about 2 mL of chloroform, collecting the rinsing with the specimen in the beaker. Allow the Aerosol to dry, weigh it, and determine the total weight of the 3 sprays. Transfer the solution to a centrifuge tube with the aid of two 3-mL portions of chloroform, and add 10.0 mL of freshly prepared sodium bisulfite solution (1 in 500). Insert the stopper, shake vigorously for 1 minute, centrifuge for 5 minutes, and use the clear supernatant as the *Assay preparation*.

Procedure—Transfer 5.0 mL each of the *Standard preparation* and the *Assay preparation* to separate test tubes. To each tube add 100 µL of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 530 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_9H_{13}NO_3 \cdot C_4H_6O_6$ in each mL of the Aerosol taken by the formula:

$$(0.01Cd/W)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*, d is the density, in g per mL, of the Aerosol, determined as directed for d in the *Procedure* in the *Assay* under *Isoproterenol Sulfate Inhalation Aerosol*, W is the weight, in g, of the specimen taken, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively. ▲ 2S (USP41)

Epinephrine Bitartrate Ophthalmic Solution

» Epinephrine Bitartrate Ophthalmic Solution is a sterile, buffered, aqueous solution of Epinephrine Bitartrate. It contains an amount of epinephrine bitartrate equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of epinephrine ($C_9H_{13}NO_3$). It contains a suitable antibacterial agent and may contain suitable preservatives.

Packaging and storage—Preserve in small, well-filled, tight, light-resistant containers.

Labeling—The label indicates that the Ophthalmic Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

USP Reference standards ⟨11⟩—

USP Epinephrine Bitartrate RS

Color and clarity—Using the Ophthalmic Solution as the *Test solution*, proceed as directed for *Color and clarity*— under *Epinephrine Injection*.

pH ⟨791⟩: between 3.0 and 3.8.

Other requirements—It responds to the *Identification*— test under *Epinephrine Nasal Solution*, and meets the requirements under *Sterility Tests* ⟨71⟩.

USP Monographs

www.webofpharma.com

Assay—

pH 2.5 Buffer—Transfer 6.8 g of monobasic potassium phosphate and 1.1 g of sodium 1-octanesulfonate to a 1-liter volumetric flask. Dissolve in water, dilute with water to volume, and mix. Adjust the solution with phosphoric acid to a pH of 2.5 ± 0.1.

Mobile phase—Prepare a filtered and degassed mixture of *pH 2.5 Buffer* and acetonitrile (850:150). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, freshly mixed and free from air bubbles, equivalent to about 50 mg of epinephrine bitartrate, to a 500-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 3.2-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the column efficiency determined from the analyte peak is not less than 2000 theoretical plates, the tailing factor for the analyte peak is not more than 2.5, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of epinephrine ($C_9H_{13}NO_3$) in each mL of the Ophthalmic Solution taken by the formula:

$$(183.20/333.29)(500C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*, V is the volume, in mL, of Ophthalmic Solution taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Epinephrine Bitartrate for Ophthalmic Solution

» Epinephrine Bitartrate for Ophthalmic Solution is a sterile, dry mixture of Epinephrine Bitartrate and suitable antioxidants, prepared by freeze-drying. It contains an amount of epinephrine bitartrate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of epinephrine ($C_9H_{13}NO_3$).

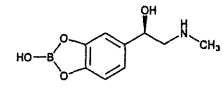
Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*

Completeness of solution ⟨641⟩—A 100-mg portion dissolves in 5 mL of water to yield a clear solution.

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

Other requirements—A solution of it responds to the *Identification*— test under *Epinephrine Nasal Solution*, and meets the requirements of the *Assay*— under *Epinephrine Bitartrate Ophthalmic Solution.* It meets also the requirements under *Sterility Tests* ⟨71⟩ and *Uniformity of Dosage Units* ⟨905⟩.

Epinephryl Borate Ophthalmic Solution



$C_9H_{12}BNO_4$ 209.01

1,3,2-Benzodioxaborole-5-methanol, 2-hydroxy-α-[(methylamino)methyl]-, (*R*)-;

(–)-3,4-Dihydroxy-α-[(methylamino)methyl]benzyl alcohol, cyclic 3,4-ester with boric acid [5579-16-8].

DEFINITION

Epinephryl Borate Ophthalmic Solution is a sterile solution in water of Epinephrine as a borate complex. It contains an amount of epinephryl borate ($C_9H_{12}BNO_4$) equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of epinephrine ($C_9H_{13}NO_3$). It contains a suitable antibacterial agent and one or more suitable preservatives and buffering agents.

IDENTIFICATION

• **A.**

Sample solution: To 5 mL of pH 4.0 acid phthalate buffer (See *Reagents, Indicators, and Solutions—Buffer Solutions*) add 0.5 mL of Ophthalmic Solution and 1 mL of 0.1 N iodine. Mix, allow to stand for 5 min, and add 2 mL of 0.1 N sodium thiosulfate.

Acceptance criteria: A deep red color is produced.

• **B.**

Sample solution: To 5 mL in a porcelain evaporating dish add 5 drops of sulfuric acid and 5 mL of methanol.

Acceptance criteria: The ignited mixture burns with a green-bordered flame.

ASSAY

• **PROCEDURE**

Sample solution: Transfer a volume of Ophthalmic Solution equivalent to 100 mg of epinephrine to a 250-mL separator. Dilute with water to 30 mL, and adjust with dilute hydrochloric acid (1 in 12) to a pH of 4.0 ± 0.2.

Analysis: Add 25 mL of carbon tetrachloride to the *Sample solution.* Shake vigorously for 1 min, allow the phases to separate, and discard the carbon tetrachloride washing. In the same manner, wash with two additional 25-mL portions of carbon tetrachloride, and discard the washings. Rinse the stopper and the mouth of the separator with 2–3 mL of water such that the rinsings enter the separator, and combine with the solution under assay. Add 0.2 mL of starch TS, and, while swirling the separator, add iodine and potassium iodide TS 1 dropwise until the blue color persists. Immediately add a volume of 0.1 N sodium thiosulfate just sufficient to discharge the blue color. Proceed with the *Assay* from this point without delay. Add 2.10 g of sodium bicarbonate through a dry powder funnel to prevent the powder from coming in contact with the mouth of the separator, and swirl to dissolve most of the solid. By means of a syringe fitted with a suitable pipet, rapidly inject 1.0 mL of acetic anhydride directly into the contents of the separator. Swirl the unstoppered separator gently for 3 min to allow carbon dioxide to escape. Insert the stopper, and

USP Monographs

www.webofpharma.com

shake gently until the evolution of carbon dioxide has ceased (7–10 min), releasing the pressure through the stopcock as necessary. Allow to stand for 5 min. Extract with six 25-mL portions of chloroform, shaking for 1 min each time, filtering each extract through a small pledget of chloroform-saturated cotton, and collecting the extracts in a 400-mL beaker. Add several glass beads, and evaporate on a steam bath to about 3 mL. With the aid of 15–20 mL of chloroform, transfer the residue to a tared 50-mL beaker, and evaporate on the steam bath to dryness. Dry the residue at 105° for 30 min, cool in a desiccator, and weigh the triacetylepinephrine. Transfer 10.0 mL of chloroform to the beaker, and gently swirl to dissolve the residue, dislodging the semisolid residue from the glass surface, if necessary, with a small metal spatula. Determine the angular rotation of the solution in a 100-mm polarimeter tube.

Calculate the percentage of epinephrine ($C_9H_{13}NO_3$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (M_{r1}/M_{r2}) \times (W/V) \times (0.5 + 0.5R/93) \times 100 \times (1/L)$$

M_{r1} = molecular weight of epinephrine, 183.20
M_{r2} = molecular weight of triacetylepinephrine, 309.32
W = weight of the isolated triacetylepinephrine (mg)
V = volume of Ophthalmic Solution taken (mL)
R = specific rotation of the triacetylepinephrine solution (°)
L = label claim (mg of epinephrine per mL of Ophthalmic Solution)

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

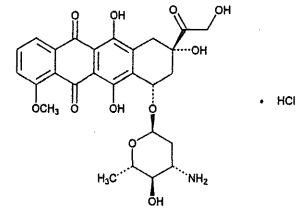
- **pH** ⟨791⟩: 5.5–7.6
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **Color and Clarity**

Standard solution: Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, and dilute with water to volume.
Sample solution: Ophthalmic Solution
Analysis: Visually examine a portion of the *Sample solution* in a suitable clear glass test tube against a white background.
Acceptance criteria: It is not pinkish, and it contains no precipitate. If any yellow color is observed in the *Sample solution*, concomitantly determine the absorbances of the *Sample solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm. The absorbance of the *Sample solution* does not exceed that of the *Standard solution*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in small, well-filled, tight, light-resistant containers.
- **Labeling:** The label indicates that the Ophthalmic Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

Epirubicin Hydrochloride



$C_{27}H_{29}NO_{11} \cdot HCl$ 579.98

5,12-Naphthacenedione, 10-[(3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-, hydrochloride, (8*S*-*cis*)-;
(1*S*,3*S*)-3-Glycoloyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthacenyl-3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside hydrochloride [56390-09-1].

DEFINITION

Epirubicin Hydrochloride contains NLT 97.0% and NMT 102.0% of epirubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General,** *Chloride* ⟨191⟩

Solution A: Nitric acid and water (1:1)
Sample solution: 10 mg/mL in *Solution A*
Acceptance criteria: Meets the requirements

ASSAY

- **Procedure**

Allow the *System suitability solution*, *Standard solution*, and *Sample solution* to stand for 3 h before use.
Solution A: Dilute 10 mL of phosphoric acid with water to 100 mL.
Solution B:
Dissolve 3.7 g of sodium lauryl sulfate in 950 mL of water. To the resulting solution, add 28 mL of *Solution A*, and dilute with water to 1000 mL.
Mobile phase: Acetonitrile, methanol, and *Solution B* (29:17:54)
System suitability solution: 0.1 mg/mL each of USP Epirubicin Hydrochloride RS and USP Doxorubicin Hydrochloride RS in *Mobile phase*
Standard solution: 1 mg/mL of USP Epirubicin Hydrochloride RS in *Mobile phase*
Sample solution: 1 mg/mL of Epirubicin Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L13
Column temperature: 35°
Flow rate: 2.5 mL/min
Injection volume: 10 µL
Run time: About 3.5 times the retention time of the epirubicin peak

USP Monographs

www.webofpharma.com

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between doxorubicin and epirubicin
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of epirubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$) in the portion of Epirubicin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of epirubicin hydrochloride in USP Epirubicin Hydrochloride RS (mg/mg)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **ORGANIC IMPURITIES**
Allow the *System suitability solution*, *Sample solution*, and *Standard solution* to stand for 3 h before use.
Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 0.01 mg/mL of USP Epirubicin Hydrochloride RS in *Mobile phase*
Peak identification solution: Dissolve 10 mg of USP Doxorubicin Hydrochloride RS in 10 mL of a mixture of water and phosphoric acid (1:1). Allow to stand for 30 min. Adjust with 2 N sodium hydroxide solution to a pH of 2.6. Add 15 mL of acetonitrile and 10 mL of methanol, and mix.
Analysis
Samples: *Sample solution*, *Standard solution*, and *Peak identification solution*
[NOTE—Use the *Peak identification solution* to identify the doxorubicinone peak.]
Calculate the percentage of each impurity in the portion of Epirubicin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of epirubicin from the *Standard solution*
C_S = concentration of USP Epirubicin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Epirubicin Hydrochloride in the *Sample solution* (mg/mL)
P = potency of epirubicin hydrochloride in USP Epirubicin Hydrochloride RS (mg/mg)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05% of the area of the epirubicin peak in the *Standard solution*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Doxorubicinone[a]	0.3	1.4	1.0
Daunorubicinone[b]	0.4	1.0	0.5
Doxorubicin	0.8	1.0	1.0
Epirubicin	1.0	—	—
Dihydrodaunorubicin[c]	1.1	1.0	0.5
Daunorubicin	1.5	1.0	0.5
Epidaunorubicin[d]	1.7	1.0	1.0
Epirubicin dimer[e]	2.1	1.0	1.0
Individual unspecified impurity	—	1.0	0.5
Total impurities	—	—	3.0

[a] (8*S*,10*S*)-6,8,10,11-Tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[b] (8*S*,10*S*)-8-Acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[c] Dihydrodaunorubicin; (8*S*,10*S*)-10-[(3-Amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-(1-hydroxyethyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione hydrochloride.
[d] (8*S*,10*S*)-8-Acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[e] 8,8′-[(2*R*,4*R*)-4-Hydroxy-2-(hydroxymethyl)-1,3-dioxolan-2,4-diyl]bis[(8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione].

• **LIMIT OF ACETONE**
Analysis: See *Residual Solvents* ⟨467⟩.
Acceptance criteria: NMT 1.5%

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 4.0%
• **pH** ⟨791⟩
Sample solution: 5 mg/mL
Acceptance criteria: 4.0–5.5
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 1.1 USP Endotoxin Units/mg, where the label states that Epirubicin Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements where the label states that Epirubicin Hydrochloride is sterile.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store in airtight containers, protected from light. Store as per labeling instructions. Possible storage conditions could include the following: Store at a temperature between 2° and 8°. Store at room temperature. If the substance is sterile, store in a sterile, airtight, tamper-proof container.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Doxorubicin Hydrochloride RS
USP Epirubicin Hydrochloride RS

Epirubicin Hydrochloride Injection

DEFINITION

Epirubicin Hydrochloride Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of epirubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$).

USP Monographs

www.webofpharma.com

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectrum of the major peak of the *Sample solution* and the *Standard solution* as obtained in the *Assay* exhibit maxima and minima at the same wavelengths.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 2.9 g of sodium lauryl sulfate in 950 mL of water. To the resulting solution add 1.4 mL of phosphoric acid and dilute with water to 1 L.
Mobile phase: Acetonitrile and *Solution A* (50:50)
Standard solution: 1 mg/mL of USP Epirubicin Hydrochloride RS in *Mobile phase*
Sample solution: Nominally 1 mg/mL of epirubicin hydrochloride from Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. When this procedure is used for *Identification* test *B*, use a diode array detector set at 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of epirubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Epirubicin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of epirubicin hydrochloride in the *Sample solution* (mg/mL)
P = potency of epirubicin hydrochloride in USP Epirubicin Hydrochloride RS (mg/mg)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: Dissolve 3.7 g of sodium lauryl sulfate in 950 mL of water. To the resulting solution add 28 mL of phosphoric acid and dilute with water to 1 L.
Solution B: Dilute 28 mL of phosphoric acid with water to 1 L.
Mobile phase: Acetonitrile, methanol, and *Solution A* (29:17:54)
Diluent: Acetonitrile, methanol, and *Solution B* (29:17:27)
System suitability solution: 0.1 mg/mL each of USP Epirubicin Hydrochloride RS and USP Doxorubicin Hydrochloride RS in *Mobile phase*
Peak identification solution: Dissolve 10 mg of USP Doxorubicin Hydrochloride RS in a mixture of 5 mL of water and 5 mL of phosphoric acid. Allow to stand for 30 min. Adjust with 2 M sodium hydroxide to a pH of 2.6. Add 15 mL of acetonitrile and 10 mL of methanol, and mix.
Standard stock solution: 0.5 mg/mL of USP Epirubicin Hydrochloride RS in *Mobile phase*
Standard solution: 0.01 mg/mL of USP Epirubicin Hydrochloride RS from *Standard stock solution* in *Diluent*
Sample solution: Nominally 1 mg/mL of epirubicin hydrochloride from Injection in *Diluent*. Store at room temperature and use within 4 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L13
Column temperature: 35°
Flow rate: 2.5 mL/min
Injection volume: 10 µL
Run time
For the Standard solution: About 2 times the retention time of the epirubicin peak
For the System suitability solution, Peak identification solution, and **Sample solution:** About 4.5 times the retention time of the epirubicin peak
System suitability
Samples: *System suitability solution*, *Peak identification solution*, and *Standard solution*. [NOTE—Use the *Peak identification solution* to identify the doxorubicinone peak.]
Suitability requirements
Resolution: NLT 2.0 between epirubicin and doxorubicin, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of epirubicin from the *Standard solution*
C_S = concentration of USP Epirubicin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of epirubicin hydrochloride in the *Sample solution* (mg/mL)
P = potency of epirubicin hydrochloride in USP Epirubicin Hydrochloride RS (mg/mg)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05% of the area of the epirubicin peak in the *Standard solution*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Doxorubicinone[a]	0.3	1.4	1.8
Daunorubicinone[b]	0.4	1.0	0.5
Doxorubicin	0.8	1.0	1.0
Epirubicin	1.0	—	—
Dihydro-daunorubicin[c]	1.1	1.0	0.5
Daunorubicin	1.5	1.0	0.5
Epidaunorubicin[d, e]	1.7	1.0	—

www.webofpharma.com

Table 1 *(continued)*

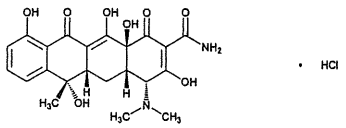
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Epirubicin dimer[e,f]	2.1	1.0	—
Any other individual impurity	—	1.0	0.5
Total impurities	—	—	3.9

[a] (8*S*,10*S*)-6,8,10,11-Tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[b] (8*S*,10*S*)-8-Acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[c] Dihydrodaunorubicin; (8*S*,10*S*)-10-[(3-Amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-(1-hydroxyethyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[d] (8*S*,10*S*)-8-Acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione.
[e] These impurities do not have individual limits; they are included in total impurities.
[f] 8,8′-[(2*R*,4*R*)-4-Hydroxy-2-(hydroxymethyl)-1,3-dioxolan-2,4-diyl]bis{(8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione}.

SPECIFIC TESTS
- **pH** ⟨791⟩: 2.5–3.5
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 1.61 USP Endotoxin Units/mg of epirubicin
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in light-resistant containers. Store in a refrigerator.
- **USP Reference Standards** ⟨11⟩
  USP Doxorubicin Hydrochloride RS
  USP Epirubicin Hydrochloride RS

Epitetracycline Hydrochloride



$C_{22}H_{24}N_2O_8 \cdot HCl$ 480.90

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride, [4*R*-(4α,4aβ,5aβ,6α,12aβ)]-;
(4*R*,4a*S*,5a*S*,6*S*,12a*S*) 4-(Dimethylamino)- 1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [23313-80-6].

DEFINITION
Epitetracycline Hydrochloride contains NLT 70.0% of epitetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**

Solution A: 1 mL of phosphoric acid diluted with water to 1 L
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
7.5	60	40
7.6	85	15
10	85	15

System suitability solution: 0.025 mg/mL each of USP Epitetracycline Hydrochloride RS, USP Tetracycline Hydrochloride RS, USP 4-Epianhydrotetracycline Hydrochloride RS, and USP Anhydrotetracycline Hydrochloride RS in *Solution A*
Standard solution: 0.1 mg/mL of USP Epitetracycline Hydrochloride RS in *Solution A*. Sonication may be needed to aid dissolution.
Sample solution: 0.1 mg/mL of Epitetracycline Hydrochloride in *Solution A*. Sonication may be needed to aid dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; 3-µm packing L60. [Note—L1 column is also suitable.]
Temperatures
Column: 50°
Autosampler: 10°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for epitetracycline, tetracycline, 4-epianhydrotetracycline, and anhydrotetracycline are 1.0, 1.2, 2.1, and 2.3, respectively.]
Suitability requirements
Resolution: NLT 2.5 between epitetracycline and tetracycline and NLT 2.5 between 4-epianhydrotetracycline and anhydrotetracycline, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of epitetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) in the portion of Epitetracycline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

USP Monographs

www.webofpharma.com

C_S = concentration of USP Epitetracycline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Epitetracycline Hydrochloride in the *Sample solution* (mg/mL)
P = potency of epitetracycline hydrochloride in USP Epitetracycline Hydrochloride RS (mg/mg)

Acceptance criteria: NLT 70.0%

IMPURITIES

• **LIMIT OF 4-EPIANHYDROTETRACYCLINE**

Solution A, Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.002 mg/mL of USP 4-Epianhydrotetracycline Hydrochloride RS in *Solution A*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.5 between epitetracycline and tetracycline and NLT 2.5 between 4-epianhydrotetracycline and anhydrotetracycline, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 4-epianhydrotetracycline hydrochloride in the portion of Epitetracycline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of 4-epianhydrotetracycline from the *Sample solution*
r_S = peak response of 4-epianhydrotetracycline from the *Standard solution*
C_S = concentration of USP 4-Epianhydrotetracycline Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Epitetracycline Hydrochloride in the *Sample solution* (mg/mL)
P = potency of 4-epianhydrotetracycline hydrochloride in USP 4-Epianhydrotetracycline Hydrochloride RS (mg/mg)

Acceptance criteria: NMT 2.0%

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: 10 mg/mL in water

Acceptance criteria: 2.3–4.0

• **LOSS ON DRYING** ⟨731⟩

Sample: 100 mg

Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

Acceptance criteria: NMT 6.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Anhydrotetracycline Hydrochloride RS
(4*S*,4a*S*,12a*S*)-4-(Dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide hydrochloride.
$C_{22}H_{23}ClN_2O_7$ 462.88

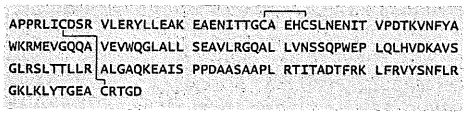
USP 4-Epianhydrotetracycline Hydrochloride RS
(4*R*,4a*S*,12a*S*)-4-(Dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide hydrochloride.
$C_{22}H_{23}ClN_2O_7$ 462.88

USP Epitetracycline Hydrochloride RS

USP Tetracycline Hydrochloride RS

Change to read:

▲Epoetin



$C_{809}H_{1301}N_{229}O_{240}S_5$ 18,236.06 Da (amino acid sequence)

DEFINITION

Epoetin is the recombinant form of human erythropoietin. It is a 165-amino acid glycoprotein manufactured using recombinant DNA technology. The presence of the impurities, host cell DNA, and host cell protein in Epoetin is process-specific, and is controlled through the purification process. The impurity levels are determined by validated methods and limits approved by the competent regulatory authority. It has a potency of NLT 140,000 and NMT 200,000 international units (IU) per absorbance unit (AU) at 280 nm.

IDENTIFICATION

• **A. ERYTHROPOIETIN BIOASSAYS** ⟨124⟩**:** Meets the requirements

• **B. PEPTIDE MAPPING**

Solution A: Trifluoroacetic acid and water (1.5: 1000)

Solution B: Acetonitrile, trifluoroacetic acid, and water (900: 1.2: 100)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
30.0	78	22
110.0	58	42
130.0	35	65
130.1	10	90
140.0	10	90
140.1	90	10

Phosphate buffer saline: 137 mM sodium chloride, 2.7 mM potassium chloride, 8.1 mM disodium hydrogen phosphate, and 1.47 mM potassium dihydrogen phosphate

Buffer A: 20 mM sodium citrate and 100 mM sodium chloride, pH 6.9, prepared as follows. Dissolve 5.80 g of sodium citrate dihydrate, 0.057 g of citric acid, and 5.84 g of sodium chloride in 800 mL of water, and mix. Adjust with hydrochloric acid to a pH of 6.9 and dilute with water to 1 L. Pass through a nylon filter of 0.2-µm pore size.

Digest stop solution: 8 M guanidine hydrochloride

USP Monographs

www.webofpharma.com

Digest buffer stock: 1.0 M tris(hydroxymethyl) aminomethane solution. Adjust with hydrochloric acid to a pH of 7.3.

Lys-C stock solution: Dissolve lysyl endopeptidase (Lys-C), containing approximately 4.5 AU/mg, in *Digest buffer stock* to a final concentration of 2 mg/mL. Expiry of reconstituted Lys-C is 2 years at –70 ± 10°, and 1 day at 5 ± 3°.

Lys-C digest solution: Add 20 µL of *Lys-C stock solution* to 180 µL of *Digest buffer stock*. Expiry is 1 day stored on ice or at 5 ± 3°.

Standard solution: 100 µg of USP Erythropoietin RS in 50 µL of water. Buffer exchange by a suitable method into *Buffer A*. Add 5 µL of *Lys-C digest solution* to 50 µL of buffer exchanged USP Erythropoietin RS. Incubate at 37° for 30 min. Add 50 µL of *Digest stop solution* and mix.

Sample solution: 100 µg of Epoetin in 50 µL of *Phosphate buffer saline*. If necessary, buffer exchange by a suitable method into *Buffer A*. Add 5 µL of *Lys-C digest solution*. Incubate at 37° for 30 min. Add 50 µL of *Digest stop solution* and mix.

Blank solution: 50 µL of *Phosphate buffered saline*. Add 5 µL of *Lys-C digest solution*. Incubate at 37° for 30 min. Add 50 µL of *Digest stop solution* and mix.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 3.0-mm × 25-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 0.2 mL/min
Injection volume: 50 µL

System suitability
Sample: *Standard solution*
Suitability requirements: 10 major peaks must be present as illustrated in the reference chromatogram provided with the lot of USP Erythropoietin RS being used. The change in retention times for each of peaks 5, 8, and 9 is NMT 1.2 min when compared to the first injection for three replicate injections. The ratio of relative heights percentage (RRH%) for each peak 8 and peak 9 in the *Standard solution* injections must be within 94%–106%.
Signal-to-noise ratio: NLT 3 for peak 6

Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank solution*
Follow each injection of *Standard solution* with a *Blank solution* injection. Record the retention time and response (peak height) of each of the 10 major peaks for both the *Standard solution* and *Sample solution*.
Calculate the relative peak height percentage (RH%) of peak 8 and peak 9 as compared to peak 5 for both the initial *Standard solution* injection and *Sample solution* taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak height of peak 8 or 9
r_S = peak height of peak 5

Calculate the ratio of RRH% for peak 8 and peak 9:

$$\text{Result} = (RH_x/RH_S) \times 100$$

RH_x = RH% of peak 8 or 9 for the *Standard solution*
RH_S = RH% of peak 8 or 9 for the *Sample solution*

Acceptance criteria: The chromatographic profile of the initial *Sample solution* injection is similar to that of the *Standard solution*. The absolute difference between the retention times of peaks 5, 8, and 9 in the *Sample solution* must be within 1.2 min when compared to the initial *Standard solution* injection. The RRH% for each peak 8 and peak 9 in the *Sample solution* must be within 94%–106% when compared to the initial *Standard solution*. There must be no new peaks greater than peak 6 in height in the *Sample solution* that are not present in the *Standard solution* injection.

ASSAY

• **Erythropoietin Bioassays** ⟨124⟩
Acceptance criteria: Meets the requirements. It has a potency of NLT 140,000 and NMT 200,000 IU/AU at 280 nm.

IMPURITIES

• **Limit of High Molecular Weight Proteins**
Mobile phase: 20 mM sodium citrate and 100 mM sodium chloride, pH 6.9, prepared as follows. Dissolve 5.80 g of sodium citrate dihydrate, 0.057 g of citric acid, and 5.84 g of sodium chloride in 800 mL of water and mix. Adjust with hydrochloric acid to a pH of 6.9 and dilute with water to 1 L. Pass through a nylon filter of 0.2-µm pore size.
System suitability solution: 2 mg/mL of USP Erythropoietin RS in *Mobile phase*. Heat at 80° for 30 min, and use immediately.
Sample solution: 2 mg/mL of Epoetin
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 7.8-mm × 30-cm; packing L20
Temperatures
Autosampler: 2°–8°
Column: 25°
Flow rate: 1.0 mL/min
Injection volume: 40 µL
Run time: 30 min
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention time for the aggregate is approximately 0.7.]
Suitability requirements: The monomer retention time must be within 8–10 min for *System suitability solution* injections. The relative retention times for the dimer and monomer are about 0.9 and 1.0, respectively.
Relative standard deviation: The precision of the monomer peak area must be <2%.
Analysis
Sample: *Sample solution*
[NOTE—Condition the *Chromatographic system* with the *Standard solution*. Inject the *Standard solution* in duplicate before, and in a single bracket after, all *Sample solutions* injected.]
Record the chromatogram, and measure the areas of the main peak and of the peaks eluting near the main peak, excluding the solvent peaks.
Acceptance criteria: The total impurities (aggregates and dimer) should be NMT 0.1%.

SPECIFIC TESTS

• ***N*-Glycan Profiling**
Solution A: Water. Degas before use.
Solution B: Dissolve 41.0 g of anhydrous sodium acetate in 900 mL of water. Transfer the solution to a 1-L volumetric

USP Monographs

www.webofpharma.com

flask, and dilute with water to volume. Pass the solution through a nylon membrane filter of NMT 0.45-µm pore size, and degas before use.

Solution C: To 900 mL of water add 26 mL of 50% (w/w) sodium hydroxide solution. Dilute the solution with water to a final volume of 1000 mL. Pass the solution through an alkaline-resistant nylon membrane filter of NMT 0.45-µm pore size, and degas before use.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	80	10	10
15	80	10	10
70	60	30	10
94	0	90	10
99	0	90	10
105	0	10	90
110	0	10	90
111	80	10	10
130	80	10	10

2-AB labeling solution: Add 150 µL of glacial acetic acid to 350 µL of dimethyl sulfoxide (DMSO) and mix. Add 110 µL of the acetic acid–DMSO mixture to a tube containing 5 mg of 2-aminobenzamide acid (2-AB) labeling dye and mix. Transfer the complete acetic-acid/DMSO/2-AB mixture to a tube containing 6 mg of sodium cyanoborohydride and mix. Use immediately and protect from light.

Buffer A: 20 mM sodium citrate, 100 mM sodium chloride, pH 6.9. Dissolve 5.80 g of sodium citrate dihydrate, 0.057 g of citric acid, and 5.84 g of sodium chloride in 800 mL of water and mix. Adjust with hydrochloric acid to a pH of 6.9 and dilute with water to 1 L. Pass through a nylon filter of 0.2-µm pore size.

0.5 M DTT: Add 77 mg of dithiothreitol to 1 mL of water and mix. Use immediately.

Enzyme reaction buffer: 0.5 M sodium phosphate, pH 7.5

Standard solution: Reconstitute 100 µg of USP Erythropoietin RS in 50 µL of water. If necessary, buffer exchange by a suitable method into *Buffer A*. Transfer the entire contents to a 1.5-mL polypropylene tube (or equivalent). Add 12.0 µL of *Enzyme reaction buffer*, 2.5 µL of *0.5 M DTT*, and 38.5 IUB milliunits of peptide-*N*-glycosidase F (PNGase F). [NOTE—1 unit of PNGase F is defined as the amount of enzyme required to catalyze the release of *N*-linked oligosaccharides from 1.0 nmol of denatured ribonuclease B per minute at a pH of 7.5 at 37°, and is equal to 1 IUB milliunit.] Dilute with water to a final volume of 100 µL, mix, and briefly centrifuge. Incubate at 37° for 30 min. Briefly centrifuge the sample, then freeze on dry ice for at least 5 min. After the sample is frozen, uncap the tube and place it in the centrifugal evaporator and operate under vacuum without heat for approximately 70–80 min or until completely dry.

Add 5.0 µL of *2-AB labeling solution*, vortex, briefly centrifuge, and incubate at 60° for 3 h. Following incubation, add 120 µL of water, vortex, and centrifuge the sample.

Remove excess 2-AB using a microcentrifuge column filled with an appropriate amount of G-10 gel filtration stationary phase for desalting a 75–150-µL sample. To prepare the resin, add 0.5 mL of water to the microcentrifuge column and swell for 15 min. Centrifuge at maximum speed for 5–10 s. Repeat wash and remove residual water. Apply labeled glycans, place the column in the microcentrifuge and spin at 200 × g for 1 min. Apply the flow through to a second column and repeat centrifugation. Transfer the repeat flow through to an HPLC vial for analysis.

Sample solution: Prepare as directed in the *Standard solution* using 100 µg of Epoetin.

Blank: Water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Fluorescence (330- and 420-nm emission wavelength)

Columns

Guard: 4.0-mm × 5-cm; 10-µm packing L46

Analytical: 4.0-mm × 25-cm; 10-µm packing L46

Temperatures

Column: 25°

Autosampler: 2°–8°

Flow rate: 0.5 mL/min

Injection volume: 25 µL

System suitability

Samples: *Standard solution* and *Blank*

Suitability requirements: The percentage interference of the *Blank* injection must be <1% of the total peak area of the initial *Standard solution*. The *Standard solution* chromatogram must be consistent with the reference chromatogram provided with the lot of USP Erythropoietin RS being used, including the presence of peaks 1–7. [NOTE—Peak 5 may exhibit splitting or shouldering but must be consistent for all *Standard solution* injections within a run.]

Relative standard deviation: Precision of total integrated peak area must be NMT 1.5%, *Standard solution*.

Analysis

Sample: *Sample solution*

Calculate the peak area response of each of the three peak groups, bi-sialylated (2 N), tri-sialylated (3 N), and tetra-sialylated (4 N) [NOTE—Refer to the reference chromatogram provided with the lot of USP Erythropoietin RS being used] :

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = total peak response for each of the three peak groups, bi-sialylated (2 N), tri-sialylated (3 N), and tetra-sialylated (4 N)

r_T = sum of the total peak responses for the three peak groups, bi-sialylated (2 N), tri-sialylated (3 N), and tetra-sialylated (4 N)

Acceptance criteria: The *N*-glycan percentages must be within the following ranges.

2 N: 4.5%–6.0%

3 N: 18%–25%

4 N: 69%–77%

• **ISOFORM DISTRIBUTION**

Cathode solution: Measure 0.31 g of L-histidine and dilute with water to a final volume of 10 mL.

Anode solution: 0.2 N sulfuric acid

Initiator: Measure 0.072 g of potassium persulfate and dilute with water to a final volume of 10 mL.

USP Monographs

www.webofpharma.com

Gel: Prepare a single horizontal gel using plate dimensions of 265-mm × 128-mm × 3-mm with a gel of 0.5-mm thickness prepared as follows. Combine 9.0 g of urea, 5.9 mL of acrylamide (30%), 2.4 mL of bis-acrylamide (2%), 450 µL of 3-10 ampholyte, and 1.1 mL of 3-5 ampholyte. Dilute with water to 28 mL, mix thoroughly, and pass the solution through a filter of 0.45-µm pore size. Add 2 mL of *Initiator* and mix by inversion. Transfer the solution to the gel cassette assembly.

Fixing solution: Prepare a solution containing 200 mL of 60% (w/v) of a mixture containing trichloroacetic acid and 17.5% 5-sulfosalicylic acid, 100 mL of glacial acetic acid, 400 mL of absolute methanol, and 300 mL of water.

Gel wash solution: Prepare a solution containing 400 mL of absolute methanol and 100 mL of glacial acetic acid. Dilute with water to 1 L.

Stain solution: Dissolve 1.25 g of Coomassie Brilliant Blue R-250 in 1 L of *Gel wash solution*. Pass through a filter of 0.45-µm pore size.

Destain solution: Prepare a solution containing 75 mL of absolute methanol and 100 mL of glacial acetic acid. Dilute with water to 1 L.

Standard stock solution: 2 µg/µL of USP Erythropoietin RS in water

Standard solution A: 0.13 µg/µL of USP Erythropoietin RS, prepared by diluting *Standard stock solution* in water

Standard solution B: 1.3 µg/µL of USP Erythropoietin RS, prepared by diluting *Standard stock solution* in water

Sample solution: 20 µg of Epoetin in 15 µL of water

Electrophoretic system

Mode: Horizontal electrophoretic system with integrated cooling apparatus capable of maintaining 2°–8°

Temperature: Cool to 2°–8° before applying voltage and maintain the temperature during electrophoresis.

Prefocusing: Prefocus the gel for 20–40 min at a constant 10 watts (maximum of 3000 V and 50 mA).

Loading profile: Load 15 µL of *Standard solution A*, *Standard solution B*, and *Sample solution* onto separate lanes of the gel at the cathode site.

Focusing: Focus the gel for 2.5 h at a constant 10 watts (maximum of 3000 V and 50 mA).

Staining: Incubate the gel in *Fixing solution* two times for 15 min each. Decant and incubate the gel in *Gel wash solution* for at least 30 min. Decant and incubate the gel in *Stain solution* for 15–60 min. Decant and rinse the gel with *Destain*. Decant and incubate the gel in *Destain* until the background is clear and *Standard solution A* is still visible.

System suitability

Samples: *Standard solution A* and *Standard solution B*

There is no artifact of staining that obscures visualization of protein lanes and the location and intensity of bands are appropriate in *Standard solution A* and *Standard solution B*. Isoforms 10, 11, 12, and 13 are present in *Standard solution A* and *Standard solution B*. No bands are present below isoform 9 in *Standard solution B*. Refer to the typical image provided with the lot of USP Erythropoietin RS being used.

Analysis

Sample: *Sample solution*

Scan the gel with a densitometer.

Using data from the densitometric scan, calculate the relative percentage of each individual isoform in the portion of the *Sample solution* taken:

$$\text{Result} = (I/T) \times 100$$

I = individual band intensity
T = total band intensity in the *Sample solution*

Acceptance criteria: Isoforms 10–13 must be present at the following percentages.

Isoform 13: NLT 13%
Isoforms 12 + 13: 46%–72%
Isoforms 10 + 11: NMT 52%
Isoforms 10–13: NLT 91%

Isoforms 9 and 14 may be present in the *Sample solution*. No isoform below isoform 9 is observed and any minor band present in the *Sample solution* must also be present at the same location in *Standard solution B*. Refer to the typical image provided with the lot of USP Erythropoietin RS being used.

• **PROTEIN CONTENT**

Sample solution: Epoetin, undiluted

Blank: Use an appropriate buffer solution consistent with the Epoetin under test.

Instrumental conditions

Mode: UV
Analytical wavelength: 280 nm
Cell: Quartz cuvette

Analysis

Samples: *Sample solution* and *Blank*

Calculate the protein concentration, in mg/mL, of the sample taken:

$$\text{Result} = (A/e) \times l$$

A = absorbance at 280 nm, corrected with the *Blank*
e = extinction coefficient, 0.74 mL/mg · cm^{-1}
l = path length (cm)

• **STERILITY TESTS** ⟨71⟩: Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 2.5 USP Endotoxin Units/mL.

ADDITIONAL REQUIREMENTS

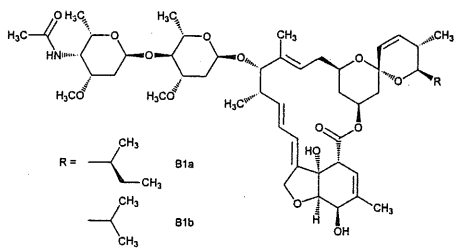
• **PACKAGING AND STORAGE:** Preserve in fluorinated ethylene propylene (FEP) or equivalent containers between 2° and 8°. Protect from light.

• **LABELING:** Label it to indicate that the material is of recombinant DNA origin.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Erythropoietin RS▲ (Postponed on 1-May-2018)

Eprinomectin



$C_{50}H_{75}NO_{14}$ (Component B_{1a}) 914.13

$C_{49}H_{73}NO_{14}$ (Component B_{1b}) 900.10

Component B_{1a}

Avermectin A_{1a}, 4″-(acetylamino)-5-*O*-demethyl-4″-deoxy-, (4″*R*)-;

www.webofpharma.com

(2a*E*,4*E*,5′*S*,6*S*,6′*R*,7*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-6′-(*S*)-*sec*-butyl-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5′,6,8,19-tetramethyl-17-oxospiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-7-yl-4-*O*-(4-acetamido-2,4,6-trideoxy-3-*O*-methyl-α-L-*lyxo*-hexopyranosyl)-2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranoside [or (4″*R*)-4″-(acetylamino)-5-*O*-demethyl-4″-deoxyavermectin A_{1a}] [133305-88-1].

Component B_{1b}

Avermectin A_{1a}, 4″-(acetylamino)-5-*O*-demethyl-25-de(1-methylpropyl)-4″-deoxy-25-(1-methylethyl)-, (4″*R*)-;
(2a*E*,4*E*,5′*S*,6*S*,6′*R*,7*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20,20b-dihydroxy-6′-isopropyl-5′,6,8,19-tetramethyl-17-oxospiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-7-yl-4-*O*-(4-acetamido-2,4,6-trideoxy-3-*O*-methyl-α-L-*lyxo*-hexopyranosyl)-2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranoside [or (4″*R*)-4″-(acetylamino)-5-*O*-demethyl-25-de(1-methyl-propyl)-4″-deoxy-25-(1-methylethyl) avermectin A_{1a}] [133305-89-2].

DEFINITION

Eprinomectin is a mixture of component B_{1a} ($C_{50}H_{75}NO_{14}$) and component B_{1b} ($C_{49}H_{73}NO_{14}$). It contains NLT 90.0% of component B_{1a} ($C_{50}H_{75}NO_{14}$) and NLT 95.0% of components B_{1a} ($C_{50}H_{75}NO_{14}$) and B_{1b} ($C_{49}H_{73}NO_{14}$), calculated on the anhydrous, solvent-free, and antioxidant-free basis. It may contain small amounts of a suitable antioxidant.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention times of the component B_{1a} peak and the component B_{1b} peak of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 0.1% (v/v) solution of perchloric acid in water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	45	55
15	45	55
25	5	95
30	45	55
35	45	55

Diluent: Methanol and water (4:1)
Standard solution: 0.500 mg/mL of USP Eprinomectin RS in *Diluent*
System suitability solution: Transfer 4 mL of *Standard solution* to an LC vial. Add 2 drops of 1 M sodium hydroxide and let stand for 20 min prior to injection.
Sample solution: 0.500 mg/mL of Eprinomectin in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 245 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 15 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—For relative retention times, see *Table 2*.]

Table 2

Components of the System Suitability Solution	Relative Retention Time
Impurity A	0.55
Component B_{1b}	0.77
Component B_{1a}	1.00
Impurities C + D	1.05
Impurity E	1.28

Suitability requirements
Resolution: NLT 3 between component B_{1b} and component B_{1a}; NLT 1 between component B_{1a} and impurities C + D, *System suitability solution*
Column efficiency: NLT 4,500 theoretical plates for component B_{1a}, *System suitability solution*
Symmetry factor: NMT 1.5 for component B_{1a}, *System suitability solution*
Relative standard deviation: NMT 1.0% from five injections for component B_{1a}, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of component B_{1a} ($C_{50}H_{75}NO_{14}$) in the portion of Eprinomectin taken:

$$\text{Result} = [r_{1a}/(r_{1a} + r_{1b})] \times 100$$

r_{1a} = peak area of component B_{1a} from the *Sample solution*
r_{1b} = peak area of component B_{1b} from the *Sample solution*

Calculate the percentage of component B_{1a} ($C_{50}H_{75}NO_{14}$) and component B_{1b} ($C_{49}H_{73}NO_{14}$) in the portion of Eprinomectin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of component B_{1a} or component B_{1b} from the *Sample solution*
r_S = peak area of component B_{1a} or component B_{1b} from the *Standard solution*
C_S = concentration of component B_{1a} or component B_{1b} in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NLT 90.0% of component B_{1a} and NLT 95.0% of components B_{1a} and B_{1b}, on the anhydrous, solvent-free, and antioxidant-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF 8A-OXO-B_{1A}**

Solution A, Solution B, Diluent, Standard solution, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.
Mobile phase: Acetonitrile and *Solution A* (13:7)

www.webofpharma.com

Butylated hydroxytoluene stock solution: 0.5 mg/mL of butylated hydroxytoluene in methanol. Sonicate to dissolve, if necessary.
Butylated hydroxytoluene solution: 0.01 mg/mL of butylated hydroxytoluene from *Butylated hydroxytoluene stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 15 µL
System suitability 1
Samples: *Standard solution* and *System suitability solution*
System suitability determination: Use the conditions as directed for *Chromatographic system* and the suitability requirements for *System suitability* as directed in the *Assay*.
System suitability 2
Samples: *Sample solution* and *Butylated hydroxytoluene solution*
Suitability requirements
Relative standard deviation: NMT 3.0% from six injections, *Butylated hydroxytoluene solution*
Analysis
Samples: *Sample solution* and *Butylated hydroxytoluene solution*
[NOTE—The retention time for 8a-oxo-B_{1a} is 4–9 min from the *Sample solution*, and the retention time for butylated hydroxytoluene is 12–17 min from *Butylated hydroxytoluene solution*.]
Calculate the percentage of 8a-oxo-B_{1a}, on the anhydrous, solvent-free, and antioxidant-free basis in the portion of Eprinomectin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times P \times 100$$

r_U = peak area of 8a-oxo-B_{1a} from the *Sample solution*
r_S = peak area of butylated hydroxytoluene from the *Butylated hydroxytoluene solution*
C_S = concentration of butylated hydroxytoluene in the *Butylated hydroxytoluene solution* (mg/mL)
C_U = concentration of Eprinomectin in the *Sample solution* (mg/mL)
F = relative response factor for butylated hydroxytoluene with respect to 8a-oxo-B_{1a}, 0.4
P = purity of butylated hydroxytoluene used to prepare the *Butylated hydroxytoluene solution*

Acceptance criteria: NMT 0.5%

Change to read:

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, Standard solution, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Eprinomectin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

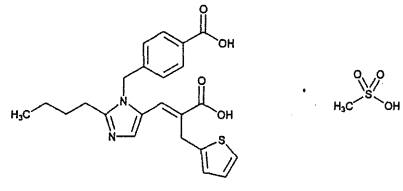
r_U = peak area of each individual related substance from the *Sample solution*
r_T = sum of the responses of all the peaks

Acceptance criteria: [NOTE—See *Table 2* for the relative retention times of impurity A and impurity E.]
Impurities with relative retention times of 0.23, 0.93, and 1.16 with respect to the B_{1a} peak: NMT 1.0%
Impurity A: NMT 1.0%
Impurity E: NMT 1.0%
All other known impurities: NMT 0.5%
Total unknown impurities: NMT 1.0▲% ▲(ERR 1-Jun-2019)
Total impurities: NMT 5.0%

SPECIFIC TESTS
• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 5 mg/mL of Eprinomectin in chloroform
Acceptance criteria: +132° to +140°, determined at 405 nm on the anhydrous, solvent-free, and antioxidant-free basis
• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia*
Sample: 0.250 g
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 2° and 8° at ambient humidity.
• **LABELING:** Label it to state the name(s) and amount(s) of any added substance(s). Label to indicate that it is for veterinary use only.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Eprinomectin RS

Eprosartan Mesylate



$C_{23}H_{24}N_2O_4S \cdot CH_4O_3S$ 520.62
2-Thiophenepropropanoic acid, α-[[2-butyl-1-[(4-carboxyphenyl)methyl]-1*H*-imidazol-5-yl]methylene]-, (*E*)-monomethanesulfonate;
(*E*)-2-Butyl-1-(*p*-carboxybenzyl)-α-2-thenylimidazole-5-acrylic acid, monomethanesulfonate [144143-96-4].
Free base [133040-01-4].

DEFINITION
Eprosartan Mesylate contains NLT 98.0% and NMT 101.5% of eprosartan mesylate ($C_{23}H_{24}N_2O_4S \cdot CH_4O_3S$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (22:78) containing 0.1% trifluoroacetic acid

www.webofpharma.com

Standard solution: 0.2 mg/mL of USP Eprosartan Mesylate RS in *Mobile phase*. Sonication may be necessary to dissolve.
Sample solution: 0.2 mg/mL of Eprosartan Mesylate in *Mobile phase*. Sonication may be necessary to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 15-cm; 5-µm packing L60
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of eprosartan mesylate ($C_{23}H_{24}N_2O_4S \cdot CH_4O_3S$) in the portion of Eprosartan Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of eprosartan from the *Sample solution*
r_S = peak area of eprosartan from the *Standard solution*
C_S = concentration of USP Eprosartan Mesylate RS in the *Standard solution* (mg/mL)
C_U = concentration of Eprosartan Mesylate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the anhydrous and solvent-free basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%
• **Organic Impurities**
Solution A: Use *Mobile phase* described in the *Assay*.
Solution B: Acetonitrile and water (40:60) containing 0.1% trifluoroacetic acid
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
35	0	100
40	0	100
40.1	100	0
55	100	0

Standard solution and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.2 µg/mL of USP Eprosartan Mesylate RS in *Solution A* from the *Standard solution*
Sample solution: 0.5 mg/mL of Eprosartan Mesylate in *Solution A*. Sonication may be necessary to dissolve.
System suitability: Proceed as directed in the *Assay* except for the *Signal-to-noise ratio*.
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Eprosartan Mesylate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_T = sum of all the peak areas from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard peaks below 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dealkyl eprosartan[a]	0.36	0.5
Hydroxy eprosartan[b]	0.79	0.3
Eprosartan	1.0	—
Any unspecified impurity	—	0.10
Total impurities	—	1.0

[a] (*E*)-4-{[2-Butyl-5-(2-carboxyvinyl)-1*H*-imidazol-1-yl]methyl}benzoic acid.
[b] 4-({2-Butyl-5-[2-carboxy-1-hydroxy-3-(thiophen-2-yl)propyl]-1*H*-imidazol-1-yl}methyl)benzoic acid.

• **Limit of Ethyl Methanesulfonate**
Standard stock solution: 0.01 mg/mL of ethyl methanesulfonate in *N,N*-dimethylformamide
Standard solution: 0.004 µg/mL of ethyl methanesulfonate prepared as follows. Weigh 20 µL of *Standard stock solution* into a 50-mL volumetric flask and add 2 mL of *N,N*-dimethylformamide. Dilute with acetonitrile to volume, mix, and add 5 boiling stones.
Sensitivity solution: 0.00054 µg/mL of ethyl methanesulfonate in *N,N*-dimethylformamide from *Standard solution*
Sample solution: 20 mg/mL of Eprosartan Mesylate prepared as follows. Weigh about 1 g of Eprosartan Mesylate into a 50-mL volumetric flask, and pipette 2.0 mL of *N,N*-dimethylformamide into the flask. Dissolve and dilute with acetonitrile to volume, and mix. Add 5 boiling stones, allow the solution to precipitate for at least 2 h, and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC-MS
Detector: Mass spectrometer, (+) ionization, a selected single-ion monitoring mode with m/z = 79 (CH_2SOOH^+ ion)
Column: 0.25-mm × 30-m; coated with a 0.25-µm layer of phase G27
Temperatures
Injection port: 160°
Column: See *Table 3*.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	20	170	0
170	10	280	2

Carrier gas: Helium
Flow rate: Constant pressure 46 kPa (0.9 mL/min at 50°)
Injection volume: 2 µL
Injection type: Split ratio, 1:4

www.webofpharma.com

System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Relative standard deviation: NMT 10%, *Standard solution*
Signal-to-noise ratio: NLT 2.5, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of ethyl methanesulfonate in the portion of Eprosartan Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S \times C_U) \times 10^6$$

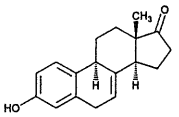
r_U = peak response of ethyl methanesulfonate from the *Sample solution*
r_S = peak response of ethyl methanesulfonate from the *Standard solution*
C_S = concentration of ethyl methanesulfonate in the *Standard solution* (µg/mL)
C_U = concentration of Eprosartan Mesylate in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.2 ppm

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 1.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers and store at NMT 25°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Eprosartan Mesylate RS

Equilin



$C_{18}H_{20}O_2$ 268.35
Estra-1,3,5(10),7-tetraen-17-one, 3-hydroxy-;
3-Hydroxyestra-1,3,5(10),7-tetraen-17-one [474-86-2].

DEFINITION
Equilin contains NLT 97.0% and NMT 103.0% of equilin ($C_{18}H_{20}O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 50 µg/mL in alcohol

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (35:65)
Internal standard solution: 35 µg/mL of phenol in acetonitrile
Standard solution: 0.2 mg/mL of USP Equilin RS in *Internal standard solution*
Sample solution: 0.2 mg/mL of Equilin in *Internal standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention times for phenol and equilin are 3 and 14 min, respectively.]
Suitability requirements
Resolution: NLT 5 between equilin and phenol. Adjust the operating parameters such that the peak of the *Standard solution* is about 0.7 full-scale.
Relative standard deviation: NMT 2.0% for five replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of equilin ($C_{18}H_{20}O_2$) in the portion of Equilin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of equilin to the internal standard from the *Sample solution*
R_S = peak response ratio of equilin to the internal standard from the *Standard solution*
C_S = concentration of USP Equilin RS in the *Standard solution* (mg/mL)
C_U = concentration of Equilin in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%

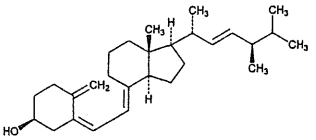
SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 20 mg/mL, in dioxane
Acceptance criteria: +300° to +316°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 105° for 1 h.
Acceptance criteria: NMT 0.5%
• **CLARITY OF SOLUTION**
Sample solution: 1 mg/mL of Equilin prepared as follows. Add 100 mg to 100 mL of 1 N sodium hydroxide in a 125 mL conical flask.
Analysis: Heat the *Sample solution* on a steam bath until solution is complete, then cool, and transfer to a 100-mL color-comparison tube.
Acceptance criteria: The solution is clear.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Equilin RS

USP Monographs

www.webofpharma.com

Ergocalciferol



$C_{28}H_{44}O$ 396.65
9,10-Secoergosta-5,7,10 (19),22-tetraen-3-ol, (3β,5*Z*,7*E*,22*E*)-;
Ergocalciferol [50-14-6].

DEFINITION
Ergocalciferol contains NLT 97.0% and NMT 103.0% of ergocalciferol ($C_{28}H_{44}O$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (USP 1-May-2020)
Wavelength range: 2–12 µm
Acceptance criteria: Meets the requirements in the chapter

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (USP 1-May-2020)
Analytical wavelength: 265 nm
Sample solution: 10 µg/mL in alcohol
Acceptance criteria: Meets the requirements in the chapter. Absorptivities do not differ by more than 3.0%.

Change to read:

• **C.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

Delete the following:

▲• **D. THIN-LAYER CHROMATOGRAPHY**
[NOTE—For the *Standard solutions* and the *Sample solution*, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and use the solutions immediately.]
Diluent: 10 mg/mL of squalane in chloroform
Standard solution A: 50 mg/mL of USP Ergocalciferol RS in *Diluent*
Standard solution B: 100 µg/mL of USP Ergosterol RS in *Diluent*
Sample solution: 50 mg/mL of Ergocalciferol in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Cyclohexane and ether (1:1)
Spray reagent: 20 mg/mL of acetyl chloride in antimony trichloride TS
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
[NOTE—Perform the development and subsequent operations in the dark.]
Place the plate in a chamber containing and equilibrated with *Developing solvent system*. Develop until the solvent front has moved about 15 cm above the line of application. Remove the plate, allow the solvent to evaporate, and spray with *Spray reagent*.
Acceptance criteria: The *Sample solution* shows a yellowish-orange area (ergocalciferol) having the same R_F value as the area of *Standard solution A* and may show a violet area below the ergocalciferol area. The color of the violet area is not more intense than that of the violet area from *Standard solution B*.▲ (USP 1-May-2020)

ASSAY

Change to read:

• **PROCEDURE**
▲▲ (USP 1-May-2020)
Mobile phase: *n*-Amyl alcohol in ▲hexane, solvent, chromatographic▲ (USP 1-May-2020) (3 in 1000)
System suitability solution: 250 mg of USP Vitamin D Assay System Suitability RS in 10 mL of a mixture of toluene and *Mobile phase* (1:1). Heat this solution, under reflux, at 90° for 45 min, and cool. [NOTE—This solution contains cholecalciferol, precholecalciferol, and *trans*-cholecalciferol.]
[NOTE—For the stock solutions, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and prepare the solutions fresh daily.]
Standard stock solution: 0.6 mg/mL of USP Ergocalciferol RS in toluene
Standard solution: 120 µg/mL of USP Ergocalciferol RS in *Mobile phase*, prepared from *Standard stock solution*
Sample stock solution: 0.6 mg/mL of Ergocalciferol in toluene
Sample solution: 120 µg/mL of Ergocalciferol in *Mobile phase*, prepared from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L3
▲Flow rate: 1.5 mL/min▲ (USP 1-May-2020)
Injection volume: 5–10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for precholecalciferol, *trans*-cholecalciferol, and cholecalciferol are 0.4, 0.5, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.0 between *trans*-cholecalciferol and precholecalciferol
Relative standard deviation: NMT 2.0% for the peak response of cholecalciferol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ergocalciferol ($C_{28}H_{44}O$) in the portion of Ergocalciferol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ergocalciferol RS in the *Standard solution* (µg/mL)
C_U = concentration of Ergocalciferol in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0%

USP Monographs

www.webofpharma.com

IMPURITIES

• **REDUCING SUBSTANCES**

Standard solution: 0.2 µg/mL of hydroquinone in dehydrated alcohol

Sample solution: 10 mg/mL of Ergocalciferol in dehydrated alcohol

Blank: Dehydrated alcohol

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

To 10 mL each of *Standard solution*, *Sample solution*, and *Blank*, add 0.5 mL of 5 mg/mL blue tetrazolium in methanol. Then add 0.5 mL of tetramethylammonium hydroxide TS in dehydrated alcohol (1 in 10). Allow the mixture to stand for 5 min, accurately timed, then add 1 mL of glacial acetic acid. Determine the absorbance of the solution at 525 nm, with a suitable spectrometer, against the *Blank*.

Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution*.

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** ⟨741⟩, *Procedures, Procedure for Class Ib, Apparatus I* and *Procedure for Class Ib, Apparatus II*: 115°–119°

• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 15 mg/mL in alcohol. [NOTE—Prepare and use the solution without delay. Use Ergocalciferol from a container opened not longer than 30 min.]

Acceptance criteria: +103° to +106°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in hermetically sealed containers under nitrogen, and store in a cool place protected from light.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ergocalciferol RS

▲▲ (USP 1-May-2020)

USP Vitamin D Assay System Suitability RS

Ergocalciferol Capsules

DEFINITION

Ergocalciferol Capsules usually consist of an edible vegetable oil solution of Ergocalciferol, encapsulated with Gelatin. Ergocalciferol Capsules contain NLT 100.0% and NMT 120.0% of the labeled amount of vitamin D as ergocalciferol ($C_{28}H_{44}O$).

ASSAY

Change to read:

• **PROCEDURE**

[NOTE—Throughout this *Assay*, protect solutions containing, and derived from, the test specimen and the Reference Standard from the atmosphere and light, preferably by the use of a blanket of inert gas and low-actinic glassware.]

▲▲ (USP 1-Aug-2019)

Butylated hydroxytoluene solution: 10 mg/mL of butylated hydroxytoluene in *n*-hexane

Aqueous potassium hydroxide solution: 1 g/mL of potassium hydroxide in freshly boiled water. [NOTE—Prepare solution fresh daily.]

Alcoholic potassium hydroxide solution: 3 g of potassium hydroxide in 50 mL of freshly boiled water. Add 10 mL of alcohol, and dilute with freshly boiled water to 100 mL. [NOTE—Prepare solution fresh daily.]

Sodium ascorbate solution: 175 mg/mL of ascorbic acid in 1 N sodium hydroxide. [NOTE—Prepare solution fresh daily.]

Sodium sulfide solution: 12 g of sodium sulfide in 20 mL of water. Dilute with glycerin to 100 mL.

Mobile phase: ▲*n*-Hexane▲ (USP 1-Aug-2019) and *n*-amyl alcohol (997:3). The ratio of components and the flow rate may be varied to meet the *System suitability* requirements.

System suitability solution: Transfer 100 mg of USP Vitamin D Assay System Suitability RS to a 10-mL volumetric flask. Add a mixture of toluene and *Mobile phase* (1 in 5) to volume, and mix. Heat a portion of this solution under reflux, at 90° for 45 min, and cool.

Standard stock solution: 0.5 mg/mL of USP Ergocalciferol RS in toluene. [NOTE—Prepare solution fresh daily.]

Standard solution A: 20 µg/mL from the *Standard stock solution* in *Mobile phase*. [NOTE—Store this solution at a temperature NMT 0°.]

Standard solution B: Pipet 4 mL of the *Standard stock solution* into a round-bottomed flask fitted with a reflux condenser, and then add 2 or 3 crystals of butylated hydroxytoluene. Displace the air with nitrogen, and heat in a water bath maintained at a temperature of 90° in subdued light under a nitrogen atmosphere for 45 min to obtain a solution containing vitamin D and pre-vitamin D. Cool, transfer with the aid of several portions of *Mobile phase* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Reflux NLT 10 Capsules with a mixture of 10 mL of *Sodium ascorbate solution* and 2 drops of *Sodium sulfide solution* on a steam bath for 10 min, crush any remaining solids with a blunt glass rod, and continue heating for 5 min. Cool, and add 25 mL of alcohol and 3 mL of *Aqueous potassium hydroxide solution*. Reflux the mixture on a steam bath for 30 min. Cool rapidly under running water, and transfer the saponified mixture to a conical separator, rinsing the saponification flask with two 15-mL portions of water, 10 mL of alcohol, and two 50-mL portions of ether. [NOTE—Use ether within 24 h after opening the container.] Shake the combined saponified mixture and rinsings vigorously for 30 s, and allow to stand until both layers are clear. Transfer the aqueous phase to a second conical separator, add a mixture of 10 mL of alcohol and 50 mL of solvent hexane, and shake vigorously. Allow to separate, transfer the aqueous phase to a third conical separator, and transfer the solvent hexane phase to the first separator, rinsing the second separator with two 10-mL portions of solvent hexane and adding the rinsings to the first separator. Shake the aqueous phase in the third separator with 50 mL of solvent hexane, and add the solvent hexane phase to the first separator. Wash the combined ether–solvent hexane extracts by shaking vigorously with three 50-mL portions of *Alcoholic potassium hydroxide solution*, and wash with 50-mL portions of water vigorously until the last washing is neutral to phenolphthalein. Drain any remaining drops of water from the combined ether–solvent hexane extracts, add 2 sheets of 9-cm filter paper, in strips, to the separator, and shake. Transfer the washed ether–solvent hexane extracts to a round-bottomed flask, rinsing the separator and paper with solvent hexane. Combine the solvent hexane rinsings with the ether–solvent hexane extracts, add 100 µL of *Butylated hydroxytoluene solution*, and mix. Evaporate under vacuum to dryness by swirling in a water bath maintained at a temperature NMT 40°. Cool under running water, and

www.webofpharma.com

introduce nitrogen sufficient to restore atmospheric pressure. Without delay, dissolve and dilute the residue in an accurately measured volume of a mixture of toluene and *Mobile phase* (1 in 5), until the nominal concentration of vitamin D is about 25 µg/mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L3
Injection volume: 20 µL

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for precholecalciferol, *trans*-cholecalciferol, and cholecalciferol are 0.4, 0.5, and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.0 between *trans*-cholecalciferol and precholecalciferol
Relative standard deviation: NMT 2.0% for cholecalciferol

Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Ergocalciferol response factor
Calculate the *Ergocalciferol response factor* (F_D):

$$F_D = C_S/r_S$$

C_S = concentration of USP Ergocalciferol RS in *Standard solution A* (µg/mL)
r_S = peak response of ergocalciferol from *Standard solution A*

Pre-ergocalciferol response factor
Calculate the concentration of ergocalciferol (C'_S), in µg/mL, in *Standard solution B*:

$$C'_S = F_D \times r'_S$$

F_D = *Ergocalciferol response factor*, as previously determined
r'_S = peak area of ergocalciferol from *Standard solution B*

Calculate the concentration of pre-ergocalciferol (C'_{pre}), in µg/mL, in *Standard solution B*:

$$C'_{pre} = C_S - C'_S$$

C_S = concentration of USP Ergocalciferol RS in *Standard solution A* (µg/mL)
C'_S = concentration of ergocalciferol in *Standard solution B* (µg/mL)

Calculate the *Pre-ergocalciferol response factor* (F_{pre}):

$$F_{pre} = C'_{pre}/r'_{pre}$$

C'_{pre} = concentration of pre-ergocalciferol in *Standard solution B* (µg/mL)
r'_{pre} = peak response of pre-ergocalciferol from *Standard solution B*

[NOTE—The value of F_{pre} determined in duplicate, on different days, can be used during the entire procedure.]

Vitamin D content
Calculate the percentage of the labeled amount of vitamin D as ergocalciferol ($C_{28}H_{44}O$) in the portion of Capsules taken:

$$\text{Result} = \{[(F_D \times r_C) + (F_{pre} \times r'_{pre})]/C_U\} \times 100$$

F_D = *Ergocalciferol response factor*, as previously determined
r_C = peak area of ergocalciferol from the *Sample solution*
F_{pre} = *Pre-ergocalciferol response factor*, as previously determined
r'_{pre} = peak area of pre-ergocalciferol from the *Sample solution*
C_U = nominal concentration of ergocalciferol in the *Sample solution* (µg/mL)

Acceptance criteria: 100.0%–120.0%

PERFORMANCE TESTS
• **DISINTEGRATION** ⟨701⟩
Buffer solution: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate and 1.66 mL of glacial acetic acid with water to obtain a 1000-mL solution having a pH of 4.5 ± 0.05
Immersion fluid: *Buffer solution*
Time: 45 min
Acceptance criteria: Meet the requirements
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Change to read:

• **LABELING:** Label the Capsules to indicate the content of ergocalciferol in ▲micrograms. Expression of the amount of ergocalciferol in terms of units may be added in parentheses after the mass units.[1] ▲ (USP 1-Aug-2019)
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ergocalciferol RS
USP Vitamin D Assay System Suitability RS

Ergocalciferol Oral Solution

DEFINITION
Ergocalciferol Oral Solution is a solution of Ergocalciferol in an edible vegetable oil, in Polysorbate 80, or in Propylene Glycol. It contains NLT 100.0% and NMT 120.0% of the labeled amount of vitamin D as ergocalciferol ($C_{28}H_{44}O$).

ASSAY
• **PROCEDURE**
Mobile phase: Chloroform containing alcohol as a preservative
Standard stock solution: 50 µg/mL of USP Ergocalciferol RS in chloroform. [NOTE—Prepare this solution fresh daily.]
Standard solution A: 5 µg/mL of USP Ergocalciferol RS from the *Standard stock solution* in chloroform. [NOTE—Store this *Standard solution A* at a temperature not above 0°.]

[1] Where articles are labeled in terms of units in addition to the required labeling, the relationship of the USP Units or International Units (IU) to mass is as follows: 1 µg of cholecalciferol or ergocalciferol = 40 USP Units or IU.

USP Monographs

www.webofpharma.com

Standard solution B: Transfer 5.0 mL of the *Standard stock solution* into a round-bottomed flask fitted with a reflux condenser. Displace the air with nitrogen, and reflux for 1 h in a water bath under a nitrogen atmosphere to obtain a solution containing ergocalciferol and pre-ergocalciferol. Cool, transfer, with the aid of several portions of chloroform, to a 50-mL volumetric flask, dilute with chloroform to volume, and mix.

Sample solution: Equivalent to 5 µg/mL of ergocalciferol from an accurately measured volume of Oral Solution in chloroform

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L3

Flow rate: 1 mL/min

Injection size: 10–20 µL

System suitability

Sample: *Standard solution B*

[NOTE—The relative retention times for pre-ergocalciferol and for ergocalciferol are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.0 between the pre-ergocalciferol peak and the ergocalciferol peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Ergocalciferol response factor

Calculate the *Ergocalciferol response factor*, F_D:

$$F_D = C_S/r_S$$

C_S = concentration of USP Ergocalciferol RS in the *Standard solution A* (µg/mL)

r_S = peak response of ergocalciferol from *Standard solution A*

Pre-ergocalciferol response factor

Calculate the concentration, C'_S, in µg/mL, of ergocalciferol in *Standard solution B*:

$$C'_S = F_D \times r'_S$$

F_D = Ergocalciferol response factor

r'_S = peak area of ergocalciferol from *Standard solution B*

Calculate the concentration, C'_{pre}, in µg/mL, of pre-ergocalciferol:

$$C'_{pre} = C_S - C'_S$$

C_S = concentration of USP Ergocalciferol RS in the *Standard solution A* (µg/mL)

C'_S = concentration of ergocalciferol in the *Standard solution B* (µg/mL)

Calculate the *Pre-ergocalciferol response factor*, F_{pre}:

$$F_{pre} = C'_{pre}/r_p$$

C'_{pre} = concentration of pre-ergocalciferol (µg/mL)

r_p = peak response of pre-ergocalciferol from *Standard solution B*

Vitamin D content

Calculate the percentage of the labeled amount of vitamin D as ergocalciferol ($C_{28}H_{44}O$) in the portion of Oral Solution taken:

$$\text{Result} = \{[(F_D \times r_C) + (F_{pre} \times r_{pre})]/C_U\} \times 100$$

F_D = ergocalciferol response factor

r_C = peak area of ergocalciferol from the *Sample solution*

F_{pre} = response factor for pre-ergocalciferol

r_{pre} = peak area of pre-ergocalciferol from the *Sample solution*

C_U = nominal concentration of ergocalciferol in the *Sample solution* (µg/mL)

Acceptance criteria: 100.0%–120.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label the Oral Solution to indicate the concentration of ergocalciferol, in mg per dosage unit. The activity may be expressed also in terms of USP Units, on the basis that 40 USP Vitamin D Units = 1 µg.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Ergocalciferol RS

Ergocalciferol Tablets

DEFINITION

Ergocalciferol Tablets contain NLT 100.0% and NMT 120.0% of the labeled amount of ergocalciferol ($C_{28}H_{44}O$).

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION**

Standard solution: 0.01 mg/mL of USP Ergocalciferol RS in alcohol

Sample solution: Evaporate 1 mL of the *Sample solution* obtained from *Identification* test *B* under a stream of nitrogen to dryness. Dissolve the residue in 50 mL of alcohol.

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*, concomitantly measured.

- **B. COLOR REACTION**

Sample solution: Triturate a quantity of powdered Tablets, equivalent to 5 mg of ergocalciferol, with 10 mL of chloroform, and filter.

Analysis: To 5 mL of the *Sample solution* add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and shake vigorously.

Acceptance criteria: A bright red color is produced and rapidly changes through violet and blue to green.

ASSAY

- **PROCEDURE:** Proceed with Tablets as directed in *Vitamin D Assay* ⟨581⟩, *Chemical Method*.

Acceptance criteria: 100.0%–120.0%

PERFORMANCE TESTS

- **DISINTEGRATION** ⟨701⟩

Time: 30 min

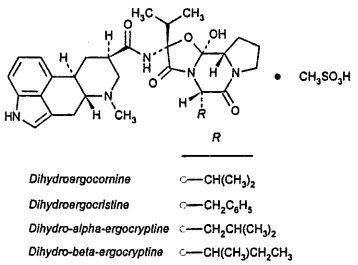
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label the Tablets to indicate the content of ergocalciferol, in mg. The activity may be expressed also in terms of USP Units, on the basis that 40 USP Vitamin D Units = 1 µg.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ergocalciferol RS

Ergoloid Mesylates



$C_{31}H_{41}N_5O_5 \cdot CH_4O_3S$ (dihydroergocornine mesylate) 659.79

$C_{35}H_{41}N_5O_5 \cdot CH_4O_3S$ (dihydroergocristine mesylate) 707.84

$C_{32}H_{43}N_5O_5 \cdot CH_4O_3S$ (dihydro-α-ergocryptine mesylate) 673.82

$C_{32}H_{43}N_5O_5 \cdot CH_4O_3S$ (dihydro-β-ergocryptine mesylate) 673.82

Ergotaman-3′,6′,18-trione, 9,10-dihydro-12′-hydroxy-2′,5′-bis(1-methylethyl)-, (5′α,10α)-, monomethanesulfonate (salt) mixture with 9,10α-dihydro-12′-hydroxy-2′-(1-methylethyl)-5′α-(phenylmethyl)ergotaman-3′,6′,18-trione monomethanesulfonate (salt), 9,10α-dihydro-12′-hydroxy-2′-(1-methylethyl)-5′α-(2-methylpropyl)ergotaman-3′,6′,18-trione monomethanesulfonate (salt), and 9,10α-dihydro-12′-hydroxy-2′-(1-methylethyl)-5′α-(1-methylpropyl) ergotaman-3′,6′,18-trione monomethanesulfonate (salt).

Dihydroergotoxine monomethanesulfonate (salt).

An equiproportional mixture of dihydroergocornine mesylate, dihydroergocristine mesylate, and ratio of dihydro-α-ergocryptine mesylate to dihydro-β-ergocryptine mesylate is (1.5-2.5:1) [8067-24-1].

» Ergoloid Mesylates is a mixture of the methanesulfonate salts of the three hydrogenated alkaloids, dihydroergocristine ($C_{35}H_{41}N_5O_5 \cdot CH_4O_3S$), dihydroergocornine ($C_{31}H_{41}N_5O_5 \cdot CH_4O_3S$), and dihydroergocryptine ($C_{32}H_{43}N_5O_5 \cdot CH_4O_3S$), in an approximate weight ratio of 1:1:1. Ergoloid Mesylates contains not less than 97.0 percent and not more than 103.0 percent of the alkaloid methanesulfonate mixture, calculated on the anhydrous basis, and not less than 30.3 percent and not more than 36.3 percent of the methanesulfonate salt of each of the individual alkaloids. Dihydroergocryptine mesylate exists as a mixture of *alpha-* and *beta-* isomers. The ratio of *alpha-* to *beta-* isomers is not less than 1.5:1.0 and not more than 2.5:1.0.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Ergoloid Mesylates RS

Identification—

A: The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar, undried preparation of USP Ergoloid Mesylates RS.

B: In a suitable chromatographic chamber, arranged for thin-layer chromatography, place a volume of a solvent system consisting of a mixture of acetone, *n*-butyl alcohol, ammonium hydroxide, and water (65:20:10:5) sufficient to develop the chromatogram. Prepare a test solution of Ergoloid Mesylates in a mixture of chloroform and methanol (9:1) containing 40 mg per mL. Apply 10 µL of this solution and 10 µL of a reference solution of methanesulfonic acid containing 0.4 mL in 100 mL of a mixture of chloroform and methanol (9:1) to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram until the solvent front has moved 10 cm. Remove the plate from the developing chamber, mark the solvent front, and dry in a current of cold air. Spray the plate with a 1 in 1000 solution of bromocresol purple in alcohol that previously has been adjusted to the purple color with 6 N ammonium hydroxide, then place in a stream of warm air until the spots appear: the R_F value of the methanesulfonic acid spot obtained from the test solution corresponds to that obtained from the reference solution.

Specific rotation ⟨781S⟩: between +11.0° and +15.0°.
Test solution: 10 mg per mL, in dilute alcohol (1 in 2).

pH ⟨791⟩: between 4.2 and 5.2 in a solution (1 in 200).

Water Determination, *Method I* ⟨921⟩: not more than 5.0%.

Limit of ergotamine—Prepare three solutions in a mixture of chloroform and methanol (9:1) containing 5 mg of Ergoloid Mesylates per mL, 5 mg of USP Ergoloid Mesylates RS per mL, and 5 mg of Ergotamine Tartrate per mL. Apply 5-µL volumes of the solutions at points about 2 cm from the bottom edge of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow the spots to dry. Add the solvent system consisting of a mixture of chloroform and methanol (9:1) and a small beaker of ammonium hydroxide to a suitable chamber, seal, and allow to equilibrate for 30 minutes. Develop the chromatogram in the equilibrated chamber until the solvent front has moved about 15 cm from the points of application. Remove the plate, air-dry, and locate the spots, first by viewing under long-wavelength UV light, and then by spraying with a reagent prepared by dissolving 800 mg of *p*-(dimethylamino) benzaldehyde in a mixture of 80 mL of alcohol and 11 mL of sulfuric acid: the chromatogram from Ergoloid Mesylates shows primary spots that correspond in size and color to the spots obtained from the USP Ergoloid Mesylates RS solution, and shows no spot corresponding to the principal spot in the chromatogram of Ergotamine Tartrate.

Limit of nonhydrogenated alkaloids—Prepare a solution in alcohol containing 0.4 mg of Ergoloid Mesylates per mL, and prepare a 1 in 10 dilution of the first solution. Determine the absorbances in 1-cm cells of the first solution at 317.5 nm and the dilution at 280 nm, using alcohol as the blank: the absorbance of the first solution is not more than 0.15 times that of the dilution.

Assay—

Mobile phase—Prepare a degassed solution containing a mixture of water, acetonitrile, and triethylamine (80: 20: 2.5). Adjust the ratio as necessary.

www.webofpharma.com

Standard preparation—Transfer about 10 mg of USP Ergoloid Mesylates RS, accurately weighed, to a 10-mL volumetric flask. Dissolve in a mixture of acetonitrile and water (1:1), dilute with the same solvent to volume, and mix. Prepare this solution fresh.

Assay preparation—Using about 10 mg of Ergoloid Mesylates, accurately weighed, proceed as directed for *Standard preparation*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing L1. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between dihydro-α-ergocryptine mesylate and dihydroergocristine mesylate is not less than 1.35, the resolution, *R*, between dihydroergocristine and dihydro-β-ergocryptine is not less than 1.0; the column efficiency determined for the dihydro-β-ergocryptine mesylate peak is not less than 950 theoretical plates; the tailing factor for dihydro-β-ergocryptine mesylate is not more than 2.5; and the relative standard deviation of the sum of the four peaks for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. The order of elution is dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine, and dihydro-β-ergocryptine. Calculate the total quantity, in mg, of these alkaloids in the portion of Ergoloid Mesylates taken by the formula:

$$10C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Ergoloid Mesylates RS in the *Standard preparation* and r_U and r_S are the sums of the responses of the four major peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Calculate the percentage of each alkaloid taken by the formula:

$$100r_i(MW)_i/\Sigma[r_i(MW)_i]$$

in which r_i is the peak response of an individual alkaloid; $(MW)_i$ is the molecular weight of that alkaloid; and $\Sigma[r_i(MW)_i]$ is the summation of the products of peak responses and molecular weights calculated for the four alkaloids.

Ergoloid Mesylates Capsules

» Ergoloid Mesylates Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ergoloid mesylates, consisting of not less than 30.3 percent and not more than 36.3 percent of the methane-sulfonate salt of each of the individual alkaloids (dihydroergocristine, dihydroergocornine, and dihydroergocryptine). The ratio of *alpha-* to *beta*-dihydroergocryptine mesylate is not less than 1.5:1.0 and not more than 2.5:1.0.

Packaging and storage—Preserve in tight, light-resistant containers between 15° and 25°. Do not freeze.

USP Reference standards ⟨11⟩—
USP Ergoloid Mesylates RS

Identification—

A: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, both relative to the internal standard as obtained in the *Assay*.

B: Using a sharp blade, carefully open 1 Capsule and transfer the entire contents into a 250-mL flask. Inspect the encapsulated liquid to ensure that crystallization or agglomeration of the drug substance has not taken place. Add 5 mL of water to the flask, and swirl to dissolve. Add 10 mL of *p*-dimethylaminobenzaldehyde TS: a blue color develops within 2 minutes and persists for not less than 10 minutes.

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—The total bacterial count does not exceed 1000 per g, and the total combined molds and yeasts count does not exceed 200 per g. Capsules meet also the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The test specimen shows an absence of the members of the *Enterobacteriaceae* family and *Pseudomonadaceae* family at levels greater than 100 per g of each.

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Time: 15 minutes.

Procedure—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and note if there is membrane formation. Record the time that each Capsule ruptures.

Tolerances—All of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 Capsules fail to rupture in 15 minutes but rupture in not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements, chloroform being used as the solvent in the procedure for *Weight Variation*.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and triethylamine (32:18:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve an accurately weighed quantity of *m*-chloroacetanilide in acetonitrile to obtain a solution having a known concentration of about 0.12 mg per mL.

Tartaric acid solution—Dissolve an accurately weighed quantity of tartaric acid in water to obtain a solution having a known concentration of about 5.6 mg per mL.

Standard preparation—Transfer about 25.0 mg of USP Ergoloid Mesylates RS, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Internal standard solution* to volume, and mix. Transfer 40.0 mL of this solution to a 200-mL volumetric flask, add 40.0 mL of *Tartaric acid solution*, and mix.

Assay preparation—Pipet 40.0 mL of *Tartaric acid solution* into a 200-mL volumetric flask, and heat in a water bath maintained at 50°. Add 10 Capsules (or the equivalent of 10 mg of ergoloid mesylates), and shake the flask by mechanical means for 10 minutes or until the gelatin has dissolved. Pipet 40.0 mL of *Internal standard solution* into the flask, and shake for an additional 10 minutes. Remove the flask from the bath and cool to room temperature. Transfer about 20 mL of the

www.webofpharma.com

solution to a 30-mL centrifuge tube, and centrifuge at 10,000 rpm for 60 minutes. Filter a portion of the supernatant through a filter having a porosity of 0.45 µm, discarding the first 5 mL of the filtrate. Use the remainder of the filtrate as the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and an 8-mm × 10-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed under *Procedure:*the relative retention times for *m*-chloroacetanilide, dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine, and dihydro-β-ergocryptine are about 1.0, 1.4, 1.8, 2.2, and 2.8, respectively; the column efficiency determined for the dihydro-β-ergocryptine peak is not less than 950 theoretical plates; the tailing factor for dihydro-β-ergocryptine is not more than 2.5 and that for dihydroergocornine is not more than 2.0; the resolution, *R,* between dihydro-α-ergocryptine and dihydroergocristine is not less than 1.35; and the relative standard deviation of the sum of the four ergoloid mesylate peaks for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the total quantity, in mg, of ergoloid mesylates in the portion of Capsules taken by the formula:

$$80C(\Sigma R_U/\Sigma R_S)$$

in which *C* is the concentration, in mg per mL, of USP Ergoloid Mesylates RS in the *Standard preparation,* and ΣR_U and ΣR_S are sums of the ratios of the peak responses of the individual alkaloids to the peak response of the internal standard obtained from the *Assay preparation* and the *Standard preparation,* respectively. Calculate the percentage of each of the individual alkaloids in the portion of Capsules taken by the formula:

$$100R_U(MW)_U/\Sigma[R_U(MW)_U]$$

in which $(MW)_U$ is the molecular weight of the individual alkaloid.

Ergoloid Mesylates Oral Solution

» Ergoloid Mesylates Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of Ergoloid Mesylates, consisting of not less than 30.3 percent and not more than 36.3 percent of the methanesulfonate salt of each of the individual alkaloids (dihydroergocristine, dihydroergocornine, and dihydroergocryptine). The ratio of *alpha-* to *beta*-dihydroergocryptine mesylate is not less than 1.5:1.0 and not more than 2.5:1.0.

Packaging and storage—Preserve in tight, light-resistant containers. Store at a temperature not exceeding 30°.

USP Reference standards ⟨11⟩—
USP Ergoloid Mesylates RS

Identification—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Alcohol Determination, *Method II* ⟨611⟩**:** between 90.0% and 110.0% of the labeled amount of C_2H_5OH.

Assay—

Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay* under *Ergoloid Mesylates.*

Standard preparation—Dissolve an accurately weighed quantity of USP Ergoloid Mesylates RS in acetonitrile solution (1 in 5) to obtain a solution having a known concentration of about 0.25 mg per mL.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 25 mg of ergoloid mesylates, to a 100-mL volumetric flask. Rinse the pipet with small portions of acetonitrile solution (1 in 5), collecting the rinsings in the volumetric flask. Dilute with acetonitrile solution (1 in 5) to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Ergoloid Mesylates* except to inject about 60-µL volumes of the *Assay preparation* and the *Standard preparation.* Calculate the quantity, in mg, of ergoloid mesylates in each mL of the Oral Solution taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which *V* is the volume, in mL, of Oral Solution taken, and the other terms are as defined therein.

Calculate the percentage of each of the individual alkaloids in the Oral Solution taken by the formula given in the *Assay* under *Ergoloid Mesylates.*

Ergoloid Mesylates Tablets

» Ergoloid Mesylates Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ergoloid mesylates, consisting of not less than 30.3 percent and not more than 36.3 percent of the methanesulfonate salt of each of the individual alkaloids (dihydroergocristine, dihydroergocornine, and dihydroergocryptine). The ratio of *alpha-* to *beta*-dihydroergocryptine mesylate is not less than 1.5:1.0 and not more than 2.5:1.0.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

Labeling—Label the Tablets to indicate that they are intended for swallowing intact.

USP Reference standards ⟨11⟩—
USP Ergoloid Mesylates RS

Identification—Mix a small amount of powdered Tablets, equivalent to about 5 mg of ergoloid mesylates, with 5 mL of water and 5 mL of a mixture of equal volumes of glacial acetic

USP Monographs

www.webofpharma.com

acid and sulfuric acid, and add 1 drop of freshly prepared ferric chloride solution (1 in 20): a violet-blue color develops within 5 minutes.

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 50 rpm, the distance between the paddle blade and the inside bottom of the vessel being maintained at 4.5 ± 0.2 cm during the test.
Time: 30 minutes.
Determine the amount of ergoloid mesylates dissolved using the following procedure.
Mobile phase—Prepare as directed in the *Assay.*
Standard solution—Dissolve an accurately weighed quantity of USP Ergoloid Mesylates RS in water to obtain a solution having a known concentration of about 50 µg per mL. Transfer 4 mL of this solution for every 0.5 mg of ergoloid mesylates contained in the Tablets to a 200-mL volumetric flask, add 1 mL of 0.1 N hydrochloric acid and 100 mL of water, mix, and dilute with water to volume.
Test solution—Transfer a 20-mL portion of the solution under test to a suitable container, add 100 µL of 0.1 N hydrochloric acid, and mix.
Chromatographic system—Proceed as directed in the *Assay* except that the sum of the relative standard deviation values for the four ergoloid mesylates peaks for replicate injections is not more than 3.0%.
Procedure—Separately inject equal volumes (about 500 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of ergoloid mesylates as directed in the *Assay.*
Tolerances—Not less than 75% *(Q)* of the labeled amount of ergoloid mesylates is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Assay—
Mobile phase—Prepare a filtered and degassed solution containing a mixture of water, acetonitrile, and triethylamine (700:300:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Transfer about 113 mg of papaverine hydrochloride to a 1-L flask. Add a mixture of 0.01 M tartaric acid and acetonitrile (2:1) to volume, and mix.
Standard preparation—Transfer about 33 mg of USP Ergoloid Mesylates RS, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with *Internal standard solution* to volume, and mix. Use a freshly prepared solution.
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of ergoloid mesylates, to a 50-mL centrifuge tube. Add 15.0 mL of *Internal standard solution,* insert the stopper into the tube, and shake for about 15 minutes. Centrifuge, filter if necessary, and use the clear supernatant.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L1. [NOTE—Use an L1 column capable of handling pH values greater than 11.] The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined for the dihydro-β-ergocryptine mesylate peak is not less than 1000 theoretical plates; the tailing factor for the dihydro-β-ergocryptine mesylate peak is not more than 2.0; the resolution, *R,* between the dihydro-α-ergocryptine mesylate and dihydroergocristine mesylate is not less than 2.0, and between dihydroergocristine and dihydro-β-ergocryptine peaks is not less than 2.0; and the relative standard deviation of the ratio of the sum of the four peaks to the internal standard for replicate injections is not more than 1.5%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The order of elution is papaverine, dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine, and dihydro-β-ergocryptine. Calculate the quantity, in mg, of ergoloid mesylates in the portion of Tablets taken by the formula:

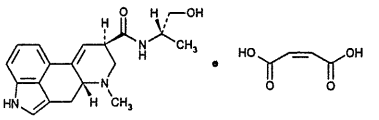
$$15C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Ergoloid Mesylates RS in the *Standard preparation;* and R_U and R_S are the sums of the ratios of responses of the four major peaks to the response of the internal standard peak obtained from the *Assay preparation* and the *Standard preparation,* respectively. Calculate the percentage of each of the individual alkaloids taken by the formula:

$$100R_i(MW)y_i/\Sigma[R_i(MW)_i]$$

in which R_i is the peak response ratio of the individual alkaloid to the internal standard; $(MW)_i$ is the molecular weight of the individual alkaloid; and $\Sigma[R_i(MW)_i]$ is the summation of the products of peak response ratios and molecular weights for the four alkaloids.

Ergonovine Maleate



$C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$ 441.48
Ergoline-8-carboxamide, 9,10-didehydro-*N*-(2-hydroxy-1-methylethyl)-6-methyl-, 8β(*S*)-, (*Z*)-2-butenedioate (1:1) (salt).
9,10-Didehydro-*N*-[(*S*)-2-hydroxy-1-methylethyl]-6-methylergoline-8β-carboxamide maleate (1:1) (salt) [129-51-1].

» Ergonovine Maleate contains not less than 97.0 percent and not more than 103.0 percent of $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, in a cold place.
USP Reference standards ⟨11⟩—
USP Ergonovine Maleate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.

www.webofpharma.com

Medium: alcohol.

Absorptivities at 311 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: The R_F value of the principal blue spot obtained from the *Test preparation* corresponds to that obtained from the *Standard preparation* in the chromatogram prepared as directed in the test for *Related alkaloids*.

Specific rotation ⟨781S⟩: between +51° and +56°.

Test solution: 5 mg per mL, in water.

Loss on drying ⟨731⟩—Dry it in vacuum at 80° for 3 hours: it loses not more than 2.0% of its weight.

Related alkaloids— [NOTE—Conduct this test promptly, without exposure to daylight and with minimum exposure to artificial light.]

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Solvent mixture—Prepare a mixture of alcohol and ammonium hydroxide (9:1).

Standard preparation—Prepare a solution of USP Ergonovine Maleate RS in *Solvent mixture* having a known concentration of about 10 mg per mL.

Standard dilutions—Prepare a series of dilutions of the *Standard preparation* in *Solvent mixture* having known concentrations of about 0.20, 0.10, and 0.05 mg per mL. Use immediately after preparation.

Test preparation—Immediately prior to use, prepare a solution of Ergonovine Maleate in *Solvent mixture* having a concentration of about 10 mg per mL.

Application volume: 5 µL.

Developing solvent system: a mixture of chloroform, methanol, and water (75:25:3), equilibrated for 30 minutes.

Procedure—Apply 5-µL portions of the *Standard preparation*, each of the three *Standard dilutions*, and the *Test preparation*, and proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. Locate the spots on the plate by spraying thoroughly and evenly with a solution prepared by dissolving 1 g of *p*-dimethylaminobenzaldehyde in a cooled mixture of 50 mL of alcohol and 50 mL of hydrochloric acid. Immediately dry in a stream of nitrogen for about 2 minutes: the R_F value of the principal spot obtained from the *Test preparation* corresponds to that obtained from the *Standard preparation*. Estimate the concentration of any other spots observed in the chromatogram of the *Test preparation* by comparison with the *Standard dilutions*. The spots from the 0.20, 0.10, and 0.05 mg per mL dilutions are equivalent to 2.0%, 1.0%, and 0.50% of impurities, respectively. The sum of the impurities is not greater than 2.0%.

Assay—

Standard preparation—Using a suitable quantity of USP Ergonovine Maleate RS, accurately weighed, prepare a solution in water having a known concentration of about 40 µg per mL.

Assay preparation—Transfer about 40 mg of Ergonovine Maleate, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix. Dilute 10.0 mL of this solution with water to 100.0 mL.

Procedure—Transfer 5.0 mL each of the *Standard preparation*, the *Assay preparation*, and water to provide a blank, to separate conical flasks. Add 10.0 mL of *p*-dimethylaminobenzaldehyde TS with constant swirling to each, and allow to stand for 20 minutes. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 555 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$ taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Ergonovine Maleate RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Ergonovine Maleate Injection

» Ergonovine Maleate Injection is a sterile solution of Ergonovine Maleate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$.

Packaging and storage—Preserve in single-dose, light-resistant containers, preferably of Type I glass, and store in a cold place.

USP Reference standards ⟨11⟩—
USP Ergonovine Maleate RS

Identification—The R_F value of the principal blue spot obtained from the *Test preparation* corresponds to that obtained from *Standard preparation A* in the chromatogram prepared as directed in the test for *Related alkaloids*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 700.0 USP Endotoxin Units per mg of ergonovine maleate.

pH ⟨791⟩: between 2.7 and 3.5.

Related alkaloids— [NOTE—Conduct this test promptly, without exposure to daylight and with minimum exposure to artificial light.]

Solvent mixture, Standard preparation, and *Standard dilutions*—Prepare as directed in the test for *Related alkaloids*— under *Ergonovine Maleate*.

Test preparation—Immediately prior to use, transfer a volume of Injection, equivalent to about 5 mg of ergonovine maleate, to a separator, and extract with three 5-mL portions of chloroform. Discard the chloroform extracts. Render alkaline to litmus with 6 N ammonium hydroxide, and extract with three 5-mL portions of chloroform. Evaporate the combined extracts with the aid of a stream of nitrogen, but without heat, to dryness. Dissolve the residue so obtained in 0.5 mL of *Solvent mixture*.

Procedure—Proceed as directed for *Procedure* in the test for *Related alkaloids*— under *Ergonovine Maleate*.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

0.05 M Phosphate buffer—Dissolve 6.8 g of monobasic potassium phosphate in 600 mL of water and adjust with phosphoric acid to a pH of 2.1. Dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a suitable and degassed solution of *0.05 M Phosphate buffer* and acetonitrile (80:20) such that the retention time is approximately 3 minutes with a flow rate of 1 mL per minute.

Standard preparation—Dissolve an accurately weighed quantity of USP Ergonovine Maleate RS in *Mobile phase*, adding sufficient water to equal 10% of the final volume, to obtain a solution having a known concentration of about 0.02 mg per mL.

Assay preparation—Quantitatively dilute an accurately measured volume of the Injection, equivalent to about 2 mg of ergonovine maleate, with *Mobile phase* and water, if necessary, to obtain a solution having a concentration of about 0.02 mg per mL in which the Injection volume plus any added water constitutes 10% of the final volume.

www.webofpharma.com

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 312-nm detector and a 3-mm × 30-cm column that contains packing L1. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure:* the relative standard deviation is not more than 3.0%.

Procedure—By means of a suitable sampling valve, introduce equal volumes (about 100 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph. Measure the peak responses of Ergonovine Maleate, at corresponding retention times, obtained from the *Assay preparation* and the *Standard preparation*. Calculate the quantity, in mg, of $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$ in each mL of the Injection taken by the formula:

$$(CD/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Ergonovine Maleate RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, D is the dilution factor, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ergonovine Maleate Tablets

» Ergonovine Maleate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Ergonovine Maleate RS

Identification—The R_F value of the principal blue spot obtained from the *Test preparation* corresponds to that obtained from the *Standard preparation* in the chromatogram prepared as directed in the test for *Related alkaloids*.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$ dissolved from fluorometric measurements, using 322 nm as the excitation wavelength and 428 nm as the emission wavelength, of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Ergonovine Maleate RS in the same *Medium*.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Related alkaloids— [NOTE—Conduct this test promptly, without exposure to daylight and with minimum exposure to artificial light.]
Solvent mixture—Mix 75 volumes of chloroform, 25 volumes of methanol, and 1 volume of ammonium hydroxide.
Detecting reagent—Cautiously dissolve 800 mg of *p*-dimethylaminobenzaldehyde in a mixture of alcohol and sulfuric acid (101:11).
Standard stock solution—Transfer 25 mg of USP Ergonovine Maleate RS, accurately weighed, to a separator, shake with 10 mL of water, render the mixture alkaline to litmus with 6 N ammonium hydroxide, and extract with three 10-mL portions of chloroform. Evaporate the combined extracts with the aid of a stream of nitrogen, but without heat, to dryness. Dissolve and dilute the residue to 10.0 mL with the *Solvent mixture*.
Standard preparations A , B, C, and *D*—Dilute accurately measured volumes of *Standard stock solution* quantitatively with *Solvent mixture* (designated below as parts by volume of *Standard stock solution* in total parts by volume of the finished *Standard preparation*) to obtain *Standard preparations*, designated below by letter, having the following compositions:

Standard Solution	Dilution	Concentration (µg RS per mL)	Percentage (%, for comparison with test specimen)
A	(1 in 20)	125	5.0
B	(1 in 33)	75	3.0
C	(1 in 100)	25	1.0
D	(1 in 200)	12.5	0.5

Test preparation—Immediately prior to use, transfer a quantity of finely powdered Tablets, equivalent to about 5 mg of ergonovine maleate, to a separator, shake with 10 mL of water, render the mixture alkaline to litmus with 6 N ammonium hydroxide, and extract with three 10-mL portions of chloroform. Evaporate the combined extracts with the aid of a stream of nitrogen, but without heat, to dryness. Dissolve the residue obtained in 2.0 mL of *Solvent mixture*.
Procedure—Apply separately 20 µL of the *Test preparation* and 20 µL of each *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Dry the plate with the aid of a current of cool air. Position the plate in a chromatographic chamber, and develop the chromatograms in *Solvent mixture* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in a current of cool air. Examine the plate under long-wavelength UV light. Mark the principal and any secondary fluorescent spots. Spray the plate with *Detecting reagent*, and mark the principal and secondary blue spots. Compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations:* the sum of the intensities of secondary spots obtained from the *Test preparation* corresponds to not more than 5.0% of related compounds.

Assay—
0.05 M Phosphate buffer, Mobile phase, and *Chromatographic system*—Proceed as directed in the *Assay*— under *Ergonovine Maleate Injection*.
Standard preparation—Dissolve an accurately weighed quantity of USP Ergonovine Maleate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.02 mg per mL.
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 mg of ergonovine maleate, to a 50-mL volumetric flask, add 25 mL of *Mobile phase*, place in a sonic bath for 5 minutes, cool to room temperature, dilute with *Mobile phase* to volume, mix, and centrifuge. Use the supernatant as directed in the *Procedure*.
Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Ergonovine Maleate Injection*. Calculate the quantity,

USP Monographs

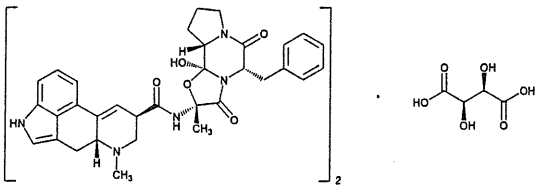
www.webofpharma.com

in mg, of $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$ in the portion of Tablets taken by the formula:

$$(50C)(r_U/r_S)$$

in which the terms are as defined therein.

Ergotamine Tartrate



$(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$ 1313.41

Ergotaman-3′,6′,18-trione, 12′-hydroxy-2′-methyl-5′-(phenylmethyl)-, (5′α)-,[*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (2:1) (salt);
Ergotamine tartrate (2:1) (salt) [379-79-3].

DEFINITION

Ergotamine Tartrate contains NLT 97.0% and NMT 100.5% of ergotamine tartrate [$(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$], calculated on the dried basis.

IDENTIFICATION

• **A.** The chromatogram of the *Sample solution* prepared as directed in the test for *Related Alkaloids*, exhibits its principal fluorescent spot and principal blue spot at the same R_F values as the corresponding principle spots of the *Standard solution*.

ASSAY

• **PROCEDURE**

Diluent: Acetic anhydride and glacial acetic acid (6:100)
Sample: 200 mg of Ergotamine Tartrate
Titrimetric system
Mode: Direct titration
Titrant: 0.05 N perchloric acid VS
Blank: 15 mL of *Diluent*
Endpoint detection: Visual
Analysis
Samples: *Sample* and *Blank*
Dissolve the *Sample* in 15 mL of *Diluent*. Add 1 drop of crystal violet TS, and titrate with *Titrant* from a 10-mL buret. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N perchloric acid is equivalent to 32.84 mg of ergotamine tartrate [$(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$].
Acceptance criteria: 97.0%–100.5% on the dried basis

IMPURITIES

• **RELATED ALKALOIDS**

Conduct this test without exposure to daylight and with the minimum necessary exposure to artificial light.
Solution A: A mixture of 5.5 mL of hydrochloric acid and 4.5 mL of water
Diluent: Chloroform and methanol (90:10)
Standard solution: 10 mg/mL of USP Ergotamine Tartrate RS in *Diluent*
Diluted standard solutions: USP Ergotamine Tartrate RS from the *Standard solution* diluted with *Diluent* as listed in *Table 1*

Table 1

Diluted standard solution	Concentration (mg/mL)	Percentage (%, for comparison with Sample)
A	0.2	2.0
B	0.1	1.0
C	0.05	0.5
D	0.025	0.25

Sample solution: 10 mg/mL of Ergotamine Tartrate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 5 µL
Developing solvent system: Ether, dimethylformamide, chloroform, and dehydrated alcohol (70:15:10:5)
Spray reagent: 200 mg of *p*-(dimethylamino) benzaldehyde in *Solution A*. Use a freshly prepared solution.
Analysis
Samples: *Standard solution*, *Diluted standard solutions*, and *Sample solution*
Line the chamber with filter paper, and allow it to equilibrate for 15 min. Place each spot over an opened bottle of ammonium hydroxide for 20 s, then allow the plate to dry in a current of cold air for 20 s. Develop the chromatogram until the solvent front has moved about 17 cm. Remove the plate from the developing chamber, allow the solvent to evaporate in a current of cold air for approximately 2 min, and spray with *Spray reagent*. Dry the plate at 60° for about 5 min, and compare the chromatograms.
Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*; the sum of the intensities of any secondary spots from the *Sample solution* is NMT the intensity of the principal spot from *Diluted standard solution A*; and the intensity of NMT one of the secondary spots is greater than that of the principal spot from *Diluted standard solution B*.

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Solution A: 10 g/L of tartaric acid in water
Solution B: Use chloroform from which any alcohol present has been removed by prior washing with water
Sample solution A: Dissolve 350 mg of Ergotamine Tartrate in 25 mL of *Solution A* contained in a separator, then add 500 mg of sodium bicarbonate, and mix gently but thoroughly. Add 10 mL of *Solution B*, and shake vigorously. After the layers have separated, draw off the chloroform phase through a small filter, previously moistened with *Solution B*, into a 50-mL volumetric flask. Rapidly continue the extraction with three 10-mL portions of *Solution B*, passing the extracts through the same filter. Place the flask in a bath at 20° for 10 min. Dilute with *Solution B* at 20° to 50.0 mL.
Sample solution B: Evaporate a 25.0-mL aliquot of *Sample solution A* on a rotary evaporator to dryness, maintaining the temperature of the bath below 45°. Dissolve the residue in 25 mL of glacial acetic acid.
Titrimetric system
Mode: Direct titration
Titrant: 0.05 N perchloric acid VS
Blank: 25 mL of glacial acetic acid
Endpoint detection: Visual

USP Monographs

www.webofpharma.com

Analysis
Samples: *Sample solution A*, *Sample solution B*, and *Blank*
Add 1 drop of crystal violet TS to *Sample solution B*, and titrate with 0.05 N perchloric acid VS to an emerald-green endpoint. Perform a blank determination, make any necessary correction, and calculate the concentration of the ergotamine base. Each mL of 0.05 N perchloric acid is equivalent to 29.08 mg of ergotamine ($C_{33}H_{35}N_5O_5$).
Determine the specific rotation of the ergotamine base from the angular rotation at 20° of *Sample solution A* and the concentration of the ergotamine base.
Acceptance criteria: −155° to −165°
• **Loss on Drying** ⟨731⟩
Sample: 100 mg of Ergotamine Tartrate
Analysis: Dry the *Sample* under vacuum at 60° for 4 h.
Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed, light-resistant containers in a cold place.
• **USP Reference Standards** ⟨11⟩
USP Ergotamine Tartrate RS

Delete the following:

▲Ergotamine Tartrate Inhalation Aerosol

» Ergotamine Tartrate Inhalation Aerosol is a suspension of microfine Ergotamine Tartrate in propellants in a pressurized container. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ergotamine tartrate [$(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$].

Packaging and storage—Preserve in small, non-reactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.
USP Reference standards ⟨ 11 ⟩ —
USP Ergotamine Tartrate RS
Identification—Place 10 mL of water in a small beaker, and deliver 2 sprays from the Inhalation Aerosol under the surface of the water, actuating the valve by pressing the tip against the bottom of the beaker. To one part of the resulting solution in a test tube add 2 parts of *p*-(dimethylamino)benzaldehyde TS, and mix: a blue color is produced.
Delivered dose uniformity over the entire contents: meets the requirements for *Metered-Dose Inhalers* under *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* ⟨601⟩.
PROCEDURE FOR DOSE UNIFORMITY—
Standard preparation—Dissolve an accurately weighed quantity of USP Ergotamine Tartrate RS in a tartaric acid solution (1 in 500), and dilute quantitatively and stepwise with the same tartaric acid solution as necessary to obtain a solution having a known concentration of about 18 µg per mL.
Test preparation—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of a tartaric acid solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of trichlorotrifluoroethane, insert the stopper, shake vigorously for 1 minute and centrifuge for 5 minutes. Use the clear supernatant as the *Test preparation*.
Procedure—Into three separate flasks transfer 6.0 mL each of the *Test preparation*, the *Standard preparation*, and a tartaric acid solution (1 in 500) to provide the blank. To each flask add 10.0 mL of *p*-dimethylaminobenzaldehyde TS, mix, and allow to stand for 30 minutes. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation*, at the wavelength of maximum absorbance at about 546 nm, against the blank. Calculate the quantity, in µg, of $(C_{33}H_{35}N_5O_5)_2.C_4H_6O_6$ contained in the minimum dose taken by the formula:

$$(20CN)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Ergotamine Tartrate RS in the *Standard preparation*; N is the number of sprays discharged to obtain the minimum recommended dose; and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.
Particle size—Prime the valve of the Inhalation Aerosol by alternately shaking and firing it several times through its oral inhalation actuator, and then actuate one measured spray onto a clean, dry microscope slide held 5 cm from the end of the actuator, perpendicular to the direction of the spray. Carefully rinse the slide with about 2 mL of carbon tetrachloride, and allow to dry. Examine the slide under a microscope, equipped with a calibrated ocular micrometer, using 450 × magnification. Focus on the particles of 25 fields of view near the center of the test specimen pattern, and note the size of the great majority of individual particles: they are less than 5 µm in diameter. Record the number and size of all individual crystalline particles (not agglomerates) more than 10 µm in length measured along the longest axis: not more than 10 such particles are observed.

Assay—
Standard preparation—Prepare as directed under *Unit spray content*.
Assay preparation— [NOTE—A suitable specimen beaker is one having a small indentation formed on its inside bottom surface having dimensions adequate to accept the aerosol valve stem during actuation, thereby preventing particle entrapment and side-of-stem leakage during the delivery of the specimen.] Place 10 mL of trichlorotrifluoroethane in a suitable 100-mL beaker. Prime the valve of Ergotamine Tartrate Inhalation Aerosol by alternately shaking and firing it 10 times through its oral inhalation actuator. Accurately weigh the Aerosol, shake it, and immediately deliver a single spray under the surface of the trichlorotrifluoroethane, actuating the valve by pressing the tip into the indentation in the bottom of the beaker. Raise the Aerosol above the surface of the trichlorotrifluoroethane, and shake it gently preparatory to delivering another spray similarly under the surface of the trichlorotrifluoroethane. Deliver a total of 2 sprays in this manner. Rinse the valve stem and ferrule with about 2 mL of trichlorotrifluoroethane, collecting the rinsing with the specimen in the beaker. Allow the Aerosol to dry, weigh it, and determine the total weight of the 2 sprays. Transfer the solution to a centrifuge tube with the aid of two 3-mL portions of trichlorotrifluoroethane, and add 10.0 mL of tartaric acid solution (1 in 500). Insert the stopper, shake vigorously for 15 minutes, centrifuge for 5 minutes, and use the clear supernatant as the *Assay preparation*.
Procedure—Transfer 5.0 mL each of the *Standard preparation*, the *Assay preparation*, and a blank consisting of

www.webofpharma.com

tartaric acid solution (1 in 500) to separate test tubes. To each tube add 10.0 mL of *p*-dimethylaminobenzaldehyde TS, mix, and allow to stand for 30 minutes. Concomitantly determine the absorbances of the solutions against the blank in 1-cm cells at the wavelength of maximum absorbance at about 546 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$ in each mL of the Aerosol taken by the formula:

$$(0.01Cd/W)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Ergotamine Tartrate RS in the *Standard preparation*; d is the density, in g per mL, of Aerosol determined as directed for d in the *Procedure* in the *Assay* under *Isoproterenol Sulfate Inhalation Aerosol*; W is the weight, in g, of the specimen taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively. ▲ 2S (USP41)

Ergotamine Tartrate Injection

DEFINITION

Ergotamine Tartrate Injection is a sterile solution of ergotamine tartrate and the tartrates of its epimer, ergotaminine, and of other related alkaloids, in Water for Injection to which tartaric acid or suitable stabilizers have been added. The total alkaloid content, in each mL, is NLT 450 µg and NMT 550 µg. The content of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$ is NLT 52.0% and NMT 74.0% of the content of total alkaloid; the content of ergotaminine tartrate is NMT 45.0% of the content of total alkaloid.

ASSAY

• **PROCEDURE**

Solution A: Use chloroform that has recently been saturated with water.

Solution B: 250 mg/mL of citric acid in water

Solution C: 10 mg/mL of tartaric acid in water

Solution D: Freshly prepared mixture of alcohol and *Solution C* (50:50)

Standard stock solution: 0.2 mg/mL of USP Ergotamine Tartrate RS prepared as follows. Dissolve 10 mg of USP Ergotamine Tartrate RS, with warming if necessary, in 50 mL of diluted alcohol.

Standard solution: 50.0 µg/mL of USP Ergotamine Tartrate RS from *Standard stock solution* prepared as follows. Dilute a suitable volume of *Standard stock solution* with water to the appropriate concentration. Pipet 5 mL of the resulting solution into a small conical flask.

Sample stock solution A: Pipet a volume of Injection, equivalent to 5 mg of ergotamine tartrate, into a beaker. Add 5 mL of *Solution A* and a portion of sodium bicarbonate, approximately equivalent in weight to one-tenth that of the portion of Injection taken, and mix.

Chromatographic system for sample preparation

Column A: Add sufficient chromatographic siliceous earth to make a fluffy mixture (1 g for each mL of the Injection taken plus an additional 3 g) to the beaker containing *Sample stock solution A*, and pack the mixture in a chromatographic tube about 2.5 cm in diameter and about 30 cm in length. Rinse the sides of the beaker with 2 mL of *Solution A*. Add sufficient chromatographic siliceous earth to make a fluffy mixture, and transfer it to the column.

Column B: Combine 9 g of siliceous earth with 7 mL of *Solution B* in a chromatographic tube about 2.5 cm in diameter and about 30 cm in length. Place a mixture of 2 g of the siliceous earth and 2 mL of water on top.

Assembly: Use *Column A* and *Column B* in series by inserting a pledget of glass wool in *Column B* and mounting *Column A* so that the eluate will drain into *Column B*.

Sample stock solution B: Add a total of 90 mL of *Solution A* to *Column A* and collect the eluent from *Column B* in a 200-mL volumetric flask. Rinse the tip of *Column A* with *Solution A*. Pass a sufficient volume of *Solution A* through *Column B* to dilute the eluent to volume.

Sample stock solution C: Extrude the adsorbent from *Column B* using a slight air pressure into a 600-mL beaker containing 10 g of sodium bicarbonate, and mix. Cautiously add 50 mL of water with continuous stirring. Wash the mixture with water into a 250-mL separator, and extract the ergotamine with four 15-mL portions of *Solution A*. Pass the extracts through a glass wool filter, combining them in a 100-mL volumetric flask. Wash the filter, and dilute with *Solution A* to volume.

Sample solution for total alkaloid content: Nominally 0.05 mg/mL of ergotamine tartrate from Injection prepared as follows. Pipet a volume of Injection, equivalent to 2.5 mg of ergotamine tartrate, into a 50-mL volumetric flask, add 25 mL of alcohol, and dilute with *Solution C* to volume. Pipet 5 mL of the resulting solution into a small conical flask.

Sample solution for ergotaminine: Pipet 20 mL of *Sample stock solution B* into a small conical flask and evaporate to dryness using a current of air. Dissolve the residue in 5.0 mL of *Solution D*.

Sample solution for ergotamine: Pipet 10 mL of *Sample stock solution C* into a small conical flask and evaporate to dryness using a current of air. Dissolve the residue in 5.0 mL of *Solution D*.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: Maximum absorbance at 545 nm

Analysis

Samples: *Standard solution*, *Sample solution for total alkaloid content*, *Sample solution for ergotaminine*, and *Sample solution for ergotamine*

In turn, place each of the flasks in an ice bath, and swirl continuously while adding, dropwise, 10.0 mL of *p*-dimethylaminobenzaldehyde TS. Allow to stand in subdued light at room temperature for NLT 90 min and NMT 2 h.

Concomitantly determine the absorbances of the samples against a reagent blank.

Calculate the concentration, in µg/mL, of total alkaloids in the portion of Injection taken:

$$\text{Result} = (A_{U1}/A_S) \times C_S \times (V_{U1}/V_U)$$

A_{U1} = absorbance of the *Sample solution for total alkaloid content*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Ergotamine Tartrate RS from the *Standard solution* (µg/mL)

V_{U1} = total volume of the *Sample solution for total alkaloid content*, 50 mL

V_U = volume of Injection taken to prepare the *Sample solution for total alkaloid content* (mL)

Calculate the percentage of the labeled amount of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$ in the portion of Injection taken:

$$\text{Result} = (A_{U2}/A_{U1}) \times 50$$

www.webofpharma.com

A_{U2} = absorbance of the *Sample solution for ergotamine*
A_{U1} = absorbance of the *Sample solution for total alkaloid content*

Calculate the percentage of ergotaminine tartrate in the portion of Injection taken:

$$\text{Result} = (A_{U3}/A_{U1}) \times 50$$

A_{U3} = absorbance of the *Sample solution for ergotaminine*
A_{U1} = absorbance of the *Sample solution for total alkaloid content*

Acceptance criteria
Total alkaloid content: 450–550 µg/mL
Ergotamine tartrate: 52.0%–74.0% of the content of total alkaloid
Ergotaminine tartrate: NMT 45.0% of the content of total alkaloid

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 357.0 USP Endotoxin Units/mg of ergotamine tartrate
- **pH** ⟨791⟩: 3.5–4.0
- **OTHER REQUIREMENTS:** It meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose, light-resistant containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Ergotamine Tartrate RS

Ergotamine Tartrate Tablets

DEFINITION
Ergotamine Tartrate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$.

IDENTIFICATION
- **PROCEDURE**

Solution A: Shake chloroform with ammonium hydroxide, draw off the chloroform layer, and use the chloroform saturated with ammonia.
Solution B: Combine equal volumes of glacial acetic acid and ethyl acetate.
Solution C: Combine equal volumes of ferric chloride TS and water.
Sample solution: Triturate a portion of finely powdered Tablets, equivalent to 5 mg of ergotamine tartrate, with 10 mL of solvent hexane for a few min. Allow to settle, and discard the solvent hexane extract. To the residue add 10 mL of *Solution A*, triturate for a few min, filter, and evaporate the filtrate on a steam bath to dryness. Dissolve the residue in 8 mL of *Solution B*.
Analysis
Sample: *Sample solution*
Part 1: To 1 mL of *Sample solution* add slowly, with continuous agitation and cooling, 1 mL of sulfuric acid.
Part 2: To the resulting solution from *Part 1*, add 0.1 mL of *Solution C*.
Acceptance criteria: Both criteria for *Part 1* and *Part 2* must be met.
Part 1: A blue color with a red tinge develops.
Part 2: The red tinge becomes less apparent and the blue color more pronounced.

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile and 0.01 M monobasic potassium phosphate (55:45)
Diluent: Acetonitrile and water (55:45)
Internal standard solution: 0.16 mg/mL of ergonovine maleate in *Diluent*
Standard stock solution: 0.2 mg/mL of USP Ergotamine Tartrate RS in *Diluent*
Standard solution: 0.02 mg/mL of USP Ergotamine Tartrate RS from *Standard stock solution* and 0.016 mg/mL of ergonovine maleate from *Internal standard solution* prepared as follows. Transfer 5.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.
Sample solution: Nominally 0.02 mg/mL of ergotamine tartrate from Tablets and 0.016 mg/mL of ergonovine maleate from *Internal standard solution* prepared as follows. Transfer a number of whole Tablets, equivalent to 10 mg of ergotamine tartrate, to a 500-mL volumetric flask. Add 50.0 mL of *Internal standard solution* and 300 mL of *Diluent*, and sonicate for 10 min. Dilute with *Diluent* to volume. Pass through a membrane disk of 0.45-µm pore size, discarding the first 25 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ergonovine maleate and ergotamine tartrate are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between ergonovine maleate and ergotamine tartrate
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0 for ergotamine tartrate
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample Solution*
Calculate the percentage of the labeled amount of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$ in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of ergotamine tartrate to the internal standard from the *Sample solution*
R_S = peak response ratio of ergotamine tartrate to the internal standard from the *Standard solution*
C_S = concentration of USP Ergotamine Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ergotamine tartrate from the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: 10 g/L of tartaric acid in water; 1000 mL
Apparatus 2: 75 rpm
Time: 30 min
Instrumental conditions
Mode: Fluorescence
Analytical wavelengths
Excitation: Maximum excitation at 327 nm

USP Monographs

www.webofpharma.com

Emission: Maximum emission at 427 nm
Standard solution: USP Ergotamine Tartrate RS in *Medium*
Sample solution: Dilute a portion of the solution under test with *Medium* to a concentration that is similar to that of the *Standard solution*.
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of ergotamine tartrate $[(C_{33}H_{35}N_2O_5)_2 \cdot C_4H_6O_6]$ dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of ergotamine tartrate $[(C_{33}H_{35}N_2O_5)_2 \cdot C_4H_6O_6]$ is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
• **LABELING:** Label the Tablets to indicate that they are intended for swallowing intact.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ergotamine Tartrate RS

Ergotamine Tartrate Sublingual Tablets

DEFINITION
Ergotamine Tartrate Sublingual Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$.

IDENTIFICATION
• **A.**
Solution A: Shake chloroform with ammonium hydroxide, draw off the chloroform layer, and use the chloroform saturated with ammonia.
Solution B: Combine equal volumes of glacial acetic acid and ethyl acetate.
Solution C: Combine equal volumes of ferric chloride TS and water.
Sample solution: Triturate a portion of finely powdered Tablets, equivalent to 5 mg of ergotamine tartrate, with 10 mL of solvent hexane for a few min. Allow to settle, and discard the solvent hexane extract. To the residue add 10 mL of *Solution A*, triturate for a few min, filter, and evaporate the filtrate on a steam bath to dryness. Dissolve the residue in 8 mL of *Solution B*.
Analysis
Sample: *Sample solution*
Part 1: To 1 mL of *Sample solution* add slowly, with continuous agitation and cooling, 1 mL of sulfuric acid.
Part 2: To the resulting solution from *Part 1*, add 0.1 mL of *Solution C*.
Acceptance criteria: Both criteria for *Part 1* and *Part 2* must be met.
Part 1: A blue color with a red tinge develops.
Part 2: The red tinge becomes less apparent and the blue color more pronounced.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and 0.01 M monobasic potassium phosphate (55:45)
Diluent: Acetonitrile and water (55:45)
Internal standard solution: 0.16 mg/mL of ergonovine maleate in *Diluent*
Standard stock solution: 0.2 mg/mL of USP Ergotamine Tartrate RS in *Diluent*
Standard solution: 0.02 mg/mL of USP Ergotamine Tartrate RS from *Standard stock solution* and 0.016 mg/mL of ergonovine maleate from *Internal standard solution* prepared as follows. Transfer 5.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.
Sample solution: Nominally 0.02 mg/mL of ergotamine tartrate from Tablets prepared as follows. Transfer a number of whole Tablets, equivalent to 10 mg of ergotamine tartrate, to a 500-mL volumetric flask. Add 50.0 mL of *Internal standard solution* and 300 mL of *Diluent*. Sonicate for 10 min, and dilute with *Diluent* to volume. Pass through a membrane disk of 0.45-µm pore size, discarding the first 25 mL of the filtrate.
Chromatographic system
(See *Chromatography, System Suitability* ⟨621⟩.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ergonovine maleate and ergotamine tartrate are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the ergotamine tartrate and ergonovine maleate
Column efficiency: NLT 3000 theoretical plates for ergotamine tartrate
Tailing factor: NMT 2.0 for ergotamine tartrate
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$ in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of ergotamine tartrate to the internal standard from the *Sample solution*
R_S = peak response ratio of ergotamine tartrate to the internal standard from the *Standard solution*
C_S = concentration of USP Ergotamine Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ergotamine tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISINTEGRATION** ⟨701⟩**:** 5 min
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
• **LABELING:** Label Tablets to indicate that they are intended for sublingual administration.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ergotamine Tartrate RS

www.webofpharma.com

Ergotamine Tartrate and Caffeine Suppositories

DEFINITION

Ergotamine Tartrate and Caffeine Suppositories contain NLT 90.0% and NMT 110.0% of the labeled amounts of ergotamine tartrate [$(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$] and caffeine ($C_8H_{10}N_4O_2$).

IDENTIFICATION

• **A.**

Solution A: 10 mg/mL of tartaric acid in water

Sample solution: Melt 1 Suppository in 10 mL of hot *Solution A*. Chill the mixture until the layer of oil has hardened, then filter, divide the filtrate into two parts, and use one part. Reserve the other part of this filtrate for use in *Identification* test *B*.

Analysis

Sample: *Sample solution*

To one portion of the *Sample solution*, add 10 mL of *p*-dimethylaminobenzaldehyde TS.

Acceptance criteria: A blue color develops (presence of ergotamine).

• **B.**

Sample solution: Use the remaining portion of the *Sample solution* from *Identification* test *A*.

Analysis

Sample: *Sample solution*

Transfer the *Sample solution* to a small evaporating dish, and evaporate to dryness on a steam bath. Add 1 mL of hydrochloric acid and 100 mg of potassium chlorate, and evaporate. Invert the dish over a vessel containing ammonium hydroxide.

Acceptance criteria: The residue acquires a purple color, which disappears upon the addition of 1 N sodium hydroxide (presence of caffeine).

ASSAY

• **PROCEDURE**

Protect all solutions from light.

Solution A: Acetonitrile, water, and triethylamine (150: 850: 0.5) adjusted by the dropwise addition of fluorometric grade sulfuric acid to a pH of 3.1 ± 0.1

Solution B: Acetonitrile, water, and triethylamine (620:1380:1) adjusted by the dropwise addition of fluorometric grade sulfuric acid to a pH of 3.1 ± 0.1. Make any necessary adjustments in pH to meet relative retention times, and make other adjustments if necessary.

Solution C: 10 mg/mL of tartaric acid in water

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
NLT 3[a]	0	100
23	100	0
25	100	0

[a] Do not switch to using 100% of *Solution B* until 3 min after the injection of a sample or until after the caffeine peak has eluted, whichever occurs last.

Diluent: Methanol and *Solution C* (50:50)

Standard stock solution: 40 µg/mL of USP Ergotamine Tartrate RS in *Diluent*

Standard solution: 20 µg/mL of USP Ergotamine Tartrate RS from *Standard stock solution* and 1 mg/mL of USP Caffeine RS in *Diluent*

System suitability solution: 10 µg/mL of USP Ergotamine Tartrate RS and 0.5 mg/mL of USP Caffeine RS from *Standard solution* and 2 µg/mL of USP Ergotaminine RS in *Diluent*

Sample solution: Nominally 20 µg/mL of ergotamine tartrate from NLT 20 Suppositories prepared as follows. Weigh NLT 20 Suppositories, and grind to a fine mesh. Transfer a portion of the ground mass, equivalent to 2 mg of ergotamine tartrate, to a suitable glass-stoppered flask. Add 100.0 mL of *Diluent*, insert the stopper in the flask, and place it in a water bath maintained at 40°. Shake vigorously for 5 min, or longer if necessary, until the specimen is completely melted. Sonicate for 30 min, and transfer to a freezer for 45 min. Pass through a glass fiber filter of 0.7-µm pore size, and discard the first 5–10 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 244 nm (for caffeine), in series with a fluorometric detector (for ergotamine tartrate) with excitation at 229 nm and detection at 435 nm

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for caffeine, ergotamine, and ergotaminine are 1.0, 4, and 4.5, respectively.]

Suitability requirements

Resolution: NLT 3.0 between ergotamine and ergotaminine, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of caffeine ($C_8H_{10}N_4O_2$) in the portion of Suppositories taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of caffeine from the *Sample solution*

r_S = peak response of caffeine from the *Standard solution*

C_S = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of caffeine in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of ergotamine tartrate [$(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$] in the portion of Suppositories taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorometric response of ergotamine from the *Sample solution*

I_S = fluorometric response of ergotamine from the *Standard solution*

C_S = concentration of USP Ergotamine Tartrate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of ergotamine tartrate in the *Sample solution* (µg/mL)

www.webofpharma.com

Acceptance criteria: 90.0%–110.0% of the labeled amounts of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$ and caffeine ($C_8H_{10}N_4O_2$)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, at a temperature not above 25°. Do not expose unwrapped Suppositories to sunlight.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Caffeine RS
  USP Ergotamine Tartrate RS
  USP Ergotaminine RS

Ergotamine Tartrate and Caffeine Tablets

DEFINITION

Ergotamine Tartrate and Caffeine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$ and of caffeine ($C_8H_{10}N_4O_2$).

[NOTE—Throughout the following procedures, protect samples, the Reference Standards, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

- **A.** The retention times of the ergotamine and caffeine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Triethylamine, sulfuric acid, and water (4: 0.5: 2000) adjusted with sulfuric acid or 1 N sodium hydroxide to a pH of 3.0

Solution A: Acetonitrile and *Buffer* (15:85)

Solution B: Acetonitrile and *Buffer* (42:58)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
12	0	100
17	0	100
17.5	100	0
25	100	0

Diluent: 10 g/L of tartaric acid in water. Use *d*- or *l*-tartaric acid. Do not use a racemic mixture. Use a freshly prepared solution.

Ergotamine tartrate stock solution: 0.2 mg/mL of USP Ergotamine Tartrate RS in *Diluent* prepared as follows. Transfer a suitable quantity of USP Ergotamine Tartrate RS to an appropriate volumetric flask, and add 66% of the flask volume of *Diluent*. Sonicate for 5 min, and allow the solution to equilibrate to room temperature. Dilute with *Diluent* to volume.

Standard stock solution: 0.01 mg/mL of USP Ergotamine Tartrate RS and 1 mg/mL of USP Caffeine RS in *Diluent* prepared as follows. Transfer a suitable quantity of USP Caffeine RS to an appropriate volumetric flask, and add 66% of the flask volume of *Diluent*. Sonicate for 5 min, and allow the solution to equilibrate to room temperature. Add a suitable volume of *Ergotamine tartrate stock solution*, and dilute with *Diluent* to volume.

Standard solution: 0.001 mg/mL of USP Ergotamine Tartrate RS and 0.1 mg/mL of USP Caffeine RS from *Standard stock solution* in *Diluent*

System suitability solution: 0.001 mg/mL of USP Ergotaminine RS in *Standard solution*

Sample stock solution: Transfer NLT 10 Tablets to a 1-L volumetric flask. Add *Diluent* to 66% of the flask volume. Shake mechanically for NLT 45 min to promote dissolution. It may be necessary to loosen and retighten the closure multiple times during the shaking to relieve pressure. Dilute with *Diluent* to volume. Use within 9 h.

Sample solution: Nominally 0.001 mg/mL of ergotamine tartrate and 0.1 mg/mL of caffeine from *Sample stock solution* in *Diluent*. Pass through a suitable filter, and use the filtrate within 9 h.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detectors: UV 254 nm (for caffeine), in series with a fluorometric detector (for ergotamine and ergotaminine), with excitation at 250 nm and detection at 435 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 60 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for caffeine, ergotamine, and ergotaminine are 1.0, 2.6, and 2.8, respectively.]

Suitability requirements

Resolution: NLT 1.5 for ergotamine and ergotaminine, *System suitability solution*

Tailing factor: NMT 2.0 for ergotamine, *Standard solution*

Relative standard deviation: NMT 2.0% each for caffeine and ergotamine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of caffeine ($C_8H_{10}N_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of caffeine at 254 nm from the *Sample solution*

r_S = peak response of caffeine at 254 nm from the *Standard solution*

C_S = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of caffeine in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of ergotamine tartrate $[(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6]$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ergotamine at 435 nm from the *Sample solution*

r_S = peak response of ergotamine at 435 nm from the *Standard solution*

C_S = concentration of USP Ergotamine Tartrate RS in the *Standard solution* (mg/mL)

www.webofpharma.com

C_U = nominal concentration of ergotamine tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of the labeled amounts of caffeine and ergotamine tartrate

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 10 g/L of tartaric acid in water. Use *d*- or *l*-tartaric acid. Do not use a racemic mixture. Use a freshly prepared solution; 900 mL.
Apparatus 2: 75 rpm
Time: 30 min
Buffer, Solution A, Solution B, Mobile phase, Diluent, Standard solution, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: Pass a portion of the solution under test through a suitable filter, and discard the first portion of the filtrate.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of caffeine ($C_8H_{10}N_4O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of caffeine at 254 nm from the *Sample solution*
r_S = peak response of caffeine at 254 nm from the *Standard solution*
C_S = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Calculate the percentage of the labeled amount of ergotamine tartrate [$(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$] dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of ergotamine at 435 nm from the *Sample solution*
r_S = peak response of ergotamine at 435 nm from the *Standard solution*
C_S = concentration of USP Ergotamine Tartrate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances
Caffeine: NLT 75% (*Q*) of the labeled amount of caffeine ($C_8H_{10}N_4O_2$) is dissolved.
Ergotamine tartrate: NLT 70% (*Q*) of the labeled amount of ergotamine tartrate [$(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$] is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **LIMIT OF ERGOTAMININE AND OTHER ERGOTAMINE-RELATED IMPURITIES**

Buffer: 0.93 g/L of ammonium carbonate in water passed through a suitable filter. Add 6.7 mL of diethylamine per L.
Mobile phase: Acetonitrile and *Buffer* (50:60)
Diluent: Acetonitrile and methanol (50:50)
Standard stock solution: 0.4 mg/mL of USP Ergotamine Tartrate RS prepared as follows. Transfer a suitable quantity of USP Ergotamine Tartrate RS to an appropriate volumetric flask, and add 10% of the flask volume of acetonitrile. Add *Diluent* to 66% of the final flask volume, and sonicate, if needed. Allow the solution to equilibrate to room temperature, and dilute with *Diluent* to volume. Store refrigerated, and use within 24 h.
Standard solution: 0.004 mg/mL of USP Ergotamine Tartrate RS from *Standard stock solution* in *Diluent*. Store refrigerated, and use within 24 h.
System suitability solution: 0.004 mg/mL of USP Ergotaminine RS in the *Standard solution*. Store refrigerated, and use within 24 h.
Sensitivity solution: 0.2 µg/mL of USP Ergotamine Tartrate RS from the *Standard solution* in *Diluent*. Store refrigerated, and use within 24 h.
Sample solution: Nominally 0.4 mg/mL of ergotamine tartrate from NLT 20 Tablets prepared as follows. Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 10 mg of ergotamine tartrate, to an appropriate container. Add 25.0 mL of *Diluent* to the container, and sonicate, if needed. Allow the solution to equilibrate to room temperature. Centrifuge a portion, pass the supernatant through a suitable filter, and discard NLT the first 3 mL of the filtrate. Store refrigerated, and use within 1 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Autosampler temperature: 5°
Flow rate: 1.3 mL/min
Injection volume: 60 µL
Run time: NLT 3.9 times the retention time of ergotamine
System suitability
Samples: *Standard solution, System suitability solution,* and *Sensitivity solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 for ergotamine and ergotaminine, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of ergotamine from the *Standard solution*
C_S = concentration of USP Ergotamine Tartrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ergotamine tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ergotamine	1.0	—
Unidentified impurity 1	1.55	1.5
Ergotaminine	2.85	2.0

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual degradation product	—	0.5
Total degradation products	—	4.0

• **LIMIT OF THEOPHYLLINE AND OTHER CAFFEINE-RELATED IMPURITIES**

Buffer: 1.6 g/L of anhydrous sodium acetate in water adjusted with glacial acetic acid to a pH of 4.5

Mobile phase: Acetonitrile and *Buffer* (95:5)

Standard stock solution: 0.2 mg/mL of USP Caffeine RS in *Mobile phase* prepared as follows. Transfer a suitable quantity of USP Caffeine RS to an appropriate volumetric flask, and add 66% of the flask volume of *Mobile phase*. Sonicate for NLT 10 min, and allow the solution to equilibrate to room temperature. Dilute with *Mobile phase* to volume.

Standard solution: 0.002 mg/mL of USP Caffeine RS from *Standard stock solution* in *Mobile phase*

System suitability solution: 0.002 mg/mL of USP Theophylline RS in the *Standard solution*

Sensitivity solution: 0.1 µg/mL of USP Caffeine RS from the *Standard solution* in *Mobile phase*

Sample stock solution: Nominally 1 mg/mL of caffeine from NLT 10 Tablets prepared as follows. Finely powder NLT 10 Tablets. Transfer a portion of the powder, equivalent to 250 mg of caffeine, to a 250-mL volumetric flask, and add 66% of the flask volume of *Mobile phase*. Sonicate for NLT 45 min with occasional shaking, and allow the solution to equilibrate to room temperature. Dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.2 mg/mL of caffeine from *Sample stock solution* prepared as follows. Transfer a suitable quantity of *Sample stock solution* to an appropriate volumetric flask, and dilute with *Mobile phase* to volume. Pass through a suitable filter, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 25-cm; 5-µm packing L3

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time: NLT 3 times the retention time of caffeine

System suitability

Samples: *Standard solution, System suitability solution,* and *Sensitivity solution*

[NOTE—See *Table 3* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between caffeine and theophylline, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of caffeine from the *Standard solution*

C_S = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of caffeine in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard peaks less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Caffeine	1.0	—	—
Theophylline	1.3	1.2	0.1
1,3,9-Trimethylxanthine[a]	1.7	1.2	0.1
Formyl 1,3-dimethyl-5,6-diaminouracil[b]	2.4	1.9	0.1
Any individual degradation product	—	1.0	0.2
Total degradation products	—	—	0.4

[a] 1,3,9-Trimethyl-3,9-dihydro-1*H*-purine-2,6-dione.

[b] *N*-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl) formamide.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Caffeine RS

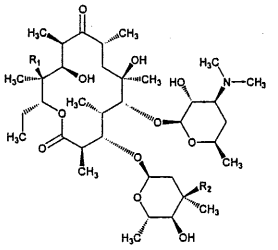
USP Ergotamine Tartrate RS

USP Ergotaminine RS

USP Theophylline RS

Erythromycin

Change to read:



Erythromycin	R_1	R_2
A	OH	OCH_3
B	H	OCH_3
C	OH	OH

$C_{37}H_{67}NO_{13}$ ▲733.94▲ (USP 1-Dec-2019)

Erythromycin;

(3*R**,4*S**,5*S**,6*R**,7*R**,9*R**,11*R**,12*R**,13*S**,14*R**)-4-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione [114-07-8].

DEFINITION

Erythromycin consists primarily of erythromycin A ($C_{37}H_{67}NO_{13}$). The sum of the percentages of erythromycin

www.webofpharma.com

A, erythromycin B, and erythromycin C is NLT 85.0% and NMT 100.5%, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

▲• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)
Standard solution: 50 mg/mL of USP Erythromycin RS, previously dried at a pressure not exceeding 5 mm of mercury at 60° for 3 h, in chloroform
Sample solution: 50 mg/mL of Erythromycin, previously dried at a pressure not exceeding 5 mm of mercury at 60° for 3 h, in chloroform
Spectral range: 4000–2050 cm^{-1} and 1980–400 cm^{-1}
Acceptance criteria: Meets the requirements▲ (USP 1-Dec-2019)

Add the following:

▲• **B.** The retention times of erythromycin A, erythromycin B, and erythromycin C in the *Sample solution* correspond to those of *Standard solution 1* and *Standard solution 2*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

• **PROCEDURE**
▲Use solutions containing erythromycin promptly or within 1 day if stored in a refrigerator.▲ (USP 1-Dec-2019)
Diluted phosphoric acid: Dilute 10 mL of phosphoric acid with water to 100 mL.
Solution A: 1.75 g of dibasic potassium phosphate in 50 mL of water. Adjust with *Diluted phosphoric acid* or 0.2 N sodium hydroxide TS to a pH of 9.0. Add 400 mL of water, 165 mL of tertiary butyl alcohol, and 30 mL of acetonitrile. Dilute with water to 1000 mL.
Solution B: 20 mL of pH 7.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Solutions, Buffer Solutions*); adjusted with phosphoric acid to a pH of 3.5
Mobile phase: Acetonitrile, *Solution A*, and water (2:5:1)
Diluent: pH 7.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Solutions, Buffer Solutions*) and methanol (15:1)
Standard solution 1: 4 mg/mL of USP Erythromycin RS prepared as follows. Transfer 100 mg of USP Erythromycin RS to a 25-mL volumetric flask, add 5 mL of methanol, swirl to dissolve and dilute with *Diluent* to volume.
Standard solution 2: 0.2 mg/mL of USP Erythromycin B RS and USP Erythromycin C RS prepared as follows. Transfer 5 mg each of USP Erythromycin B RS and USP Erythromycin C RS to a 25-mL volumetric flask, add 5 mL of methanol, swirl to dissolve, and dilute with *Diluent* to volume.
▲▲ (USP 1-Dec-2019)
System suitability solution: Transfer 2 mg of USP Erythromycin Related Compound N RS to a 10-mL volumetric flask, add 0.4 mL of *Standard solution 1* and dilute with *Standard solution 2* to volume.
Sample solution: 4 mg/mL of Erythromycin prepared as follows. Transfer 100 mg of the sample to a 25-mL volumetric flask, add 5 mL of methanol, swirl to dissolve, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; packing L21 (1000 Å)
Column temperature: 65°
Flow rate: 2 mL/min
Injection volume: 100 µL
Run time: NLT 5 times the retention time of the erythromycin A peak
System suitability
Samples: *Standard solution 1* and *System suitability solution*
[NOTE—The relative retention times for erythromycin related compound N (*N*-demethylerythromycin A), erythromycin C, erythromycin A, and erythromycin B are about 0.56, 0.61, 1.0, and 1.6, respectively.]
Suitability requirements
Resolution: NLT 0.8 between erythromycin related compound N and erythromycin C; NLT 5.5 between erythromycin related compound N and erythromycin A, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution 1*
Analysis
Samples: *Standard solution 1*, *Standard solution 2*, ▲▲ (USP 1-Dec-2019)and *Sample solution*
▲▲ (USP 1-Dec-2019)
Calculate the percentage of erythromycin A in the portion of Erythromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of erythromycin A from the *Sample solution*
r_S = peak response of erythromycin A from *Standard solution 1*
C_S = concentration of USP Erythromycin RS in *Standard solution 1* (mg/mL)
C_U = concentration of Erythromycin in the *Sample solution* (mg/mL)
P = percentage of erythromycin A in USP Erythromycin RS

Calculate the percentages of erythromycin B and erythromycin C in the portion of Erythromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of the relevant analyte from the *Sample solution*
r_S = peak response of the relevant analyte from *Standard solution 2*
C_S = concentration of the corresponding Reference Standard in *Standard solution 2* (mg/mL)
C_U = concentration of Erythromycin in the *Sample solution* (mg/mL)
P = ▲potency of erythromycin B or erythromycin C in the corresponding Reference Standard (mg/mg)▲ (USP 1-Dec-2019)

Acceptance criteria: The sum of the percentages of erythromycin A, erythromycin B, and erythromycin C is 85.0%–100.5% on the anhydrous basis. ▲The percentage of erythromycin B is NMT 12.0%, and the percentage of erythromycin C is NMT 5.0%.▲ (USP 1-Dec-2019)

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

Change to read:

• **LIMIT OF THIOCYANATE**
▲Use the *Standard solutions*, *Sample solution*, and *Blank solution* within 30 min.▲ (USP 1-Dec-2019)

USP Monographs

www.webofpharma.com

Standard stock solutions 1 and 2: 0.2 mg/mL of potassium thiocyanate prepared in duplicate as follows. Transfer 100 mg of potassium thiocyanate, previously dried at 105° for 1 h and cooled, to a 50-mL volumetric flask. Add about 20 mL of methanol to each flask, swirl to dissolve, and dilute with methanol to volume. Transfer 5.0 mL of this solution to a 50-mL volumetric flask and dilute with methanol to volume.

Standard solutions 1 and 2: 0.02 mg/mL of potassium thiocyanate prepared in duplicate as follows. Transfer 5.0 mL of each of the *Standard stock solutions* to separate 50-mL low-actinic volumetric flasks, add 1.0 mL of ferric chloride TS, dilute with methanol to volume.

Sample solution: 2 mg/mL of Erythromycin prepared as follows. Transfer 100 mg of Erythromycin to a 50-mL low-actinic volumetric flask, add 20 mL of methanol, and swirl to dissolve. Add 1.0 mL of ferric chloride TS and dilute with methanol to volume.

Blank solution: Add 1.0 mL of ferric chloride TS to a 50-mL low-actinic volumetric flask. Dilute with methanol to volume.

Instrumental conditions

▲(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)▲ (USP 1-Dec-2019)

Mode: UV-Vis

Analytical wavelength: 492 nm

System suitability

Samples: *Standard solution 1*, *Standard solution 2*, and *Blank solution*

Use the *Blank solution* to zero the instrument. Measure the absorbance of the two *Standard solutions*.

Suitability: 0.985–1.015

Calculate the suitability, *S*:

$$\text{Result} = (A_1/W_1) \times (W_2/A_2)$$

A_1 = absorbance of *Standard solution 1*
W_1 = weight of the potassium thiocyanate taken to prepare *Standard solution 1* (mg)
W_2 = weight of the potassium thiocyanate taken to prepare *Standard solution 2* (mg)
A_2 = absorbance of *Standard solution 2*

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of thiocyanate in the portion of Erythromycin taken:

$$\text{Result} = (M_{r1}/M_{r2}) \times (A_U/W_U) \times 0.5 \times [(W_1/A_1) + (W_2/A_2)] \times 100$$

M_{r1} = molecular weight of thiocyanate, 58.08
M_{r2} = molecular weight of potassium thiocyanate, 97.18
A_U = absorbance of the *Sample solution*
W_U = weight of Erythromycin taken to prepare the *Sample solution* (mg)
W_1 = weight of the potassium thiocyanate taken to prepare *Standard solution 1* (mg)
A_1 = absorbance of *Standard solution 1*
W_2 = weight of the potassium thiocyanate taken to prepare *Standard solution 2* (mg)
A_2 = absorbance of *Standard solution 2*

Acceptance criteria: NMT 0.3%

Change to read:

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, Standard solution 1, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

▲Use solutions containing erythromycin promptly, or within 1 day if stored in a refrigerator.

Diluted standard solution: 0.12 mg/mL of USP Erythromycin RS prepared as follows. Transfer 3.0 mL of *Standard solution 1* to a 100-mL volumetric flask and dilute with *Diluent* to volume.

Peak identification solution: Dissolve 10 mg of USP Erythromycin RS in 2 mL of methanol, add 10 mL of *Solution B*, and allow to stand for about 30 min.▲ (USP 1-Dec-2019)

Analysis

Samples: *Sample solution*, ▲*Diluted standard solution*, and *Peak identification solution*

Identify the erythromycin A enol ether peak using the *Peak identification solution*.▲ (USP 1-Dec-2019)

Calculate the percentage of the largest single impurity (the largest peak other than erythromycin A, erythromycin B, erythromycin C, and erythromycin A enol ether) in the portion of Erythromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of the largest single impurity (the largest peak other than erythromycin A, erythromycin B, erythromycin C, and erythromycin A enol ether) from the *Sample solution*
r_S = peak response of erythromycin A from the *Diluted standard solution*
C_S = concentration of USP Erythromycin RS in the *Diluted standard solution* (mg/mL)
C_U = concentration of Erythromycin in the *Sample solution* (mg/mL)
P = percentage of erythromycin A in USP Erythromycin RS

Calculate the percentage of erythromycin A enol ether in the portion of Erythromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

r_U = peak response of erythromycin A enol ether from the *Sample solution*
r_S = peak response of erythromycin A from the *Diluted standard solution*
C_S = concentration of USP Erythromycin RS in the *Diluted standard solution* (mg/mL)
C_U = concentration of Erythromycin in the *Sample solution* (mg/mL)
P = percentage of erythromycin A in the USP Erythromycin RS
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.▲▲ (USP 1-Dec-2019)

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Erythromycin A enol ether▲[a]▲ (USP 1-Dec-2019)	3.2	11	3.0
Erythromycin A	1.0	—	—

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Largest single impurity[b]	—	—	3.0

▲[a] (2*R*,3*R*,4*S*,5*R*,8*R*,9*S*,10*S*,11*R*)-11-{[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-5-ethyl-3,4-dihydroxy-9-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-2,4,8,10,12,14-hexamethyl-6,15-dioxabicyclo[10.2.1]pentadec-1(14)-en-7-one.▲ (USP 1-Dec-2019)

[b] The largest peak other than erythromycin A, erythromycin B, erythromycin C, and erythromycin A enol ether.

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 20 mg/mL in dehydrated alcohol
  Acceptance criteria: −71° to −78°, determined after standing for 30 min
- **WATER DETERMINATION** ⟨921⟩, *Method I*
  Sample solution: Use 20 mL of methanol containing 10% of imidazole in place of methanol in the titration vessel.
  Acceptance criteria: NMT 10.0%
- **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Erythromycin RS
  USP Erythromycin B RS
  ▲12-Deoxyerythromycin;
  (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*S*,13*R*,14*R*)-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-14-ethyl-7,12-dihydroxy-4-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione.
  $C_{37}H_{67}NO_{12}$ 717.94▲ (USP 1-Dec-2019)
  USP Erythromycin C RS
  ▲3″-*O*-Demethylerythromycin;
  (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-{[(2*R*,4*R*,5*S*,6*S*)-4,5-Dihydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione.
  $C_{36}H_{65}NO_{13}$ 719.91▲ (USP 1-Dec-2019)
  USP Erythromycin Related Compound N RS
  N-Demethylerythromycin A;
  ▲(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-14-Ethyl-7,12,13-trihydroxy-4-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-6-{[(2*S*,3*R*,4*S*,6*R*)-3-hydroxy-6-methyl-4-(methylamino)tetrahydro-2*H*-pyran-2-yl]oxy}-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione.▲ (USP 1-Dec-2019)
  $C_{36}H_{65}NO_{13}$ 719.91

Erythromycin Delayed-Release Capsules

» Erythromycin Delayed-Release Capsules contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Erythromycin RS

Identification—Prepare a test solution by mixing a quantity of finely ground Capsule contents with methanol to obtain a concentration of about 2.5 mg of erythromycin per mL. Prepare a Standard solution of USP Erythromycin RS in methanol containing 2.5 mg per mL. Apply separately 10 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in an unlined chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about 7 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and examine the chromatogram, in which erythromycin appears as a black-to-purple spot: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Dissolution ⟨711⟩—Proceed as directed for *Procedure* for *Method B* under *Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms.*

Apparatus 1: 50 rpm.

Times: 60 minutes for *Acid Stage;* 60 minutes for *Buffer Stage.*

Procedure—Transfer the contents of 1 Capsule to the apparatus. Proceed as directed for *Acid Stage,* 900 mL of 0.06 N hydrochloric acid being placed in the vessel instead of 1000 mL of 0.1 N hydrochloric acid, and the apparatus operated for 60 minutes instead of 2 hours. Do not perform an analysis at the end of the *Acid stage.* Continue as directed for *Buffer Stage,* 900 mL of the pH 6.8 phosphate buffer being used instead of 1000 mL. Determine the amount of $C_{37}H_{67}NO_{13}$ dissolved after 120 minutes by assaying a filtered portion of the solution under test as directed under *Antibiotics—Microbial Assays* ⟨81⟩.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{37}H_{67}NO_{13}$ is dissolved in 120 minutes.

Water Determination, *Method I* ⟨921⟩: not more than 7.5%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using not less than 5 Capsules blended for about 3 minutes in a high-speed glass blender jar containing 200 mL of methanol. Add 300 mL of *Buffer B.3,* and blend again for about 3 minutes. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Erythromycin Topical Gel

» Erythromycin Topical Gel is Erythromycin in a suitable gel vehicle. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of $C_{37}H_{67}NO_{13}$.

Packaging and storage—Preserve in tight containers.

USP Monographs

www.webofpharma.com

USP Reference standards ⟨11⟩—
USP Erythromycin RS
Identification—Prepare a test solution as follows. To 1 g of Topical Gel in a 50-mL screw-capped tube, add 20 mL of 0.01 N hydrochloric acid, and heat in a water bath to reflux. Remove the tube from the water bath and shake it. Place the tube in the water bath again, and heat to reflux. Remove the tube from the water bath, and immediately decant a portion of the hot clear supernatant into a test tube. Allow to cool, add an equal volume of a mixture of methanol, water, and triethylamine (90:9:1), and mix. Concomitantly prepare a Standard solution as directed above, except to use 5 mg of USP Erythromycin RS and 5 mL of 0.01 N hydrochloric acid instead of 1 g of Topical Gel and 20 mL of 0.01 N hydrochloric acid. Separately apply 5 µL of the test solution and the Standard solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in an unlined chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of methanol, water, and triethylamine (90:9:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and examine the chromatograms. The spots due to erythromycin are black to purple in color: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.
Minimum fill ⟨755⟩: meets the requirements.
Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed quantity of Topical Gel, equivalent to about 20 mg of erythromycin, blended for about 3 minutes in a high-speed glass blender jar containing 200.0 mL of *Buffer B.3* to which has been added 0.5% of polysorbate 80. Dilute an accurately measured volume of the blended solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Erythromycin Intramammary Infusion

» Erythromycin Intramammary Infusion is a solution of Erythromycin in a suitable vegetable oil vehicle. It contains one or more suitable preservatives. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{37}H_{67}NO_{13}$.

Packaging and storage—Preserve in single-dose disposable syringes that are well-closed containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
Identification—Transfer a quantity of Intramammary Infusion, equivalent to about 5 mg of erythromycin, to a separator containing 50 mL of solvent hexane. Shake until dissolved. Extract with three separate 20-mL portions of methanol. Combine the methanol extracts in a beaker, and evaporate to dryness. Dissolve the residue in 2 mL of methanol (test solution). Proceed as directed in the *Identification*— test under *Erythromycin Delayed-Release Capsules*, beginning with "Prepare a Standard solution of USP Erythromycin RS."
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay—Transfer the contents of a syringe of Intramammary Infusion to a separator containing 50 mL of solvent hexane. Shake until dissolved. Wash with four separate 20-mL portions of a mixture of methanol and water (4:1). Combine the washings in a 100-mL volumetric flask, dilute with the methanol and water solution to volume, and mix. Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this stock solution diluted quantitatively with *Buffer B.3* to yield a *Test Dilution* having a concentration of erythromycin assumed to be equal to the median dose level of the Reference Standard.

Erythromycin Injection

» Erythromycin Injection is a sterile solution of Erythromycin in a polyethylene glycol vehicle. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{37}H_{67}NO_{13}$.

Packaging and storage—Preserve in multiple-dose containers.
Labeling—Label it to indicate that it is for veterinary use only. Label it to state that it is for intramuscular administration only.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
Identification—Transfer a quantity of Injection, equivalent to about 5 mg of erythromycin, to a separator containing 50 mL of solvent hexane. Shake until dissolved. Extract with three separate 20-mL portions of methanol. Combine the methanol extracts in a beaker, and evaporate to dryness. Dissolve the residue in 2 mL of methanol (test solution). Proceed as directed in the *Identification*— test under *Erythromycin Delayed-Release Capsules*, beginning with "Prepare a Standard solution of USP Erythromycin RS."
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined.*
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of erythromycin, to a separator containing 50 mL of solvent hexane. Shake until dissolved. Wash with four separate 20-mL portions of a mixture of methanol and water (4:1). Combine the washings in a 100-mL volumetric flask, dilute with the methanol and water solution to volume, and mix. Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this stock solution diluted quantitatively with *Buffer B.3* to yield a *Test Dilution* having a concentration of erythromycin assumed to be equal to the median dose level of the Standard.

www.webofpharma.com

Erythromycin Ointment

» Erythromycin Ointment is Erythromycin in a suitable ointment base. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve in collapsible tubes or in other tight containers, preferably at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin B RS
USP Erythromycin C RS
USP Erythromycin Related Compound N RS
N-Demethylerythromycin A.
$C_{36}H_{65}NO_{13}$ 719.91

Identification—

A: Transfer a quantity of Ointment, equivalent to about 5 mg of erythromycin, to a separator containing 50 mL of solvent hexane. Shake until dissolved. Extract with three separate 20-mL portions of methanol. Combine the methanol extracts in a beaker, and evaporate to dryness. Dissolve the residue in 2 mL of methanol (test solution). Proceed as directed in the *Identification* test under *Erythromycin Delayed-Release Capsules*, beginning with "Prepare a Standard solution of USP Erythromycin RS."

B: The retention times of the peaks for erythromycin A, erythromycin B, and erythromycin C in the chromatogram of the *Assay preparation* correspond to those in the chromatograms of the *Standard preparation* and *Erythromycins B and C standard preparation*, as obtained in the *Assay*.

Minimum fill ⟨755⟩: meets the requirements.

Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay—

Solution A—Prepare a degassed mixture of acetonitrile and water (90:10). Store in a reservoir protected from air by sparging with helium.

Solution B—To 1000 mL of degassed water add 0.5 mL of sodium hydroxide solution (1 in 2), using a suitable syringe and needle to minimize exposure to air. Degas, and store in a reservoir protected from air by sparging with helium.

Solution C—Use degassed water, and store in a reservoir protected from air by sparging with helium.

Mobile phase—Using a suitable pumping system, pump *Solution A*, *Solution C*, and *Solution B* from the respective reservoirs in the ratio of 56:37:7. Make any necessary adjustments (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of methanol and water (50:50).

Standard preparation—Quantitatively prepare a solution of USP Erythromycin RS in *Diluent* having a known concentration of about 0.66 mg per mL.

Erythromycins B and C standard preparation—Quantitatively prepare a solution in *Diluent* having known concentrations of about 34 µg each of USP Erythromycin B RS and USP Erythromycin C RS per mL.

System suitability solution—Transfer about 2 mg of USP Erythromycin Related Compound N RS to a 10-mL volumetric flask, add 0.4 mL of the *Standard preparation* and 6 mL of *Erythromycins B and C standard preparation*, and mix. Dilute with *Erythromycins B and C standard preparation* to volume, and mix.

Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 60 mg of erythromycin, to a 125-mL separator. Add 50 mL of solvent hexane, and shake until dissolved. Extract with four separate 20-mL portions of *Diluent*, collecting the extracts in a 100-mL volumetric flask. Dilute the combined extracts with *Diluent* to volume, mix, and pass a portion of the solution through a filter having a 0.45-µm porosity. Use the clear filtrate as the *Assay preparation*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with an electrochemical detector, a glassy carbon electrode fitted with three gaskets, a 4-mm × 5-cm guard column that contains 8-µm packing L50, and a 4-mm × 25-cm analytical column that contains 8-µm packing L50. The electrochemical detector is used in the pulsed integrated amperometric mode with a range of 10 nC, an output of 1 V full scale, a rise time of 0.6 second, positive polarity, E = 0.9 V; t1 = 400 ms; E2 = 0.9 V; t2 = 100 ms; E3 = –0.9 V; t3 = 100 ms. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.4 for erythromycin related compound N, 0.5 for erythromycin C, 1.0 for erythromycin A, and 1.6 for erythromycin B; the resolution, *R*, between erythromycin related compound N and erythromycin C is not less than 0.6, between erythromycin C and erythromycin A not less than 2.5, and between erythromycin A and erythromycin B not less than 2.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 3%.

[NOTE—Turn off the electrochemical detector before stopping the flow of the *Mobile phase*.]

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation*, *Erythromycins B and C standard preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of erythromycin A in the portion of Ointment taken by the formula:

$$0.1(CP/W)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Erythromycin RS in the *Standard preparation*; *P* is the designated percentage of erythromycin A in USP Erythromycin RS; *W* is the quantity, in g, of Ointment taken to prepare the *Assay preparation*; and r_U and r_S are the erythromycin A peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentages of erythromycin B and erythromycin C in the portion of Ointment taken by the formula:

$$0.0001(CP/W)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of the relevant USP Reference Standard in the *Erythromycins B and C standard preparation*; *P* is the designated percentage of erythromycin B or erythromycin C in the relevant USP Reference Standard; *W* is the quantity, in g, of Ointment taken to prepare the *Assay preparation*; and r_U and r_S are the peak areas for the relevant analyte obtained from the *Assay preparation* and the *Erythromycins B and C standard preparation*, respectively. Calculate the percentage content of erythromycin in the Ointment by adding the percentages of erythromycin A, erythromycin B, and erythromycin C found.

www.webofpharma.com

Erythromycin Ophthalmic Ointment

DEFINITION

Erythromycin Ophthalmic Ointment is a sterile preparation of Erythromycin in a suitable ointment base. It contains NLT 90.0% and NMT 120.0% of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 2.5 mg/mL of USP Erythromycin RS in methanol

Sample solution: 2.5 mg/mL of erythromycin from Ophthalmic Ointment in methanol prepared as follows. Transfer an amount of Ophthalmic Ointment containing nominally 5 mg of erythromycin to a separator containing 50 mL of solvent hexane. Shake until dissolved. Extract with three separate 20-mL portions of methanol. Combine the methanol extracts in a beaker, and evaporate to dryness. Dissolve the residue in 2 mL of methanol.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 µL

Developing solvent system: Methanol and chloroform (85:15)

Spray reagent: Alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5)

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* to the plate. Place the plate in an unlined chromatographic chamber, and develop the chromatogram using the *Developing solvent system* until the solvent front has moved about 7 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with *Spray reagent*. Heat the plate at 100° for 10 min, and examine the chromatogram, in which erythromycin appears as a black-to-purple spot.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Solution A: Acetonitrile and water (90:10). Store in a reservoir protected from air by sparging with helium.

Solution B: 0.04 mg/mL of sodium hydroxide in water

Mobile phase: *Solution A* and *Solution B* (56:44)

Diluent: Methanol and water (50:50)

Standard solution 1: 0.66 mg/mL of USP Erythromycin RS in *Diluent*

Standard solution 2: 0.034 mg/mL of USP Erythromycin B RS and USP Erythromycin C RS in *Diluent*

System suitability solution: Transfer 2 mg of USP Erythromycin Related Compound N RS to a 10-mL volumetric flask, add 0.4 mL of *Standard solution 1* and 6 mL of *Standard solution 2*, and mix. Dilute with *Standard solution 2* to volume.

Sample solution: Nominally 0.6 mg/mL of erythromycin from Ophthalmic Ointment in *Diluent* prepared as follows. Transfer an amount of Ophthalmic Ointment containing nominally 60 mg of erythromycin to a 125-mL separator. Add 50 mL of solvent hexane, and shake until dissolved. Extract with four separate 20-mL portions of *Diluent*, collecting the extracts in a 100-mL volumetric flask. Dilute the combined extracts with *Diluent* to volume, and pass a portion of the solution through a suitable filter. Use the clear filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Pulsed amperometric electrochemical detector

Electrode: Glassy carbon

Waveform: See *Table 1*.

Table 1

Time (s)	Potential (V)	Integration
0.00	+0.9	—
0.40	+0.9	Begin
0.50	+0.9	End
0.60	−0.9	—

Columns

Guard: 4-mm × 5-cm; 8-µm packing L50

Analytical: 4-mm × 25-cm; 8-µm packing L50

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution 1* and *System suitability solution*

[NOTE—For relative retention times, see *Table 2*.]

Table 2

Peak	Relative Retention Time
Erythromycin related compound N	0.4
Erythromycin C	0.5
Erythromycin A	1.0
Erythromycin B	1.6

Suitability requirements

Resolution: NLT 0.6 between erythromycin related compound N and erythromycin C; NLT 2.5 between erythromycin C and erythromycin A; NLT 2.5 between erythromycin A and erythromycin B, *System suitability solution*

Tailing factor: NMT 2, *Standard solution 1*

Relative standard deviation: NMT 3%, *Standard solution 1*

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of erythromycin A relative to the labeled amount of erythromycin in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area of erythromycin A from the *Sample solution*

r_S = peak area of erythromycin A from *Standard solution 1*

C_S = concentration of USP Erythromycin RS in *Standard solution 1* (mg/mL)

C_U = nominal concentration of erythromycin in the *Sample solution* (mg/mL)

P = potency of erythromycin A in USP Erythromycin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

USP Monographs

www.webofpharma.com

Calculate the percentage of erythromycin B relative to the labeled amount of erythromycin in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak area of erythromycin B from the *Sample solution*
r_S = peak area of erythromycin B from *Standard solution 2*
C_S = concentration of USP Erythromycin B RS in *Standard solution 2* (mg/mL)
C_U = nominal concentration of erythromycin in the *Sample solution* (mg/mL)
P = potency of erythromycin B in USP Erythromycin B RS (mg/mg)

Calculate the percentage of erythromycin C relative to the labeled amount of erythromycin in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak area of erythromycin C from the *Sample solution*
r_S = peak area of erythromycin C from *Standard solution 2*
C_S = concentration of USP Erythromycin C RS in *Standard solution 2* (mg/mL)
C_U = nominal concentration of erythromycin in the *Sample solution* (mg/mL)
P = potency of erythromycin C in USP Erythromycin C RS (mg/mg)

Calculate the percentage of the labeled amount of erythromycin in the Ophthalmic Ointment by adding the percentages of erythromycin A, erythromycin B, and erythromycin C.

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩**:** It meets the requirements.
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Erythromycin RS
  USP Erythromycin B RS
  USP Erythromycin C RS
  USP Erythromycin Related Compound N RS

Erythromycin Pledgets

» Erythromycin Pledgets are suitable absorbent pads impregnated with Erythromycin Topical Solution. Pledgets contain not less than 90.0 percent of the labeled volume of Erythromycin Topical Solution.

Packaging and storage—Preserve in tight containers.
Labeling—Label Pledgets to indicate that each Pledget is to be used once and then discarded. Label Pledgets also to indicate the volume, in mL, of Erythromycin Topical Solution contained in each Pledget, and the concentration, in mg of erythromycin per mL, of the Erythromycin Topical Solution.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
Identification—Prepare a test solution by shaking one Pledget with methanol, and diluting appropriately to obtain a concentration of about 2.5 mg of erythromycin per mL. Prepare a Standard solution of USP Erythromycin RS in methanol containing 2.5 mg per mL. Apply separately 10 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in an unlined chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about 7 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and examine the chromatogram, in which erythromycin appears as a black-to-purple spot: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.
Other requirements—The Erythromycin Topical Solution expressed from Pledgets meets the requirements for *Water* and *Alcohol Determination, Method II 611:* under *Erythromycin Topical Solution.*
Topical solution assay—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, using the solution expressed from not less than 10 Pledgets. Dilute an accurately measured volume of this solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.
Assay and minimum volume—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, assaying 10 Pledgets individually. Transfer 1 Pledget to a suitable container, add about 50 mL of *Buffer B.3*, and shake for 2 minutes. Transfer the solution so obtained to a 100-mL volumetric flask. Wash the Pledget with two 20-mL portions of *Buffer B.3*, add the washings to the 100-mL volumetric flask, dilute with *Buffer B.3* to volume, and mix. Dilute an accurately measured volume of this solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. Calculate the volume, in mL, of Erythromycin Topical Solution in each Pledget taken by dividing the number of mg of erythromycin in each individual Pledget by the number of mg of erythromycin in each mL of Erythromycin Topical Solution as obtained in the *Topical Solution assay*. The volume of Erythromycin Topical Solution in each Pledget is not less than 90.0% of the labeled amount. If this requirement is not met, determine the volume of Erythromycin Topical Solution in each of 20 additional Pledgets. The volume of Erythromycin Topical Solution in not more than 1 of the 30 Pledgets is less than 90.0% of the labeled amount.

Erythromycin Topical Solution

» Erythromycin Topical Solution is a solution of Erythromycin in a suitable vehicle. It contains not

USP Monographs

www.webofpharma.com

less than 90.0 percent and not more than 125.0 percent of the labeled amount of $C_{37}H_{67}NO_{13}$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
Identification—Prepare a test solution by mixing a portion of the Topical Solution with methanol to obtain a concentration of about 2.5 mg of erythromycin per mL. Proceed as directed in the *Identification*— test under *Erythromycin Delayed-Release Capsules*, beginning with "Prepare a Standard solution of USP Erythromycin RS."
Water Determination, *Method I* ⟨921⟩: not more than 8.0% if it contains 20 mg per mL, or not more than 5.0% if it contains 15 mg per mL, or not more than 2.0% if it contains acetone, 20 mL of a mixture of pyridine and methanol (1:1) being used in place of methanol in the titration vessel.
Alcohol Determination, *Method II* ⟨611⟩: between 92.5% and 107.5% of the labeled amount of C_2H_5OH.
Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Topical Solution diluted quantitatively with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Erythromycin Tablets

» Erythromycin Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{37}H_{67}NO_{13}$.
[NOTE—Tablets that are enteric-coated meet the requirements for *Erythromycin Delayed-Release Tablets*.]

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
Identification—Prepare a test solution by mixing a quantity of finely powdered Tablets with methanol to obtain a concentration of about 2.5 mg of erythromycin per mL. Proceed as directed in the *Identification*— test under *Erythromycin Delayed-Release Capsules*, beginning with "Prepare a Standard solution of USP Erythromycin RS."

Dissolution ⟨711⟩—
Medium: 0.05 M pH 6.8 phosphate buffer (see *Buffer solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.
Apparatus 2: 50 rpm.
Time: 60 minutes.
Test solution—If necessary, dilute a filtered portion of the solution under test with *Medium* to obtain a solution having a concentration of about 0.28 mg of erythromycin per mL, and mix.
Standard solution—Dissolve an accurately weighed quantity of USP Erythromycin RS in methanol (not more than 1 mL of methanol for each 14 mg of the Reference Standard), and dilute with water, quantitatively and with mixing, to obtain a stock solution containing about 0.56 mg per mL. Immediately prior to use, dilute the stock solution quantitatively with water to obtain a *Standard solution* having a known concentration of about 0.28 mg per mL.
Procedure—Transfer 5.0-mL portions of the *Test solution* and the *Standard solution* to separate 25-mL volumetric flasks, and treat each as follows: Add 2.0 mL of water, and allow to stand for 5 minutes with intermittent swirling. Add 15.0 mL of 0.25 N sodium hydroxide, dilute with *Medium* to volume, and mix. Heat to 60° for 5 minutes, and allow to cool. Concomitantly determine the absorbances of these solutions at the wavelength of maximum absorbance at about 236 nm, with a suitable spectrophotometer, using blank solutions similarly prepared, except that 2.0 mL of 0.5 N sulfuric acid is substituted for the 2.0 mL of water. Calculate the amount of $C_{37}H_{67}NO_{13}$ dissolved.
Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{37}H_{67}NO_{13}$ is dissolved in 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Loss on drying ⟨731⟩—Dry about 100 mg of powdered Tablets in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 5.0% of its weight.
Assay—Place not less than 4 Tablets in a high-speed glass blender jar with 200 mL of methanol, and blend for 3 minutes. Add 300 mL of *Buffer B.3*, and blend for 3 minutes. Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this stock test solution diluted quantitatively with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Erythromycin Delayed-Release Tablets

DEFINITION
Erythromycin Delayed-Release Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHY**
Standard solution: 2.5 mg/mL of USP Erythromycin RS in methanol
Sample solution: Nominally 2.5 mg/mL of erythromycin from powdered Tablets in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 10 µL
Developing solvent system: Methanol and chloroform (85:15)
Spray reagent: Alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5)
Analysis
Samples: *Standard solution* and *Sample solution*
Place the plate in an unlined chromatographic chamber, and develop the chromatogram until the solvent front has moved about 7 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with *Spray reagent*. Heat the plate at 100° for 10 min, and examine the chromatogram, in which erythromycin appears as a black-to-purple spot.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY
• **ANTIBIOTICS—MICROBIAL ASSAYS** ⟨81⟩
Sample solution: Place NLT 4 Tablets in a high-speed glass blender jar with 200 mL of methanol, and blend for 3 min. Add 300 mL of *Buffer B.3*, and blend for 3 min.
Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution*

www.webofpharma.com

having a concentration that is nominally equivalent to the median level of the standard.
Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩**:** Proceed as directed for *Procedure* for *Method B* in *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms.*
Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1.*
Acid stage
Medium: Simulated gastric fluid TS (without pepsin); 900 mL
Apparatus 1: 100 rpm
Time: 60 min
Analysis: Do not analyze the sample at this stage.
Buffer stage
Medium: 0.05 M pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)
Apparatus 1: 100 rpm
Time: 60 min
Buffer: pH 1.2 buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)
Solution A: 1 g/L of bromocresol purple in pH 4.5 phosphate buffer
Standard solution: Dissolve USP Erythromycin RS in *Medium* to obtain a concentration similar to that of the *Sample solution.*
Sample solution: If necessary, dilute a filtered portion of the solution under test with *Medium* to obtain a solution containing about 0.28 mg/mL of erythromycin.
Detector: UV 410 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Transfer 2.0 mL of the *Standard solution* and the *Sample solution* to individual separators of a suitable size. Add 6 mL of *Buffer* and 8 mL of *Solution A,* and mix. Extract with 40.0 mL of chloroform. Determine the amount of erythromycin ($C_{37}H_{67}NO_{13}$) dissolved from UV absorbances of the chloroform extracts.
Tolerances: NLT 75% (*Q*) of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.* Proceed as directed under *Test 1,* except to use *Apparatus 2* at 75 rpm.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩
Analysis: Use 20 mL of methanol containing 10% of imidazole in place of methanol in the titration vessel.
Acceptance criteria: NMT 6.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** The labeling indicates the *Dissolution Test* with which the product complies.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Erythromycin RS

Erythromycin and Benzoyl Peroxide Topical Gel

» Erythromycin and Benzoyl Peroxide Topical Gel is a mixture of Erythromycin in a suitable gel vehicle containing benzoyl peroxide and one or more suitable dispersants, stabilizers, and wetting agents. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amounts of erythromycin ($C_{37}H_{67}NO_{13}$) and benzoyl peroxide ($C_{14}H_{10}O_4$).

Packaging and storage—Before mixing, preserve the Erythromycin and the vehicle containing benzoyl peroxide in separate, tight containers. After mixing, preserve the mixture in tight containers.
USP Reference standards ⟨11⟩**—**
USP Erythromycin RS
Identification—Prepare *Standard preparation* and an *Assay preparation* as directed in the *Assay,* except to omit the *Internal standard solution,* and chromatograph as directed in the *Assay:* the *Assay preparation* exhibits a major peak for benzoyl peroxide, the retention time of which corresponds to that exhibited by the *Standard preparation.*
Minimum fill ⟨755⟩: meets the requirements.

Limit of benzoyl peroxide related substances—
Mobile phase A, Mobile phase B, Standard preparation A, Standard preparation B, Standard preparation C, Standard preparation D, Resolution solution, and *Chromatographic system*—Proceed as directed in the test for *Related compounds* under *Benzoyl Peroxide Gel.*
Test preparation—Transfer an accurately weighed quantity of Topical Gel, equivalent to about 100 mg of benzoyl peroxide, to a 50-mL volumetric flask, add 25 mL of acetonitrile, shake vigorously to disperse the specimen, sonicate for 5 minutes, dilute with acetonitrile to volume, mix, and filter.
Procedure—Proceed as directed for *Procedure* in the test for *Related compounds* under *Benzoyl Peroxide Gel:* it meets the limits stated.
Assay for erythromycin—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Topical Gel blended for 3 to 5 minutes in a high-speed glass blender jar containing 0.5 mL of polysorbate 80 and an accurately measured volume of *Buffer B.3* sufficient to obtain a stock solution having a convenient concentration of erythromycin. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration of erythromycin assumed to be equal to the median dose level of the Standard.

Assay for benzoyl peroxide—
Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *ASSAY* under *Benzoyl Peroxide Gel.*
Assay preparation—Prepare as directed for *Assay preparation* in the *ASSAY* under *Benzoyl Peroxide Gel,* using Topical Gel.
Procedure—Proceed as directed for *Procedure* in the *ASSAY* under *Benzoyl Peroxide Gel.* Calculate the quantity, in mg, of benzoyl peroxide ($C_{14}H_{10}O_4$) in the portion of Topical Gel taken by the formula:

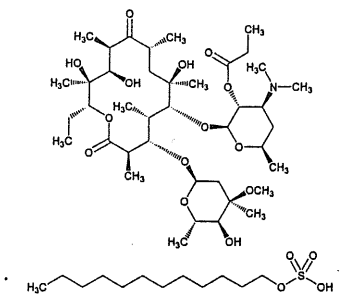
$$125C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of benzoyl peroxide in the *Standard preparation,* and R_U and R_S are the ratios of benzoyl peroxide peak response to ethyl benzoate peak response obtained from the *Assay preparation* and the *Standard preparation,* respectively.

USP Monographs

www.webofpharma.com

Erythromycin Estolate



$C_{40}H_{71}NO_{14} \cdot C_{12}H_{26}O_4S$ 1056.39
Erythromycin, 2′-propanoate, dodecyl sulfate (salt).
Erythromycin 2′-propionate dodecyl sulfate (salt) [3521-62-8].

» Erythromycin Estolate has a potency equivalent to not less than 600 µg of erythromycin ($C_{37}H_{67}NO_{13}$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Estolate RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197K***▲ (CN 1-May-2020)
Crystallinity ⟨695⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 4.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.
Free erythromycin—Prepare a test solution of it in methanol containing 10.0 mg per mL. Prepare a Standard solution of USP Erythromycin RS in methanol containing 0.3 mg per mL. [NOTE—Prepare these solutions immediately before use.] Prepare a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Before using, place the plate in an unlined developing chamber containing about 100 mL of methanol, and allow the solvent front to move to the top of the plate, marking the direction of travel. Remove the plate, and allow to dry. Apply separate 1-µL volumes of the test solution and the Standard solution on the plate, allow the spots to dry, and develop the chromatograms in a freshly prepared solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about one-half of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to dry. Place the plate under a hood, and spray uniformly with a solution consisting of 150 mg of xanthydrol dissolved in a mixture of hydrochloric acid and glacial acetic acid (92.5: 7.5). Heat the sprayed plate in an oven at 100° for 5 minutes. [**CAUTION**—Avoid exposure to acid fumes when removing the plate from the oven.] Examine the plate for reddish violet spots: free erythromycin has an R_F value of about 0.3, and erythromycin estolate has an R_F value of about 0.7. Any spot corresponding to free erythromycin obtained from the test solution does not exceed in size or intensity that of the principal spot obtained from the Standard solution (3.0%).

Assay—Proceed with Erythromycin Estolate as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed quantity of Erythromycin Estolate dissolved in methanol to obtain a solution containing the equivalent of 1.0 mg of erythromycin per mL. Immediately dilute quantitatively with 9 volumes of *Buffer B.3*, and allow to stand at room temperature for 18 hours. Dilute a portion of this solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Erythromycin Estolate Capsules

» Erythromycin Estolate Capsules contain the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Estolate RS
Identification—Prepare a test solution by mixing a quantity of Capsule contents with methanol to obtain a concentration equivalent to about 20 mg of erythromycin per mL. Prepare a Standard solution of USP Erythromycin Estolate RS in methanol containing the equivalent of 20 mg of erythromycin per mL. Apply separately 3 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Proceed as directed in the *Identification*— test under *Erythromycin Delayed-Release Capsules*, beginning with "Place the plate in an unlined chromatographic chamber."
Disintegration ⟨701⟩: 30 minutes, proceeding as directed for *Hard Gelatin Capsules*, except to use disks and to use simulated gastric fluid TS as the immersion fluid instead of water.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 5.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.
Assay—Place not less than 4 Capsules in a high-speed glass blender jar with 200.0 mL of methanol, and blend for 3 minutes. Add 300.0 mL of *Buffer B.3*, and blend for 3 minutes. Allow this solution to stand at room temperature for 18 hours. Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this stock test solution diluted quantitatively with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Erythromycin Estolate Oral Suspension

» Erythromycin Estolate Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$). It contains one or more suitable buffers, colors, diluents, dispersants, and flavors.

www.webofpharma.com

Packaging and storage—Preserve in tight containers, in a cool place.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Estolate RS
Identification—Transfer a quantity of Oral Suspension, equivalent to about 20 mg of erythromycin, to a separator. Add 15 mL of 0.02 N sodium hydroxide, and swirl to mix. Add 2 g of sodium chloride and 25 mL of chloroform, and shake for 3 minutes. Drain the chloroform phase through a small amount of chloroform-washed anhydrous sodium sulfate, and collect the chloroform extract in a beaker, rinsing the sodium sulfate with an additional 10 mL of chloroform. Evaporate the chloroform to dryness. Dissolve the residue in 1 mL of methanol (test solution). Prepare a Standard solution by transferring a quantity of USP Erythromycin Estolate RS, equivalent to 20 mg of erythromycin, to a separator and carrying out the extraction procedure described for preparation of the test solution. Apply separately 3 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Proceed as directed in the *Identification* test under *Erythromycin Delayed-Release Capsules*, beginning with "Place the plate in an unlined chromatographic chamber."

Uniformity of dosage units ⟨905⟩—
F *OR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS:* meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 3.5 and 6.5.
Assay—Dilute an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, quantitatively with methanol to obtain a solution containing the equivalent of 2.5 mg of erythromycin per mL. Dilute with 1.5 volumes of *Buffer B.3*, and allow to stand at room temperature for 18 hours. Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this stock test solution diluted quantitatively with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Erythromycin Estolate for Oral Suspension

» Erythromycin Estolate for Oral Suspension is a dry mixture of Erythromycin Estolate with one or more suitable buffers, colors, diluents, dispersants, and flavors. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Estolate RS
Identification—Prepare a test solution by mixing a quantity of Erythromycin Estolate for Oral Suspension with methanol to obtain a concentration equivalent to about 20 mg of erythromycin per mL. Prepare a Standard solution of USP Erythromycin Estolate RS in methanol containing the equivalent of 20 mg of erythromycin per mL. Apply separately 3 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Proceed as directed in the *Identification*— test under *Erythromycin Delayed-Release Capsules*, beginning with "Place the plate in an unlined chromatographic chamber."

Uniformity of dosage units ⟨905⟩—
F *OR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS* : meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 5.0 and 7.0 (if pediatric drops, between 5.0 and 5.5), in the suspension constituted as directed in the labeling.
Water Determination, *Method I* ⟨921⟩: not more than 2.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.
Assay—Constitute Erythromycin Estolate for Oral Suspension as directed in the labeling, and proceed as directed in the *Assay* — under *Erythromycin Estolate Oral Suspension.*

Erythromycin Estolate Tablets

» Erythromycin Estolate Tablets contain the equivalent of not less than 90.0 percent and not more than 120.0 percent (115.0 percent, if chewable) of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve in tight containers.
Labeling—Label the Tablets to indicate whether they are to be chewed before swallowing.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Estolate RS
Identification—Prepare a test solution by mixing a quantity of finely powdered Tablets with methanol to obtain a concentration equivalent to about 20 mg of erythromycin per mL. Prepare a Standard solution of USP Erythromycin Estolate RS in methanol containing the equivalent of 20 mg of erythromycin per mL. Apply separately 3 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Proceed as directed in the *Identification*— test under *Erythromycin Delayed-Release Capsules*, beginning with "Place the plate in an unlined chromatographic chamber."
[NOTE—Use the following procedure for chewable Tablets. Transfer a quantity of finely powdered Tablets, equivalent to about 20 mg of erythromycin, to a separator, and proceed as directed in the *Identification* test under *Erythromycin Estolate Oral Suspension*, beginning with "Add 15 mL of 0.02 *N* sodium hydroxide."]
Disintegration ⟨701⟩: 30 minutes, proceeding as directed for *Uncoated Tablets*, except to use disks and to use simulated gastric fluid as the immersion fluid instead of water. [NOTE—Chewable tablets are exempt from this requirement.]
Uniformity of dosage units ⟨905⟩: meet the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 5.0%; or if chewable tablets, not more than 4.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.
Assay—Proceed with Tablets as directed in the *Assay* under *Erythromycin Estolate Capsules.*

www.webofpharma.com

Erythromycin Estolate and Sulfisoxazole Acetyl Oral Suspension

» Erythromycin Estolate and Sulfisoxazole Acetyl Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$) and the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of sulfisoxazole ($C_{11}H_{13}N_3O_3S$). It contains one or more suitable buffers, colors, diluents, emulsifiers, flavors, preservatives, and suspending agents.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Estolate RS
USP Sulfisoxazole Acetyl RS

Identification—To a quantity of the Oral Suspension add a volume of methanol sufficient to yield a solution having a concentration equivalent to about 2.5 mg of erythromycin per mL. Shake this mixture by mechanical means for about 30 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the test solution. Prepare a solution of USP Erythromycin Estolate RS in methanol containing about 3 mg per mL (*Standard solution A*). Prepare a solution of USP Sulfisoxazole Acetyl RS in methanol containing about 8.7 mg per mL (*Standard solution B*). Apply separately 10 µL each of the test solution and the two Standard solutions to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow to dry. Place the plate in an unlined chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about 9 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of dehydrated alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and examine the chromatograms, in which the erythromycin estolate appears as a black-to-purple spot and the sulfisoxazole acetyl appears as a yellow spot: the R_F value of the principal black-to-purple spot obtained from the test solution corresponds to that obtained from *Standard solution A*, and the R_F value of the principal yellow spot obtained from the test solution corresponds to that obtained from *Standard solution B*.

Uniformity of dosage units ⟨905⟩—
F *OR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS* : meets the requirements.

Deliverable volume ⟨698⟩: meets the requirements.

pH ⟨791⟩: between 3.5 and 6.5.

Assay for erythromycin—Dilute an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, quantitatively with methanol to obtain a solution containing the equivalent of 2.5 mg of erythromycin per mL. Dilute with 1.5 volumes of *Buffer B.3*, and allow to stand at room temperature for 18 hours. Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of this stock test solution diluted quantitatively with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (1.0 µg of erythromycin per mL).

Assay for sulfisoxazole—
Mobile solvent—Mix 40 volumes of acetonitrile and 60 volumes of water. The acetonitrile concentration may be varied to meet system suitability requirements and to provide a suitable elution time for sulfisoxazole acetyl. Filter the solution through a membrane filter (1-µm or finer porosity).

Internal standard solution—Prepare a solution of benzanilide in acetonitrile having a concentration of about 0.33 mg per mL. Filter the solution through a membrane filter (1-µm or finer porosity).

Standard preparation—Prepare a solution of USP Sulfisoxazole Acetyl RS in *Internal standard solution* having a known concentration of about 1 mg per mL.

Assay preparation—Transfer an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 600 mg of sulfisoxazole, to a 125-mL separator, and extract with three 75-mL portions of chloroform. Collect the chloroform extracts in a 250-mL volumetric flask, dilute with chloroform to volume, and mix. Filter a portion of this solution through a membrane filter (1-µm or finer porosity). Pipet 4 mL of the filtrate into a glass-stoppered, 25-mL conical flask, and evaporate with the aid of a current of dry air to dryness. Add 10.0 mL of *Internal standard solution*, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure:* the resolution factor between sulfisoxazole acetyl and benzanilide is not less than 3.0.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of sulfisoxazole ($C_{11}H_{13}N_3O_3S$) in each mL of the Oral Suspension taken by the formula:

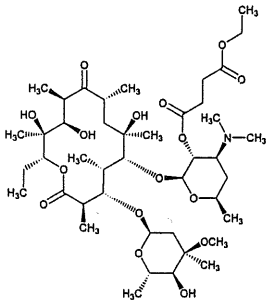
$$(267.31/309.35)(625C/V)(R_U/R_S)$$

in which 267.31 and 309.35 are the molecular weights of sulfisoxazole and sulfisoxazole acetyl, respectively; C is the concentration, in mg, of USP Sulfisoxazole Acetyl RS in each mL of the *Standard preparation*; V is the volume, in mL, of Oral Suspension taken; and R_U and R_S are the ratios of peak responses of sulfisoxazole acetyl peak to benzanilide peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

www.webofpharma.com

Erythromycin Ethylsuccinate

Change to read:



$C_{43}H_{75}NO_{16}$ ▲862.06▲ (USP 1-Dec-2019)

Erythromycin 2′-(ethyl butanedioate);
Erythromycin 2′-(ethyl succinate) [1264-62-6].

DEFINITION

Erythromycin Ethylsuccinate consists primarily of the 2′-ethylsuccinate ester of erythromycin A. The sum of the percentages of erythromycin A, erythromycin B, and erythromycin C is NLT 76.5%, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• ▲**A.**▲ (USP 1-Dec-2019) ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)
Sample solution: 10 mg/mL in chloroform
Cell: 1.0 mm
Acceptance criteria: Meets the requirements

Add the following:

▲• **B.** The retention times of the erythromycin A, erythromycin B, and erythromycin C peaks in the *Sample solution* correspond to those of *Standard solution 1* and *Standard solution 2*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

• **PROCEDURE**
▲Use solutions containing erythromycin promptly, or within 1 day if stored in a refrigerator.▲ (USP 1-Dec-2019)
Solution A: 20 mg/mL of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 8.0.
Solution B: 35 mg/mL of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 8.0.
Solution C: Adjust 20 mL of *Solution A* with phosphoric acid to a pH of 3.5.
Mobile phase: Acetonitrile, tertiary butyl alcohol, *Solution B*, and water (6:35:10:149)
Standard solution 1: 50 mg of USP Erythromycin RS in a 25-mL volumetric flask. Add 12.5 mL of methanol, and swirl to dissolve. Dilute with *Solution A* to volume.
Standard solution 2: 5 mg each of USP Erythromycin B RS and USP Erythromycin C RS, in a 50-mL volumetric flask. Add 25.0 mL of methanol, and swirl to dissolve. Add 2.5 mL of *Standard solution 1*, and dilute with *Solution A* to volume.
▲▲ (USP 1-Dec-2019)
System suitability solution: 0.1 mg/mL of USP Erythromycin Related Compound N RS in *Standard solution 2*
Sample solution: 115 mg of Erythromycin Ethylsuccinate in a 50-mL volumetric flask. Add 25.0 mL of methanol, and swirl to dissolve. Add about 20 mL of *Solution A* and allow to stand at room temperature for about 12 h to effect hydrolysis. Dilute with *Solution A* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; packing L21 (1000Å)
Column temperature: 70°
Flow rate: 2 mL/min
Injection volume: 200 µL
Run time: NLT 5 times the retention time of erythromycin A
System suitability
Samples: *Standard solution 1* and *System suitability solution*
The order of elution of the components is erythromycin related compound N, erythromycin C, erythromycin A, and erythromycin B.
Suitability requirements
Resolution: NLT 0.8 between erythromycin related compound N and erythromycin C; NLT 5.5 between erythromycin related compound N and erythromycin A, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution 1*
Analysis
Samples: *Standard solution 1*, *Standard solution 2*, ▲▲ (USP 1-Dec-2019) and *Sample solution*
▲▲ (USP 1-Dec-2019)
Calculate the percentage of erythromycin A in the portion of Erythromycin Ethylsuccinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of erythromycin A from the *Sample solution*
r_S = peak response of erythromycin A from *Standard solution 1*
C_S = concentration of USP Erythromycin RS in *Standard solution 1* (mg/mL)
C_U = concentration of Erythromycin Ethylsuccinate in the *Sample solution* (mg/mL)
P = percentage of erythromycin A in USP Erythromycin RS

Calculate the percentage of erythromycin B and erythromycin C in the portion of Erythromycin Ethylsuccinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of the relevant analyte from the *Sample solution*
r_S = peak response of the relevant analyte from *Standard solution 2*
C_S = concentration of the corresponding Reference Standard in *Standard solution 2* (mg/mL)
C_U = concentration of Erythromycin Ethylsuccinate in the *Sample solution* (mg/mL)
P = ▲potency of erythromycin B or erythromycin C in the corresponding Reference Standard (mg/mg)▲ (USP 1-Dec-2019)

USP Monographs

www.webofpharma.com

Acceptance criteria: NLT 76.5% for the sum of the percentages of erythromycin A, erythromycin B, and erythromycin C, on the anhydrous basis. The percentage of erythromycin B is NMT 12.0%, and the percentage of erythromycin C is NMT 5.0%.

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩

Sample: After ignition at 550 ± 50°, the charred residue is moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

Acceptance criteria: NMT 1.0%

Change to read:

• **ORGANIC IMPURITIES**

Solution B, Solution C, Mobile phase, Standard solution 1, Standard solution 2, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

▲**Peak identification solution:** 10 mg of USP Erythromycin RS in 2 mL of methanol. Add 10 mL of *Solution C*, and allow to stand for about 30 min. Refrigerate this solution until used, and discard 8 h after preparation.▲ (USP 1-Dec-2019)

Analysis

Samples: *Standard solution 2*, *Sample solution*, and ▲*Peak identification solution*

Identify the erythromycin A enol ether peak using the *Peak identification solution*.▲ (USP 1-Dec-2019) Begin peak integration of the *Sample solution* after the two peaks for succinates that elute just after the solvent front.

Calculate the percentage of the largest single impurity (the largest peak other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, and erythromycin *N*-ethylsuccinate) in the portion of Erythromycin Ethylsuccinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of the largest single impurity (the largest peak other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, and erythromycin *N*-ethylsuccinate) from the *Sample solution*

r_S = peak response of erythromycin A from *Standard solution 2*

C_S = concentration of USP Erythromycin RS in *Standard solution 2* (mg/mL)

C_U = concentration of Erythromycin Ethylsuccinate in the *Sample solution* (mg/mL)

P = percentage of erythromycin A in USP Erythromycin RS

Calculate the percentage of erythromycin A enol ether in the portion of Erythromycin Ethylsuccinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

r_U = peak response of erythromycin A enol ether from the *Sample solution*

r_S = peak response of erythromycin A from *Standard solution 2*

C_S = concentration of USP Erythromycin RS in *Standard solution 2* (mg/mL)

C_U = concentration of Erythromycin Ethylsuccinate in the *Sample solution* (mg/mL)

P = percentage of erythromycin A in USP Erythromycin RS

F = relative response factor (see *Table 1*)

Calculate the percentage of erythromycin *N*-ethylsuccinate in the portion of Erythromycin Ethylsuccinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

r_U = peak response of erythromycin *N*-ethylsuccinate from the *Sample solution*

r_S = peak response of erythromycin A from *Standard solution 2*

C_S = concentration of USP Erythromycin RS in *Standard solution 2* (mg/mL)

C_U = concentration of Erythromycin Ethylsuccinate in the *Sample solution* (mg/mL)

P = percentage of erythromycin A in USP Erythromycin RS

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Erythromycin *N*-ethylsuccinate▲[a]▲ (USP 1-Dec-2019)	1.3	7.4	3.0
Erythromycin A enol ether▲[b]▲ (USP 1-Dec-2019)	4.3–4.7	11	3.0
Erythromycin A	1.0	—	—
Largest single impurity[c]	—	—	3.0

▲[a] Ethyl 4-{[(2*S*,3*R*,4*S*,6*R*)-2-{[(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-14-ethyl-7,12,13-trihydroxy-4-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-3,5,7,9,11,13-hexamethyl-2,10-dioxooxacyclotetradecan-6-yl]oxy}-3-hydroxy-6-methyltetrahydro-2*H*-pyran-4-yl](methyl)amino}-4-oxobutanoate.

[b] (2*R*,3*R*,4*S*,5*R*,8*R*,9*S*,10*S*,11*R*)-11-[[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-5-ethyl-3,4-dihydroxy-9-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-2,4,8,10,12,14-hexamethyl-6,15-dioxabicyclo[10.2.1]pentadec-1(14)-en-7-one.▲ (USP 1-Dec-2019)

[c] The largest peak other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, and erythromycin *N*-ethylsuccinate.

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*

Sample solution: Use 20 mL of methanol containing 10% of imidazole in place of methanol in the titration vessel.

Acceptance criteria: NMT 3.0%

Change to read:

• **CRYSTALLINITY** ⟨695⟩**:** ▲Meets the requirements except, where it is labeled as amorphous, most of the particles do not exhibit birefringence and extinction positions.▲ (USP 1-Dec-2019)

• **X-RAY DIFFRACTION** ⟨941⟩**:** Where labeled as being amorphous, its X-ray diffraction pattern performed at high sensitivity for angles of diffraction between 2° and 20° exhibits no reflection, and between 7° and 10° exhibits a more intense hachured baseline, creating a halo.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Erythromycin Ethylsuccinate that is noncrystalline is labeled to indicate that it is amorphous. Any preparation containing the amorphous form of Erythromycin Ethylsuccinate is so labeled.

USP Monographs

www.webofpharma.com

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Erythromycin RS
  USP Erythromycin B RS
  ▲12-Deoxyerythromycin;
  (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*S*,13*R*,14*R*)-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-14-ethyl-7,12-dihydroxy-4-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione.
  $C_{37}H_{67}NO_{12}$ 717.94▲ (USP 1-Dec-2019)
  USP Erythromycin C RS
  ▲3″-*O*-Demethylerythromycin;
  (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-{[(2*R*,4*R*,5*S*,6*S*)-4,5-Dihydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione.
  $C_{36}H_{65}NO_{13}$ 719.91▲ (USP 1-Dec-2019)
  USP Erythromycin Related Compound N RS
  N-Demethylerythromycin A;
  ▲(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-14-Ethyl-7,12,13-trihydroxy-4-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-6-{[(2*S*,3*R*,4*S*,6*R*)-3-hydroxy-6-methyl-4-(methylamino)tetrahydro-2*H*-pyran-2-yl]oxy}-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione.▲ (USP 1-Dec-2019)
  $C_{36}H_{65}NO_{13}$ 719.91
  USP Erythromycin Ethylsuccinate RS

Erythromycin Ethylsuccinate Injection

» Erythromycin Ethylsuccinate Injection is a sterile solution of Erythromycin Ethylsuccinate in Polyethylene Glycol 400, and contains 2 percent of butyl aminobenzoate and a suitable preservative. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, using a membrane filter resistant to the solvent effect of polyethylene glycol 400.
Water Determination, *Method I* ⟨921⟩: not more than 1.5%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Injection diluted quantitatively with methanol to yield a solution containing the equivalent of about 1 mg of erythromycin per mL. Dilute a portion of this stock solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Sterile Erythromycin Ethylsuccinate

» Sterile Erythromycin Ethylsuccinate is Erythromycin Ethylsuccinate suitable for parenteral use. It has a potency equivalent to not less than 765 µg of erythromycin ($C_{37}H_{67}NO_{13}$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Ethylsuccinate RS

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197S*▲ (CN 1-May-2020)—
Solution: 1 in 100.
Medium: chloroform.
Cell size: 1.0 mm.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined.*
Other requirements—It conforms to the Definition and meets the requirements for *SPECIFIC TESTS, Water Determination 921, Method I, Residue on Ignition 281*, and the *X-Ray Diffraction 941:* under *Erythromycin Ethylsuccinate.*

Erythromycin Ethylsuccinate Oral Suspension

» Erythromycin Ethylsuccinate Oral Suspension is a suspension of Erythromycin Ethylsuccinate containing one or more suitable buffers, colors, dispersants, flavors, and preservatives. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve in tight containers, and store in a cold place.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Ethylsuccinate RS
Identification—To a quantity of the Oral Suspension add a volume of methanol sufficient to yield a solution having a concentration equivalent to about 2.5 mg of erythromycin per mL. Shake this mixture by mechanical means for about 30 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the test solution. Prepare a Standard solution of USP Erythromycin Ethylsuccinate RS in methanol containing about 3 mg per mL. Apply separately 10 µL each of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated

www.webofpharma.com

with a 0.25-mm layer of chromatographic silica gel mixture, and allow to dry. Place the plate in an unlined chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about 9 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of dehydrated alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and examine the chromatograms, in which the erythromycin and succinic acid moieties appear as black-to-purple spots: the R_F values of the principal spots obtained from the test solution correspond to those obtained from the Standard solution.

Uniformity of dosage units ⟨905⟩—
FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS : meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 6.5 and 8.5.
Assay—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, blended for 4 ± 1 minutes in a high-speed glass blender jar with sufficient methanol to give a stock solution containing the equivalent of about 1 mg of erythromycin per mL. Dilute this stock solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Erythromycin Ethylsuccinate for Oral Suspension

» Erythromycin Ethylsuccinate for Oral Suspension is a dry mixture of Erythromycin Ethylsuccinate with one or more suitable buffers, colors, diluents, dispersants, and flavors. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Ethylsuccinate RS
Identification—To a quantity of Erythromycin Ethylsuccinate for Oral Suspension add a volume of methanol sufficient to yield a solution containing the equivalent of about 2.5 mg of erythromycin per mL, and stir for 30 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the test solution. Proceed as directed in the *Identification—* test under *Erythromycin Ethylsuccinate Oral Suspension*, beginning with "Prepare a Standard solution."

Uniformity of dosage units ⟨905⟩—
FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS : meets the requirements.
Deliverable volume ⟨698⟩: meets the requirements.
pH ⟨791⟩: between 7.0 and 9.0, in the suspension constituted as directed in the labeling.
Loss on drying ⟨731⟩—Dry about 100 mg in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.
Assay—Constitute Erythromycin Ethylsuccinate for Oral Suspension as directed in the labeling, and proceed as directed in the *Assay—* under *Erythromycin Ethylsuccinate Oral Suspension*.

Erythromycin Ethylsuccinate Tablets

DEFINITION
Erythromycin Ethylsuccinate Tablets contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHY**
Standard solution: 3 mg/mL of USP Erythromycin Ethylsuccinate RS in methanol
Sample solution: Nominally 2.5 mg/mL of erythromycin from powdered Tablets in methanol. Shake this mixture by mechanical means for about 30 min. Centrifuge a portion of this mixture, and use the clear supernatant as the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Methanol and chloroform (85:15)
Spray reagent: Dehydrated alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5)
Analysis
Samples: *Standard solution* and *Sample solution*
Place the plate in an unlined chromatographic chamber, and develop in the *Developing solvent system* until the solvent front has moved about 9 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with the *Spray reagent*. Heat it at 100° for 10 min, and examine the chromatograms. The erythromycin and succinic acid moieties appear as black-to-purple spots.
Acceptance criteria: The R_F values of the principal spots from the *Sample solution* correspond to those from the *Standard solution*.

ASSAY
• **PROCEDURE**
(See *Antibiotics—Microbial Assays* ⟨81⟩.)
Test dilution: Blend NLT 4 Tablets for 4 ± 1 min in a high-speed glass blender jar with a sufficient volume of methanol to give a stock solution containing nominally NMT 5 mg/mL of erythromycin. Dilute this stock solution quantitatively with *Buffer B.3* to obtain a *Test dilution* having a concentration assumed to be equal to the median dose level of the standard.
Analysis: Proceed as directed in the chapter.
Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
FOR NONCHEWABLE TABLETS
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Solution A: 25 mg/mL of ferric chloride
Solution B: Slowly, and with constant mechanical stirring, add 325 mL of sulfuric acid to 173 mL of cold water. Allow the solution to cool, add 2 mL of *Solution A* and 1 g of

USP Monographs

www.webofpharma.com

p-dimethylaminobenzaldehyde, and stir to dissolve. Store in a low-actinic flask. Prepare *Solution B* on the day of use.

Standard solution: 0.44 mg/mL of USP Erythromycin RS in *Medium*. Sonicate as necessary to dissolve. Use the *Standard solution* within 5.5 h.

Sample solution: Pass a portion of the solution under test through a filter having a pore size of 0.5 µm or less, discarding the first 5 mL of the filtrate.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: Vis

Analytical wavelength: 480 nm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

To three separate 50-mL glass-stoppered conical flasks add 2.0 mL of *Standard solution*, 2.0 mL of *Sample solution*, and 2.0 mL of the *Blank*. Place the flasks in an ice bath for about 15 min. At precise 1-min intervals add 10.0 mL of *Solution B* to the *Standard solution*, the *Sample solution*, and the *Blank*, in turn. Immediately after adding *Solution B*, remove each flask from the ice bath, insert the stopper, mix, and allow to stand at room temperature for exactly 30 min. Sequentially determine the absorbance at 480 nm of the *Standard solution* and the *Sample solution* at precise 1-min intervals using the *Blank* to set the spectrophotometer to zero.

Calculate the percentage of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times P \times F \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Erythromycin RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
P = potency of erythromycin in USP Erythromycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg
L = label claim (mg/Tablet)

Acceptance criteria: NLT 75% (*Q*) of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$) is dissolved.

FOR TABLETS LABELED AS CHEWABLE

Medium: 0.1 M acetate buffer, pH 5.0; 900 mL

Apparatus 2: 75 rpm

Time: 60 min

Buffer A: 33.25 g/L of sodium acetate in water. Adjust with glacial acetic acid to a pH of 4.8 ± 0.1 before diluting to final volume.

Buffer B: pH 7.6 phosphate buffer (see *Reagents, Indicators, and Solutions—Solutions*)

Standard stock solution: 0.5 mg/mL of USP Erythromycin RS in *Buffer A*

Standard solutions: Transfer 8.0-, 4.0-, and 1.0-mL volumes of the *Standard stock solution* to separate 100-mL volumetric flasks, add 6.0 mL of *Buffer B* and 6.0 mL of *Medium* to each flask, and dilute with *Buffer A* to volume. The *Standard solutions* have known concentrations of about 40, 20, and 5 µg/mL of USP Erythromycin RS, respectively.

Sample solution: Transfer 6.0 mL of the solution under test to a 50-mL volumetric flask, add 6.0 mL of *Buffer B*, and heat in boiling water for 30 min. Cool to room temperature, and dilute with *Buffer A* to volume.

Arsenomolybdate stock solution: Dissolve 100 g of ammonium molybdate in 1.7 L of water in a 2-L volumetric flask. Slowly add, with mixing, 84 mL of sulfuric acid. Add 12 g of sodium arsenate dissolved in 100 mL of water. Dilute with water to volume. Store in an amber bottle for 24 h before using. This solution should not come in contact with rubber.

Arsenomolybdate solution: Water and *Arsenomolybdate stock solution* (2:1). Prepare freshly on the day of use.

4.5 M sulfuric acid: Add 1.5 L of water to a 2-L volumetric flask, and place the flask in an ice bath. Slowly add, with stirring, 300 mL of sulfuric acid. Allow the solution to cool, dilute with water to volume, and mix. At the time of use, add 0.5 g of sodium dodecyl sulfate to each L.

Apparatus: To determine the absorbance values, use an automated analyzer consisting of a liquid sampler; a proportioning pump; a manifold; and a spectrophotometer equipped with matched flow cells, suitable recording devices, and analysis capability at 660 nm. See *Figure 1*.

Analysis

Samples: *Standard solutions* and *Sample solution*

Adjust the system until a steady baseline is achieved. Start the sampler, and conduct determinations at a rate of about 40–60/h with a 3:1 (sample/wash) ratio. Adjust the analyzer flow rates, if necessary, to optimize system performance.

Calculate the percentage of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times P \times F \times (1/L) \times 100$$

A_U = response of the *Sample solution*
A_S = response of the *Standard solution*
C_S = concentration of USP Erythromycin RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
P = potency of erythromycin in USP Erythromycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg
L = label claim (mg/Tablet)

Acceptance criteria: NLT 75% (*Q*) of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry 100 mg in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

Acceptance criteria: NMT 4.0%; Chewable Tablets are exempt from this requirement.

- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 5.0%; Chewable Tablets only

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers,protect from light and moisture, and store at a temperature below 30°.
- **LABELING:** Label the Chewable Tablets to indicate that they are to be chewed before swallowing.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Erythromycin RS
USP Erythromycin Ethylsuccinate RS

www.webofpharma.com

Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension

DEFINITION

Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension is a dry mixture of Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl with one or more suitable buffers, colors, flavors, surfactants, and suspending agents. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$) and the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of sulfisoxazole ($C_{11}H_{13}N_3O_3S$).

[NOTE—Where Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension is prescribed, without reference to the quantity of erythromycin or sulfisoxazole contained therein, a product containing 40 mg/mL of erythromycin and 120 mg/mL of sulfisoxazole when constituted as directed in the labeling shall be dispensed.]

IDENTIFICATION

Change to read:

• **▲A.▲** (USP 1-May-2019) **THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**

Standard solution A: 3 mg/mL of USP Erythromycin Ethylsuccinate RS in methanol

Standard solution B: 8.7 mg/mL of USP Sulfisoxazole Acetyl RS in methanol

Sample solution: Nominally 2.5 mg/mL of erythromycin from Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension in methanol. Shake this mixture by mechanical means for about 30 min. Centrifuge a portion of this mixture, and use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Methanol and chloroform (85:15)

Spray reagent: Dehydrated alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Place the plate in an unlined chromatographic chamber, and develop the plate in the *Developing solvent system* until the solvent front has moved about 9 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with *Spray reagent*. Heat the plate at 100° for 10 min, and examine the chromatograms, in which the erythromycin and succinic acid moieties appear as black-to-purple spots and the sulfisoxazole acetyl appears as a yellow spot.

Acceptance criteria: The R_F values of the principal black-to-purple spots of the *Sample solution* correspond to those of *Standard solution A*; and the R_F value of the principal yellow spot of the *Sample solution* corresponds to that of *Standard solution B*.

ASSAY

• **ERYTHROMYCIN**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample stock solution: Constitute Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension as directed in the labeling, and allow to stand for 1 h. Gently shake the suspension, transfer 5.0 mL to a high-speed blender jar containing 195.0 mL of methanol, and blend for 4 ± 1 min.

Sample solution: Dilute the *Sample stock solution* quantitatively with *Buffer B.3* to obtain a *Sample solution* having a concentration assumed to be equal to the median dose level of the Standard (1.0 µg/mL of erythromycin).

Analysis: Proceed as directed in *Antibiotics—Microbial Assays* ⟨81⟩.

Acceptance criteria: 90.0%–120.0%

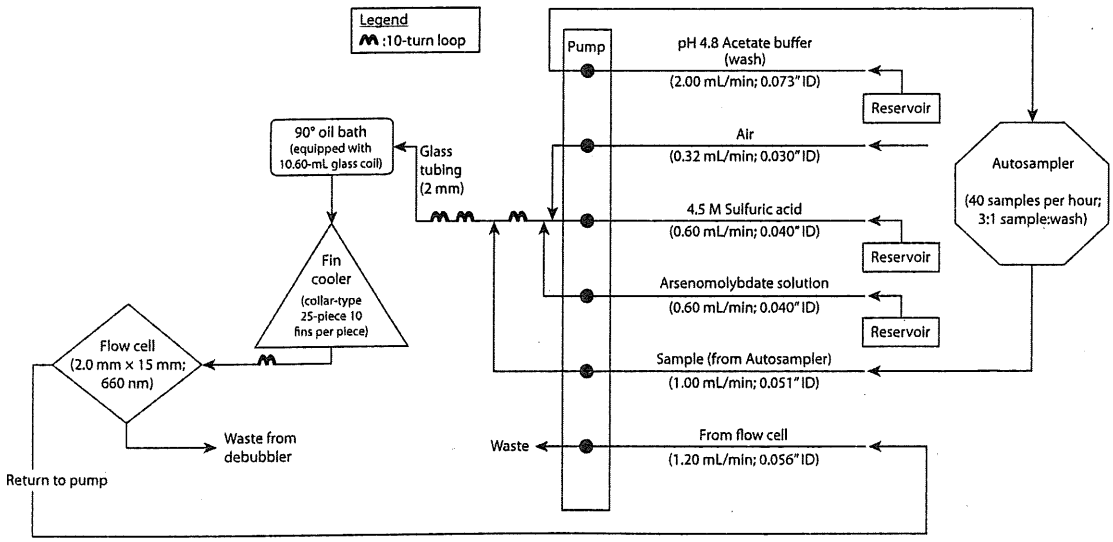


Figure 1 (Erythromycin Ethylsuccinate Tablets)

www.webofpharma.com

Change to read:

• **SULFISOXAZOLE**
Mobile phase: Acetonitrile and water (40:60) ▲▲ (USP 1-May-2019)
Internal standard solution: 0.33 mg/mL of benzanilide in acetonitrile▲▲ (USP 1-May-2019)
Standard solution: 1 mg/mL of USP Sulfisoxazole Acetyl RS in *Internal standard solution*
Sample stock solution: Constitute Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension as directed in the labeling, and allow to stand for 1 h. Gently shake Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension, transfer a volume equivalent to 600 mg of sulfisoxazole to a 125-mL separator, and extract with three 75-mL portions of chloroform. Collect the extracts in a 250-mL volumetric flask and dilute with chloroform to volume. Pass a portion of this solution through a suitable filter of 1-µm or finer pore size.
Sample solution: Pipet 4 mL of the filtrate from the *Sample stock solution* into a glass-stoppered, 25-mL conical flask, and evaporate with the aid of a current of dry air to dryness. Add 10.0 mL of *Internal standard solution*, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; ▲5-µm▲ (USP 1-May-2019) packing L1
Flow rate: 1.2 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between sulfisoxazole acetyl and benzanilide
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sulfisoxazole ($C_{11}H_{13}N_3O_3S$) in the portion of Erythromycin Ethylsuccinate and Sulfisoxazole Acetyl for Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of sulfisoxazole acetyl to benzanilide from the *Sample solution*
R_S = peak response ratio of sulfisoxazole acetyl to benzanilide from the *Standard solution*
C_S = concentration of USP Sulfisoxazole Acetyl RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of sulfisoxazole, 267.31
M_{r2} = molecular weight of sulfisoxazole acetyl, 309.35

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*
For solids packaged in single-unit containers: Meets the requirements with respect to erythromycin and sulfisoxazole
• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: Suspension constituted as directed in the labeling
Acceptance criteria: 5.0–7.2

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry about 100 mg in a capillary-stoppered bottle under vacuum at 60° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Erythromycin RS
USP Erythromycin Ethylsuccinate RS
USP Sulfisoxazole Acetyl RS

Sterile Erythromycin Gluceptate

$C_{37}H_{67}NO_{13} \cdot C_7H_{14}O_8$ 960.11
Erythromycin monoglucoheptonate (salt).
Erythromycin glucoheptonate (1:1) (salt). [23067-13-2].

» Sterile Erythromycin Gluceptate is erythromycin gluceptate suitable for parenteral use. It has a potency equivalent to not less than 600 µg of erythromycin ($C_{37}H_{67}NO_{13}$) per mg, calculated on the anhydrous basis. In addition, where packaged for dispensing, it contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Gluceptate RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: ***197M*** ▲ (CN 1-May-2020).
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.0 USP Endotoxin Unit per mg of erythromycin.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 6.0 and 8.0, in a solution containing 25 mg per mL.
Water Determination, *Method I* ⟨921⟩: not more than 5.0%.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—Where packaged for dispensing it meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions* and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*
Assay—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed quantity of Sterile Erythromycin Gluceptate dissolved in methanol to yield a solution containing the equivalent of about 10 mg of erythromycin per mL. Dilute this solution quantitatively with 9 volumes of *Buffer B.3* to obtain a stock solution containing the equivalent of about 1 mg of erythromycin per mL. Where it is packaged for dispensing, constitute Sterile Erythromycin Gluceptate as directed in the labeling. Withdraw all of the withdrawable contents where the

www.webofpharma.com

package is represented as being a single-dose container; or where the labeling specifies the amount of potency in a given volume of the resultant preparation, withdraw an accurately measured volume. Dilute quantitatively with *Buffer B.3* to obtain a stock solution having a convenient concentration. Dilute a portion of the stock solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Erythromycin Lactobionate for Injection

$C_{37}H_{67}NO_{13}.C_{12}H_{22}O_{12}$ 1092.22
Erythromycin mono(4-*O*-β-D-galactopyranosyl-D-gluconate) (salt).
Erythromycin lactobionate (1:1) (salt) [3847-29-8].

» Erythromycin Lactobionate for Injection is a sterile, dry mixture of erythromycin lactobionate and a suitable preservative. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Lactobionate RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: ***197M***. ▲(CN 1-May-2020) The specimen and the Reference Standard being previously dried in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 1.0 USP Endotoxin Unit per mg of erythromycin.
pH ⟨791⟩: between 6.5 and 7.5, in a solution containing the equivalent of 50 mg of erythromycin per mL.
Water Determination, *Method I* ⟨921⟩: not more than 5.0%.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections when the constituted solution is diluted with filtered water to a concentration of not more than 5 mg of erythromycin base per mL before the test is performed.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩, using Erythromycin Lactobionate for Injection constituted as directed in the labeling. Withdraw all of the withdrawable contents where the package is represented as being a single-dose container; or, where the labeling specifies the amount of erythromycin equivalent in a given volume of the resultant preparation, withdraw an accurately measured volume. Dilute quantitatively with water to obtain a stock solution containing the equivalent of about 10 mg of erythromycin per mL. Dilute this stock solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Sterile Erythromycin Lactobionate

» Sterile Erythromycin Lactobionate has a potency equivalent to not less than 525 µg of erythromycin ($C_{37}H_{67}NO_{13}$) per mg, calculated on the anhydrous basis. In addition, where packaged for dispensing, it contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Lactobionate RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy* ***197M***. ▲(CN 1-May-2020) The specimen and the Reference Standard being previously dried in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 1.0 USP Endotoxin Unit per mg of erythromycin.
Sterility Tests ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 6.5 and 7.5, in a solution containing the equivalent of 50 mg of erythromycin per mL.
Water Determination, *Method I* ⟨921⟩: not more than 5.0%.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections when it is diluted with filtered water to a concentration of not more than 5 mg of erythromycin per mL before the test is performed.
Residue on Ignition ⟨281⟩: not more than 2.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.
Other requirements—Where packaged for dispensing, it meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
Standard preparation—Prepare as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩.
Assay preparation 1—Dissolve an accurately weighed quantity of Sterile Erythromycin Lactobionate quantitatively in methanol to obtain a stock solution containing the equivalent of about 10 mg of erythromycin per mL. Dilute this stock solution quantitatively with *Buffer B.3* (see *Antibiotics—Microbial Assays* ⟨81⟩, *Media and Solutions, Solutions, Buffers*) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.
Assay preparation 2 (where it is packaged for dispensing and is represented as being in a single-dose container)—Constitute Sterile Erythromycin Lactobionate in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Buffer B.3* to

www.webofpharma.com

obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay preparation 3 (where the label states the quantity of erythromycin in a given volume of constituted solution)—Constitute 1 container of Sterile Erythromycin Lactobionate in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Procedure—Proceed as directed for erythromycin under *Antibiotics—Microbial Assays* ⟨81⟩.

Erythromycin Stearate

$C_{37}H_{67}NO_{13} \cdot C_{18}H_{36}O_2$ 1018.40

Erythromycin octadecanoate (salt);
Erythromycin stearate (salt) [643-22-1].

DEFINITION

Erythromycin Stearate is the stearic acid salt of Erythromycin, with an excess of Stearic Acid. The sum of the percentages of erythromycin A, erythromycin B, and erythromycin C is NLT 55.0%, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• ▲**A.**▲ (USP 1-Dec-2019) ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Add the following:

▲• **B.** The retention times of the erythromycin A, erythromycin B, and erythromycin C peaks in the *Sample solution* correspond to those of *Standard solution 2*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

• **PROCEDURE**

▲Use solutions containing erythromycin promptly, or within 1 day if stored in a refrigerator.▲ (USP 1-Dec-2019)

Diluted phosphoric acid: Dilute 10 mL of phosphoric acid with water to 100 mL.

Solution A: 20 mg/mL of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 8.0.

Solution B: 35 mg/mL of dibasic potassium phosphate in water. Adjust with potassium hydroxide TS or *Diluted phosphoric acid*, as appropriate, to a pH of 9.0.

Solution C: 20 mL of *Solution A*. Adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Acetonitrile, tertiary butyl alcohol, *Solution B*, and water (6:35:10:149)

Standard solution 1: 40 mg of USP Erythromycin RS in a conical flask. Add 5.0 mL of methanol, and swirl to dissolve. Add 5.0 mL of *Solution A*.

Standard solution 2: 6 mg each of USP Erythromycin RS, USP Erythromycin B RS, USP Erythromycin C RS, and USP Erythromycin Related Compound N RS in a 50-mL conical flask. Add 15.0 mL of methanol and swirl to dissolve. Add 15.0 mL of *Solution A*.

▲▲ (USP 1-Dec-2019)

Sample solution: 165 mg of Erythromycin Stearate in a 100-mL conical flask. Add 15.0 mL of methanol and swirl to dissolve. Add 15.0 mL of *Solution A* and mix. Allow the resulting suspension to settle, and pass a portion of the supernatant through a filter of 0.2-µm or finer pore size. Use the clear filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; packing L21 (1000Å)

Column temperature: 70°

Flow rate: 2 mL/min

Injection volume: 100 µL

Run time: NLT 5 times the retention time of erythromycin A

System suitability

Samples: *Standard solution 1* and *Standard solution 2*

The elution order of the components is erythromycin related compound N, erythromycin C, erythromycin A, and erythromycin B.

Suitability requirements

Resolution: NLT 0.8 between erythromycin related compound N and erythromycin C; NLT 5.5 between erythromycin related compound N and erythromycin A, *Standard solution 2*

Relative standard deviation: NMT 2.0%, *Standard solution 1*

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, ▲▲ (USP 1-Dec-2019)and *Sample solution* ▲▲ (USP 1-Dec-2019)

Calculate the percentage of erythromycin A in the portion of Erythromycin Stearate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of erythromycin A from the *Sample solution*

r_S = peak response of erythromycin A from *Standard solution 1*

C_S = concentration of USP Erythromycin RS in *Standard solution 1* (mg/mL)

C_U = concentration of Erythromycin Stearate in the *Sample solution* (mg/mL)

P = percentage of erythromycin A in USP Erythromycin RS

Calculate the percentage of erythromycin B and erythromycin C in the portion of Erythromycin Stearate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of the relevant analyte from the *Sample solution*

r_S = peak response of the relevant analyte from *Standard solution 2*

C_S = concentration of the corresponding Reference Standard in *Standard solution 2* (mg/mL)

C_U = concentration of Erythromycin Stearate in the *Sample solution* (mg/mL)

P = ▲potency of erythromycin B or erythromycin C in the corresponding Reference Standard (mg/mg)▲ (USP 1-Dec-2019)

Acceptance criteria: The sum of the percentages of erythromycin A, erythromycin B, and erythromycin C is NLT 55.0% on the anhydrous basis. The percentage of erythromycin B is NMT 12.0%, and the percentage of erythromycin C is NMT 5.0%.

www.webofpharma.com

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩
Sample: The charred residue is moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.
Acceptance criteria: NMT 1.0%

Change to read:

• **ORGANIC IMPURITIES**
Diluted phosphoric acid, Solution A, Solution B, Solution C, Mobile phase, Standard solution 2, Sample solution, Chromatographic system, and **System suitability:**
Proceed as directed in the Assay.
▲**Peak identification solution:** 5 mg of USP Erythromycin RS in 1 mL of methanol. Add 5 mL of *Solution C*, mix, and allow to stand for about 30 min.▲ (USP 1-Dec-2019)
Analysis
Samples: *Standard solution 2*, *Sample solution*, and ▲*Peak identification solution*
Identify the erythromycin A enol ether peak using the *Peak identification solution*.▲ (USP 1-Dec-2019)
Calculate the percentage of the largest single impurity (the largest peak other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, erythromycin *N*-ethylsuccinate, and pseudoerythromycin A enol ether) in the portion of Erythromycin Stearate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of the largest single impurity (the largest peak other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, erythromycin *N*-ethylsuccinate, and pseudoerythromycin A enol ether) from the *Sample solution*
r_S = peak response of erythromycin A from *Standard solution 2*
C_S = concentration of USP Erythromycin RS in *Standard solution 2* (mg/mL)
C_U = concentration of Erythromycin Stearate in the *Sample solution* (mg/mL)
P = percentage of erythromycin A in USP Erythromycin RS

Calculate the percentage of erythromycin A enol ether in the portion of Erythromycin Stearate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

r_U = peak response of erythromycin A enol ether from the *Sample solution*
r_S = peak response of erythromycin A from *Standard solution 2*
C_S = concentration of USP Erythromycin RS in *Standard solution 2* (mg/mL)
C_U = concentration of Erythromycin Stearate in the *Sample solution* (mg/mL)
P = percentage of erythromycin A in USP Erythromycin RS
F = relative response factor (see *Table 1*)

Calculate the percentage of pseudoerythromycin A enol ether in the portion of Erythromycin Stearate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

r_U = peak response of pseudoerythromycin A enol ether from the *Sample solution*
r_S = peak response of erythromycin A from *Standard solution 2*
C_S = concentration of USP Erythromycin RS in *Standard solution 2* (mg/mL)
C_U = concentration of Erythromycin Stearate in the *Sample solution* (mg/mL)
P = percentage of erythromycin A in USP Erythromycin RS
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Pseudoerythromycin A enol ether[▲a▲ (USP 1-Dec-2019)]	1.5	6.6	3.0
Erythromycin A enol ether[▲b▲ (USP 1-Dec-2019)]	4.3–4.7	11	3.0
Erythromycin A	1.0	—	—
Largest single impurity[c]	—	—	3.0

[▲a (2*R*,3*S*,6*R*,7*S*,8*S*,9*R*,10*R*)-3-[(2*R*,3*R*)-2,3-Dihydroxypentan-2-yl]-9-{[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-7-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-2,6,8,10,12-pentamethyl-4,13-dioxabicyclo[8.2.1]tridec-1(12)-en-5-one.
[b (2*R*,3*R*,4*S*,5*R*,8*R*,9*S*,10*S*,11*R*)-11-{[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-5-ethyl-3,4-dihydroxy-9-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-2,4,8,10,12,14-hexamethyl-6,15-dioxabicyclo[10.2.1]pentadec-1(14)-en-7-one.▲ (USP 1-Dec-2019)
[c The largest peak other than erythromycin A, erythromycin B, erythromycin C, erythromycin A enol ether, erythromycin *N*-ethylsuccinate, and pseudoerythromycin A enol ether.

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*
Sample solution: Use 20 mL of methanol containing 10% of imidazole in place of methanol in the titration vessel.
Acceptance criteria: NMT 4.0%
• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Erythromycin RS
USP Erythromycin B RS
▲12-Deoxyerythromycin;
(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*S*,13*R*,14*R*)-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-14-ethyl-7,12-dihydroxy-4-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione.
$C_{37}H_{67}NO_{12}$ 717.94▲ (USP 1-Dec-2019)
USP Erythromycin C RS
▲3″-*O*-Demethylerythromycin;
(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-{[(2*R*,4*R*,5*S*,6*S*)-4,5-Dihydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione.
$C_{36}H_{65}NO_{13}$ 719.91▲ (USP 1-Dec-2019)

www.webofpharma.com

USP Erythromycin Related Compound N RS
N-Demethylerythromycin A;
▲(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-14-Ethyl-7,12,13-trihydroxy-4-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl]oxy}-6-{[(2*S*,3*R*,4*S*,6*R*)-3-hydroxy-6-methyl-4-(methylamino)tetrahydro-2*H*-pyran-2-yl]oxy}-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione.▲ (USP 1-Dec-2019)
$C_{36}H_{65}NO_{13}$ 719.91
USP Erythromycin Stearate RS

Erythromycin Stearate Tablets

» Erythromycin Stearate Tablets contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Erythromycin RS
USP Erythromycin Stearate RS
Identification—To a quantity of powdered Tablets add a volume of methanol sufficient to yield a solution containing the equivalent of about 5 mg of erythromycin per mL. Shake this mixture by mechanical means for about 30 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the test solution. Prepare a Standard solution of USP Erythromycin Stearate RS in methanol containing about 8 mg per mL. Apply separately 20 µL each of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow to dry. Place the plate in an unlined chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about 9 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a methanolic solution of 2′,7′-dichlorofluorescein (1 in 500), and examine the plate under long-wavelength UV light: the R_F values of the principal fluorescent spots obtained from the test solution correspond to those obtained from the Standard solution. Then spray the plate with a mixture of dehydrated alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100° for 10 minutes, and examine the chromatograms, in which the erythromycin appears as a black-to-purple spot: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Dissolution ⟨711⟩—
Medium: 0.05 M pH 6.8 phosphate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.
Apparatus 2: 100 rpm.
Time: 120 minutes.
Stock standard solution—Dissolve an accurately weighed quantity of USP Erythromycin RS in methanol to obtain a solution containing about 14 mg per mL. Dilute quantitatively with water, and mix to obtain a solution having a known concentration of about 0.56 mg of USP Erythromycin RS per mL.
Working standard solution—On the day of use, dilute 25.0 mL of *Stock standard solution* with water to 50.0 mL, and mix.
Test solution—After 120 minutes, withdraw a portion of the solution under test, filter, and dilute with *Medium*, if necessary, to obtain a solution having an estimated concentration of about 0.28 mg of erythromycin per mL.
Procedure—Transfer 5.0-mL portions of the *Working standard solution* to two 25-mL volumetric flasks, one of which serves as a working standard blank. Similarly, transfer 5.0-mL portions of the *Test solution* to two 25-mL volumetric flasks, one of which serves as a blank for that *Test solution*. To each of the flasks designated as a blank add 2.0 mL of 0.5 N sulfuric acid and to the remaining flasks add 2.0 mL of water. Allow to stand for 5 minutes with intermittent swirling. To all flasks add 15.0 mL of 0.25 N sodium hydroxide, dilute with *Medium* to volume, and mix. Heat the flasks in a water bath at 60 ± 0.5° for 5 minutes, and allow to cool. Using a suitable spectrophotometer, determine the absorbance of each solution, corrected for its blank solution, at the wavelength of maximum absorbance at about 236 nm. Determine the amount of $C_{37}H_{67}NO_{13}$ dissolved from the *Test solution* in comparison with the solution obtained from the *Working standard solution*.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{37}H_{67}NO_{13}$ is dissolved in 120 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Loss on drying ⟨731⟩—Dry about 100 mg of powdered Tablets in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 5.0% of its weight.
Assay—Proceed with Tablets as directed in the *Assay*— under *Erythromycin Tablets*.

Escitalopram Oral Solution

DEFINITION
Escitalopram Oral Solution contains an amount of escitalopram oxalate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of escitalopram ($C_{20}H_{21}FN_2O$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 6.1 g/L of monobasic potassium phosphate. To each L of this solution add 1.5 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile and *Buffer* (32:68)
Diluent: Acetonitrile and *Buffer* (25:75)
Standard solution: 33 µg/mL of USP Escitalopram Oxalate RS in *Diluent*
Sample solution: Nominally, 25 µg/mL of escitalopram from a suitable volume of Oral Solution, prepared as follows. Fill 50% of the flask volume with *Diluent*, and sonicate for 10 min with intermittent shaking. Allow the solution to cool, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
Run time: 2.1 times the retention time of escitalopram

www.webofpharma.com

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of escitalopram ($C_{20}H_{21}FN_2O$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of escitalopram from the *Sample solution*
r_S = peak response of escitalopram from the *Standard solution*
C_S = concentration of USP Escitalopram Oxalate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of escitalopram in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of escitalopram free base, 324.39
M_{r2} = molecular weight of escitalopram oxalate, 414.43

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirement

IMPURITIES

• **LIMIT OF CITALOPRAM RELATED COMPOUND B**
Diluted phosphoric acid: Dilute 5 mL of phosphoric acid with water to 50 mL.
Solution A: 6.8 g/L of monobasic potassium phosphate adjusted with *Diluted phosphoric acid* to a pH of 3.0.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
13	60	40
15	60	40
16	90	10
20	90	10

Diluent: Acetonitrile and *Solution A* (30:70)
Standard solution: 0.6 µg/mL of USP Escitalopram Oxalate RS in *Diluent*
System suitability solution: 0.6 µg/mL of USP Citalopram Related Compound B RS in *Standard solution*
Sample solution: Nominally, 0.25 mg/mL of escitalopram from a suitable volume of Oral Solution in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 237 nm
Column: 2.1-mm × 7.5-cm; 3-µm packing L1
Column temperature: 45°
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for citalopram related compound B and escitalopram are about 0.81 and 1.0, respectively. Use the *System suitability solution* to identify the citalopram related compound B peak.]
Suitability requirements
Resolution: NLT 2.0 between citalopram related compound B and escitalopram, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of citalopram related compound B in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of citalopram related compound B from the *Sample solution*
r_S = peak response of escitalopram from the *Standard solution*
C_S = concentration of USP Escitalopram Oxalate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of escitalopram in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of escitalopram free base, 324.39
M_{r2} = molecular weight of escitalopram oxalate, 414.43

Acceptance criteria: NMT 0.2%

• **ORGANIC IMPURITIES**
Solution A: 6.6 g/L of dibasic ammonium phosphate in water. To each L of this solution add 2 mL of triethylamine. Adjust with phosphoric acid to a pH of 3.0.
Solution B: Acetonitrile and methanol (60:40)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
10	70	30
20	60	40
35	60	40
36	80	20
45	80	20

Diluent: Acetonitrile and *Solution A* (30:70)
Standard stock solution: 0.33 mg/mL of USP Escitalopram Oxalate RS in *Diluent*
System suitability solution: 1.0 µg/mL of USP Citalopram Related Compound C RS in *Standard stock solution*
Standard solution: 3.3 µg/mL of USP Escitalopram Oxalate RS in *Diluent* from the *Standard stock solution*
Sample solution: Nominally, 0.25 mg/mL of escitalopram from a suitable volume of Oral Solution in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for escitalopram, *System suitability solution*

www.webofpharma.com

Resolution: NLT 2.0 between citalopram related compound C and escitalopram, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of escitalopram from the *Standard solution*
C_S = concentration of USP Escitalopram Oxalate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of escitalopram in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of escitalopram free base, 324.39
M_{r2} = molecular weight of escitalopram oxalate, 414.43
F = relative response factor for each individual impurity (see *Table 3*)

Acceptance criteria: See *Table 3*.
[NOTE—Disregard any peaks less than 0.05%.]

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxalic acid[a]	0.06	—	—
5-Dimethylaminobutyryl citalopram[b, c]	0.42	—	—
Citalopram related compound A[d]	0.48	1.0	0.2
Citalopram related compound B[e]	0.75	—	—
Desfluorocitalopram[f, c]	0.90	—	—
Citalopram related compound C[g]	0.92	2.56	0.3
Citalopram related compound D[h, c]	0.99	—	—
Escitalopram	1.0	—	—
Citalopram related compound E[i]	1.09	1.0	0.2
Citalopram chloromethyl quaternary ammonium salt[j, c]	1.1	—	—
Citalopram alkene dimer[k, c]	1.6	—	—
Citalopram related compound H[l, c]	1.7	—	—
Any individual unspecified degradation product	—	1.0	0.2
Total impurities[m]	—	—	0.7

[a] Not included in total impurities. For identification purposes only.
[b] 1-(3-Dimethylaminopropyl)-1-(4′-fluorophenyl)-5-(4-dimethylaminobutyryl)-1,3-dihydroisobenzofuran.
[c] Process impurity listed for information only. Do not include in total impurities.
[d] 1-(3-(Dimethylamino)propyl)-1-(4′-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.
[e] This impurity is monitored and controlled using the test for *Limit of Citalopram Related Compound B*. It is listed here for identification purposes only.
[f] 1-[3-(Dimethylamino)propyl]-1-phenyl-1,3-dihydroisobenzofuran-5-carbonitrile.
[g] 3-[3-(Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3*H*)-isobenzofuranone.
[h] 1-(4′-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile.
[i] 1-(3-Dimethylaminopropyl)-1-(4-fluororphenyl)-1,3-dihydroisobenzofuran-5-carbonitrile-*N*-oxide.
[j] *N*-Chloromethyl-3-[5-cyano-1-(4-flurorphenyl)-1,3-dihydroisobenzofuran-1-yl]-*N*,*N*-dimethylpropan-1-ammonium chloride.
[k] 3-[5-Cyano-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-*N*-{5-cyano-2-[4-(dimethylamino)-1-(4-fluorophenyl)but-1-enyl]benzyl}-*N*,*N*-dimethylpropan-1-ammonium chloride.
[l] 3-[5-Bromo-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-*N*,*N*-dimethylpropan-1-amine.
[m] Sum of all impurities from *Organic Impurities* and *Limit of Citalopram Related Compound B*.

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.0–5.0
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/mL. The total yeasts and molds count does not exceed 10^1 cfu/mL. It meets the requirement of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Citalopram Related Compound B RS
  1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydroisobenzofuran-5-carbonitrile oxalate.
  $C_{20}H_{21}FN_2O_2$ 340.39
  USP Citalopram Related Compound C RS
  3-[3-(Dimethylamino)-1-propyl](4-fluorophenyl)-6-cyano-1(3*H*)-isobenzofuranone oxalate.
  $C_{22}H_{21}FN_2O_6$ 428.41
  USP Escitalopram Oxalate RS

Escitalopram Tablets

DEFINITION

Change to read:

Escitalopram Tablets contain an amount of Escitalopram Oxalate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of escitalopram ($C_{20}H_{21}FN_2O$). ▲▲2S (USP41)

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

• **PROCEDURE**

Buffer: 1.5 g of anhydrous sodium acetate and 0.4 mL of glacial acetic acid in 1 L of water. Adjust with ▲1 N sodium hydroxide VS▲ 2S (USP41) to a pH of 5.2.

Mobile phase: Methanol, acetonitrile, and *Buffer* (33:7:60)

System suitability solution: ▲6.4 µg/mL of USP Escitalopram Oxalate RS (equivalent to 5 µg/mL of escitalopram)▲ 2S (USP41) and 1 µg/mL of USP Citalopram Related Compound C RS in *Mobile phase*

Standard solution: ▲510 µg/mL of USP Escitalopram Oxalate RS (equivalent to 400 µg/mL of escitalopram) in *Mobile phase*▲ 2S (USP41)

Sample solution: ▲Nominally 400 µg/mL of escitalopram from Tablets prepared as follows.▲ 2S (USP41) Transfer ▲NLT▲ 2S (USP41) 10 Tablets to a suitable volumetric flask, add *Buffer* to 10% of the ▲total▲ 2S (USP41) volume, and shake vigorously for 10 min. Add methanol to 50% of the ▲total▲ 2S (USP41) volume, shake for 1 additional min, sonicate for 10 min, and dilute with *Mobile phase* to volume. ▲▲ 2S (USP41)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 239 nm. ▲For *Identification B*, use a diode array detector in the range of 210–400 nm.▲ 2S (USP41)

Column: 4.6-mm × 10-cm; 3-µm packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 10 µL

▲Run time: NLT 3 times the retention time of escitalopram▲ 2S (USP41)

System suitability

Samples: *System suitability solution* and *Standard solution*

▲[NOTE—See *Table 1* for the relative retention times.]▲ 2S (USP41)

Suitability requirements

Resolution: NLT 3.0 between ▲escitalopram▲ 2S (USP41) and citalopram related compound C, *System suitability solution*

Relative standard deviation: ▲NMT 1.0%,▲ 2S (USP41) *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of escitalopram ($C_{20}H_{21}FN_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of ▲USP Escitalopram Oxalate RS in the *Standard solution* (µg/mL)▲ 2S (USP41)

C_U = nominal concentration of ▲escitalopram in the *Sample solution* (µg/mL)▲ 2S (USP41)

M_{r1} = molecular weight of ▲escitalopram,▲ 2S (USP41) 324.39

M_{r2} = molecular weight of ▲escitalopram oxalate, 414.43▲ 2S (USP41)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

Test 1

▲[NOTE—USP Citalopram Hydrobromide RS is used as a quantitative standard with an appropriate molecular weight correction. Escitalopram is an optical isomer of citalopram.]▲ 2S (USP41)

Medium: *0.1 N hydrochloric acid* ▲*VS*;▲ 2S (USP41) 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution 1: 3 µg/mL of USP Citalopram Hydrobromide RS▲(equivalent to 2.4 µg/mL of citalopram)▲ 2S (USP41) in *Medium*

Standard solution 2: 15 µg/mL of USP Citalopram Hydrobromide RS▲(equivalent to 12 µg/mL of citalopram)▲ 2S (USP41) in *Medium*

Standard solution 3: 30 µg/mL of USP Citalopram Hydrobromide RS▲(equivalent to 24 µg/mL of citalopram)▲ 2S (USP41) in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 239 nm

▲Cell:▲ 2S (USP41) 0.5 cm

Blank: *Medium*

System suitability

Samples: *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*

Suitability requirements

Correlation coefficient: NLT 0.995, determined using *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*, three replicates of each solution

Relative standard deviation: NMT 2.0%, determined using *Standard solution 3*, six replicates

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and *Sample solution*

Calculate the concentration, in ▲µg/mL, of citalopram▲ 2S (USP41) for each *Standard solution* (*i*):

$$\text{Result}_i = C_{Si} \times (M_{r1}/M_{r2})$$

C_{Si} = concentration of USP Citalopram Hydrobromide RS in the *Standard solution* (*i*) ▲(µg/mL)▲ 2S (USP41)

M_{r1} = molecular weight of citalopram, 324.39

M_{r2} = molecular weight of citalopram hydrobromide, 405.30

Plot the absorbances of *Standard solution 1*, *Standard solution 2*, and *Standard solution 3* versus the corresponding citalopram ▲▲ 2S (USP41) concentrations.

Determine the concentration, C_U, in ▲µg/mL,▲ 2S (USP41) of escitalopram in the *Sample solution* using the calibration curve.

Calculate the percentage of the labeled amount of escitalopram ($C_{20}H_{21}FN_2O$) dissolved:

www.webofpharma.com

$$\text{Result} = C_U \times V \times (1/L) \times F \times 100$$

C_U = nominal concentration of escitalopram in the *Sample solution* (µg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

F = conversion factor, 0.001 mg/µg

Tolerances: NLT 80% (*Q*) of the labeled amount of escitalopram ($C_{20}H_{21}FN_2O$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: *0.1 N hydrochloric acid VS*; 900 mL

Apparatus 2: 75 rpm

Time: 30 min

Buffer: 3.4 g/L of monobasic potassium phosphate in water. To each 1 L of the mixture, add 1.0 mL of *triethylamine*, and adjust with 1.5 M phosphoric acid TS to a pH of 3.8.

Mobile phase: Acetonitrile, methanol, and *Buffer* (28:5:67)

Standard solution: (*L*/900) mg/mL of escitalopram from USP Escitalopram Oxalate RS in *Medium*, where *L* is the label claim of escitalopram

Sample solution: Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 238 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of escitalopram ($C_{20}H_{21}FN_2O$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_U = peak response of escitalopram from the *Sample solution*

r_S = peak response of escitalopram from the *Standard solution*

C_S = concentration of USP Escitalopram Oxalate RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of escitalopram, 324.39

M_{r2} = molecular weight of escitalopram oxalate, 414.43

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of escitalopram is dissolved.

• **Uniformity of Dosage Units** ⟨**905**⟩: Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**

Buffer, Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 1.0 µg/mL of USP Escitalopram Oxalate RS (equivalent to 0.8 µg/mL of escitalopram) in *Mobile phase*

System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between escitalopram and citalopram related compound C, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of escitalopram from the *Standard solution*

C_S = concentration of USP Escitalopram Oxalate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of escitalopram in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 1*)

M_{r1} = molecular weight of escitalopram, 324.39

M_{r2} = molecular weight of escitalopram oxalate, 414.43

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citalopram related compound A[a]	0.33	0.84	0.3
Citalopram related compound B[b]	0.56	0.78	0.5
Citalopram related compound C	0.80	0.51	0.5
Escitalopram	1.0	—	—
Citalopram related compound E[c]	1.4	0.94	0.2
Any other individual, unspecified degradation product	—	1.0	0.20

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total ▲degradation products▲ 2S (USP41)	—	—	2.0

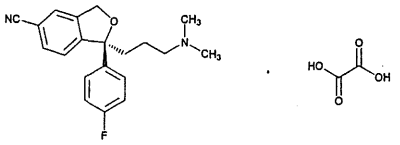
[a] 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.
[b] 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydroisobenzofuran-5-carbonitrile.▲▲ 2S (USP41)
[c] 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile-*N*-oxide.

ADDITIONAL REQUIREMENTS

Change to read:

- **LABELING:** ▲▲ 2S (USP41) The labeling states the *Dissolution* test used only if *Test 1* is not used.
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Citalopram Hydrobromide RS
  USP Citalopram Related Compound C RS
  3-(3-Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3*H*)-isobenzofuranone oxalate.
  $C_{20}H_{19}FN_2O_2 \cdot C_2H_2O_4$ 428.42
  USP Escitalopram Oxalate RS

Escitalopram Oxalate



$C_{20}H_{21}FN_2O \cdot C_2H_2O_4$ 414.43
S-(+)-5-Isobenzofurancarbonitrile, 1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydro-, oxalate;
S-(+)-1-[3-(Dimethylamino)propyl]-1-(*p*-fluorophenyl)-5-phthalancarbonitrile oxalate [219861-08-2].

DEFINITION

Escitalopram Oxalate contains NLT 98.0% and NMT 102.0% of escitalopram oxalate ($C_{20}H_{21}FN_2O \cdot C_2H_2O_4$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 3.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid or sodium hydroxide solution to a pH of 3.0 before final dilution.
Solution A: Acetonitrile and *Buffer* (10:90)
Solution B: Acetonitrile and *Buffer* (65:35)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	95	5	1
35	65	35	1
45	0	100	1
45.1	0	100	2
60	0	100	2
60.1	95	5	1
68	95	5	1

[NOTE—The gradient was established on an HPLC system with a dwell volume of approximately 1.6 mL.]
System suitability solution: 2 µg/mL each of USP Escitalopram Oxalate RS and USP Citalopram Related Compound D RS in *Solution A*
Standard solution: 0.5 mg/mL of USP Escitalopram Oxalate RS in *Solution A*
Sample solution: 0.5 mg/mL of Escitalopram Oxalate in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 237 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: See *Table 1*.
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between escitalopram and citalopram related compound D, *System suitability solution*
Tailing factor: 0.8–3, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of escitalopram oxalate ($C_{20}H_{21}FN_2O \cdot C_2H_2O_4$) in the portion of Escitalopram Oxalate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ENANTIOMERIC PURITY**

Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, add 150 mL of 0.2 N sodium hydroxide, adjust with phosphoric acid or sodium hydroxide solution to a pH of 7.0, and dilute with water to 1 L.
Mobile phase: Acetonitrile and *Buffer* (15:85)
System suitability solution: 125 µg/mL each of USP R-Citalopram Oxalate RS and USP Escitalopram Oxalate RS in *Mobile phase*
Sample solution: 125 µg/mL of Escitalopram Oxalate in *Mobile phase*

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L57
Column temperature: 30°
Flow rate: 0.6 mL/min
Injection volume: 15 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.3 between *R*-citalopram and escitalopram
Tailing factor: 0.8–2.5 for escitalopram
Analysis
Sample: *Sample solution*
Calculate the percentage of *R*-citalopram oxalate in the portion of Escitalopram Oxalate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of *R*-citalopram from the *Sample solution*
r_T = sum of peak responses of *R*-citalopram and escitalopram from the *Sample solution*

Acceptance criteria: NMT 3.0%

• **ORGANIC IMPURITIES**
Buffer, Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution A: 2 µg/mL each of USP Escitalopram Oxalate RS and USP Citalopram Related Compound D RS in *Solution A*
System suitability solution B: 0.5 mg/mL of USP Escitalopram Oxalate RS in *Solution A*
System suitability
Samples: *System suitability solution A* and *System suitability solution B*
Suitability requirements
Resolution: NLT 1.5 between escitalopram and citalopram related compound D, *System suitability solution A*
Tailing factor: 0.8–3, *System suitability solution B*
Relative standard deviation: NMT 2.0%, *System suitability solution B*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Escitalopram Oxalate taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = peak response of escitalopram from the *Sample solution*
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

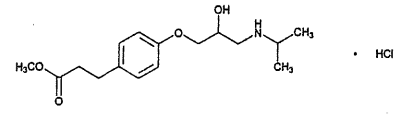
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxalic acid[a]	0.075	—	—
5-Dimethylamino-butyryl citalopram[b]	0.40	0.34	0.2
Citalopram related compound A[c]	0.50	0.79	0.1
Citalopram related compound B (3-hydroxycitalopram)[d]	0.74	1.0	0.1
Citalopram related compound C (3-oxocitalopram)[e]	0.90	0.79	0.1
Citalopram related compound D (desmethyl citalopram)	0.97	1.0	0.1
Escitalopram	1.0	—	—
Citalopram related compound E (citalopram *N*-oxide)[f]	1.1	1.0	0.1
Any other individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] Included for identification only. This peak is due to the oxalate counterion and hence is not an impurity.
[b] 1-(3-Dimethylaminopropyl)-1-(4′-fluorophenyl)-5-(4-dimethylaminobutyryl)-1,3-dihydroisobenzofuran.
[c] 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.
[d] 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydroisobenzofuran-5-carbonitrile.
[e] 3-(3-Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3*H*)-isobenzofuranone.
[f] 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile-*N*-oxide.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP R-Citalopram Oxalate RS
(*R*)-1-[3-(Dimethylamino)propyl]-1-(*p*-fluorophenyl)-5-phthalancarbonitrile oxalate.
$C_{20}H_{21}FN_2O \cdot C_2H_2O_4$ 414.43
USP Citalopram Related Compound D RS.
[NOTE—May be available as a hydrochloride or a hydrobromide salt.]
1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrochloride.
$C_{19}H_{19}FN_2O \cdot HCl$ 346.83
1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrobromide.
$C_{19}H_{19}FN_2O \cdot HBr$ 391.28
USP Escitalopram Oxalate RS

Esmolol Hydrochloride



$C_{16}H_{25}NO_4 \cdot HCl$ 331.83

www.webofpharma.com

Benzenepropanoic acid, 4-[2-hydroxy-3-[(1-methylethyl) amino]propoxy]-, methyl ester, hydrochloride, (±)-; (±)-Methyl *p*-[2-hydroxy-3-(isopropylamino)propoxy] hydrocinnamate hydrochloride [81161-17-3].

DEFINITION

Esmolol Hydrochloride contains NLT 98.0% and NMT 102.0% of esmolol hydrochloride ($C_{16}H_{25}NO_4 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: Dissolve 3.0 g of potassium dihydrogen phosphate in 650 mL of water.

Mobile phase: Acetonitrile, methanol, and *Buffer* (150:200:650)

System suitability stock solution: 1 mg/mL of USP Esmolol Hydrochloride RS prepared as follows. Transfer a suitable quantity of USP Esmolol Hydrochloride RS to an appropriate volumetric flask, and dissolve in and dilute with 1 N hydrochloric acid to volume. Allow the contents to stand for at least 30 min. [Note—This results in the partial degradation of the esmolol resulting in the production of esmolol free acid (see *System suitability* for the relative retention times).]

System suitability solution: 0.2 mg/mL of USP Esmolol Hydrochloride RS in water from the *System suitability stock solution*

Standard solution: 200 µg/mL of USP Esmolol Hydrochloride RS in water

Sample solution: 200 µg/mL of Esmolol Hydrochloride in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 222 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for esmolol free acid and esmolol are 0.41 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between esmolol free acid and esmolol, *System suitability solution*

Tailing factor: NMT 2.0 for the esmolol peak, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of esmolol hydrochloride ($C_{16}H_{25}NO_4 \cdot HCl$) in the portion of the Esmolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of esmolol from the *Sample solution*

r_S = peak response of esmolol from the *Standard solution*

C_S = concentration of USP Esmolol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Esmolol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%
- **Organic Impurities**

Buffer and **System suitability solution:** Prepare as directed in the *Assay*.

Solution A: Methanol

Solution B: Prepare as directed for *Mobile phase* in the *Assay*.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	0	100
20	0	100
25	25	75
35	25	75
36	0	100
40	0	100

Sample solution: 1 mg/mL of Esmolol Hydrochloride in water

Chromatographic system: Proceed as directed in the *Assay*, except include a column temperature of 30°.

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 4.0 between esmolol free acid and esmolol

Tailing factor: NMT 2.0 for the esmolol peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual impurity in the portion of Esmolol Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Esmolol free acid[a]	0.43	0.4
Esmolol *N*-isopropylamide analog[b] (if present)	0.65	0.25
N-Ethyl esmolol[c] (if present)	0.84	0.15
Esmolol	1.0	—
Esmolol dimer[d]	6.5	0.5

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any other individual unspecified impurity	—	0.10
Total impurities	—	1.0

[a] 3-{4-[2-Hydroxy-3-(isopropylamino)propoxy]phenyl}propionic acid.
[b] 3-{4-[2-Hydroxy-3-(isopropylamino)propoxy]phenyl}-*N*-isopropylpropionamide.
[c] Methyl 3-{4-[3-(ethylamino)-2-hydroxypropoxy]phenyl}propionate.
[d] Methyl 3-{4-[2-hydroxy-3-(3-{4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl}-*N*-isopropylpropionamido)propoxy]phenyl}propionate.

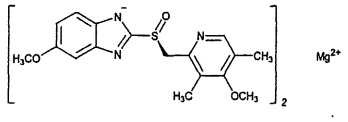
SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample solution: 250 mg/mL of Esmolol Hydrochloride in water
  Acceptance criteria: 3.0–5.0
- **Water Determination** ⟨921⟩, *Method I*, *Method Ia*: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Protect from freezing, and store at room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Esmolol Hydrochloride RS

Esomeprazole Magnesium



$C_{34}H_{36}MgN_6O_6S_2 \cdot 3H_2O$ Trihydrate: 767.17

$C_{34}H_{36}MgN_6O_6S_2 \cdot 2H_2O$ Dihydrate: 749.15

$C_{34}H_{36}MgN_6O_6S_2$ Anhydrous: 713.12

1*H*-Benzimidazole,5-methoxy-2-[(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-, magnesium salt (2:1)
5-Methoxy-2-[(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridyl)methyl]sulfinyl]benzimidazole, magnesium salt (2:1)
Trihydrate: [217087-09-7].
Dihydrate: [217087-10-0].

DEFINITION

Esomeprazole Magnesium contains NLT 98.0% and NMT 102.0% of esomeprazole magnesium ($C_{34}H_{36}MgN_6O_6S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020): [Note—If a difference appears in the IR spectra of the analyte and the Standard, separately dissolve equal portions of the sample specimen and the USP Reference Standard in equal volumes of methanol, evaporate the solution to dryness in similar containers under identical conditions, and repeat the test on the residues.]
- **B.** The *Sample solution*, prepared and tested as directed in the test for *Content of Magnesium*, exhibits a significant absorption at 285.2 nm.

ASSAY

- **Procedure**
  Solution A: Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, and dilute with water to 1000 mL. Dilute 250 mL of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.
  Solution B: Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.
  Mobile phase: Acetonitrile and *Solution A* (7:13)
  Standard solution: Transfer 10 mg of USP Omeprazole RS to a 200-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 10 mL of *Solution B*, and dilute with water to volume. [Note—This solution contains 0.05 mg/mL of omeprazole.]
  Sample solution: Transfer 10 mg of Esomeprazole Magnesium to a 200-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 10 mL of *Solution B*, and dilute with water to volume. [Note—This solution contains 0.05 mg/mL of esomeprazole magnesium.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 4.0-mm × 12.5-cm or 4.6-mm × 15-cm; 5-µm packing L7. [Note—Alternatively, a 3.9-mm × 15-cm column that contains 4-µm packing L1 may be used.]
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 2000 theoretical plates
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of esomeprazole magnesium ($C_{34}H_{36}MgN_6O_6S_2$) in the portion of Esomeprazole Magnesium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M_{r1}/(2 \times M_{r2})] \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Esomeprazole Magnesium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of esomeprazole magnesium, 713.12
M_{r2} = molecular weight of omeprazole, 345.42

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

OTHER COMPONENTS

- **Content of Magnesium**
  Lanthanum solution: Transfer 58.7 g of lanthanum oxide to a 1000-mL volumetric flask, wet the substance with some water, and dissolve by cautious addition of 250 mL of hydrochloric acid in 20- to 30-mL portions, cooling between the additions. Add water while stirring, cool to room temperature, and dilute with water to volume. [Note—Store the solution in a plastic bottle.]
  Standard stock solution: 1000 µg/mL of magnesium in water, from a commercially prepared atomic absorption standard solution. [Note—Store the solution in a plastic bottle.]

USP Monographs

www.webofpharma.com

Standard solution A: Transfer 10.0 mL of *Standard stock solution* to a 500-mL volumetric flask, add 50 mL of 1 N hydrochloric acid, and dilute with water to volume. Transfer 20.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume. [NOTE—This solution contains 2 µg/mL of magnesium.]
Standard solution B: Combine 5.0 mL of *Standard solution A* and 4.0 mL of *Lanthanum solution*, and dilute with water to 100.0 mL (0.1 µg/mL).
Standard solution C: Combine 10.0 mL of *Standard solution A* and 4.0 mL of *Lanthanum solution*, and dilute with water to 100.0 mL (0.2 µg/mL).
Standard solution D: Combine 15.0 mL of *Standard solution A* and 4.0 mL of *Lanthanum solution*, and dilute with water to 100.0 mL (0.3 µg/mL).
Standard solution E: Combine 20.0 mL of *Standard solution A* and 4.0 mL of *Lanthanum solution*, and dilute with water to 100.0 mL (0.4 µg/mL).
Standard solution F: Combine 25.0 mL of *Standard solution A* and 4.0 mL of *Lanthanum solution*, and dilute with water to 100.0 mL (0.5 µg/mL). [NOTE—Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the instrument. When using instruments with a linear calibration graph, the number of *Standard solutions* can be reduced.]
Sample solution: Transfer 250 mg of Esomeprazole Magnesium to a 100-mL volumetric flask, add 20 mL of 1 N hydrochloric acid, swirl until dissolved, and dilute with water to volume. Allow to stand for 30 min. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of the solution to another 100-mL volumetric flask, add 4.0 mL of *Lanthanum solution*, and dilute with water to volume.
Blank: Transfer 4.0 mL of *Lanthanum solution* to a 100-mL volumetric flask, and dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Flame: Air–acetylene
Analytical wavelength: 285.2 nm
Analysis
Samples: *Standard solution B, Standard solution C, Standard solution D, Standard solution E, Standard solution F, Sample solution,* and *Blank*
Determine the concentration, C_S, in µg/mL, of magnesium in the *Sample solution* using the calibration graph.
Calculate the percentage of magnesium in the portion of Esomeprazole Magnesium taken:

$$\text{Result} = (C_S/C_U) \times [100/(100 - F)] \times 100$$

C_S = concentration of magnesium in the *Sample solution* as calculated above (µg/mL)
C_U = concentration of Esomeprazole Magnesium in the *Sample solution* (µg/mL)
F = content of water in Esomeprazole Magnesium, as determined in *Specific Tests, Water Determination* (%)

Acceptance criteria: 3.30%–3.55% on the anhydrous basis

IMPURITIES

• ORGANIC IMPURITIES

Solution A: Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, and dilute with water to 1000 mL. Dilute 250 mL of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.
Mobile phase: Acetonitrile and *Solution A* (11:29). [NOTE—To improve the resolution, the composition may be changed to 1:3, if necessary.]
System suitability solution: 1 mg each of USP Omeprazole RS and USP Omeprazole Related Compound A RS in 25 mL of *Mobile phase*. [NOTE—Omeprazole related compound A is omeprazole sulfone.]
Sample solution: 4 mg of Esomeprazole Magnesium in 25 mL of *Mobile phase*. [NOTE—Prepare this solution fresh.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 12.5-cm or 4.6-mm × 15-cm; 5-µm packing L7. [NOTE—Alternatively, a 3.9-mm × 15-cm column that contains 4-µm packing L1 may be used.]
Flow rate: 0.8–1 mL/min
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*
[NOTE—For relative retention times, see *Table 1*.]
Suitability requirements
Resolution: NLT 3 between omeprazole related compound A and omeprazole
Analysis
Sample: *Sample solution*
Record the chromatogram for at least 4.5 times the retention time of the omeprazole peak, and measure the peak responses. Identify the impurities based on the retention times shown in *Table 1*.
Calculate the percentage of any individual impurity in the portion of Esomeprazole Magnesium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria
Individual impurities: See *Table 1*.
Total impurities: NMT 0.5%

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Omeprazole *N*-oxide[a]	0.45	0.1
Omeprazole sulfone (omeprazole related compound A)	0.8	0.2
Any other individual impurities	—	0.1
Omeprazole	1.0	—

[a] 4-Methoxy-2-[[(*RS*)-(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl]-3,5-dimethylpyridine 1-oxide.

• ENANTIOMERIC PURITY

Solution A: Mix 70 mL of 1 M monobasic sodium phosphate with 20 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 1000 mL. Dilute 250 mL of this solution with water to 1000 mL.
Diluent: Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 1000 mL.
Mobile phase: Acetonitrile and *Solution A* (3:17)

USP Monographs

www.webofpharma.com

System suitability solution: 2 mg of USP Omeprazole RS in 10 mL of *Diluent*. Dilute 1.0 mL of this solution with *Diluent* to 50 mL.
Sample solution: Dissolve 40 mg of Esomeprazole Magnesium in 5 mL of methanol, and dilute with *Diluent* to 25 mL. Dilute 1 mL of this solution with *Diluent* to 50 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 302 nm
Column: 4.0-mm × 10-cm; packing L41
Flow rate: 0.6 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 3 between the enantiomer peaks.
[NOTE—The elution order is the *R*-enantiomer, followed by the esomeprazole peak, which is the *S*-enantiomer.]
Analysis
Sample: *Sample solution*
Calculate the percentage of the *R*-enantiomer in the portion of Esomeprazole Magnesium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of the *R*-enantiomer from the *Sample solution*
r_T = sum of the peak responses for esomeprazole and *R*-enantiomer from the *Sample solution*

Acceptance criteria: NMT 0.2% of the *R*-enantiomer

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*: 6.0%–8.0% for the trihydrate form
If labeled as dihydrate: 4.5%–7.0%
If labeled as amorphous: 7.0%–10.0%
• **CRYSTALLINITY** ⟨695⟩: If it is labeled as amorphous, most of the particles do not exhibit birefringence and extinction positions.
• **COLOR OF SOLUTION**
Sample solution: 20 mg/mL of Esomeprazole Magnesium in methanol, filtered
Analysis: Determine the absorbance of this solution at 440 nm, in 1-cm cells, using methanol as the blank.
Acceptance criteria: NMT 0.2

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at room temperature. If it is labeled as amorphous, store at 2°–8° under nitrogen atmosphere.
• **LABELING:** Where it is a dihydrate form, the label so indicates. Where it is an amorphous form, the label so indicates.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Esomeprazole Magnesium RS
USP Omeprazole RS
USP Omeprazole Related Compound A RS
Omeprazole sulfone;
5-Methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulfonyl]-1*H*-benzimidazole.
$C_{17}H_{19}N_3O_4S$ 361.42
[CAS-88546-55-8].

Esomeprazole Magnesium Delayed-Release Capsules

DEFINITION
Esomeprazole Magnesium Delayed-Release Capsules contain an amount of Esomeprazole Magnesium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$).

IDENTIFICATION
• **A.**
Buffer: Prepare a pH 6.0 phosphate buffer containing 26.6 g/L of dibasic sodium phosphate dihydrate and 55.2 g/L of monobasic sodium phosphate monohydrate in water.
Diluent: Prepare a pH 11.0 diluent as follows. Dissolve 5.24 g of tribasic sodium phosphate dodecahydrate in water. Add 110 mL of 0.5 M dibasic sodium phosphate solution, and dilute with water to 1000 mL.
Mobile phase: Transfer 150 mL of acetonitrile and 85 mL of the *Buffer* to a 1000-mL volumetric flask, and dilute with water to volume.
Standard stock solution: Prepare a solution containing 0.2 mg/mL of USP Omeprazole RS by dissolving a suitable amount first in alcohol, using 20% of the final volume, and then diluting with *Diluent* to volume.
Standard solution: 0.02 mg/mL of USP Omeprazole RS from the *Standard stock solution* in water
Sample stock solution: Transfer a portion of the Capsule content, equivalent to 20 mg of esomeprazole, to a 200-mL volumetric flask, add 120 mL of *Diluent*, and shake for 20 min to dissolve the pellets. Sonicate for a few min, if needed, to completely dissolve. Add 40 mL of alcohol, and sonicate for a few min. Cool, and dilute with *Diluent* to volume. Pass a portion of the solution through a filter of 1-µm pore size.
Sample solution: 0.01 mg/mL of esomeprazole from the *Sample stock solution* in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 302 nm
Column: 4.0-mm × 10-cm; 5-µm packing L41
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The elution order is the *R*-enantiomer, followed by the esomeprazole peak, which is the *S*-enantiomer.]
Suitability requirements
Resolution: NLT 1.0 between the enantiomer peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the ratio of the retention times of the esomeprazole peak in the *Standard solution* and the *Sample solution*:

$$\text{Result} = (t_U/t_S)$$

t_U = retention time of esomeprazole from the *Sample solution*
t_S = retention time of esomeprazole from the *Standard solution*

Acceptance criteria: 0.98–1.02

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: Prepare a pH 7.3 phosphate buffer by mixing 10.5 mL of 1.0 M monobasic sodium phosphate buffer and 60 mL of 0.5 M dibasic sodium phosphate buffer, and diluting with water to 1000 mL.

Diluent: Prepare as directed in *Identification* test *A*.

Mobile phase: Mix 350 mL of acetonitrile and 500 mL of the *Buffer*. Dilute with water to 1000 mL.

Standard solution: Transfer 10 mg of USP Omeprazole RS to a 250-mL volumetric flask, and dissolve in about 10 mL of alcohol. Add 40 mL of *Diluent*, and dilute with water to volume. This solution contains 0.04 mg/mL of USP Omeprazole RS.

Sample stock solution: Mix the contents of NLT 20 Capsules. Transfer a portion of the Capsule content, equivalent to 20 mg of esomeprazole, to a 100-mL volumetric flask, add 60 mL of *Diluent*, and shake for 20 min to dissolve the pellets. Sonicate for a few min, if needed, to completely dissolve. Add 20 mL of alcohol, and sonicate for a few min. Cool, and dilute with *Diluent* to volume. Pass a portion of the solution through a filter of 1-µm pore size.

Sample solution: 0.04 mg/mL of esomeprazole from the *Sample stock solution* in water. Store this solution protected from light.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 302 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) in the portion of the Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of esomeprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.1 N hydrochloric acid; 300 mL. After 2 h, continue with a pH 6.8 phosphate buffer as follows. To the vessel, add 700 mL of 0.086 M dibasic sodium phosphate, and adjust with 2 N hydrochloric acid or 2 N sodium hydroxide, if necessary, to a pH of 6.8 ± 0.05.

Apparatus 2: 100 rpm

Time: 30 min in a pH 6.8 phosphate buffer

Standard solution: Prepare a solution containing 2 mg/mL of USP Omeprazole RS in alcohol. Dilute this solution with pH 6.8 phosphate buffer to obtain a solution containing (*L*/1000) mg/mL, where *L* is the label claim, in mg/Capsule. Immediately add 2.0 mL of 0.25 M sodium hydroxide to 10.0 mL of this solution, and mix. [NOTE—Do not allow the solution to stand before adding the sodium hydroxide solution.]

Sample solution: After 30 min in pH 6.8 phosphate buffer, pass a portion of the solution under test through a suitable filter. Transfer 5.0 mL of the filtrate to a suitable glassware containing 1.0 mL of 0.25 M sodium hydroxide. Mix well. Protect from light.

Buffer, Mobile phase, System suitability, and **Chromatographic system:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of esomeprazole ($C_{17}H_{19}N_3O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of *Medium*, 1000 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Acid resistance stage

Acid stage medium: 0.1 N hydrochloric acid; 300 mL

Apparatus 2: 100 rpm

Time: 2 h

Solution A: Prepare a 0.05 M ammonium acetate buffer pH 7.6 as follows. Dissolve 3.85 g of ammonium acetate in 1000 mL of water, and adjust with a diluted ammonia solution to a pH of 7.6.

Solution B: Use acetonitrile.

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for 5 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	77	23
8	77	23
10	50	50

Diluent: Dissolve 7.6 g of sodium borate in about 800 mL of water. Add 1.0 g of edetate disodium, and adjust with 50% sodium hydroxide solution to a pH of 11.0 ± 0.1. Transfer the solution to a 2000-mL volumetric flask, add 400 mL of dehydrated alcohol, and dilute with water to volume.

Standard solution: 0.12 mg/mL of USP Omeprazole RS in *Diluent*, using sonication at a temperature between 10° and 15° to dissolve. Protect this solution from light.

Sample solution: After 2 h, drain the *Acid stage medium* from each vessel and carefully transfer the pellets into a suitable volumetric flask (use a 100-mL flask for 20-mg Capsules and a 200-mL flask for 40-mg Capsules). Add *Diluent* to about 70% of the final volume, and sonicate at a temperature between 10° and 15° for about 20 min with intermittent shaking. Allow to cool, dilute with *Diluent* to volume, mix, and pass through a PVDF or other suitable filter of 0.45-µm or finer pore size. Further

www.webofpharma.com

dilute 5 mL of this solution with *Diluent* to 10 mL. Protect this solution from light.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 302 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage, *T*, of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) retained:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times (1/L) \times 100$$

r_U = peak response of esomeprazole from the *Sample solution*
r_S = peak response of omeprazole from the *Standard solution*
C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)
D = dilution factor used in preparing the *Sample solution*
L = label claim (mg/Capsule)

Calculate the percentage of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) dissolved:

$$\text{Result} = A - T$$

A = esomeprazole content as a percentage of the labeled amount, as determined in the *Assay*
T = percentage of the labeled amount of esomeprazole retained, as determined above

[NOTE—If T is greater than A, then consider the result to be zero.]

Tolerances: NMT 10% of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) is dissolved.

Buffer stage
Buffer stage medium: pH 6.8 phosphate buffer. Proceed as directed in *Acid resistance stage* with a new set of Capsules. After 2 h with *Acid stage medium*, continue with a pH 6.8 phosphate buffer as follows. To the vessel, add 700 mL of 0.086 M dibasic sodium phosphate, and adjust with 2 N hydrochloric acid or 2 N sodium hydroxide, if necessary, to a pH of 6.8 ± 0.05.
Apparatus 2: 100 rpm
Time: 30 min
Solution A: Prepare a 0.05 M ammonium acetate buffer pH 7.6 as follows. Dissolve 3.85 g of ammonium acetate in 1000 mL of water, and adjust with a diluted ammonia solution to a pH of 7.6 ± 0.05.
Mobile phase: Acetonitrile and *Solution A* (27:73)
Diluent: 0.086 M dibasic sodium phosphate buffer and 0.1 N hydrochloric acid (70:30). Adjust with 2 N hydrochloric acid or 2 N sodium hydroxide, if necessary, to a pH of 6.8 ± 0.05.
Standard stock solution: Prepare a solution containing 0.4 mg/mL of USP Omeprazole RS as follows. Dissolve first in alcohol, using 10% of the final volume, and then dilute with *Diluent* to volume. Protect this solution from light.
Standard solution: Dilute the *Standard stock solution* with *Diluent* to obtain a solution containing (L/1000) mg/mL, where L is the label claim, in mg/Capsule. Immediately transfer 10 mL of this solution to a test tube containing 2.0 mL of 0.25 M sodium hydroxide, and mix. Protect this solution from light.
Sample solution: After 30 min, pass a portion of the solution under test through a PVDF or other suitable filter of 0.45-µm pore size. Immediately transfer 5.0 mL of the filtrate to a test tube containing 1.0 mL of 0.25 M sodium hydroxide. Mix well. Protect this solution from light.
Chromatographic system: Proceed as directed in *Acid resistance stage*, except use a flow rate of 1.0 mL/min.

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
D = dilution factor used to prepare the *Sample solution*
V = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (Q) of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
[NOTE—Use only glass bowls.]

Acid resistance stage
Acid stage medium: 0.1 N hydrochloric acid; 300 mL
Apparatus 2: 100 rpm (*Acid stage medium*)
Time: 2 h
Buffer: Prepare a 25 mM potassium phosphate buffer pH 8.0 as follows. Dissolve 3.4 g of monobasic potassium phosphate in 1000 mL of water, add 8.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 8.0.
Solution A: *Buffer* : methanol (90:10)
Solution B: Acetonitrile : methanol (50:50)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	85	15
3	65	35
4	65	35
4.5	20	80
5.5	20	80
6	85	15
8	85	15

Diluent 1: 0.3 N sodium hydroxide : methanol (10:90)
Diluent 2: 0.1 N sodium hydroxide : methanol (75:25)
[NOTE—Protect all standard and sample solutions from light.]

www.webofpharma.com

Standard stock solution: 0.4 mg/mL of USP Omeprazole RS prepared as follows. Dissolve a suitable amount of USP Omeprazole RS in a suitable volumetric flask containing 30% volume of 0.3N sodium hydroxide, sonicate as needed to dissolve, and dilute to volume with *Diluent 1*.

Standard solution: Dilute the *Standard stock solution* with *Diluent 2* to obtain a solution containing (*L*/500) mg/mL, where *L* is the label claim, in mg/Capsule.

Sample solution: After 2 h, drain the *Acid stage medium* from each vessel carefully without losing any pellet. Add 250 mL of 0.25N sodium hydroxide to each vessel and run the dissolution apparatus at 200 rpm for 30 min or until the pellet is completely dissolved. Centrifuge a portion of this solution at 3000 rpm for 10 min. Transfer 5.0 mL of this solution to a 10-mL volumetric flask and dilute to volume with *Diluent 2*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 305 nm
Column: 4.6-mm × 20-mm; 5-µm packing L1
[NOTE—A suitable L1 guard column may be used.]
Column temperature: 30°
Flow rate: 1.2 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage, *T*, of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) retained:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times (1/L) \times V \times 100$$

r_U = peak response of esomeprazole from the *Sample solution*
r_S = peak response of omeprazole from the *Standard solution*
C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)
D = dilution factor used to prepare the *Sample solution*
L = label claim (mg/Capsule)
V = volume of 0.25 N sodium hydroxide, 250 mL

Calculate the percentage of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) dissolved:

$$\text{Result} = A - T$$

A = esomeprazole content as a percentage of the labeled amount, as determined in the *Assay*
T = percentage of the labeled amount of esomeprazole retained, as determined above

[NOTE—If *T* is greater than *A*, then consider the result to be zero.]

Tolerances: NMT 10% of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) is dissolved.

Buffer stage
Buffer stock solution: Prepare a 76 g/L solution of sodium phosphate tribasic in water.
Buffer stage medium: 0.1 N hydrochloric acid : *Buffer stock solution* (3:1). Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide, if necessary, to pH 6.8.
Apparatus 2: 100 rpm
Time: 30 min

Standard solution: Dilute the *Standard stock solution* with *Buffer stage medium* to obtain a solution containing (*L*/1000) mg/mL, where *L* is the label claim, in mg/Capsule. Immediately transfer 5 mL of this solution to a test tube containing 1.0 mL of 0.25 ▲N▲ (ERR 1-Jan-2019) sodium hydroxide, and mix.

Sample solution: Proceed as directed in *Acid resistance stage* with a new set of Capsules. After 2h with *Acid stage medium*, continue with *Buffer stage medium* as follows. Completely drain the vessel of *Acid stage medium* carefully without losing any pellet. Add 1000 mL of preheated *Buffer stage medium* to each vessel. After 30 min, pass a portion of the solution under test through a full flow or other suitable filter of 10-µm pore size. Immediately transfer 5 mL of the filtrate to a test tube containing 1 mL of 0.25 ▲N▲ (ERR 1-Jan-2019) sodium hydroxide, and mix.

Chromatographic system: Proceed as directed in *Acid resistance stage*, except use *Injection volume* of 20 µL.

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times (1/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Omeprazole RS from the *Standard solution* (mg/mL)
D = dilution factor used to prepare the *Sample solution*
L = label claim (mg/Capsule)
V = volume of *Buffer stage medium*, 1000 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer: Prepare a pH 7.6 phosphate buffer by mixing 5.2 mL of 1.0 M monobasic sodium phosphate buffer and 63 mL of 0.5 M dibasic sodium phosphate buffer, and diluting with water to 1000 mL.
Solution A: Mix 100 mL of acetonitrile and 100 mL of the *Buffer*. Dilute with water to 1000 mL.
Solution B: Mix 800 mL of acetonitrile and 10 mL of the *Buffer*. Dilute with water to 1000 mL.
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	80	20
30	0	100
31	100	0
45	100	0

Diluent: Prepare as directed in *Identification* test *A*.

USP Monographs

www.webofpharma.com

System suitability stock solution: 1 mg/mL each of USP Omeprazole RS and USP Omeprazole Related Compound A RS in methanol
System suitability solution: 1 µg/mL each of USP Omeprazole RS and USP Omeprazole Related Compound A RS from *System suitability stock solution*, in a mixture of *Diluent* and water (1:4)
Sample solution: Transfer a portion of the powdered pellets (about 80–90 mg), from the Capsule content, to a 200-mL volumetric flask, add 20 mL of methanol, and shake for 30 s. Add 40 mL of *Diluent*, shake for 30 s by hand, and sonicate for a few min. Cool, and dilute with water to volume. Pass a portion of the solution through a filter of 0.45-µm pore size. [NOTE—The solution is stable for 3 h if stored protected from light.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 302 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 4* for the relative retention times.]
Suitability requirements
Resolution: NLT 2.5 between omeprazole related compound A and omeprazole
Analysis
Sample: *Sample solution*
Calculate the percentage of any individual impurity in the portion of the Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all peak responses

Acceptance criteria: See *Table 4*.

Table 4

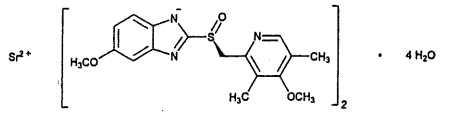
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Omeprazole sulfone[a]	0.93	0.5
Omeprazole	1.00	—
Any other individual impurity	—	0.2
Total impurities	—	2

[a] Omeprazole related compound A.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Omeprazole RS
USP Omeprazole Related Compound A RS
Omeprazole sulfone; 5-Methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfonyl]-1*H*-benzimidazole.
$C_{17}H_{19}N_3O_4S$ 361.42

Esomeprazole Strontium



$C_{34}H_{36}N_6O_6S_2Sr \cdot 4H_2O$ Tetrahydrate: 848.50
$C_{34}H_{36}N_6O_6S_2Sr$ Anhydrous: 776.44
1*H*-Benzimidazole, 5-methoxy-2-[(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl], strontium salt, hydrate (2:1:4);
(–)-5-Methoxy-2-{(*S*)-[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulfinyl}-1*H*-benzimidazole strontium salt tetrahydrate [934714-36-0].

DEFINITION

Esomeprazole Strontium contains NLT 98.0% and NMT 102.0% of esomeprazole strontium ($C_{34}H_{36}N_6O_6S_2Sr$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A OR 197K▲** (CN 1-May-2020) or ⟨197A⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.**
Sample solution: 1 mg/mL of Esomeprazole Strontium in 20% acetonitrile (v/v)
Acceptance criteria: The *Sample solution* imparts a red color to the nonluminous flame.

ASSAY

- **PROCEDURE**
Buffer: Dissolve 1.12 g of dibasic sodium phosphate anhydrous and 0.18 g of monobasic sodium phosphate anhydrous in 1000 mL of water. If necessary, adjust with phosphoric acid to a pH of 7.6.
Solution A: Acetonitrile and *Buffer* (10:30)
Solution B: Acetonitrile and *Buffer* (20:30)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
25	0	100
50	0	100

Return to the original conditions, and re-equilibrate the system for about 5 min.
System suitability solution: 0.5 mg/mL of USP Omeprazole RS and 0.5 µg/mL of USP Omeprazole Related Compound A RS in *Solution A*
Standard solution: 0.5 mg/mL of USP Omeprazole RS in *Solution A*
Sample solution: 0.5 mg/mL of Esomeprazole Strontium in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm

USP Monographs

www.webofpharma.com

Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between omeprazole related compound A and omeprazole, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of esomeprazole strontium ($C_{34}H_{36}N_6O_6S_2Sr$) in the portion of Esomeprazole Strontium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M_{r1}/(2 \times M_{r2})] \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Esomeprazole Strontium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of esomeprazole strontium, 776.44
M_{r2} = molecular weight of omeprazole, 345.42

Acceptance criteria: 98.0%–102.0% on the dried basis

OTHER COMPONENTS

• **CONTENT OF STRONTIUM**
Solution A: 37.2 mg/mL of edetate sodium in water
Solution B: 24.9 mg/mL of cupric sulfate in water
Solution C: *Solution A* and *Solution B* (1:1)
Buffer: Transfer 54 g of ammonium chloride to a 1000-mL volumetric flask, add 350 mL of ammonium hydroxide, and dilute with water to volume.
Sample: 400 mg of Esomeprazole Strontium
Analysis: Transfer the *Sample* to a suitable flask, dissolve in 30 mL of methanol, add 40 mL of *Buffer*, and mix. Add 0.5 mL of pH 7.0 phosphate buffer and 1 mL of *Solution C*, and mix. Titrate with 0.05 M edetate disodium VS to a potentiometric endpoint (see *Titrimetry* ⟨541⟩). Perform a blank determination. Each mL of 0.05 M edetate disodium VS is equivalent to 4.381 mg of strontium.
Acceptance criteria: 10.8%–11.8% on the dried basis

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer, Solution A, Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 500 µg/mL of USP Omeprazole RS and 0.5 µg/mL of USP Omeprazole Related Compound A RS in *Solution A*
Standard solution: 0.5 µg/mL each of USP Omeprazole RS and USP Omeprazole Related Compound A RS, and 0.25 µg/mL of (*S*)-binol in *Solution A*
Sample solution: 500 µg/mL of Esomeprazole Strontium in *Solution A*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between omeprazole related compound A and omeprazole, *System suitability solution*
Relative standard deviation: NMT 10% for each peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of omeprazole related compound A and (*S*)-binol in the portion of Esomeprazole Strontium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of omeprazole related compound A or (*S*)-binol from the *Sample solution*
r_S = peak response of omeprazole related compound A or (*S*)-binol from the *Standard solution*
C_S = concentration of USP Omeprazole Related Compound A RS or (*S*)-binol in the *Standard solution* (µg/mL)
C_U = concentration of Esomeprazole Strontium in the *Sample solution* (µg/mL)

Calculate the percentage of each unspecified impurity in the portion of Esomeprazole Strontium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Omeprazole sulfone (omeprazole related compound A)	0.7	0.1
Esomeprazole	1.0	—
(*S*)-Binol[a]	3.9	0.05
Any individual unspecified impurity	—	0.10
Total impurities	—	0.3

[a] 1-(2-Hydroxynaphthalen-1-yl)naphthalene-2-ol.

• **ENANTIOMERIC PURITY**
Solution A: Dissolve 1.42 g of dibasic sodium phosphate anhydrous in 900 mL of water. Adjust with phosphoric acid to a pH of 6.5 and dilute with water to 1000 mL.
Solution B: Acetonitrile
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20	90	10
25	70	30
30	70	30

Return to the original conditions, and re-equilibrate the system for about 5 min.
Buffer: Prepare as directed in the *Assay*.
Diluent: Acetonitrile and *Buffer* (10:30)

www.webofpharma.com

System suitability solution: 0.005 mg/mL of USP Omeprazole RS in *Diluent*
Sample solution: 0.5 mg/mL of Esomeprazole Strontium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.0-mm × 15-cm; 5-µm packing L41
Column temperature: 30°
Flow rate: 0.8 mL/min
Injection volume: 5 µL
Run time: 4 times the retention time of esomeprazole
System suitability
[NOTE—The elution order is the *R*-enantiomer, followed by the esomeprazole peak, which is the *S*-enantiomer.]
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 5 between the esomeprazole and *R*-enantiomer peaks
Relative standard deviation: NMT 10% for each peak
Analysis
Sample: *Sample solution*
Calculate the percentage of the *R*-enantiomer in the portion of Esomeprazole Strontium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of the *R*-enantiomer from the *Sample solution*
r_T = sum of the peak responses of esomeprazole and *R*-enantiomer from the *Sample solution*

Acceptance criteria: NMT 0.1% of the *R*-enantiomer

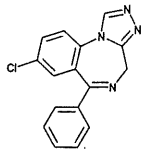
SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 125° to constant weight.
Acceptance criteria: 7.5%–9.5%
- **COLOR OF SOLUTION**
Sample solution: 20 mg/mL of Esomeprazole Strontium in acetone
Analysis: Determine the absorbance of the *Sample solution* at 440 and 650 nm, in 1-cm cells, using acetone as the blank.
Acceptance criteria: NMT 0.2 at 440 and 650 nm

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Esomeprazole Strontium RS
USP Omeprazole RS
USP Omeprazole Related Compound A RS
Omeprazole sulfone;
5-Methoxy-2-{[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulfonyl}-1*H*-benzimidazole.
$C_{17}H_{19}N_3O_4S$ 361.42

Estazolam



$C_{16}H_{11}ClN_4$ 294.74
4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine, 8-chloro-6-phenyl-;
8-Chloro-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,4]benzodiazepine [29975-16-4].

DEFINITION

Estazolam contains NLT 98.0% and NMT 102.0% of estazolam ($C_{16}H_{11}ClN_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
Buffer: 2.8 g/L of monobasic potassium phosphate in water. Adjust with 1 N sodium hydroxide to a pH of 6.5.
Mobile phase: Acetonitrile, methanol, and *Buffer* (10:35:55)
Standard stock solution: 0.5 mg/mL of USP Estazolam RS in *Mobile phase*
Standard solution: 0.02 mg/mL of USP Estazolam RS in water from *Standard stock solution*
Sample stock solution: 0.5 mg/mL of Estazolam in *Mobile phase*
Sample solution: 0.02 mg/mL of Estazolam in water from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3-µm packing L11
Flow rate: 1 mL/min
Injection volume: 25 µL
Run time: 2.5 times the retention time of the estazolam peak
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of estazolam ($C_{16}H_{11}ClN_4$) in the portion of Estazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Estazolam RS in the *Standard solution* (mg/mL)
C_U = concentration of Estazolam in the *Sample solution* (mg/mL)

USP Monographs

www.webofpharma.com

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A: Acetonitrile
Solution B: Water
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	40	60
20	90	10
23	90	10
30	40	60
35	40	60

[NOTE—The gradient was established on an HPLC system with a dwell volume of approximately 1.0 mL.]
System suitability solution: 1 µg/mL each of USP Estazolam RS, USP Nordazepam RS, and USP Estazolam Related Compound A RS in acetonitrile
Standard solution: 1 µg/mL of USP Estazolam RS in acetonitrile
Sample solution: 1 mg/mL of Estazolam in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between nordazepam and estazolam related compound A, *System suitability solution*
Tailing factor: NMT 1.2 for estazolam, *Standard solution*
Relative standard deviation: NMT 2.0% for estazolam, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Estazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of estazolam from the *Standard solution*
C_S = concentration of USP Estazolam RS in the *Standard solution* (µg/mL)
C_U = concentration of Estazolam in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Estazolam	1.0	—	—
Nordazepam[a]	1.4	1.3	0.1
Estazolam related compound A[b]	1.6	1.0	0.1
Formamido chlorobenzophenone[c]	2.0	1.5	0.1
Bischloroacetylbenzophenone[d]	2.2	1.5	0.1
Aminochlorobenzophenone[e]	2.6	1.4	0.1
Chloroacetamido chlorobenzophenone[f]	2.7	1.2	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] 7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.
[b] 5-Chloro-2-(3-chloromethyl-4*H*-1,2,4-triazol-4-yl)-benzophenone.
[c] *N*-(2-Benzoyl-4-chlorophenyl) formamide.
[d] 2-(*N*-Chloroacetyl-*N*-chloroacetylhydrazonomethyl)amino-5-chlorobenzophenone.
[e] (2-Amino-5-chlorophenyl) phenyl-methanone.
[f] *N*-(2-Benzoyl-4-chlorophenyl)-2-chloroacetamide.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Estazolam RS
4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine, 8-chloro-6-phenyl-;
8-Chloro-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,4] benzodiazepine.
$C_{16}H_{11}ClN_4$ 294.74
USP Estazolam Related Compound A RS
5-Chloro-2-(3-chloromethyl-4*H*-1,2,4-triazol-4-yl)-benzophenone.
$C_{16}H_{11}Cl_2N_3O$ 332.18
USP Nordazepam RS
7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.
$C_{15}H_{11}ClN_2O$ 270.71

Estazolam Tablets

DEFINITION
Estazolam Tablets contain an amount of Estazolam equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of estazolam ($C_{16}H_{11}ClN_4$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: 2.8 g/L of potassium phosphate, monobasic in water. Adjust to a pH of 6.5 with 1 N NaOH.

Mobile phase: Acetonitrile, methanol, and *Buffer* (10:35:55)

Standard solution: 0.02 mg/mL of USP Estazolam RS in *Mobile phase*

Sample solution: 0.02 mg/mL of Estazolam in *Mobile phase*, from NLT 20 finely powdered Tablets. [NOTE—Sonicate for 5 min. Pass a portion through a suitable filter with no glass prefilter.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 3-µm packing L11

Flow rate: 1 mL/min

Injection size: 25 µL

Run time: 2.7 times the retention time of the estazolam peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{16}H_{11}ClN_4$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Estazolam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of estazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL, deaerated

Apparatus 2: 50 rpm

Time: 30 min

Buffer and **Mobile phase:** Proceed as directed in the *Assay*.

Standard stock solution: 0.1 mg/mL of USP Estazolam RS in methanol

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of about (L/1000) mg/mL, where L is the Tablet label claim in mg.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first few mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Proceed as directed in the *Assay*, except for the injection size and run time.

Injection size: 100 µL

Run time: 1.6 times the retention time of estazolam

System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of estazolam dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of estazolam from the *Sample solution*

r_S = peak response of estazolam from the *Standard solution*

C_S = concentration of USP Estazolam RS in the *Standard solution* (mg/mL)

L = Tablet label claim (mg)

V = volume of *Medium* (mL), 900

Tolerances: NLT 80% (Q) of the labeled amount of estazolam is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Buffer, Mobile phase, Standard solution, and **Sample solution:** Proceed as directed in the *Assay*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Proceed as directed in the *Assay*, except for the injection size and run time.

Injection size: 25 µL for the *Standard solution* and 50 µL for the *Sample solution*

Run time: 4 times the retention time of estazolam

System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_T = sum of the responses of all peaks from the *Sample solution*

Acceptance criteria

Any individual unspecified degradation product: NMT 0.5%

Total impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

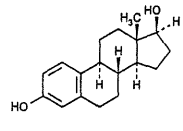
USP Estazolam RS

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine, 8-chloro-6-phenyl-.

8-Chloro-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,4]benzodiazepine.

$C_{16}H_{11}ClN_4$ 294.74

Estradiol



$C_{18}H_{24}O_2$ 272.38

Estra-1,3,5(10)-triene-3,17-diol, (17β)-.

www.webofpharma.com

Estra-1,3,5(10)-triene-3,17β-diol [50-28-2].
Hemihydrate 281.39
[35380-71-3].

» Estradiol contains not less than 97.0 percent and not more than 103.0 percent of $C_{18}H_{24}O_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
Labeling—The hemihydrate form is so labeled.
USP Reference standards ⟨11⟩—
USP Estradiol RS
USP Estrone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 50 µg per mL.
Medium: alcohol.
Absorptivities at 280 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.
Melting range, *Class I* ⟨741⟩**:** between 173° and 179°.
[NOTE—Dry over silica gel for not less than 16 hours prior to testing.]
Specific rotation ⟨781S⟩**:** between +76° and +83°.
Test solution: 10 mg per mL, in dioxane.
Water Determination, *Method I* ⟨921⟩**:** not more than 3.5%.
Chromatographic purity— [NOTE—Make all solutions fresh daily.]
Mobile phase—Prepare a filtered and degassed mixture of 2,2,4-trimethylpentane, *n*-butyl chloride, and methanol (45:4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluting solution—Prepare a filtered and degassed mixture of *n*-butyl chloride and methanol (5:1).
Test solution—Transfer about 70 mg of Estradiol, accurately weighed, to a 10-mL volumetric flask, dissolve in *Diluting solution,* shake vigorously to aid dissolution, dilute with *Diluting solution* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains packing L3. The flow rate is about 2 mL per minute. Chromatograph the *Test solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between estradiol and any impurity is not less than 1.0; the column efficiency is not less than 800 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Estradiol taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks: not more than 0.5% of any individual impurity is found; and not more than 1.0% of total impurities is found.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Transfer about 300 mg of ethylparaben to a 500-mL volumetric flask, add methanol to volume, and mix.
Standard preparation—Dissolve accurately weighed quantities of USP Estradiol RS and USP Estrone RS in methanol to obtain a solution containing 0.40 mg and 0.24 mg, respectively, in each mL. Pipet 10 mL of this solution and 5 mL of the *Internal standard solution* into a 200-mL volumetric flask. Add 100 mL of methanol, dilute with water to volume, and mix to obtain a solution having a known concentration of about 20 µg of USP Estradiol RS per mL.
Assay preparation—Transfer about 100 mg of Estradiol, accurately weighed, to a 250-mL volumetric flask, add methanol to volume, and mix. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, add 5.0 mL of *Internal standard solution* and 100 mL of methanol, dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for the internal standard, about 1.3 for estrone, and 1.0 for estradiol; the resolution, *R,* between the analyte and estrone is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{24}O_2$ in the portion of Estradiol taken by the formula:

$$5C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Estradiol RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Estradiol Vaginal Cream

DEFINITION
Estradiol Vaginal Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of estradiol ($C_{18}H_{24}O_2$) in a suitable cream base.

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHY**
Standard solution: 0.5 mg/mL of USP Estradiol RS in chloroform
Sample solution: Transfer a portion of Vaginal Cream, equivalent to 1 mg of estradiol, to a 150-mL beaker. Add 25 mL of acetonitrile, and gently heat to boiling. Boil for 45 s, and cool to room temperature. Add 25 mL of water, and swirl. Filter with the aid of suction. Transfer the filtrate to a 125-mL separator, add 50 mL of chloroform, and shake. Allow the layers to separate, drain the chloroform layer into a flask, and evaporate in a rotary evaporator to dryness. Dissolve the residue in 2 mL of chloroform.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 50 µL. Dry the applications with the aid of a stream of nitrogen.
Developing solvent system: Toluene and acetone (4:1)
Spray reagent: Sulfuric acid and methanol (1:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Spray the plate with a fine mist of *Spray reagent*, then heat the plate for 3–5 min at 90°. Observe the plate under visible light.
Acceptance criteria: The R_F value and color of the principal spot of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (1:1)
Internal standard solution: 60 µg/mL of dydrogesterone in acetonitrile. Use a freshly prepared solution.
Standard solution: 10 µg/mL of USP Estradiol RS and 7.5 µg/mL of USP Estrone RS, prepared as follows. Transfer 10 mg of USP Estradiol RS and 7.5 mg of USP Estrone RS to a 1000-mL volumetric flask. Add 50.0 mL of *Internal standard solution* and 450 mL of acetonitrile, and mix. Dilute with water to volume.
Sample solution: Nominally 10 µg/mL of estradiol, prepared as follows. Transfer a portion of Vaginal Cream, equivalent to 0.5 mg of estradiol, to a 150-mL beaker. Add 2.5 mL of *Internal standard solution*, 22.5 mL of acetonitrile, and a few boiling chips. Cover with a watch glass, and heat gently until the Vaginal Cream melts, swirling occasionally. Heat to boiling for about 45 s. Allow to cool to room temperature, add 25.0 mL of water, and mix. Pass first through paper and then through a microdisk filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for estradiol, estrone, and the internal standard are 1.0, 1.25, and 2.0, respectively.]
Suitability requirements
Resolution: NLT 1.9 between estradiol and estrone
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of estradiol ($C_{18}H_{24}O_2$) in the portion of Vaginal Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of estradiol to the internal standard from the *Sample solution*
R_S = peak response ratio of estradiol to the internal standard from the *Standard solution*
C_S = concentration of USP Estradiol RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of estradiol in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*
• **PH** ⟨791⟩**:** 3.5–6.5

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Estradiol RS
USP Estrone RS

Estradiol Vaginal Inserts

DEFINITION
Estradiol Vaginal Inserts contain NLT 90% and NMT 107% of the labeled amount of estradiol ($C_{18}H_{24}O_2$).

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
[NOTE—When two different concentrations are given for a solution, the lower concentration is for Inserts labeled to contain 0.01 mg of estradiol, and the high value is for Inserts labeled to contain 0.025 mg of estradiol.]
Standard solution: 2.5 mg/mL of USP Estradiol RS in absolute alcohol
Sample solution: Place a number of Inserts, equivalent to 0.38 or 0.95 mg of estradiol, into a vessel. Add 50 mL of isopropyl alcohol, and allow to disintegrate by stirring overnight. Centrifuge the suspension. Evaporate an aliquot of 40 mL of the supernatant to dryness, and dissolve the residue in 3 mL of isopropyl alcohol. Evaporate to dryness, reconstitute with 300 µL of absolute alcohol to obtain a solution containing 1.0 or 2.5 mg/mL of estradiol, and centrifuge.
Adsorbent: Use a suitable, high-performance thin-layer chromatographic plate.
Application volume: NLT 5 µL (equivalent to 10 µg of estradiol)
Developing solvent system: Chloroform and acetone (9:1)
Analysis: Proceed as directed in the chapter, using the *Developing solvent system* described above. Develop the chromatogram over a path of a minimum of 8 cm, and allow the plate to air-dry. Remove the plate, mark the solvent front, and allow solvent evaporation as described in the chapter. Heat at 100° for about 15 min. Allow the plate to cool, and then immerse it in a mixture of absolute alcohol and concentrated sulfuric acid (95:5). Remove it immediately, place the plate on absorbing paper, and allow it to air-dry. Heat the plate at 100° until the sulfuric acid has evaporated. Examine under UV light at λ = 365 nm.
Acceptance criteria: The principal spot obtained from the *Sample solution* has the same color and R_F value as that from the *Standard solution*.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**

USP Monographs

www.webofpharma.com

[NOTE—When two different concentrations are given for a solution, the lower concentration is for Inserts labeled to contain 0.01 mg of estradiol, and the high value is for Inserts labeled to contain 0.025 mg of estradiol.]

Mobile phase: Acetonitrile and water (11:9)

Diluent: Absolute alcohol and water (1:1)

Estrone standard stock solution: 0.1 mg/mL of USP Estrone RS in absolute alcohol

Estradiol standard stock solution: 0.25 mg/mL of USP Estradiol RS in absolute alcohol

System suitability solution: 0.6 and 2.0 µg/mL of USP Estrone RS and USP Estradiol RS in *Diluent* from *Estrone standard stock solution* and *Estradiol standard stock solution*, respectively

Standard solution: 1.0 or 2.5 µg/mL of USP Estradiol RS in *Diluent* from *Estradiol standard stock solution*

Sample solution: 1.0 or 2.5 µg/mL of estradiol prepared using 10 Inserts in *Diluent*. Stir the mixture overnight with a magnetic stirrer, shake thoroughly, and centrifuge if necessary.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 3.9-mm × 30-cm; 4-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between estradiol and estrone
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of estradiol ($C_{18}H_{24}O_2$) in the portion of Inserts taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Estradiol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of estradiol in the *Sample solution* (mg/mL)

Acceptance criteria: 90%–107%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Phosphate buffer pH 4.75 ± 0.05 (100 g of potassium dihydrogen phosphate in 10 L of water, adjusted with 1 N sodium hydroxide to a pH of 4.75 ± 0.05); 500 mL

Apparatus 1: 40 rpm

Time: 3, 5, and 10 h

Mobile phase: Methanol, acetonitrile, and water (27.5:27.5:45)

Standard stock solution: 0.1 mg/mL of USP Estradiol RS in absolute alcohol

Standard solutions: Quantitatively dilute with water the *Standard stock solution* to obtain solutions with final concentrations equal to approximately 20%, 60%, and 160% of the expected concentration of estradiol in the *Medium* for Inserts containing 0.025 mg, assuming complete dissolution, and approximately 20%, 50%, 150%, and 400% of the expected concentration of estradiol in the *Medium* for Inserts containing 0.01 mg of estradiol, assuming complete dissolution.

Sample solutions: Use the solution under test, unfiltered.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorescence
Excitation wavelength: 230 nm
Emission wavelength: 310 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1; or 4.6-mm × 7.5-cm; 5.0-µm packing L1
Flow rate: 1 mL/min
Injection size: 200 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8
Relative standard deviation: NMT 2%

Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms, and measure the responses for the estradiol peak. Construct a calibration curve by plotting the peak response versus concentration of the *Standard solutions*. Determine the amount of estradiol ($C_{18}H_{24}O_2$) in the *Sample solutions* from a linear regression analysis of the calibration curve.
Calculate the amount of estradiol ($C_{18}H_{24}O_2$) dissolved:

$$\text{Result} = \left\{A_n \times V_n + \left[\sum_1^n \left(\Delta V_{(n-1)} \times A_{(n-1)}\right)\right]\right\} / V$$

A_n = percentage of estradiol, at the sample point n (e.g., A_2 at the second sampling point)
V_n = volume of *Medium* in the vessel before the sample is taken (mL)
$\Delta V_{(n-1)}$ = volume of sample taken at the sampling point ($n - 1$)
$A_{(n-1)}$ = amount of estradiol (uncorrected) at the sample point ($n - 1$)
V = volume of the medium, 500 mL

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
3	25–50
5	40–80
10	NLT 80

The percentage of the labeled amount of estradiol dissolved at the specified times conforms to *Acceptance Table 2* in ⟨711⟩.

IMPURITIES

• **ORGANIC IMPURITIES**

[NOTE—When two different concentrations are given for a solution, the lower concentration is for Inserts labeled to contain 0.01 mg of estradiol, and the high value is for Inserts labeled to contain 0.025 mg of estradiol.]

Solution A: Acetonitrile

Solution B: Water

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	16	84
35	68	32

www.webofpharma.com

[NOTE—Before the next injection, run the system at the initial condition until equilibration is achieved.]

System suitability solution: 100 µg/mL of USP Estradiol RS, 0.5 µg/mL of USP Estradiol Related Compound B RS, and 0.5 µg/mL of USP Estradiol Related Compound C RS in absolute alcohol

Sample solution: Place a number of Inserts into a measured volume of absolute alcohol to obtain a solution having an estradiol concentration of 2.4 or 6.0 µg/mL. Stir for a minimum of 16 h, shake thoroughly, and centrifuge if necessary. Evaporate 10.0 mL of the supernatant to dryness. Dissolve the residue in 1.0 mL of water and add 7.0 mL of a mixture of toluene and acetone (5:2), mix on a whirl mixer, allow to stand for 1 h, and evaporate 5 mL of the organic phase to dryness. The residue is reconstituted in 450 µL of absolute alcohol to obtain a solution containing 38 or 95 µg/mL of estradiol. Centrifuge, and use the supernatant as the *Sample solution.*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 25 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for estradiol related compound B and estradiol are about 0.96 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between estradiol related compound B and estradiol

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the Inserts taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of estradiol from the *Sample solution*

Acceptance criteria: See *Table 3. For Inserts labeled to contain 0.025 mg of estradiol* and *Table 4. For Inserts labeled to contain 0.01 mg of estradiol.*

Table 3. For Inserts labeled to contain 0.025 mg of estradiol

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Estradiol related compound C (6-ketoestradiol)[a]	0.71	2.4
Estradiol related compound B (6-dehydroestradiol)[b]	0.96	1.4
Estradiol	1.0	—
Any other individual impurity	—	0.8
Total impurities	—	4.4

[a] 1,3,5(10)-Estratrien-3,17β-diol-6 one.
[b] 1,3,5(10),6-Estratetraen-3,17β-diol.

Table 4. For Inserts labeled to contain 0.01 mg of estradiol

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Estradiol related compound C (6-ketoestradiol)[a]	0.71	1.5
Estradiol related compound B (6-dehydroestradiol)[b]	0.96	1.3
Estradiol	1.0	—
Any other individual impurity	—	1.3
Total impurities	—	4.0

[a] 1,3,5(10)-Estratrien-3,17β-diol-6-one.
[b] 1,3,5(10),6-Estratetraen-3,17β-diol.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 100 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g. Inserts meet the requirements of the tests for absence of *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Candida albicans.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in a tight container, and store at controlled room temperature. Do not refrigerate.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Estradiol RS

USP Estradiol Related Compound B RS
6-Dehydroestradiol.

USP Estradiol Related Compound C RS
1,3,5(10)-Estratrien-3,17β-diol-6 one.
$C_{18}H_{22}O_3$

USP Estrone RS

Estradiol Transdermal System

DEFINITION

Estradiol Transdermal System contains NLT 85.0% and NMT 120.0% of the labeled amount of estradiol ($C_{18}H_{24}O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Diluent: Acetonitrile and water (1:1)

Mobile phase: Acetonitrile and water (55:45)

Standard solution: 0.1 mg/mL of USP Estradiol RS in *Diluent*

Sample solutions: Equivalent to 0.1 mg/mL of estradiol in *Diluent,* prepared as follows. Cut 10 Transdermal Systems into pieces, and keep the pieces from each system separate. Remove and discard the protective liners, if present, from the strips. Transfer the pieces of each system into separate stoppered flasks of suitable size, and add a measured volume of *Diluent* to each flask to provide the target estradiol concentration. Shake by mechanical means for about 3 h, and sonicate for 15 min.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

USP Monographs

www.webofpharma.com

Detector: UV 280 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: 0.9–1.6
Relative standard deviation: NMT 2.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of estradiol ($C_{18}H_{24}O_2$) in each Transdermal System taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Estradiol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of estradiol in the *Sample solution* (mg/mL)

Use the individual assays to determine *Uniformity of Dosage Units*.
Acceptance criteria: 85.0%–120.0%

OTHER COMPONENTS

• **ALCOHOL CONTENT** (if present)
Diluent: Acetonitrile and water (1:1)
Internal standard solution: Prepare by diluting 4.0 mL of dehydrated methanol with water to 100 mL.
Standard stock solution: 5.0 mg/mL of ethanol in *Diluent*. Prepare by weighing by difference 1.6 mL of dehydrated alcohol into a tared 50-mL volumetric flask containing 15 mL of water, and dilute with *Diluent* to volume. Pipet 10.0 mL of this solution into a 50-mL volumetric flask, and dilute with *Diluent* to volume.
Standard solution: 2.5 mg/mL of ethanol. Prepare by pipeting 25.0 mL of the *Standard stock solution* into a 50-mL volumetric flask. Add 5.0 mL of the *Internal standard solution*, and dilute with water to volume.
Sample solutions: Prepare as directed for the *Sample solutions* in the *Assay*, with the following changes. Pipet 25.0 mL of each solution into individual 50-mL volumetric flasks. Add 5.0 mL of the *Internal standard solution*, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 2-mm × 2-m glass; support S2
Temperature
Column: 100°
Injection port: 200°
Detector: 200°
Carrier gas: Helium
Flow rate: 30 mL/min
Injection size: 2 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for the methanol and alcohol peaks are 0.4 and 1.0, respectively.]
Suitability requirements
Relative standard deviation: NMT 1.5% from the peak response ratio of alcohol to methanol
Analysis
Samples: *Standard solution* and *Sample solutions*
Calculate the percentage of alcohol (C_2H_5OH) in each Transdermal System taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of alcohol to methanol from the *Sample solution*
R_S = peak response ratio of alcohol to methanol from the *Standard solution*
C_S = concentration of dehydrated alcohol in the *Standard solution* (mg/mL)
C_U = nominal concentration of alcohol in the *Sample solution* (mg/mL)

Average the percentage of alcohol found in the Transdermal Systems analyzed.
Acceptance criteria: 80%–120% of the labeled amount of C_2H_5OH

PERFORMANCE TESTS

• **DRUG RELEASE** ⟨724⟩
Test 1: For products labeled for dosing every 84 h
Medium: Water; 900 mL, deaerated
Apparatus 5: 50 rpm
Times: 24, 48, and 96 h
Mobile phase: Water and acetonitrile (3:2)
Standard solution: 9 µg/mL of USP Estradiol RS in dehydrated alcohol. Dilute this solution with *Medium* to obtain solutions having concentrations of about 0.9, 0.45, and 0.045 µg/mL.
Sample solution: At each sampling time interval, withdraw a 10-mL aliquot of the solution under test.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorimetric, with excitation at 220 nm and emission at 270 nm
Column: 4.6-mm × 3-cm; packing L1
Temperature: 40°
Flow rate: 1.0 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Tailing factor: 0.9–2.5
Relative standard deviation: NMT 3.0%, using 0.45 µg/mL of the *Standard solution*
Analysis: Plot the peak responses of the *Standard solutions* versus concentration, in µg/mL, of estradiol. From the graph determine the amount, in µg/mL, of estradiol released. Calculate the cumulative release rate as percentage of the labeled amount of estradiol:
At 24 h:

$$\text{Result} = \{[900(A_1 - b)]/(1000 \times m \times L)\} \times 100$$

At 48 h:

$$\text{Result} = \{[890(A_2 - b) + 10(A_1 - b]/(1000 \times m \times L)\} \times 100$$

At 96 h:

$$\text{Result} = \{[880(A_3 - b) + 10(A_2 - b) + 10(A_1 - b)]/(1000 \times m \times L)\} \times 100$$

A_1 = peak area of estradiol in the *Sample solution* at the first time interval
A_n = peak area of estradiol in the *Sample solution* at the release interval n
m = slope of the calibration curve
b = y-intercept of the calibration curve

USP Monographs

www.webofpharma.com

L = Transdermal System label claim (mg)

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (release rate)
24	1.2%–6.0%
48	3.0%–11.4%
96	5.0%–16.3%

The percentages of the labeled amount of estradiol ($C_{18}H_{24}O_2$) released at the times specified conform to *Drug Release* ⟨724⟩, *Acceptance Table 1*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 2*.

Medium: 0.005 M phosphate buffer, pH 5.5, containing 0.3% sodium lauryl sulfate; 500 mL

Apparatus 5: 100 rpm. Use a 76-mm stainless steel disk assembly. Adhere the patch to the disk assembly using transfer tape. [NOTE—A suitable tape is available as 3M adhesive transfer tape 927, www.mmm.com.]

Times: 1, 4, 8, and 24 h

Mobile phase: Acetonitrile and water (1:1)

Standard stock solution: 800 µg/mL of USP Estradiol RS in acetone

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a solution having a known concentration close to that expected in the solution under test, assuming 100% drug release.

Sample solution: At each sampling time interval, withdraw a known volume aliquot of the solution under test.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.0 mL/min
Injection size: 100 µL

System suitability
Sample: *Standard solution*
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%

Analysis: Calculate the amount of estradiol released at each sampling time:

$$M_i = (r_U/r_S) \times C_S \times V_i$$

$$m_1 = M_1$$

$$m_2 = M_2 + M_1(V_a/V_1)$$

$$m_3 = M_3 + M_2(V_a/V_2) + M_1(V_a/V_1)$$

$$m_4 = M_4 + M_3(V_a/V_3) + M_2(V_a/V_2) + M_1(V_a/V_1)$$

Calculate the percentage of the labeled amount of estradiol released at each sampling time:

$$\text{Result} = (m_i/L) \times 100$$

M_i = amount of estradiol released into the *Medium* at a given sampling time (mg)
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V_i = corrected volume of the *Medium* at a given sampling time (mL)
m_1, m_2, m_3, m_4 = total amounts of estradiol released from the patch at given sampling times (mg)
M_1, M_2, M_3, M_4 = amounts of estradiol released into the *Medium* at given sampling times (mg)
V_a = volume of the aliquot taken from the dissolution vessel at each sampling time (mL)
V_1, V_2, V_3 = volumes of *Medium* at given sampling times (mL)
L = Transdermal System label claim (mg)

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (release rate)
1	15%–40%
4	45%–70%
8	70%–90%
24	NLT 80%

The percentages of the labeled amount of estradiol ($C_{18}H_{24}O_2$) released at the times specified conform to *Drug Release* ⟨724⟩, *Acceptance Table 1*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 3*.

Medium: 1% (v/v) polysorbate 40 in water; 900 mL

Apparatus 5: 50 rpm

Times: 4, 8, and 24 h

Standard stock solution: Known concentration (mg/mL) of USP Estradiol RS in methanol

Standard solution: Five different concentrations within the range of the expected release amounts of estradiol, prepared as follows. Add 1.0 mL of polysorbate 40 into a 100-mL volumetric flask, and then add the required amount of *Standard stock solution*. Mix well to dissolve the polysorbate 40, and dilute with water to volume.

Sample solution: At each sampling time interval, withdraw a known volume aliquot of the solution under test.

Mobile phase: Acetonitrile and water (2:3)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm, 5-µm packing L1 for 9-cm² systems; 4.6-mm × 12.5-cm, 5-µm packing L1 for 18-, 27-, or 36-cm² systems. In any case, a guard column containing packing L1 is used.
Flow rate: 1.0 mL/min
Injection size: 50 µL

System suitability
Sample: *Standard solution*
Relative standard deviation: NMT 2.0%

Analysis: Calculate the cumulative release rate as a percentage of the labeled amount of estradiol:

$$\text{Result} = \{[900(A - b)]/(1000 \times m \times L)\} \times 100$$

A = peak area of estradiol in the *Sample solution* at each time interval
b = y-intercept of the calibration curve
m = slope of the calibration curve
L = Transdermal System label claim (mg)

USP Monographs

www.webofpharma.com

Tolerances: The percentages of the labeled amount of estradiol ($C_{18}H_{24}O_2$) released at the times specified conform to *Table 3*, *Table 4*, and *Table 5*.

L1 (6 units)

Table 3

Time (h)	Amount Dissolved (individual values)
4	40%–71%
8	58%–94%
24	NLT 75%

L2 (12 units)

Table 4

Time (h)	Amount Dissolved (average of 12)	Amount Dissolved (individual values)
4	40%–71%	34%–77%
8	58%–94%	50%–102%
24	NLT 75%	NLT 68%

L3 (24 units)

Table 5

Time (h)	Amount Dissolved (average of 24)	Amount Dissolved (individual for 22 units of 24)	Amount Dissolved (individual for 24)
4	40%–71%	34%–77%	29%–82%
8	58%–94%	50%–102%	43%–109%
24	NLT 75%	NLT 68%	NLT 60%

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 4*.

Medium: Water; 500 mL for 0.025 mg/day and 0.0375 mg/day dosage; 900 mL for 0.05 mg/day, 0.075 mg/day, and 0.1 mg/day dosage

Apparatus 6: 50 rpm. Use a stainless steel cylinder assembly. Adhere the Transdermal System to the cylinder assembly using a strip of suitable double-sided transfer tape.

Times: 2, 6, and 12 h

Buffer solution: 25 mM of monobasic sodium phosphate, adjusted with phosphoric acid to a pH of 3.0

Mobile phase: Acetonitrile and *Buffer solution* (40:60)

Standard stock solution: 0.2 mg/mL of USP Estradiol RS in methanol

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a solution having a known concentration that is approximately 90% of the concentration expected from complete release in the solution under test.

Sample solution: At each sampling time interval, withdraw about 1.5 mL of the solution under test. Place each sample aliquot into an amber HPLC vial.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.0-mm × 10-cm; 3.5-µm packing L1

Flow rate: 0.5 mL/min

Injection volume: 15 µL

Run time: 2.5 times the retention time of estradiol

System suitability

Sample: *Standard solution*

Tailing factor: NMT 1.8

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of estradiol ($C_{18}H_{24}O_2$) in the sample withdrawn from the vessel at time point *i*:

$$C_i = (r_i/r_S) \times C_S$$

r_i = peak response of estradiol from the *Sample solution* at time point *i*

r_S = peak response of estradiol from the *Standard solution*

C_S = concentration of USP Estradiol RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of estradiol ($C_{18}H_{24}O_2$) released at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of estradiol in the portion of the sample withdrawn at each time point (*i*) (mg/mL)

V = volume of *Medium*, 900 or 500 mL

L = Transdermal System label claim (mg)

V_S = volume of *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Dissolved (release rate, %)
2	20–40
6	48–68
12	70–90

The percentages of the labeled amount of estradiol ($C_{18}H_{24}O_2$) released at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: The results from the Transdermal Systems used in the *Assay* meet the requirements.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in hermetic, light-resistant, unit-dose pouches.
- **LABELING:** The label states the total amount of estradiol in the Transdermal System and the release rate, in mg/day, for the duration of application of one system. When more than one *Drug Release* test is given, the labeling states the *Drug Release* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Estradiol RS

www.webofpharma.com

Estradiol Tablets

» Estradiol Tablets contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{18}H_{24}O_2$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Estradiol RS
USP Estrone RS

Identification—Place a quantity of finely powdered Tablets, equivalent to about 4 mg of estradiol, in a screw-capped, 20-mL vial. Add 10 mL of chloroform, and sonicate for 2 minutes. Filter through medium-porosity filter paper. Apply 20 µL each of this solution and a Standard solution of USP Estradiol RS in chloroform containing 0.4 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a lined chamber with a solvent system consisting of a mixture of toluene and acetone (4:1) until the solvent front has moved 10 cm beyond the starting line. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Spray the plate with a mixture of methanol and sulfuric acid (1:1), and heat at 100° for about 5 minutes: the principal spots obtained from the test solution and the Standard solution have the same color and R_F value.

Dissolution ⟨711⟩—
Medium: 0.3% sodium lauryl sulfate in water; 500 mL.
Apparatus 2: 100 rpm.
Time: 60 minutes.
Mobile phase—Prepare a suitable degassed and filtered solution of water and acetonitrile (55:45).
Standard solution—Prepare a solution of USP Estradiol RS in methanol having an accurately known concentration of about 0.02 mg per mL. Dilute aliquots of this solution with *Medium* to obtain a final solution having a concentration approximately equal to the expected concentration of drug in the *Medium*, assuming 100% dissolution.
Test solution—Use a filtered portion of the solution under test from the dissolution vessel.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm × 7.5-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak areas as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation is not more than 2.0%.
Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity of $C_{18}H_{24}O_2$ dissolved by comparison of the peak areas obtained from the *Test solution* and the *Standard solution*.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{18}H_{24}O_2$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Chromatographic purity—
Solution A—Prepare a degassed mixture of water and acetonitrile (8:2).
Solution B—Prepare a degassed mixture of acetonitrile and water (8:2).
Diluent—Prepare a mixture of water and acetonitrile (6:4).
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve accurately weighed quantities of USP Estradiol RS and USP Estrone RS in acetonitrile to obtain a solution having concentrations of 0.5 mg per mL and 0.3 mg per mL, respectively. Pipet 2 mL of this solution into a 50-mL volumetric flask, and dilute with *Diluent* to volume.
Test solution—Transfer a number of Tablets, containing a combined amount of 4 to 8 mg of estradiol based on the label claim, to a suitable flask, add a volume of *Diluent* equivalent to about 5 mL per each mg of estradiol, swirl until the Tablets are completely disintegrated, then shake for 15 minutes, and allow the solids to settle. Pass a portion of this solution through a 0.45-µm PVDF filter, discarding the first 2 mL of the filtrate. This solution contains about 0.2 mg of estradiol per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 200-nm detector and a 4.0-mm × 12.5-cm column that contains 5-µm packing L7. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–5	100→90	0→10	linear gradient
5–15	90→50	10→50	linear gradient
15–25	50→0	50→100	linear gradient
25–35	0	100	isocratic
35–35.1	0→100	100→0	linear gradient
35.1–40	100	0	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*. Identify the components based on their relative retention times which are 1.0 for estradiol and about 1.1 for estrone: the resolution, R, between estradiol and estrone is not less than 3.0; the tailing factors for estradiol and estrone peaks are not more than 1.5; and the relative standard deviation for replicate injections is not more than 2% for each peak.
Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks: not more than 5% of total impurities is found. Disregard any peaks observed in the blank.

Assay—
Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay*— under *Estradiol*.
Assay preparation—Weigh and finely powder not fewer than 10 Tablets. Transfer a portion of the powder, equivalent to about 8 mg of estradiol, to a 100-mL volumetric flask. Add 4 mL of water, and swirl. Add 10.0 mL of *Internal standard solution* and about 60 mL of methanol. Shake by mechanical means for 15 minutes, dilute with methanol to volume, mix, and allow the solids to settle. Filter a portion, discarding the first 10 mL of the filtrate. Mix 5.0 mL of the subsequent filtrate with 5.0 mL of methanol and 10.0 mL of water.

www.webofpharma.com

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Estradiol*. Calculate the quantity, in mg, of $C_{18}H_{24}O_2$ in the portion of Tablets taken by the formula:

$$0.4C(R_U/R_S)$$

in which the terms are as defined therein.

Estradiol and Norethindrone Acetate Tablets

DEFINITION

Estradiol and Norethindrone Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of estradiol ($C_{18}H_{24}O_2$) and NLT 90.0% and NMT 110.0% of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$).

IDENTIFICATION

• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩

Standard solution: 0.5 mg/mL of USP Estradiol RS and 0.25 mg/mL of USP Norethindrone Acetate RS in dehydrated alcohol

Sample solution: Place 2 Tablets into a 10-mL vial, and add 0.2 mL of water. When the Tablets are partially disintegrated, add a few glass beads, and shake vigorously to disintegrate. Add 4.0 mL of dehydrated alcohol, and shake. [Note—Centrifuge until the supernatant is clear before application to the plate.]

Adsorbent: 0.25-mm chromatographic silica gel plate

Application volume: 2 µL

Developing solvent system: Chloroform and acetone (9:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter, using the *Developing solvent system*. Apply the *Samples* and develop the plate. After removal of the plate, mark the solvent front, and allow the solvent to evaporate. Place the plate on a heating plate at 100° for 15 min. Allow the plate to cool, and then immerse it in a mixture of dehydrated alcohol and concentrated sulfuric acid (95:5). Place the plate on a piece of thick horizontal paper until it is almost dry. Heat the plate at 100° until it has fully developed. Examine under UV light at 365 nm.

Acceptance criteria: The color and R_F value of the principal spots of the *Sample solution* correspond to those of the *Standard solution*.

• **B.** The retention time of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Mobile phase: Acetonitrile and water (11:9)

Diluent: Dehydrated alcohol and water (1:1)

Estrone standard stock solution: 0.12 mg/mL of USP Estrone RS in dehydrated alcohol

Estradiol standard stock solution: 0.25 mg/mL of USP Estradiol RS in dehydrated alcohol

Norethindrone acetate standard stock solution: 0.15 mg/mL of USP Norethindrone Acetate RS in dehydrated alcohol

Standard solution: 20 µg/mL of USP Estradiol RS from the *Estradiol standard stock solution* and 10 µg/mL of USP Norethindrone Acetate RS from the *Norethindrone acetate standard stock solution* in *Diluent*

System suitability solution: Combine 800 µL of the *Estradiol standard stock solution*, 600 µL of the *Norethindrone acetate standard stock solution*, 200 µL of the *Estrone standard stock solution*, and 10.0 mL of *Diluent*.

Sample solution: Add 12 Tablets into a measured amount of *Diluent* to obtain a solution having an estradiol concentration of 20 µg/mL and a norethindrone acetate concentration of 10 µg/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV dual wavelength (254 nm/280 nm) or equivalent

[Note—The absorption of estradiol at 280 nm and norethindrone acetate at 254 nm can be included in a single run by altering the wavelength.]

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1 mL/min

[Note—Perform an investigational run to determine the retention times for estradiol and norethindrone acetate.]

Injection size: 50 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 1.8 between estradiol and estrone, *System suitability solution*

Relative standard deviation: NMT 3%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—Measure the areas for the estradiol and norethindrone acetate peaks.]

Calculate the quantity, as a percentage, of $C_{18}H_{24}O_2$ in each of the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Estradiol RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of estradiol in the *Sample solution* (µg/mL)

Calculate the quantity, as a percentage, of $C_{22}H_{28}O_3$ in each of the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Norethindrone Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of norethindrone acetate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of $C_{18}H_{24}O_2$ and 90.0%–110.0% of the labeled amount of $C_{22}H_{28}O_3$

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Test 1

Medium: 0.3% sodium lauryl sulfate; 500 mL

Apparatus 2: 50 rpm

Time: 30 min for Tablets labeled to contain 1 mg of estradiol and 0.5 mg of norethindrone acetate, and 50

www.webofpharma.com

min for Tablets labeled to contain 0.5 mg of estradiol and 0.1 mg of norethindrone acetate
Mobile phase: Acetonitrile and water (11:9)
Standard stock solution A: 20 µg/mL of USP Estradiol RS in alcohol or in a mixture of alcohol and water
Standard stock solution B: 10 µg/mL of USP Norethindrone Acetate RS in alcohol or in a mixture of alcohol and water
Standard solution: Dilute suitable quantities of *Standard stock solution A* and *Standard stock solution B* in *Medium* or a mixture of alcohol and water to obtain a final concentration of both analytes similar to the expected concentration of the *Sample solution*.
Sample solution: Pass a portion of the solution through a filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 241 nm for norethindrone acetate and 280 nm for estradiol
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 150 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{18}H_{24}O_2$ and of $C_{22}H_{28}O_3$ dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of estradiol or norethindrone acetate from the *Sample solution*
r_S = peak response of estradiol or norethindrone acetate from the *Standard solution*
C_S = concentration of USP Estradiol RS or USP Norethindrone Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of estradiol or norethindrone acetate in the *Sample solution* (mg/mL) (based on the label claim)

Tolerances: For Tablets labeled to contain 1 mg of estradiol and 0.5 mg of norethindrone acetate: NLT 75% (*Q*) of the labeled amounts of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ is dissolved in 30 min. For Tablets labeled to contain 0.5 mg of estradiol and 0.1 mg of norethindrone acetate: NLT 75% (*Q*) of the labeled amounts of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ is dissolved in 50 min.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.3% sodium lauryl sulfate; 500 mL
Apparatus 2: 50 rpm
Time: 20 min
Mobile phase: Acetonitrile and water (55:45)
Standard stock solution A: 0.5 mg/mL of USP Estradiol RS in methanol. Sonicate as necessary.
Standard stock solution B: 0.5 mg/mL of USP Norethindrone Acetate RS in methanol. Sonicate as necessary.
Standard solution: (L_1/500) mg/mL of USP Estradiol RS and (L_2/500) mg/mL of USP Norethindrone Acetate RS prepared from *Standard stock solution A* and *Standard stock solution B* in *Medium*, where L_1 is the label claim of estradiol and L_2 is the label claim of norethindrone acetate, in mg/Tablet
Sample solution: Pass a portion of the solution through a filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 241 nm for norethindrone acetate and 210 nm for estradiol
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 20°
Column: 30°
Flow rate: 1 mL/min
Injection volume: 100 µL
Run time: NMT 1.3 times the retention time of norethindrone acetate
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for estradiol and norethindrone acetate
Relative standard deviation: NMT 2.0% for estradiol and norethindrone acetate
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of estradiol ($C_{18}H_{24}O_2$) and norethindrone acetate ($C_{22}H_{28}O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of estradiol or norethindrone acetate from the *Sample solution*
r_S = peak response of estradiol or norethindrone acetate from the *Standard solution*
C_S = concentration of USP Estradiol RS or USP Norethindrone Acetate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim of estradiol or norethindrone acetate (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of estradiol ($C_{18}H_{24}O_2$) and norethindrone acetate ($C_{22}H_{28}O_3$) is dissolved in 20 min.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Organic Impurities

• **Procedure**
Solution A: Tetrahydrofuran and water (1:200)
Solution B: Acetonitrile, tetrahydrofuran, and water (160:1:40)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	80	20
2	65	35
35	20	80
49	20	80
50	80	20
60	80	20

www.webofpharma.com

Diluent: Dehydrated alcohol and water (1:1)
System suitability solution: 240 µg/mL of USP Estradiol RS, 60 µg/mL of USP Norethindrone Acetate RS, and 1 µg/mL of USP Estrone RS in *Diluent*
Estradiol standard stock solution: 250 µg/mL of USP Estradiol RS in alcohol
Norethindrone acetate standard stock solution: 150 µg/mL of USP Norethindrone Acetate RS in alcohol
Standard solution: Combine 250 µL of the *Estradiol standard stock solution* and 100 µL of the *Norethindrone acetate standard stock solution*, and dilute with 50.0 mL of *Diluent*.
Sample solution: A quantity equivalent to 240 µg/mL of estradiol and 120 µg/mL of norethindrone acetate from NLT 20 finely ground Tablets in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 and 254 nm
Column: 3.9-mm × 30-cm; 4-µm packing L1
Flow rate: 0.8 mL/min
Injection size: 100 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for estradiol, estrone, and norethindrone acetate are about 1.0, 1.1, and 1.7, respectively.]
Suitability requirements
Resolution: NLT 1.3 between estrone and estradiol, measured at 254 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any estradiol impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response area at 235 nm for each impurity from the *Sample solution*
r_S = peak response area at 235 nm from the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)
F = relative response factor (see *Impurity Table 1. Tablets Labeled as Containing 1 mg of Estradiol and 0.5 mg of Norethindrone Acetate* or *Impurity Table 2. Tablets Labeled as Containing 0.5 mg of Estradiol and 0.1 mg of Norethindrone Acetate*)

Calculate the percentage of any norethindrone acetate related impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response area at 254 nm for each impurity from the *Sample solution*
r_S = peak response area at 254 nm from the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)
F = relative response factor (see *Impurity Table 1. Tablets Labeled as Containing 1 mg of Estradiol and 0.5 mg of Norethindrone Acetate* or *Impurity Table 2. Tablets Labeled as Containing 0.5 mg of Estradiol and 0.1 mg of Norethindrone Acetate*)

Acceptance criteria: The Tablets meet the requirements given in either *Impurity Table 1. Tablets Labeled as Containing 1 mg of Estradiol and 0.5 mg of Norethindrone Acetate* or *Impurity Table 2. Tablets Labeled as Containing 0.5 mg of Estradiol and 0.1 mg of Norethindrone Acetate.*

Impurity Table 1. Tablets Labeled as Containing 1 mg of Estradiol and 0.5 mg of Norethindrone Acetate

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Estradiol related impurities			
6α-Hydroxyestradiol	0.47	1.0	1.0
6β-Hydroxyestradiol	0.51	1.0	1.0
6-Ketoestradiol	0.62	1.0	1.0
6-Dehydroestradiol	0.95	1.0	1.0
Estradiol	1.0	—	—
Any other single estradiol related impurity	—	1.0	0.5
Total estradiol related impurities	—	—	2.0
Norethindrone acetate related impurities			
6β-Hydroxynorethindrone acetate	0.58	1.0	1.0
Norethindrone	0.66	1.0	1.0
6-Ketonorethindrone acetate	0.79	0.56	1.0
6-Dehydronorethindrone acetate	0.97	0.45	1.0
Norethindrone acetate	1.0	—	—
Any other single norethindrone acetate related impurity	—	1.0	0.5
Total norethindrone acetate related impurities	—	—	2.0

Impurity Table 2. Tablets Labeled as Containing 0.5 mg of Estradiol and 0.1 mg of Norethindrone Acetate

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Estradiol related impurities			
6β-Hydroxy-estradiol	0.51	1.0	1.0
Estradiol	1.0	—	—
Any other single estradiol related impurity	—	1.0	1.0
Total estradiol related impurities	—	—	2.5
Norethindrone acetate related impurities			
6β-Hydroxy-norethindrone acetate	0.58	1.0	1.5
Norethindrone	0.66	1.0	1.0
6-Keto-norethindrone acetate	0.79	0.56	2.5
6-Dehydro-norethindrone acetate	0.97	0.45	1.0

USP Monographs

www.webofpharma.com

Impurity Table 2. Tablets Labeled as Containing 0.5 mg of Estradiol and 0.1 mg of Norethindrone Acetate *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Norethindrone acetate	1.0	—	—
Any other single norethindrone acetate related impurity	—	1.0	1.0
Total norethindrone acetate related impurities	—	—	4.0

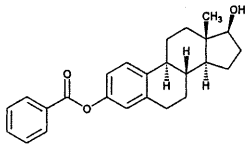
SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 1000 cfu/g, and the total combined molds and yeasts count does not exceed 100 cfu/g. The Tablets meet the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Estradiol RS
  USP Estrone RS
  USP Norethindrone Acetate RS

Estradiol Benzoate



$C_{25}H_{28}O_3$ 376.49

Estra-1,3,5(10)-triene-3,17-diol, (17β)-, 3-benzoate; Estradiol 3-benzoate [50-50-0].

DEFINITION

Estradiol Benzoate contains NLT 97.0% and NMT 103.0% of estradiol benzoate ($C_{25}H_{28}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K.▲ (CN 1-May-2020) [NOTE—If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in acetone, evaporate to dryness, and record new spectra using the residues.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and water (7:3)

System suitability solution: Transfer 20.0 mg each of USP Estradiol Benzoate RS and estradiol 17-acetate to a 100-mL volumetric flask. Add 70 mL of acetonitrile, and sonicate until dissolved. Add 25 mL of water, mix well, and allow to equilibrate to ambient temperature. Dilute with water to volume.

Standard solution: Transfer 20.0 mg of USP Estradiol Benzoate RS to a 100-mL volumetric flask. Add 70 mL of acetonitrile, and sonicate until dissolved. Add 25 mL of water, mix well, and allow to equilibrate to ambient temperature. Dilute with water to volume.

Sample solution: Transfer 20.0 mg of Estradiol Benzoate to a 100-mL volumetric flask. Add 70 mL of acetonitrile, and sonicate until dissolved. Add 25 mL of water, mix well, and allow to equilibrate to ambient temperature. Dilute with water to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Columns
Guard: 4.6-mm × 4.5-cm; packing L1
Analytical: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for estradiol 17-acetate and estradiol benzoate are about 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6.0 between estradiol 17-acetate and estradiol benzoate, *System suitability solution*
Tailing factor: NMT 2.0, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of estradiol benzoate ($C_{25}H_{28}O_3$) in the portion of Estradiol Benzoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Estradiol Benzoate RS in the *Standard solution* (mg/mL)
C_U = concentration of Estradiol Benzoate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩
  Sample: 250 mg
  Acceptance criteria: NMT 0.2%
- **LIMIT OF METHANOL AND DICHLOROMETHANE:** Proceed as directed in *Residual Solvents* ⟨467⟩.
  Acceptance criteria: The sum of methanol and methylene chloride is NMT 0.20%.
- **ORGANIC IMPURITIES**
  Diluent: Methylene chloride and alcohol (2:1)
  Standard solution: 5 mg/mL of USP Estradiol Benzoate RS in *Diluent*
  Sample solution A: 5 mg/mL of Estradiol Benzoate in *Diluent*
  Sample solution B: 0.1 mg/mL of Estradiol Benzoate in *Diluent* from *Sample solution A*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)

www.webofpharma.com

Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Developing solvent system: Toluene and ethyl acetate (70:30)
Spray reagent: 50 mg/mL of ammonium molybdate in 10% sulfuric acid

Analysis
Samples: *Standard solution*, *Sample solution A*, and *Sample solution B*
To the plate apply 20-µL aliquots of the *Standard solution* and *Sample solution A* and 20-, 15-, 10-, 5-, and 2-µL aliquots of *Sample solution B*. The volumetric series of *Sample solution B* represents 2.0%, 1.5%, 1.0%, 0.5%, and 0.2% of the concentration of Estradiol Benzoate within the *Sample solution A* spot.
Allow the spots to dry, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Spray the plate thoroughly with *Spray reagent*, and dry. Heat the plate in a drying oven for 10 min at about 115°.
Calculate the relative retardation factor (relative to estradiol benzoate) of all spots within the lanes for *Sample solution A* and *Sample solution B*.
Determine the percentages of each impurity by comparing the intensity of the impurity spots within *Sample solution A* to those of the main spots obtained from the series of *Sample solution B*, ignoring any impurity peak less intense than the main spots found in the *Sample solution B* lane containing 0.2% of the amount of estradiol benzoate of *Sample solution A*.
Acceptance criteria: See *Table 1*.

Table 1

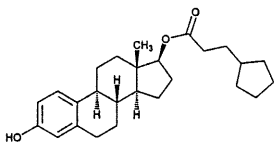
Name	Relative Retardation Factor	Acceptance Criteria, NMT (%)
Estradiol[a]	0.84	1.0
Estradiol benzoate	1.00	—
17a-Estradiol benzoate[b]	1.15	1.0
Estrone[c]	1.21	1.0
Any individual unspecified impurity	—	1.0
Total impurities	—	2.0

[a] Estra-1,3,5(10)-triene-3,17b-diol.
[b] Estra-1,3,5(10)-triene-3,17a-diol 3-benzoate.
[c] Estra-1,3,5(10)-triene-17-one, 3-hydroxy.

SPECIFIC TESTS
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL, previously dried in dioxane
Acceptance criteria: +57.0° to +63.0°
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 100°–105° for 3 h.
Acceptance criteria: NMT 0.5%
- **PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING** ⟨786⟩
Suspension fluid: To a mixture of glycerin and water (60:40, w/w) add a sufficient quantity of polysorbate 20 to obtain a solution having a concentration of 125 µL of polysorbate 20 per 100 g of solution.
Sample suspension: Saturate the *Suspension fluid* by adding 100 mg of fine Estradiol Benzoate per 100 g of *Suspension fluid*, and sonicate for 10 min. Pass the resulting suspension through a nylon filter of 0.45-µm pore size. To the filtrate, add 50 mg of Estradiol Benzoate per mL of the filtered, saturated *Suspension fluid*, and mix on a vortex mixer until dispersed (about 1 min).
Analysis: Using a suitable multi-wavelength particle size analyzer,[1] determine the particle size distribution within the *Sample suspension*, analyzing the results in the range of 5–600 µm.
Acceptance criteria: NMT 50% of the particles are less than 30 µm, and NLT 90% of the particles are less than 450 µm. The mean diameter of fine grade Estradiol Benzoate is NMT 100 µm, and the mean diameter of coarse grade Estradiol Benzoate is 100–200 µm.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label it to indicate that it is for veterinary use only. Label it to indicate whether it is coarse grade or fine grade.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Estradiol Benzoate RS

Estradiol Cypionate



$C_{26}H_{36}O_3$ 396.56
Estra-1,3,5(10)-triene-3,17-diol, (17β)-, 17-cyclopentanepropanoate;
Estradiol 17-cyclopentanepropionate [313-06-4].

DEFINITION
Estradiol Cypionate contains NLT 97.0% and NMT 103.0% of estradiol cypionate ($C_{26}H_{36}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Solution A: Acetonitrile
Solution B: Water
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	70	30
30	100	0
40	100	0
41	70	30

[1] A suitable multi-wavelength particle size analyzer is model LS 13 320, obtained from Beckman Coulter, Inc., Fullerton, CA, or equivalent.

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
50	70	30

System suitability solution: 1 mg/mL of USP Estradiol Cypionate System Suitability Mixture RS in acetonitrile
Standard solution: 1 mg/mL of USP Estradiol Cypionate RS in acetonitrile
Sample solution: 1 mg/mL of Estradiol Cypionate in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L26
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between estradiol-9-ene cypionate and estradiol cypionate, *System suitability solution*
Relative standard deviation: NMT 1.10%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of estradiol cypionate ($C_{26}H_{36}O_3$) in the portion of Estradiol Cypionate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Estradiol Cypionate RS in the *Standard solution* (mg/mL)
C_U = concentration of Estradiol Cypionate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%
- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 1 mg/mL of USP Estradiol Cypionate System Suitability Mixture RS and 2 µg/mL USP Estradiol RS in acetonitrile
Sensitivity solution: 0.5 µg/mL of USP Estradiol Cypionate RS in acetonitrile
Sample solution: 1 mg/mL of Estradiol Cypionate in acetonitrile
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
[NOTE—See *Table 2* for the relative retention times.]
Resolution: NLT 1.5 between estradiol-9-ene cypionate and estradiol cypionate, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Estradiol Cypionate taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response for each impurity
r_T = sum of the responses of all the peaks
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.05% of the estradiol cypionate peak.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Estradiol	0.22	1.0	0.15
Estradiol-9-ene cypionate[a]	0.93	2.5	0.15
Estradiol cypionate	1.0	—	—
4-Methylestradiol cypionate[b]	1.3	1.0	0.15
Estradiol dicypionate[c]	1.9	1.0	0.15
Any other individual unspecified impurity	—	1.0	0.10

[a] 3-Hydroxyestra-1,3,5(10),9(11)-tetraen-17β-yl cyclopentanepropanoate.
[b] 3-Hydroxy-4-methylestra-1,3,5(10)-trien-17β-yl cyclopentanepropanoate.
[c] Estra-1,3,5(10)-trien-3,17β-diyl di(cyclopentanepropanoate).

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 20 mg/mL in dioxane
Acceptance criteria: +39° to +44°
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Estradiol RS
USP Estradiol Cypionate RS
USP Estradiol Cypionate System Suitability Mixture RS
This mixture contains:
Estradiol cypionate.
Estradiol-9-ene cypionate.
3-Hydroxyestra-1,3,5(10),9(11)-tetraen-17β-yl cyclopentanepropanoate.
$C_{26}H_{34}O_3$ 394.55
Estradiol dicypionate.
Estra-1,3,5(10)-trien-3,17β-diyl di(cyclopentanepropanoate).
$C_{34}H_{48}O_4$ 520.74
4-Methylestradiol cypionate.
3-Hydroxy-4-methylestra-1,3,5(10)-trien-17β-yl cyclopentanepropanoate.
$C_{27}H_{38}O_3$ 410.59

Estradiol Cypionate Injection

DEFINITION

Estradiol Cypionate Injection is a sterile solution of Estradiol Cypionate in a suitable oil. It contains NLT 90.0% and NMT 110.0% of the labeled amount of estradiol cypionate ($C_{26}H_{36}O_3$).

USP Monographs

www.webofpharma.com

IDENTIFICATION

• **A.**

Sulfanilic acid solution: Mix 50 mg of sulfanilic acid with 2 mL of 3 N hydrochloric acid, warm the mixture, then cool it in ice water, and slowly add, with agitation, 0.3 mL of sodium nitrite solution (1 in 10).

Sample solution: Transfer a volume of Injection, equivalent to 5 mg of estradiol cypionate, to a glass-stoppered, 50-mL test tube, and add 30 mL of alcohol.

Analysis: Shake the *Sample solution* vigorously for 5 min, centrifuge until the two layers have separated, and transfer the alcohol layer, with the aid of a hypodermic syringe, to a 50-mL beaker. Evaporate on a steam bath to dryness, add 5 mL of potassium hydroxide solution (1 in 10), and heat on the steam bath for 15 min. Add the *Sulfanilic acid solution* to the saponified estradiol cypionate.

Acceptance criteria: A red color is produced.

ASSAY

• **PROCEDURE**

Mobile phase: 0.8 g/L of ammonium nitrate. Dissolve in 300 mL of water, and combine with 700 mL of acetonitrile.

Internal standard solution: 2.0 mg/mL of testosterone benzoate in tetrahydrofuran

Standard solution: 0.1 mg/mL of USP Estradiol Cypionate RS in tetrahydrofuran, prepared as follows. Transfer 10 mg of USP Estradiol Cypionate RS to a 100-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with tetrahydrofuran to volume. Shake vigorously to dissolve.

Sample solution: Using a "to contain" pipet, transfer an accurately measured volume, in mL, of Injection, equivalent to 10 mg of estradiol cypionate, to a 100-mL volumetric flask. Rinse the pipet with small portions of tetrahydrofuran, collecting the washings in the volumetric flask. Add 10.0 mL of the *Internal standard solution*, and dilute with tetrahydrofuran to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between the peaks for estradiol cypionate and the internal standard

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of estradiol cypionate ($C_{26}H_{36}O_3$) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of estradiol cypionate to the internal standard from the *Sample solution*

R_S = peak response ratio of estradiol cypionate to the internal standard from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩**:** Meets the requirements

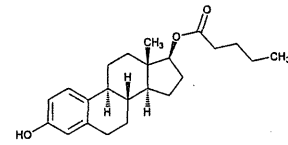
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose, light-resistant containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Estradiol Cypionate RS

Estradiol Valerate



$C_{23}H_{32}O_3$ 356.50

Estra-1,3,5(10)-triene-3,17-diol(17β)-, 17-pentanoate; Estradiol 17-valerate [979-32-8].

DEFINITION

Estradiol Valerate contains NLT 98.0% and NMT 102.0% of estradiol valerate ($C_{23}H_{32}O_3$).

IDENTIFICATION

Change to read:

• **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	48	52
1	48	52
10	35	65
17.5	0	100
26	0	100

Standard solution: 1.2 mg/mL of USP Estradiol Valerate RS in acetonitrile

Sample solution: 1.2 mg/mL of Estradiol Valerate in acetonitrile

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 2.6-µm packing L7

Flow rate: 2.0 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of estradiol valerate ($C_{23}H_{32}O_3$) in the portion of Estradiol Valerate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Estradiol Valerate RS in the *Standard solution* (mg/mL)
C_U = concentration of Estradiol Valerate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	48	52
1	48	52
10	35	65
17.5	0	100
26	0	100

System suitability solution: 1.25 mg/mL of USP Estradiol Valerate Identification Mixture RS in acetonitrile
Standard solution: 5 µg/mL of USP Estradiol Valerate RS in acetonitrile
Sample solution: 5 mg/mL of Estradiol Valerate in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 10-cm; 2.6-µm packing L7
Flow rate: 2.0 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 3* for relative retention times.]
Suitability requirements
Resolution: NLT 2.5 between estradiol-9-ene valerate and estradiol valerate, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Estradiol Valerate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of estradiol valerate from the *Standard solution*
C_S = concentration of USP Estradiol Valerate RS in the *Standard solution* (mg/mL)
C_U = concentration of Estradiol Valerate in the *Sample solution* (mg/mL)
F = relative response factor for each impurity

Acceptance criteria: See *Table 3*. Disregard peaks that are less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Estradiol	0.1	1.0	0.5
Estradiol-9-ene valerate	0.9	2.0	0.5
Estradiol valerate	1.0	—	—
4-Methylestradiol valerate	1.3	1.0	0.5
Estradiol divalerate	1.7	1.0	0.15
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

• **FREE ACID**
Diluent: Neutralize 25 mL of alcohol, in a conical flask, with 0.01 N sodium hydroxide VS to a faint blue color, using bromothymol blue TS.
Sample solution: 500 mg of Estradiol Valerate in 25 mL of *Diluent*
Analysis: Rapidly titrate the *Sample solution* with 0.01 N sodium hydroxide VS to a faint blue color. Each mL of 0.01 N sodium hydroxide is equivalent to 1.021 mg of valeric acid.
Acceptance criteria: NMT 0.5%, as valeric acid

SPECIFIC TESTS

• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 25 mg/mL, uncorrected for moisture, in methanol
Acceptance criteria: +41° to +47°
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.1%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Estradiol Valerate RS
USP Estradiol Valerate Identification Mixture RS
It contains Estradiol Valerate and a small amount of the following impurities:
Estradiol;
Estra-1,3,5(10)-triene-3,17β-diol.
$C_{18}H_{24}O_2$ 272.38
Estradiol-9-ene valerate;
3-Hydroxyestra-1,3,5(10),9(11)-tetraen-17β-yl valerate.
$C_{23}H_{30}O_3$ 354.48
4-Methylestradiol valerate;
3-Hydroxy-4-methylestra-1,3,5(10)-trien-17β-yl valerate.
$C_{24}H_{34}O_3$ 370.52
Estradiol divalerate;
Estra-1,3,5(10)-trien-3,17β-diyl divalerate.
$C_{28}H_{40}O_4$ 440.61

USP Monographs

www.webofpharma.com

Estradiol Valerate Injection

DEFINITION
Estradiol Valerate Injection is a sterile solution of Estradiol Valerate in a suitable vegetable oil. It contains NLT 90.0% and NMT 115.0% of the labeled amount of estradiol valerate ($C_{23}H_{32}O_3$).

IDENTIFICATION
• **A.**
Phenol reagent (Folin-Ciocalteu reagent): In a 1500-mL flask connected by a standard taper joint to a reflux condenser, dissolve 100 g of sodium tungstate and 25 g of sodium molybdate in 700 mL of water. Add 50 mL of phosphoric acid and 100 mL of hydrochloric acid, and reflux gently for 10 h. Cool, and add 150 g of lithium sulfate, 50 mL of water, and 4–6 drops of bromine. Boil the mixture without the condenser for 15 min to remove the excess bromine. Cool, transfer to a 1-L volumetric flask, dilute with water to volume, and filter. The filtrate is golden yellow in color, and has no greenish tint. Store the filtrate in a tight container in a refrigerator. Mix the filtrate with water (1:2) before use as the *Phenol reagent*.
Analysis: Transfer 0.5 mL of Injection to a separator containing 10 mL of solvent hexane and 10 mL of 80% methanol. Shake the contents for 2 min, and allow the phases to separate. Add 1 mL of *Phenol reagent* and 3 mL of sodium carbonate solution (1 in 5) to 1 mL of the bottom layer, and mix.
Acceptance criteria: A blue color develops.

ASSAY
• **PROCEDURE**
Mobile phase: Dissolve 0.8 g of ammonium nitrate in 300 mL of water, add 700 mL of acetonitrile, and mix.
Internal standard solution: 8.0 mg/mL of testosterone benzoate in tetrahydrofuran
Standard solution: 0.8 mg/mL of USP Estradiol Valerate RS, prepared as follows. Transfer 20 mg of USP Estradiol Valerate RS to a 25-mL volumetric flask. Add 5.0 mL of the *Internal standard solution*, and dilute with tetrahydrofuran to volume.
Sample solution: Nominally 0.8 mg/mL of estradiol valerate, prepared as follows. Using a "to contain" pipet, transfer an accurately measured volume of Injection, equivalent to 20 mg of estradiol valerate, to a 25-mL volumetric flask. Rinse the pipet with small portions of tetrahydrofuran, collecting the washings in the volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with tetrahydrofuran to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for estradiol valerate and testosterone benzoate are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 3.0 between estradiol valerate and the internal standard
Column efficiency: NLT 1100 theoretical plates for estradiol valerate
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of estradiol valerate ($C_{23}H_{32}O_3$) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of estradiol valerate to the internal standard from the *Sample solution*
R_S = peak response ratio of estradiol valerate to the internal standard from the *Standard solution*
C_S = concentration of USP Estradiol Valerate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

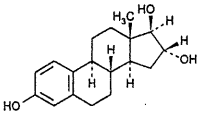
Acceptance criteria: 90.0%–115.0%

IMPURITIES
• **LIMIT OF ESTRADIOL**
Standard solution: A solution of estradiol in acetone containing 30.0% of the labeled concentration of the Injection. Dilute 1.0 mL of this solution with oil labeled as vehicle for Injection to 10.0 mL.
Sample solution: Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 5 µL
Developing solvent system: Cyclohexane and ethyl acetate (7:3)
Spray reagent: A 3-in-10 solution of methanol in sulfuric acid, prepared as follows. To 30 mL of methanol in a 100-mL volumetric flask in an ice bath, cautiously add sulfuric acid to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Apply the samples at spots 2.5 cm from the bottom edge of the plate. Allow the applications to be absorbed by the layer without air-drying. Develop the chromatogram in an unlined chamber until the solvent front has moved about 15 cm above the point of application. Remove the plate, dry at 90° for 30 min, and spray the plate lightly with *Spray reagent*. Heat the plate at 90° for 30 min.
Acceptance criteria: NMT 3.0%; any spot from the *Sample solution* close to the origin and corresponding to the estradiol spot is not larger or more intense than that from the *Standard solution*.

SPECIFIC TESTS
• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose, light-resistant containers, preferably of Type I or Type III glass.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Estradiol Valerate RS

Estriol



$C_{18}H_{24}O_3$ 288.38

www.webofpharma.com

Estra-1,3,5(10)-triene-3,16,17-triol, (16α,17β)-;
Estriol [50-27-1].

DEFINITION

Estriol contains NLT 97.0% and NMT 102.0% of estriol ($C_{18}H_{24}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K.**▲ (CN 1-May-2020) Meets the requirements

Change to read:

• **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**

▲**Solution A:** Water
Solution B: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	65	35
3.0	65	35
14.0	56	44
17.0	10	90
17.1	65	35
20.0	65	35

Diluent: Methanol and water (1:1)
System suitability solution: 100 µg/mL of USP Estriol RS and 5 µg/mL of USP Estriol Related Compound A RS in *Diluent*. Sonicate to dissolve as needed.
Standard solution: 100 µg/mL of USP Estriol RS in *Diluent*. Sonicate to dissolve as needed.
Sample solution: 100 µg/mL of Estriol in *Diluent*. Sonicate to dissolve as needed.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 2.6-µm packing L1
Column temperature: 32°
Flow rate: 1.0 mL/min
Injection volume: 25 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.2 between estriol related compound A and estriol, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of estriol ($C_{18}H_{24}O_3$) in the portion of Estriol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Estriol RS in the *Standard solution* (µg/mL)
C_U = concentration of Estriol in the *Sample solution* (µg/mL)▲ (USP 1-May-2019)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

• **ORGANIC IMPURITIES**

▲**Solution A, Solution B, Diluent,** and **Chromatographic system:** Proceed as directed in the *Assay*.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	65	35
3.0	65	35
40.0	35	65
41.0	65	35
45.0	65	35

System suitability stock solution A: 200 µg/mL of USP Estriol RS prepared as follows. Transfer a suitable quantity of USP Estriol RS to an appropriate volumetric flask. Add 50% of the total flask volume of methanol to dissolve, and sonicate to fully dissolve the solids. Dilute with water to volume and mix well.
System suitability stock solution B: 100 µg/mL each of USP Estriol Related Compound A RS, USP Estrone RS, and USP Estradiol RS prepared as follows. Transfer a suitable quantity of each Reference Standard to an appropriate volumetric flask. Add 80% of the total flask volume of methanol to dissolve, and sonicate to fully dissolve the solids. Dilute with methanol to volume and mix well.
System suitability solution: 100 µg/mL of USP Estriol RS and 5 µg/mL each of USP Estriol Related Compound A RS, USP Estrone RS, and USP Estradiol RS prepared as follows. Transfer 10-mL of *System suitability stock solution A* into a 20-mL volumetric flask. Add 1 mL of *System suitability stock solution B* and 1 mL of water. Dilute with *Diluent* to volume and mix well. Sonicate to dissolve as needed.
Standard solution: 0.4 µg/mL of USP Estriol RS and 2 µg/mL each of USP Estriol Related Compound A RS, USP Estrone RS, and USP Estradiol RS in *Diluent*. Sonicate to dissolve as needed.
Sample solution: 400 µg/mL of Estriol prepared as follows. Transfer a suitable quantity of Estriol to an appropriate volumetric flask. Add 50% of the total flask volume of methanol to dissolve, and sonicate to fully dissolve the solids. Dilute with water to volume and mix well.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 3* for the relative retention times.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 1.2 between estriol related compound A and estriol; NLT 1.2 between estrone and estradiol, *System suitability solution*
Relative standard deviation: NMT 10.0%; estriol, estriol related compound A, estrone, and estradiol, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of estriol related compound A, estrone, and estradiol in the portion of Estriol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of estriol related compound A, estrone, or estradiol from the *Sample solution*
r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (µg/mL)
C_U = concentration of Estriol in the *Sample solution* (µg/mL)

Calculate the percentage of any individual impurity in the portion of Estriol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of estriol from the *Standard solution*
C_S = concentration of USP Estriol RS in the *Standard solution* (µg/mL)
C_U = concentration of Estriol in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
16β,17α-Estriol[a]	0.94	0.5
Estriol related compound A	0.97	0.5
Estriol	1.00	—
16β-Estriol[b]	1.53	0.5
17α-Estriol[c]	1.60	0.5
Estrone	2.30	0.5
Estradiol	2.34	0.5
3-*O*-Methyl estriol[d]	2.64	0.5
Any individual unspecified impurity	—	0.10
Total impurities[e]	—	1

[a] Estra-1,3,5(10)-triene-3,16β,17α-triol.
[b] Estra-1,3,5(10)-triene-3,16β,17β-triol.
[c] Estra-1,3,5(10)-triene-3,16α,17α-triol.
[d] 3-Methoxy-(16α,17β)-estra-1,3,5(10)-triene-16,17-diol.
[e] Total impurities exclude estriol related compound A. ▲(USP 1-May-2019)

SPECIFIC TESTS
• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 4 mg/mL, in dioxane
Acceptance criteria: +54° to +62°

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.5% of its weight

Delete the following:

▲• **COMPLETENESS OF SOLUTION**
Sample solution: 50 mg/mL in pyridine
Acceptance criteria: The solution is clear and free from undissolved solid. ▲(USP 1-May-2019)

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲USP Estradiol RS ▲(USP 1-May-2019)
USP Estriol RS
▲USP Estriol Related Compound A RS
Estra-1,3,5(10),9(11)-tetraene-3,16α,17β-triol.
$C_{18}H_{22}O_3$ 286.37
USP Estrone RS ▲(USP 1-May-2019)

Add the following:

▲Estriol Compounded Vaginal Cream

DEFINITION
Estriol Compounded Vaginal Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of estriol ($C_{18}H_{24}O_3$). Prepare Estriol Compounded Vaginal Cream, 0.5 mg/g (0.05%), as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Estriol	50 mg
Propylene Glycol	5 mL
Emollient Cream Base,[a] a sufficient quantity to make	100 g

[a] PCCA, Houston, TX.

Wet the *Estriol* with the *Propylene Glycol* in a suitable container and mix until smooth and homogenous. Add the *Emollient Cream Base* by geometric dilution until final weight is achieved. Mix after each addition of the *Emollient Cream Base*.

ASSAY
• **PROCEDURE**
Solution A: 25 mM monobasic potassium phosphate
Solution B: Acetonitrile and methanol (50:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
30	40	60
31	80	20
35	80	20

USP Monographs

www.webofpharma.com

Diluent: Methanol and water (80:20)
Standard stock solution: Transfer about 20 mg of USP Estriol RS to a 250-mL volumetric flask and dilute with *Diluent* to final volume.
Standard solution: 0.005 mg/mL of estriol prepared from *Standard stock solution* and *Diluent*
Sample solution: Weigh about 3 mL of Vaginal Cream and transfer into a 100-mL volumetric flask, add about 5 mL of chloroform, and sonicate until the cream is broken down. Dilute with *Diluent* to final volume and pass through a filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 3-µm packing L11
Column temperature: 60°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for estriol is about 17.2 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of estriol ($C_{18}H_{24}O_3$) in the portion of Vaginal Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of estriol from the *Sample solution*
r_S = peak response of estriol from the *Standard solution*
C_S = concentration of USP Estriol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of estriol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in a tight, light-resistant suitable calibrated dispenser. Store at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature
- **LABELING:** Label it to indicate that it is for vaginal use only, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Estriol RS▲ (USP 1-Aug-2019)

Conjugated Estrogens

DEFINITION

Conjugated Estrogens is a mixture of sodium estrone sulfate and sodium equilin sulfate, derived wholly or in part from equine urine or synthetically from estrone and equilin. It contains other conjugated estrogenic substances of the type excreted by pregnant mares. It is a dispersion of the estrogenic substances on a suitable powdered diluent.

Conjugated Estrogens contains NLT 52.5% and NMT 61.5% of sodium estrone sulfate and NLT 22.5% and NMT 30.5% of sodium equilin sulfate, and the total of sodium estrone sulfate and sodium equilin sulfate is NLT 79.5% and NMT 88.0% of the labeled content of Conjugated Estrogens. Conjugated Estrogens contains concomitant components as sodium sulfate conjugates NLT 13.5% and NMT 19.5% of 17α-dihydroequilin, NLT 2.5% and NMT 9.5% of 17α-estradiol, and NLT 0.5% and NMT 4.0% of 17β-dihydroequilin of the labeled content of Conjugated Estrogens.

IDENTIFICATION

- **A.** The relative retention times of the 17α-dihydroequilin peak, the estrone peak, and the equilin peak from the *Sample solution* correspond to those from the *Standard solution*, as obtained in the *Assay*.
- **B.** The chromatogram of Conjugated Estrogens from the *Sample solution* in the *Assay* exhibits additional peaks or shoulders, corresponding to 17α-estradiol and 17β-dihydroequilin at retention times of about 0.24 and 0.35, relative to that of 3-*O*-methylestrone.

ASSAY

- **PROCEDURE**

Buffer: Sodium acetate TS, 1 N acetic acid, and water (79:21:400). Adjust with 1 N acetic acid or sodium acetate TS to a pH of 5.2 ± 0.1, if necessary.
Internal standard solution: 150 µg/mL of 3-*O*-methylestrone in methanol
Standard stock solution: 160 µg/mL, 70 µg/mL, and 50 µg/mL each of USP Estrone RS, USP Equilin RS, and USP 17α-Dihydroequilin RS, respectively, in alcohol
Standard solution: Pipet 1.0 mL of the *Internal standard solution* and 1.0 mL of the *Standard stock solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Evaporate the mixture with the aid of a stream of nitrogen to dryness, maintaining the temperature below 50°. To the dry residue, add 15 µL of dried pyridine and 65 µL of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. Immediately cover the tube tightly, mix, and allow to stand for 15 min. Add 0.5 mL of toluene, and mix.
System suitability solution: Pipet 1.0 mL of a 2.0-µg/mL solution of USP Estradiol RS (17β-estradiol) in alcohol, 1.0 mL of *Internal standard solution*, and 1.0 mL of *Standard stock solution* into a centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution*, beginning with "Evaporate the mixture...".
Sample solution: Transfer a quantity of Conjugated Estrogens, equivalent to 2 mg of total conjugated estrogens, to a 50-mL centrifuge tube, fitted with a polytef-lined screw cap, containing 15 mL of *Buffer* and 1 g of barium chloride. Cap the tube tightly, and shake for 30 min. If necessary, adjust the solution with 1 N acetic acid or sodium acetate to a pH of 5.0 ± 0.5. Sonicate for 30 s, then shake for an additional 30 min. Add a suitable sulfatase enzyme solution equivalent to 2500 Units, and shake for 20 min in a water bath maintained at 50°. Add 15.0 mL of ethylene dichloride to the warm mixture, cap the tube again, and shake by mechanical means for 15 min. Centrifuge for 10 min or until the lower layer is clear. Transfer as much of the organic phase as possible, and dry by rapidly passing through a filter consisting of a pledget of dry glass wool and 5 g of anhydrous sodium sulfate in a small funnel. Protect from loss by evaporation. Transfer 3.0 mL of the solution to a suitable centrifuge tube fitted with a tight screw cap or stopper. Add 1.0 mL of *Internal standard solution*. Proceed as directed for *Standard solution*, beginning with "Evaporate the mixture...".

USP Monographs

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 15-m fused silica capillary column, bonded with a 0.25-µm layer of phase G19
Temperature
Column: 208°
Injector port: 260°
Detector: 260°
Carrier gas: Hydrogen
Flow rate: 2 mL/min
Injection mode: Split
Split flow rate: 40–60 mL/min
Injection size: 1 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—Adjust the operating conditions as necessary to maintain the elution time of the 3-*O*-methylestrone peak between 17 and 25 min.]
[NOTE—The relative retention times for 17β-estradiol, 17α-dihydroequilin, estrone, equilin, and 3-*O*-methylestrone are 0.29, 0.30, 0.80, 0.87, and 1.00, respectively.]
Suitability requirements
Resolution: NLT 1.2 between estrone and equilin, *System suitability solution*
Tailing factor: NMT 1.3 for the estrone peak, *System suitability solution*
Relative standard deviation: NMT 2.0% for the estrone peak ratios for NLT four injections of the *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of sodium estrone sulfate and sodium equilin sulfate in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

R_U = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Sample solution*
R_S = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Standard solution*
C_S = concentration of USP Estrone RS or USP Equilin RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)
F = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

Acceptance criteria: 52.5%–61.5% of sodium estrone sulfate and 22.5%–30.5% of sodium equilin sulfate

OTHER COMPONENTS

• **CONTENT OF 17α-DIHYDROEQUILIN, 17β-DIHYDROEQUILIN, AND 17α-ESTRADIOL** (concomitant components)
Buffer, Internal standard solution, Standard stock solution, Standard solution, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for 17α-estradiol, 17α-dihydroequilin, and 17β-dihydroequilin are about 0.82, 1.00, and 1.11, respectively.]
Identify the peaks for 17α-estradiol, 17α-dihydroequilin, and 17β-dihydroequilin from the *Sample solution*.
Calculate the percentages of 17α-estradiol, 17α-dihydroequilin, and 17β-dihydroequilin as their sodium sulfate salts in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

R_U = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Sample solution*
R_S = ratio of the peak response of 17α-dihydroequilin to that of the internal standard from the *Standard solution*
C_S = concentration of USP 17α-Dihydroequilin RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)
F = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

Acceptance criteria: NLT 13.5% and NMT 19.5% of 17α-dihydroequilin, NLT 2.5% and NMT 9.5% of 17α-estradiol, and NLT 0.5% and NMT 4.0% of 17β-dihydroequilin, as their sodium sulfate conjugates

IMPURITIES

• **LIMITS OF 17α-DIHYDROEQUILENIN, 17β-DIHYDROEQUILENIN, AND EQUILENIN** (signal impurities)
Buffer, Internal standard solution, Standard stock solution, Standard solution, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for dihydroequilenin, 17β-dihydroequilenin, 3-*O*-methylestrone, and equilenin are 0.56, 0.64, 1.0, and 1.3, respectively.]
Identify any peaks for dihydroequilenin, 17β-dihydroequilenin, 3-*O*-methylestrone, and equilenin from the *Sample solution*. Calculate the percentages of 17α-dihydroequilenin, 17β-dihydroequilenin, and equilenin as their sodium sulfate salts in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

R_U = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Sample solution*
R_S = ratio of the peak response of estrone to that of the internal standard from the *Standard solution*
C_S = concentration of USP Estrone RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)
F = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

Acceptance criteria: NMT 3.25%, NMT 2.75%, and NMT 5.5% of the labeled content of Conjugated Estrogens for 17α-dihydroequilenin, 17β-dihydroequilenin, and equilenin, respectively, as their sodium sulfate salts

• **LIMITS OF 17β-ESTRADIOL AND $\Delta^{8,9}$-DEHYDROESTRONE**
Buffer, Internal standard solution, Standard stock solution, Standard solution, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

[NOTE—The relative retention times of 17β-estradiol, 3-*O*-methylestrone, and $\Delta^{8,9}$-dehydroestrone are about 0.29, 1.0, and 0.9, respectively.]

Identify any peaks for 17β-estradiol, 3-*O*-methylestrone, and $\Delta^{8,9}$-dehydroestrone from the *Sample solution*.

Calculate the percentages of 17β-estradiol and $\Delta^{8,9}$-dehydroestrone as their sodium sulfate salts in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

R_U = ratio of the peak response of the appropriate analyte to that of the internal standard from the *Sample solution*

R_S = ratio of the peak response of estrone to that of the internal standard from the *Standard solution*

C_S = concentration of USP Estrone RS in the *Standard solution* (µg/mL)

C_U = concentration of the *Sample solution* (µg/mL)

F = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

Acceptance criteria: NMT 2.25% and NMT 6.25% of the labeled content of Conjugated Estrogens for 17β-estradiol and $\Delta^{8,9}$-dehydroestrone, respectively, as their sodium sulfate salts

- **LIMIT OF ESTRONE, EQUILIN, AND 17α-DIHYDROEQUILIN** (free steroids)

Buffer, Internal standard solution, Standard stock solution, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Free steroids standard solution: Dilute the *Standard stock solution* tenfold. Pipet 1.0 mL of the resulting solution and 1.0 mL of the *Internal standard solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution* in the *Assay*, beginning with "Evaporate the mixture...".

Sample solution: Proceed as directed for *Sample solution* in the *Assay* with the following exceptions: do not add the sulfatase enzyme solution, and transfer 6.0 mL of the solution, instead of 3.0 mL, to the centrifuge tube.

Blank solution: Prepare a reagent blank in the same manner as the *Sample solution*.

System suitability: Proceed as directed in the *Assay* with the additional requirement that the relative standard deviation for the ratio of the peak response of estrone to that of the internal standard in the *Free steroids standard solution* is NMT 5.5%, from NLT two replicate injections.

Analysis

Samples: *Free steroids standard solution* and *Sample solution*

Calculate the total percentage of estrone, equilin and 17α-dihydroequilin (free steroids) in the portion of Conjugated Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the sum of the estrone, equilin, and 17α-dihydroequilin peak areas (corrected for any peaks found in the *Blank solution*) to the internal standard peak area from the *Sample solution*

R_S = ratio of the estrone peak area to the internal standard peak area from the *Standard solution*

C_S = concentration of USP Estrone RS in the *Standard solution* (µg/mL)

C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria: NMT 1.3% of free steroids

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature, or under refrigeration.
- **LABELING:** Label it to state the content of Conjugated Estrogens on a weight-to-weight basis.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP 17α-Dihydroequilin RS
Estra-1,3,5(10),7-tetraene-3,17α-diol.
$C_{18}H_{22}O_2$ 270.37
USP Equilin RS
USP Estradiol RS
USP Estrone RS

Conjugated Estrogens Tablets

DEFINITION

Conjugated Estrogens Tablets contain NLT 73.0% and NMT 95.0% of the labeled amount of conjugated estrogens as the total of sodium estrone sulfate and sodium equilin sulfate. The ratio of sodium equilin sulfate to sodium estrone sulfate in the Tablets is NLT 0.35 and NMT 0.65.

IDENTIFICATION

- **A.** The relative retention times of the 17α-dihydroequilin peak, the estrone peak, and the equilin peak from the *Sample solution* correspond to those from the *Standard solution*, as obtained in the *Assay*.
- **B.** The chromatogram of the *Sample solution* in the *Assay* exhibits additional peaks or shoulders, corresponding to 17α-estradiol and 17β-dihydroequilin at retention times of about 0.24 and 0.35, relative to that of 3-*O*-methylestrone.

ASSAY

- **PROCEDURE**

Buffer: Sodium acetate TS, 1 N acetic acid, and water (79:21:400). Adjust with 1 N acetic acid or sodium acetate TS to a pH of 5.2 ± 0.1, if necessary.

Internal standard solution: 150 µg/mL of 3-*O*-methylestrone in methanol

Standard stock solution: 160, 70, and 50 µg/mL each of USP Estrone RS, USP Equilin RS, and USP 17α-Dihydroequilin RS, respectively, in alcohol

Standard solution: Pipet 1.0 mL of the *Internal standard solution* and 1.0 mL of the *Standard stock solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Evaporate the mixture with the aid of a stream of nitrogen to dryness, maintaining the temperature below 50°. To the dry residue add 15 µL of dried pyridine and 65 µL of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. Immediately cover the tube tightly, mix, and allow to stand for 15 min. Add 0.5 mL of toluene, and mix.

System suitability solution: Pipet 1.0 mL of a 2.0-µg/mL solution of USP Estradiol RS (17β-estradiol) in alcohol, 1.0 mL of *Internal standard solution*, and 1.0 mL of *Standard stock solution* into a centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for the *Standard solution*, beginning with "Evaporate the mixture...".

Sample solution: If the Tablets are sugar coated, carefully remove the color and sugar coatings with water, leaving the shellac coating intact, and dry under nitrogen. Transfer an equivalent to 2 mg of total conjugated estrogens from NLT 20 finely powdered Tablets, to a 50-mL centrifuge tube fitted with a polytef-lined screw-cap and containing 15 mL of *Buffer* and 1 g of barium chloride. Cap the tube tightly, and shake for 30 min. If necessary, adjust the

USP Monographs

www.webofpharma.com

solution with 1 N acetic acid or sodium acetate to a pH of 5.0 ± 0.5. Sonicate for 30 s, then shake for an additional 30 min. Add a suitable sulfatase enzyme solution equivalent to 2500 Units, and shake for 20 min in a water bath maintained at 50°. Add 15.0 mL of ethylene dichloride to the warm mixture, cap the tube again, and shake by mechanical means for 15 min. Centrifuge for 10 min or until the lower layer is clear. Transfer as much of the organic phase as possible, and dry by rapidly passing through a filter consisting of a pledget of dry glass wool and 5 g of anhydrous sodium sulfate in a small funnel. Protect from loss by evaporation. Transfer 3.0 mL of the solution to a suitable centrifuge tube fitted with a tight screw cap or stopper. Add 1.0 mL of *Internal standard solution*. Proceed as directed for the *Standard solution*, beginning with "Evaporate the mixture...".

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 15-m fused silica capillary; bonded with a 0.25-µm layer of phase G19
Temperatures
Column: 208°
Detector: 260°
Injection port: 260°
Carrier gas: Hydrogen
Flow rate: 2 mL/min
Injection mode: Split
Split flow rate: 40–60 mL/min
Injection volume: 1 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—Adjust the operating conditions as necessary to maintain the elution time of the 3-*O*-methylestrone peak at 17–25 min.]
[NOTE—The relative retention times for 17β-estradiol, 17α-dihydroequilin, estrone, equilin, and 3-*O*-methylestrone are about 0.29, 0.30, 0.80, 0.87, and 1.00, respectively.]
Suitability requirements
Resolution: NLT 1.2 between estrone and equilin, *System suitability solution*
Tailing factor: NMT 1.3 for the estrone peak, *System suitability solution*
Relative standard deviation: NMT 2.0% of the estrone peak ratios for NLT four injections of the *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of sodium estrone sulfate and sodium equilin sulfate in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

R_U = peak response ratio of the appropriate analyte to that of the internal standard from the *Sample solution*
R_S = peak response ratio of the appropriate analyte to that of the internal standard from the *Standard solution*
C_S = concentration of USP Estrone RS or USP Equilin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of estrone or equilin in the *Sample solution* (µg/mL)
F = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

Acceptance criteria: 73.0%–95.0% of the labeled amount of conjugated estrogens as the total of sodium estrone sulfate and sodium equilin sulfate. The ratio of sodium equilin sulfate to sodium estrone sulfate is NLT 0.35 and NMT 0.65.

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩**:** Proceed as directed for *Extended-Release* articles.
Test 1 (for products labeled as 0.3-, 0.45-, and 0.625-mg tablets)**:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Times: 2, 5, and 8 h
Buffer: 0.025 M monobasic potassium phosphate
Mobile phase: Acetonitrile and *Buffer* (1:3)
Standard solution: Transfer 10 Tablets to a 1000-mL volumetric flask, dilute with water to volume, and stir vigorously by mechanical means for at least 3 h. Pipet a filtered 100-mL aliquot of the solution into a 900-mL volumetric flask, and dilute with water to volume.
Sample solution: Filter a portion of the solution under test. It is recommended that the filters selected be tested for binding affinity.
Chromatographic system
(see *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 3.0-cm; 3-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20–200 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for equilin sulfate and estrone sulfate are about 0.9 and 1.0, respectively. The estrone sulfate peak is the last major peak in the chromatogram. If estrone is present, it may be retained on the column for a period longer than 50 min and interfere in later chromatographic runs.]
Suitability requirements
Resolution: NLT 1.5 between equilin sulfate and estrone sulfate
Relative standard deviation: NMT 1.5% for the estrone sulfate peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of estrone sodium sulfate dissolved:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = estrone sulfate peak response from the *Sample solution*
r_S = estrone sulfate peak response from the *Standard solution*

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
2	19–49
5	66–96
8	NLT 80

USP Monographs

www.webofpharma.com

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 2 (for products labeled as 0.9-mg tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Apparatus, Times, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed for *Test 1*.

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
2	12–37
5	57–85
8	NLT 80

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 3 (for products labeled as 1.25- and 2.50-mg tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium, Apparatus, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed for *Test 1*.

Times: 2, 5, 8, and 12 h

Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved (%)
2	3–22
5	37–67
8	66–96
12	NLT 80

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 4 (for products labeled as 1.25-mg tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: Acetate buffer, pH 4.5; 900 mL

Apparatus 2: 50 rpm, with sinkers

Times: 2, 4, 8, and 12 h

Buffer: 0.025 M monobasic potassium phosphate

Mobile phase: Acetonitrile and *Buffer* (22:78)

Standard solution: Weigh 20 Tablets and determine the average tablet weight. Grind the Tablets to a uniform fine powder. Weigh a portion of the powdered Tablets equivalent to the average tablet weight, transfer to a 900-mL volumetric flask, and dilute with *Medium* to volume. Stir vigorously by mechanical means for at least 2 h or until the dissolution of the powder is complete. Pass a portion of the extract through a suitable filter of 10-µm pore size.

Sample solution: Pass a portion of the solution under test through a suitable filter of 10-µm pore size. It is recommended that the filters selected be tested for binding affinity.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.2-mm × 5.0-cm; 5-µm packing L1

Flow rate: 0.8 mL/min

Injection volume: 20–200 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for equilin sulfate and estrone sulfate are about 0.9 and 1.0, respectively. The estrone sulfate peak is the last major peak in the chromatogram. If estrone is present, it may be retained on the column for a period longer than 50 min and interfere in later chromatographic runs.]

Suitability requirements

Resolution: NLT 1.2 between equilin sulfate and estrone sulfate

Relative standard deviation: NMT 2.0% for the estrone sulfate peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of estrone sulfate dissolved:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = estrone sulfate peak response from the *Sample solution*

r_S = estrone sulfate peak response from the *Standard solution*

Tolerances: See *Table 4*.

Table 4

Time (h)	Amount Dissolved (%)
2	11–31
4	43–63
8	75–95
12	NLT 87

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 5 (for products labeled as 0.3-, 0.45-, and 0.625-mg tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium, Apparatus, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed for *Test 4*.

Times: 1, 3, and 8 h

Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved (%)
1	6–26
3	48–68
8	NLT 87

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

www.webofpharma.com

Test 6 (for products labeled as 0.9-mg tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium, Apparatus, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed for *Test 4*.
Times: 1, 3, and 8 h
Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Dissolved (%)
1	3–23
3	41–61
8	NLT 80

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

• **UNIFORMITY OF DOSAGE UNITS**
Analysis: Assay 10 individual Tablets as directed in the *Assay*, and calculate the average content of conjugated estrogens, as the average of the total contents of sodium estrone sulfate and sodium equilin sulfate, in the 10 Tablets.
Acceptance criteria: The requirements are met if the content of each of the Tablets is 85.0%–115.0% of the average content of conjugated estrogens. If the content of NMT 2 Tablets falls outside the range of 85.0%–115.0% of the average content but not outside the range of 75.0%–125.0%, assay an additional 20 Tablets. The requirements are met if the content of NMT 2 of the 30 Tablets falls outside the limits of 85.0%–115.0% of that average, and no unit is outside the range of 75.0%–125.0% of the average content.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** The labeling indicates the Tablet strength and states with which in vitro *Dissolution Test* the product complies.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP 17α-Dihydroequilin RS
Estra-1,3,5(10),7-tetraene-3,17α-diol.
$C_{18}H_{22}O_2$ 270.37
USP Equilin RS
USP Estradiol RS
USP Estrone RS

Esterified Estrogens

DEFINITION

Esterified Estrogens is a mixture of the sodium salts of the sulfate esters of the estrogenic substances, principally estrone. It is a dispersion of the estrogenic substances on a suitable powdered diluent. The content of total esterified estrogens is NLT 90.0% and NMT 110.0% of the labeled amount.
Esterified Estrogens contains NLT 75.0% and NMT 85.0% of sodium estrone sulfate, and NLT 6.0% and NMT 15.0% of sodium equilin sulfate, in such proportion that the total of these two components is NLT 90.0%, of the labeled amount of esterified estrogens.

IDENTIFICATION

• **A.** The *Sample solution* exhibits peaks for estrone and equilin at relative retention times corresponding to those from the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Internal standard solution: 150 µg/mL of 3-*O*-methylestrone in methanol
Standard stock solution: 160 and 70 µg/mL each of USP Estrone RS and USP Equilin RS in alcohol
Standard solution: Pipet 1.0 mL of the *Standard stock solution* and 1.0 mL of *Internal standard solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Evaporate the mixture with the aid of a stream of nitrogen to dryness, maintaining the temperature below 50°. To the dry residue add 15 µL of dried pyridine and 65 µL of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. Immediately cover the tube tightly, and allow to stand for 15 min. Add 0.5 mL of toluene.
System suitability stock solution: 2 µg/mL of USP Estradiol RS (17β-estradiol) in alcohol
System suitability solution: Pipet 1.0 mL of *System suitability stock solution*, 1.0 mL of *Standard stock solution*, and 1.0 mL of *Internal standard solution* into a centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution*, beginning with "Evaporate the mixture...".
Buffer: Mix 79 mL of sodium acetate TS with 21 mL of 1 N acetic acid, and dilute with water to 500 mL. If necessary, adjust the solution with 1 N acetic acid or sodium acetate TS to a pH of 5.2 ± 0.1.
Sample solution: Transfer the equivalent to 2 mg of total esterified estrogens from Esterified Estrogens, to a 50-mL centrifuge tube, fitted with a polytef-lined screw cap, containing 15 mL of *Buffer* and 1 g of barium chloride. Cap the tube tightly, and shake for 30 min. If necessary, adjust the solution with 1 N acetic acid or sodium acetate to a pH of 5.0 ± 0.5. Sonicate for 30 s, then shake for an additional 30 min. Add a suitable sulfatase enzyme solution equivalent to 2500 Units, and shake for 20 min in a water bath maintained at 50°. Add 15.0 mL of ethylene dichloride to the warm mixture, cap the tube again, and shake by mechanical means for 15 min. Centrifuge for 10 min or until the lower layer is clear. Transfer as much of the organic phase as possible, and dry by passing rapidly through a filter consisting of a pledget of dry glass wool and 5 g of anhydrous sodium sulfate in a small funnel. Protect from loss by evaporation. Transfer 3.0 mL of the solution to a suitable centrifuge tube fitted with a tight screw cap or stopper. Add 1.0 mL of *Internal standard solution*. Proceed as directed under *Standard solution*, beginning with "Evaporate the mixture...".
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 15-m fused silica capillary column; with a 0.25-µm layer of phase G19
Temperature
Column: 208°
Detector: 260°
Injector port: 260°
Carrier gas: Hydrogen
Flow rate: 2 mL/min
Injection mode: Split
Split flow rate: 40–60 mL/min
Injection size: 1 µL
System suitability
Samples: *Standard solution* and *System suitability solution*

www.webofpharma.com

[NOTE—Adjust the operating conditions as necessary to maintain the elution time of the 3-*O*-methylestrone peak at between 17 and 25 min.]
[NOTE—The relative retention times for 17β-estradiol, estrone, equilin, and 3-*O*-methylestrone are 0.29, 0.80, 0.87, and 1.00, respectively.]

Suitability requirements

Resolution: NLT 1.2 between estrone and equilin, *System suitability solution*

Tailing factor: NMT 1.3 for the estrone peak, *System suitability solution*

Relative standard deviation: NMT 2.0% for the estrone peak ratios for NLT 4 injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Separately calculate the percentage of the labeled amount of sodium estrone sulfate and sodium equilin sulfate in the portion of Esterified Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times 100$$

R_U = ratio of the estrone or equilin peak area to the 3-*O*-methylestrone peak area from the *Sample solution*

R_S = ratio of the estrone or equilin peak area to the 3-*O*-methylestrone peak area from the *Standard solution*

C_S = concentration of USP Estrone RS or USP Equilin RS in the *Standard solution* (µg/mL)

C_U = concentration of the *Sample solution* (µg/mL)

F = factor converting free estrogen to the conjugate ester sodium salt, 1.377 for estrone and 1.380 for equilin

Acceptance criteria: 90.0%–110.0% of the labeled amount of total esterified estrogens; 75.0%–85.0% of sodium estrone sulfate; 6.0%–15.0% of sodium equilin sulfate, in such proportion that the total of these two components is NLT 90.0%, of the labeled amount of esterified estrogens

IMPURITIES

• **FREE STEROIDS**

Internal standard solution, Buffer, Standard stock solution, Chromatographic system, and **System suitability solution:** Proceed as directed in the *Assay*.

Free steroids standard solution: Dilute the *Standard stock solution* tenfold. Pipet 1.0 mL of the resulting solution and 1.0 mL of the *Internal standard solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution* in the *Assay*, beginning with "Evaporate the mixture...".

Sample solution: Proceed as directed for *Sample solution* in the *Assay* with the following exceptions: do not add the sulfatase enzyme solution, and transfer 6.0 mL of the filtrate instead of 3.0 mL to the *Sample solution*.

Blank solution: Prepare a reagent blank in the same manner as the *Sample solution*.

System suitability: Proceed as directed in the *Assay* with the additional requirement that the relative standard deviation for the ratio of the peak response of estrone to that of the internal standard in the *Free steroids standard solution* is NMT 5.5%, on the basis of NLT two replicate injections.

Analysis

Samples: *Free steroids standard solution* and *Sample solution*

Calculate the total percentage of estrone and equilin (free stereoids) in the portion of Esterified Estrogens taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the sum of the estrone and equilin peak areas (corrected for any peaks found in the *Blank solution*) to the 3-*O*-methylestrone peak area from the *Sample solution*

R_S = ratio of the estrone peak area to the 3-*O*-methylestrone peak area from the *Free steroids standard solution*

C_S = concentration of USP Estrone RS in the *Free steroids standard solution* (µg/mL)

C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria: NMT 3.0% of free steroids

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label it to state the content of Esterified Estrogens on a weight-to-weight basis.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Equilin RS
USP Estradiol RS
USP Estrone RS

Esterified Estrogens Tablets

DEFINITION

Esterified Estrogens Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of esterified estrogens as the total of sodium estrone sulfate and sodium equilin sulfate. The ratio of sodium equilin sulfate to sodium estrone sulfate is NLT 0.071 and NMT 0.20.

IDENTIFICATION

• **A.** The *Sample solution* exhibits peaks for estrone and equilin at relative retention times corresponding to those from the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Internal standard solution: 150 µg/mL of 3-*O*-methylestrone in methanol

Standard stock solution: 160 and 70 µg/mL each of USP Estrone RS and USP Equilin RS in alcohol

Standard solution: Pipet 1.0 mL of the *Standard stock solution* and 1.0 mL of the *Internal standard solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Evaporate the mixture with the aid of a stream of nitrogen to dryness, maintaining the temperature below 50°. To the dry residue add 15 µL of dried pyridine and 65 µL of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. Immediately cover the tube tightly, and allow to stand for 15 min. Add 0.5 mL of toluene.

System suitability stock solution: 2 µg/mL of USP Estradiol RS (17β-estradiol) in alcohol

System suitability solution: Pipet 1.0 mL of *System suitability stock solution*, 1.0 mL of *Standard stock solution*, and 1.0 mL of *Internal standard solution* into a centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution*, beginning with "Evaporate the mixture...".

Buffer: Mix 79 mL of sodium acetate TS with 21 mL of 1 N acetic acid, and dilute with water to 500 mL. If necessary, adjust the solution with 1 N acetic acid or sodium acetate TS to a pH of 5.2 ± 0.1.

Sample solution: Transfer an equivalent to 2 mg of total esterified estrogens from powdered Tablets (NLT 20), to a 50-mL centrifuge tube, fitted with a polytef-lined screw cap, containing 15 mL of *Buffer* and 1 g of barium chloride. Cap the tube tightly, and shake for 30 min. If necessary,

www.webofpharma.com

adjust the solution with 1 N acetic acid or sodium acetate to a pH of 5.0 ± 0.5. Sonicate for 30 s, then shake for an additional 30 min. Add a suitable sulfatase enzyme solution equivalent to 2500 Units, and shake for 20 min in a water bath maintained at 50°. Add 15.0 mL of ethylene dichloride to the warm mixture, cap the tube again, and shake by mechanical means for 15 min. Centrifuge for 10 min or until the lower layer is clear. Transfer as much of the organic phase as possible, and dry by passing rapidly through a filter consisting of a pledget of dry glass wool and 5 g of anhydrous sodium sulfate in a small funnel. Protect from loss by evaporation. Transfer 3.0 mL of the solution to a suitable centrifuge tube fitted with a tight screw cap or stopper. Add 1.0 mL of *Internal standard solution*. Proceed as directed under *Standard solution*, beginning with "Evaporate the mixture...".

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 15-m fused silica capillary column; with a 0.25-µm layer of phase G19
Temperature
Column: 208°
Detector: 260°
Injector port: 260°
Carrier gas: Hydrogen
Flow rate: 2 mL/min
Injection mode: Split
Split flow rate: 40–60 mL/min
Injection size: 1 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—Adjust the operating conditions as necessary to maintain the elution time of the 3-*O*-methylestrone peak between 17 and 25 min.]
[NOTE—The relative retention times for 17β-estradiol, estrone, equilin, and 3-*O*-methylestrone are about 0.29, 0.80, 0.87, and 1.00, respectively.]
Suitability requirements
Resolution: NLT 1.2 between estrone and equilin, *System suitability solution*
Tailing factor: NMT 1.3 for the estrone peak, *System suitability solution*
Relative standard deviation: NMT 2.0% for the estrone peak ratios for NLT 4 injections, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Separately calculate the percentage of the labeled amount of sodium estrone sulfate and sodium equilin sulfate in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times F \times (100)$$

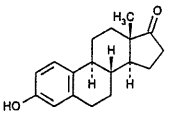
R_U = ratio of the estrone or equilin peak to the 3-*O*-methylestrone peak area from the *Sample solution*
R_S = ratio of the estrone or equilin peak to the 3-*O*-methylestrone peak area from the *Standard solution*
C_S = concentration of USP Estrone RS or USP Equilin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of estrone or equilin in the *Sample solution* (µg/mL)
F = factor converting free estrogen to the conjugate sodium salt, 1.377 for estrone and 1.380 for equilin

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **DISINTEGRATION** ⟨701⟩
Simulated intestinal fluid: Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, mix, add 190 mL of 0.2 N sodium hydroxide and 400 mL of water. Add 10.0 g of pancreatin, mix, and adjust the resulting solution with 0.2 N sodium hydroxide to a pH of 7.5 ± 0.1. Dilute with water to 1000 mL.
Analysis: Place 1 Tablet in each of the six tubes of the basket, and immerse the basket in water at 25 ± 0.5° for 5 min to remove the coating. Add a disk to each tube, and operate the apparatus using simulated gastric fluid TS, maintained at 37 ± 2°, as the immersion fluid. After 30 min in simulated gastric fluid TS, lift the basket from the fluid, and observe the Tablets.
Acceptance criteria: All the Tablets have disintegrated. If all the Tablets have not disintegrated completely, substitute *Simulated intestinal fluid*, maintained at 37 ± 2°, as the immersion fluid, and continue the test so that the total period of time, including previous exposure to water and simulated gastric fluid TS, does not exceed 90 min.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Analysis: Test 10 individual Tablets as directed in the *Assay*, and calculate the average content of esterified estrogens, as the average of the total contents of sodium estrone sulfate and sodium equilin sulfate, in the 10 Tablets.
Acceptance criteria: The requirements are met if the content of each of the Tablets is 85.0%–115.0% of the average content of esterified estrogens. If the content of NMT 2 Tablets falls outside the range of 85.0%–115.0% of the average content but not outside the range of 75.0%–125.0%, test an additional 20 Tablets. The requirements are met if the content of NMT 2 of the 30 Tablets falls outside the limits of 85.0%–115.0% of the average, and no unit is outside the range of 75.0%–125.0% of the average content.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Equilin RS
USP Estradiol RS
USP Estrone RS

Estrone



$C_{18}H_{22}O_2$ 270.37
Estra-1,3,5(10)-trien-17-one, 3-hydroxy-.
3-Hydroxyestra-1,3,5(10)-trien-17-one [53-16-7].

» Estrone contains not less than 97.0 percent and not more than 103.0 percent of $C_{18}H_{22}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Estrone RS

www.webofpharma.com

Clarity of solution—Add 100 mg to 100 mL of 1 N sodium hydroxide in a 125-mL conical flask, heat on a steam bath until solution is complete, then cool, and transfer to a 100-mL color-comparison tube: the solution is clear.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 50 µg per mL.
Medium: alcohol, heated on a steam bath and cooled to room temperature.
Specific rotation ⟨781S⟩: between +158° and +165°.
Test solution: 10 mg, previously dried, per mL, in dioxane.
Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.5%.
Limit of equilenin and equilin—Dissolve 10 mg in sufficient alcohol to make 50 mL. Transfer 5 mL of the solution to a small beaker. Add 5 mL of a buffer solution prepared by dissolving 2 mL of glacial acetic acid and 13.3 g of anhydrous sodium acetate in water to make 100 mL, warm to about 50°, and add 1 mL of a freshly prepared 1 in 200 solution of 2,6-dibromoquinone-chlorimide in alcohol. Mix, and allow to stand for 30 minutes. Transfer the solution to a small separator, add 10 mL of chloroform and 20 mL of 1 N sodium hydroxide, and shake vigorously for 2 minutes. Separate the chloroform layer, and filter rapidly through a dry filter paper into a dry test tube, discarding the first 2 mL of the filtrate. Viewed transversely against a white background, the chloroform filtrate shows no more red color than that produced by similarly treating 5 mL of an alcohol solution containing 20 µg of equilenin.

Ordinary impurities ⟨466⟩—
Test solution: acetone.
Standard solution: acetone.
Eluant: a mixture of chloroform and acetone (9:1), in a nonequilibrated chamber.
Visualization: 5.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and 0.05 M monobasic potassium phosphate (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer about 20 mg of USP Estrone RS, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. If necessary, sonicate to aid solution. Transfer 5 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 40 µg of USP Estrone RS per mL.
Assay preparation—Transfer about 20 mg of Estrone, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. If necessary, sonicate to aid solution. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 1500 theoretical plates, the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{22}O_2$ in the portion of Estrone taken by the formula:

$$0.5C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Estrone RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Estrone Injectable Suspension

» Estrone Injectable Suspension is a sterile suspension of Estrone in Water for Injection. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{18}H_{22}O_2$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Estrone RS
USP Progesterone RS
Identification—Transfer a volume of Injectable Suspension, equivalent to about 5 mg of estrone, to a glass-stoppered centrifuge tube, and add 2.5 mL of a mixture of ether and benzene (1:1). Shake for 2 minutes, and allow insoluble matter to settle, centrifuging, if necessary, to obtain a clear supernatant. Apply 5 µL each of this supernatant and a 1 in 500 solution of USP Estrone RS in a mixture of ether and benzene (1:1) to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of benzene and acetone (4:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of dehydrated alcohol and sulfuric acid (3:1), and heat in an oven at 105° for 10 minutes: the R_F value and appearance (pale orange to amber by direct observation in daylight, and fluorescing pale yellow-green under long-wavelength UV light) of the principal spot obtained from the test solution correspond to those obtained from the Standard solution.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 88.0 USP Endotoxin Units per mg of estrone.
Uniformity of dosage units ⟨905⟩: meets the requirements.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay*— under *Estrone.*
Assay preparation—Transfer an accurately measured volume of the well-mixed Injectable Suspension, equivalent to about 10 mg of estrone to a 50-mL volumetric flask. Add 30 mL of methanol and swirl for 5 minutes. Dilute with methanol

USP Monographs

www.webofpharma.com

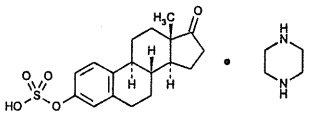
to volume, and mix. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Estrone*. Calculate the quantity, in mg, of $C_{18}H_{22}O_2$ in each mL of Injectable Suspension taken by the formula:

$$0.25(C/V)(r_U/r_S)$$

in which V is the volume, in mL, of the Injectable Suspension taken, and the other terms are as defined therein.

Estropipate



$C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$ 436.56

Estra-1,3,5(10)-trien-17-one, 3-(sulfooxy)-, compd. with piperazine (1:1).

Estrone hydrogen sulfate compound with piperazine (1:1) [7280-37-7].

» Estropipate contains not less than 97.0 percent and not more than 103.0 percent of $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Estrone RS
USP Estropipate RS

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Loss on drying ⟨731⟩—Dry it at 105° for 1 hour: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.5%.

Free estrone—

Stock impurity standard preparation—Weigh accurately 25.0 mg of USP Estrone RS into a 100-mL volumetric flask, dilute with spectrophotometric-grade methanol to volume, and sonicate to achieve complete solution.

Impurity standard preparation—Weigh accurately 25.0 mg of USP Estropipate RS into a 25-mL volumetric flask, add 2.0 mL of *Stock impurity standard preparation*, dilute with spectrophotometric-grade methanol to volume, and sonicate to achieve complete solution.

Standard preparation—Weigh accurately 25.0 mg of USP Estropipate RS into a 25-mL volumetric flask, dilute with spectrophotometric-grade methanol to volume, and sonicate to achieve complete solution.

Test preparation—Using a portion of Estropipate, accurately weighed, prepare as directed under *Standard preparation*.

Mobile phase—Mix 650 mL of 0.025 M potassium dihydrogen phosphate with 350 mL of spectrophotometric-grade acetonitrile. Filter the solution through a membrane filter having a porosity of 1 µm or less, and degas at a pressure of less than 100 mm of mercury until no further bubbles appear. The concentration of acetonitrile may be varied to meet system suitability requirements and to provide a suitable elution time for all components.

Chromatographic system—Typically, a high-pressure liquid chromatograph, operated at room temperature, is fitted with a 30-cm × 3.9-mm stainless steel column that contains packing L1. The mobile phase is maintained at a pressure and flow rate (approximately 1.5 mL per minute) capable of giving the required resolution (see *System suitability test*) and a suitable elution time. An UV detector that monitors absorption at a wavelength of 213 nm is used with a recorder adjusted such that approximately 0.04 absorbance unit gives a full-scale reading.

System suitability test—Chromatograph two injections of the *Impurity standard preparation*, and determine that after the injection front the small peak (estrone) after the major peak does not differ in peak response between the duplicate injections by more than 4%. Also determine that the small peak after the major component has a retention time relative to the major component of approximately 5.5. (For a particular column, resolution may be increased by decreasing the amount of acetonitrile in the *Mobile phase*.)

Procedure—Inject separately 5.0-µL portions of the *Standard preparation*, the *Impurity standard preparation*, and the *Test preparation* into the high-pressure liquid chromatograph by means of a suitable sampling valve or high-pressure microsyringe. Measure the peak responses for the estrone peak relative to the estropipate peak obtained with the *Standard preparation*, the *Impurity standard preparation*, and the *Test preparation*. Calculate the percentage of free estrone taken by the formula:

$$2.5(C/W)(H_U/H_S)$$

in which H_U and H_S are the measured peak heights of the impurity (estrone) in the *Test preparation* and the *Impurity standard preparation* corrected for the peak height of estrone in the *Standard preparation*, respectively, W is the weight, in mg, of estropipate in the *Test preparation*, and C is the concentration, in µg per mL, of USP Estrone RS in the *Impurity standard preparation*. Not more than 2.0% is found.

Assay—

Standard preparation—Prepare as directed under *Free estrone*.

Assay preparation—Prepare as directed for *Test preparation* under *Free estrone*.

Chromatographic system—Use the same system as in test for *Free estrone*. Adjust the recorder so that approximately 0.4 absorbance unit gives a full-scale reading.

System suitability test—Chromatograph two injections of the *Standard preparation*, and determine that only one major peak is observed after the injection front. The peak responses between the duplicate injections for the major peak do not differ by more than 3%.

Procedure—Inject 5.0 µL of the *Assay preparation* and the *Standard preparation* into the high-pressure liquid chromatograph by means of a suitable sampling valve or high-pressure microsyringe. Measure the peak heights for the respective estropipate peak (it is actually an estrone sulfate peak) obtained with the *Assay preparation* and the *Standard preparation*. Calculate the quantity, in mg, of $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$ in the portion of Estropipate taken by the formula:

$$25C(H_U/H_S)$$

in which H_U and H_S are the peak heights obtained with the *Assay preparation* and the *Standard preparation*, respectively, and C is the concentration, in mg per mL, of USP Estropipate RS in the *Standard preparation*.

www.webofpharma.com

Estropipate Vaginal Cream

» Estropipate Vaginal Cream contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of estropipate ($C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$), in a suitable cream base.

Packaging and storage—Preserve in collapsible tubes.
USP Reference standards ⟨11⟩—
USP Estropipate RS

Identification—
Arsenomolybdate spray reagent—Dissolve 25 g of ammonium molybdate in 450 mL of water, add 21 mL of sulfuric acid, and mix. Add 3 g of dibasic sodium arsenate heptahydrate dissolved in 25 mL of water, mix, and incubate at 37 ± 2° for 24 to 48 hours. Store, protected from light.
Procedure—Transfer a portion of Vaginal Cream, equivalent to about 8 mg of estropipate, to a container. Add 20 mL of methanol, stir to obtain a homogeneous mixture, and pass through filter paper. Apply 30 µL of the filtrate and 30 µL of a Standard solution of USP Estropipate RS in methanol containing about 400 µg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and methanol (10:8) until the solvent front has moved about 14 cm from the origin. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots by lightly spraying with *Arsenomolybdate spray reagent* and by heating at 105° for about 20 minutes: the R_F values of the principal spots obtained from the test solution correspond to those obtained from the Standard solution.
Minimum fill ⟨755⟩: meets the requirements.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of 0.1 M monobasic potassium phosphate, methanol, and acetonitrile (215:59:59). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluent—Prepare a mixture of water and methanol (1:1).
Resolution solution—Dissolve a suitable quantity of 4′-nitroacetophenone in methanol to obtain a solution having a known concentration of about 1.0 mg per mL. Transfer 5.0 mL of the solution so obtained to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Estropipate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 1.0 mg per mL. Transfer 5.0 mL of the solution so obtained to a 100-mL volumetric flask, dilute with 0.05 M piperazine to volume, and mix. Transfer 5.0 mL of the solution so obtained to a 25-mL volumetric flask, add 5.0 mL of the *Resolution solution*, dilute with 0.05 M piperazine to volume, mix, and filter.
Assay preparation—Transfer an accurately weighed portion of Vaginal Cream, equivalent to about 1.5 mg of estropipate, to a 125-mL separator, add 50 mL of chloroform and 20 mL of 0.05 M piperazine, and extract for 3 minutes. Collect the organic extract in another separator, and extract the aqueous layer with an additional 20-mL portion of chloroform. Combine the organic phases, wash with a 20-mL portion of 0.05 M piperazine for 3 minutes, and collect the aqueous washings. Discard the organic layer. Rinse the separators with 0.05 M piperazine, and add the rinse to the aqueous washings. Transfer the aqueous extract and the aqueous washings to a 100-mL volumetric flask, dilute with 0.05 M piperazine to volume, and mix. Transfer 15.0 mL of the solution so obtained to a 25-mL volumetric flask, dilute with 0.05 M piperazine to volume, mix, and filter.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 213-nm detector and 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. [NOTE—The injection of a diluted solution of 4′-nitroacetophenone can be used to better identify the peak locus.] Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*:the relative retention times are about 0.8 for 4′-nitroacetophenone and 1.0 for estropipate; the resolution, *R*, between 4′-nitroacetophenone and estropipate is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for not less than 30 minutes for the *Standard preparation* and 90 minutes for the *Assay preparation*, and measure the responses for the major peaks. Calculate the quantity, in mg, of estropipate ($C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$) in the portion of Vaginal Cream taken by the formula:

$$166.67C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Estropipate RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Estropipate Tablets

» Estropipate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of estropipate ($C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Estropipate RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: 0.05 M pH 6.8 phosphate buffer; 900 mL.
Apparatus 2: 75 rpm.
Time: 30 minutes.
Determine the amount of $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$ dissolved by employing the following method.
Diluent and *Mobile phase*—Proceed as directed in the *Assay*.
System suitability solution—Transfer about 100 mg of 4′-nitroacetophenone, accurately weighed, to a 100-mL volumetric flask, and dissolve in methanol, sonicating if necessary. Dilute with methanol to volume, and mix. Pipet 2 mL of this solution into a 250-mL volumetric flask, dilute with water to volume, and mix.
Standard solution—Dissolve an accurately weighed quantity of USP Estropipate RS in *Diluent*, sonicating if necessary, and dilute quantitatively with *Diluent* to obtain a solution having a known concentration of about 1 mg per mL. Pipet 2 mL of this

www.webofpharma.com

solution into a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 4 mL of the solution so obtained into a second 100-mL volumetric flask, add 8.0 mL of the *System suitability solution*, dilute with water to volume, and mix.

Test solution—Transfer an accurately measured volume of a filtered portion of the solution under test, equivalent to about 20 µg of estropipate, to a 25-mL volumetric flask. Dilute with water to volume if the contents are less than the nominal volume of the flask, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay*. To evaluate the system suitability requirements, use the *Standard solution*.

Procedure—Separately inject equal volumes (about 300 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$ dissolved.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

Diluent, Mobile phase, System suitability solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay*.

Test preparation—Transfer 1 Tablet to a 50-mL volumetric flask, add 20 mL of water, insert the stopper, and shake by mechanical means for about 30 minutes or until the Tablet disintegrates. Add 20 mL of methanol, insert the stopper, and shake by mechanical means for about 60 minutes. Dilute with *Diluent* to volume, and mix. Transfer an accurately measured volume of this stock solution, equivalent to about 0.25 mg of estropipate, to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass this solution through a solvent-resistant membrane filter having a 1-µm or finer porosity, discarding the first portion of the filtrate.

Procedure—Proceed as directed in the *Assay*, except to use the *Test preparation* instead of the *Assay preparation*.

Assay—

Diluent—Prepare a mixture of methanol and water (1:1).

Mobile phase—Dissolve 13.6 g of monobasic potassium phosphate in 1000 mL of water, and mix. Mix 600 mL of the resulting solution with 200 mL of methanol, add 200 mL of acetonitrile with stirring, and mix. Filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Estropipate RS in *Diluent*, sonicating if necessary, and dilute quantitatively with *Diluent* to obtain a solution having a known concentration of about 1 mg per mL. Pipet 1.0 mL of this solution into a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

System suitability solution—Transfer about 100 mg of 4′-nitroacetophenone, accurately weighed, to a 100-mL volumetric flask, and dissolve in methanol, sonicating if necessary. Dilute with methanol to volume, and mix. Pipet 5 mL of this solution into a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pipet 5 mL of the solution so obtained into a 25-mL volumetric flask, dilute with *Standard preparation* to volume, and mix.

Assay preparation—Transfer 20 Tablets to a 1000-mL volumetric flask, add 200 mL of water, insert the stopper, and shake by mechanical means for about 30 minutes or until the Tablets disintegrate completely. Add 200 mL of methanol, insert the stopper, and shake by mechanical means for about 60 minutes. Dilute with *Diluent* to volume, and mix. Transfer an accurately measured volume of this stock solution, equivalent to about 0.25 mg of estropipate, to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass this solution through a solvent-resistant membrane filter having a 1-µm or finer porosity, discarding the first portion of the filtrate.

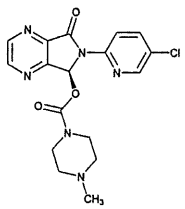
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 213-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.85 for 4′-nitroacetophenone and 1.0 for estropipate; the resolution, *R*, between estropipate and 4′-nitroacetophenone is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of estropipate ($C_{18}H_{22}O_5S \cdot C_4H_{10}N_2$) in each Tablet taken by the formula:

$$(25C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Estropipate RS in the *Standard preparation; V* is the volume, in mL, of the stock solution taken to prepare the *Assay preparation;* and r_U and r_S are the peak responses for estropipate obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Eszopiclone



$C_{17}H_{17}ClN_6O_3$ 388.81

1-Piperazinecarboxylic acid, 4-methyl-, 6-(5-chloro-2-pyridinyl)-6,7-dihydro-7-oxo-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl ester, (5*S*)-;

(+)-(5*S*)-6-(5-Chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl 4-methylpiperazine-1-carboxylate [138729-47-2].

DEFINITION

Eszopiclone contains NLT 98.5% and NMT 101.0% of eszopiclone ($C_{17}H_{17}ClN_6O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the second peak of the *System suitability solution*, as obtained in the *Limit of R-Isomer* test.

ASSAY

- **PROCEDURE**

Solution A: 1.4 g/L of anhydrous dibasic sodium phosphate in water

www.webofpharma.com

Mobile phase: Acetonitrile and *Solution A* (25:75) adjusted with phosphoric acid to a pH of 6.5
Standard stock solution: 1 mg/mL of USP Eszopiclone RS prepared as follows. Transfer a suitable quantity of USP Eszopiclone RS to an appropriate volumetric flask and add 15% of the final flask volume of acetonitrile. Add 15% of the final flask volume of *Mobile phase*. Sonication may be used to promote dissolution. Dilute with *Mobile phase* to volume.
Standard solution: 0.2 mg/mL of USP Eszopiclone RS from *Standard stock solution* in *Mobile phase*
Sample stock solution: 1 mg/mL of Eszopiclone prepared as follows. Transfer a suitable quantity of Eszopiclone to an appropriate volumetric flask and add 15% of the final flask volume of acetonitrile. Add 15% of the final flask volume of *Mobile phase*. Sonication may be used to promote dissolution. Dilute with *Mobile phase* to volume.
Sample solution: 0.2 mg/mL of Eszopiclone from *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 303 nm
Column: 4.6-mm × 15.0-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: NLT 2.5 times the retention time of eszopiclone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of eszopiclone ($C_{17}H_{17}ClN_6O_3$) in the portion of Eszopiclone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Eszopiclone RS in the *Standard solution* (mg/mL)
C_U = concentration of Eszopiclone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.10%
- **Organic Impurities**

Solution A: 8.1 g/L of sodium lauryl sulfate and 2.1 g/L of monobasic sodium phosphate in water
Mobile phase: Acetonitrile and *Solution A* (628:1000) adjusted with phosphoric acid to a pH of 4.0
System suitability solution: 0.04 mg/mL each of USP Eszopiclone Related Compound A RS and USP Eszopiclone RS in *Mobile phase*. Sonication may be used to promote dissolution.
Standard stock solution: 0.4 mg/mL of USP Eszopiclone RS in *Mobile phase*. Sonication may be used to promote dissolution.
Standard solution: 0.004 mg/mL of USP Eszopiclone RS from *Standard stock solution* in *Mobile phase*
Sample solution: 4 mg/mL of Eszopiclone in *Mobile phase*. Sonication may be used to promote dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: 2.7 times the retention time of eszopiclone
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between eszopiclone related compound A and eszopiclone, *System suitability solution*
Tailing factor: NMT 1.5 for eszopiclone, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Eszopiclone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of eszopiclone from the *Standard solution*
C_S = concentration of USP Eszopiclone RS in the *Standard solution* (mg/mL)
C_U = concentration of Eszopiclone in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard peaks below 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Zopiclone alcohol[a]	0.14	0.10
Eszopiclone related compound A	0.93	0.10
Eszopiclone	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.3

[a] 6-(5-Chloropyridin-2-yl)-7-hydroxy-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-one.

- **Limit of *R*-Isomer**

Mobile phase: 1 mL of diethylamine in 1000 mL of absolute alcohol. Sonication may be used to promote dissolution.
System suitability solution: 0.5 mg/mL of USP Eszopiclone RS and 0.1 mg/mL of USP Zopiclone R-Isomer RS prepared as follows. Transfer suitable amounts of USP Eszopiclone RS and USP Zopiclone R-Isomer RS to an appropriate volumetric flask. Add 20% of the flask volume of dichloromethane. Sonication may be used to promote dissolution. Dilute with absolute alcohol to volume.
Sample solution: 0.5 mg/mL of Eszopiclone prepared as follows. Transfer a suitable amount of Eszopiclone to an appropriate volumetric flask. Add 20% of the flask volume of dichloromethane. Sonication may be used to promote dissolution. Dilute with absolute alcohol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 303 nm
Column: 4.6-mm × 25.0-cm; 5-µm packing L40

www.webofpharma.com

Flow rate: 0.5 mL/min
Injection volume: 20 µL
Run time: NLT 1.3 times the retention time of eszopiclone
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times of zopiclone *R*-isomer and eszopiclone are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between zopiclone *R*-isomer and eszopiclone
Analysis
Sample: *Sample solution*
Calculate the percentage of zopiclone *R*-isomer in the portion of Eszopiclone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of zopiclone *R*-isomer from the *Sample solution*
r_T = sum of the peak responses from the *Sample solution*

Acceptance criteria: NMT 0.30%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° under vacuum for 3 h.
Acceptance criteria: NMT 0.50%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Eszopiclone RS
USP Eszopiclone Related Compound A RS
[NOTE—This material may be available in the free base or salt form.]
6-(5-Chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl 4-methylpiperazine-1-carboxylate 4-oxide.
$C_{17}H_{17}ClN_6O_4$ 404.81
6-(5-Chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl 4-methylpiperazine-1-carboxylate 4-oxide, 3-▲chlorobenzoate▲ (ERR 1-Jun-2018) salt (1:1).
$C_{17}H_{17}ClN_6O_4 \cdot$ ▲$C_7H_5ClO_2$▲ (ERR 1-Jun-2018) 561.38
USP Zopiclone R-Isomer RS
(*R*)-6-(5-Chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl 4-methylpiperazine-1-carboxylate.
$C_{17}H_{17}ClN_6O_3$ 388.81

Eszopiclone Tablets

DEFINITION
Eszopiclone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of eszopiclone ($C_{17}H_{17}ClN_6O_3$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Standard: USP Eszopiclone RS
Sample: Nominally 37.5 mg of eszopiclone from Tablets prepared as follows. Powder a number of Tablets, and mix the resulting powder. Transfer a portion of powder, equivalent to 37.5 mg of eszopiclone, to a suitable container, add 30 mL of *acetone*, and shake. Dilute with *acetone* to 50 mL and pass the resulting solution through a suitable filter. Evaporate the filtrate to dryness on a water bath and dry the residue in an oven at 60° for 2 h.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 1.4 g/L of anhydrous dibasic sodium phosphate in water
Mobile phase: *Acetonitrile* and *Solution A* (25:75) adjusted with dilute phosphoric acid to a pH of 6.5 ± 0.05
Standard stock solution: 0.5 mg/mL of USP Eszopiclone RS prepared as follows. Transfer a suitable quantity of USP Eszopiclone RS to an appropriate volumetric flask and add 50% of the final flask volume of acetonitrile. Sonication may be used to promote dissolution. Dilute with acetonitrile to volume.
Standard solution: 0.03 mg/mL of USP Eszopiclone RS from *Standard stock solution* in *Mobile phase* passed through a suitable filter of 0.45-µm pore size. Use the filtrate.
Sample stock solution: Nominally 0.2 mg/mL of eszopiclone from Tablets prepared as follows. Transfer NLT 5 intact Tablets to a suitable volumetric flask. Add 5% of the final flask volume of *Solution A* and sonicate in cool water for 5 min with constant shaking. Add 30% of the final flask volume of acetonitrile and sonicate for 15 min. Dilute with acetonitrile to volume. Centrifuge the resulting solution and use the supernatant.
Sample solution: Nominally 0.03 mg/mL of eszopiclone from *Sample stock solution* in *Mobile phase* passed through a suitable filter of 0.45-µm pore size. Use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 303 nm
Column: 4.6-mm × 15.0-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
Run time: NLT 1.9 times the retention time of eszopiclone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of eszopiclone ($C_{17}H_{17}ClN_6O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Eszopiclone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of eszopiclone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm
Time: 30 min
Solution A: 1.4 g/L of anhydrous dibasic sodium phosphate in water
Mobile phase: Acetonitrile and *Solution A* (30:70) adjusted with dilute phosphoric acid (1 in 10) to a pH of 6.5 ± 0.05
Standard stock solution: 0.1 mg/mL of USP Eszopiclone RS in acetonitrile. Sonication may be used to promote dissolution.
Standard solution: (*L*/500) mg/mL of USP Eszopiclone RS from *Standard stock solution* in *Medium*, where *L* is the Tablet label claim in mg. Pass the resulting solution through a suitable filter of 0.45-µm pore size and use the filtrate.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 303 nm
Column: 4.6-mm × 15.0-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.2 mL/min
Injection volume: 100 µL
Run time: NLT 1.5 times the retention time of eszopiclone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of eszopiclone ($C_{17}H_{17}ClN_6O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Eszopiclone RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)

Acceptance criteria: NLT 80% (*Q*) of the labeled amount of eszopiclone ($C_{17}H_{17}ClN_6O_3$) is dissolved.

Test 2

Medium: *0.1 N hydrochloric acid VS*; 500 mL
Apparatus 2: 50 rpm
Time: 20 min
Buffer: To each liter of water add 1.0 mL of *phosphoric acid* and adjust with *2 N sodium hydroxide TS* to a pH of 4.0.
Mobile phase: Acetonitrile and *Buffer* (20:80)
Standard stock solution: 0.1 mg/mL of USP Eszopiclone RS in *Medium*. Sonication may be used to promote dissolution.
Standard solution: (*L*/500) mg/mL of USP Eszopiclone RS from *Standard stock solution* in *Medium*, where *L* is the label claim in mg/Tablet. Pass the resulting solution through a suitable filter of 0.45-µm pore size and use the filtrate.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 303 nm
Column: 4.6-mm × 15.0-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 80 µL
Run time: NLT 1.5 times the retention time of eszopiclone
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of eszopiclone ($C_{17}H_{17}ClN_6O_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Eszopiclone RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of eszopiclone ($C_{17}H_{17}ClN_6O_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Protect all solutions from light.
Solution A: 8.1 g/L of sodium dodecyl sulfate and 6.9 g/L of monobasic sodium phosphate in water. Sonicate for NLT 15 min and do not let the temperature of the water bath exceed 25°. Pass the resulting solution through a suitable filter of 0.45-µm pore size. Foam may form during filtration.
Mobile phase: Acetonitrile and *Solution A* (37:63) adjusted with dilute phosphoric acid (1 in 100) to a pH of 4.8 ± 0.05
Diluent: Acetonitrile and *Solution A* (37:63) adjusted with dilute phosphoric acid (1 in 100) to a pH of 2.5 ± 0.05
System suitability solution: 0.008 mg/mL each of USP Eszopiclone Related Compound A RS and USP Eszopiclone RS in *Diluent*. Sonication may be used to promote dissolution.
Standard solution: 0.008 mg/mL of USP Eszopiclone RS in *Diluent* passed through a suitable membrane filter of 0.45-µm pore size. Use the filtrate. Sonication may be used to promote dissolution.
Sample solution: Nominally 0.8 mg/mL of eszopiclone in *Diluent* prepared as follows. Crush NLT 20 Tablets to a fine powder and transfer a suitable portion to an appropriate volumetric flask. Add 60% of the final flask volume of *Diluent*, sonicate for 15 min in cold water with periodic shaking, and dilute with *Diluent* to volume. Pass the

USP Monographs

www.webofpharma.com

resulting solution through a suitable membrane filter of 0.45-µm pore size, and use the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 303 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
Run time: NLT 2 times the retention time of eszopiclone

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 10 between eszopiclone related compound A and eszopiclone, *System suitability solution*
Tailing factor: NMT 2.0 for eszopiclone, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of eszopiclone from the *Standard solution*
C_S = concentration of USP Eszopiclone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of eszopiclone in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.04%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zopiclone alcohol[a]	0.11	1.7	1.0
2-Amino-5-chloropyridine	0.21	0.76	1.0
Eszopiclone related compound A	0.44	0.86	1.0
Eszopiclone	1.0	—	—
Any individual unspecified degradation product	—	1.0	0.50
Total degradation products	—	—	2.0

[a] 6-(5-Chloropyridin-2-yl)-7-hydroxy-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-one.

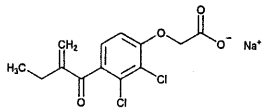
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** The labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
USP Eszopiclone RS
USP Eszopiclone Related Compound A RS
[NOTE—This material may be available in the free base or salt form.]
6-(5-Chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl 4-methylpiperazine-1-carboxylate 4-oxide.
$C_{17}H_{17}ClN_6O_4$ 404.81
6-(5-Chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl 4-methylpiperazine-1-carboxylate 4-oxide, 3-▲chlorobenzoate▲ (ERR 1-Jun-2018) salt (1:1).
$C_{17}H_{17}ClN_6O_4 \cdot$ ▲$C_7H_5ClO_2$▲ (ERR 1-Jun-2018) 561.38

Ethacrynate Sodium for Injection



$C_{13}H_{11}Cl_2NaO_4$ 325.12
Acetic acid, [2,3-dichloro-4-(2-methylene-1-oxobutyl) phenoxy]-, sodium salt;
Sodium [2,3-dichloro-4-(2-methylenebutyryl)phenoxy] acetate [6500-81-8].

DEFINITION

Ethacrynate Sodium for Injection is a sterile, freeze-dried powder prepared by the neutralization of Ethacrynic Acid with the aid of Sodium Hydroxide. It contains an amount of ethacrynate sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ethacrynic acid ($C_{13}H_{12}Cl_2O_4$).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Diluent: Acidified methanol prepared by adding 9 mL of hydrochloric acid to 100 mL of methanol
Sample solution: 50 µg/mL in *Diluent*
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
Buffer: Mix 10 mL of triethylamine and about 900 mL of water in a 1-L volumetric flask. Adjust with phosphoric acid to a pH of 6.8 ± 0.1, and dilute with water to volume.
Diluent: Acetonitrile and water (20:30)
Mobile phase: Acetonitrile and *Buffer* (400:600)
Standard solution: 0.5 mg/mL of USP Ethacrynic Acid RS in *Diluent*
Sample stock solution: Nominally equivalent to 2.5 mg/mL of ethacrynic acid from a suitable number of containers of Ethacrynate Sodium for Injection prepared as follows. Combine the contents of a suitable number of containers of Ethacrynate Sodium for Injection, equivalent to about 500 mg of ethacrynic acid, in a 200-mL volumetric flask. Add 5 mL of *Diluent* to each container, mix to dissolve the contents, and combine the resulting solutions in the volumetric flask taken. Rinse each container with two additional 5-mL portions of *Diluent*, add the rinsings to the solution in the volumetric flask, and dilute with *Diluent* to volume.

USP Monographs

www.webofpharma.com

Sample solution: 0.5 mg/mL of ethacrynic acid in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1200 theoretical plates
Tailing factor: NMT 2
Capacity factor, *k'*: NLT 0.8
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ethacrynic acid ($C_{13}H_{12}Cl_2O_4$) in the portion of Ethacrynate Sodium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ethacrynic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ethacrynic acid in the *Sample solution* (mg/mL)

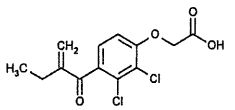
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Uniformity of Dosage Units** ⟨905⟩**:** Meets the requirements

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: Equivalent to 1 mg/mL of ethacrynic acid in Sterile Water for Injection
Acceptance criteria: 5.0–7.0
• **Sterility Tests** ⟨71⟩**:** Meets the requirements
• **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 5.0 USP Endotoxin Units/mg of ethacrynate sodium
• **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution* .
• **Labeling:** Label it to indicate that it was prepared by freeze-drying, having been filled into its container in the form of a true solution.
• **USP Reference Standards** ⟨11⟩
USP Ethacrynic Acid RS

Ethacrynic Acid



$C_{13}H_{12}Cl_2O_4$ 303.14
Acetic acid, [2,3-dichloro-4-(2-methylene-1-oxobutyl) phenoxy]-.
[2,3-Dichloro-4-(2-methylenebutyryl)phenoxy]acetic acid [58-54-8].

» Ethacrynic Acid contains not less than 97.0 percent and not more than 102.0 percent of $C_{13}H_{12}Cl_2O_4$, calculated on the dried basis. [**Caution**—Use care in handling Ethacrynic Acid, since it irritates the skin, eyes, and mucous membranes.]

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Ethacrynic Acid RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 50 µg per mL.
Medium: methanol.
Absorptivities at 271 nm, calculated on the dried basis, do not differ by more than 3.0%.
C: Add 2 mL of 1 N sodium hydroxide to about 25 mg of it, and heat for several minutes in a boiling water bath. Cool the solution, acidify with 0.25 mL of 18 N sulfuric acid, add 0.5 mL of chromotropic acid sodium salt solution (1 in 10), then add, cautiously, 2 mL of sulfuric acid TS: a deep violet color is produced.
Loss on drying ⟨731⟩—Dry it at a pressure not exceeding 5 mm of mercury at 60° for 2 hours: it loses not more than 0.25% of its weight.
Residue on ignition ⟨281⟩**:** not more than 0.1%.
Toluene extractives—Accurately weigh about 1 g into a glass-stoppered, 100-mL cylinder. Add 50 mL of sodium sulfite solution (2 in 25), and agitate until the solid dissolves. Allow to stand for 20 minutes, add 5 mL of hydrochloric acid, and mix. Divide the solution between two centrifuge tubes, each of which contains 15 mL of toluene. Close each tube tightly, using a polyethylene stopper, and shake vigorously during 2 minutes, occasionally relieving the pressure from the sulfur dioxide by loosening the stoppers. Centrifuge the tubes, withdraw most of the upper layer by means of a syringe, avoiding withdrawal of any of the lower, aqueous phase, and transfer the toluene extracts to a tared evaporating dish. Repeat the extraction twice with additional 15-mL portions of toluene, and evaporate the combined extracts on a steam bath to dryness. Dry the residue at a pressure not exceeding 5 mm

www.webofpharma.com

of mercury at 60° for 2 hours. Cool, and weigh: not more than 2.0% of extractives is found.

Equivalent weight—Dissolve about 400 mg, accurately weighed, in 100 mL of methanol, add 5 mL of water, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically, using a calomel–glass electrode system. Perform a blank determination, and make any necessary correction. Calculate the equivalent weight on the dried basis: it is between 294 and 309.

Assay—Dissolve about 100 mg of Ethacrynic Acid, accurately weighed, in 20 mL of glacial acetic acid in an iodine flask. Pipet 20 mL of 0.1 N bromine VS into the flask, add 3 mL of hydrochloric acid, immediately insert the stopper, and seal the flask with a few mL of water in the stopper well. Swirl the flask, and allow to stand in the dark for 1 hour. Add 50 mL of water and 15 mL of potassium iodide TS, and immediately titrate with 0.1 N sodium thiosulfate VS, adding 2.0 mL of starch TS as the endpoint is approached. Perform a blank determination (see *Residual Titrations* under *Titrimetry* ⟨541⟩). Each mL of 0.1 N bromine is equivalent to 15.16 mg of $C_{13}H_{12}Cl_2O_4$.

Ethacrynic Acid Tablets

DEFINITION

Ethacrynic Acid Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ethacrynic acid ($C_{13}H_{12}Cl_2O_4$).

IDENTIFICATION

• **A. Ultraviolet Absorption**

Diluent: A mixture of hydrochloric acid and anhydrous methanol (9 in 1000)

Standard solution: 50 µg/mL of USP Ethacrynic Acid RS in *Diluent*

Sample solution: Nominally 50 µg/mL of ethacrynic acid in *Diluent* prepared as follows. Weigh a portion of finely powdered Tablets, equivalent to 50 mg of ethacrynic acid, and transfer to a separator containing 25 mL of 0.1 N hydrochloric acid. Extract with two 40-mL portions of methylene chloride, filter the extracts into a 100-mL volumetric flask, and dilute with methylene chloride to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, evaporate in a gentle current of air to dryness, and promptly dissolve the residue in a portion of *Diluent*, then dilute with *Diluent* to volume.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, concomitantly measured.

• **B.**

Sample solution: Nominally 12.5 mg/mL of ethacrynic acid prepared as follows. Add 2 mL of 1 N sodium hydroxide to a portion of the powdered Tablets equivalent to 25 mg of ethacrynic acid.

Analysis

Sample: *Sample solution*

Heat the *Sample solution* for several minutes in a boiling water bath. Cool the solution, acidify with 0.25 mL of 18 N sulfuric acid, add 0.5 mL of chromotropic acid sodium salt solution (1 in 10), then cautiously add 2 mL of sulfuric acid TS.

Acceptance criteria: A deep violet color is produced.

ASSAY

• **Procedure**

Solution A: Mix 10 mL of triethylamine and about 900 mL of water in a 1-L volumetric flask. Adjust with phosphoric acid to a pH of 6.8 ± 0.1, dilute with water to volume, mix, and filter.

Mobile phase: Acetonitrile and *Solution A* (40:60). Filter and degas.

Diluent: Acetonitrile and water (40:60)

Standard solution: 0.5 mg/mL of USP Ethacrynic Acid RS in *Diluent*

Sample solution: Nominally 0.5 mg/mL of ethacrynic acid in *Diluent* prepared as follows. Transfer a portion of the powder from NLT 20 finely powdered Tablets, equivalent to about 50 mg of ethacrynic acid, to a 100-mL volumetric flask, add about 80 mL of *Diluent*, and shake or sonicate to dissolve the ethacrynic acid. Dilute with *Diluent* to volume, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor, *k'*: NLT 0.8

Column efficiency: NLT 1200 theoretical plates

Tailing factor: NMT 2

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ethacrynic acid ($C_{13}H_{12}Cl_2O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ethacrynic acid from the *Sample solution*

r_S = peak response of ethacrynic acid from the *Standard solution*

C_S = concentration of USP Ethacrynic Acid RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ethacrynic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: 0.1 M phosphate buffer, prepared by mixing 13.6 g of monobasic potassium phosphate and 92.2 mL of 1 N sodium hydroxide with water to obtain 1000 mL of a solution having a pH of 8.0 ± 0.05; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: A known concentration of USP Ethacrynic Acid RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute suitably with *Medium*.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 277 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ethacrynic acid ($C_{13}H_{12}Cl_2O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

USP Monographs

www.webofpharma.com

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Ethacrynic Acid RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of ethacrynic acid ($C_{13}H_{12}Cl_2O_4$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

Procedure for content uniformity (if applicable)

Diluent: A mixture of hydrochloric acid and methanol (9 in 1000)

Standard solution: 50 µg/mL of USP Ethacrynic Acid RS in *Diluent*

Sample stock solution: Add 1 Tablet to a 100-mL volumetric flask containing 10 mL of water, and allow to stand for 15 min, shaking occasionally until the Tablet is disintegrated. Add *Diluent* to volume and mix. Pass a portion of the mixture through a suitable filter.

Sample solution: Nominally 50 µg/mL of ethacrynic acid in *Diluent* from the *Sample stock solution* prepared as follows. Pipet a volume of the *Sample stock solution*, equivalent to 5 mg of ethacrynic acid, into a 100-mL volumetric flask. Dilute with *Diluent* to volume and mix.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 269 nm

Cell: 1 cm

Blank: *Diluent*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ethacrynic acid ($C_{13}H_{12}Cl_2O_4$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

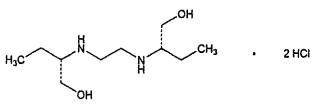
A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Ethacrynic Acid RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of ethacrynic acid in the *Sample solution* (µg/mL)

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards** ⟨11⟩
USP Ethacrynic Acid RS

Ethambutol Hydrochloride



$C_{10}H_{24}N_2O_2 \cdot 2HCl$ 277.23

1-Butanol, 2,2′-(1,2-ethanediyldiimino)bis-, dihydrochloride, [*S*-(*R**,*R**)]-;
(+)-2,2′-(Ethylenediimino)-di-1-butanol dihydrochloride;
(2*S*,2′*S*)-2,2′-[Ethane-1,2-diylbis(azanediyl)]dibutan-1-ol
[1070-11-7].

DEFINITION

Ethambutol Hydrochloride contains NLT 98.0% and NMT 100.5% of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

• **B. Identification Tests—General** ⟨191⟩, *Chemical Identification Tests, Chloride*

Sample solution: 100 mg/mL in water

Acceptance criteria: Meets the requirements

ASSAY

• **Procedure**

Sample solution: 200 mg of Ethambutol Hydrochloride in a mixture of 100 mL of glacial acetic acid and 5 mL of mercuric acetate TS. Add crystal violet TS.

Analysis: Titrate with 0.1 N perchloric acid VS (the color change at the endpoint is from blue to blue-green). Perform a blank determination, and make any necessary corrections (see *Titrimetry* ⟨541⟩). Each milliliter of 0.1 N perchloric acid is equivalent to 13.86 mg of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$).

Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES

• **Organic Impurities**

Protect all solutions containing ethambutol from light.

Solution A: Methanol and water (50:50)

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	82	18
30	82	18
35	0	100
37	0	100
38	82	18
50	82	18

Impurity stock solution A: To 4 mg of USP Ethambutol Related Compound A RS, add 4 mL of acetonitrile and 0.1 mL of triethylamine. Sonicate for 5 min and add 15 µL of (*R*)-(+)-alpha-methylbenzyl isocyanate. Heat the mixture at 70° for 20 min.

Impurity stock solution B: To 4 mg of USP Ethambutol Related Compound B RS, add 4 mL of acetonitrile and 0.1 mL of triethylamine. Sonicate for 5 min and add 15 µL of (*R*)-(+)-alpha-methylbenzyl isocyanate. Heat the mixture at 70° for 20 min.

System suitability solution: To 4 mg of USP Ethambutol Hydrochloride RS, add 4 mL of acetonitrile and 0.1 mL of triethylamine. Sonicate for 5 min and add 15 µL of (*R*)-(+)-alpha-methylbenzyl isocyanate. Heat the mixture at 70° for 20 min. Add 40 µL each of *Impurity stock solution A* and *Impurity stock solution B*.

Standard stock solution: To 4 mg of USP Ethambutol Hydrochloride RS, add 4 mL of acetonitrile and 0.1 mL of triethylamine. Sonicate for 5 min and add 15 µL of (*R*)-(+)-alpha-methylbenzyl isocyanate. Heat the mixture at 70° for 20 min.

USP Monographs

www.webofpharma.com

Standard solution: Dilute 0.5 mL of *Standard stock solution* with acetonitrile to 100.0 mL.
Sample solution: To 4 mg of Ethambutol Hydrochloride, add 4 mL of acetonitrile and 0.1 mL of triethylamine. Sonicate for 5 min and add 15 µL of (*R*)-(+)-alpha-methylbenzyl isocyanate. Heat the mixture at 70° for 20 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Temperatures
Autosampler: 10°
Column: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for ethambutol related compound B, ethambutol, and ethambutol related compound A are 0.91, 1.0, and 1.3, respectively.]
Suitability requirements
Resolution: NLT 1.2 between ethambutol related compound B and ethambutol; NLT 3.0 between ethambutol and ethambutol related compound A, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Ethambutol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of ethambutol from the *Standard solution*
C_S = concentration of USP Ethambutol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Ethambutol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ethambutol	1.0	—
Ethambutol related compound A	1.3	1.0
Any individual impurity[a]	—	0.10
Total impurities[a]	—	1.0

[a] Calculate any individual impurity and total impurities from the relative retention times of 0.75 to 1.5 with reference to ethambutol.

• **LIMIT OF AMINOBUTANOL**
Solution A: 12.4 mg/mL of boric acid in water. Adjust with 5 N sodium hydroxide to a pH of 9.0 prior to final dilution.
Fluorescamine solution: 0.1 mg/mL of fluorescamine in acetone
Standard solution: 5 µg/mL of USP Aminobutanol RS
Sample solution: 0.5 mg/mL of Ethambutol Hydrochloride
Instrumental conditions
(See *Fluorescence Spectroscopy* ⟨853⟩.)
Mode: Fluorometry
Analytical wavelength: 485 nm, with the excitation wavelength at about 385 nm
Cell: 1 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Pipet a 10-mL portion of the *Sample solution* into a glass-stoppered, 100-mL conical flask, and add 10 mL of water and 20 mL of *Solution A*. To another 100-mL flask, add 10.0 mL of the *Sample solution*, 10.0 mL of the *Standard solution*, and 20 mL of *Solution A*. Place the flasks on a magnetic stirrer, and while the contents are being stirred rapidly, add 10 mL of *Fluorescamine solution* rapidly. Insert the stoppers in the flasks, invert, and shake briefly. After 1 min, accurately timed, determine the relative fluorescence intensities of both solutions in a suitable fluorometer.
Acceptance criteria: The fluorescence intensity of the solution from the *Sample solution* is NMT 1.0% of the difference between the intensities of the two solutions.

SPECIFIC TESTS
• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 100 mg/mL in water
Acceptance criteria: +6.0° to +6.7°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Aminobutanol RS
$C_4H_{11}NO$ 89.14
USP Ethambutol Hydrochloride RS
USP Ethambutol Related Compound A RS
((2*R*,2′*S*)-2,2′-[Ethane-1,2-diylbis(azanediyl)]dibutan-1-ol)dihydrochloride.
$C_{10}H_{24}N_2O_2 \cdot 2HCl$ 277.23
USP Ethambutol Related Compound B RS
((2*R*,2′*R*)-2,2′-[Ethane-1,2-diylbis(azanediyl)]dibutan-1-ol)dihydrochloride.
$C_{10}H_{24}N_2O_2 \cdot 2HCl$ 277.23

Ethambutol Hydrochloride Compounded Oral Suspension

DEFINITION
Ethambutol Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$).
Prepare Ethambutol Hydrochloride Compounded Oral Suspension 100 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Ethambutol Hydrochloride tablets[a] equivalent to	3 g of ethambutol hydrochloride
Vehicle: a 1:1 mixture of Ora-Plus[b] and Ora-Sweet SF[b], a sufficient quantity to make	30 mL

[a] Ethambutol Hydrochloride 100-mg tablet(s), Lupin Pharmaceuticals, Baltimore, MD.
[b] Perrigo Laboratories, Allegan, MI.

www.webofpharma.com

Place the required number of tablets in a suitable container and comminute to a fine powder. Wet the powder with a small amount of *Vehicle* and triturate to make a smooth paste. Add the *Vehicle* to make the contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container using the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: 0.1% triethylamine in water, adjusted with phosphoric acid to a pH of 7.0. Pass through a nylon filter of 0.45-µm pore size and degas.

Mobile phase: Acetonitrile and *Solution A* (50:50)

Diluent: 1.4 g/L of sodium phosphate in water adjusted with phosphoric acid to a pH of 6.8

Standard stock solution: 10 mg/mL of USP Ethambutol Hydrochloride RS in *Diluent*. Sonicate to mix well. Store at 2°–8°.

Standard solution: Transfer 0.25 mL of the *Standard stock solution* to a 5-mL volumetric flask, dilute with *Diluent* to volume, and mix well. Transfer an aliquot to a centrifuge tube, and centrifuge for 5 min at 14,000 rpm at 2°–8°. Transfer the supernatant to an amber vial and store at 2°–8°.

Sample solution: Transfer 2 mL of Oral Suspension to a 20-mL volumetric flask, dilute with *Diluent* to volume, and sonicate to mix well. Transfer 0.25 mL of the resultant solution to a 5-mL volumetric flask, dilute with *Diluent* to volume, and mix well. Transfer an aliquot to a centrifuge tube, and centrifuge for 5 min at 14,000 rpm at 2°–8°. Transfer the supernatant to an amber vial and store at 2°–8°.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV-Vis 200 nm

Column: 4.6-mm × 25-cm; 5-µm packing L10

Temperatures

Autosampler: 5°

Column: 30°

Flow rate: 1.3 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for ethambutol hydrochloride is about 3.7 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ethambutol hydrochloride from the *Sample solution*

r_S = peak response of ethambutol hydrochloride from the *Standard solution*

C_S = concentration of USP Ethambutol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ethambutol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 3.5–5.6

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant plastic containers. Store in a refrigerator or at controlled room temperature.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator or at controlled room temperature

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ethambutol Hydrochloride RS

Ethambutol Hydrochloride Tablets

DEFINITION

Ethambutol Hydrochloride Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$).

IDENTIFICATION

SAMPLE: Triturate a quantity equivalent to 100 mg of ethambutol from powdered Tablets, with 3 mL of methanol in a glass mortar. Add 5 mL of methanol to obtain a suspension, then pass through a funnel lined with a suitable filter paper (Whatman No. 42 or equivalent) previously moistened with methanol, and collect the filtrate in a beaker containing 100 mL of acetone. Stir the mixture, and allow crystallization to proceed for 15 min. Decant the liquid, and gently dry the crystals with the aid of a current of air until the odor of methanol is no longer detectable: a portion of the crystals so obtained responds to the following tests.

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩

Sample solution: 100 mg/mL in water

Acceptance criteria: Meet the requirement

ASSAY

• **PROCEDURE**

Buffer: Mix 1.0 mL of triethylamine with 1 L of water, and adjust with phosphoric acid to pH 7.0.

Mobile phase: Acetonitrile and *Buffer* (1:1)

Standard solution: 0.30 mg/mL of USP Ethambutol Hydrochloride RS

Sample solution: Equivalent to 0.30 mg/mL of ethambutol hydrochloride from powdered Tablets (NLT 20). Filter the solution, discarding the first 10-mL portion.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 15-cm base-deactivated; 5-µm packing L10

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Stop Printing Here

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$) present in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ethambutol Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ethambutol hydrochloride from the *Sample solution* (mg/mL)

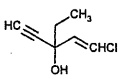
Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Buffer: 38.0 g/L of monobasic sodium phosphate and 2.0 g/L of anhydrous dibasic sodium phosphate
Bromocresol green solution: Dissolve 200 mg of bromocresol green in 30 mL of water and 6.5 mL of 0.1 N sodium hydroxide. Dilute with *Buffer* to 500 mL, and add 0.1 N hydrochloric acid to adjust to a pH of 4.6 ± 0.1.
Standard solution: 0.1 mg/mL of USP Ethambutol Hydrochloride RS
Sample solution: Pass a portion of solution under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Vis
Analytical wavelength: 415 nm
Analysis
Samples: *Standard solution*, *Sample solution*, and water (blank)
Into 3 separate, glass-stoppered, 50-mL centrifuge tubes, pipet 1 mL of water to provide the blank, 1 mL of *Standard solution*, and 1 mL of *Sample solution*. Add 5.0 mL of *Bromocresol green solution* to each tube, add 10.0 mL of chloroform to each, insert the stoppers, and shake the mixtures vigorously. Allow the mixtures to separate, discard the upper aqueous layers, and filter the 3 chloroform layers through separate pledgets of cotton.
Determine the the percentage of the labeled amount of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$) dissolved, using the blank to set the instrument.
Tolerances: NLT 75% (*Q*) of the labeled amount of $C_{10}H_{24}N_2O_2 \cdot 2HCl$ is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES
• **Limit of Aminobutanol**
Buffer: 1.24 g of boric acid to a 100-mL volumetric flask. Dissolve in 90 mL of water, adjust with 5 N sodium hydroxide to a pH of 9.0, and dilute with water to volume.
Fluorescamine solution: 0.1 mg/mL of fluorescamine in acetone
Standard solution: 5.0 µg/mL of USP Aminobutanol RS in water
Sample solution: Place a number of Tablets, equivalent to 400 mg of ethambutol hydrochloride, in a beaker, cover with acetone, and allow to stand for 15 min. Decant the acetone, dry the Tablets, and remove the coating. Grind the Tablet cores in a mortar to a fine powder, moisten with methanol, and triturate to a fine paste. Transfer the mixture with the aid of methanol to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Pass the mixture through a dry, folded filter paper. Pipet 25 mL of the filtrate into a 200-mL volumetric flask, and dilute with water to volume. Allow to stand for 15 min, and pass through a dry, folded filter paper, discarding the first cloudy portions of the filtrate. The clear filtrate is the *Sample solution*.
Instrumental conditions
(See *Fluorescence Spectroscopy* ⟨853⟩.)
Mode: Fluorometry
Analytical wavelength: Excitation wavelength at about 385 nm; emission wavelength at 485 nm
Cell: 1 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Pipet a 10-mL portion of the *Sample solution* into a glass-stoppered, 100-mL conical flask, and add 10 mL of water and 20 mL of *Buffer*. To another 100-mL flask, add 10.0 mL of the *Sample solution*, 10.0 mL of the *Standard solution*, and 20 mL of *Buffer*. Place the flasks on a magnetic stirrer, and while the contents are being stirred rapidly, add 10 mL of *Fluorescamine solution* rapidly. Insert the stoppers in the flasks, invert, and shake briefly. After 1 min, accurately timed, determine the relative fluorescence intensities of both solutions.
Acceptance criteria: The fluorescence intensity of the solution from the *Sample solution* is NMT the difference between the intensities of the two solutions; NMT 1.0%.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers.
• **USP Reference Standards** ⟨11⟩
USP Aminobutanol RS
USP Ethambutol Hydrochloride RS

Ethchlorvynol



C_7H_9ClO 144.60
1-Penten-4-yn-3-ol, 1-chloro-3-ethyl-.
1-Chloro-3-ethyl-1-penten-4-yn-3-ol [113-18-8].

» Ethchlorvynol contains not less than 98.0 percent and not more than 100.0 percent of *E*-ethchlorvynol (C_7H_9ClO), calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant glass or polyethylene containers, using polyethylene-lined closures.
USP Reference standards ⟨11⟩—
USP Ethchlorvynol RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197F*▲ (CN 1-May-2020).
B: Dissolve about 1 g in 20 mL of methanol. To 1 mL of the solution, add about 4 drops of 6 N ammonium hydroxide,

www.webofpharma.com

mix, then add silver nitrate TS, a few drops at a time: a yellowish white precipitate is formed, and it at first redissolves, but becomes insoluble when an excess of silver nitrate TS has been added.

C: To 10 mL of the solution prepared in *Identification* test *B* add 5 mL of freshly prepared *m*-phenylenediamine hydrochloride-oxalic acid solution (prepared by dissolving 1 g of *m*-phenylenediamine hydrochloride and 1 g of oxalic acid in 35 mL of water and filtering, if necessary): a reddish orange color is produced in about 3 minutes.

Refractive index ⟨831⟩: between 1.476 and 1.480.

Acidity—Dissolve 5.0 mL of Ethchlorvynol in 50 mL of a mixture of equal volumes of water and methanol that has been neutralized to the phenolphthalein endpoint with 0.1 N sodium hydroxide. Add 1 mL of phenolphthalein TS, and titrate with 0.10 N sodium hydroxide to a pink endpoint: not more than 1.7 mL of 0.10 N sodium hydroxide is required for neutralization.

Water Determination, *Method I* ⟨921⟩: not more than 0.2%.

Chromatographic purity—Calculate the percentage of each peak, other than the *E*-ethchlorvynol peak, observed in the chromatogram of the Ethchlorvynol obtained as directed in the *Assay* taken by the same formula:

$$100r_i/r_t$$

in which r_i is the response of each secondary peak and r_t is the sum of all of the peaks observed in the chromatogram: not more than 0.2% of toluene, not more than 1.5% of *Z*-ethchlorvynol, and not more than 0.3% of any other impurity is found, and the total of all observed impurities is not more than 2.0%.

Assay—

Resolution solution—Add 2.5 µL of toluene to 0.5 mL of USP Ethchlorvynol RS, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a thermal conductivity detector and a 1.8-m × 4-mm glass column (pretreated with 10% dimethyldichlorosilane in toluene) packed with 10% phase G16 on 60- to 80-mesh support S1AB. The column is maintained at about 160°, and the injector and the detector are maintained at about 200°. The carrier gas is dry helium, flowing at a rate of about 30 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times for toluene, β-chlorovinylethyl ketone (if present), *Z*-ethchlorvynol, and *E*-ethchlorvynol are about 0.1, 0.2, 0.8, and 1.0, respectively; the resolution, *R*, between the *Z*- and *E*-ethchlorvynol peaks is not less than 1.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Inject about 3 µL of Ethchlorvynol into the chromatograph, record the chromatogram, and measure the responses for all of the peaks. Calculate the percentage of *E*-ethchlorvynol (C_7H_9ClO) in the specimen of Ethchlorvynol taken by the formula:

$$100r_e/r_t$$

in which r_e is the response of the *E*-ethchlorvynol peak obtained in the chromatogram of the Ethchlorvynol and r_t is the sum of the responses of all of the peaks observed in the chromatogram.

Ethchlorvynol Capsules

» Ethchlorvynol Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of *E*-ethchlorvynol (C_7H_9ClO).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Ethchlorvynol RS

Identification—

A: The retention time of the Ethchlorvynol peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: Dissolve an amount of the contents of the Capsules, equivalent to about 1 g of ethchlorvynol, in 20 mL of methanol: the solution responds to *Identification*—tests *B:* and *C:* under *Ethchlorvynol.*

Dissolution ⟨711⟩—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 15 minutes.

Procedure—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each capsule shell to rupture.

Tolerances—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements, chloroform being used as the solvent in the procedure for *Weight Variation.*

Assay—

Methanol solution—Add 600 mL of methanol to 1400 mL of water in a suitable container, and mix. Allow the solution to equilibrate to room temperature.

Internal standard solution—Dissolve a quantity of chlorobutanol in *Methanol solution* to obtain a solution having a known concentration of about 6 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Ethchlorvynol RS in *Methanol solution* to obtain a solution having a known concentration of about 4 mg per mL. Transfer 5.0 mL of this solution to a suitable flask, add 5.0 mL of *Internal standard solution*, and mix to obtain a *Standard preparation* having a known concentration of about 2 mg of USP Ethchlorvynol RS per mL.

Assay preparation—Transfer not less than 20 Capsules, accurately counted, to a 500-mL volumetric flask. Add 250 mL of *Methanol solution*, heat at 70° to 80° for not more than 3 hours, and stir until the Capsules burst. Cool the solution to room temperature, add *Methanol solution* to volume, and mix. Quantitatively dilute a portion of this solution if necessary with *Methanol solution* to obtain a solution containing about 4 mg of *E*-ethchlorvynol per mL, and mix. Transfer 5.0 mL of this solution to a suitable flask, add 5.0 mL of *Internal standard solution*, and mix to obtain an *Assay preparation* containing about 2 mg of *E*-ethchlorvynol per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 1.8-m × 4-mm glass column (pretreated with 10% dimethyldichlorosilane in toluene) packed with 10% phase G16 on 60- to 80-mesh support S1AB. The column is

USP Monographs

www.webofpharma.com

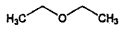
maintained at about 160°, and the injector and the detector are maintained at about 200°. The carrier gas is dry helium, flowing at a rate of about 30 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*:the resolution, *R*, between the ethchlorvynol and chlorobutanol peaks is not less than 4.0, and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Assay preparation* into the gas chromatograph, record the chromatographs, and measure the responses for the major peaks. The relative retention times are about 0.55 for chlorobutanol and 1.0 for *E*-ethchlorvynol. Calculate the quantity, in mg, of *E*-ethchlorvynol (C_7H_9ClO) in each Capsule taken by the formula:

$$(CL/D)(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Ethchlorvynol RS in the *Standard preparation*, *L* is the labeled quantity, in mg, of ethchlorvynol in each Capsule, *D* is the concentration, in mg per mL, of ethchlorvynol in each mL of the *Assay preparation* based on the number of Capsules taken, the labeled quantity, in mg, of ethchlorvynol in each Capsule, and the extent of dilution, and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ether



$C_4H_{10}O$ 74.12

Ethane, 1,1′-oxybis-;
Ethyl ether [60-29-7].

DEFINITION

Ether contains NLT 96.0% and NMT 98.0% of ether ($C_4H_{10}O$), the remainder consisting of alcohol and water.

[**CAUTION**—Ether is highly volatile and flammable. Its vapor, when mixed with air and ignited, may explode.]

[NOTE—Ether to be used for anesthesia must be preserved in tight containers of NMT 3-kg capacity, and is not to be used for anesthesia if it has been removed from the original container longer than 24 h. Ether to be used for anesthesia may, however, be shipped in larger containers for repackaging in containers as directed above, provided the ether at the time of repackaging meets the requirements of the tests of this Pharmacopeia.]

IMPURITIES

• **LIMIT OF NONVOLATILE RESIDUE**

Sample: 50 mL

Analysis: Allow the *Sample* to evaporate spontaneously from a tared dish, and dry at 105° for 1 h.

Acceptance criteria: 0.003%; the weight of the residue is NMT 1 mg.

• **LIMIT OF LOW-BOILING HYDROCARBONS**

Standard solution: Anhydrous ethyl ether, previously tested for absence of hydrocarbons, as directed for *Analysis*, and pentane (99.8: 0.2)

Sample solution: Ether

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 3.7-m stainless steel; with 30% phase G22 on 30- to 60-mesh support S1D

Temperatures

Column: 80°

Injection port: 230°

Detector: 250°

Flow rate: 30 mL/min

Carrier gas: Nitrogen

Injection volume: 1 µL

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—The retention times are about 2.3 min for isopentane, 2.7 min for pentane, and 4.3 min for 2-methylpentane.]

Measure the total area under the hydrocarbon peaks in the *Sample solution*.

Acceptance criteria: 0.2%; the total area of the hydrocarbon peaks does not exceed that of the peak for pentane in the *Standard solution*.

• **LIMIT OF ALDEHYDE**

Solution A: A mixture of 1 mL of alkaline mercuric–potassium iodide TS and 17 mL of a saturated solution of sodium chloride

Sample: 20 mL

Analysis: Place the *Sample* in a glass-stoppered cylinder, and add 7 mL of *Solution A*. Insert the stopper in the cylinder, shake vigorously for 10 s, and then set aside for 1 min.

Acceptance criteria: The water layer shows no turbidity.

• **LIMIT OF PEROXIDE**

Solution A: Cool separately, in small beakers surrounded by crushed ice, 10 mL of 6 N hydrochloric acid and 10 mL of titanium tetrachloride. Add the titanium tetrachloride dropwise to the chilled acid. Allow the mixture to stand at ice-bath temperature until all of the yellow solid dissolves, and dilute the solution with 6 N hydrochloric acid to 1000 mL.

Standard solution: 0.011 mg/mL of H_2O_2, prepared as follows. Pipet 25 mL of hydrogen peroxide solution into a 1000-mL volumetric flask, and dilute with water to volume. Pipet 15 mL of this solution into a 1000-mL volumetric flask, and dilute with water to volume.

Sample solution: Ether

Instrumental conditions

Mode: Vis

Analytical wavelength: 410 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

To 50 mL of the *Sample solution*, in a separator, add 5.0 mL of *Solution A*. Shake vigorously, allow the layers to separate, and drain the lower layer into a glass-stoppered, 25-mL graduated cylinder. Dilute with water to 10.0 mL. Prepare a second solution by adding 5.0 mL of *Solution A* and 1.0 mL of *Standard solution* to a glass-stoppered, 25-mL graduated cylinder, and diluting with water to 10.0 mL. Use a suitable spectrophotometer to determine the colors.

Acceptance criteria: NMT 0.3 ppm; any yellow color in the *Sample solution* does not exceed that in the *Standard solution*.

SPECIFIC TESTS

• **SPECIFIC GRAVITY** ⟨841⟩: 0.713–0.716, indicating 96.0%–98.0% of $C_4H_{10}O$

• **ACIDITY**

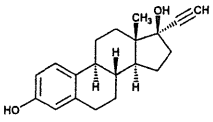
Analysis: Exercise great care to avoid contamination from carbon dioxide when adding the Ether and titrating. To 10 mL of water in a glass-stoppered flask, add 0.10 mL of bromothymol blue TS and 0.010 N sodium hydroxide until

www.webofpharma.com

a blue color persists after vigorous shaking. Add 25 mL of Ether, and shake briskly to mix the two layers. If no blue color remains, titrate with 0.010 N sodium hydroxide until the blue color is restored and persists for several min.
Acceptance criteria: 0.003% as CH_3COOH; NMT 0.80 mL of 0.010 N sodium hydroxide is required.
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%; except where labeled as intended for anesthetic use, it contains NMT 0.2%.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in partly filled, tight, light-resistant containers, at a temperature not exceeding 30° remote from fire.
- **LABELING:** Where Ether is intended for anesthetic use, the label so states.

Ethinyl Estradiol



$C_{20}H_{24}O_2$ 296.40
19-Norpregna-1,3,5(10)-trien-20-yne-3, 17-diol, (17α)-; 19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol [57-63-6].

DEFINITION
Ethinyl Estradiol contains NLT 97.0% and NMT 102.0% of $C_{20}H_{24}O_2$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 50 µg/mL in alcohol
Wavelength: 281 nm
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile and water (1:1)
Internal standard solution: 0.5 mg/mL of ethylparaben in *Mobile phase*
Standard solution: 0.2 mg/mL of USP Ethinyl Estradiol RS in *Mobile phase*, prepared as follows. Transfer 10 mg of USP Ethinyl Estradiol RS to a 50-mL volumetric flask, and add 10 mL of *Mobile phase* and 5.0 mL of *Internal standard solution*. Dilute with *Mobile phase* to volume.
Sample stock solution: 1.0 mg/mL of Ethinyl Estradiol in *Mobile phase*
Sample solution: 0.2 mg/mL of Ethinyl Estradiol, prepared as follows. Combine 10.0 mL of the *Sample stock solution* and 5.0 mL of *Internal standard solution* in a 50-mL volumetric flask. Dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ethylparaben and ethinyl estradiol are about 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.5 between the ethylparaben and ethinyl estradiol peaks
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ethinyl estradiol ($C_{20}H_{24}O_2$) taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio from the *Sample solution*
R_S = peak response ratio from the *Standard solution*
C_S = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (mg/mL)
C_U = concentration of Ethinyl Estradiol in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES
- **COMPLETENESS OF SOLUTION:** Dissolve 100 mg in 5 mL of alcohol; the solution is clear and free from undissolved solid.

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 180°–186°. It may exist also in a polymorphic modification, melting at 142°–146°.
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 50 mg/mL, using sonication if necessary, in colorless pyridine from a freshly opened container
Acceptance criteria: −28.0° to −29.5°
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 3 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, nonmetallic, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Ethinyl Estradiol RS

Ethinyl Estradiol Tablets

DEFINITION
Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of $C_{20}H_{24}O_2$.

IDENTIFICATION
- **THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 0.03 mg/mL of USP Ethinyl Estradiol RS in methanol
Sample solution: Transfer 25 Tablets to a suitable container, add 50 mL of water, and sonicate until the Tablets disintegrate (if needed, remove any coating with water before sonication). Place the sample in a separatory funnel, add 25 mL of ether, and shake well to extract the actives. Using a glass pipet, transfer the ether layer to a clean beaker, and evaporate to 10 mL.

www.webofpharma.com

Spray reagent: Methanol and sulfuric acid (1:1)
Application volume: 30 µL
Developing solvent system: Chloroform and alcohol (24:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed under *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*. Spray the plate with *Spray reagent*, place in an oven at 105° for about 5 min, and examine the plate.
Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and 20 mM potassium phosphate buffer, pH 6.0 (1:1)
Diluent: Acetonitrile and water (1:1)
Standard stock solution: 0.3 mg/mL of USP Ethinyl Estradiol RS in methanol
Standard solution: 0.12 µg/mL of USP Ethinyl Estradiol RS from *Standard stock solution* in *Diluent*
Sample solution: 0.12 µg/mL of ethinyl estradiol from NLT 20 Tablets in *Diluent*. [NOTE—Shake for about 30 min before makeup to volume. Centrifuge a portion of the solution, and makeup with *Diluent*.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Spectrofluorometric, with an excitation wavelength of 285 nm and an emission wavelength of 310 nm
Column: 4.6-mm × 15-cm; packing L11
Guard column: 4.6-mm × 12.5-mm; packing L11
Flow rate: 2 mL/min
Injection size: 200 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for ethinyl estradiol
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{20}H_{24}O_2$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ethinyl estradiol from the *Sample solution*
r_S = peak response of ethinyl estradiol from the *Standard solution*
C_S = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

• **DISSOLUTION**

[NOTE—Care must be taken not to expose any of the solutions to plastic or rubber. Fluorescent material will leach into the solutions and interfere with the quantitation of ethinyl estradiol. Also, adsorption may occur.]
Test 1
Medium: 0.3% sodium lauryl sulfate in water; 500 mL, degassed
Apparatus 2: 100 rpm
Time: 30 min
pH 6.0 phosphate buffer: Transfer 2.7 g of monobasic potassium phosphate to a 1-L volumetric flask. Dissolve in 900 mL of water. Adjust with 1 N sodium hydroxide to a pH of 6.0, and dilute with water to volume.
Mobile phase: *pH 6.0 phosphate buffer* and acetonitrile (1:1)
Standard stock solution: 0.25 mg/mL of USP Ethinyl Estradiol RS in methanol. This solution is stable for 14 days.
Standard solution: Dilute the *Standard stock solution* with *Medium* to a final concentration of 0.06 µg/mL. Add 1 or 2 drops of methanol to dissipate the bubbles, if necessary. This solution is stable for 24 h.
Sample solution: Centrifuge the solution under test for 10 min at 2000 rpm. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorescence, excitation at 285 nm, emission at 310 nm
Column: 4.6-mm x 15-cm, 5-µm packing L11
Guard column: 4.6-mm × 1.25-cm, 5-µm packing L11
Flow rate: 2.0 mL/min
Injection size: 200 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0%
Analysis: Calculate the percentage of ethinyl estradiol dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of ethinyl estradiol is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 5 ppm of polysorbate 80 in water; 500 mL, deaerated with helium
Apparatus 2: 75 rpm
Time: 45 min
Standard stock solution: Transfer 10 mg of USP Ethinyl Estradiol RS and 50 mg of USP Norgestrel RS to a 500-mL volumetric flask. Add 250 mL of acetonitrile, and sonicate until dissolved. Cool to room temperature, and dilute with water to volume. The final concentration is about 20 µg/mL of ethinyl estradiol and 100 µg/mL of norgestrel. This solution is stable for 15 days.
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of 0.02 µg/mL of ethinyl estradiol. This solution is stable for 6 days.
Sample solution: Centrifuge the solution under test at about 3000 rpm for 20 min. Use the supernatant. This solution is stable for 12 h.
Mobile phase: Water, acetonitrile, and methanol (55:40:5)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 10-cm, 3-µm packing L1
Column temperature: 30°
Flow rate: 1.2 mL/min
Injection size: 200 µL

www.webofpharma.com

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 6.0 between the ethinyl estradiol and norgestrel peaks
Relative standard deviation: NMT 3.0%, for ethinyl estradiol
Analysis: Calculate the percentage of ethinyl estradiol dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of ethinyl estradiol is dissolved.
• **UNIFORMITY OF DOSAGE UNITS:** Meet the requirements

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**
Solution A: Acetonitrile and 20 mM potassium phosphate buffer, pH 6.0 (1:1)
Solution B: Acetonitrile and 20 mM potassium phosphate buffer, pH 6.0 (4:1)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	100	0	2
20	100	0	2
20.1	100	0	2.5
25.0	0	100	2.5
25.1	0	100	3
30.0	0	100	3
30.1	0	100	2
32.0	100	0	2
35.0	100	0	2

Diluent: Acetonitrile and water (1:1)
Standard stock solution: 0.3 mg/mL of USP Ethinyl Estradiol RS in methanol
Standard solution: 0.12 µg/mL of USP Ethinyl Estradiol RS from *Standard stock solution* in *Diluent*
Sample solution A: Transfer 20 Tablets into a 200-mL volumetric flask. Add 120 mL of *Diluent*, and shake for about 30 min. Dilute with *Diluent* to volume. Centrifuge a portion of the dissolution sample, and use the clear supernatant.
Sample solution B: 0.6 µg/mL of ethinyl estradiol from *Sample solution A* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors: UV detector at 210 nm; and a spectrofluorometric detector with an excitation wavelength of 285 nm and an emission wavelength of 310 nm
Column: 4.6-mm × 15-cm; packing L11
Guard column: 4.6-mm × 12.5-mm; packing L11
Flow rate: See the gradient table above.
Injection size: 200 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for ethinyl estradiol
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Sample solution A* and *Sample solution B*
[NOTE—Measure the peak heights for the major peaks obtained within 20 min. Use the response from *Sample solution A* for estrone and all other impurities. Use *Sample solution B* for 17β-ethinyl estradiol.]
Calculate the percentage of 17β-ethinyl estradiol in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = height of any peak at the relative retention time of 1.16, using the spectrofluorometric detector
r_S = peak height of ethinyl estradiol, using the spectrofluorometric detector

Calculate the percentage of estrone in the portion of Tablets taken:

$$\text{Result} = [(r_U/r_S) \times 100] - E$$

r_U = height of any peak at the relative retention time of 1.2, using the UV detector at 210 nm
r_S = peak height of ethinyl estradiol, using the UV detector at 210 nm
E = percentage of 17β-ethinyl estradiol

Calculate the percentage of any other impurity:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = height of any peak other than those mentioned above
r_S = peak height of ethinyl estradiol, using the UV detector at 210 nm

Acceptance criteria
17β-Ethinyl estradiol: NMT 0.5%
Estrone: NMT 0.5%
Any other impurity: NMT 0.5%
Total impurities: NMT 2.0 %

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ethinyl Estradiol RS
USP Norgestrel RS

Ethiodized Oil Injection

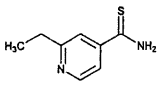
» Ethiodized Oil Injection is an iodine addition product of the ethyl ester of the fatty acids of poppyseed oil, containing not less than 35.2 percent and not more than 38.9 percent of organically combined iodine. It is sterile.

USP Monographs

www.webofpharma.com

Packaging and storage—Preserve in well-filled, light-resistant, single-dose or multiple-dose containers.
Identification—Place 1 drop in a test tube, and heat directly in a flame: the violet color of iodine vapors is observed.
Specific gravity ⟨841⟩: between 1.280 and 1.293, at 15°.
Viscosity—Capillary Methods ⟨911⟩ or ***Viscosity—Rotational Methods*** ⟨912⟩: between 50 centipoises and 100 centipoises, at 15°.
Sterility Tests ⟨71⟩: meets the requirements.
Acidity—Dissolve 1.0 mL in 10 mL of chloroform in a glass-stoppered cylinder, add phenolphthalein TS and 0.30 mL of sodium hydroxide solution (1 in 250), insert the stopper, and shake vigorously: a red color is produced.
Free iodine—Dissolve 1.0 mL in 5 mL of chloroform, add 20 mL of potassium iodide solution (1 in 20), agitate vigorously, and add 2 drops of starch TS: no blue color is produced.
Assay— [**Caution**—Observe rigorously the precautions set forth for Procedure under *Oxygen Flask Combustion* ⟨471⟩.] Weigh accurately about 30 mg (1 drop) of Injection in a tared cellulose acetate capsule, and proceed as directed for *Procedure* under *Oxygen Flask Combustion* ⟨471⟩, beginning with "Place the specimen," and using a thick-walled, 500-mL combustion flask. Use 10 mL of sodium hydroxide solution (1 in 100) and 1 mL of freshly prepared sodium bisulfite solution (1 in 100) as the absorbing liquid. Pipet 1 mL of bromine-sodium acetate TS into the cup of the flask, loosen the stopper, and allow the solution to be sucked into the flask. Wash down the cup and the ground joint with water, insert the stopper in the flask, shake it vigorously, then add 5 drops of formic acid, and again shake the flask. Remove the stopper, and rinse the stopper and the specimen holder with water, collecting the rinsings in the flask. Bubble nitrogen through the solution to displace all of the oxygen from the solution and the flask. Add 0.50 g of potassium iodide and 3 mL of 2 N sulfuric acid, allow the mixture to stand for 2 minutes, add 3 mL of starch TS, and titrate the liberated iodine with 0.05 N sodium thiosulfate VS. Each mL of 0.05 N sodium thiosulfate is equivalent to 1.058 mg of iodine (I).

Ethionamide



$C_8H_{10}N_2S$ 166.24
4-Pyridinecarbothioamide, 2-ethyl-;
2-Ethylthioisonicotinamide [536-33-4].

DEFINITION
Ethionamide contains NLT 98.0% and NMT 102.0% of ethionamide ($C_8H_{10}N_2S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Standard solution and **Sample solution:** Prepare as directed in the *Assay*.
Acceptance criteria: Meets the requirements

ASSAY
- **Procedure**

Standard solution: 10 µg/mL of USP Ethionamide RS in methanol
Sample solution: 10 µg/mL of Ethionamide in methanol
Instrumental conditions
Mode: UV
Analytical wavelength: 290 nm
Cell: 1 cm
Blank: Methanol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ethionamide ($C_8H_{10}N_2S$) in the portion of Ethionamide taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Ethionamide RS in the *Standard solution* (µg/mL)
C_U = concentration of Ethionamide in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.2%
- **Selenium** ⟨291⟩

Sample: 200 mg
Acceptance criteria: NMT 30 ppm

SPECIFIC TESTS
- **Melting Range or Temperature** ⟨741⟩: 158°–164°
- **pH** ⟨791⟩: 6.0–7.0, 1 in 100 slurry in water
- **Water Determination,** *Method I* ⟨921⟩: NMT 2.0%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** ⟨11⟩

USP Ethionamide RS

Ethionamide Tablets

DEFINITION
Ethionamide Tablets contain NLT 95.0% and NMT 110.0% of the labeled amount of ethionamide ($C_8H_{10}N_2S$).

IDENTIFICATION
- **A.**

Sample solution: The solution of Tablets employed for measurement of absorbance in the *Assay*
Acceptance criteria:
The *Sample solution* exhibits an absorbance maximum at 290 ± 2 nm.
- **B.**

Sample solution: Nominally 20 mg/mL of ethionamide from digested powdered Tablets in methanol
Analysis: Filter the *Sample solution* through a sintered-glass funnel of medium pore size. Evaporate the filtrate on a steam bath to dryness.
Acceptance criteria: The residue so obtained melts at 155°–164°.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Standard solution: 10 µg/mL of USP Ethionamide RS in methanol

Sample stock solution: Nominally 0.4 mg/mL of ethionamide prepared as follows. Transfer nominally 100 mg of ethionamide from powdered Tablets (NLT 20), to a sintered-glass funnel of medium pore size that is fitted into a 250-mL suction flask. Extract the sample by stirring it with successive 10-mL portions of methanol, using a total of about 100 mL of solvent, and drawing off each portion of liquid with gentle suction before adding the next portion. Transfer the combined methanol extracts to a 250-mL volumetric flask, and dilute with methanol to volume.

Sample solution: Nominally 10 µg/mL of ethionamide in methanol from the *Sample stock solution*

Blank: Methanol

Instrumental conditions

Mode: UV

Analytical wavelength: 290 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ethionamide ($C_8H_{10}N_2S$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Ethionamide RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of ethionamide in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Detector: UV 274 nm

Standard solution: USP Ethionamide RS in *Medium*

Sample solution: Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: NLT 75% (*Q*) of the labeled amount of ethionamide ($C_8H_{10}N_2S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

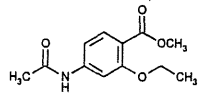
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ethionamide RS

Ethopabate



$C_{12}H_{15}NO_4$ 237.25

Benzoic acid, 4-(acetylamino)-2-ethoxy-, methyl ester.

Methyl 4-acetamido-2-ethoxybenzoate [59-06-3].

» Ethopabate contains not less than 96.0 percent and not more than 101.0 percent of $C_{12}H_{15}NO_4$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, protected from light.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Ethopabate RS

USP Ethopabate Related Compound A RS

Methyl-4-acetamido-2-hydroxybenzoate.

$C_{10}H_{11}NO_4$ 209.20

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—

Solution: 10 µg per mL.

Medium: methanol.

Loss on drying ⟨731⟩—Dry 1.0 g of it in vacuum at 60° for 2 hours: it loses not more than 1.0% of its weight.

Melting range ⟨741⟩: between 146° and 151°.

Residue on ignition ⟨281⟩: not more than 0.5%.

Chromatographic purity—Examine the chromatogram of the *Assay preparation*, as obtained in the *Assay*, for peaks that elute at the following retention times in relation to ethopabate: 0.33, *p*-aminosalicylic acid (ethopabate related compound B); 0.64, methyl 2-ethoxy-4-aminobenzoate (ethopabate related compound C); 0.68, methyl 2-hydroxy-4-aminobenzoate (ethopabate related compound D); 0.9, methyl 4-acetamido-2-hydroxybenzoate (ethopabate related compound A); and 1.6, ethyl 4-acetamido-2-ethoxybenzoate (ethopabate related compound E). Calculate the percentage of diazotizable substances, represented by peaks for ethopabate related compounds B, C, and D, if present, by the formula:

$$(0.72r_B + 0.68r_C + 0.74r_D)/0.01r_U$$

in which 0.72, 0.68, and 0.74 are the response factors of ethopabate related compounds B, C, and D, respectively, relative to that of ethopabate, r_B, r_C, and r_D are the responses of the peaks observed for ethopabate related compounds B, C, and D, respectively, and r_U is the ethopabate peak response obtained from the *Assay preparation:* not more than 0.5% of diazotizable substances is found. Calculate the percentage of any other impurities by the formula:

$$100 - A_E - A_s$$

in which A_E is the percentage of total peak area represented by the main ethopabate peak in the chromatogram obtained from the *Assay preparation*, and A_s is the percentage of peak area represented by the sum of the peaks for ethopabate related compounds B, C, and D: not more than 2.0% of other impurities is found. [NOTE—Exclude from the total peak area the responses of any minor peaks that are 0.01% or less than that of the main ethopabate peak.]

Assay—

Mobile phase—Dissolve 3 g of sodium 1-hexanesulfonate in 1 L of water, and adjust with phosphoric acid to a pH of 2.5.

www.webofpharma.com

Prepare a filtered and degassed mixture of this solution, methanol, and acetonitrile (450:150:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of methanol and water (50:50).

Standard preparation—Prepare a solution of USP Ethopabate RS in *Diluent* having a known concentration of about 0.4 mg per mL. If necessary, filter this solution through a filter having a porosity of 0.5 µm or finer, and use the filtrate as the *Standard preparation*. Use this solution on the day prepared.

Assay preparation—Transfer about 40 mg of Ethopabate, accurately weighed, to a 100-mL volumetric flask, add about 80 mL of *Diluent*, and dissolve with the aid of sonication. Mix and dilute to volume with *Mobile phase*. If necessary, filter this solution through a filter having a porosity of 0.5 µm or finer, and use the filtrate as the *Assay preparation*. Use this solution on the day prepared.

Resolution solution—Prepare a solution in *Diluent* containing about 0.4 mg of USP Ethopabate RS and 0.1 mg of USP Ethopabate Related Compound A RS per mL.

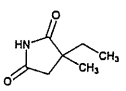
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 268-nm detector and a 3.9-mm × 30-cm column that contains packing L11 and is maintained at about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure:* the relative retention times are about 0.9 for methyl 4 acetamido-2-hydroxybenzoate (ethopabate related compound A) and 1.0 for ethopabate, the column efficiency is not less than 4000 theoretical plates, the resolution, *R*, between the ethopabate related compound A peak and the ethopabate peak is not less than 1.2, and the tailing factor is not more than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the responses for the major peaks. Calculate the quantity, in mg, of $C_{12}H_{15}NO_4$ in the portion of Ethopabate taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Ethopabate RS in the *Standard preparation*, and r_U and r_S are the ethopabate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ethosuximide



$C_7H_{11}NO_2$ 141.17

2,5-Pyrrolidinedione, 3-ethyl-3-methyl-, (±)-; (±)-2-Ethyl-2-methylsuccinimide [77-67-8].

DEFINITION

Ethosuximide contains NLT 98.0% and NMT 101.0% of ethosuximide ($C_7H_{11}NO_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)
Standard solution: 66.7 mg/mL of USP Ethosuximide RS in chloroform
Sample solution: ▲66.7 mg/mL of Ethosuximide in chloroform▲ (USP 1-May-2019)
Acceptance criteria: Meets the requirements

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**
Buffer: Add 4.1 mL of phosphoric acid to 1000 mL of water, and adjust with sodium hydroxide TS to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (10:90)
System suitability solution: 2 mg/mL of 2-ethyl-2-methylsuccinic acid and 10 mg/mL of USP Ethosuximide RS in *Mobile phase*
Standard solution: 10.0 mg/mL of USP Ethosuximide RS in *Mobile phase*
Sample solution: 10.0 mg/mL of Ethosuximide in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 30.0-cm; ▲10-µm▲ (USP 1-May-2019) packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
▲Run time: NLT 2 times the retention time of ethosuximide▲ (USP 1-May-2019)
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 6.6 between 2-ethyl-2-methylsuccinic acid and ethosuximide
▲▲ (USP 1-May-2019)
Tailing factor: NMT 2.0 for ethosuximide
Relative standard deviation: NMT 0.4% for ethosuximide
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ethosuximide ($C_7H_{11}NO_2$) in the portion of Ethosuximide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ethosuximide RS in the *Standard solution* (mg/mL)
C_U = concentration of Ethosuximide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the anhydrous basis

USP Monographs

www.webofpharma.com

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%

Change to read:

• **▲ORGANIC IMPURITIES▲** (USP 1-May-2019)

Buffer, Mobile phase, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard solution: 0.1 mg/mL each of USP Ethosuximide RS and 2-ethyl-2-methylsuccinic acid in *Mobile phase*

Sample solution: 100 mg/mL of Ethosuximide in *Mobile phase*. Sonicate as needed.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 2-ethyl-2-methylsuccinic acid in the portion of Ethosuximide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 2-ethyl-2-methylsuccinic acid from the *Sample solution*

r_S = peak response of 2-ethyl-2-methylsuccinic acid from the *Standard solution*

C_S = concentration of 2-ethyl-2-methylsuccinic acid in the *Standard solution* (mg/mL)

C_U = concentration of Ethosuximide in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Ethosuximide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity, other than 2-ethyl-2-methylsuccinic acid, from the *Sample solution*

r_S = peak response of ethosuximide from the *Standard solution*

C_S = concentration of USP Ethosuximide RS in the *Standard solution* (mg/mL)

C_U = concentration of Ethosuximide in the *Sample solution* (mg/mL)

Acceptance criteria: ▲See *Table 1*. The reporting threshold for impurities is 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ethosuximide	1.0	—
2-Ethyl-2-methylsuccinic acid	1.5	0.1
Any individual unspecified impurity	—	0.1
Total impurities	—	0.5

▲ (USP 1-May-2019)

SPECIFIC TESTS

Delete the following:

▲• **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 47°–52° ▲ (USP 1-May-2019)

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.5%

Change to read:

• **LIMIT OF CYANIDE**

Sample solution: 100 mg/mL in alcohol

Analysis: To 10 mL of *Sample solution*, add 3 drops of ferrous sulfate TS, 1 mL of 1 N sodium hydroxide ▲VS,▲ (USP 1-May-2019) and a few drops of ferric chloride TS. Warm gently, and acidify with 2 N sulfuric acid ▲TS.▲ (USP 1-May-2019)

Acceptance criteria: No blue precipitate or blue color is formed within 15 min.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ethosuximide RS

Ethosuximide Capsules

DEFINITION

Ethosuximide Capsules contain NLT 93.0% and NMT 107.0% of the labeled amount of ethosuximide ($C_7H_{11}NO_2$), present in the form of a solution of Ethosuximide in Polyethylene Glycol 400 or other suitable solvent.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S▲** (CN 1-May-2020)

Standard solution: ▲66.7 mg/mL▲ (USP 1-May-2019) of USP Ethosuximide RS in chloroform

Sample solution: Nominally 60 mg/mL of ethosuximide in chloroform prepared as follows. Transfer the equivalent of about 300 mg of ethosuximide from Capsules to a separator containing 50 mL of ethyl ether. Shake with three 10-mL portions of water, discarding the aqueous extracts. Add about 5 g of anhydrous sodium sulfate, swirl for 3 min, and filter through a small pledget of cotton that previously has been washed with ethyl ether into a small flask. Evaporate the ethyl ether solution at room temperature in a current of air to dryness, and dissolve the residue in 5 mL of chloroform.

Wavelength range: 3000–1650 cm^{-1}

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: Meet the requirements

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: Acetonitrile and water (12.5: 87.5). To each liter, add 1.0 mL of glacial acetic acid.

System suitability solution: 0.062 mg/mL of USP Ethosuximide RS and 0.064 mg/mL of 2-ethyl-2-methylsuccinic acid in *Mobile phase*

Standard solution: 0.062 mg/mL of USP Ethosuximide RS in *Mobile phase*

www.webofpharma.com

Sample solution: Nominally 0.062 mg/mL of ethosuximide from Capsules in *Mobile phase* prepared as follows. Transfer 20 Capsules into a 2-L volumetric flask, dissolve in 1800 mL of *Mobile phase*, and dilute with *Mobile phase* to volume. Transfer 5.0 mL of the resulting solution to a 200-mL volumetric flask and dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 3.9-mm × 15-cm; ▲4-µm▲ (USP 1-May-2019) packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
▲Run time: NLT 2 times the retention time of ethosuximide▲ (USP 1-May-2019)

System suitability
Sample: *System suitability solution*
▲[NOTE—The relative retention times for ethosuximide and 2-ethyl-2-methylsuccinic acid are 1.0 and 1.3, respectively.]▲ (USP 1-May-2019)
Suitability requirements
Resolution: NLT 3.5 between ethosuximide and 2-ethyl-2-methylsuccinic acid
Tailing factor: NMT 1.5
Relative standard deviation: NMT ▲1.0%▲ (USP 1-May-2019) for ethosuximide and NMT 5.0% for 2-ethyl-2-methylsuccinic acid

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ethosuximide ($C_7H_{11}NO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ethosuximide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ethosuximide in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

▲Test 1: Use *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms, Procedure for a pooled sample for immediate-release dosage forms.*▲ (USP 1-May-2019)
Medium: pH 6.8 phosphate buffer; 900 mL
Apparatus 1: 50 rpm
Time: 30 min
Mobile phase: Acetonitrile and water (20:80)
Standard solution: USP Ethosuximide RS in *Medium*
Sample solution: Prepare as indicated in *Dissolution* ⟨711⟩, *Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms, Procedure for a pooled sample for immediate-release dosage forms.* Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 50 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
▲Calculate the percentage of the labeled amount of ethosuximide ($C_7H_{11}NO_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ethosuximide RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)▲ (USP 1-May-2019)

Tolerances: NLT 80% (Q) of the labeled amount of ethosuximide ($C_7H_{11}NO_2$) is dissolved.

▲Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: pH 6.8 phosphate buffer (prepared by adding 0.9 g sodium hydroxide per liter of 6.8-g/L monobasic potassium phosphate in water; adjust with sodium hydroxide to a pH of 6.8, if necessary; sonicate for about 15 min); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Mobile phase: Acetonitrile and water (12.5: 87.5). To each liter, add 1.0 mL of glacial acetic acid.
Standard solution: (L/900) mg/mL of USP Ethosuximide RS prepared as follows, where L is the label claim in mg/Capsule. Transfer a suitable portion of USP Ethosuximide RS to an appropriate volumetric flask. Add 80% of the total flask volume of *Medium* and sonicate for NLT 5 min or until the solids are dissolved. Allow the resulting solution to cool to room temperature, and dilute with *Medium*.
Sample solution: Centrifuge a 10-mL aliquot of the solution under test for about 15 min. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: NLT 4 times the retention time of ethosuximide

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ethosuximide ($C_7H_{11}NO_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

USP Monographs

www.webofpharma.com

C_S = concentration of USP Ethosuximide RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of ethosuximide ($C_7H_{11}NO_2$) is dissolved. ▲ (USP 1-May-2019)

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **LIMIT OF 2-ETHYL-2-METHYLSUCCINIC ACID**

Mobile phase, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard solution: 0.026 mg/mL of 2-ethyl-2-methylsuccinic acid in *Mobile phase*

Sample solution: Nominally 2.5 mg/mL of ethosuximide from Capsules in *Mobile phase* prepared as follows. Transfer 20 Capsules into a 2-L volumetric flask, dissolve in 1800 mL of *Mobile phase*, dilute with *Mobile phase* to volume, and mix.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 2-ethyl-2-methylsuccinic acid in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 2-ethyl-2-methylsuccinic acid from the *Sample solution*
r_S = peak response of 2-ethyl-2-methylsuccinic acid from the *Standard solution*
C_S = concentration of 2-ethyl-2-methylsuccinic acid in the *Standard solution* (mg/mL)
C_U = nominal concentration of ethosuximide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight containers. ▲Store at controlled room temperature.▲ (USP 1-May-2019)

Add the following:

▲• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.▲ (USP 1-May-2019)

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ethosuximide RS

Ethosuximide Oral Solution

DEFINITION

Ethosuximide Oral Solution contains NLT 90.0% and NMT 105.0% of the labeled amount of ethosuximide ($C_7H_{11}NO_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S** ▲ (CN 1-May-2020)

Standard solution: 30.0 mg/mL of USP Ethosuximide RS in chloroform

Sample solution: Nominally 30 mg/mL of ethosuximide in chloroform prepared as follows. Transfer the equivalent of about 150 mg of ethosuximide from Oral Solution to a 125-mL separatory funnel, add 50 mL of ether, and shake well. Allow the layers to separate, and retain the ether layer. Wash the ether layer with three 10-mL portions of water. Transfer the ether layer to a suitable beaker, add 5 g of anhydrous sodium sulfate, and swirl. Filter the mixture into a 50-mL volumetric flask through a small pledget of cotton that has been previously washed with ether. Evaporate to dryness. Dissolve the residue in 5 mL of chloroform.

Wavelength range: 3000–1650 cm^{-1}

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: Acetonitrile and water (12.5: 87.5). To each liter, add 1.0 mL of glacial acetic acid.

System suitability solution: 0.062 mg/mL of USP Ethosuximide RS and 0.064 mg/mL of 2-ethyl-2-methylsuccinic acid in *Mobile phase*

Standard solution: 0.062 mg/mL of USP Ethosuximide RS in *Mobile phase*

Sample solution: Nominally 0.062 mg/mL of ethosuximide from Oral Solution in *Mobile phase* prepared as follows. Transfer a volume of Oral Solution, equivalent to 250 mg of ethosuximide, to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume. Transfer 5.0 mL of this solution to a 200-mL volumetric flask and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 3.9-mm × 15-cm; ▲4-µm▲ (USP 1-May-2019) packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

▲Run time: NLT 2 times the retention time of ethosuximide▲ (USP 1-May-2019)

System suitability

Sample: *System suitability solution*

▲[NOTE—The relative retention times for ethosuximide and 2-ethyl-2-methylsuccinic acid are 1.0 and 1.3, respectively.]▲ (USP 1-May-2019)

Suitability requirements

Resolution: NLT 3.5 between ethosuximide and 2-ethyl-2-methylsuccinic acid

Tailing factor: NMT 1.5

Relative standard deviation: NMT ▲1.0%▲ (USP 1-May-2019) for ethosuximide and NMT 5.0% for 2-ethyl-2-methylsuccinic acid

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ethosuximide ($C_7H_{11}NO_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ethosuximide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ethosuximide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–105.0%

IMPURITIES
• **LIMIT OF 2-ETHYL-2-METHYLSUCCINIC ACID**
Mobile phase, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Standard solution: 0.05 mg/mL of 2-ethyl-2-methylsuccinic acid in *Mobile phase*
Sample solution: Nominally 2.5 mg/mL of ethosuximide from Oral Solution in *Mobile phase* prepared as follows. Using a "to contain" pipet, transfer a volume of Oral Solution, equivalent to 250 mg of ethosuximide, to a 100-mL volumetric flask. Rinse the pipet several times with *Mobile phase*, and dilute with *Mobile phase* to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 2-ethyl-2-methylsuccinic acid in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 2-ethyl-2-methylsuccinic acid from the *Sample solution*
r_S = peak response of 2-ethyl-2-methylsuccinic acid from the *Standard solution*
C_S = concentration of 2-ethyl-2-methylsuccinic acid in the *Standard solution* (mg/mL)
C_U = nominal concentration of ethosuximide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.0%

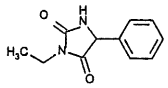
SPECIFIC TESTS
• **pH** ⟨791⟩**:** 4.5–5.8

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in tight containers. ▲Store at controlled room temperature. Protect from freezing and light.▲ (USP 1-May-2019)
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ethosuximide RS

Ethotoin



$C_{11}H_{12}N_2O_2$ 204.23
2,4-Imidazolidinedione, 3-ethyl-5-phenyl-, (±)-.
(±)-3-Ethyl-5-phenylhydantoin [86-35-1].

» Ethotoin contains not less than 97.5 percent and not more than 102.0 percent of $C_{11}H_{12}N_2O_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Ethotoin RS
USP 5-Phenylhydantoin RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 1 mg per mL.
Medium: alcohol.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chloride—Transfer 1.0 g of Ethotoin to a suitable separator, and dissolve in 50 mL of ether. Extract with three 15-mL portions of water, collect the combined extracts in a beaker, heat on a steam bath to expel any traces of ether, and allow to cool to room temperature. Transfer the solution to a 50-mL color-comparison tube, add 2 N nitric acid until the solution is acidic, add 1 mL of 2 N nitric acid in excess, mix, add 1 mL of silver nitrate TS, dilute with water to 50 mL, and allow to stand for 5 minutes, protected from direct sunlight. The turbidity produced does not exceed that of a solution prepared by mixing 2 mL of freshly prepared 0.002 N hydrochloric acid, 1 mL of 2 N nitric acid, 1 mL of silver nitrate TS, and 46 mL of water (0.014%).

Related compounds—
Buffer solution—Dissolve about 1 g of monobasic sodium phosphate in 1 L of water. Adjust the solution with 1.5 M phosphoric acid to a pH of 3.5 ± 0.1.
Diluent—Prepare a mixture of *Buffer solution* and methanol (65:35).
Solution A—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (80:20).
Solution B—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (60:40).
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard solution—Dissolve an accurately weighed quantity of USP Ethotoin RS, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 2.5 µg of ethotoin per mL.
Test solution—Transfer about 50 mg of Ethotoin, accurately weighed, to a 200-mL volumetric flask, dissolve in about 100

www.webofpharma.com

mL of *Diluent* with sonication, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The column temperature is maintained at 40°. The flow rate is about 0.8 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–10	100	0	isocratic
10–30	100→0	0→100	linear gradient
30–40	0	100	isocratic
40–42	0→100	100→0	linear gradient
42–55	100	0	re-equilibration

Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0 for ethotoin; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [NOTE—Discard any peak due to the *Diluent*.] Calculate the percentage of any impurity in the portion of Ethotoin taken by the formula:

$$(20{,}000/F)(C/W)(r_i/r_s)$$

in which C is the concentration, in mg per mL, of USP Ethotoin RS in the *Standard solution; F* is the response factor for the impurity as shown in the table below; W is the weight, in mg, of the portion of Ethotoin taken; r_i is the peak area for any impurity in the *Test solution;* and r_s is the peak area for ethotoin in the *Standard solution*. The impurities meet the requirements given in the table below.

Assay—

Mobile phase—Dissolve 0.65 g of monobasic potassium phosphate in 600 mL of water, adjust with phosphoric acid solution (1 in 10) to a pH of 3.5 ± 0.1, and dilute with water to 650 mL. Add 350 mL of methanol, mix, filter through a membrane filter of 0.5 µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

5-Phenylhydantoin stock solution—Dissolve, with the aid of sonication if necessary, an accurately weighed quantity of USP 5-Phenylhydantoin RS in *Mobile phase* to obtain a solution having a known concentration of about 0.37 mg per mL.

Standard preparation—Dissolve, with the aid of sonication if necessary, an accurately weighed quantity of USP Ethotoin in *Mobile phase* to obtain a solution having a known concentration of about 0.25 mg of ethotoin per mL.

System suitability solution—Transfer 25 mg of USP Ethotoin RS, accurately weighed, to a 100-mL volumetric flask, add about 1.0 mL of *5-Phenylhydantoin stock solution*, add *Mobile phase* to volume, and sonicate to dissolve.

Assay preparation—Transfer about 50 mg of Ethotoin, accurately weighed, to a 200-mL volumetric flask, dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and sonicate to dissolve.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the 5-phenylhydantoin and ethotoin peaks is not less than 6.0. The relative retention times are about 0.4 for 5-phenylhydantoin and 1.0 for ethotoin. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{11}H_{12}N_2O_2$ in the portion of Ethotoin taken by the formula:

$$200C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Ethotoin RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ethotoin Tablets

» Ethotoin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{11}H_{12}N_2O_2$.

Compound name	RRT[1]	RRF[2]	Limit (%)
5-Phenylhydantoin	about 0.4	1.0	1.5
3-Methyl-5-phenylhydantoin	about 0.6	1.0	0.9
Ethotoin	1.0	—	—
Ethotoin/5-Phenylhydantoin dimer	about 1.9	1.0	0.3
Ethotoin dimer	about 2.5	0.58	0.4
Unknown impurities	—	1.0	0.1 Individual
			1.0 Total Unknown
Total	—	—	2.0

[1] RRT—Relative retention time.
[2] RRF—Relative response factor.

USP Monographs

www.webofpharma.com

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Ethotoin RS
USP Ethylparaben RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 2: 100 rpm.
Time: 60 minutes.
Standard solution—Transfer about 100 mg of USP Ethotoin RS, accurately weighed, to a 25-mL volumetric flask. Dissolve in methanol, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, add *Dissolution Medium* to volume, and mix.
Procedure—Determine the amount of $C_{11}H_{12}N_2O_2$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 257 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with the *Standard solution*.
Tolerances—Not less than 80% (*Q*) of the labeled amount of $C_{11}H_{12}N_2O_2$ is dissolved in 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Diluent—Prepare a mixture of water, acetonitrile, and phosphoric acid (750:250:1).
Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (3:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Prepare a solution of USP Ethylparaben RS in *Diluent* having a concentration of 0.02 mg per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Ethotoin RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL. Immediately transfer 5 mL of this solution and 5 mL of the *Internal standard solution* to a suitable container, and mix well.
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of ethotoin, to a 100-mL volumetric flask. Add 75 mL of *Mobile phase*, shake vigorously for 60 minutes, dilute with *Mobile phase* to volume, mix, and immediately filter. Without delay, transfer 5 mL of the filtrate and 5 mL of the *Internal standard solution* to a suitable container, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the analyte and internal standard peaks is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [NOTE—For the purpose of identification, the relative retention times are about 0.5 for ethotoin and 1.0 for ethylparaben.] Calculate the quantity, in mg, of ethotoin ($C_{11}H_{12}N_2O_2$) in the portion of Tablets taken by the formula:

$$200C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Ethotoin RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ethyl Chloride

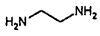


C_2H_5Cl 64.51
Ethane, chloro-.
Chloroethane [75-00-3].

» Ethyl Chloride contains not less than 99.5 percent and not more than 100.5 percent of C_2H_5Cl. [**CAUTION**—Ethyl Chloride is highly flammable. Do not use where it may be ignited.]

Packaging and storage—Preserve in tight containers, preferably hermetically sealed, and remote from fire.
Reaction—Shake 10 mL with 10 mL of water, both having been previously cooled to 0°, and allow the supernatant layer of ethyl chloride to volatilize spontaneously: the remaining liquid is neutral to litmus.
Limit of nonvolatile residue and odor—Allow 5 mL to evaporate spontaneously from a tared, shallow dish: no foreign odor is perceptible while the last portions evaporate, and the weight of the residue is negligible.
Chloride—Add a few drops of silver nitrate TS to 10 mL of alcohol, cool to 0°, and add to the clear liquid about 500 µL of Ethyl Chloride cooled to the same temperature: no turbidity is produced immediately.
Assay—Introduce about 1.5 mL of cold Ethyl Chloride into a tared glass-stoppered pressure bottle containing 25.0 mL of 1 N alcoholic potassium hydroxide VS, rapidly replace the stopper, and weigh accurately. Tie down the stopper, insert the bottle in a wire basket, and immerse in a water bath at room temperature. [**CAUTION**—Before raising the bath temperature, take adequate precautions to cover the bottle or erect a suitable safety shield to prevent injury in case the bottle should burst.] Heat the water bath to boiling, maintain at this temperature for 30 minutes, and then cool gradually to room temperature before handling the bottle. Remove the stopper, add phenolphthalein TS, and titrate the excess alkali with 1 N hydrochloric acid VS. Perform a blank determination (see *Residual Titrations* ⟨541⟩). Each mL of 1 N alcoholic potassium hydroxide is equivalent to 64.51 mg of C_2H_5Cl.

Ethylenediamine



$C_2H_8N_2$ 60.10
1,2-Ethanediamine.
Ethylenediamine [107-15-3].

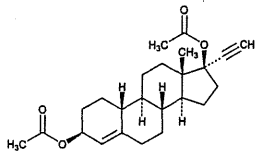
» Ethylenediamine contains not less than 98.0 percent and not more than 100.5 percent, by weight, of $C_2H_8N_2$.

www.webofpharma.com

[**CAUTION**—Use care in handling Ethylenediamine because of its caustic nature and the irritating properties of its vapor.]
[NOTE—Ethylenediamine is strongly alkaline and may readily absorb carbon dioxide from the air to form a nonvolatile carbonate. Protect Ethylenediamine against undue exposure to the atmosphere.]

Packaging and storage—Preserve in well-filled, tight, glass containers.
Identification—To 2 mL of cupric sulfate solution (1 in 100) add 3 drops of a solution of Ethylenediamine (1 in 6), and shake: a purplish blue color is produced.
Assay—Weigh accurately about 1 mL of Ethylenediamine in a tared, glass-stoppered flask containing about 25 mL of water. Dilute with water to about 75 mL, add a mixed indicator of bromocresol green TS and methyl red TS (5 in 6), mix, and titrate with 1 N hydrochloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 1 N hydrochloric acid is equivalent to 30.05 mg of $C_2H_8N_2$.

Ethynodiol Diacetate



$C_{24}H_{32}O_4$ 384.51
19-Norpregn-4-en-20-yne-3,17-diol, diacetate, (3β,17α)-;
19-Nor-17α-pregn-4-en-20-yne-3β,17-diol diacetate [297-76-7].

DEFINITION
Ethynodiol Diacetate contains NLT 97.0% and NMT 102.0% of ethynodiol diacetate ($C_{24}H_{32}O_4$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (41:59)
Standard stock solution: 2.5 mg/mL of USP Ethynodiol Diacetate RS, prepared as follows. Transfer a sufficient amount of USP Ethynodiol Diacetate RS into a suitable volumetric flask and dissolve, by sonication, in a volume of acetonitrile equivalent to 50% of the flask volume. Dilute with water to volume.
Standard solution: 0.25 mg/mL of USP Ethynodiol Diacetate RS in *Mobile phase* from *Standard stock solution*
Sample stock solution: 2.5 mg/mL of Ethynodiol Diacetate, prepared as follows. Transfer a sufficient amount of Ethynodiol Diacetate into a suitable volumetric flask and dissolve, by sonication, in a volume of acetonitrile equivalent to 50% of the flask volume. Dilute with water to volume.
Sample solution: 0.25 mg/mL of Ethynodiol Diacetate in *Mobile phase* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 15-cm; packing L11
Column temperature: 40°
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for ethynodiol diacetate is NLT 18 min.]
Suitability requirements
Tailing factor: 0.75–2.0
Relative standard deviation: NMT 0.7%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ethynodiol diacetate ($C_{24}H_{32}O_4$) in the portion of Ethynodiol Diacetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ethynodiol Diacetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Ethynodiol Diacetate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0%

IMPURITIES
• **PROCEDURE 1**
Mobile phase, Sample stock solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the area percentages of the individual impurities in the portion of Ethynodiol Diacetate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual peak between the solvent front and the ethynodiol diacetate peak
r_T = sum of the areas of all peaks appearing after the solvent front

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
α-Ethynodiol diacetate	0.87	1.5
Ethynodiol diacetate	1.0	—
Any other individual impurity	—	0.5
Total impurities	—	2.0

• **PROCEDURE 2: LIMIT OF CONJUGATED DIENE**
Sample solution: 0.5 mg/mL in methanol
Blank: Methanol
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: about 236 nm
Cell: 1 cm
Acceptance criteria: Absorbance is NMT 0.500.

USP Monographs

www.webofpharma.com

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in chloroform
Acceptance criteria: –70° to –76°

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ethynodiol Diacetate RS

Ethynodiol Diacetate and Ethinyl Estradiol Tablets

DEFINITION
Ethynodiol Diacetate and Ethinyl Estradiol Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of ethynodiol diacetate ($C_{24}H_{32}O_4$), and NLT 90.0% and NMT 110.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION
• The retention times of the ethynodiol diacetate and ethinyl estradiol peaks from the *Sample solution* correspond to those from the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol, acetonitrile, and water (3:7:10)
Standard solution: Dissolve, with the aid of sonication if necessary, quantities of USP Ethynodiol Diacetate RS and USP Ethinyl Estradiol RS in *Mobile phase*. Dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations, in mg/mL, of the Reference Standards, corresponding to about 1/25 of the labeled amounts of ethynodiol diacetate and ethinyl estradiol in the Tablets.
Sample solution: Place 10 Tablets in a 250-mL volumetric flask. Add a portion of *Mobile phase*, and sonicate until the Tablets are completely disintegrated. Cool to room temperature, dilute with *Mobile phase* to volume, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L10
Flow rate: 2 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates for ethynodiol diacetate
Tailing factor: NMT 1.5 for ethynodiol diacetate
Relative standard deviation: NMT 2.0% for each peak due to ethynodiol diacetate and ethinyl estradiol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{24}H_{32}O_4$ in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ethynodiol diacetate from the *Sample solution*
r_S = peak response of ethynodiol diacetate from the *Standard solution*
C_S = concentration of USP Ethynodiol Diacetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ethynodiol diacetate in the *Sample solution*

Calculate the percentage of $C_{20}H_{24}O_2$ in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ethinyl estradiol from the *Sample solution*
r_S = peak response of ethinyl estradiol from the *Standard solution*
C_S = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ethinyl estradiol in the *Sample solution*

Acceptance criteria: 93.0%–107.0% of the labeled amount of $C_{24}H_{32}O_4$ and 90.0%–110.0% of the labeled amount of $C_{20}H_{24}O_2$

PERFORMANCE TESTS
• **DISINTEGRATION** ⟨701⟩**:** 15 min, the use of disks being omitted
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Content Uniformity* with respect to ethynodiol diacetate and to ethinyl estradiol

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ethinyl Estradiol RS
USP Ethynodiol Diacetate RS

Ethynodiol Diacetate and Mestranol Tablets

DEFINITION
Ethynodiol Diacetate and Mestranol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of ethynodiol diacetate ($C_{24}H_{32}O_4$) and mestranol ($C_{21}H_{26}O_2$).

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHY**
Standard solution A: 5 mg/mL of USP Ethynodiol Diacetate RS in chloroform
Standard solution B: 0.5 mg/mL of USP Mestranol RS in chloroform
Sample solution: Transfer an amount equivalent to 10 mg of ethynodiol diacetate from powdered Tablets to a stoppered, 15-mL centrifuge tube. Add 10 mL of acetonitrile, insert the stopper in the tube, and mix by shaking and inversion for 2 min. Centrifuge at 1200 rpm for 10 min, and decant the supernatant through filter paper into a suitable container. Evaporate a 5-mL aliquot of the filtrate on a steam bath with the aid of a stream of nitrogen, and dissolve the residue in 1 mL of chloroform.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 5 µL
Developing solvent: Cyclohexane and ethyl acetate (70:30)
Spray reagent: A 1-in-2 solution of sulfuric acid in water

www.webofpharma.com

Analysis
Samples: *Standard solution A, Standard solution B,* and *Sample solution*
Remove the plate when the solvent has moved about 15 cm above the initial spots. Spray the plate with *Spray reagent,* and heat it at 80° for 10 min: the ethynodiol diacetate spots appear yellowish tan and the mestranol spots appear pink when viewed under white light.
Acceptance criteria: The ethynodiol diacetate and mestranol spots from the *Sample solution* have the same relative positions on the plate as the spots from the respective *Standard solutions.* The R_F values for ethynodiol diacetate and mestranol are 0.8 and 0.6, respectively.

ASSAY
• **PROCEDURE**
Diluent: Methanol and water (4:1)
Mobile phase: Methanol, tetrahydrofuran, and water (63:7:30)
Standard solution: 0.2 mg/mL of USP Ethynodiol Diacetate RS and 0.02 mg/mL of USP Mestranol RS in *Diluent*
Sample solution: Transfer 10 Tablets to a glass-stoppered flask, and pipet a sufficient volume of *Diluent* into the flask to obtain a solution having a final nominal concentration of 0.2 mg/mL of ethynodiol diacetate and 0.02 mg/mL of mestranol. Agitate vigorously until the Tablets are completely disintegrated. Shake vigorously by mechanical means for 30 min. Allow the solids to settle for 10 min, and pass the solution through a filter of 0.5-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 204 nm
Column: 4.6-mm × 25-cm; packing L13
Flow rate: 1.2 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for mestranol and ethynodiol diacetate are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between mestranol and ethynodiol diacetate
Relative standard deviation: NMT 2.0% for both mestranol and ethynodiol diacetate
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ethynodiol diacetate ($C_{24}H_{32}O_4$) and mestranol ($C_{21}H_{26}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

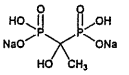
r_U = peak response of the corresponding analyte from the *Sample solution*
r_S = peak response of the corresponding analyte from the *Standard solution*
C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of ethynodiol diacetate ($C_{24}H_{32}O_4$) and mestranol ($C_{21}H_{26}O_2$)

PERFORMANCE TESTS
• **DISINTEGRATION** ⟨701⟩
Time: 15 min, omitting the use of disks
Acceptance criteria: Meet the requirements
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity: Proceed as directed in the *Assay,* except reduce by half the concentrations in the *Standard solution* and the *Sample solution* and inject twice the volume into the chromatograph.
Acceptance criteria: Meet the requirements for *Content Uniformity* with respect to ethynodiol diacetate and to mestranol

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ethynodiol Diacetate RS
USP Mestranol RS

Etidronate Disodium



$C_2H_6Na_2O_7P_2$ 249.99
Phosphonic acid, (1-hydroxyethylidene)bis-, disodium salt; Disodium dihydrogen (1-hydroxyethylidene)diphosphonate [7414-83-7].

DEFINITION
Etidronate Disodium contains NLT 97.0% and NMT 101.0% of etidronate disodium ($C_2H_6Na_2O_7P_2$), calculated on the anhydrous basis.

IDENTIFICATION
• **A. INFRARED ABSORPTION** ⟨197⟩
Standard: Recrystallize USP Etidronate Disodium RS from water and dry it at 105° for 1 h.
Sample: Recrystallize the sample from water and dry it at 105° for 1 h.
Analysis and **Acceptance criteria:** The spectra of trifluorovinyl chloride polymer and mineral oil dispersions of the *Sample,* separately prepared, exhibit maxima in the regions of 4000–1350 cm^{-1} and 1350–450 cm^{-1}, respectively, only at the same wavelengths as those of similar preparations of the *Standard.* The analysis and acceptance criteria described in ⟨197A⟩ may also be used.
• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*: Meets the requirements of test *A*
• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Mobile phase: 35–40 mM ammonium nitrate solution in water. Adjust with dilute ammonium hydroxide to a pH of 7.0.
Standard solution: 0.73–0.75 mg/mL of USP Etidronic Acid Monohydrate RS in 1 N sodium hydroxide VS and *Mobile phase* (1:150)
Sample solution: 0.84–0.86 mg/mL of Etidronate Disodium in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: Refractive index
Column: 4.6-mm × 15-cm; 10-µm packing L23

www.webofpharma.com

Temperatures
Column: 32°
Detector: 32°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.5% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of etidronate disodium ($C_2H_6Na_2O_7P_2$) in the portion of Etidronate Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Etidronic Acid Monohydrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Etidronate Disodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of etidronate disodium, 249.99
M_{r2} = molecular weight of etidronic acid monohydrate, 224.04

Acceptance criteria: 97.0%–101.0% on the anhydrous basis

IMPURITIES

• **Limit of Phosphite**
Solution A: 0.65 mg/mL of anhydrous sodium carbonate and 0.40 mg/mL of sodium bicarbonate in water
Solution B: 4.68 mg/mL of anhydrous sodium carbonate and 2.89 mg/mL of sodium bicarbonate in water
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
6.0	100	0
6.1	0	100
8.0	0	100
8.1	100	0
15	100	0

Suppressor regenerant solution: 12.5 mM sulfuric acid. [Note—This solution is needed only if the chemical suppression option is used.]
Standard solution: Equivalent to 0.016 mg/mL of dibasic sodium phosphite on the anhydrous basis from USP Etidronate Disodium Related Compound A RS and 0.015 mg/mL of dibasic sodium phosphate in *Solution A*. [Note—USP Etidronate Disodium Related Compound A RS is sodium phosphite dibasic pentahydrate.]
Sample solution: 5.0 mg/mL of Etidronate Disodium in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity
Columns
Guard: 4-mm × 50-mm; packing L61. [Note—Use either a 4-mm anion self-regenerating suppressor or a suitable chemical suppressor.]
Analytical: 4-mm × 25-cm; packing L61
Flow rate: 1.0 mL/min. [Note—When a chemical suppressor is used, the flow rate is 3–5 mL/min for the *Suppressor regenerant solution*.]
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[Note—The elution order is phosphite, followed by phosphate.]
Suitability requirements
Resolution: NLT 2.5 between phosphite and phosphate
Relative standard deviation: NMT 10% for each peak, for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phosphite, determined as monobasic sodium phosphite, in the portion of Etidronate Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of phosphite from the *Sample solution*
r_S = peak response of phosphite from the *Standard solution*
C_S = concentration of USP Etidronate Disodium Related Compound A RS on the anhydrous basis in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of monobasic sodium phosphite, 103.98
M_{r2} = molecular weight of dibasic sodium phosphite, 125.96

Acceptance criteria: NMT 1.0% of phosphite, determined as monobasic sodium phosphite

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: 10 mg/mL
Acceptance criteria: 4.2–5.2

Change to read:

• **Water Determination** ⟨921⟩, *Method I, Method Ic*
Sample solution: 100 mg of finely powdered Etidronate Disodium in 10 mL of a mixture of ▲glacial acetic acid▲ (ERR 1-Jul-2019) and formamide (1:1)
Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.
• **USP Reference Standards** ⟨11⟩
USP Etidronate Disodium RS
USP Etidronate Disodium Related Compound A RS
Sodium phosphite dibasic pentahydrate.
$Na_2HPO_3 \cdot 5H_2O$ 216.04
USP Etidronic Acid Monohydrate RS
Phosphonic acid, (1-hydroxyethylidene)bis-, monohydrate
$C_2H_8O_7P_2 \cdot H_2O$ 224.04

USP Monographs

www.webofpharma.com

Etidronate Disodium Tablets

DEFINITION

Etidronate Disodium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of etidronate disodium ($C_2H_6Na_2O_7P_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*:** 197A or 197M▲ (CN 1-May-2020)

Standard: Recrystallize USP Etidronate Disodium RS from water and dry it at 105° for 1 h.

Sample: Recrystallize the sample from water and dry it at 105° for 1 h.

Analysis and **Acceptance criteria ▲for ⟨197M⟩:▲** (CN 1-May-2020) The spectra of trifluorovinyl chloride polymer and mineral oil dispersions of the *Sample*, separately prepared, exhibit maxima in the regions of 4000–1350 cm^{-1} and 1350–450 cm^{-1}, respectively, only at the same wavelengths as those of similar preparations of the *Standard*. ▲▲ (CN 1-May-2020)

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: 35–40 mM ammonium nitrate solution in water. Adjust with dilute ammonium hydroxide to a pH of 7.0.

Standard solution: 0.73–0.75 mg/mL of USP Etidronic Acid Monohydrate RS in 1 N sodium hydroxide VS and *Mobile phase* (1:150)

Sample solution: Transfer a quantity of finely powdered Tablets (NLT 20), nominally equivalent to 160–170 mg of etidronate disodium, to a 200-mL volumetric flask, and dilute with *Mobile phase* to volume. Agitate the solution for at least 5 min, and pass through a nylon filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4.6-mm × 15-cm; 10-µm packing L23
Temperatures
Detector: 32°
Column: 32°
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.5%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of etidronate disodium ($C_2H_6Na_2O_7P_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Etidronic Acid Monohydrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of etidronate disodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of etidronate disodium, 249.99
M_{r2} = molecular weight of etidronic acid monohydrate, 224.04

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION ⟨711⟩**

Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Mobile phase: Prepare as directed in the *Assay*.
Standard solution
For products labeled to contain 200 mg of etidronate disodium: Transfer 20 mg of USP Etidronic Acid Monohydrate RS to a 100-mL volumetric flask, dissolve in 50 mL of water, add 2.0 mL of 0.1 N sodium hydroxide VS, and dilute with water to volume.
For products labeled to contain 400 mg of etidronate disodium: Transfer 20 mg of USP Etidronic Acid Monohydrate RS to a 50-mL volumetric flask, dissolve in 25 mL of water, add 2.0 mL of 0.1 N sodium hydroxide VS, and dilute with water to volume.
Sample solution: Transfer a portion of the solution under test to an HPLC vial.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4.6-mm × 15-cm; 10-µm packing L23
Temperatures
Detector: 32°
Column: 32°
Flow rate: 1.5 mL/min
Injection volume: See *Table 1*.

Table 1

Tablet Strength (mg)	Injection Volume (µL)
200	100
400	50

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.5%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of etidronate disodium ($C_2H_6Na_2O_7P_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Etidronic Acid Monohydrate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
M_{r1} = molecular weight of etidronate disodium, 249.99
M_{r2} = molecular weight of etidronic acid monohydrate, 224.04
L = label claim (mg/Tablet)

Tolerances: NLT 70% (Q) of the labeled amount of etidronate disodium ($C_2H_6Na_2O_7P_2$) is dissolved.

www.webofpharma.com

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Limit of Phosphite and Phosphate**

Mobile phase: 20 mM ammonium nitrate solution in water. Adjust with dilute ammonium hydroxide to a pH of 7.0.

Standard solution: A mixture of 0.04 mg/mL of dibasic sodium phosphite, on the anhydrous basis from USP Etidronate Disodium Related Compound A RS, and anhydrous dibasic sodium phosphate in *Mobile phase*

Sample solution: Nominally equivalent to 4.0 mg/mL of etidronate disodium in *Mobile phase* prepared as follows. Transfer a quantity of finely powdered Tablets (NLT 20), equivalent to 200 mg of etidronate disodium, to a 50-mL volumetric flask. Add about 40 mL of *Mobile phase*, dissolved by sonication, and dilute with *Mobile phase* to volume. Centrifuge a portion and use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 4.6-mm × 15-cm; 10-µm packing L23

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

[NOTE—The typical relative retention times are about 0.7 for the phosphate, relative to the phosphite, and 1.0 for the phosphite.]

Suitability requirements

Resolution: NLT 1.5 between the phosphite and phosphate peaks

Relative standard deviation: NMT 5% for both the phosphite and phosphate peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of phosphite, determined as monobasic sodium phosphite, in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of phosphite from the *Sample solution*

r_S = peak response of phosphite from the *Standard solution*

C_S = concentration of USP Etidronate Disodium Related Compound A RS on the anhydrous basis in the *Standard solution* (mg/mL)

C_U = nominal concentration of etidronate disodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of monobasic sodium phosphite, 103.98

M_{r2} = molecular weight of dibasic sodium phosphite, 125.96

Calculate the percentage of phosphate, determined as dibasic sodium phosphate, in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phosphate from the *Sample solution*

r_S = peak response of phosphate from the *Standard solution*

C_S = concentration of dibasic sodium phosphate in the *Standard solution* (mg/mL)

C_U = nominal concentration of etidronate disodium in the *Sample solution* (mg/mL)

Acceptance criteria

Phosphite: NMT 1.0%, determined as monobasic sodium phosphite

Phosphate: NMT 1.0%, determined as dibasic sodium phosphate

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **USP Reference Standards** ⟨11⟩

USP Etidronate Disodium RS

USP Etidronate Disodium Related Compound A RS

Sodium phosphite dibasic pentahydrate.

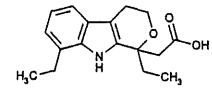
$Na_2HPO_3 \cdot 5H_2O$ 216.04

USP Etidronic Acid Monohydrate RS

Phosphonic acid, (1-hydroxyethylidene)bis-, monohydrate

$C_2H_8O_7P_2 \cdot H_2O$ 224.04

Etodolac



$C_{17}H_{21}NO_3$ 287.35

Pyrano[3,4-*b*]indole-1-acetic acid, 1,8-diethyl-1,3,4,9-tetrahydro-(±)-.

(±)-1,8-Diethyl-1,3,4,9-tetrahydropyrano [3,4-*b*]indole-1-acetic acid [41340-25-4].

» Etodolac contains not less than 98.0 percent and not more than 102.0 percent of $C_{17}H_{21}NO_3$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Etodolac RS

USP Etodolac Related Compound A RS

(±)-8-Ethyl-1-methyl-1,3,4,9-tetrahydropyrano [3,4-*b*]-indole-1-acetic acid.

$C_{16}H_{19}NO_3$ 273.33

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197K***▲ (CN 1-May-2020)

Water Determination, *Method I* ⟨921⟩: not more than 0.5%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Limit of chloride—Dissolve 1.0 g of Etodolac in 60 mL of methanol, dilute with 10 mL of water, add 20 mL of 2 N nitric acid, and titrate with 0.01 N silver nitrate determining the endpoint potentiometrically (see *Titrimetry* ⟨541⟩): the limit is not more than 0.3 mg per g.

Limit of alcohol and methanol—

Internal standard stock solution—Dissolve suitable quantities of isopropyl alcohol in dimethylformamide to obtain a solution containing about 2.5 µL per mL.

Internal standard solution—Pipet 5.0 mL of *Internal standard stock solution* into a 100-mL volumetric flask. Dilute with dimethylformamide to volume, and mix. This solution contains 0.125 µL of isopropyl alcohol.

USP Monographs

www.webofpharma.com

Standard stock solution—Transfer 5.0 mL of methanol and 5.0 mL of alcohol to a 200-mL volumetric flask. Dilute with dimethylformamide to volume, and mix. Pipet 5.0 mL of this solution into a 100-mL volumetric flask, dilute with dimethylformamide to volume, and mix.

Standard solution—Pipet 10.0 mL of *Standard stock solution* and 5.0 mL of *Internal standard stock solution* into a 100-mL volumetric flask. Dilute with dimethylformamide to volume, and mix. This solution contains 0.101 mg of methanol and 0.099 mg of alcohol per mL.

Test solution—Transfer about 0.5 g of Etodolac, accurately weighed, to a 10-mL flask. Pipet 5.0 mL of *Internal standard solution* into the flask. Insert a stopper into the flask, and sonicate to dissolve.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm × 25-m fused silica capillary column coated with a 5-µm film of phase G36. The carrier gas is helium, with a split flow rate of 50 mL per minute. The injection port temperature is maintained at 200°, and the detector is maintained at 300°. The column temperature is maintained at 45° for 5 minutes, then programmed to increase at the rate of 30° per minute to 280° and to maintain this temperature for 27 minutes. Chromatograph the *Standard solution* and the *Test solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for methanol, 0.8 for alcohol, and 1.0 for isopropyl alcohol; and the resolution, *R*, between peaks is not less than 1.

Procedure—Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the peak responses. Identify by their retention times any peaks present in the chromatogram obtained from the *Test solution* that correspond to those in the chromatogram obtained from the *Standard solution*. Calculate the percentages of C_2H_5OH and CH_3OH in the portion of Etodolac taken by the formula:

$$500(C/W)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of C_2H_5OH or CH_3OH in the *Standard solution*; W is the weight, in mg, of Etodolac taken to prepare the *Test solution*; and R_U and R_S are the peak area ratios of the relevant analyte to the internal standard obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.1% of each is found.

Chromatographic purity—

Solution A—Mix 0.6 mL of phosphoric acid with 100 mL of water.

Solution B—Mix 0.6 mL of phosphoric acid with 100 mL of acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve suitable quantities of USP Etodolac Related Compound A RS and USP Etodolac RS in acetonitrile to obtain a solution containing about 0.01 mg per mL and 0.2 mg per mL, respectively.

Test solution—Transfer about 25 mg of Etodolac, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4-mm × 25-cm column that contains packing L7. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0-5	60	40	isocratic
5-35	60→20	40→80	linear gradient
	60	40	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for etodolac related compound A and 1.0 for etodolac; the resolution, *R*, between etodolac related compound A and etodolac is not less than 3; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 3%.

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Etodolac taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all of the peaks: not more than 0.5% of any individual impurity is found; and the total of all impurities is not more than 2.0%.

Assay—Dissolve about 230 mg of Etodolac in 60 mL of methanol. Titrate with 0.1 N tetrabutylammonium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 28.74 mg of $C_{17}H_{21}NO_3$.

Etodolac Capsules

» Etodolac Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of etodolac ($C_{17}H_{21}NO_3$).

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—

USP Etodolac RS

USP Etodolac Related Compound A RS

(±)-8-Ethyl-1-methyl-1,3,4,9-tetrahydropyrano [3,4-*b*]-indole-1-acetic acid.

$C_{16}H_{19}NO_3$ 273.33

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—

Medium: pH 6.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 1000 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{17}H_{21}NO_3$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 274 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Etodolac RS in the same *Medium*.

Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{17}H_{21}NO_3$ is dissolved in 30 minutes.

www.webofpharma.com

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and phosphoric acid (500: 500: 0.25). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Etodolac RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL. Prepare this solution fresh daily.

System suitability solution—Dissolve suitable quantities of USP Etodolac Related Compound A RS and USP Etodolac RS in *Mobile phase* to obtain a solution containing about 0.01 mg of etodolac related compound A and 0.2 mg of etodolac per mL.

Assay preparation—Weigh not fewer than 20 Capsules, and transfer the contents as completely as possible to a suitable container. Remove any residual powder from the empty capsules with the aid of a current of air, and weigh the capsule shells, determining the weight of the contents by difference. Mix the contents of the Capsules, and transfer an accurately weighed portion of the powder, equivalent to about 1000 mg of etodolac, to a 500-mL volumetric flask, add 300 mL of *Mobile phase*, shake for 15 minutes, sonicate for 5 minutes, cool, dilute with *Mobile phase* to volume, and mix. Allow to settle for 10 minutes. Pipet 10.0 mL of the solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass the solution through a filter having a 0.45-µm or finer porosity, prior to use.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 274-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for etodolac related compound A and 1.0 for etodolac; the resolution, *R*, between etodolac related compound A and etodolac is not less than 2; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of etodolac ($C_{17}H_{21}NO_3$) in the portion of Capsule contents taken by the formula:

$$5000C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Etodolac RS in the *Standard preparation;* and r_U and r_S are the etodolac peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Etodolac Tablets

» Etodolac Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of etodolac ($C_{17}H_{21}NO_3$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Etodolac RS

USP Etodolac Related Compound A RS

(±)-8-Ethyl-1-methyl-1,3,4,9-tetrahydropyrano [3,4-*b*]-indole-1-acetic acid.

$C_{16}H_{19}NO_3$ 273.33

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Dissolution ⟨711⟩—

Medium: pH 6.8 Phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 1000 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{17}H_{21}NO_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 274 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Etodolac RS in the same *Medium*.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{17}H_{21}NO_3$ is dissolved in 30 minutes.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and phosphoric acid (500: 500: 0.25). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve suitable quantities of USP Etodolac Related Compound A RS and USP Etodolac RS in *Mobile phase* to obtain a solution containing about 0.01 mg of etodolac related compound A and 0.2 mg of etodolac per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Etodolac RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL. Prepare this solution fresh daily.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1000 mg of etodolac, to a 500-mL volumetric flask, add 300 mL of *Mobile phase*, shake for 15 minutes, sonicate for 5 minutes, cool, dilute with *Mobile phase* to volume, and mix. Allow to settle for 10 minutes. Pipet 10.0 mL of the solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass the solution through a filter having a porosity of 0.45 µm or less.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 274-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for etodolac related compound A and 1.0 for etodolac; the resolution, *R*, between etodolac related compound A and etodolac is not less than 2; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of etodolac ($C_{17}H_{21}NO_3$) in the portion of Tablets taken by the formula:

$$5000C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Etodolac RS in the *Standard preparation;* and r_U and r_S are the etodolac

www.webofpharma.com

peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Etodolac Extended-Release Tablets

» Etodolac Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of etodolac ($C_{17}H_{21}NO_3$).

Packaging and storage—Preserve in well-closed containers. Store at controlled room temperature, protected from light.
Labeling—When more than one *Dissolution Test* is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.
USP Reference standards ⟨11⟩—
USP Etodolac RS
USP Etodolac Related Compound A RS
(±)-8-Ethyl-1-methyl-1,3,4,9-tetrahydropyrano [3,4-*b*]-indole-1-acetic acid.
$C_{16}H_{19}NO_3$ 273.33
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—
TEST 1—
Medium: 0.05 M phosphate buffer, pH 7.4; 1000 mL.
Apparatus 2: 75 rpm, with USP sinker.
Times: 3, 6, 10, and 16 hours.
Procedure—Determine the amount of $C_{17}H_{21}NO_3$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 279 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Etodolac RS in the same *Medium*. Use *Medium* as the blank.
Tolerances—The percentages of the labeled amount of $C_{17}H_{21}NO_3$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
3	between 15% and 40%
6	between 35% and 70%
10	between 60% and 95%
16	not less than 80%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.05 M phosphate buffer, pH 7.5; 1000 mL.
Apparatus 2: 100 rpm.
Times: 2, 4, 8, and 14 hours.
Procedure—Determine the amount of $C_{17}H_{21}NO_3$ dissolved by comparing the difference between the absorbances of the filtered portions of the solution under test determined at 278 nm and 245 nm with the difference between the absorbances of a Standard solution having a known concentration of USP Etodolac RS in the same *Medium* determined at the same wavelengths. Use *Medium* as the blank, and use a 0.05-cm silica cell.
Tolerances—The percentages of the labeled amount of $C_{17}H_{21}NO_3$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
2	between 10% and 35%
4	between 30% and 55%
8	between 60% and 90%
14	not less than 85%

TEST 3—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.05 M phosphate buffer, pH 6.8; 1000 mL.
Apparatus 2: 75 rpm.
Times: 2, 4, 8, and 14 hours.
Procedure—Determine the amount of $C_{17}H_{21}NO_3$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 278 nm on portions of the solution under test passed through a 10-µm polyethylene filter, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Etodolac RS in the same *Medium*. Use *Medium* as the blank, and use a 0.05-cm silica cell.
Tolerances—The percentages of the labeled amount of $C_{17}H_{21}NO_3$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
2	between 10% and 30%
4	between 30% and 50%
8	between 55% and 75%
14	not less than 80%

TEST 4—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.05 M phosphate buffer, pH 6.8; 900 mL.
Apparatus 2: 75 rpm, with a wire helix sinker.
Times: 2, 4, 8, and 18 hours.
Test solution—Pass a portion of the solution under test through a suitable 70-µm filter.
Standard solution—From a solution containing about 4 mg per mL of USP Etodolac RS in *Mobile phase*, make dilutions with *Medium* to obtain a solution with a final concentration of about *L*/1500 mg per mL, where *L* is the tablet label claim, in mg.
Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and concentrated phosphoric acid (500: 500: 0.25). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Chromatographic system (see Chromatography ⟨621⟩*)*—The liquid chromatograph is equipped with a 274-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. The column is maintained at 25°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of etodolac dissolved at the times specified.

USP Monographs

www.webofpharma.com

Tolerances—The percentages of the labeled amount of $C_{17}H_{21}NO_3$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
2	between 10% and 30%
4	between 20% and 45%
8	between 40% and 65%
18	not less than 80%

Uniformity of dosage units ⟨905⟩: meet the requirements.

Chromatographic purity—

Diluent, Mobile phase, and *System suitability solution*—Proceed as directed in the *Assay*.

Test solution—Use the *Assay preparation*.

Chromatographic system—Prepare as directed in the *Assay*. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for etodolac related compound A and 1.0 for etodolac; the resolution, *R,* between etodolac related compound A and etodolac is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak areas. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak area for each impurity, and r_s is the sum of the areas of all the peaks: not more than 0.2% of any individual impurity is found; and not more than 0.75% of total impurities is found.

Assay—

Diluent—Use acetonitrile.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and phosphoric acid (500: 500: 0.25). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve accurately weighed quantities of USP Etodolac RS and USP Etodolac Related Compound A RS in *Diluent,* and quantitatively dilute with *Diluent* to obtain a solution having known concentrations of about 0.48 mg per mL and 0.05 mg per mL, respectively.

Standard preparation—Dissolve an accurately weighed quantity of USP Etodolac RS in *Diluent,* and quantitatively dilute with *Diluent* to obtain a solution having a known concentration of about 0.6 mg per mL.

Assay preparation— [NOTE—Do not finely powder Tablets.] Weigh and powder not fewer than 20 Tablets, and transfer an accurately weighed portion of the powder, equivalent to about 600 mg of etodolac, to a 200-mL volumetric flask. Add about 100 mL of *Diluent,* mix, and shake for 40 minutes by mechanical means. Dilute with *Diluent* to volume, and mix. Pass through a filter having a 0.45-µm porosity, discarding the first 3 mL of the filtrate. Transfer 2.0 mL of the filtrate to a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix.

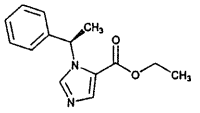
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 274-nm detector, a 4.0-mm × 4.0-cm guard column that contains 5-µm packing L7, and a 4.0-mm × 25-cm column that contains 5-µm packing L7. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for etodolac related compound A and 1.0 for etodolac; the resolution, *R,* between etodolac related compound A and etodolac is not less than 2.5; and the tailing factor is not more than 2.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of etodolac ($C_{17}H_{21}NO_3$) in the portion of Tablets taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Etodolac RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Etomidate



$C_{14}H_{16}N_2O_2$ 244.29

1*H*-Imidazole-5-carboxylic acid, 1-(1-phenylethyl)-, ethyl ester, (+)-;

(+)-Ethyl 1-(α-methylbenzyl)imidazole-5-carboxylate [33125-97-2].

DEFINITION

Etomidate contains NLT 98.0% and NMT 102.0% of etomidate ($C_{14}H_{16}N_2O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.7 g/L of monobasic sodium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (40:60)

Standard solution: 0.16 mg/mL of USP Etomidate RS in acetonitrile

Sample solution: 0.16 mg/mL of Etomidate in acetonitrile

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2.3 mL/min

Injection volume: 20 µL

Run time: NLT 3 times the retention time of etomidate

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of etomidate ($C_{14}H_{16}N_2O_2$) in the portion of Etomidate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Etomidate RS in the *Standard solution* (mg/mL)
C_U = concentration of Etomidate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **ORGANIC IMPURITIES**
Solution A: Dissolve 6 g of sodium citrate dihydrate and 4 g of anhydrous citric acid in 1 L of water.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	30	70
21	95	5
30	95	5

Diluent: Weigh 6 g of sodium citrate dihydrate and 4 g of anhydrous citric acid into a 1000-mL volumetric flask. Add 500 mL of water, and shake to dissolve. Add 110 mL of acetonitrile and 50 mL of methanol, and dilute with water to volume.
System suitability solution: 0.02 mg/mL each of USP Etomidate RS and USP Metomidate Hydrochloride RS in *Diluent*
Standard solution: 0.004 mg/mL of USP Metomidate Hydrochloride RS in *Diluent*
Sensitivity solution: 0.8 µg/mL of USP Metomidate Hydrochloride RS from the *Standard solution* in *Diluent*
Sample solution: 0.8 mg/mL of Etomidate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2.0 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between etomidate and metomidate, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Etomidate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of metomidate from the *Standard solution*
C_S = concentration of USP Metomidate Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Etomidate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of metomidate free base, 230.26
M_{r2} = molecular weight of metomidate hydrochloride, 266.72

Acceptance criteria: See *Table 2*. [NOTE—Disregard any impurity peaks less than 0.05%.]

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Etomidate acid[a]	0.34	0.1
Metomidate[b]	0.90	0.1
Etomidate	1.0	—
Any unspecified impurity	—	0.1
Total impurities	—	1.0

[a] 1-(1-Phenylethyl)-1*H*-imidazole-5-carboxylic acid.
[b] Methyl 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Sample: 1 g of Etomidate
Analysis: Dry the *Sample* over phosphorus pentoxide for 16 h.
Acceptance criteria: NMT 0.5%
• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL of Etomidate in dehydrated alcohol
Acceptance criteria: 67.0°–70.0° (t = 20°), calculated on the dried basis

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well closed, light-resistant containers, and store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Etomidate RS
USP Metomidate Hydrochloride RS
Methyl 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate hydrochloride.
$C_{13}H_{14}N_2O_2 \cdot HCl$ 266.72

Etomidate Injection

DEFINITION
Etomidate Injection is a sterile, nonpyrogenic solution. It contains NLT 90.0% and NMT 110.0% of the labeled amount of etomidate ($C_{14}H_{16}N_2O_2$). It may contain suitable buffers and preservatives.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
  Sample solution: 10 µg/mL of etomidate in isopropyl alcohol
  Medium: Isopropyl alcohol
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Buffer: 0.7 g/L of monobasic sodium phosphate in water
  Mobile phase: Acetonitrile and *Buffer* (2:3)
  Standard solution: 0.16 mg/mL of USP Etomidate RS in acetonitrile
  Sample solution: Nominally 0.16 mg/mL of etomidate from Injection in acetonitrile
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 3.9-mm × 30-cm; packing L1
  Flow rate: 2.3 mL/min
  Injection volume: 20 µL
  Run time: NLT 3 times the retention time of etomidate
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of etomidate ($C_{14}H_{16}N_2O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Etomidate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of etomidate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES, PROCEDURE 1: RELATED COMPOUNDS**
  Solution A: Dissolve 6 g of sodium citrate dihydrate and 4 g of anhydrous citric acid in 1 L of water.
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	30	70
21	95	5
30	95	5

Diluent: Weigh 6 g of sodium citrate dihydrate and 4 g of anhydrous citric acid into a 1000-mL volumetric flask. Add 500 mL of water, and shake to dissolve. Add 110 mL of acetonitrile and 50 mL of methanol, and dilute with water to volume.
System suitability solution: 0.02 mg/mL each of USP Etomidate RS and USP Metomidate Hydrochloride RS in *Diluent*
Standard stock solution: 0.1 mg/mL of USP Metomidate Hydrochloride RS in methanol
Standard solution: 0.004 mg/mL of USP Metomidate Hydrochloride RS from the *Standard stock solution* in *Diluent*
Sensitivity solution: 0.8 µg/mL of USP Metomidate Hydrochloride RS from the *Standard solution* in *Diluent*
Sample solution: Nominally 0.8 mg/mL of etomidate from Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2.0 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between etomidate and metomidate, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of metomidate from the *Standard solution*
C_S = concentration of USP Metomidate Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of etomidate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of metomidate free base, 230.26
M_{r2} = molecular weight of metomidate hydrochloride, 266.72

Acceptance criteria: See *Table 2*. [NOTE—Disregard any impurity peaks less than 0.05%.]

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Etomidate acid[a]	0.34	1.4
Propylene glycol ester[b]	0.77	—
Metomidate[c]	0.90	0.1
Etomidate	1.0	—
Any unspecified impurity	—	0.1

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	1.6

[a] 1-(1-Phenylethyl)-1*H*-imidazole-5-carboxylic acid.
[b] This is quantitated in *Organic Impurities, Procedure 2: Total Propylene Glycol Esters.*
[c] Methyl 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate.

• **ORGANIC IMPURITIES, PROCEDURE 2: TOTAL PROPYLENE GLYCOL ESTERS:** [NOTE—Perform this test if propylene glycol is used in the formulation.]
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 1.6 µg/mL of USP Etomidate RS in acetonitrile
Standard solution: 0.025 mg/mL of USP Etomidate RS in acetonitrile
Sample solution: Nominally 1.6 mg/mL of etomidate from Injection in acetonitrile
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 3.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of etomidate from the *Standard solution*
C_S = concentration of USP Etomidate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of etomidate in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any impurity peaks less than 0.05%.

Table 3

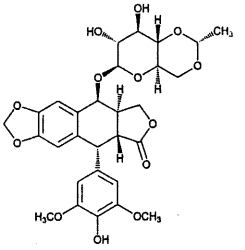
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Etomidate acid[a]	0.16	—	—
Total propylene glycol esters[b]	0.40	0.9	2.8
Etomidate	1.0	1.0	—
Any unspecified impurity	—	1.0	0.1
Total impurities[c]	—	—	4.0

[a] This is quantitated in *Organic Impurities, Procedure 1: Related Compounds.*
[b] 2-Hydroxypropyl ester and 2-hydroxy-1-methylethyl ester.
[c] Total impurities include all impurities from *Table 2* and *Table 3*.

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 8.35 USP Endotoxin Units/mg of etomidate.
• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
• **pH** ⟨791⟩: Between 4.0 and 7.0
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements for small-volume injections.
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Etomidate RS
USP Metomidate Hydrochloride RS
Methyl 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate hydrochloride.
$C_{13}H_{14}N_2O_2 \cdot HCl$ 266.72

Etoposide



$C_{29}H_{32}O_{13}$ 588.56
Furo[3′,4′:6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one-, 9-[(4,6-*O*-ethylidene-β-D-glucopyranosyl)oxy]5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl), [5*R*-[5α, 5aβ,8aα,9β(*R**)]]-;
4′-Demethylepipodophyllotoxin 9-[4,6-*O*-(*R*)-ethylidene-β-D-glucopyranoside] [33419-42-0].

DEFINITION
Etoposide contains NLT 95.0% and NMT 105.0% of etoposide ($C_{29}H_{32}O_{13}$), calculated on the anhydrous basis.
[**CAUTION**—Etoposide is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 2.72 g/L of sodium acetate in water. Adjust with glacial acetic acid to a pH of 4.0.
Mobile phase: Acetonitrile and *Buffer* (26:74)
System suitability solution: 0.3 mg/mL of USP Etoposide Resolution Mixture RS in *Mobile phase*
Standard stock solution: 2.0 mg/mL of USP Etoposide RS in acetonitrile

USP Monographs

www.webofpharma.com

Standard solution: 0.2 mg/mL of USP Etoposide RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: 2.0 mg/mL of Etoposide in acetonitrile
Sample solution: 0.2 mg/mL of Etoposide in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L11
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of etoposide
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.35 between the etoposide and α-etoposide peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of etoposide ($C_{29}H_{32}O_{13}$) in the portion of Etoposide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of etoposide from the *Sample solution*
r_S = peak response of etoposide from the *Standard solution*
C_S = concentration of USP Etoposide RS in the *Standard solution* (mg/mL)
C_U = concentration of Etoposide in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.1%
- **Organic Impurities**

Buffer: Prepare as directed in the *Assay*.
Solution A: Acetonitrile and *Buffer* (20:80)
Solution B: Acetonitrile and *Buffer* (60:40)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
30	40	60
40	40	60
42	0	100
45	0	100
47	100	0
50	100	0

Diluent: Acetonitrile and 0.02 M sodium acetate previously adjusted with acetic acid to a pH of 4.0 (30:70)
Standard stock solution: 2.0 mg/mL of USP Etoposide RS in *Diluent*
System suitability stock solution: 0.2 mg/mL of *n*-propylparaben in *Diluent*
System suitability solution: Transfer 5.0 mL of the *System suitability stock solution* and 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume.
Standard solution: 10 µg/mL of USP Etoposide RS from the *Standard stock solution* in *Diluent*
Sample solution: 2.0 mg/mL of Etoposide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 15-cm; less than 5-µm packing L11
Flow rate: 1.5 mL/min
Injection volume: 25 µL
Run time: NLT 40 min
System suitability
[NOTE—Run time is 15 min in isocratic conditions.]
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.1 between propylparaben and etoposide
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Etoposide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of etoposide from the *Standard solution*
C_S = concentration of USP Etoposide RS in the *Standard solution* (mg/mL)
C_U = concentration of Etoposide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lignan P	0.20	0.5
Etoposide	1.0	—
Picroetoposide	1.43	1.0
Any unspecified impurity	—	—
Total impurities	—	2.0

SPECIFIC TESTS

- **Optical Rotation,** *Specific Rotation* ⟨781S⟩

Sample solution: 5 mg/mL in chloroform and methanol (9:1)
Acceptance criteria: −110° to −118° (t = 20°)

- **Water Determination,** *Method I* ⟨921⟩: NMT 6.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩

USP Etoposide RS
USP Etoposide Resolution Mixture RS

www.webofpharma.com

Etoposide Capsules

DEFINITION

Etoposide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of etoposide ($C_{29}H_{32}O_{13}$).

[**CAUTION**—Etoposide is potentially cytotoxic. Great care should be taken to prevent inhaling particles of Etoposide and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Transfer a suitable quantity of the contents of Capsules, equivalent to 100 mg of etoposide, to a separator containing 100 mL of water. Extract twice with 20-mL portions of chloroform, separate and combine the organic layers, dry over anhydrous sodium sulfate, and filter. Transfer the dried filtrate to a second separator, extract with 30 mL of water, and allow the layers to separate. Drain the chloroform layer through a bed of anhydrous sodium sulfate contained in a filter funnel into a round-bottom flask, and evaporate the chloroform at a temperature of 30 ± 5° using a rotary evaporator. Dissolve the oily residue obtained in 5 mL of water, shake gently, and allow to stand for 30 min. Filter, collecting the precipitate formed on a glass filter funnel, wash the precipitate with three 20-mL portions of water, and allow the precipitate to dry on the filter for about 90 min in a vacuum oven at 40°. Prepare a dispersion of the precipitate in potassium bromide at a ratio of 1 in 100.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 2.72 g/L of sodium acetate in water. Adjust with glacial acetic acid to a pH of 4.0.

Mobile phase: Acetonitrile and *Buffer* (26:74)

System suitability solution: 0.3 mg/mL of USP Etoposide Resolution Mixture RS in *Mobile phase*

Standard stock solution: 2.0 mg/mL of USP Etoposide RS in acetonitrile

Standard solution: 0.2 mg/mL of USP Etoposide RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: Transfer a sufficient number of Capsules, equivalent to 500 mg of etoposide, to a 500-mL volumetric flask, add about 400 mL of *Mobile phase*, and stir using a magnetic bar for about 15 min, followed by sonication for about 1 h with occasional shaking. Cool, dilute with *Mobile phase* to volume, stir for an additional 5 min, and filter.

Sample solution: Equivalent to 0.2 mg/mL of Etoposide in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L11

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 1.5 times the retention time of etoposide

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.35 between the etoposide and α-etoposide peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of etoposide ($C_{29}H_{32}O_{13}$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of etoposide from the *Sample solution*

r_S = peak response of etoposide from the *Standard solution*

C_S = concentration of USP Etoposide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of etoposide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: pH 4.5 acetate buffer; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Buffer: 2.72 g/L of sodium acetate in water. Adjust with acetic acid to a pH of 4.0.

Mobile phase: Acetonitrile and *Buffer* (26:74)

Standard solution: 55 µg/mL of USP Etoposide RS prepared as follows. Transfer a quantity of USP Etoposide RS to a suitable volumetric flask, and dissolve with sonication in methanol equivalent to 2% of the final volume. Dilute with *Medium* to volume.

Sample solution: Withdraw a 10-mL aliquot from the dissolution vessel.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 3.9-mm × 30-cm; packing L11

Flow rate: 2 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of etoposide ($C_{29}H_{32}O_{13}$) dissolved.

Tolerances: NLT 80% (*Q*) of the labeled amount of etoposide ($C_{29}H_{32}O_{13}$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer: Prepare as directed in the *Assay*.

Solution A: Acetonitrile and *Buffer* (20:80)

Solution B: Acetonitrile and *Buffer* (60:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
30	40	60

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
40	40	60
42	0	100
45	0	100
47	100	0
50	100	0

Diluent: Acetonitrile and 0.02 M sodium acetate previously adjusted with acetic acid to a pH of 4.0 (30:70)
Standard stock solution: 2.0 mg/mL of USP Etoposide RS in *Diluent*
System suitability stock solution: 0.2 mg/mL of *n*-propylparaben in *Diluent*
System suitability solution: Transfer 5.0 mL of the *System suitability stock solution* and 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume.
Standard solution: 10 µg/mL of USP Etoposide RS from the *Standard stock solution* in *Diluent*
Sample solution: Nominally equivalent to 2.0 mg/mL of etoposide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 15-cm; less than 5-µm packing L11
Flow rate: 1.5 mL/min
Injection volume: 25 µL
Run time: NLT 40 min
System suitability
Run time is 15 min under isocratic conditions.
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.1 between propylparaben and etoposide
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of etoposide from the *Standard solution*
C_S = concentration of USP Etoposide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of etoposide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Etoposide	1.0	—
Picroetoposide	1.43	2.0
Any unspecified impurity	—	—
Total impurities	—	3.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers in a cold place. Do not freeze.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Etoposide RS
  USP Etoposide Resolution Mixture RS

Etoposide Injection

DEFINITION
Etoposide Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of etoposide ($C_{29}H_{32}O_{13}$) in a sterile solution in a nonaqueous medium intended for dilution with a suitable parenteral vehicle before intravenous infusion.

IDENTIFICATION
- **A.**
Diluent: Chloroform and methanol (9:1)
Standard solution: 0.8 mg/mL of USP Etoposide RS in *Diluent*
Sample solution: Equivalent to 0.8 mg/mL of etoposide in *Diluent* from the Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, acetone, alcohol, and water (80: 25: 2.5: 0.5)
Spray reagent: Add 10 mL of sulfuric acid with cooling and stirring to 70 mL of dehydrated alcohol in a 100-mL volumetric flask. Dilute with dehydrated alcohol to volume, and mix.
Analysis:
Samples: *Standard solution* and *Sample solution*
Allow the chromatogram to develop until the solvent front has moved 17 cm from the origin. Remove the plate, and allow it to air-dry in a fume hood for 5 min. Replace the plate in the tank, and develop again to a distance of 17 cm from the origin. Remove the plate, and air-dry it in a fume hood for about 20 min. Spray the plate with the *Spray reagent*, and heat in a forced-air oven at 120° for about 15 min.
Acceptance criteria: The appearance and R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Buffer: 2.72 g/L of sodium acetate in water. Adjust with glacial acetic acid to a pH of 4.0.
Mobile phase: Acetonitrile and *Buffer* (26:74)
System suitability solution: 0.3 mg/mL of USP Etoposide Resolution Mixture RS in *Mobile phase*
Standard stock solution: 2.0 mg/mL of USP Etoposide RS in acetonitrile
Standard solution: 0.2 mg/mL of USP Etoposide RS in *Mobile phase* from the *Standard stock solution*
Sample solution: Equivalent to 0.2 mg/mL of etoposide in *Mobile phase* from the Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

USP Monographs

www.webofpharma.com

Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L11
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of etoposide
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.35 between the etoposide and α-etoposide peaks, *System suitability solution*
Relative standard deviation: NMT 2.0% for etoposide, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of etoposide ($C_{29}H_{32}O_{13}$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of etoposide from the *Sample solution*
r_S = peak response of etoposide from the *Standard solution*
C_S = concentration of USP Etoposide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of etoposide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer: Prepare as directed in the *Assay*.
Solution A: Acetonitrile and *Buffer* (20:80)
Solution B: Acetonitrile and *Buffer* (60:40)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
30	40	60
40	40	60
42	0	100
45	0	100
47	100	0
50	100	0

Diluent: Acetonitrile and 0.02 M sodium acetate previously adjusted with acetic acid to a pH of 4.0 (30:70)
Standard stock solution: 2.0 mg/mL of USP Etoposide RS in *Diluent*
System suitability stock solution: 0.2 mg/mL of *n*-propylparaben in *Diluent*
System suitability solution: Transfer 5.0 mL of the *System suitability stock solution* and 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume.
Standard solution: 10 µg/mL of USP Etoposide RS from the *Standard stock solution* in *Diluent*
Sample solution: Nominally equivalent to 2.0 mg/mL of etoposide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 15-cm; less than 5-µm packing L11
Flow rate: 1.5 mL/min
Injection volume: 25 µL
Run time: NLT 40 min
System suitability
[NOTE—Run time is 15 min in isocratic conditions.]
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.1 between propylparaben and etoposide
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of etoposide from the *Standard solution*
C_S = concentration of USP Etoposide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of etoposide in the *Sample solution* (mg/mL)

Acceptance criteria
Total impurities: NMT 3.0%

SPECIFIC TESTS

• **pH** ⟨791⟩
Sample solution: 5.0 mL of Injection in 45 mL of water
Acceptance criteria: 3.0–4.0
• **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩ (if present)**:** 90.0%–110.0% of the labeled amount of alcohol (C_2H_5OH), using *n*-propyl alcohol as the internal standard
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩
Sample solution: Dilute the Injection with sterile water to obtain 0.31 mg/mL of etoposide activity
Acceptance criteria: NMT 2.0 USP Endotoxin Units/mg of etoposide
• **BENZYL ALCOHOL CONTENT** (if present)
Buffer, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: Transfer 0.75 mL of freshly distilled benzyl alcohol, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of the labeled amount of benzyl alcohol in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzyl alcohol from the *Sample solution*
r_S = peak response of benzyl alcohol from the *Standard solution*
C_S = concentration of benzyl alcohol in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

USP Monographs

www.webofpharma.com

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

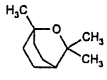
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers of Type I glass.

• **LABELING:** Label it to indicate that it must be diluted with suitable parenteral vehicle before intravenous infusion.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Etoposide RS
USP Etoposide Resolution Mixture RS

Eucalyptol



$C_{10}H_{18}O$ 154.25
1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane;
1,8-Epoxy-*p*-menthane [470-82-6].

DEFINITION
Eucalyptol is obtained from oil of eucalyptus and from other sources. It contains NLT 98.0% and NMT 100.0% of $C_{10}H_{18}O$.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

• **B.** Add 1 mL of phosphoric acid to 1 mL of Eucalyptol contained in a test tube maintained in an ice bath. A solid white crystalline mass is formed, from which eucalyptol separates upon addition of warm water.

ASSAY

• **PROCEDURE**

System suitability solution: 0.2 mg/mL of limonene and 0.9 mg/mL of USP Eucalyptol RS in methanol
Sample solution: 0.9 mg/mL of Eucalyptol in methanol
Blank: Methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 60-m fused-silica capillary column coated with phase G16
Temperature
Injector port: 250°
Detector: 250°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	6	200	—

Split flow rate: 50 mL/min
Carrier gas: Helium
Column head pressure: 30 psi
Injection size: 1 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between limonene and eucalyptol
Column efficiency: NLT 150,000 theoretical plates, eucalyptol
Analysis
Samples: *Sample solution* and *Blank*
[NOTE—Identify any peaks from the *Sample solution* that correspond to those in the *Blank* by their retention times.]
Calculate the percentage of eucalyptol ($C_{10}H_{18}O$) in the portion of Eucalyptol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of eucalyptol from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*, other than the responses corresponding to those of the *Blank*

Acceptance criteria: 98.0%–100.0%

IMPURITIES

• **LIMIT OF PHENOLS**
Analysis 1: Shake 5 mL with 5 mL of sodium hydroxide TS.
Acceptance criteria 1: The volume of Eucalyptol is not diminished.
Analysis 2: Shake 1 mL with 20 mL of water and allow the liquids to separate. To 10 mL of the water layer add 1 drop of ferric chloride TS.
Acceptance criteria 2: The mixture develops no violet color.

SPECIFIC TESTS

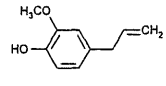
• **SPECIFIC GRAVITY** ⟨841⟩: 0.921–0.924
• **CONGEALING TEMPERATURE** ⟨651⟩: NLT 0°
• **OPTICAL ROTATION,** *Angular Rotation* ⟨781A⟩: −0.5° to +0.5°
• **DISTILLING RANGE,** *Method I* ⟨721⟩: 174°–177°
• **REFRACTIVE INDEX** ⟨831⟩: 1.455–1.460 at 20°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Eucalyptol RS

Eugenol



$C_{10}H_{12}O_2$ 164.20
Phenol, 2-methoxy-4-(2-propenyl)-;
4-Allyl-2-methoxyphenol [97-53-0].

DEFINITION
Eugenol is obtained from clove oil and from other sources.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

IMPURITIES

• **HYDROCARBONS**
Analysis: Dissolve 1 mL in 20 mL of 0.5 N sodium hydroxide in a 50-mL stoppered tube, add 18 mL of water, and mix.

USP Monographs

www.webofpharma.com

Acceptance criteria: A clear mixture results immediately, but it may become turbid when exposed to air.

- **LIMIT OF PHENOL**

Analysis: Shake 1 mL with 20 mL of water. Filter, and add 1 drop of ferric chloride TS to 5 mL of the clear filtrate.

Acceptance criteria: The mixture exhibits a transient grayish-green color but not a blue or violet color.

SPECIFIC TESTS

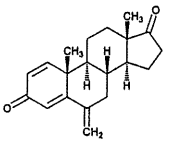
- **SPECIFIC GRAVITY** ⟨841⟩: 1.064–1.070
- **DISTILLING RANGE,** *Method II* ⟨721⟩: NLT 95% distills from 250°–255°
- **REFRACTIVE INDEX** ⟨831⟩: 1.540–1.542 at 20°
- **SOLUBILITY IN 70% ALCOHOL:** One volume dissolves in 2 volumes of 70% alcohol.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Eugenol RS

Exemestane



$C_{20}H_{24}O_2$ 296.40

Androsta-1,4-diene-3,17-dione, 6-methylene-;
6-Methyleneandrosta-1,4-diene-3,17-dione [107868-30-4].

DEFINITION

Exemestane contains NLT 97.0% and NMT 102.0% of exemestane ($C_{20}H_{24}O_2$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
15	60	40
18	10	90
28	10	90
30	60	40
35	60	40

Diluent: Acetonitrile and water (1:1)
Standard solution: 0.1 mg/mL of USP Exemestane RS in *Diluent*
Sample solution: 0.1 mg/mL of Exemestane in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 247 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 45°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of exemestane ($C_{20}H_{24}O_2$) in the portion of Exemestane taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of exemestane from the *Sample solution*
r_S = peak response of exemestane from the *Standard solution*
C_S = concentration of USP Exemestane RS in the *Standard solution* (mg/mL)
C_U = concentration of Exemestane in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Solution A: Water
Solution B: Acetonitrile
Mobile phase: See *Table 2*. Return to original conditions and re-equilibrate the system.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	75	25
30	55	45
40	5	95
45	5	95

Diluent: Acetonitrile and water (3:1)
System suitability solution: 1 mg/mL of USP Exemestane RS, 0.01 mg/mL of USP Exemestane Related Compound B RS, and 0.01 mg/mL of USP Exemestane Related Compound C RS in *Diluent*
Sensitivity solution: 0.5 µg/mL each of USP Exemestane RS, USP Exemestane Related Compound B RS, and USP Exemestane Related Compound C RS in *Diluent*
Standard solution: 5 µg/mL of USP Exemestane RS in *Diluent*
Sample solution: 1 mg/mL of Exemestane in *Diluent*. The concentration is calculated on the anhydrous and solvent-free basis.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

USP Monographs

www.webofpharma.com

Detector: UV 247 nm
Column: 4.6-mm × 25-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between exemestane related compound B and exemestane related compound C; NLT 2.0 between exemestane related compound C and exemestane, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10 for the exemestane, exemestane related compound B, and exemestane related compound C peaks, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Exemestane taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of exemestane from the *Standard solution*
C_S = concentration of USP Exemestane RS in the *Standard solution* (mg/mL)
C_U = concentration of Exemestane in the *Sample solution* (mg/mL) (the concentration is calculated on the anhydrous and solvent-free basis)
F = relative response factor for each individual impurity (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any impurity peaks less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Exemestane related compound B	0.34	0.95	0.15
Exemestane related compound C	0.77	1.1	1.0
Exemestane	1.0	—	—
Any unspecified impurity	—	1.0	0.1

• **LIMIT OF EXEMESTANE RELATED COMPOUND D**
Mobile phase: Hexane, isopropyl alcohol, and diethylamine (90: 10: 0.1)
System suitability solution: 8 mg/mL of USP Exemestane System Suitability Mixture RS in anhydrous alcohol
Standard solution: 0.04 mg/mL of USP Exemestane RS in anhydrous alcohol
Sensitivity solution: 4 µg/mL of USP Exemestane RS in anhydrous alcohol, from the *Standard solution*
Sample solution: 8 mg/mL of Exemestane in anhydrous alcohol. The concentration is calculated on the anhydrous and solvent-free basis.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 247 nm
Column: 4.6-mm × 25-cm; 10-µm packing L80
Column temperature: 30°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT than 2.0 between exemestane and exemestane related compound D, *System suitability solution*
Relative standard deviation: NMT 5%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of exemestane related compound D in the portion of Exemestane taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of exemestane related compound D from the *Sample solution*
r_S = peak response of exemestane from the *Standard solution*
C_S = concentration of USP Exemestane RS in the *Standard solution* (mg/mL)
C_U = concentration of Exemestane in the *Sample solution* (mg/mL) (the concentration is calculated on the anhydrous and solvent-free basis)
F = relative response factor for exemestane related compound D (see *Table 4*)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Exemestane	1.0	1.0	—
Exemestane related compound D	1.55	1.1	0.10

Total impurities: NMT 2.5%. Total impurities include the impurities in *Table 3* and *Table 4*.

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.3%
• **OPTICAL ROTATION** ⟨781S⟩, *Specific Rotation*
Sample solution: 10 mg/mL in methanol
Acceptance criteria: +290° to +300° on the anhydrous basis

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Exemestane RS
USP Exemestane Related Compound B RS
6-Hydroxymethylandrosta-1,4-diene-3,17-dione.
$C_{20}H_{26}O_3$ 314.42
USP Exemestane Related Compound C RS
Androsta-1,4-diene-3,17-dione.
$C_{19}H_{24}O_2$ 284.39

USP Monographs

www.webofpharma.com

USP Exemestane System Suitability Mixture RS
Exemestane containing a small amount of exemestane related compound D (16-Methyleneandrosta-1,4-diene-3,17-dione).
$C_{20}H_{24}O_2$ 296.40

Exenatide

HGEGTFTSDL SKQMEEEAVR LFIEWLKNGG PSSGAPPPS—NH_2

$C_{184}H_{282}N_{50}O_{60}S$ 4186.57

L-Histidylglycyl-L-glutamylglycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-aspartyl-L-leucyl-L-seryl-L-lysyl-L-glutaminyl-L-methionyl-L-glutamyl-L-glutamyl-L-glutamyl-L-alanyl-L-valyl-L-arginyl-L-leucyl-L-phenylalanyl-L-isoleucyl-L-glutamyl-L-tryptophyl-L-leucyl-L-lysyl-L-asparaginylglycylglycyl-L-prolyl-L-seryl-L-serylglycyl-L-alanyl-L-prolyl-L-prolyl-L-prolyl-L-serinamide [141758-74-9].

DEFINITION

Exenatide is a 39 amino acid synthetic peptide agonist for glucagon-like peptide-1 (GLP-1) receptor. Exenatide contains NLT 95% and NMT 105% of exenatide ($C_{184}H_{282}N_{50}O_{60}S$), calculated on the anhydrous, acetic acid-free basis. Exenatide is a white to off-white powder. [NOTE—Exenatide is very hygroscopic. Protect from exposure to moisture.]

IDENTIFICATION

• **A. HPLC**

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Identity sample solution: Mix equal volumes of the *Standard solution* and the *Sample solution*.

Analysis

Samples: *Standard solution*, *Sample solution*, and *Identity sample solution*

Examine the chromatograms of the *Standard solution*, *Sample solution*, and *Identity sample solution*.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, and the major peak from the *Identity sample solution* elutes as a single peak.

• **B. AMINO ACID ANALYSIS**

For further discussion of the theory and applications, see *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩, which may be a helpful, but not mandatory, resource. [NOTE—Use a suitable, validated hydrolysis and separation and calculation procedure including amino acids used in the calculation (see *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩.)]

Standardize the instrument with a mixture containing equal molar per volume amounts (except for L-cystine which is half the molar amount) of glycine and the L-form of the following amino acids: alanine, arginine, aspartic acid, cystine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, norleucine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan, and valine.

Hydrolysis solution: 6 N hydrochloric acid containing 4% of phenol

Sample solution: Accurately weigh out between 0.4 and 1.0 mg of Exenatide in glass ampuls. Add a minimum of 1.0 mL of *Hydrolysis solution*, freeze the sample ampul, and flame seal under vacuum. Hydrolyze at 110° for about 22 h. After hydrolysis, dry the test sample under vacuum to remove any residual acid. To the ampul add 2 mL of a buffer solution that is suitable for the amino acid analyzer, and pass through a filter of 0.45-µm pore size.

Procedure: Prepare a co-injection of the *Standard solution* and the test sample. Inject a suitable volume into the amino acid analyzer, and record and measure the responses for each amino acid peak in the *Standard solution*. Express the content of each amino acid in nanomoles.

Calculate the mean nanomole of the amino acids:

$$\text{Result} = (\text{nmol found in the } \textit{Analysis} \text{ for Ala, Arg, Asx, Glx, Gly, His, Ile, Leu, Lys, Phe, Pro, Trp, Val})/30$$

Divide the nanomole of each amino acid by the *Result* to determine the amino acid ratios that must meet the *Acceptance criteria*.

Acceptance criteria: See *Table 1*.

Table 1

Name	Acceptance Criteria (amino acid ratio)
Aspartic acid, threonine, phenylalanine, lysine, alanine	1.5–2.5
Serine	4.2–5.5
Glutamic acid	5.2–6.8
Proline	3.5–4.5
Glycine	4.5–5.5
Valine	0.5–1.5
Methionine, isoleucine, histidine, arginine	0.5–1.5
Leucine	2.5–3.5
Tryptophan	0.5–1.5

• **C.** The average mass by *Mass Spectrometry* ⟨736⟩ is 4186.6 ± 1.0 mass units.

ASSAY

• **PROCEDURE**

Solution A: 10 mM ammonium hydrogen carbonate, pH 9.5, 0.0375% ammonia

Solution B: Acetonitrile and *Solution A* (90:10)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	74	26
0.5	74	26
33	63	37
35	10	90
36	10	90
36.1	74	26
42	74	26

System suitability solution: 1.0 mg/mL of USP Exenatide RS and 0.005 mg/mL each of USP [Glu^{13}]-Exenatide RS and USP [$Met(O)^{14}$]-Exenatide RS

Standard solutions: 1.0 mg/mL of USP Exenatide RS in water, prepared in duplicate. [NOTE—The retention time for the exenatide peak is approximately 14–20 min.]

Sample solutions: 1.0 mg/mL of Exenatide in water, prepared in duplicate

USP Monographs

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Temperatures
Column: 60°
Autosampler: 10°
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solutions*
Suitability requirements
Mean peak area: Maximum difference between the mean peak area of the two *Standard solutions* is ±2%.
Resolution: NLT 1.0 between the [Glu[13]]-exenatide and [Met(O)[14]]-exenatide peaks, *System suitability solution*
Relative standard deviation: NMT 2.0% for the exenatide peak area and retention time from three replicate injections of the two *Standard solutions*
Analysis
Samples: *Standard solutions* and *Sample solutions*
Calculate the percentage of exenatide ($C_{184}H_{282}N_{50}O_{60}S$) in the portion of Exenatide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of exenatide from the *Sample solutions*
r_S = peak response of exenatide from the *Standard solutions*
C_S = concentration of USP Exenatide RS in the *Standard solutions* (mg/mL)
C_U = concentration of Exenatide in the *Sample solutions* (mg/mL), calculated on the anhydrous, acetic acid-free basis

Acceptance criteria: 95%–105% on the anhydrous, acetic acid-free basis

IMPURITIES

• PROCEDURE 1: EXENATIDE RELATED SUBSTANCES AND IMPURITIES

[NOTE—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

Mobile phase, System suitability solution, Standard solutions, Sample solutions, and Chromatographic system: Proceed as directed in the *Assay*.
Acceptance criteria
Individual impurities: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
[Glu[13]]-exenatide	0.65–0.68	0.50
Sum of [Asp[28]]-exenatide and [Met(O)[14]]-exenatide	0.68–0.76	0.50
Exenatide	1.00	—
Unspecified impurities	—	0.50

Total impurities: NMT 3.0% from *Procedure 1*
Reporting limit: 0.05%

• PROCEDURE 2: *N*-ACETYL HIS[1]-EXENATIDE

[NOTE—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]
Solution A: 0.1 M sodium perchlorate, pH 2.7
Solution B: Acetonitrile
Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	63	37
40	59	41
60	39	61

System suitability solution: 1.0 mg/mL of USP Exenatide RS and 0.005 mg/mL of USP [*N*-Acetyl-His[1]]-Exenatide RS
Standard solution: 1.0 mg/mL of USP Exenatide RS in water, prepared in duplicate
Sample solution: 1.0 mg/mL of Exenatide in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 3.0-mm × 15-cm; 3-µm packing L1
Temperatures
Column: 55°
Autosampler: 10°
Flow rate: 0.6 mL/min
Injection volume: 15 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between the main exenatide peak and the [*N*-acetyl-His[1]]-exenatide impurity peak, *System suitability solution*
Retention time variability: NMT ±5% of mean for the exenatide peak, *Standard solution*
Relative standard deviation: NMT 2.0% for the exenatide peak from three replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Exenatide taken, disregarding any peak with an area less than 0.05% of the main peak:

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = peak area of each individual impurity from the *Sample solution*, other than the solvent peak and the main exenatide acetate peak
r_T = sum of the areas of all the peaks from the *Sample solution*, excluding that of the solvent peak

Acceptance criteria
Individual impurities: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Exenatide	1.00	—
[*N*-Acetyl-His[1]]-exenatide	1.10–1.13	1.0

www.webofpharma.com

Table 5 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Unspecified impurities	—	0.50

Total impurities: NMT 3.0% from *Procedure 2*

• **PROCEDURE 3: LIMIT OF D-HIS[1] EXENATIDE**

System suitability solution A: 100 mg of L-amino acids and 0.5 mg of corresponding D-enantiomers are dissolved in 500 mL of water. An aliquot of 700 µL is dried in a SpeedVac and derivatized as described in the *Sample solution*.

Sample solution: Hydrolyze 1 mg of the sample in 350 µL of 6 N deuterium chloride (DCl) in heavy water (D_2O) at 110° for 8 h. After removal of excess reagent by a stream of nitrogen, the sample is esterified with 250 µL of 4 M deuterochloric acid-ethyl alcohol at 110° for 20 min. After cooling to about 40°, open the vial and evaporate the reagent with a gentle stream of nitrogen at moderate temperature. Dissolve the residue in 250 µL of trifluoroacetic anhydride in trifluoroacetic acid ethyl ester (1:2). Tightly close the vials and heat to 130° for 10 min. After cooling to room temperature, remove the excess reagent by a stream of nitrogen. Add 50 µL of isobutyl chloroformate to the sample and heat the closed vial to 110° for 10 min. After removal of the excess reagent under a nitrogen stream, dissolve the residue in 250 µL of dichloromethane.

System suitability solution B: About 100 µg of each D- and L-amino acid is derivatized as described in *Sample solution*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC–MS (see *Mass Spectrometry* ⟨736⟩)
Detector: Mass
Column: 20-m × 0.28-mm; 0.28-µm packing G49
Temperatures
Oven: 155°, 3 min isotherm, 4°/min to 190°, 5 min
Injection port: 220°
Carrier gas: Hydrogen
Flow rate: 26 mL/min
Injection volume: 1.0 µL

System suitability
Samples: *System suitability solution A* and *System suitability solution B*
Suitability requirements
Resolution: NLT 1.0 for enantiomers of the analyte and for any additional peak next to the analyte, *System suitability solution B*
Tailing factor: NMT 2.0 for the representative enantiomer, *System suitability solution B*
Relative standard deviation: NMT ±0.1% from the calculated content of D-Ala, D-Pro, D-Asp, D-Glu, D-Lys, and D-Arg, *System suitability solution A*

Analysis
Sample: *Sample solution*. The mass fragment of 379 Da is used for detection.
Calculate the percentage of D-His[1]-exenatide impurity in the portion of Exenatide taken:

$$\text{Result} = [A_D/(A_D + A_L)] \times 100$$

A_D = peak response of D-His from the *Sample solution*
A_L = peak response of L-His from the *Sample solution*

Acceptance criteria: NMT 1.0%

PROCESS-RELATED IMPURITIES

• **PROCEDURE 4: LIMIT OF PHOSPHATE**
[NOTE—Limit of phosphate should be performed if phosphate is used in manufacture.]
Solution A: 50 mM potassium hydroxide in water
Solution B: Water
Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	2	98
3	2	98
20	50	50
21	100	0

Diluent: 1 mM sodium hydroxide in water
Standard solution: 2 µg/mL of phosphate in *Diluent*
Sample solution: 2 mg/mL of Exenatide in *Diluent*
System suitability solution: *Standard solution* and *Sample solution* (1:1)
Chromatographic system
(See *Ion Chromatography* ⟨1065⟩.)
Mode: IC
Detector: Conductivity
Column: 2-mm × 25-cm; 10.5-µm packing L83
Temperatures
Column: 30°
Sample: 15°
Suppressor: Anion self-regenerating suppressor
Suppressant: Autosuppression
Flow rate: 0.25 mL/min
Injection volume: 25 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Tailing factor: NMT 3%, *Standard solution*
Relative standard deviation: NMT 10%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
The peak area for the phosphate ion peak in the chromatograms obtained with the *Standard solution* shows a peak corresponding to phosphate ion at a retention time of 15–21 min. The identity of the phosphate peak in the exenatide sample is confirmed by the *System suitability solution* showing one single peak. The phosphate content in exenatide is >0.1%, if the mean peak area of the *Sample solution* is higher than the mean peak area of the *Standard solution*. The phosphate content in exenatide is ≤0.1%, if the mean peak area of the *Sample solution* is equal to or smaller than the mean peak area for the *Standard solution*.

Acceptance criteria: NMT 0.1%

• **PROCEDURE 5: TRIFLUOROACETIC ACID (TFA) IN PEPTIDES** ⟨503.1⟩**:** Exenatide must contain NMT 0.25% trifluoroacetic acid.

OTHER COMPONENTS

• **ACETIC ACID IN PEPTIDES** ⟨503⟩**:** NMT 5.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 10 USP Endotoxin Units/mg of Exenatide.
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is less than 200 cfu/g and the total

USP Monographs

www.webofpharma.com

combined yeasts and molds count does not exceed 200 cfu/g.

• **Water Determination** ⟨921⟩, *Method I, Method Ic*: NMT 7.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers at −20 ± 5°.

• **USP Reference Standards** ⟨11⟩
USP [N-Acetyl-His[1]]-Exenatide RS
USP Exenatide RS
USP [Glu[13]]-Exenatide RS
USP [Met(O)[14]]-Exenatide RS

Add the following:

▲Exenatide Injection

DEFINITION

Exenatide Injection is a sterile solution of exenatide acetate in a suitable diluent. It may contain suitable preservatives. It possesses, in each milliliter, an activity of NLT 90.0% and NMT 110.0% of the labeled amount of exenatide ($C_{184}H_{282}N_{50}O_{60}S$).

IDENTIFICATION

• **A. HPLC**

Diluent, Buffer solution A, Solution A, Buffer solution B, Solution B, Mobile phase, Standard solution, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in *Product-Related Substances and Impurities*.

Identity sample solution: 0.25 mg/mL of USP Exenatide RS in *Diluent* mixed with 0.25 mg/mL of Injection

Analysis

Samples: *Standard solution*, *Sample solution*, and *Identity sample solution*

Acceptance criteria: The retention time of the exenatide peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Product-Related Substances and Impurities*. In addition, the major peaks of the *Identity sample solution* coelute.

• **B.** The average mass by *Mass Spectrometry* ⟨736⟩ is 4186.6 ± 1.0 Da.

ASSAY

• **Procedure**

Diluent: A 2-L aqueous solution containing 3.26 g of sodium acetate trihydrate, 2.98 g of L-methionine, and 2.06 mL of glacial acetic acid, pH 4.5. Pass through a membrane filter of 0.2-µm pore size.
[Note—Alternative suitable diluents may also be used.]

Buffer solution: 0.13 M sodium sulfate solution

Mobile phase: *Buffer solution*, acetonitrile, and trifluoroacetic acid (1500:500:2). Pass through a membrane filter of 0.45-µm pore size.

Standard solution: 0.25 mg/mL of USP Exenatide RS in *Diluent*

Soybean trypsin inhibitor stock solution: 1 mg/mL of soybean trypsin inhibitor in water. Prepare in duplicate.

System suitability solution: Transfer 10–50 µL of *Soybean trypsin inhibitor stock solution* to 1 mL of the *Standard solution* and mix with a vortex mixer for at least 30 s. Determine the soybean trypsin inhibitor peak level before initiating sample analysis. The soybean trypsin inhibitor peak level should be ≥2.0% but ≤5.0% relative to the total peak area. If the peak level is <2.0%, transfer additional *Soybean trypsin inhibitor stock solution* to the *System suitability solution*. If the peak level is >5.0%, discard and prepare a new *System suitability solution*.

Sample solution: Transfer an adequate quantity of each Injection (0.25 mg/mL) from its container directly to an HPLC vial, without contact with any intermediate container or transfer device.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 7.8-mm × 30-cm; 5-µm packing L20
Flow rate: 0.8 mL/min
Injection volume: 20 µL
Temperatures
Autosampler: 2°–8°
Column: 25°

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.3 between the exenatide peak and soybean trypsin inhibitor peak, *System suitability solution*
Tailing factor: NLT 0.8 and NMT 1.4, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of exenatide ($C_{184}H_{282}N_{50}O_{60}S$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = mean peak response from the *Standard solution*
C_S = concentration of USP Exenatide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of exenatide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **Procedure**

Diluent: A 2-L aqueous solution containing 3.26 g of sodium acetate trihydrate, 2.98 g of L-methionine, and 2.06 mL of glacial acetic acid, pH 4.5. Pass through a membrane filter of 0.2-µm pore size.
[Note—Alternative suitable diluents may also be used.]

Buffer solution A: Transfer 27.20 g of anhydrous monobasic potassium phosphate to a 2-L flask or beaker. Add approximately 1900 mL of water and mix well until dissolved. Adjust with phosphoric acid to a pH of 3.30. Transfer the buffer solution to a 2-L volumetric flask, dilute with water to volume, and mix well.

Solution A: Transfer 1670 mL of *Buffer solution A* to a glass container, add 1673 mL of acetonitrile, and mix well. Pass through a membrane filter of 0.45-µm pore size.

Buffer solution B: Transfer 27.20 g of anhydrous monobasic potassium phosphate and 56.20 g of sodium perchlorate monohydrate to a 2-L flask or beaker. Add approximately 1950 mL of water, heat to 65° for 5 min, and mix well until dissolved. Adjust with phosphoric acid to a pH of 3.30. Transfer the buffer solution to a 2-L volumetric flask, dilute with water to volume, and mix well.

www.webofpharma.com

Solution B: Transfer 1083 mL of *Buffer solution B* to a glass container, add 1115 mL of acetonitrile, and mix well. Pass through a membrane filter of 0.45-µm pore size.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5.0	100	0
45.0	40	60
50.0	0	100
52.0	0	100
54.0	100	0
65.0	100	0

0.3% Hydrogen peroxide: Transfer 1 mL of 3% hydrogen peroxide to a 10-mL volumetric flask and dilute with water to volume. Stopper and mix well.

Standard solution: 0.25 mg/mL of USP Exenatide RS in *Diluent*

System suitability solution: Transfer 5 µL of *0.3% Hydrogen peroxide* to 1 mL of *Standard solution* and mix well by mixing with a vortex mixer for at least 30 s. Incubate the tightly capped vial in a boiling water bath for 30 min. Cool the vial rapidly to room temperature and transfer the contents to a glass HPLC vial without any contact with an intermediate transfer device.

Sample solution: Transfer an adequate quantity of each Injection (0.25 mg/mL) from its container directly to an HPLC vial, without contact with any intermediate container or transfer device.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 10-cm; 3-µm packing L52
Flow rate: 1.2 mL/min
Injection volume: 40 µL
Temperatures
Autosampler: 2°–8°
Column: 40°

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between the exenatide peak and the peak at an approximate relative retention time of 1.09, *System suitability solution*
Column efficiency: NLT 15,000 theoretical plates, *Standard solution*
Tailing factor: Between 0.8 and 1.5 for the exenatide peak, *Standard solution*
Relative standard deviation: NMT 2.0% for 5 replicate injections, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms and measure the response for each peak. Determine the corrected total peak area by excluding system peaks and peaks below 0.10% of the total peak area.
Calculate the percentage of each impurity in the portion of Injection taken by using the corrected total peak area:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = peak response of each impurity from the *Sample solution*
r_T = corrected total peak area

Acceptance criteria: See *Table 2*.

Table 2

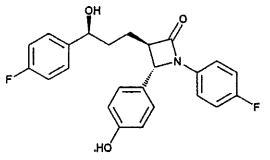
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
[3-39]-Exenatide[a]	0.32	1.00
Exenatide	1.00	—
[Asu[9]]-exenatide	1.22	1.00
Unspecified impurities	—	1.00
Total impurities	—	10.0

[a] Peptide fragment peak resulting from degradation.

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 50 USP Endotoxin Units/mL
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **SUBVISIBLE PARTICULATE MATTER IN THERAPEUTIC PROTEIN INJECTIONS** ⟨787⟩: NMT 6000 counts/container ≥10 µm, NMT 600 counts/container ≥25 µm
- **PH** ⟨791⟩: 4.2–4.8
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Store at 2°–8°. Do not freeze.
- **LABELING:** Meets the requirements in *Labeling* ⟨7⟩.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Exenatide RS▲ 2S (USP41)

Ezetimibe



$C_{24}H_{21}F_2NO_3$ 409.43
2-Azetidinone, 1-(4-fluorophenyl)-3-[3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-, [3*R*-[3α(*S**),4β]]-;
(3*R*,4*S*)-1-(*p*-Fluorophenyl)-3-[(3*S*)-3-(*p*-fluorophenyl)-3-hydroxypropyl]-4-(*p*-hydroxyphenyl)-2-azetidinone;
(3*R*,4*S*)-1-(4-Fluorophenyl)-3-[(*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-azetidin-2-one
[163222-33-1].

DEFINITION
Ezetimibe contains NLT 98.0% and NMT 102.0% of ezetimibe ($C_{24}H_{21}F_2NO_3$), calculated on the anhydrous and solvent-free basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*:** ⟨197M⟩ or ⟨197A⟩▲ (USP 1-Dec-2019)
- **B.** The ratio of the retention time of the ezetimibe peak of the *Sample solution* to that of the *System suitability solution* obtained in *Organic Impurities, Procedure 2* is between 0.97 and 1.03.

ASSAY

Change to read:

- **PROCEDURE**

Solution A: Water
Solution B: Acetonitrile
Solution C: Methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0.0	63	27	10
37.0	63	27	10
60.0	40	50	10
70.0	40	50	10
80.0	10	80	10
90.0	10	80	10
90.1	63	27	10
100.0	63	27	10

Diluent: Acetonitrile, methanol, and water (270:100:630). Add 1.0 mL of glacial acetic acid per liter of the mixture.
Standard solution: 0.25 mg/mL of USP Ezetimibe RS prepared as follows. Dissolve a suitable amount of USP Ezetimibe RS in acetonitrile at about 1%–2% of the total volume in a suitable volumetric flask, and dilute with *Diluent* to volume. ▲ [NOTE—The *Standard solution* contains a detectable level of *o*-fluorobenzene isomer (approximately 0.04%) from USP Ezetimibe RS.]▲ (USP 1-Dec-2019)
Sample solution: 0.25 mg/mL of Ezetimibe prepared as follows. Dissolve a suitable amount of Ezetimibe in acetonitrile at about 1%–2% of the total volume in a suitable volumetric flask, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
0–5 min: UV 215 nm
5–100 min: UV 248 nm
Column: 4.6-mm × 15-cm; 5-µm packing L43
Flow rate: 2 mL/min
Injection volume: 60 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 ▲for ezetimibe▲ (USP 1-Dec-2019)
Relative standard deviation: NMT 0.73% ▲for ezetimibe▲ (USP 1-Dec-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ezetimibe ($C_{24}H_{21}F_2NO_3$) in the portion of Ezetimibe taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ezetimibe from the *Sample solution*
r_S = peak response of ezetimibe from the *Standard solution*
C_S = concentration of USP Ezetimibe RS in the *Standard solution* (mg/mL)
C_U = concentration of Ezetimibe in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.2%

Change to read:

- **ORGANIC IMPURITIES, PROCEDURE 1**

Mobile phase, Diluent, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
▲▲ (USP 1-Dec-2019)
Sensitivity solution: 0.125 µg/mL of USP Ezetimibe RS in *Diluent* from the *Standard solution*
System suitability
Samples: *Standard solution* ▲▲ (USP 1-Dec-2019) and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between ezetimibe and *o*-fluorobenzene isomer, ▲*Standard solution*▲ (USP 1-Dec-2019)
Tailing factor: NMT 1.5 ▲for ezetimibe,▲ (USP 1-Dec-2019) *Standard solution*
Relative standard deviation: NMT 10% ▲for ezetimibe,▲ (USP 1-Dec-2019) *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each specified and any unspecified impurity in the portion of Ezetimibe taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*
F = relative response factor from *Table 2*

Acceptance criteria: See *Table 2*. ▲The reporting threshold is▲ (USP 1-Dec-2019) 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desfluoroaniline analog[a]	0.72	1.0	0.2
Ezetimibe diastereomers[b]	0.77	1.0	—
o-Fluorobenzene isomer▲▲ (USP 1-Dec-2019)	0.89	1.0	0.2
Ezetimibe	1.0	—	—
▲*m*-Fluoroaniline▲ (USP 1-Dec-2019) analog[c]	1.13	1.0	0.2
Ezetimibe ketone[d]	1.42	1.5	0.1
Any unspecified impurity	—	1.0	0.10

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total ▲▲ (USP 1-Dec-2019) impurities	—	—	0.6

▲▲ (USP 1-Dec-2019)
[a] (3*R*,4*S*)-3-[(*S*)-3-(4-Fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-1-phenylazetidin-2-one.
[b] The two ezetimibe diastereomers, *R,R,R*- and *S,S,S*-, elute as one peak and are quantitated using *Procedure 2*.
[c] (3*R*,4*S*)-1-(3-Fluorophenyl)-3-[(*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one.
[d] (3*R*,4*S*)-1-(4-Fluorophenyl)-3-[3-(4-fluorophenyl)-3-oxopropyl]-4-(4-hydroxyphenyl)azetidin-2-one.

Change to read:

- **ORGANIC IMPURITIES, PROCEDURE 2**

Mobile phase: Acetonitrile and water (45:55)
Diluent: Acetonitrile with 0.1% glacial acetic acid (v/v)
System suitability solution: 0.4 mg/mL of USP Ezetimibe System Suitability Mixture RS in *Diluent*
Sensitivity solution: 0.2 µg/mL of USP Ezetimibe RS in *Diluent*
Sample solution: 0.4 mg/mL of Ezetimibe in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 248 nm
Column: Two columns in series of 4.6-mm × 15-cm; 5-µm packing L93
Column temperature: 5°
Flow rate: 0.35 mL/min
Injection volume: 10 µL
Run time: NLT 1.4 times the retention time of the ezetimibe peak
System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 1.5 between ezetimibe and *R,R,S*-ezetimibe diastereomer, *System suitability solution*
Tailing factor: NMT 1.5 for ezetimibe, *System suitability solution*
Relative standard deviation: NMT 10% ▲for ezetimibe,▲ (USP 1-Dec-2019) *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each enantiomeric or diastereomeric impurity in the portion of Ezetimibe taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 3*. ▲The reporting threshold is 0.05%.▲ (USP 1-Dec-2019)

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
S,S,S-Ezetimibe[a]	0.76	0.2
R,R,R-Ezetimibe[b]	0.82	0.1
Desfluoroaniline analog[c]	0.89	—

Table 3 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
R,R,S-Ezetimibe▲▲ (USP 1-Dec-2019)	0.93	0.4
Ezetimibe	1.00	—
S,S,R-Ezetimibe[d]	1.12	0.1
R,S,R-Ezetimibe[e]	1.22	0.1
Total ▲▲ (USP 1-Dec-2019) impurities	—	0.5
▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)	▲▲ (USP 1-Dec-2019)

▲▲ (USP 1-Dec-2019)
[a] (3*S*,4*S*)-1-(4-Fluorophenyl)-3-[(*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one.
[b] (3*R*,4*R*)-1-(4-Fluorophenyl)-3-[(*R*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one.
[c] Desfluoroaniline analog impurity is quantitated using *Procedure 1*.
[d] (3*S*,4*R*)-1-(4-Fluorophenyl)-3-[(*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one.
[e] (3*S*,4*R*)-1-(4-Fluorophenyl)-3-[(*R*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one.

▲**Total impurities:** NMT 0.9% for the sum of all impurities from *Organic Impurities, Procedure 1* and *Procedure 2*▲ (USP 1-Dec-2019)

SPECIFIC TESTS

- **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia* or *Method Ic*: NMT 0.6%
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 10 mg/mL of Ezetimibe previously dried for NLT 16 h over anhydrous calcium sulfate in methanol
Temperature: 20°
Acceptance criteria: −25.0° to −30.0°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Protect from moisture and store below 25°.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Ezetimibe RS
▲It also contains a very small amount of *o*-fluorobenzene isomer.
(3*R*,4*S*)-1-(4-Fluorophenyl)-3-[(*S*)-3-(2-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one.
$C_{24}H_{21}F_2NO_3$ 409.43▲ (USP 1-Dec-2019)
USP Ezetimibe System Suitability Mixture RS
It contains ezetimibe and the following impurities (it may also contain desfluoroaniline analog):
o-Fluorobenzene isomer;
▲▲ (USP 1-Dec-2019)
R,R,S-Ezetimibe;
(3*R*,4*S*)-1-(4-Fluorophenyl)-3-[(*R*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one.
$C_{24}H_{21}F_2NO_3$ 409.43

Ezetimibe Tablets

DEFINITION
Ezetimibe Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of ezetimibe ($C_{24}H_{21}F_2NO_3$).

USP Monographs

www.webofpharma.com

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **B.** The UV absorption spectrum of the ezetimibe peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 1 L of water.

Mobile phase: Tetrahydrofuran, acetonitrile, and *Buffer* (100:250:650)

Diluent: Acetonitrile, glacial acetic acid, and water (600:1:400)

Standard solution: 0.2 mg/mL of USP Ezetimibe RS in *Diluent.* Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of the filtrate.

Sample solution: Nominally 0.2 mg/mL of ezetimibe in *Diluent* prepared as follows. Place NLT 10 powdered Tablets in a suitable volumetric flask, add *Diluent* to fill about 60% of the total volume, sonicate for about 30 min, and shake on a wrist shaker for about 45 min. Dilute with *Diluent* to volume, pass through a suitable filter of 0.45-µm pore size, and discard the first 3 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 232 nm. For *Identification B,* use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 30 µL

Run time: NLT 2.4 times the retention time of the ezetimibe peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ezetimibe ($C_{24}H_{21}F_2NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ezetimibe from the *Sample solution*

r_S = peak response of ezetimibe from the *Standard solution*

C_S = concentration of USP Ezetimibe RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ezetimibe in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1

Do not refrigerate solutions containing ezetimibe.

Medium: 0.45% sodium lauryl sulfate in 0.05 M sodium acetate buffer, pH 4.5, prepared as follows. To 6 L of water in a suitable flask, add about 27 g of sodium lauryl sulfate and 24.6 g of sodium acetate. Dissolve the reagents by stirring until the solution is clear. Adjust with either hydrochloric acid or sodium hydroxide to a pH of 4.5; 500 mL.

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: 0.02 mg/mL of USP Ezetimibe RS in *Medium* prepared as follows. To a suitable amount of USP Ezetimibe RS in an appropriate volumetric flask, add methanol to fill about 1% of the total volume, and shake until completely dissolved. Dilute with *Medium* to volume.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Discard the first 3 mL of the filtrate.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 233 nm

Cell: 1.0 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ezetimibe ($C_{24}H_{21}F_2NO_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Ezetimibe RS in the *Standard solution* (mg/mL)

V = volume of *Medium,* 500 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of ezetimibe ($C_{24}H_{21}F_2NO_3$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.*

Apparatus 2, Standard solution, Sample solution, Instrumental conditions, and **Analysis:** Proceed as directed in *Test 1.*

Buffer: 6.8 g/L of sodium acetate pH 4.5 prepared as follows. Dissolve 6.8 g of sodium acetate in 1 L of water. Add 3 mL of glacial acetic acid and mix. If necessary, adjust with 2 N acetic acid or 0.2 N sodium hydroxide to a pH of 4.5.

Medium: 0.45% (w/v) sodium dodecyl sulfate in *Buffer;* 500 mL

Time: 20 min

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0% for 5 replicate readings

Tolerances: NLT 80% (*Q*) of the labeled amount of ezetimibe ($C_{24}H_{21}F_2NO_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*

System suitability solution: Weigh about 20 mg of USP Ezetimibe RS into a 100-mL volumetric flask. Dissolve in 10 mL of 0.01 N alcoholic sodium hydroxide. Place the capped volumetric flask into a 55° oven for 15 min. Remove from the oven and immediately add 2 mL of 0.1 N hydrochloric acid and about 50 mL of *Diluent.* Mix, allow to cool to room temperature, and dilute with *Diluent* to

www.webofpharma.com

volume. Pass through a suitable filter of 0.45-µm pore size and discard the first 3 mL of the filtrate. [NOTE—Unidentified peak 2 with a relative retention time of about 1.14 is generated during hydrolysis.]

Sensitivity solution: 0.1 µg/mL of USP Ezetimibe RS in *Diluent*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.5 between the ezetimibe peak and unidentified peak 2, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Unidentified peak 1	0.64	—
S,S,S-Ezetimibe and *R,R,R*-Ezetimibe[a,b]	0.78	—
Ezetimibe	1.00	—
Unidentified peak 2	1.14	—
Ezetimibe tetrahydropyran analog[c]	1.53	0.2
Ezetimibe ketone[d]	1.75	0.2
Any unspecified impurity	—	0.2
Total impurities[e]	—	0.5

[a] (3*S*,4*S*)-1-(4-Fluorophenyl)-3-[(*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one and (3*R*,4*R*)-1-(4-Fluorophenyl)-3-[(*R*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one.

[b] Process-related impurity and controlled in the drug substance.

[c] *N*,6-Bis(4-fluorophenyl)-2-(4-hydroxyphenyl)tetrahydro-2*H*-pyran-3-carboxamide.

[d] (3*R*,4*S*)-1-(4-Fluorophenyl)-3-[3-(4-fluorophenyl)-3-oxopropyl]-4-(4-hydroxyphenyl)azetidin-2-one.

[e] Total impurities include specified and unspecified degradation products. Process impurities are not included.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Protect from moisture. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ezetimibe RS

Factor IX Complex

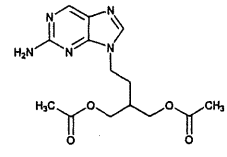
» Factor IX Complex conforms to the regulations of the federal Food and Drug Administration concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile, freeze-dried powder consisting of partially purified Factor IX fraction, as well as concentrated Factors II, VII, and X fractions, of venous plasma obtained from healthy human donors. It contains no preservative. It meets the requirements of the test for potency in having not less than 80 percent and not more than 120 percent of the potency stated on the label in Factor IX Units by comparison with the U.S. Factor IX Standard or with a working reference that has been calibrated with it.

Packaging and storage—Preserve in hermetic containers in a refrigerator.

Expiration date—The expiration date is not later than 2 years from the date of manufacture.

Labeling—Label it with a warning that it is to be used within 4 hours after constitution, and to state that it is for intravenous administration and that a filter is to be used in the administration equipment.

Famciclovir



$C_{14}H_{19}N_5O_4$ 321.33

1,3-Propanediol, 2-[2-(2-amino-9*H*-purin-9-yl)ethyl]-, diacetate (ester);
2-[2-(2-Amino-9*H*-purin-9-yl)ethyl]-1,3-propanediol diacetate (ester) [104227-87-4].

DEFINITION

Famciclovir contains NLT 98.0% and NMT 102.0% of famciclovir ($C_{14}H_{19}N_5O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Dilute acid: Dilute 5 mL of phosphoric acid with water to 50 mL.

Buffer: 2.72 g/L of monobasic potassium phosphate in water. Adjust with *Dilute acid* to a pH of 4.0 ± 0.05.

Mobile phase: Acetonitrile and *Buffer* (35:65)

Diluent: Water

Standard solution: 25 µg/mL of USP Famciclovir RS in *Diluent*

Sample solution: 25 µg/mL of Famciclovir in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

USP Monographs

www.webofpharma.com

Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: 5 times the retention time of famciclovir
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2500 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of famciclovir ($C_{14}H_{19}N_5O_4$) in the portion of Famciclovir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Famciclovir RS in the *Standard solution* (mg/mL)
C_U = concentration of Famciclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—On the basis of the synthetic route resulting in different impurity profiles, perform *Procedure 1* or *Procedure 2*. *Procedure 1* is recommended when dimethylaminopyridine, penciclovir, deoxychloro famciclovir, propionyl famciclovir, and 6-alkylamino famciclovir may be present (see *Table 2*). *Procedure 2* is recommended when famciclovir malonate, acetoxybutyl aminopurine, famciclovir methoxycarbonyl analog, famciclovir related compound C, famciclovir 8, *N*²-dimer, and famciclovir 6,6-dimer may be present (see *Table 4*).]

Dilute acid, Buffer, and **Diluent:** Proceed as directed in the *Assay*.
Solution A: *Buffer*
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
35	70	30
40	70	30
42	95	5
50	95	5

System suitability solution: 0.5 mg/mL of USP Famciclovir System Suitability Mixture RS in *Diluent*
Standard solution: 0.5 µg/mL of USP Famciclovir RS, 1 µg/mL of USP Famciclovir Related Compound A RS, and 3 µg/mL of USP Famciclovir Related Compound B RS in *Diluent*
Sample solution: 500 µg/mL of Famciclovir in *Diluent*. [NOTE—The solution is stable for 15 h at 6°.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between the propionyl famciclovir and 6-chloro famciclovir peaks, *System suitability solution*
Column efficiency: NLT 20,000 theoretical plates for the famciclovir peak, *System suitability solution*
Tailing factor: NMT 1.5 for the famciclovir peak, *System suitability solution*
Relative standard deviation: NMT 5.0% for the famciclovir peak; NMT 10.0% for the famciclovir related compound A and famciclovir related compound B peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Famciclovir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of famciclovir from the *Standard solution*
C_S = concentration of USP Famciclovir RS in the *Standard solution* (µg/mL)
C_U = concentration of Famciclovir in the *Sample solution* (µg/mL)
F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dimethylaminopyridine[a]	0.12	0.59	0.05
Penciclovir[b]	0.16	0.29	0.50
Famciclovir related compound A[c]	0.19	1.3	0.20
Famciclovir related compound B[d]	0.51	1.1	0.60
N-7 Isomer of famciclovir[e]	0.89	0.92	0.10
Famciclovir	1.0	—	—
N-Acetyl famciclovir[f]	1.05	0.56	0.10
Deoxychloro famciclovir[g]	1.26	0.87	0.20
Propionyl famciclovir[h]	1.32	0.88	0.15
6-Chloro famciclovir[i]	1.36	0.85	0.15
6-Alkylamino famciclovir[i]	1.83	0.46	0.10
Any other unspecified impurity	—	1.0	0.10

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	1.0

[a] *N,N*-Dimethylpyridin-4-amine.
[b] 9-[4-Hydroxy-3-(hydroxymethyl)butyl]guanine.
[c] 2-[2-(2-Amino-9*H*-purin-9-yl)ethyl]propane-1,3-diol.
[d] 4-(2-Amino-9*H*-purin-9-yl)-2-(hydroxymethyl)butyl acetate.
[e] 2-[2-(2-Amino-7*H*-purin-7-yl)ethyl]propane-1,3-diyl diacetate.
[f] 2-[2-(2-Acetamido-9*H*-purin-9-yl)ethyl]propane-1,3-diyl diacetate.
[g] 4-(2-Amino-9*H*-purin-9-yl)-2-(chloromethyl)butyl acetate.
[h] 2-(Acetoxymethyl)-4-(2-amino-9*H*-purin-9-yl)butyl propionate.
[i] 2-[2-(2-Amino-6-chloro-9*H*-purin-9-yl)ethyl]propane-1,3-diyl diacetate.
[j] 2-(2-{6-[4-Acetoxy-3-(acetoxymethyl)butylamino]-2-amino-9*H*-purin-9-yl} ethyl)propane-1,3-diyl diacetate.

• **Organic Impurities, Procedure 2**

Buffer: 3.85 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 5.0.
Solution A: 4.14 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 2.2.
Solution B: Acetonitrile and *Solution A* (25:75)
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	99.5	0.5
5	92	8
30	92	8
65	20	80
70	20	80
70.1	99.5	0.5
79	99.5	0.5

Diluent: Acetonitrile and *Buffer* (5:95)
System suitability solution: 1 mg/mL of USP Famciclovir RS, 1 µg/mL of USP Famciclovir Related Compound A RS, 5 µg/mL of USP Famciclovir Related Compound B RS, and 4 µg/mL of USP Famciclovir Related Compound C RS prepared as follows. Dissolve an appropriate amount of Standard in 5% of the final volume of acetonitrile, and dilute with *Buffer* to final volume.
Standard stock solution: 1 mg/mL of USP Famciclovir RS prepared as follows. Dissolve USP Famciclovir RS in 5% of the final volume of acetonitrile, and dilute with *Buffer* to final volume.
Standard solution: 1 µg/mL of USP Famciclovir RS in *Diluent* from the *Standard stock solution*
Sensitivity solution: 0.3 µg/mL of USP Famciclovir RS in *Diluent* from the *Standard solution*
Sample solution: 1 mg/mL of Famciclovir prepared as follows. Dissolve Famciclovir in 5% of the final volume of acetonitrile, and dilute with *Buffer* to final volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L60
Temperatures
Column: 35°
Sample: 6°
Flow rate: 1.5 mL/min
Injection volume: 25 µL

System suitability
Samples: *System suitability solution* and *Sensitivity solution*
Suitability requirements
Resolution: NLT 0.6 between the famciclovir and famciclovir related compound C peaks; the famciclovir related compound A peak is resolved from peaks due to the solvent front, *System suitability solution*
Relative standard deviation: NMT 20% for famciclovir related compound A; NMT 10% for famciclovir related compound B; and NMT 10% for famciclovir related compound C, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Famciclovir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of famciclovir from the *Standard solution*
C_S = concentration of USP Famciclovir RS in the *Standard solution* (µg/mL)
C_U = concentration of Famciclovir in the *Sample solution* (µg/mL)
F = relative response factor for each individual impurity (see *Table 4*)

Acceptance criteria: See *Table 4*. Disregard peaks less than 0.03%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Famciclovir related compound A[a]	0.09	1.4	0.1
Famciclovir related compound B[b]	0.28	1.1	0.5
Famciclovir malonate[c]	0.35	1.1	0.1
Acetoxybutyl aminopurine[d]	0.41	1.3	0.1
Hydroxy famciclovir[e]	0.43	1.0	—[f]
Famciclovir methoxy-carbonyl analog[g]	0.54	0.96	0.1
N-7 Isomer of famciclovir[h]	0.88	0.69	0.1
Famciclovir related compound C[i]	0.94	1.2	0.4
Famciclovir	1.00	—	—
N 7-Acetyl famciclovir[j]	1.50	0.46	—[f]
N 2 Acetyl famciclovir[k]	1.67	0.55	—[f]
6-Methoxy famciclovir[l]	1.76	0.18	—[f]
6-Chloro famciclovir[m]	2.01	0.66	0.1
Famciclovir 8,*N* 2-dimer[n]	2.18	1.0	0.2
Famciclovir 6,6-dimer[o]	2.29	0.53	0.07

USP Monographs

www.webofpharma.com

Table 4 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other unspecified impurity	—	1.0	0.06
Total impurities	—	—	0.8

[a] 2-[2-(2-Amino-9*H*-purin-9-yl)ethyl]propane-1,3-diol.
[b] 4-(2-Amino-9*H*-purin-9-yl)-2-(hydroxymethyl)butyl acetate.
[c] Dimethyl 2-[2-(2-amino-9*H*-purin-9-yl)ethyl]malonate.
[d] 4-(2-Amino-9*H*-purin-9-yl)butyl acetate.
[e] 2-[2-(2-Amino-9*H*-purin-9-yl)ethyl]-2-hydroxypropane-1,3-diyl diacetate.
[f] No individual limit but included in total impurities.
[g] Methyl 2-(acetoxymethyl)-4-(2-amino-9*H*-purin-9-yl)butanoate.
[h] 2-[2-(2-Amino-7*H*-purin-7-yl)ethyl]propane-1,3-diyl diacetate.
[i] 4-(2-Amino-9*H*-purin-9-yl)-2-methylbutyl acetate.
[j] 2-{2-[7-Acetyl-2-amino-7*H*-purin-9(8*H*)-yl]ethyl}propane-1,3-diyl diacetate.
[k] 2-[2-(2-Acetamido-9*H*-purin-9-yl)ethyl]propane-1,3-diyl diacetate.
[l] 2-[2-(2-Amino-6-methoxy-9*H*-purin-9-yl)ethyl]propane-1,3-diyl diacetate.
[m] 2-[2-(2-Amino-6-chloro-9*H*-purin-9-yl)ethyl]propane-1,3-diyl diacetate.
[n] 2-{2-[2-(1-{9-[4-Acetoxy-3-(acetoxymethyl)butyl]-2-amino-9*H*-purin-8-yl} ethylamino)-9*H*-purin-9-yl]ethyl}propane-1,3-diyl diacetate.
[o] 2,2′-[2,2′-(2,2′-Diamino-6,6′-bipurine-9,9′-diyl)bis(ethane-2,1-diyl)] bis(propane-3,2,1-triyl) tetraacetate.

- **FAMCICLOVIR RELATED COMPOUND E AND FAMCICLOVIR RELATED COMPOUND F**

Perform this test if *Organic Impurities, Procedure 2* is used.
Buffer: 15.6 g/L of monobasic sodium phosphate in water. Adjust with 0.1 M sodium hydroxide to a pH of 4.5 ± 0.1 before final dilution.
Solution A: Methanol and *Buffer* (20:80)
Solution B: Methanol and *Buffer* (60:40)
Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	100	0
6	100	0
10	0	100
14	0	100
18	100	0
25	100	0

Standard stock solution: 28 µg/mL each of USP Famciclovir Related Compound E RS and USP Famciclovir Related Compound F RS prepared as follows. Transfer 7 mg of USP Famciclovir Related Compound E RS and USP Famciclovir Related Compound F RS to a 250-mL volumetric flask. Add 1 mL of 0.1 M sodium hydroxide and 50 mL of *Solution A*. Sonicate to dissolve, cool to room temperature, and dilute with *Solution A* to volume.
Standard solution: 0.28 µg/mL each of USP Famciclovir Related Compound E RS and USP Famciclovir Related Compound F RS in *Solution A* from the *Standard stock solution*
Sample solution: 30 mg/mL of Famciclovir in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.7 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between famciclovir related compound E and famciclovir related compound F
Tailing factor: NMT 2.0 for the famciclovir related compound F peak
Relative standard deviation: NMT 5.0% for famciclovir related compound F
Signal-to-noise ratio: NLT 30 for the famciclovir related compound E and famciclovir related compound F peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate, in ppm, the amount of famciclovir related compound E and famciclovir related compound F in the portion of Famciclovir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 10^6$$

r_U = peak response of famciclovir related compound E or famciclovir related compound F from the *Sample solution*
r_S = peak response of famciclovir related compound E or famciclovir related compound F from the *Standard solution*
C_S = concentration of USP Famciclovir Related Compound E RS or USP Famciclovir Related Compound F RS in the *Standard solution* (µg/mL)
C_U = concentration of Famciclovir in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 10 ppm for famciclovir related compound E and NMT 5 ppm for famciclovir related compound F

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum at a pressure not exceeding 20 mm of mercury at 60° for 2 h.
Acceptance criteria: NMT 0.5%

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in a well-closed container at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Famciclovir RS
USP Famciclovir Related Compound A RS
[2-[2-(2-Amino-9*H*-purin-9-yl)ethyl]propane-1,3-diol] hydrochloride.
$C_{10}H_{15}N_5O_2 \cdot HCl$ 273.72
USP Famciclovir Related Compound B RS
4-(2-Amino-9*H*-purin-9-yl)-2-(hydroxymethyl)butyl acetate.
$C_{12}H_{17}N_5O_3$ 279.30
USP Famciclovir Related Compound C RS
4-(2-Amino-9*H*-purin-9-yl)-2-methylbutyl acetate.
$C_{12}H_{17}N_5O_2$ 263.30
USP Famciclovir Related Compound E RS
2-Aminopurine.
$C_5H_5N_5$ 135.13
USP Famciclovir Related Compound F RS
2-Amino-6-chloropurine.
$C_5H_4ClN_5$ 169.57
USP Famciclovir System Suitability Mixture RS

www.webofpharma.com

Contains famciclovir, propionyl famciclovir, and 6-chloro famciclovir.

Famciclovir Compounded Oral Suspension

DEFINITION

Famciclovir Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of famciclovir ($C_{14}H_{19}N_5O_4$).

Prepare Famciclovir Compounded Oral Suspension 100 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Famciclovir tablets[a] equivalent to	10 g of famciclovir
Vehicle: a 1:1 mixture of Ora-Plus[b] and Ora-Sweet SF,[b] a sufficient quantity to make	100 mL

[a] Famvir 500-mg tablets, Novartis Pharmaceuticals Corporation, East Hanover, NJ.
[b] Perrigo Laboratories, Allegan, MI.

Place the *Famciclovir tablets* in a suitable container and comminute to a fine powder. Wet the powder with a small amount of *Vehicle* and triturate to make a smooth paste. Add the *Vehicle* to make the contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Solution A: 5 mM monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0. Pass through a nylon filter of 0.45-µm pore size, and degas.

Mobile phase: Methanol and *Solution A* (30:70)

Diluent: Water. Adjust with phosphoric acid to a pH of 3.0.

Standard stock solution: 10 mg/mL of USP Famciclovir RS in *Diluent*. Sonicate to mix well. Store at 2°–8°.

Standard solution: Transfer 1.0 mL of the *Standard stock solution* to a 250-mL volumetric flask, dilute with *Diluent* to volume, and mix well. Transfer an aliquot to a centrifuge tube, and centrifuge at 2°–8° for 5 min at 14,000 rpm. Transfer the supernatant to an amber vial and store at 2°–8°.

Sample solution: Transfer 1.0 mL of Oral Suspension to a 10-mL volumetric flask, dilute with *Diluent* to volume, and sonicate to mix well. Transfer 1.0 mL of the resultant solution to a 250-mL volumetric flask, dilute with *Diluent* to volume, and mix well. Transfer an aliquot to a centrifuge tube, and centrifuge at 2°–8° for 5 min at 14,000 rpm. Transfer the supernatant to an amber vial and store at 2°–8°.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV-Vis 310 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Temperatures
Column: 30°
Autosampler: 5°
Flow rate: 1.0 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for famciclovir is about 4.5 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of famciclovir ($C_{14}H_{19}N_5O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of famciclovir from the *Sample solution*
r_S = peak response of famciclovir from the *Standard solution*
C_S = concentration of USP Famciclovir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of famciclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.3–6.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant, plastic containers. Store in a refrigerator (2°–8°) or at controlled room temperature.
• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator (2°–8°) or at controlled room temperature.
• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Famciclovir RS

Add the following:

▲Famciclovir Tablets

DEFINITION

Famciclovir Tablets contain Famciclovir Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of famciclovir ($C_{14}H_{19}N_5O_4$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
Sample: Transfer a portion of finely powdered Tablets, equivalent to 1000 mg of famciclovir, to a 250-mL volumetric flask, and add 100 mL of acetonitrile. Sonicate for 5 min and centrifuge for 10 min. Pass a portion through a filter of 0.45-µm or finer pore size into a 250-mL evaporating flask. Evaporate the solution to dryness at low heat (about 70°).
Acceptance criteria: Meet the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer A: 13.8 g/L of monobasic sodium phosphate in water. Adjust with 1 M sodium hydroxide or phosphoric acid to a pH of 4.5 ± 0.05.

Buffer B: 13.8 g/L of monobasic sodium phosphate in water. Adjust with 1 M sodium hydroxide to a pH of 7.0 ± 0.05.

Mobile phase: Methanol and *Buffer B* (30:70)

Standard solution: 0.1 mg/mL of USP Famciclovir RS in *Buffer A*

Sample stock solution: Nominally 1 mg/mL of famciclovir prepared as follows. Transfer an amount equivalent to 500 mg of famciclovir, from NLT 20 finely powdered Tablets, to an appropriate volumetric flask. Add about 50% of the flask volume of *Buffer A* and sonicate for about 15 min with intermittent shaking. Dilute with *Buffer A* to volume.

Sample solution: Nominally 0.1 mg/mL of famciclovir from *Sample stock solution* in *Buffer A*. Pass through a suitable filter of 0.45-µm or finer pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 222 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Run time: NLT 2 times the retention time of famciclovir

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of famciclovir ($C_{14}H_{19}N_5O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Famciclovir RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of famciclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0. Pass through a suitable filter of 0.45-µm pore size.

Mobile phase: Methanol and *Buffer* (30:70)

Standard stock solution: 0.55 mg/mL of USP Famciclovir RS prepared as follows. Transfer 55 mg of USP Famciclovir RS to a 100-mL volumetric flask, dissolve in about 50 mL of *Medium*, and dilute with *Buffer* to volume.

Standard solution: 0.055 mg/mL of USP Famciclovir RS in *Buffer* from *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and dilute with *Buffer* to obtain a concentration similar to that of the *Standard solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 310 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of famciclovir ($C_{14}H_{19}N_5O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Famciclovir RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

D = dilution factor for the *Sample solution*, if applicable

L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of famciclovir ($C_{14}H_{19}N_5O_4$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: 0.55 mg/mL of USP Famciclovir RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter and dilute with *Medium*, if necessary.

Instrumental conditions

Mode: UV

Analytical wavelength: 262 nm

Cell: 5 mm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of famciclovir ($C_{14}H_{19}N_5O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Famciclovir RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

D = dilution factor for the *Sample solution*, if applicable

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of famciclovir ($C_{14}H_{19}N_5O_4$) is dissolved.

www.webofpharma.com

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: 0.55 mg/mL of USP Famciclovir RS prepared as follows. Transfer a suitable amount of USP Famciclovir RS to a suitable volumetric flask, add methanol to about 5% of the flask volume, and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and dilute with *Medium*, if necessary, to obtain a concentration similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: 314 nm
Cell: 0.1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of famciclovir ($C_{14}H_{19}N_5O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Famciclovir RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*, if applicable
L = label claim (mg/Tablet)

Tolerances: NLT 85% (Q) of the labeled amount of famciclovir ($C_{14}H_{19}N_5O_4$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**

Solution A: 2.72 g/L of monobasic potassium phosphate in water. Adjust with 1 M phosphoric acid to a pH of 4.0 ± 0.05.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
50	75	25
60	75	25
65	95	5
75	95	5

Peak identification solution: 4 µg/mL of USP Famciclovir Related Compound A RS and 10 µg/mL of USP Famciclovir Related Compound B RS in *Solution A*
System suitability solution: 0.5 mg/mL of USP Famciclovir System Suitability Mixture RS in *Solution A*
Standard solution: 1 µg/mL of USP Famciclovir RS in *Solution A*
Sample solution: Nominally 1 mg/mL of famciclovir in *Solution A* prepared as follows. Transfer an amount equivalent to 250 mg of famciclovir, from NLT 10 finely powdered Tablets, to a 250-mL volumetric flask. Add about 125 mL of *Solution A*, and sonicate for 30 min with intermittent shaking. Dilute with *Solution A* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Temperatures
Autosampler: 6°
Column: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between propionyl famciclovir and 6-chloro famciclovir, *System suitability solution*
Relative standard deviation: NMT 5.0% for famciclovir, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of famciclovir from the *Standard solution*
C_S = concentration of USP Famciclovir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of famciclovir in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Penciclovir[a]	0.16	—[b]
Famciclovir related compound A	0.2	0.2
4-Dimethylaminopyridine	0.23	—[b]
Famciclovir related compound B	0.5	1.0
N-7 isomer of famciclovir[c]	0.85	—[b]
Famciclovir	1.0	—
N-Acetyl famciclovir[d]	1.1	—[b]
Chloroimpurity of famciclovir	1.2	—[b]
6-Chloro famciclovir[e]	1.32	—[b]
Propionyl famciclovir[f]	1.35	—[b]
6-Amino derivative of famciclovir	2.0	—[b]

www.webofpharma.com

Table 2 *(continued)*

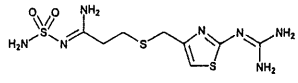
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any unspecified individual impurity	—	0.2
Total impurities	—	1.5

[a] 2-Amino-9-[4-hydroxy-3-(hydroxymethyl)butyl]-1*H*-purin-6(9*H*)-one; 9-[4-Hydroxy-3-(hydroxymethyl)butyl]guanine.
[b] This is a process impurity controlled in the drug substance and is included in the table for peak identification only. This impurity is excluded from the total impurities calculation.
[c] 2-[2-(2-Amino-7*H*-purin-7-yl)ethyl]propane-1,3-diyl diacetate.
[d] 2-[2-(2-Acetamido-9*H*-purin-9-yl)ethyl]propane-1,3-diyl diacetate.
[e] 2-[2-(2-Amino-6-chloro-9*H*-purin-9-yl)ethyl]propane-1,3-diyl diacetate.
[f] 2-(Acetoxymethyl)-4-(2-amino-9*H*-purin-9-yl)butyl propionate.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Famciclovir RS
  USP Famciclovir Related Compound A RS
  [2-[2-(2-Amino-9*H*-purin-9-yl)ethyl]propane-1,3-diol] hydrochloride.
  $C_{10}H_{15}N_5O_2 \cdot HCl$ 273.72
  USP Famciclovir Related Compound B RS
  4-(2-Amino-9*H*-purin-9-yl)-2-(hydroxymethyl)butyl acetate.
  $C_{12}H_{17}N_5O_3$ 279.30
  USP Famciclovir System Suitability Mixture RS
  Mixture of famciclovir, propionyl famciclovir, and 6-chloro famciclovir. (Other impurities may also be present.)▲ (USP 1-Aug-2019)

Famotidine



$C_8H_{15}N_7O_2S_3$ 337.45
Propanimidamide, *N*′-(aminosulfonyl)-3-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]-;
[1-Amino-3-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]propylidene]sulfamide;
3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]-*N*′-sulfamoylpropanimidamide [76824-35-6].

DEFINITION

Famotidine contains NLT 98.5% and NMT 101.0% of famotidine ($C_8H_{15}N_7O_2S_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy* 197A, 197K, or 197M▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**
  Sample: Dissolve 250 mg of Famotidine in 80 mL of glacial acetic acid.
  Analysis: Titrate with 0.1 N perchloric acid VS (see *Titrimetry* ⟨541⟩), using a suitable anhydrous electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 16.87 mg of $C_8H_{15}N_7O_2S_3$.
  Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Buffer: 1.882 g/L of sodium 1-hexanesulfonate in water, adjusted with acetic acid to a pH of 3.5
  Solution A: Acetonitrile, methanol, and *Buffer* (94:6:900)
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*. [NOTE—If necessary, adjust the *Mobile phase* to achieve a retention time of 19–23 min for the famotidine peak and a maximum of 48 min for the famotidine related compound E peak.]

Table 1

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	100	0	1
23	96	4	1
27	96	4	2
47	78	22	2
48	100	0	2
54	100	0	1

Standard stock solution: 0.5 mg/mL of USP Famotidine RS in *Solution A*
Standard solution: 0.5 µg/mL of USP Famotidine RS in *Solution A*
System suitability stock solution: 0.25 mg/mL of USP Famotidine Related Compound D RS in methanol
System suitability solution: Transfer 1 mL of the *System suitability stock solution* and 0.5 mL of the *Standard stock solution* into a 100-mL volumetric flask, and dilute with *Solution A* to volume.
Sample solution: 0.5 mg/mL of Famotidine in *Solution A*
Identification solution: 0.5 mg/mL of USP Famotidine RS and 1.5 µg/mL of each of USP Famotidine Related Compound B RS, USP Famotidine Related Compound C RS, USP Famotidine Related Compound D RS, USP Famotidine Related Compound E RS, and USP Famotidine Related Compound F RS in *Solution A*
[NOTE—To address the poor solubility of USP Famotidine Related Compound F RS in *Solution A*, it may be first dissolved in a minimal amount of 0.1 N sodium hydroxide.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 265 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: See *Table 1*.
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*

www.webofpharma.com

Suitability requirements
Resolution: NLT 3.5 between famotidine and famotidine related compound D
Analysis
Samples: *Standard solution*, *Sample solution*, and *Identification solution*
Chromatograph the *Identification solution*, and identify the components on the basis of their relative retention times, given in *Table 2*.
Calculate the percentage of each impurity in the portion of Famotidine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of famotidine from the *Standard solution*
C_S = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)
C_U = concentration of Famotidine in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Famotidine	1.0	—	—
Famotidine related compound D[a]	1.1	1.0	0.3
Famotidine related compound C[b]	1.2	0.53	0.3
Famotidine cyanoamidine[c]	1.4	0.71	0.2
Famotidine related compound F[d]	1.5	0.59	0.1
Famotidine amidine[e]	1.6	0.53	0.2
Famotidine related compound B[f]	2.0	0.40	0.3
Famotidine related compound E[g]	2.1	1.0	0.3
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	1.0

[a] Famotidine propanamide.
[b] Famotidine sulfamoyl propanamide.
[c] *N*-Cyano-3-[[2-(diaminomethyleneamino)thiazol-4-yl]methylthio] propanimidamide.
[d] Famotidine propionic acid.
[e] 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanimidamide.
[f] Famotidine dimer.
[g] Famotidine disulfide.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample at a pressure not exceeding 5 mm of mercury at 80° for 5 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Famotidine RS
USP Famotidine Related Compound B RS
3,5-Bis[2-[[2-[(diaminomethylene)amino]thiazol-4-yl] methylthio]ethyl]-4*H*-1,2,4,6-thiatriazine 1,1-dioxide.
$C_{16}H_{23}N_{11}O_2S_5$ 561.73
USP Famotidine Related Compound C RS
3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]-*N*-sulfamoylpropanamide hydrochloride.
$C_8H_{15}ClN_6O_3S_3$ 374.88
USP Famotidine Related Compound D RS
3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio] propanamide.
$C_8H_{13}N_5OS_2$ 259.35
USP Famotidine Related Compound E RS
2,2′-[4,4′-Disulfanediylbis(methylene)bis(thiazole-4,2-diyl)]diguanidine.
$C_{10}H_{14}N_8S_4$ 374.53
USP Famotidine Related Compound F RS
3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio] propanoic acid.
$C_8H_{12}N_4O_2S_2$ 260.34

Famotidine Injection

DEFINITION
Famotidine Injection is a sterile, concentrated solution of Famotidine. It contains NLT 90.0% and NMT 110.0% of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$). It may contain suitable preservatives.

IDENTIFICATION
• **A.** The retention time of the famotidine peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 13.8 g/L of monobasic sodium phosphate
Mobile phase: Methanol, water, and *Buffer* (5:32:3). Adjust with 1 N sodium hydroxide to a pH of 5.3.
Diluent: Dissolve 1.36 g of monobasic potassium phosphate in 800 mL of water, adjust with 1 N sodium hydroxide to a pH of 7.0, and dilute with water to 1 L.
Standard solution
If benzyl alcohol is present: 0.1 mg/mL of USP Famotidine RS and 0.09 mg/mL of USP Benzyl Alcohol RS in *Diluent*
If benzyl alcohol is not present: 0.1 mg/mL of USP Famotidine RS in *Diluent*
Sample solution: Transfer a volume of Injection, equivalent to 20 mg of famotidine based on the label claim, to a 200-mL volumetric flask, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Flow rate: 1 mL/min
Injection size: 30 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0% for the famotidine peak
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of famotidine from the *Sample solution*
r_S = peak response of famotidine from the *Standard solution*
C_S = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of famotidine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **CONTENT OF BENZYL ALCOHOL** (if present)

Buffer, Mobile phase, Diluent, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability stock solution: Proceed as directed in the *Organic Impurities* test.

System suitability solution: Transfer 25 mL of *System suitability stock solution* to a 50-mL volumetric flask. Add 1 drop (approximately 20 mg) of USP Benzyl Alcohol RS, and dilute with *Diluent* to volume.

System suitability

Samples: *Standard solution* and *System suitability solution*
[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.3 between adjacent peaks of benzyl alcohol and famotidine propionic acid; the benzyl alcohol peak is resolved from the solvent front, *System suitability solution*

Relative standard deviation: Less than 2.0% for each peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzyl alcohol in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of benzyl alcohol from the *Sample solution*
r_S = peak area of benzyl alcohol from the *Standard solution*
C_S = concentration of USP Benzyl Alcohol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of benzyl alcohol in the *Sample solution* (mg/mL)

Acceptance criteria: The content of benzyl alcohol meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Vehicles and added substances*.

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability stock solution: Transfer 10 mg of USP Famotidine RS to a 50-mL volumetric flask. Add 1 mL of 0.1 N hydrochloric acid. Heat at 80° for 30 min. Allow to cool, add 2 mL of 0.1 N sodium hydroxide, and heat at 80° for an additional 30 min. Allow to cool, and neutralize by adding 1 mL of 0.1 N hydrochloric acid. Dilute with *Diluent* to volume (*Solution A*). Transfer 5 mg of USP Famotidine RS to a separate 50-mL volumetric flask, add 8 mL of methanol, and sonicate to dissolve. Add 10 mL of *Solution A*, and dilute with *Diluent* to volume.

System suitability solution

If benzyl alcohol is present: Transfer 25 mL of *System suitability stock solution* to a 50-mL volumetric flask. Add 1 drop (approximately 20 mg) of USP Benzyl Alcohol RS, and dilute with *Diluent* to volume.

If benzyl alcohol is not present: Transfer 25 mL of *System suitability stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

System suitability

Sample: *System suitability solution*
[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.3 between adjacent peaks of famotidine propionic acid, famotidine sulfamoyl propanamide, famotidine, and famotidine propanamide for each pair of peaks

Analysis

Sample: *Sample solution*

Calculate the percentage of the total of famotidine propionic acid, famotidine sulfamoyl propanamide, and famotidine propanamide in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = sum of the peak areas for famotidine propionic acid, famotidine sulfamoyl propanamide, and famotidine propanamide from the *Sample solution*
r_T = sum of the peak areas for famotidine, famotidine propionic acid, famotidine sulfamoyl propanamide, and famotidine propanamide from the *Sample solution*

Acceptance criteria

Total impurities: NMT 5.0%

Table 1

Name	Relative Retention Time
Benzyl alcohol (if present)	0.4
Famotidine propionic acid (famotidine related compound F)[a]	0.7
Famotidine sulfamoyl propanamide (famotidine related compound C)[b]	0.8
Famotidine	1.0
Famotidine propanamide (famotidine related compound D)[c]	1.3

[a] 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanoic acid.
[b] 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]-*N*-sulfamoylpropanamide.
[c] 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio] propanamide.

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩: Meets the requirements
• **pH** ⟨791⟩: 5.0–5.6
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 16.67 USP Endotoxin Units/mg of famotidine
• **OTHER REQUIREMENTS:** It meets the requirements under *Container Content for Injections* ⟨697⟩.

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store in a refrigerator.
- **LABELING:** It meets the requirements under *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. Label it to indicate that the Injection is to be diluted with a suitable parenteral vehicle prior to administration. Label it to indicate the name and the quantity of any added preservative.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzyl Alcohol RS
  USP Famotidine RS

Famotidine for Oral Suspension

DEFINITION

Famotidine for Oral Suspension contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$) when constituted as directed. It contains one or more suitable buffers, colors, diluents, flavors, and preservatives.

IDENTIFICATION

- **A.** The retention time of the famotidine peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer A: Dissolve 13.6 g of sodium acetate trihydrate in 900 mL of water, and adjust with glacial acetic acid to a pH of 6.0 ± 0.1, before final dilution to 1 L.
Buffer B: Dissolve 13.6 g of monobasic sodium phosphate in 900 mL of water, adjust with 1 M sodium hydroxide to a pH of 7.0 ± 0.1, and dilute with water to 1 L.
Solution A: Acetonitrile and *Buffer A* (7:93)
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
42	52	48
43	100	0
45	100	0

Diluent: Acetonitrile and *Buffer B* (7:93)
Standard solution: 0.16 mg/mL of USP Famotidine RS in *Diluent*
Sample solution: Transfer to a 100-mL volumetric flask a portion of Famotidine for Oral Suspension, equivalent to about 40 mg of famotidine, freshly mixed and free from air bubbles and constituted as directed in the labeling. Add 10 mL of methanol, sonicate for 5 min, add 70 mL of *Diluent*, sonicate for an additional 5 min, and dilute with *Diluent* to volume. Dilute 10.0 mL of this solution with *Diluent* to 25.0 mL, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 268 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: Greater than 2000 theoretical plates
Tailing factor: NMT 2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$) in the portion of Famotidine for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of famotidine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements for *Content Uniformity*. For the product in multi-dose containers, the unit is a 5-mL aliquot of the suspension, constituted as directed in the labeling.

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, Diluent, Standard solution, Sample solution and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability stock solution: Transfer 16 mg of famotidine to a 50-mL volumetric flask. Dissolve in 1.0 mL of 1 N hydrochloric acid, heat at 80° for 30 min, and cool to room temperature. Add 2.0 mL of 1 N sodium hydroxide, heat at 80° for 30 min, and cool to room temperature. Add 1.0 mL of 1 N hydrochloric acid to neutralize, and dilute with *Diluent* to volume.
System suitability solution: Transfer 16 mg of famotidine to a 50-mL volumetric flask. Add 10 mL of *Diluent*, and sonicate to dissolve. Add 5 drops of hydrogen peroxide solution, heat at 80° for 15 min, and cool to room temperature. Add 20 mL of *System suitability stock solution*, and dilute with *Diluent* to volume.
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—Identify the components of the *System suitability solution* based on the relative retention times listed in *Table 2*.]
Suitability requirements
Resolution: Greater than 1.5 between famotidine and famotidine propanamide, *System suitability solution*
Column efficiency: Greater than 2000 theoretical plates, *Standard solution*
Tailing factor: NMT 2, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the total of famotidine sulfamoyl propanamide and famotidine propanamide in the portion of Famotidine for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

www.webofpharma.com

r_U = sum of the peak areas for famotidine sulfamoyl propanamide and famotidine propanamide from the *Sample solution*
r_S = peak area of famotidine from the *Standard solution*
C_S = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of famotidine in the *Sample solution* (mg/mL)

Acceptance criteria
Total of famotidine sulfamoyl propanamide and famotidine propanamide: Less than 2.0%

Table 2

Name	Relative Retention Time
Famotidine sulfoxide[a]	0.3
Famotidine propionic acid (famotidine related compound F)[b]	0.5
Famotidine sulfamoyl propanamide (famotidine related compound C)[c]	0.7
Famotidine	1.0
Famotidine propanamide (famotidine related compound D)[d]	1.2

[a] 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylsulfinyl]-*N'*-sulfamoylpropanimidamide.
[b] 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanoic acid.
[c] 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]-*N*-sulfamoylpropanamide.
[d] 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanamide.

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 10^2 cfu/g. The total combined molds and yeasts count is NMT 10^2 cfu/g. It meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*.
- **pH** ⟨791⟩**:** 6.5–7.5, in the suspension constituted as directed in the labeling

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Famotidine RS

Famotidine Tablets

DEFINITION
Famotidine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$).

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 4 mg/mL of USP Famotidine RS in glacial acetic acid
Sample solution: Transfer a portion of finely powdered Tablets, equivalent to about 40 mg of famotidine, to a 10-mL volumetric flask. Dissolve in glacial acetic acid with the aid of sonication, dilute with glacial acetic acid to volume, and centrifuge to get a clear liquid.
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Ethyl acetate, methanol, toluene, and ammonium hydroxide (40:25:20:2)
Analysis: Allow the spots to dry, and develop the plate in a paper-lined chromatographic chamber equilibrated with *Developing solvent system* for about 1 h before use. Allow the chromatogram to develop until the solvent front has moved about 15 cm. Remove the plate, air-dry, and examine the plate under short-wavelength UV light.
Acceptance criteria: The principal spot from the *Sample solution* corresponds in appearance and R_F value to that of the *Standard solution*.
- **B.** The retention time of the major peak in the *Sample solution* corresponds to that in the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Buffer: Dissolve 13.6 g of sodium acetate trihydrate in 750 mL of water. Add 1 mL of triethylamine, adjust with glacial acetic acid to a pH of 6.0, and dilute with water to 1 L.
Mobile phase: Acetonitrile and *Buffer* (7:93)
Diluent: Dissolve 6.8 g of monobasic potassium phosphate in 750 mL of water, adjust with 1 M potassium hydroxide to a pH of 6.0, and dilute with water to 1 L.
System suitability stock solution: Transfer 10 mg of famotidine to a 50-mL volumetric flask. Add 1 mL of 0.1 N hydrochloric acid, heat at 80° for 30 min, and cool to room temperature. Add 2 mL of 0.1 N sodium hydroxide, heat at 80° for 30 min, cool to room temperature, and neutralize by adding 1 mL of 0.1 N hydrochloric acid. Dilute with *Diluent* to volume. Transfer 10 mL of this solution to a separate 50-mL volumetric flask containing 5 mg of famotidine dissolved in 8 mL of methanol. Dilute with *Diluent* to volume. Transfer 25 mL of this solution to a 50-mL volumetric flask, and dilute with *Diluent* to volume. [NOTE—This solution is stable for up to 1 month.]
System suitability solution: Transfer 1–1.5 mL of the *System suitability stock solution* to a suitable container, add 1 drop of hydrogen peroxide solution, and mix well. [NOTE—Prepare fresh daily.]
Standard solution: Transfer 10 mg of USP Famotidine RS to a 100-mL volumetric flask, add 20 mL of methanol, and sonicate for 5 min. Dilute with *Diluent* to volume.
Sample solution: Transfer NLT 10 Tablets to a 1-L volumetric flask. Add 200 mL of *Diluent*, and swirl to erode the Tablets. Add 200 mL of methanol, and stir by mechanical means at 300 rpm for 1 h. Dilute with *Diluent* to volume, mix, and filter. Quantitatively dilute a portion of the clear filtrate with *Diluent* to obtain a solution containing about 0.1 mg/mL of famotidine.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 40°
Flow rate: 1.4 mL/min
Injection size: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
[NOTE—Identify peaks using *Table 1*.]
Resolution: NLT 1.3 between the famotidine sulfamoyl propanamide and famotidine peaks, and NLT 1.3

USP Monographs

www.webofpharma.com

between the famotidine and famotidine propanamide peaks, *System suitability solution*
Capacity factor: NLT 2.0 for the famotidine peak, *System suitability solution*
Relative standard deviation: Less than 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of famotidine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: pH 4.5, 0.1 M phosphate buffer (13.6 g/L of monobasic potassium phosphate); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Determine the amount of $C_8H_{15}N_7O_2S_3$ dissolved using one of the following methods.
Spectrophotometric method
Standard solution: USP Famotidine RS in *Medium* in a concentration similar to the one expected in the *Sample solution*
Sample solution: Pass a portion of the sample under test through a suitable filter.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 265 nm
Chromatographic method
Buffer, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.14 mg/mL of USP Famotidine RS in *Medium*. Dilute this solution with *Medium* to obtain a solution containing (*L*/900) mg/mL, where *L* is the Famotidine Tablet label claim, in mg.
Sample solution: Pass a portion of the solution under test through a suitable filter.
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor: Greater than 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of famotidine ($C_8H_{15}N_7O_2S_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)
L = label claim, mg/Tablet
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$) is dissolved.
For Tablets labeled as chewable
Proceed as directed for either of the methods specified above, except for the following:
Time: 45 min
Tolerances: NLT 80% (*Q*) of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$) is dissolved.
For Tablets labeled as film-coated
Proceed as directed for either of the methods specified above, except for the following:
Time: 30 min
Tolerances: NLT 80% (*Q*) of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of famotidine from the *Standard solution*
C_S = concentration of USP Famotidine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of famotidine in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
Famotidine sulfoxide[a]	0.4	1.0	1.0
Famotidine propionic acid (famotidine related compound F)[b]	0.7	1.0	0.5
Famotidine sulfamoyl propanamide (famotidine related compound C)[c]	0.8	1.0	0.5
Famotidine	1.0	—	—
Famotidine propanamide (famotidine related compound D)[d]	1.2	1.3	0.5
Total impurities	—	—	1.5

[a] 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylsulfinyl]-*N'*-sulfamoylpropanimidamide.
[b] 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanoic acid.
[c] 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]-*N*-sulfamoylpropanamide.
[d] 3-[[2-(Diaminomethyleneamino)thiazol-4-yl]methylthio]propanamide.

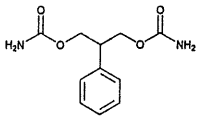
USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Famotidine RS

Felbamate



$C_{11}H_{14}N_2O_4$ 238.24

1,3-Propanediol, 2-phenyl-, dicarbamate;
2-Phenyl-1,3-propanediol dicarbamate [25451-15-4].

DEFINITION

Felbamate contains NLT 98.0% and NMT 102.0% of felbamate ($C_{11}H_{14}N_2O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (126:84:790)
Diluent: Acetonitrile, methanol, and water (222:148:630)
System suitability solution: 0.05 mg/mL of USP Felbamate Related Compound A RS and 0.2 mg/mL of USP Felbamate RS in *Mobile phase*
Standard stock solution: 1.0 mg/mL of USP Felbamate RS prepared as follows. Dissolve a suitable quantity of USP Felbamate RS in 10% of the volumetric flask volume of methanol. Sonicate and shake to completely dissolve, and dilute with *Diluent*.
Standard solution: 0.2 mg/mL of USP Felbamate RS from *Standard stock solution* in *Mobile phase*
Sample stock solution: 1.0 mg/mL of Felbamate prepared as follows. Dissolve a suitable quantity of Felbamate in 10% of the volumetric flask volume of methanol. Sonicate and shake to completely dissolve, and dilute with *Diluent*.
Sample solution: 0.2 mg/mL of Felbamate from *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.8 mL/min
Injection volume: 20 µL
Run time: 3 times the retention time of felbamate
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between felbamate related compound A and felbamate, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of felbamate ($C_{11}H_{14}N_2O_4$) in the portion of Felbamate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of felbamate from the *Sample solution*
r_S = peak response of felbamate from the *Standard solution*
C_S = concentration of USP Felbamate RS in the *Standard solution* (mg/mL)
C_U = concentration of Felbamate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF METHYLCARBAMATE**

Mobile phase: Water
Standard solution: 0.1 mg/mL of methylcarbamate in water
Sample solution: Suspend 1 g of Felbamate in 5 mL of water, and mix on a vortex mixer for 1 min followed by sonication for 5 min. Filter the slurry, and use as the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 10%
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The peak response for methylcarbamate in the *Sample solution* does not exceed the peak response for methylcarbamate in the *Standard solution* (0.05%).

- **EARLY ELUTING ORGANIC IMPURITIES**

Mobile phase, Diluent, Standard stock solution, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1 µg/mL of USP Felbamate RS in *Mobile phase* from *Standard stock solution*
Sample solution: 1.0 mg/mL of Felbamate prepared as follows. Dissolve a suitable quantity of Felbamate in 10% of the volumetric flask volume of methanol. Sonicate and shake to completely dissolve, and dilute with *Diluent*.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between felbamate related compound A and felbamate, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 10%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Identify the impurities using the relative retention times shown in *Table 1*.
Calculate the percentage of each impurity in the portion of Felbamate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of felbamate from the *Standard solution*
C_S = concentration of USP Felbamate RS in the *Standard solution* (µg/mL)
C_U = concentration of Felbamate in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Phenylpropanediol[a]	0.43	1.7	0.15
Felbamate related compound A	0.65	1.3	0.15
Felbamate	1.0	—	—
N-Aminocarbonyl felbamate[b]	1.43	0.89	0.15
Felbamate related compound B[c]	2.23	—	—
Individual unspecified impurity[d]	—	1.0	0.1

[a] 2-Phenylpropane-1,3-diol.
[b] 3-Carbamoyloxy-2-phenylpropyl allophanate.
[c] This impurity is quantified using the test for *Late Eluting Organic Impurities*.
[d] Quantify individual unspecified impurities eluting before felbamate related compound B.

• **LATE ELUTING ORGANIC IMPURITIES**
Mobile phase: Acetonitrile, methanol, and water (222:148:630)
System suitability solution: 1 µg/mL each of USP Felbamate RS and USP Felbamate Related Compound B RS in *Mobile phase*
Standard solution: 1 µg/mL of USP Felbamate RS in *Mobile phase*
Sample solution: 1.0 mg/mL of Felbamate prepared as follows. Dissolve a suitable quantity of Felbamate in 10% of the volumetric flask volume of methanol. Sonicate and shake to completely dissolve, and dilute with *Mobile phase*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.8 mL/min
Injection volume: 20 µL
Run time: 10 times the retention time of felbamate
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3 between felbamate and felbamate related compound B, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 10%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Identify the impurities using the relative retention times shown in *Table 2*.
Calculate the percentage of each impurity in the portion of Felbamate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of felbamate from the *Standard solution*
C_S = concentration of USP Felbamate RS in the *Standard solution* (µg/mL)
C_U = concentration of Felbamate in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Felbamate	1.0	—	—
Felbamate related compound B	1.9	1.29	0.15
Felbamate dimer[a]	9.1	1.0	0.15
Individual unspecified impurity[b]	—	1.0	0.1
Total impurities[c]	—	—	0.75

[a] 3,3′-Carbonylbis(oxy)bis(2-phenylpropane-3,1-diyl) dicarbamate.
[b] Quantify individual unspecified impurities eluting after felbamate related compound B.
[c] Sum of all impurities from *Table 1* and *Table 2*.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Felbamate RS
USP Felbamate Related Compound A RS
3-Hydroxy-2-phenylpropyl carbamate.
$C_{10}H_{13}NO_3$ 195.22
USP Felbamate Related Compound B RS
Phenethyl carbamate.
$C_9H_{11}NO_2$ 165.19

Felbamate Oral Suspension

DEFINITION
Felbamate Oral Suspension is a viscous liquid containing NLT 90.0% and NMT 110.0% of the labeled amount of felbamate ($C_{11}H_{14}N_2O_4$). The product may contain suitable preservatives.

USP Monographs

www.webofpharma.com

IDENTIFICATION

• **A.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 28.5 g/L of tribasic sodium phosphate dodecahydrate in water

Mobile phase: Acetonitrile and *Buffer* (23:77). Adjust with phosphoric acid to a pH of 9.9.

Diluent A: Methanol and water (1:1)

Diluent B: Acetonitrile and water (23:77)

System suitability solution: 0.16 mg/mL of USP Felbamate RS and 0.02 mg/mL of USP Felbamate Related Compound A RS in *Diluent B* and methanol prepared as follows. Dissolve the required amounts of the Reference Standards in a suitable volumetric flask in 8% of the flask volume of methanol. Dilute with *Diluent B* to volume.

Standard solution: 0.16 mg/mL of USP Felbamate RS prepared as follows. Dissolve a weighed quantity in USP Felbamate RS in a suitable volumetric flask containing 16% of the flask volume of *Diluent A*. Add 50% of the flask volume of *Diluent B*. Sonication may be used to aid dissolution. Dilute with *Diluent B* to volume.

Sample stock solution: Nominally 1 mg/mL of felbamate in *Diluent A* prepared as follows. Transfer an amount of the Oral Suspension to a suitable volumetric flask. Add 60% of the flask volume of *Diluent A*, and sonicate for 15 min. Shake mechanically for 30 min, and dilute with *Diluent A* to volume.

Sample solution: Nominally 0.16 mg/mL of felbamate from *Sample stock solution* in *Diluent B*. Pass through a filter of 45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.1-mm × 15-cm; 5-µm packing L21

Column temperature: 40°

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between felbamate related compound A and felbamate, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of felbamate ($C_{11}H_{14}N_2O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of felbamate from the *Sample solution*

r_S = peak response of felbamate from the *Standard solution*

C_S = concentration of USP Felbamate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of felbamate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 15 min

Mobile phase: Acetonitrile and water (35:65)

System suitability solution: Prepare a solution of 0.1 mg/mL of USP Methylparaben RS in methanol. Transfer 10 mL of this solution to a 100-mL volumetric flask containing 40 mg of USP Felbamate RS. Sonicate for 5 min, and dilute with *Medium* to volume.

Standard solution: (L/1000) mg/mL of USP Felbamate RS in *Medium*, where L is the Oral Suspension label claim in mg/mL. An amount of methanol, not exceeding 10% of the final volume, can be used to help in solubilizing felbamate.

Sample solution: Using a syringe, accurately weigh by difference approximately 5 mL of the well-mixed Oral Suspension. Introduce the sample into the dissolution vessel with the paddles rotating, and avoid getting the sample on the paddle or shaft. At the time specified, withdraw an aliquot of the solution under test, and pass through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 10-µm packing L7

Flow rate: 2 mL/min

Injection size: 25 µL for *System suitability* and 15 µL for *Analysis*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for felbamate and methylparaben are about 1.0 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 2.0 between methylparaben and felbamate, *System suitability solution*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 2%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of felbamate ($C_{11}H_{14}N_2O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (D/W) \times V \times 100$$

r_U = peak response of felbamate from the *Sample solution*

r_S = peak response of felbamate from the *Standard solution*

C_S = concentration of USP Felbamate RS in the *Standard solution* (mg/mL)

L = label claim of the Oral Suspension (mg/mL)

D = density of the Oral Suspension (g/mL)

W = weight of the Oral Suspension taken (g)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of felbamate ($C_{11}H_{14}N_2O_4$) is dissolved.

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent A, Diluent B, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 0.2 mg/mL of USP Felbamate RS and 0.02 mg/mL of USP Felbamate Related Compound A RS in *Diluent B* prepared as follows. Transfer suitable quantities of USP Felbamate RS and USP Felbamate Related

www.webofpharma.com

Compound A RS into a suitable volumetric flask. Dissolve in 8% of the flask volume of methanol. Dilute with *Diluent B* to volume.
Standard stock solution: 0.2 mg/mL of USP Felbamate RS in *Diluent A*
Standard solution: 0.2 µg/mL of USP Felbamate RS from *Standard stock solution* in *Diluent B* prepared as follows. Transfer a suitable volume of *Standard stock solution* to a suitable volumetric flask containing 0.8% of the flask volume of methanol, and dilute with *Diluent B* to volume.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between felbamate related compound A and felbamate, *System suitability solution*
Tailing factor: NMT 2.0 for the felbamate peak, *Standard solution*
Relative standard deviation: NMT 10% for the felbamate peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of felbamate from the *Standard solution*
C_S = concentration of USP Felbamate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of felbamate in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Phenylpropanediol[a]	0.63	2.6	0.2
Felbamate related compound A[b]	0.78	1.8	0.2
Felbamate	1.0	—	—
Individual unspecified degradation product	—	1.0	0.2
Total impurities	—	—	0.75

[a] 2-Phenylpropane-1,3-diol.
[b] 3-Hydroxy-2-phenylpropyl carbamate.

SPECIFIC TESTS
- **PH** ⟨791⟩**:** 4.5–6.0, in the well-stirred Oral Suspension
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 10^2 cfu/mL. The total yeasts and molds count is NMT 10^1 cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Felbamate RS
USP Felbamate Related Compound A RS
3-Hydroxy-2-phenylpropyl carbamate.
$C_{10}H_{13}NO_3$ 195.22
USP Methylparaben RS

Felbamate Tablets

DEFINITION
Felbamate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of felbamate ($C_{11}H_{14}N_2O_4$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*▲ (CN 1-May-2020)
Sample: Transfer a weighed quantity of finely powdered Tablets, equivalent to 12 mg of felbamate, to a centrifuge tube. Add 10 mL of methanol, and mix on a vortex mixer for 3 min. Centrifuge, and transfer 1 mL of the clear supernatant to a mortar with 200 mg of potassium bromide. Evaporate at 105° in an oven for 15 min. Cool the dried residue in a desiccator, and blend by grinding. Prepare a pellet with 100 mg of the dried residue.
Standard: Transfer 1 mL of 1.2 mg/mL of USP Felbamate RS in methanol to a mortar with 200 mg of potassium bromide. Evaporate at 105° in an oven for 15 min. Cool the dried residue in a desiccator, and blend by grinding. Prepare a pellet with 100 mg of the dried residue.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Diluent: Methanol and water (80:20)
Mobile phase: Acetonitrile, methanol, and water (126:84:790)
System suitability solution: 0.05 mg/mL of USP Felbamate Related Compound A RS and 0.2 mg/mL of USP Felbamate RS in *Mobile phase*
Standard stock solution: 2 mg/mL of USP Felbamate RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Felbamate RS from *Standard stock solution* in *Mobile phase*
Sample stock solution: Nominally 2 mg/mL of felbamate from NLT 20 finely powdered Tablets, prepared as follows. Transfer a weighed quantity of the powder to a suitable volumetric flask. Add 50% of the flask volume of the *Diluent*. Sonicate for 30 min with intermittent shaking. Shake the flask vigorously for NLT 30 min. Dilute with *Diluent* to volume. Pass a portion through a suitable membrane filter.
Sample solution: 0.2 mg/mL of felbamate from *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.8 mL/min
Injection size: 20 µL
Run time: 3 times the retention time of felbamate
System suitability
Samples: *Standard solution* and *System suitability solution*

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 2 between felbamate related compound A and felbamate, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of felbamate ($C_{11}H_{14}N_2O_4$) in the portion of the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of felbamate from the *Sample solution*
r_S = peak response of felbamate from the *Standard solution*
C_S = concentration of USP Felbamate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of felbamate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase: Prepare as directed in the *Assay*.
Standard solution: (L/1000) mg/mL of USP Felbamate RS, where L is the Tablet label claim, in mg. Transfer a suitable weighed quantity of USP Felbamate RS to a suitable volumetric flask. Add 10% of the flask volume of methanol, and sonicate for 5 min to dissolve. Dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.0-mm × 15-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of felbamate ($C_{11}H_{14}N_2O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Felbamate RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 65% (Q) of the labeled amount of felbamate ($C_{11}H_{14}N_2O_4$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**
Diluent, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability stock solution: 0.4 mg/mL of USP Felbamate Related Compound A RS and 0.6 mg/mL of USP Felbamate RS in *Diluent*
System suitability solution: 0.4 µg/mL of USP Felbamate Related Compound A RS and 0.6 µg/mL of USP Felbamate RS from *System suitability stock solution* in *Mobile phase*
Standard stock solution: 0.6 mg/mL of USP Felbamate RS in *Diluent*
Standard solution: 0.6 µg/mL of USP Felbamate RS from *Standard stock solution* in *Mobile phase*
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2 between felbamate related compound A and felbamate, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 10%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Identify the impurities using the relative retention times shown in *Table 1*. Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from *Sample solution*
r_S = peak response of felbamate from the *Standard solution*
C_S = concentration of USP Felbamate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of felbamate in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Phenylpropanediol[a]	0.43	1.7	0.2
Felbamate related compound A[b]	0.65	1.3	0.2
Felbamate	1.0	—	—
N-Aminocarbonyl-felbamate[c]	1.43	—	—
Felbamate related compound B[d]	2.23	—	—
Individual unspecified degradation product	—	1.0	0.2
Total impurities	—	—	0.75

[a] 2-Phenylpropane-1,3-diol.
[b] 3-Hydroxy-2-phenylpropyl carbamate.
[c] 3-Carbamoyloxy-2-phenylpropyl allophanate.
[d] Phenylethyl carbamate. No limit. This is a process impurity.

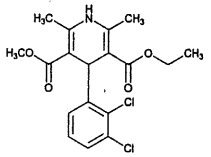
ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature.

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Felbamate RS
USP Felbamate Related Compound A RS
3-Hydroxy-2-phenylpropyl carbamate.
$C_{10}H_{13}NO_3$ 195.22

Felodipine



$C_{18}H_{19}Cl_2NO_4$ 384.25
3,5-Pyridinedicarboxylic acid 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-, ethyl methyl ester, (±)-;
(±)-Ethyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate [72509-76-3; 86189-69-7].

DEFINITION
Felodipine contains NLT 98.0% and NMT 101.0% of felodipine ($C_{18}H_{19}Cl_2NO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: Dissolve 6.9 g of monobasic sodium phosphate in 400 mL of water, add 8.0 mL of 1 M phosphoric acid, and dilute with water to 1 L.
Mobile phase: Acetonitrile, methanol, and *Buffer* (40:20:40)
System suitability stock solution: 0.05 mg/mL of USP Felodipine RS and 0.1 mg/mL of felodipine oxidation product in *Mobile phase* prepared as follows. Dissolve 150 mg of Felodipine in a mixture of 50 mL of tertiary butyl alcohol and 1 N perchloric acid (1:1). Add 10 mL of 0.1 M ceric sulfate, and allow to stand for 15 min. Add 3.5 mL of 10 N sodium hydroxide, and neutralize with 2 N sodium hydroxide. Shake the mixture with 25 mL of methylene chloride in a separator. Draw off the lower layer, and evaporate it to dryness under a stream of nitrogen on a water bath. Dissolve and dilute 10 mg of the residue (felodipine oxidation product) and 5 mg of USP Felodipine RS, with *Mobile phase* to 100 mL.
System suitability solution: 0.5 µg/mL of USP Felodipine RS and 1 µg/mL of felodipine oxidation product in *Mobile phase* from the *System suitability stock solution*
Standard solution: 0.3 mg/mL of USP Felodipine RS in *Mobile phase*. [NOTE—Prepare fresh before analysis.]
Sample solution: 0.3 mg/mL of Felodipine in *Mobile phase*. [NOTE—Prepare fresh before analysis.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL for the *System suitability solution* and 40 µL for the *Standard solution* and the *Sample solution*
System suitability
Samples: *System suitability solution* and *Standard solution*
Adjust the sensitivity of the system so that the heights of the two peaks in the chromatogram are 20% of the recorder full scale.
Suitability requirements
Resolution: NLT 2.5 between the first peak (felodipine oxidation product) and the second peak (felodipine), *System suitability solution*
Capacity factor, *k'*: NLT 5.0, *Standard solution*
Column efficiency: NLT 1500 theoretical plates, *Standard solution*
Tailing factor: NMT 1.5, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of felodipine ($C_{18}H_{19}Cl_2NO_4$) in the portion of Felodipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of felodipine from the *Sample solution*
r_S = peak response of felodipine from the *Standard solution*
C_S = concentration of USP Felodipine RS in the *Standard solution* (mg/mL)
C_U = concentration of Felodipine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **ORGANIC IMPURITIES**
Buffer, Mobile phase, System suitability solution, and **Sample solution:** Prepare as directed in the *Assay*.
Chromatographic system: Proceed as directed in the *Assay*, except use a run time of NLT 2 times the retention time of felodipine.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Felodipine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria
Individual impurities: NMT 1.0%
Total impurities: NMT 1.5%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry the sample at 105° for 3 h.
Acceptance criteria: NMT 0.5%
• **COLOR OF SOLUTION**
Sample solution: 20 mg/mL in methanol
Instrumental conditions
Mode: Vis
Cell: 5 cm
Analytical wavelength: 440 nm
Blank: Methanol
Acceptance criteria: NMT 0.2 absorbance

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Felodipine RS

Felodipine Extended-Release Tablets

DEFINITION

Felodipine Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of felodipine ($C_{18}H_{19}Cl_2NO_4$).

IDENTIFICATION

Change to read:

- **▲A.▲ (USP 1-May-2019)** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**▲ (USP 1-May-2019)**

ASSAY

Change to read:

- **PROCEDURE**

▲Buffer solution:▲ (USP 1-May-2019) 6.9 mg/mL of monobasic sodium phosphate in water. Adjust with 1 M phosphoric acid to a pH of 3.0 ± 0.05.

Mobile phase: Acetonitrile, methanol, and *▲Buffer solution▲ (USP 1-May-2019)* (40:20:40)

Standard stock solution: 2 mg/mL of USP Felodipine RS in methanol. ▲Sonication may be necessary for complete dissolution.▲ (USP 1-May-2019)

Standard solution: 0.02 mg/mL ▲of USP Felodipine RS▲ (USP 1-May-2019) from the *Standard stock solution* in *Mobile phase*

Sample ▲stock▲ (USP 1-May-2019) solution: ▲Nominally 0.1 mg/mL of felodipine prepared as follows.▲ (USP 1-May-2019) Dissolve a quantity equivalent to 10 mg of felodipine from powdered Tablets (NLT 10) in 40 mL of acetonitrile and 20 mL of methanol in a 100-mL volumetric flask, and sonicate for 5 min. Add about 30 mL of *▲Buffer solution,▲ (USP 1-May-2019)* and shake by mechanical means for 30 min. Allow the solution to cool to room temperature, and dilute with *▲Buffer solution▲ (USP 1-May-2019)* to volume. Centrifuge a portion of the solution at high speed for 15 min.

▲Sample solution: Nominally 0.02 mg/mL of felodipine prepared as follows. Transfer 10 mL of the supernatant from *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass a portion of this solution through a filter of 0.5-µm or finer pore size, discarding the first 4 mL of the filtrate.▲ (USP 1-May-2019)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 362 nm. ▲For *Identification B*, use a diode array detector in the range of 210–400 nm.▲ (USP 1-May-2019)

Column: 4.6-mm × 15-cm; ▲4-µm▲ (USP 1-May-2019) packing L1

Flow rate: 1 mL/min

Injection volume: 40 µL

▲Run time: NLT 2 times the retention time of felodipine▲ (USP 1-May-2019)

System suitability

Sample: *Standard solution*

Suitability requirements

▲Tailing factor: NMT 2.0▲ (USP 1-May-2019)

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ▲the labeled amount of▲ (USP 1-May-2019) felodipine ($C_{18}H_{19}Cl_2NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response ▲of felodipine▲ (USP 1-May-2019) from the *Sample solution*

r_S = peak response ▲of felodipine▲ (USP 1-May-2019) from the *Standard solution*

C_S = concentration of USP Felodipine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of felodipine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Test 1

Medium: pH 6.5 phosphate buffer with 1% sodium lauryl sulfate (Transfer 206 mL of 1 M monobasic sodium phosphate monohydrate, 196 mL of 0.5 M dibasic sodium phosphate anhydrous, and 50.0 g of sodium lauryl sulfate to a 5000-mL volumetric flask. Add about 4000 mL of water, and mix well. If necessary, adjust with 1 N sodium hydroxide to a pH of 6.5. Dilute with water to volume); 500 mL

Apparatus 2: 50 rpm

Times: 2, 6, and 10 h

Buffer solution: 6.9 mg/mL of monobasic sodium phosphate. Adjust with 1 M phosphoric acid to a pH of 3.0 ± 0.05.

Mobile phase: Acetonitrile, methanol, and *Buffer solution* (25:10:20)

Standard stock solution: 0.25 mg/mL of USP Felodipine RS in alcohol

Standard solution: (0.6 *L*/500) mg/mL of USP Felodipine RS from the *Standard stock solution* in *Medium*, where *L* is the label claim in mg/Tablet. **▲▲ (USP 1-May-2019)**

Sample solution: Place each Tablet in a specially made quadrangular basket of stainless steel wire gauze, soldered in one of its upper, narrow sides to the end of a steel rod (see *Figure 1. Stationary Tablet basket.*). Place the Tablet cover in the horizontal diagonal of the basket. Put the rod assembly up through the cover of the dissolution vessel, and fix it by means of two Teflon nuts, 3.2 cm from the center of the vessel, or by any other appropriate means. Adjust the lower edge of the bottom of the basket to about 1 cm above the top of the paddle blade (see *Figure 2. Drug release stationary Tablet basket configuration diagram.*). Orient the large side of the basket tangentially to the flow stream with the Tablet standing on its edge.

www.webofpharma.com

Pass a 10-mL portion of the solution under test, obtained at each time interval, through a suitable filter.

Chromatographic system: Proceed as directed in the *Assay*, except for the *Injection volume*.

Injection volume: 100 µL

Analysis

Samples: *Standard solution* and *Sample solution*

▲Calculate the concentration (C_i) of felodipine ($C_{18}H_{19}Cl_2NO_4$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of felodipine from the *Sample solution* at each time point, *i*

r_S = peak response of felodipine from the *Standard solution*

C_S = concentration of USP Felodipine RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of felodipine ($C_{18}H_{19}Cl_2NO_4$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_i \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_i) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of felodipine in the *Sample solution* at the specified time point (mg/mL)

V = volume of *Medium*, 500 mL

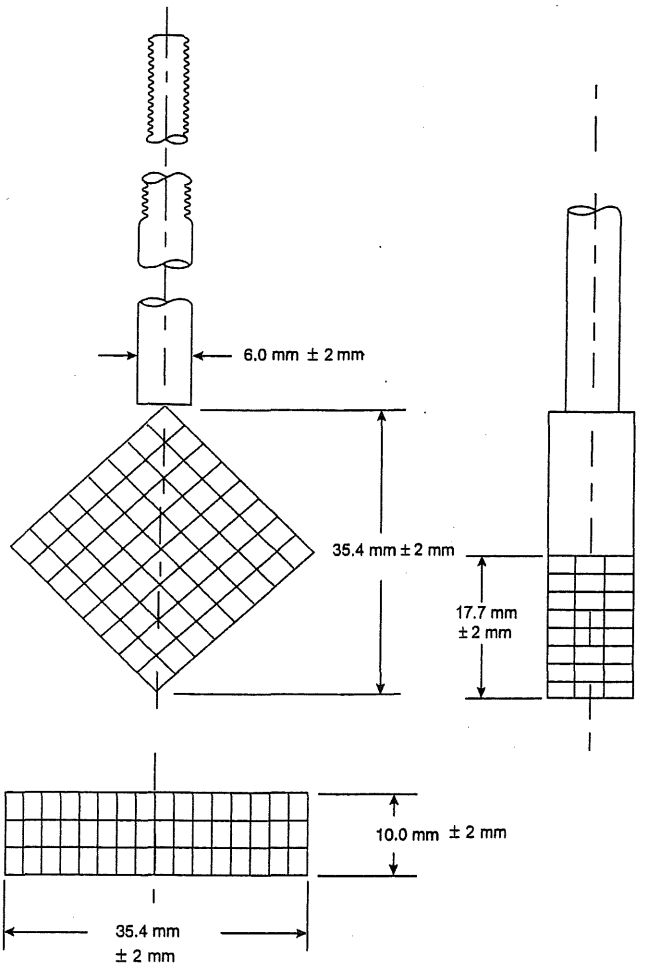
L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point, *i* (mL) ▲(USP 1-May-2019)

Tolerances: See *Table 1*.

Table 1

▲Time Point (*i*)▲(USP 1-May-2019)	Time (h)	Amount Dissolved (%)
▲1▲(USP 1-May-2019)	2	10–30
▲2▲(USP 1-May-2019)	6	42–68



NOTES

1. Rod and Basket with a Tablet cover placed in the horizontal diagonal of the basket.
2. Basket and Tablet cover material; stainless steel.
3. Basket gauze wire size: 8 mesh.

Figure 1. Stationary Tablet basket. (Felodipine Extended-Release Tablets)

USP Monographs

www.webofpharma.com

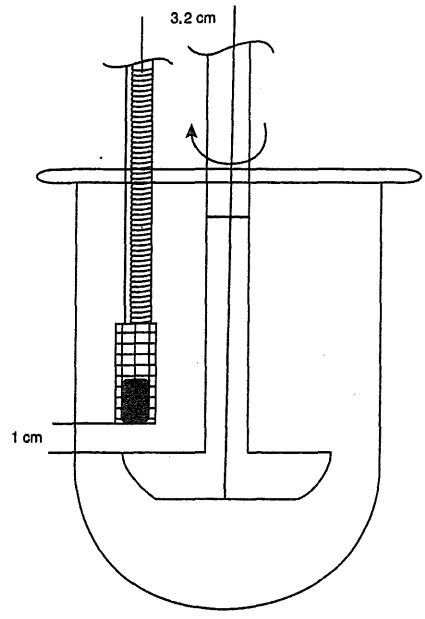


Figure 2. Drug release stationary Tablet basket configuration diagram. (Felodipine Extended-Release Tablets)

Table 1 *(continued)*

▲Time Point (*i*)▲ (USP 1-May-2019)	Time (h)	Amount Dissolved (%)
▲3▲ (USP 1-May-2019)	10	NLT 75

The percentages of the labeled amount of felodipine ($C_{18}H_{19}Cl_2NO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 1% (w/v) polysorbate 80; 500 mL

Apparatus 1: 100 rpm

Times: 1, 4, and 8 h

Buffer solution: Dissolve 6.9 g of monobasic sodium phosphate in 400 mL of water, add 8.0 mL of 1 M phosphoric acid, and dilute with water to ▲1000▲ (USP 1-May-2019) mL.

Mobile phase: Acetonitrile, methanol, and *Buffer solution* (40:20:40)

Standard stock solution: 0.2 mg/mL of USP Felodipine RS in methanol. Sonicate for 2 min, cool, and dilute with methanol to final concentration.

Standard solution: ▲(*L*/500) mg/mL of USP Felodipine RS in *Medium*, from *Standard stock solution*, where *L* is the label claim in mg/Tablet▲ (USP 1-May-2019)

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Replace the withdrawn amount with *Medium*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; ▲5-µm▲ (USP 1-May-2019) packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

▲▲ (USP 1-May-2019)

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

▲Calculate the concentration (C_i) of felodipine ($C_{18}H_{19}Cl_2NO_4$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of felodipine from the *Sample solution* at each time point, *i*

r_S = peak response of felodipine from the *Standard solution*

C_S = concentration of USP Felodipine RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of felodipine ($C_{18}H_{19}Cl_2NO_4$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of felodipine in the *Sample solution* at the specified time point (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

www.webofpharma.com

V_S = volume of the *Sample solution* withdrawn at each time point, *i* ▲(mL)▲ (ERR 1-May-2019)

▲ (USP 1-May-2019)

Tolerances: See *Table 2*.

Table 2

▲Time Point (*i*)▲ (USP 1-May-2019)	Time (h)	Amount Dissolved (%)
▲1▲ (USP 1-May-2019)	1	5–30
▲2▲ (USP 1-May-2019)	4	45–70
▲3▲ (USP 1-May-2019)	8	NLT 80

The percentages of the labeled amount of felodipine ($C_{18}H_{19}Cl_2NO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

Medium: pH 6.5 phosphate buffer with 1% sodium lauryl sulfate prepared as follows. 5.7 g/L of monobasic sodium phosphate monohydrate, 2.8 g/L of dibasic sodium phosphate, and 10 g/L of sodium lauryl sulfate in water, adjusted, if necessary, with diluted sodium hydroxide to a pH of 6.5 ± 0.05; 500 mL

Apparatus 2: 50 rpm

Times: 2, 6, and 10 h

Buffer solution: 6.9 g/L of monobasic sodium phosphate monohydrate in water. Adjust with phosphoric acid to a pH of 3.0 ± 0.05.

Mobile phase: Acetonitrile, methanol, and *Buffer solution* (45:25:30)

Standard stock solution: 0.5 mg/mL of USP Felodipine RS in methanol. ▲Sonication may be necessary for complete dissolution.▲ (USP 1-May-2019)

Standard solution: ▲0.01 mg/mL of USP Felodipine RS from the *Standard stock solution* in *Medium* prepared as follows.▲ (USP 1-May-2019) Transfer 2.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with *Medium* to volume. This solution is stable for 5 days at room temperature.

Sample solution: Place each Tablet in a specially made quadrangular basket of stainless steel wire gauze, soldered in one of its upper, narrow sides to the end of a steel rod (see *Figure 1. Stationary Tablet basket.*, in *Dissolution Test 1*). Place the Tablet cover in the horizontal diagonal of the basket. Put the rod assembly up through the cover of the dissolution vessel, and fix it by means of two Teflon nuts, 3.2 cm from the center of the vessel, or by any other appropriate means. Adjust the lower edge of the bottom of the basket to approximately 1 cm above the top of the paddle blade (see *Figure 2. Drug release stationary Tablet basket configuration diagram.*, in *Dissolution Test 1*). Orient the large side of the basket tangentially to the flow stream with the Tablet standing on its edge. Pass a portion of the solution under test, obtained at each time interval, through a suitable filter, discarding the first few milliliters.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 362 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 2.0 mL/min
Injection volume: 40 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1500 theoretical plates
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
▲Calculate the concentration (C_i) of felodipine ($C_{18}H_{19}Cl_2NO_4$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of felodipine from the *Sample solution* at each time point, *i*
r_S = peak response of felodipine from the *Standard solution*
C_S = concentration of USP Felodipine RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of felodipine ($C_{18}H_{19}Cl_2NO_4$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_i \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_i) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of felodipine in the *Sample solution* at the specified time point (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn at each time point, *i* (mL)▲ (USP 1-May-2019)

Tolerances: See *Table 3*.

Table 3

▲Time Point (*i*)▲ (USP 1-May-2019)	Time (h)	Amount Dissolved (%)
▲1▲ (USP 1-May-2019)	2	10–30
▲2▲ (USP 1-May-2019)	6	50–80
▲3▲ (USP 1-May-2019)	10	NLT 80

The percentages of the labeled amount of felodipine ($C_{18}H_{19}Cl_2NO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Change to read:

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements
▲▲ (USP 1-May-2019)

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**
▲**Buffer solution**▲ (USP 1-May-2019) and **Mobile phase:** Prepare as directed in the *Assay*.
▲**Standard stock solution:** 150 µg/mL each of USP Felodipine RS and USP Felodipine Related Compound A RS in methanol. Sonication may be necessary for complete dissolution.

www.webofpharma.com

Standard solution: 1.5 µg/mL each of USP Felodipine RS and USP Felodipine Related Compound A RS from the *Standard stock solution* in *Mobile phase*
Sensitivity solution: 0.075 µg/mL each of USP Felodipine RS and USP Felodipine Related Compound A RS from the *Standard solution* in *Mobile phase*
Sample solution: Nominally 150 µg/mL of felodipine prepared as follows. Dissolve a quantity equivalent to 30 mg of felodipine from powdered Tablets (NLT 20) in a 200-mL volumetric flask. Add 80 mL of acetonitrile and 40 mL of methanol, and sonicate for 15 min. Add about 60 mL of *Buffer solution*, and stir for 30 min. Allow the solution to cool to room temperature, and dilute with *Buffer solution* to volume. Centrifuge a portion of the solution at high speed for 15 min, and pass a portion of the supernatant through a filter of 0.5-µm or finer pore size, discarding the first 4 mL of the filtrate.▲ (USP 1-May-2019)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; ▲5-µm▲ (USP 1-May-2019) packing L1
Flow rate: 1 mL/min
Injection volume: ▲80▲ (USP 1-May-2019) µL
▲Run time: NLT 7 times the retention time of felodipine▲ (USP 1-May-2019)

System suitability
Samples: ▲*Standard solution and Sensitivity solution*
[NOTE—See *Table 4* for relative retention times.▲ (USP 1-May-2019)]
Suitability requirements
Resolution: NLT 1.5 between felodipine related compound A and felodipine, ▲*Standard solution*
Relative standard deviation: NMT 5.0% for both felodipine and felodipine related compound A peaks, *Standard solution*
Signal-to-noise ratio: NLT 10 for the felodipine peak, *Sensitivity solution*▲ (USP 1-May-2019)

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of felodipine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of felodipine related compound A from the *Sample solution*
r_S = peak response of felodipine related compound A from the *Standard solution*
C_S = concentration of USP Felodipine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of felodipine in the *Sample solution* (mg/mL)

▲Calculate the percentage of any unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of felodipine from the *Standard solution*
C_S = concentration of USP Felodipine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of felodipine in the *Sample solution* (µg/mL)▲ (USP 1-May-2019)

Acceptance criteria: ▲See *Table 4*. The reporting threshold is 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Felodipine related compound B[a,b]	0.7	—
Felodipine related compound A	0.8	2.0
Felodipine	1.0	—
Felodipine related compound C[b,c]	1.4	—
Any unspecified impurity	—	0.2
Total impurities	—	3.0

[a] Dimethyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.
[b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities for the drug product.
[c] Diethyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.▲ (USP 1-May-2019)

ADDITIONAL REQUIREMENTS

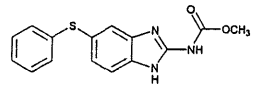
Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers. ▲Store at controlled room temperature. Protect from light.▲ (USP 1-May-2019)
- **LABELING:** When more than one test for *Dissolution* is given, the *Labeling* section states the test for *Dissolution* used only if *Test 1* is not used.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Felodipine RS
  USP Felodipine Related Compound A RS
  ▲3-Ethyl 5-methyl 4-(2,3-dichlorophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.▲ (USP 1-May-2019)
  $C_{18}H_{17}Cl_2NO_4$ 382.24

Fenbendazole



$C_{15}H_{13}N_3O_2S$ 299.35
Carbamic acid, [5-(phenylthio)-1*H*-benzimidazol-2-yl]-, methyl ester;
Methyl 5-(phenylthio)-2-benzimidazolecarbamate [43210-67-9].

DEFINITION
Fenbendazole contains NLT 98.0% and NMT 101.0% of fenbendazole ($C_{15}H_{13}N_3O_2S$), calculated on the dried basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A, 197K, or 197M▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Sample: 200 mg

Analysis: Dissolve the *Sample* in 30 mL of glacial acetic acid, warming if necessary to effect solution. Allow to cool, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 29.94 mg of fenbendazole ($C_{15}H_{13}N_3O_2S$).

Acceptance criteria: NLT 98.0%–101.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%

• **ORGANIC IMPURITIES**

Solution A: Methanol, acetic acid, and water (30:1:70)

Solution B: Methanol, acetic acid, and water (70:1:30)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	0	100
40	0	100
50	100	0

Diluent: Methanol and hydrochloric acid (99:1)

System suitability stock solution: 0.1 mg/mL each of USP Fenbendazole RS and USP Mebendazole RS in methanol

System suitability solution: 0.01 mg/mL each of USP Fenbendazole RS and USP Mebendazole RS from the *System suitability stock solution* in *Diluent*

Standard stock solution A: 0.025 mg/mL of USP Fenbendazole RS prepared as follows. Dissolve a weighed quantity of USP Fenbendazole RS in *Diluent* to obtain a solution with a known concentration of 5 mg/mL. Dilute 1.0 mL of this solution with methanol to 200.0 mL.

Standard solution A: 0.0125 mg/mL of USP Fenbendazole RS from *Standard stock solution A* in *Diluent*

Standard stock solution B: 0.1 mg/mL of USP Fenbendazole Related Compound A RS in methanol

Standard solution B: 0.01 mg/mL of USP Fenbendazole Related Compound A RS from *Standard stock solution B* in *Diluent*

Standard stock solution C: 0.1 mg/mL of USP Fenbendazole Related Compound B RS in methanol

Standard solution C: 0.01 mg/mL of USP Fenbendazole Related Compound B RS from *Standard stock solution C* in *Diluent*

Sample solution: Dissolve 5.0 mg/mL of Fenbendazole in *Diluent*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for mebendazole and fenbendazole are about 0.85 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between mebendazole and fenbendazole

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Calculate the percentages of fenbendazole related compound A and fenbendazole related compound B in the portion of Fenbendazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each related compound from the *Sample solution*

r_S = peak response of the corresponding related compound from *Standard solution B* or *Standard solution C*

C_S = concentration of the corresponding related compound in *Standard solution B* or *Standard solution C* (mg/mL)

C_U = concentration of Fenbendazole in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Fenbendazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any impurity from the *Sample solution*

r_S = peak response of USP Fenbendazole RS from *Standard solution A*

C_S = concentration of USP Fenbendazole RS in *Standard solution A* (mg/mL)

C_U = concentration of Fenbendazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fenbendazole related compound A	0.25	0.5
Fenbendazole related compound B	0.65	0.5
Fenbendazole	1.0	—
Any individual impurity	—	0.5
Total impurities	—	1

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 100°–105° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at room temperature.

• **LABELING:** Label to indicate that it is for veterinary use only.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Fenbendazole RS

USP Fenbendazole Related Compound A RS

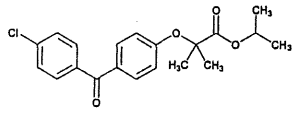
Methyl (1*H*-benzimidazole-2-yl)carbamate.

$C_9H_9N_3O_2$ 191.19

www.webofpharma.com

USP Fenbendazole Related Compound B RS
Methyl [5(6)-chlorobenzimidazole-2-yl]carbamate.
$C_9H_8ClN_3O_2$ 225.63
USP Mebendazole RS

Fenofibrate



$C_{20}H_{21}ClO_4$ 360.83
Isopropyl 2-[*p*-(*p*-chlorobenzoyl)phenoxy]-2-methylpropanoate [49562-28-9].

DEFINITION
Fenofibrate contains NLT 98.0% and NMT 102.0% of $C_{20}H_{21}ClO_4$, calculated on the dried basis.

IDENTIFICATION

Change to read:

▲• **SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water acidified with phosphoric acid to a pH of 2.5 (7:3)
Standard solution: 1 mg/mL of USP Fenofibrate RS in *Mobile phase*
Sample solution: 1 mg/mL of Fenofibrate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 286 nm
Column: 4.0-mm × 25-cm; packing L1
Flow rate: 1.0 mL/min
Injection size: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0% for six replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{20}H_{21}ClO_4$ in the portion of Fenofibrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fenofibrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Fenofibrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
INORGANIC IMPURITIES
• **Residue on Ignition** ⟨281⟩: NMT 0.1%, determined on 1.0 g
• **Chloride and Sulfate,** *Chloride* ⟨221⟩
Sample solution: Add 25 mL of water to 5.0 g of Fenofibrate, and heat at 50° for 10 min. Cool, dilute with water to 50.0 mL, filter, and use the filtrate. [NOTE—Retain the remaining portion of the *Sample solution* for the test for *Chloride and Sulfate, Sulfate*.]
Analysis: Use 10 mL of the *Sample solution*.
Acceptance criteria: It shows no more chloride than corresponds to 0.15 mL of 0.020 N hydrochloric acid (0.01%).
• **Chloride and Sulfate,** *Sulfate* ⟨221⟩
Sample: Use the *Sample solution* prepared in the test for *Chloride and Sulfate, Chloride*.
Analysis: Use 10 mL of the *Sample*.
Acceptance criteria: It shows no more sulfate than corresponds to 0.15 mL of 0.020 N sulfuric acid (0.01%).
ORGANIC IMPURITIES
• **Procedure**
Mobile phase: Acetonitrile and water acidified with phosphoric acid to a pH of 2.5 (7:3)
Impurity standard solution: 1 µg/mL each of USP Fenofibrate RS, USP Fenofibrate Related Compound A RS, and USP Fenofibrate Related Compound B RS, and 2 µg/mL of USP Fenofibrate Related Compound C RS in *Mobile phase*
Sample solution: 1 mg/mL of Fenofibrate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 286 nm
Column: 4.0-mm × 25-cm; packing L1
Flow rate: 1.0 mL/min
Injection size: 20 µL
System suitability
Sample: *Impurity standard solution*
Suitability requirements
Resolution: NLT 1.5 between fenofibrate related compound A and fenofibrate related compound B
Analysis
Samples: *Impurity standard solution* and *Sample solution*
Identify the fenofibrate peak and the peaks due to the impurities and degradation products listed in *Impurity Table 1*.
Measure the responses for the major peaks, and calculate the percentage of each of fenofibrate related compound A, fenofibrate related compound B, and fenofibrate related compound C in the portion of Fenofibrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of appropriate fenofibrate related compound from the *Sample solution*
r_S = peak response of appropriate fenofibrate related compound from the *Impurity standard solution*
C_S = concentration of the appropriate fenofibrate related compound in the *Impurity standard solution* (µg/mL)
C_U = concentration of Fenofibrate in the *Sample solution* (µg/mL)

Calculate the percentage of any other impurity in the portion of Fenofibrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of fenofibrate from the *Impurity standard solution*
C_S = concentration of fenofibrate in the *Impurity standard solution* (µg/mL)

www.webofpharma.com

C_U = concentration of Fenofibrate in the *Sample solution* (µg/mL)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
(4-Chlorophenyl)(4-hydroxyphenyl) methanone[a]	0.34	0.1
2-[4-(4-Chlorobenzoyl)phenoxy]-2-methyl-propanoic acid (fenofibric acid)[b]	0.36	0.1
(3*RS*)-3-[4-(4-Chlorobenzoyl) phenoxy]butan-2-one	0.50	0.1
Methyl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoate	0.65	0.1
Ethyl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoate	0.80	0.1
(4-Chlorophenyl)[4-(1-methylethoxy) phenyl]methanone	0.85	0.1
1-Methylethyl 2-[[2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoyl]oxy]-2-methylpropanoate[c]	1.35	0.2
Any other impurity	—	0.1

[a] Fenofibrate related compound A.
[b] Fenofibrate related compound B.
[c] Fenofibrate related compound C.

SPECIFIC TESTS
- **Melting Range or Temperature,** *Class Ia* ⟨741⟩: 79°–82°
- **Acidity**
  Sample: 1.0 g
  Analysis: Dissolve the *Sample* in 50 mL of alcohol previously neutralized to phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS.
  Acceptance criteria: NMT 0.2 mL is required to change the color of the indicator to pink.
- **Loss on Drying** ⟨731⟩
  Analysis: Dry a sample in a vacuum over phosphorus pentoxide at 60° to constant weight.
  Acceptance criteria: NMT 0.5%
- **Color and Achromicity** ⟨631⟩
  Reference solution: Mix 5 mL of *Matching Fluid G* and 95 mL of dilute hydrochloric acid (1 in 40).
  Sample solution: 50 mg/mL of Fenofibrate in acetone
  Analysis: Proceed as directed in the chapter.
  Acceptance criteria: The *Sample solution* is not more intensely colored than the *Reference solution*.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed, light-resistant containers. Store at room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Fenofibrate RS
  USP Fenofibrate Related Compound A RS
  (4-Chlorophenyl)(4-hydroxyphenyl)methanone.
  USP Fenofibrate Related Compound B RS
  2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoic acid, or fenofibric acid.
  USP Fenofibrate Related Compound C RS
  1-Methylethyl 2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl]oxy]-2-methylpropanoate.

Fenofibrate Capsules

DEFINITION
Fenofibrate Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲ (USP 1-May-2019)

ASSAY

Change to read:

- **Procedure**
  Use *Sample stock solution 2* for Capsules labeled to meet the requirements of *Dissolution Test 2*. For all other products, use *Sample stock solution 1*.
  Solution A: 136 mg/L of monobasic potassium phosphate in water. Adjust with dilute phosphoric acid (1 in 10) to a pH of 2.9 ± 0.05.
  Mobile phase: Methanol and *Solution A* (4:1)
  Standard solution: 67 µg/mL of USP Fenofibrate RS in *Mobile phase*
  Sample stock solution 1: Accurately weigh the contents of NLT 20 Capsules. Mix the contents, and transfer a weighed portion of the powder, equivalent to about 67 mg of fenofibrate, to a 100-mL volumetric flask. Add 80 mL of *Mobile phase*, sonicate for 10 min, stir for 15 min, and dilute with *Mobile phase* to volume.
  Sample stock solution 2 (for Capsules labeled to meet the requirements of *Dissolution Test 2*): Weigh the contents of NLT 20 Capsules. Mix the contents, melt in an oven at 80° for NLT 30 min, and homogenize. Allow the sample to solidify. Transfer a weighed portion of the sample, equivalent to about 67 mg of fenofibrate, to a 100-mL volumetric flask, dissolve in 30 mL of methanol with the aid of a mechanical shaker for NLT 4 h, and dilute with *Mobile phase* to volume.
  Sample solution: Nominally 67 µg/mL of fenofibrate from the designated *Sample stock solution*, in *Mobile phase*. Pass a portion of this solution through a polyvinylidene difluoride (PVDF) filter of 0.45-µm pore size, discarding the first 5 mL.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 285 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2019)
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  ▲**Run time:** NLT 1.5 times the retention time of the fenofibrate peak▲ (USP 1-May-2019)
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  ▲▲ (USP 1-May-2019)
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fenofibrate from the *Sample solution*
r_S = peak response of fenofibrate from the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = nominal concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.05 M sodium lauryl sulfate in water; 1000 mL, deaerated
Apparatus 2: 75 rpm
Time: 40 min
Solution A and **Mobile phase:** Prepare as directed in the *Assay*.
Standard solution: (0.001 × *L*) mg/mL of USP Fenofibrate RS in *Mobile phase*, where *L* is the label claim, in mg/Capsule
Sample solution: Pass a portion of the solution under test through a suitable PVDF filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL for Capsules labeled to contain 67 mg; 5 µL for Capsules labeled to contain 134 or 200 mg
System suitability
Sample: *Standard solution*
Suitability requirements
▲ ▲(USP 1-May-2019)
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Capsule)

Tolerances: NLT 70% (*Q*) of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Phosphate buffer pH 6.8 ± 0.1 containing 0.1% pancreatin and 2% polysorbate 80; 900 mL, deaerated by vacuum
Apparatus 2: 75 rpm with sinkers (see *Dissolution* ⟨711⟩, *Figure 2a*)
Time: 2 h
Standard solution: (*L*/1000) mg/mL of USP Fenofibrate RS in *Medium*, where *L* is the label claim in mg/Capsule. A volume of methanol, not exceeding 10%, can be used in the first dilution to solubilize fenofibrate.
Sample solution: Pass 20 mL of the solution under test through a suitable PVDF filter of 0.45-µm pore size, discarding the first 2 mL.
Blank: *Medium*
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Spectrophotometry
Detector: UV 288 nm
Path length: 0.1-cm flow cell
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (*Q*) of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
Medium: 0.72% sodium lauryl sulfate in water; 1000 mL, deaerated
Apparatus 2: 75 rpm, with sinkers with three prongs
Time: 30 min
Standard solution: (*L*/10) mg/mL of USP Fenofibrate RS in methanol, where *L* is the label claim in mg/Capsule. Transfer 10.0 mL of this solution to a 1000-mL volumetric flask, and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable PVDF filter of 0.45-µm pore size. Dilute with *Medium*, if necessary.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Spectrophotometry
Detector: UV 290 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 1000 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (*Q*) of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) is dissolved.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.05 M sodium lauryl sulfate in water; 1000 mL
Apparatus 2: 75 rpm, with helix sinkers or hoseclamp sinkers

www.webofpharma.com

Times: 30 min for products labeled to contain 67, 134, and 200 mg; 40 min for products labeled to contain 43 and 130 mg
Standard stock solution: 0.5 mg/mL of USP Fenofibrate RS in *Medium* prepared as follows. Dissolve a suitable quantity of USP Fenofibrate RS, taken in a suitable volumetric flask, in about 6% of the total volume of methanol, and dilute with *Medium* to volume.
Standard solution: Prepare solutions of USP Fenofibrate RS in *Medium* as per *Table 1* from *Standard stock solution*.

Table 1

Capsule Strength (mg)	Concentration (mg/mL)
67	0.065
130 and 134	0.13
200	0.2
43	0.045

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of the filtrate.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Spectrophotometry
Detector: UV 291 nm
Path length: 0.1-cm flow cell
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) is dissolved.
Test 5: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 5*.
Medium: 0.025 M sodium lauryl sulfate in water; 1000 mL, deaerated
Apparatus 2: 75 rpm, with suitable sinkers
Time: 20 min
Standard stock solution: 0.5 mg/mL of USP Fenofibrate RS in methanol. Sonicate if necessary.
Standard solution: 12.5 µg/mL of USP Fenofibrate RS prepared by diluting quantitatively from *Standard stock solution* with *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size and discard the first few milliliters. Dilute quantitatively with *Medium* to the nominal concentration as per *Table 2*.

Table 2

Capsule Strength (mg)	Concentration (µg/mL)
30	12.0
90	13.5

Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Spectrophotometry
Detector: UV 290 nm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (Q) of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) is dissolved.
▲**Test 6:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Dissolution Test 6 is suitable for products labeled to contain 200 mg of fenofibrate.
Medium, Solution A, Mobile phase, and **System suitability:** Proceed as directed in *Test 1*.
Apparatus 2: 75 rpm, with suitable sinkers
Time: 60 min
Standard solution: 0.2 mg/mL of USP Fenofibrate RS prepared as follows. Transfer a suitable amount of USP Fenofibrate RS into a suitable volumetric flask. Add methanol to 2% of the total volume of the flask and sonicate to dissolve. Dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable PVDF filter of 0.45-µm pore size. Discard the first few milliliters of filtrate.
Chromatographic system: Proceed as directed in *Test 1* except for *Run time*.
Run time: NLT 2 times the retention time of the fenofibrate
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of fenofibrate from the *Sample solution*
r_S = peak response of fenofibrate from the *Standard solution*
C_S = concentration of USP Fenofibrate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) is dissolved.▲ (RB 1-May-2019)

Change to read:

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: ▲Meet the requirements▲ (USP 1-May-2019)

Procedure for content uniformity
Solution A, Mobile phase, Standard solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed in the *Assay*, except to

www.webofpharma.com

prepare the *Sample stock solution* and *Sample solution* as follows.

Sample stock solution: Place 1 Capsule in a suitable volumetric flask, add *Solution A* to 10%–20% of the final volume, and stir for 20 min to disintegrate the Capsule. Fill the flask to about 80% with methanol, sonicate for 10 min, and stir for 15 min. Dilute with methanol to volume to obtain a solution having a known concentration of about 0.4–0.7 mg/mL of fenofibrate, based on the label claim.

Sample solution: Nominally 60–70 µg/mL of fenofibrate, from the *Sample stock solution*, in *Mobile phase*. Pass a portion of this solution through a PVDF filter of 0.45-µm pore size, discarding the first 5 mL.

▲▲ (USP 1-May-2019)

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Use *Sample solution 2* for Capsules labeled to meet the requirements of *Dissolution Test 2*. For all other products, use *Sample solution 1*.

Solution A: 136 mg/L of potassium phosphate. Adjust with dilute phosphoric acid (1 in 10) to a pH of 2.9 ± 0.05.

Mobile phase: Methanol and *Solution A* (4:1)

System suitability solution: 0.67 mg/mL of USP Fenofibrate RS and 3.35 µg/mL of USP Fenofibrate Related Compound B RS in *Mobile phase*

Standard solution: 3.35 µg/mL of USP Fenofibrate RS and 3.35 µg/mL of USP Fenofibrate Related Compound B RS in *Mobile phase*

Sensitivity solution: 0.67 µg/mL of USP Fenofibrate RS and 0.67 µg/mL of USP Fenofibrate Related Compound B RS in *Mobile phase*, from the *Standard solution*

Sample solution 1: Nominally 0.67 mg/mL of fenofibrate prepared as follows. Accurately weigh the contents of NLT 20 Capsules. Mix the contents, and transfer a weighed portion of the powder, equivalent to about 67 mg of fenofibrate, to a 100-mL volumetric flask. Add 80 mL of *Mobile phase*, sonicate for 10 min, stir for 15 min, and dilute with *Mobile phase* to volume. Pass a portion of this solution through a PVDF filter of 0.45-µm pore size, discarding the first 5 mL.

Sample solution 2 (for Capsules labeled to meet the requirements of *Dissolution Test 2*): Nominally 0.67 mg/mL of fenofibrate prepared as follows. Weigh the contents of NLT 20 Capsules. Mix the contents, melt in an oven at 80° for NLT 30 min, and homogenize. Allow the sample to solidify. Transfer a weighed portion of the sample, equivalent to about 67 mg of fenofibrate, to a 100-mL volumetric flask, dissolve in 30 mL of methanol with the aid of a mechanical shaker for NLT 4 h, and dilute with *Mobile phase* to volume. Pass through a PVDF filter of 0.45-µm pore size, discarding the first 1–2 mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

▲Run time: NLT 3 times the retention time of the fenofibrate peak▲ (USP 1-May-2019)

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 3.0 between fenofibrate and fenofibrate related compound B, *System suitability solution*

▲▲ (USP 1-May-2019)

Tailing factor: NMT 2.0 for fenofibrate related compound B, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10 for the fenofibrate peak, *Sensitivity solution*

Analysis

Samples: *Standard solution* and designated *Sample solution*

Calculate the percentage of fenofibrate related compound B in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fenofibrate related compound B from the *Sample solution*

r_S = peak response of fenofibrate related compound B from the *Standard solution*

C_S = concentration of fenofibrate related compound B in the *Standard solution* (mg/mL)

C_U = nominal concentration of fenofibrate in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response of fenofibrate from the *Standard solution*

C_S = concentration of fenofibrate in the *Standard solution* (mg/mL)

C_U = nominal concentration of fenofibrate in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurities: NMT 0.5% for fenofibrate related compound B; NMT 0.2% for any other unspecified impurity

Total impurities: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Fenofibrate RS

USP Fenofibrate Related Compound B RS

2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoic acid, or fenofibric acid.

$C_{17}H_{15}ClO_4$ 318.75

Fenofibrate Tablets

DEFINITION

Fenofibrate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**

Acidified water: Adjust the pH of water with phosphoric acid to 2.5 ± 0.1.

Mobile phase: Acetonitrile and *Acidified water* (70:30)

System suitability stock solution: 0.1 mg/mL each of USP Fenofibrate Related Compound A RS and USP Fenofibrate Related Compound B RS in acetonitrile

System suitability solution: 0.5 µg/mL each of USP Fenofibrate Related Compound A RS and USP Fenofibrate Related Compound B RS in *Mobile phase* from the *System suitability stock solution*

Standard solution: 0.05 mg/mL of USP Fenofibrate RS in *Mobile phase*

Sample stock solution: Prepare a solution containing a nominal concentration of approximately 2–4 mg/mL of fenofibrate by disintegrating a suitable number of Tablets with sonication in *Acidified water*, using 30% of the final volume of the flask. Add acetonitrile to approximately 90% of flask volume, and sonicate with periodic swirling. Dilute with acetonitrile to volume.

Sample solution: Nominally 0.05 mg/mL of fenofibrate in *Mobile phase*, from the *Sample stock solution*. Filter a portion of this solution, discarding the first few milliliters of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 286 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2019)

Column: 4.0-mm × 25-cm or 4.6-mm × 25-cm; 5-µm or 4-µm packing L1

Column temperature: 35°

Flow rate: 1.2 mL/min

Injection volume: 10 µL

▲**Run time:** NLT 2 times the retention time of the fenofibrate peak▲ (USP 1-May-2019)

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between fenofibrate related compound A and fenofibrate related compound B peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% for the fenofibrate peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fenofibrate from the *Sample solution*

r_S = peak response of fenofibrate from the *Standard solution*

C_S = concentration of USP Fenofibrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fenofibrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.025 M sodium dodecyl sulfate in water; 1000 mL

Apparatus 2: 50 rpm

Time: 30 min

Acidified water: Adjust the pH of water with phosphoric acid to 2.5 ± 0.1.

Mobile phase: Acetonitrile and *Acidified water* (70:30)

Standard stock solution: 2.5 mg/mL of USP Fenofibrate RS in acetonitrile

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of about (0.001 × *L*) mg/mL, where *L* is the label claim, in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 286 nm

Column: 2-mm × 3-cm; 3-µm packing L1

Column temperature: 35°

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NLT 0.9 and NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = volume of *Medium*, 1000 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.05 M sodium dodecyl sulfate in water; 1000 mL

Apparatus 2: 50 rpm

Time: 30 min

Buffer: 136 mg/L of monobasic potassium phosphate in water. Adjust with diluted phosphoric acid to a pH of 2.9 ± 0.05.

Mobile phase: Methanol and *Buffer* (80:20)

Standard solution: (0.001 × *L*) mg/mL of USP Fenofibrate RS in *Mobile phase*, where *L* is the label claim, in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 286 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

USP Monographs

www.webofpharma.com

Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of fenofibrate from the *Sample solution*
r_S = peak response of fenofibrate from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
For Tablet strengths other than 40 and 120 mg of fenofibrate
Medium: 0.05 M sodium lauryl sulfate in water; 1000 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: 0.012 mg/mL of USP Fenofibrate RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate and appropriately diluting with *Medium* to a concentration similar to that of the *Standard solution*.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 292 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
D = dilution factor for the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) is dissolved.
For Tablets labeled to contain 40 and 120 mg of fenofibrate
Medium: 0.75% sodium lauryl sulfate in water; 900 mL
Apparatus 2: 75 rpm
Time: 45 min
Buffer: 2.72 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.9 ± 0.05.
Mobile phase: Methanol and *Buffer* (85:15)
Standard stock solution: 2.22 mg/mL of USP Fenofibrate RS prepared as follows. Transfer a suitable amount of USP Fenofibrate RS into a suitable volumetric flask. Add 50% of the flask volume of acetonitrile, sonicate to dissolve, and dilute with *Medium* to volume.
Standard solution: (*L*/900) mg/mL of USP Fenofibrate RS in *Medium* from *Standard stock solution*, where *L* is the label claim, in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 285 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 2 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of fenofibrate
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of fenofibrate from the *Sample solution*
r_S = peak response of fenofibrate from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of fenofibrate ($C_{20}H_{21}ClO_4$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**
Acidified water, Mobile phase, System suitability solution, Sample stock solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
▲**Sensitivity solution:** 0.25 µg/mL of USP Fenofibrate RS in *Mobile phase*▲ (USP 1-May-2019)
Standard solution: 0.5 µg/mL of USP Fenofibrate RS in *Mobile phase*
Sample solution: Nominally 0.5 mg/mL of fenofibrate in *Mobile phase*, from the *Sample stock solution*. Filter a portion of this solution, discarding the first few milliliters of filtrate.
System suitability
Samples: *System suitability solution*, ▲*Sensitivity solution*,▲ (USP 1-May-2019) and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between fenofibrate related compound A and fenofibrate related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
▲**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*▲ (USP 1-May-2019)

www.webofpharma.com

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each ▲degradation product▲ (USP 1-May-2019) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each ▲degradation product▲ (USP 1-May-2019) from the *Sample solution*

r_S = peak response of fenofibrate from the *Standard solution*

C_S = concentration of USP Fenofibrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fenofibrate in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fenofibrate related compound A	0.34	1.3	0.2
Fenofibrate related compound B	0.36	1.0	0.50
(3*RS*)-3-[4-(4-Chlorobenzoyl) phenoxy]butan-2-one[a]	0.50	—	—
Methyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methyl-propanoate[a]	0.65	—	—
Ethyl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoate[a]	0.80	—	—
(4-Chlorophenyl)[4-(1-methylethoxy)phenyl]methanone[a]	0.85	—	—
Fenofibrate	1.00	—	—
Fenofibrate related compound C[a, b]	1.35	—	—
Any unspecified ▲degradation product▲ (USP 1-May-2019)	—	1.0	0.2
Total ▲degradation products▲ (USP 1-May-2019) (includes fenofibrate related compounds A and B, and unspecified ▲degradation products)▲ (USP 1-May-2019)	—	—	1.0

[a] Disregard this impurity. It is a process impurity and is controlled in the drug substance monograph.

[b] 1-Methylethyl 2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl]oxy]-2-methylpropanoate.

ADDITIONAL REQUIREMENTS

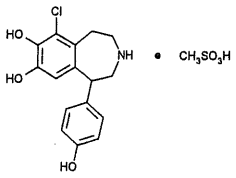
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Fenofibrate RS

USP Fenofibrate Related Compound A RS
(4-Chlorophenyl)(4-hydroxyphenyl)methanone.
$C_{13}H_9ClO_2$ 232.66

USP Fenofibrate Related Compound B RS
2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoic acid, or fenofibric acid.
$C_{17}H_{15}ClO_4$ 318.75

Fenoldopam Mesylate



$C_{16}H_{16}ClNO_3 \cdot CH_4SO_3$ 401.86

1*H*-3-Benzazepine-7,8-diol, 6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-, methanesulfonate (salt).

6-Chloro-2,3,4,5-tetrahydro-1-(*p*-hydroxyphenyl)-1*H*-3-benzazepine-7,8-diol methanesulfonate (salt) [67227-57-0].

» Fenoldopam Mesylate contains not less than 98.0 percent and not more than 102.0 percent of $C_{16}H_{16}ClNO_3 \cdot CH_4SO_3$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, protected from moisture. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—

USP Fenoldopam Mesylate RS

USP Fenoldopam Related Compound A RS
6-Chloro-1-(4-hydroxyphenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine-7,8-diol hydrochloride (*N*-methyl-6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1*H*-3-benzazepine-7,8-diol hydrochloride).
$C_{17}H_{19}ClNO_3 \cdot HCl$ 356.24

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Water Determination, *Method I* ⟨921⟩**:** not more than 1.0%.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Limit of iodide—

Mobile phase—Prepare a filtered and degassed solution containing about 0.94 g of sodium bicarbonate, 0.952 g of sodium carbonate, 0.38 g of 4-cyanophenol, and 80 mL of acetonitrile in 4 L of water. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock solution—Transfer about 118.1 mg of sodium iodide, accurately weighed, to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume, and mix to obtain a solution containing the equivalent of 100 µg of iodide per mL.

Standard solutions—Pipet 2.0 mL, 4.0 mL, 6.0 mL, and 8.0 mL of the *Standard stock solution* into separate 100-mL

USP Monographs

www.webofpharma.com

volumetric flasks, dilute with water to volume, and mix to obtain solutions having known concentrations of about 2 µg, 4 µg, 6 µg, and 8 µg of iodide per mL.

Test solution—Transfer about 300 mg of Fenoldopam Mesylate, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with water to volume, and mix.

Chromatographic system—The ion chromatograph is equipped with a conductivity detector, a 4-mm × 3.5-cm anion-exchange guard column, a 4-mm × 15-cm anion-exchange analytical column, and a micromembrane anion suppressor column. The flow rate is about 2.0 mL per minute. The regeneration solution for the suppressor column is a 0.050 M sulfuric acid solution, flowing at a rate of 5 mL per minute. Chromatograph the 6 µg per mL *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 100 µL) of each of the *Standard solutions* and the *Test solution* into the chromatograph, record the chromatograms, and measure the heights of peak responses. Plot the response of each of the *Standard solutions* versus the concentration, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the quantity of iodide in the portion of Fenoldopam Mesylate taken: not more than 0.2% is found.

Related compounds—

Buffer solution, System suitability stock solution, and *System suitability solution*—Proceed as directed in the *Assay.*

Solution A—Use *Mobile phase* as prepared in the *Assay.*

Solution B—Use filtered and degassed methanol.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Test solution—Use the *System suitability stock solution.*

Chromatographic system—Proceed as directed in the *Assay,* except to program the chromatograph as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–30	100	0	isocratic
30–60	100→70	0→30	linear gradient

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Fenoldopam Mesylate taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks: not more than 0.3% of fenoldopam related compound A is found; not more than 0.1% of any other individual impurity is found; and not more than 1.0% of total impurities is found.

Limit of residual solvents—

Internal standard solution—Prepare a solution, in organic-free water, containing 10 mg of *n*-butanol per mL. Transfer 100 µL of this solution to a 10-mL volumetric flask, dilute with dimethylsulfoxide to volume, and mix.

Standard solution—Prepare a solution, in organic-free water, containing 10 mg each of *n*-propanol, isopropyl alcohol, and dimethylformamide per mL. Transfer 100 µL of this solution to a 10-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

Test solution—Transfer about 50 mg of Fenoldopam Mesylate, accurately weighed, to a 1-mL volumetric flask. Dilute with *Internal standard solution* to volume, and sonicate to dissolve completely.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, a 0.32-mm × 30-m fused-silica capillary column coated with a 1.8-µm film of stationary phase G43, and a split injection system. The carrier gas is helium, flowing at a rate of about 1 mL per minute through the column and a split ratio of about 50:1. The injection port and the detector temperatures are maintained at 140° and 260°, respectively. The column temperature is programmed as follows. It is maintained for 12 minutes at 40°, then increased at a rate of 8° per minute to 120°, held for 0.1 minute, then increased at a rate of 25° per minute to 180°, and maintained at that temperature for 8 minutes.

Procedure—Separately inject equal volumes (about 1 µL) of the *Standard solution,* dimethylsulfoxide, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Identify, based on retention time, any peaks present in the chromatogram of the *Test solution.* Calculate the response factor, *F,* for each solvent in the *Standard solution* by the formula:

$$(W_R/W_I)(r_I/r_R)$$

in which W_R is the weight, in mg, of the solvent of interest; W_I is the weight, in mg, of the internal standard taken to prepare the *Internal standard solution;* and r_I and r_R are the peak responses for the internal standard and the solvent of interest, respectively, obtained from the *Standard solution.* Calculate the percentage, by weight, of each solvent found in the *Test solution* by the formula:

$$100FD(r_I/r_S)(W_I/W_D)$$

in which *F* is the average response factor for the solvent of interest obtained from all injections of the *Standard solution; D* is the dilution factor for the internal standard in the *Test solution* (i.e., 0.0001); r_I and r_S are the peak responses for the solvent of interest and the internal standard, respectively, obtained from the *Test solution;* W_I is the weight, in mg, of the internal standard taken to prepare the *Internal standard solution;* and W_D is the weight, in mg, of Fenoldopam Mesylate taken to prepare the *Test solution:* not more than 0.2% of total residual solvents is found.

Assay—

Buffer solution—Transfer about 16.33 g of monobasic potassium phosphate and 2 mL of triethylamine to a 2-L volumetric flask, and dissolve in 1800 mL of water. Adjust with phosphoric acid to a pH of 2.5, dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (19:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability stock solution—Transfer about 50 mg of Fenoldopam Mesylate, accurately weighed, to a 50-mL volumetric flask. With the aid of an ultrasonic bath, dissolve in and dilute with *Mobile phase* to volume, and mix.

System suitability solution—Transfer about 5 mg of USP Fenoldopam Related Compound A RS, accurately weighed, to a 50-mL volumetric flask. Add about 25 mL of *Mobile phase,* and sonicate to dissolve. Add 5 mL of the *System suitability stock solution,* dilute with *Mobile phase* to volume, and mix.

USP Monographs

www.webofpharma.com

Standard preparation—Dissolve an accurately weighed quantity of USP Fenoldopam Mesylate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer 5.0 mL of the *System suitability stock solution,* accurately measured, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 225-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The flow rate is about 1.7 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between fenoldopam and fenoldopam related compound A is not less than 1.5; the column efficiency is not less than 2000 theoretical plates; the tailing factor is not more than 1.3; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of $C_{16}H_{16}ClNO_3 \cdot CH_4SO_3$ in the portion of Fenoldopam Mesylate taken by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Fenoldopam Mesylate RS in the *Standard preparation;* and r_U and r_S are the peak responses for fenoldopam obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Fenoldopam Mesylate Injection

DEFINITION

Change to read:

Fenoldopam Mesylate Injection is a sterile solution of fenoldopam mesylate ($C_{16}H_{16}ClNO_3 \cdot CH_4SO_3$). It contains ▲an amount of fenoldopam mesylate, equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of fenoldopam ($C_{16}H_{16}ClNO_3$).▲ (USP 1-Dec-2019)

IDENTIFICATION

Delete the following:

▲• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩

Standard solution: 1 mg/mL in methanol

Sample solution: Pipet 1.0 mL of Injection into a 10-mL volumetric flask. Dilute to volume with methanol, and mix.

Application volume: 20 µL

Developing solvent system: Acetone, chloroform, acetic acid, and water (6:2:1:1)

Place the mixture in a paper-lined chromatographic chamber, and equilibrate for about 15 min before use.

Analysis: Proceed as directed in the chapter, and then dry the plate under a current of warm air until completely dry. Place the plate into a second chromatographic chamber containing iodine crystals, and examine the plate.

Acceptance criteria: Meets the requirements.▲ (USP 1-Dec-2019)

Add the following:

▲• **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*▲ (USP 1-Dec-2019)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

Change to read:

• **Procedure**

Solution A: 1.38 g/L of monobasic sodium phosphate monohydrate and 1.88 g/L of sodium hexanesulfonate in water. Adjust with a ▲10% v/v phosphoric acid▲ (USP 1-Dec-2019) solution to a pH of 2.5.

Mobile phase: Methanol and *Solution A* (33:67)

Diluent: 1.38 g/L of monobasic sodium phosphate monohydrate in water. Adjust with a ▲10% v/v phosphoric acid▲ (USP 1-Dec-2019) solution to a pH of 2.5.▲▲ (USP 1-Dec-2019)

Standard solution: 0.263 mg/mL of USP Fenoldopam Mesylate RS▲▲ (USP 1-Dec-2019) in *Diluent*

Sample solution: ▲Nominally 0.2 mg/mL of fenoldopam in *Diluent* from Injection▲ (USP 1-Dec-2019)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)▲▲ (USP 1-Dec-2019)

Mode: LC

Detector: UV 225 nm. ▲For *Identification A,* use a diode array detector in the range of 200–400 nm.▲ (USP 1-Dec-2019)

Column: 4.6-mm × 25-cm; ▲5-µm▲ (USP 1-Dec-2019) packing L1

▲Column temperature: 33°▲ (USP 1-Dec-2019)

Flow rate: 1 mL/min

Injection volume: 10 µL

▲Run time: NLT 2 times the retention time of fenoldopam▲ (USP 1-Dec-2019)

System suitability

Sample: ▲▲ (USP 1-Dec-2019)*Standard solution*

Suitability requirements▲▲ (USP 1-Dec-2019)

Tailing factor: ▲▲ (USP 1-Dec-2019)NMT 2.0 for the fenoldopam peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fenoldopam ▲▲ (USP 1-Dec-2019) ($C_{16}H_{16}ClNO_3$▲▲ (USP 1-Dec-2019)) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of fenoldopam from the *Sample solution*

r_S = peak response of fenoldopam from the *Standard solution*

C_S = concentration of USP Fenoldopam Mesylate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fenoldopam in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of fenoldopam, 305.76

M_{r2} = molecular weight of fenoldopam mesylate, 401.86

Acceptance criteria: 90.0%–110.0%

USP Monographs

www.webofpharma.com

OTHER COMPONENTS

Change to read:

- **CONTENT OF SODIUM METABISULFITE ▲(if present)▲** (USP 1-Dec-2019)

Sample: Transfer 10.0 mL of Injection to a glass-stoppered conical flask containing 5.0 mL of 0.1 N iodine VS, and swirl to dissolve. Allow to stand for exactly 5 min, protected from light.

▲Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Endpoint detection: Visual▲ (USP 1-Dec-2019)

Analysis: Add 0.5 mL of hydrochloric acid. Titrate the excess iodine with 0.05 N sodium thiosulfate VS ▲until the solution is pale yellow.▲ (USP 1-Dec-2019) Add 0.5 mL of starch TS▲as indicator and complete the titration to a colorless endpoint.▲ (USP 1-Dec-2019) Perform a blank determination ▲using 10 mL of water,▲ (USP 1-Dec-2019) and make any necessary correction. Each milliliter of 0.05 N sodium thiosulfate is equivalent to 2.3763 mg of sodium metabisulfite.

Acceptance criteria: NLT 0.25 mg/mL of Injection

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Solution A, Mobile phase, Diluent, ▲▲ (USP 1-Dec-2019) **Sample solution,** and **Chromatographic system:** Proceed as directed in the *Assay*.

▲Standard solution: 0.263 mg/mL of USP Fenoldopam Mesylate RS and 0.274 µg/mL of USP Fenoldopam Related Compound B RS in *Diluent*▲ (USP 1-Dec-2019)

System suitability
Sample: ▲▲ (USP 1-Dec-2019) *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5 for the fenoldopam related compound B peak; ▲▲ (USP 1-Dec-2019) NMT 2.0 for the fenoldopam peak
Relative standard deviation: NMT 2.0% ▲for the fenoldopam related compound B and fenoldopam peaks▲ (USP 1-Dec-2019)

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fenoldopam related compound B ▲free base▲ (USP 1-Dec-2019) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times {}^{\blacktriangle}(M_{r1}/M_{r2})^{\blacktriangle}_{\text{(USP 1-Dec-2019)}} \times 100$$

r_U = peak response of fenoldopam related compound B from the *Sample solution*
r_S = peak response of fenoldopam related compound B from the *Standard solution*
C_S = concentration of USP Fenoldopam Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fenoldopam in the *Sample solution* (mg/mL)
$^{\blacktriangle}M_{r1}$ = molecular weight of fenoldopam related compound B free base, 271.32
M_{r2} = molecular weight of fenoldopam related compound B, 367.42▲ (USP 1-Dec-2019)

Acceptance criteria: NMT 0.6%

SPECIFIC TESTS

Change to read:

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲Meets the requirements▲ (USP 1-Dec-2019)
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **PH** ⟨791⟩: 2.8–3.8
- **OTHER REQUIREMENTS:** Meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩

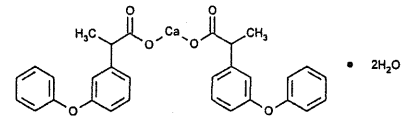
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*, preferably of Type I glass. Store in a refrigerator or at controlled room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
USP Fenoldopam Mesylate RS
USP Fenoldopam Related Compound B RS
1*H*-3-Benzazepine-7,8-diol, 2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-, methanesulfonate (salt);
▲Also known as
1-(4-Hydroxyphenyl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine-7,8-diol methanesulfonate.
$C_{16}H_{17}NO_3 \cdot CH_4SO_3$ 367.42▲ (USP 1-Dec-2019)

Fenoprofen Calcium



$C_{30}H_{26}CaO_6 \cdot 2H_2O$ 558.63
Benzeneacetic acid, α-methyl-3-phenoxy-, calcium salt dihydrate, (±)-.
Calcium (±)-*m*-phenoxyhydratropate dihydrate [53746-45-5].
Anhydrous 522.61
[34597-40-5].

» Fenoprofen Calcium contains not less than 97.0 percent and not more than 103.0 percent of $(C_{15}H_{13}O_3)_2Ca$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Fenoprofen Calcium RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Heat a 1 in 50 mixture of it with acetic acid, filter, and add 2 mL of ammonium oxalate TS to the filtrate: a white precipitate, which is soluble in 3 N hydrochloric acid, is formed.

www.webofpharma.com

Water Determination, *Method I* ⟨921⟩: between 5.0% and 8.0%.

Chromatographic purity—

Solution A—Prepare a filtered and degassed mixture of water and acetic acid (98:2).

Solution B—Prepare a filtered and degassed mixture of acetonitrile and acetic acid (98:2).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent: a mixture of water and acetonitrile (1:1).

System suitability solution—Dissolve accurately weighed quantities of 3-phenoxybenzoic acid and USP Fenoprofen Calcium RS in *Diluent* to obtain a solution containing about 0.02 mg of each per mL.

Standard solution—Dissolve an accurately weighed quantity of USP Fenoprofen Calcium RS in *Diluent* to obtain a solution having a known concentration of about 0.02 mg per mL.

Test solution—Transfer about 200 mg of Fenoprofen Calcium, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 270-nm detector and a suitable 4.6-mm × 25-cm column[1] that contains 5-µm packing L7. The flow rate is 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	70	30	equilibration
0–3	70	30	isocratic
3–41	70→10	30→90	linear gradient
41–42	10	90	isocratic
42–43	10→70	90→30	linear gradient
43–55	70	30	re-equilibration

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.89 for 3-phenoxybenzoic acid and 1.0 for fenoprofen; the resolution, *R,* between 3-phenoxybenzoic acid and fenoprofen is not less than 9.0; and the tailing factor for the fenoprofen peak is not more than 2.0. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor for the fenoprofen peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Fenoprofen Calcium taken by the formula:

$$10{,}000(C/W)(r_i/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Fenoprofen Calcium RS in the *Standard solution; W* is the quantity, in mg, of Fenoprofen Calcium taken to prepare the *Test solution;* r_i is the response for each impurity peak obtained from the *Test solution;* and r_S is the response of the fenoprofen peak obtained from the *Standard solution:* not more than 0.5% of any individual impurity is found; and not more than 2.0% of total impurities is found.

[1] One suitable column brand is the Zorbax Eclipse XDB-C8.

Content of calcium—

Test solution—Transfer about 750 mg of Fenoprofen Calcium, accurately weighed, to a 50-mL volumetric flask, dissolve in alcohol with the aid of heat, if necessary, cool, dilute with alcohol to volume, and mix.

Procedure—In a 150-mL beaker, mix 70 mL of water, 2 mL of sodium hydroxide solution (1 in 10), and about 0.3 g of hydroxy naphthol blue. Add about 1 mL of the *Test solution,* and titrate to the blue endpoint with 0.05 M edetate disodium. Transfer 10.0 mL of the *Test solution* to the solution so obtained, and titrate to the blue endpoint with 0.05 M edetate disodium VS. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of Ca: not less than 7.3% and not more than 8.0% of Ca, calculated on the anhydrous basis, is found.

Assay—

Mobile phase—Prepare a suitable degassed mixture of acetonitrile, water, and phosphoric acid (50: 49.6: 0.4). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluting solution—Prepare a mixture of methanol and water (700:300).

Resolution solution—Prepare a solution in *Diluting solution* containing about 1 mg of Fenoprofen Calcium and 1 mg of gemfibrozil per mL.

Standard preparation—Transfer about 70 mg of USP Fenoprofen Calcium RS, accurately weighed, to a 100-mL volumetric flask, add 0.5 mL of 0.5 N hydrochloric acid and 2 mL of acetone, and dissolve by shaking. Dilute with *Diluting solution* to volume, and mix.

Assay preparation—Transfer about 70 mg of Fenoprofen Calcium, accurately weighed, to a 100-mL volumetric flask, add 0.5 mL of 0.5 N hydrochloric acid and 2 mL of acetone, and dissolve by shaking. Dilute with *Diluting solution* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 272-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.5 for fenoprofen and 1.0 for gemfibrozil; the tailing factor for the fenoprofen peak is not more than 2; and the resolution, *R,* between the fenoprofen peak and the gemfibrozil peak is not less than 8. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the fenoprofen peak is not less than 3000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $(C_{15}H_{13}O_3)_2Ca$ in the portion of Fenoprofen Calcium taken by the formula:

$$100C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of anhydrous fenoprofen calcium in the *Standard preparation,* as determined from the concentration of USP Fenoprofen Calcium RS, corrected for moisture content by a titrimetric water determination; and r_U and r_S are the fenoprofen peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

USP Monographs

www.webofpharma.com

Fenoprofen Calcium Capsules

» Fenoprofen Calcium Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fenoprofen ($C_{15}H_{14}O_3$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Fenoprofen Calcium RS
USP Fenoprofen Sodium RS

Identification—
A: Transfer a portion of Capsule contents, equivalent to about 85 mg of fenoprofen, to a 125-mL separator containing 5 mL of acetone and 2 mL of 6 N hydrochloric acid. Swirl to dissolve, add 15 mL of water, and extract with three 15-mL portions of chloroform, draining each chloroform extract through a layer of about 2 g of anhydrous sodium sulfate, supported on glass wool and previously washed with chloroform, into a 50-mL volumetric flask. Rinse the sodium sulfate filter with about 2 mL of chloroform, collect the rinsing with the combined chloroform extracts, dilute with chloroform to volume, and mix. Transfer this solution to a suitable flask, and evaporate on a water bath with the aid of a current of air to dryness: the IR absorption spectrum of a film of the liquid residue thus obtained between sodium chloride plates exhibits maxima only at the same wavelengths as that of a similar preparation of USP Fenoprofen Calcium RS.
B: Place an amount of Capsule contents, equivalent to about 300 mg of fenoprofen, in a suitable container, and dissolve in 10 mL of acetone. Filter the solution through paper, and collect the filtrate in a crucible. Carefully evaporate to dryness, and ignite the crucible and its contents. Dissolve the residue in 10 mL of 1 N hydrochloric acid, transfer the solution to a beaker, add 2 drops of methyl red TS, neutralize with 6 N ammonium hydroxide, and add 3 N hydrochloric acid dropwise until the solution is acid to the indicator. Upon the addition of ammonium oxalate TS, a white precipitate is formed. The residue so obtained is insoluble in acetic acid but dissolves in hydrochloric acid.

Dissolution ⟨711⟩—
Medium: pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 1000 mL.
Apparatus 1: 10-mesh basket; 100 rpm.
Time: 60 minutes.
Procedure—Filter 20 mL of the solution under test, and transfer 5.0 mL of the filtrate to a 25-mL volumetric flask. Dilute with *Dissolution Medium* to volume, and mix. Determine the absorbances of this solution and a Standard solution prepared from USP Fenoprofen Sodium RS, in the same medium having a known concentration of about 60 µg per mL, at the wavelength of maximum absorbance at about 270 nm, using *Dissolution Medium* as the blank. Calculate the amount of $C_{15}H_{14}O_3$ dissolved, in mg, by the formula:

$$(242.28/264.26)(5C)(A_U/A_S)$$

in which 242.28 is the molecular weight of fenoprofen; 264.26 is the molecular weight of anhydrous fenoprofen sodium; C is the concentration of anhydrous fenoprofen sodium in the Standard solution, as determined from the concentration of USP Fenoprofen Sodium RS corrected for moisture content by a titrimetric water determination; and A_U and A_S are the absorbances of the solutions obtained from the substance under test and the USP Reference Standard, respectively.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{15}H_{14}O_3$ is dissolved in 60 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase, Diluting solution, Resolution solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay*— under *Fenoprofen Calcium.*
Assay preparation—Weigh the contents of not fewer than 20 Capsules, and calculate the average weight per Capsule. Mix the combined contents of the Capsules, and transfer an accurately weighed portion, equivalent to about 150 mg of fenoprofen, to a 250-mL volumetric flask. Add about 200 mL of *Diluting solution,* and sonicate for about 15 minutes. Allow to cool, dilute with *Diluting solution* to volume, mix, and pass through a suitable filter, discarding the first 10 mL of the filtrate. Use the clear filtrate as the *Assay preparation.*
Procedure—Proceed as directed in the *Assay*— under *Fenoprofen Calcium.* Calculate the quantity, in mg, of fenoprofen ($C_{15}H_{14}O_3$) in the portion of Capsules taken by the formula:

$$(484.55/522.61)(1000C)(r_U/r_S)$$

in which 484.55 is two times the molecular weight of fenoprofen; 522.61 is the molecular weight of fenoprofen calcium; and the other terms are as defined therein.

Fenoprofen Calcium Tablets

» Fenoprofen Calcium Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fenoprofen ($C_{15}H_{14}O_3$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Fenoprofen Calcium RS
USP Fenoprofen Sodium RS
Identification—An amount of finely powdered Tablets responds to the *Identification*— tests under *Fenoprofen Calcium Capsules.*

Dissolution ⟨711⟩—
Medium: pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 1000 mL.
Apparatus 1: 10-mesh basket; 100 rpm.
Time: 60 minutes.
Procedure—Filter 20 mL of the solution under test, and transfer 10.0 mL of the filtrate to a 100-mL volumetric flask. Dilute with *Dissolution Medium* to volume, and mix. Determine the absorbances of this solution and a Standard solution prepared from USP Fenoprofen Sodium RS, in the same medium having a known concentration of about 60 µg per mL at the wavelength of maximum absorbance at about 270 nm, using *Dissolution Medium* as the blank. Calculate the amount of $C_{15}H_{14}O_3$ dissolved, in mg, by the formula:

$$(242.28/264.26)(10C)(A_U/A_S)$$

in which 242.28 is the molecular weight of fenoprofen; 264.26 is the molecular weight of anhydrous fenoprofen sodium; C is the concentration of anhydrous fenoprofen sodium in the Standard solution, as determined from the concentration of USP Fenoprofen Sodium RS corrected for moisture content by a titrimetric water determination; and A_U and A_S are the

www.webofpharma.com

absorbances of the solutions obtained from the substance under test and the USP Reference Standard, respectively.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{15}H_{14}O_3$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase, Diluting solution, Resolution solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay*— under *Fenoprofen Calcium.*

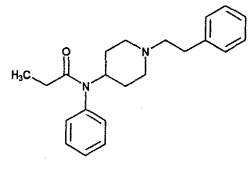
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 6000 mg of fenoprofen, to a 250-mL volumetric flask, add 50 mL of 0.5 N hydrochloric acid, and sonicate for 10 minutes, shaking the solution occasionally. Dilute with acetone to volume, sonicate for 10 minutes, and stir for an additional 30 minutes. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with *Diluting solution* to volume, and mix. Pass a portion of this solution through a suitable filter having a 0.5-µm or finer porosity, discarding the first 8 mL of the filtrate, and use the clear filtrate as the *Assay preparation.*

Procedure—Proceed as directed in the *Assay*— under *Fenoprofen Calcium.* Calculate the quantity, in mg, of fenoprofen ($C_{15}H_{14}O_3$) in the portion of Tablets taken by the formula:

$$(484.55/522.61)(10{,}000C)(r_U/r_S)$$

in which 484.55 is two times the molecular weight of fenoprofen; 522.61 is the molecular weight of fenoprofen calcium; and the other terms are as defined therein.

Fentanyl



$C_{22}H_{28}N_2O$ 336.47

Propanamide, *N*-phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]; *N*-(1-Phenethylpiperidin-4-yl)-*N*-phenylpropionamide [437-38-7].

DEFINITION

Change to read:

Fentanyl contains NLT 98.0% and NMT 102.0% of ▲▲ (USP 1-May-2020) fentanyl ($C_{22}H_{28}N_2O$), calculated on the dried basis.

[**Caution**—Great care should be taken to prevent inhaling particles of Fentanyl and skin exposure.]

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy:* 197K or 197A▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

Change to read:

- **Procedure**

Solution A: Add 3 mL of triethylamine to about 950 mL of water in a 1000-mL volumetric flask. Adjust with perchloric acid to a pH of 2.62 ± 0.02, and dilute with water to volume.

Solution B: Acetonitrile

Diluent: *Solution A* and *Solution B* (9:1)

Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
2	90	10
3.5	85	15
5.1	82	18
7.6	72	28
11.5	63	37
15	40	60
19	40	60
20	90	10
25	90	10

System suitability stock solution: ▲0.1 mg/mL of USP Fentanyl Related Compound E RS prepared as follows. Transfer a suitable amount of USP Fentanyl Related Compound E RS to an adequate volumetric flask and dissolve in 50% of the final volume using acetonitrile. Dilute with water to volume.▲ (USP 1-May-2020)

Standard stock solution: 1 mg/mL of USP Fentanyl RS prepared by dissolving in a suitable volumetric flask 40% filled with acetonitrile and diluting with water to volume

Standard solution: 0.1 mg/mL of USP Fentanyl RS from the *Standard stock solution* in *Diluent*

System suitability solution: ▲0.3 µg/mL of USP Fentanyl Related Compound E RS and 0.1 mg/mL of USP Fentanyl RS prepared by diluting *System suitability stock solution* and *Standard stock solution* with *Diluent*▲ (USP 1-May-2020)

Sample stock solution: ▲1 mg/mL of Fentanyl prepared as follows. Transfer a suitable amount of Fentanyl to an adequate volumetric flask. Add 25% of final volume of acetonitrile, dissolve by shaking, and dilute with *Diluent* to volume.▲ (USP 1-May-2020)

Sample solution: 0.1 mg/mL of Fentanyl from the *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 206 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 30 µL

System suitability

Samples: *System suitability solution*▲ and *Standard solution*▲ (USP 1-May-2020)

www.webofpharma.com

Suitability requirements
Resolution: NLT 1.2 between fentanyl and fentanyl related compound E peaks, ▲*System suitability solution*▲ (USP 1-May-2020)
Tailing factor: 0.5–2.0, ▲*Standard solution*▲ (USP 1-May-2020)
Relative standard deviation: NMT ▲0.73%, *Standard solution*▲ (USP 1-May-2020)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲▲ (USP 1-May-2020) fentanyl ($C_{22}H_{28}N_2O$) in the portion of Fentanyl taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fentanyl from the *Sample solution*
r_S = peak response of fentanyl from the *Standard solution*
C_S = concentration of ▲USP Fentanyl RS▲ (USP 1-May-2020) in the *Standard solution* (mg/mL)
C_U = concentration of Fentanyl in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%

Change to read:

• **ORGANIC IMPURITIES**
▲[NOTE—The use of high-purity (such as HPLC-grade) ammonium formate and ultratrace ammonium hydroxide is recommended.]
Solution A: Acetonitrile and 10 mM ammonium formate (5:95). Adjust with ammonium hydroxide to a pH of 9.5.
Solution B: Acetonitrile and 10 mM ammonium formate (95:5). Adjust with ammonium hydroxide to a pH of 9.5.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	85	15
9	65	35
33	50	50
38	50	50
38.1	85	15
45	85	15

Diluent: 0.025% trifluoroacetic acid in a mixture of acetonitrile and water (5:95)
Sensitivity solution: 0.5 µg/mL of USP Fentanyl RS prepared as follows. Transfer a suitable amount of USP Fentanyl RS to an adequate volumetric flask. Dissolve in 10% of final volume using acetonitrile. Sonicate to dissolve, if necessary. Dilute with *Diluent* to volume.
Standard solution: 2.0 µg/mL each of USP Fentanyl Related Compound E RS and USP Fentanyl Related Compound G RS prepared as follows. Transfer suitable amounts of USP Fentanyl Related Compound E RS and USP Fentanyl Related Compound G RS to an adequate volumetric flask. Dissolve in 10% of final volume using acetonitrile. Sonicate to dissolve, if necessary. Dilute with *Diluent* to volume.
Sample solution: 1.0 mg/mL of Fentanyl prepared as follows. Transfer a suitable amount of Fentanyl to an adequate volumetric flask. Dissolve in 10% of final volume using acetonitrile. Sonicate to dissolve, if necessary. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 45°
Flow rate: 1.0 mL/min
Injection volume: 40 µL
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fentanyl related compound E in the portion of Fentanyl taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fentanyl related compound E from the *Sample solution*
r_S = peak response of fentanyl related compound E from the *Standard solution*
C_S = concentration of USP Fentanyl Related Compound E RS in the *Standard solution* (mg/mL)
C_U = concentration of Fentanyl in the *Sample solution* (mg/mL)

Calculate the percentage of each other impurity in the portion of Fentanyl taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each other impurity from the *Sample solution*
r_S = peak response of fentanyl related compound G from the *Standard solution*
C_S = concentration of USP Fentanyl Related Compound G RS in the *Standard solution* (mg/mL)
C_U = concentration of Fentanyl in the *Sample solution* (mg/mL)
F = relative response factor (see Table 3)

Acceptance criteria: See Table 3. The reporting threshold is 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor[a]	Acceptance Criteria, NMT (%)
Fentanyl *N*-oxide[b]	0.26	0.69	0.15
Fentanyl related compound G	0.78	1.0	0.15
Fentanyl pyruvyl analog[c]	0.92	3.0	0.15
Fentanyl	1.00	—	—

www.webofpharma.com

Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor[a]	Acceptance Criteria, NMT (%)
Fentanyl related compound E	1.14	—	0.15
Fentanyl butyryl analog[d]	1.21	1.5	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.50

[a] The relative response factor is calculated against fentanyl related compound G.
[b] 1-Phenethyl-4-(*N*-phenylpropionamido)piperidine 1-oxide.
[c] 2-Oxo-*N*-(1-phenethylpiperidin-4-yl)-*N*-phenylpropanamide.
[d] *N*-(1-Phenethylpiperidin-4-yl)-*N*-phenylbutyramide. ▲(USP 1-May-2020)

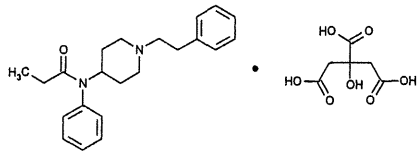
SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 60° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tightly closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fentanyl RS
▲▲(USP 1-May-2020)
USP Fentanyl Related Compound E RS
▲1-Phenethyl-*N*-phenylpiperidin-4-amine.
$C_{19}H_{24}N_2$ 280.41▲(USP 1-May-2020)
USP Fentanyl Related Compound G RS
▲*N*-(1-Phenethylpiperidin-4-yl)-*N*-phenylacetamide.
$C_{21}H_{26}N_2O$ 322.44▲(USP 1-May-2020)

Fentanyl Citrate



$C_{22}H_{28}N_2O \cdot C_6H_8O_7$ 528.59
Propanamide, *N*-phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1).
N-(1-Phenethyl-4-piperidyl)propionanilide citrate (1:1) [990-73-8].

» Fentanyl Citrate contains not less than 98.0 percent and not more than 102.0 percent of $C_{22}H_{28}N_2O \cdot C_6H_8O_7$, calculated on the dried basis. [**CAUTION**—Great care should be taken to prevent inhaling particles of Fentanyl Citrate and exposing the skin to it.]

Packaging and storage—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Fentanyl Citrate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲(CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲(CN 1-May-2020)—
Solution: 500 µg per mL.
Medium: dilute hydrochloric acid in methanol (1 in 10).
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 2 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.5%.

Ordinary impurities ⟨466⟩—
Test solution: a mixture of chloroform and methanol (4:1).
Standard solution: a mixture of chloroform and methanol (4:1) except to eliminate the 0.01 mg per mL solution and add a 0.02 mg per mL solution.
Procedure—Use a thin-layer chromatographic plate coated with chromatographic silica gel with a calcium sulfate binder.
Eluant: a mixture of chloroform, methanol, and formic acid (85:10:5).
Visualization: 3.
Assay—Dissolve about 500 mg of Fentanyl Citrate, accurately weighed, in 30 mL of glacial acetic acid. Add 3 drops of *p*-naphtholbenzein TS, and titrate with 0.05 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N perchloric acid is equivalent to 26.43 mg of $C_{22}H_{28}N_2O \cdot C_6H_8O_7$.

Fentanyl Citrate Compounded Injection

DEFINITION
Fentanyl Citrate Compounded Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of fentanyl ($C_{22}H_{28}N_2O$). It contains no bacteriostat or other preservative.
Prepare Fentanyl Citrate Compounded Injection, 50 mcg/mL, as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Fentanyl (as fentanyl citrate)	5000 mcg (7870 mcg)
Sodium hydroxide solution or Hydrochloric acid solution	To adjust pH
Sodium Chloride for Injection (0.9%), a sufficient quantity to make	100 mL

Dissolve *Fentanyl Citrate* in sufficient *Sodium Chloride for Injection (0.9%)* to bring to final volume. Adjust with *Sodium hydroxide solution* or *Hydrochloric acid solution* to a pH of 4–7.5. Pass the solution through a suitable sterile filter of 0.22-µm pore size into appropriate sterile syringes or container(s). [NOTE—Do not use a cellulose ester filter membrane for sterilization due to potential for adsorption.]

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: Add 2.72 g of dibasic potassium phosphate to 800 mL of water. Adjust with phosphoric acid to a pH of 5.8. Add 200 mL of methanol and mix well.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Methanol (%)	Solution A (%)
0.0	20	80
3.0	20	80
28.0	50	50
28.5	55	45
34.0	55	45
34.5	20	80
40	20	80

Standard solution: 2 mcg/mL of fentanyl prepared from USP Fentanyl Citrate RS in water

Sample solution: Transfer 1 mL of Injection into a 25-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; 2.6-µm packing L1

Column temperature: 40°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for fentanyl is about 24.6 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fentanyl ($C_{22}H_{28}N_2O$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fentanyl from the *Sample solution*

r_S = peak response of fentanyl from the *Standard solution*

C_S = concentration of fentanyl in the *Standard solution* (mcg/mL)

C_U = nominal concentration of fentanyl in the *Sample solution* (mcg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 4.0–7.5

• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 50.0 USP Endotoxin Units/mg of fentanyl

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in a light-resistant single-dose container. Store at controlled room temperature or in a refrigerator.

Change to read:

• **BEYOND-USE DATE:** ▲In the absence of passing a sterility and endotoxins test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test and endotoxins test is passed, NMT 90 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator.▲ (CN 1-May-2020)

• **LABELING:** Label it to indicate that it is for use in a single patient only, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Fentanyl Citrate RS

Fentanyl Citrate Injection

» Fentanyl Citrate Injection is a sterile solution of Fentanyl Citrate in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fentanyl ($C_{22}H_{28}N_2O$), present as the citrate.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—

USP Fentanyl Citrate RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 50.0 USP Endotoxin Units per mg.

pH ⟨791⟩: between 4.0 and 7.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Prepare a filtered and degassed mixture containing 4 volumes of ammonium acetate solution (1 in 100) and 6 volumes of a mixture of methanol, acetonitrile, and glacial acetic acid (400: 200: 0.6). Adjust this solution to a pH of 6.6 ± 0.1 by the dropwise addition of glacial acetic acid, and make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩), to obtain a retention time of about 5 minutes for the fentanyl peak.

Standard preparation—Dissolve an accurately weighed quantity of USP Fentanyl Citrate RS in water, and quantitatively dilute with water to obtain a solution having a known concentration of about 80 µg per mL.

Assay preparation—If necessary, dilute the Injection with water so that each mL contains the equivalent of about 50 µg of fentanyl.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak response as directed for *Procedure:* the tailing factor for the fentanyl peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

www.webofpharma.com

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of fentanyl ($C_{22}H_{28}N_3O$) in each mL of the Injection taken by the formula:

$$(336.48/528.59)CD(r_U/r_S)$$

in which 336.48 and 528.59 are the molecular weights of fentanyl and fentanyl citrate, respectively; C is the concentration, in µg per mL, of USP Fentanyl Citrate RS in the *Standard preparation*; D is the dilution factor used to obtain the *Assay preparation*; and r_U and r_S are the peak responses for the fentanyl peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Fentanyl Citrate and Bupivacaine Hydrochloride Compounded Injection

DEFINITION

Fentanyl Citrate and Bupivacaine Hydrochloride Compounded Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of fentanyl ($C_{22}H_{28}N_2O$) and bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$). It contains no bacteriostat or other preservative.

Prepare Fentanyl Citrate and Bupivacaine Hydrochloride Compounded Injection containing 2000 mcg/mL of fentanyl and 10 mg/mL of bupivacaine hydrochloride as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Fentanyl (as fentanyl citrate)	200 mg (314.6 mg)
Bupivacaine Hydrochloride	1000 mg
Sodium Chloride	695.5 mg
Sterile Water for Injection, a sufficient quantity to make	100 mL

Dissolve *Fentanyl Citrate*, *Bupivacaine Hydrochloride*, and *Sodium Chloride* in *Sterile Water for Injection*. Pass the solution through a suitable sterile filter of 0.22-µm pore size into appropriate sterile container(s).

[NOTE—Do not use a cellulose ester filter membrane for sterilization due to potential for adsorption.]

ASSAY

• **PROCEDURE**

Solution A: Add 2.72 g of dibasic potassium phosphate to 800 mL of water. Adjust with phosphoric acid to a pH of 5.8. Add 200 mL of methanol and mix well.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Methanol (%)	Solution A (%)
0.0	20	80
3.0	20	80
28.0	50	50
28.5	55	45
34.0	55	45
34.5	20	80
40	20	80

Standard solution: 2 mcg/mL of fentanyl and 0.075 mg/mL of bupivacaine hydrochloride prepared from USP Fentanyl Citrate RS and USP Bupivacaine Hydrochloride RS in water

Bupivacaine hydrochloride sample solution: Transfer 0.75 mL of Injection into a 100-mL volumetric flask, and dilute with water to volume.

Fentanyl sample solution: Transfer 0.1 mL of Injection into a 100-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; 2.6-µm packing L1

Column temperature: 40°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention times for bupivacaine hydrochloride and fentanyl are about 21.5 and 24.6 min, respectively.]

Suitability requirements

Tailing factor: NMT 2.0 for both fentanyl and bupivacaine hydrochloride

Relative standard deviation: NMT 2.0% for both fentanyl and bupivacaine hydrochloride from replicate injections

Analysis

Samples: *Standard solution* and sample solution

Calculate the percentage of the labeled amount of fentanyl ($C_{22}H_{28}N_2O$) and bupivacaine hydrochloride ($C_{18}H_{28}N_2O \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fentanyl or bupivacaine hydrochloride from the sample solution

r_S = peak response of fentanyl or bupivacaine hydrochloride from the *Standard solution*

C_S = concentration of fentanyl or bupivacaine hydrochloride in the *Standard solution* (mcg/mL or mg/mL, respectively)

C_U = nominal concentration of fentanyl or bupivacaine hydrochloride in the sample solution (mcg/mL or mg/mL, respectively)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.1–4.1

• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 50.0 USP Endotoxin Units/mg of fentanyl and NMT 2.5 USP Endotoxin Units/mg of bupivacaine hydrochloride.

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in a light-resistant, single-dose container. Store at controlled room temperature or in a refrigerator.

USP Monographs

www.webofpharma.com

Change to read:

- **BEYOND-USE DATE:** ▲In the absence of passing a sterility and endotoxins test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test and endotoxins test is passed, NMT 90 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator.▲ (CN 1-May-2020)
- **LABELING:** The label bears a warning that this is a very high-strength injection. [NOTE—This is a high-strength injection.] Label it to indicate that it is for use in a single patient only, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Bupivacaine Hydrochloride RS
  USP Fentanyl Citrate RS

Fentanyl Citrate and Ropivacaine Hydrochloride Compounded Injection

DEFINITION

Fentanyl Citrate and Ropivacaine Hydrochloride Compounded Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of fentanyl ($C_{22}H_{28}N_2O$) and ropivacaine hydrochloride ($C_{17}H_{26}N_2O \cdot HCl$).

Prepare Fentanyl Citrate and Ropivacaine Hydrochloride Compounded Injection containing 2 mcg/mL of fentanyl and 1 mg/mL of ropivacaine hydrochloride as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Fentanyl (as fentanyl citrate injection[a]), equivalent to	200 mcg of fentanyl
Ropivacaine Hydrochloride (as ropivacaine hydrochloride injection[b]), equivalent to	100 mg of ropivacaine hydrochloride
Sodium Chloride for Injection (0.9%), a sufficient quantity to make	100 mL

[a] Fentanyl Citrate 50-mcg/mL Injection, Hospira, Lake Forest, IL.
[b] Ropivacaine Hydrochloride 0.5% (5 mg/mL) Injection, Fresenius Kabi, Lake Zurich, IL.

Aseptically withdraw and combine 4 mL of the *Fentanyl Citrate Injection* (50 mcg/mL) (equivalent to 200 mcg of fentanyl) and 20 mL of *Ropivacaine Hydrochloride Injection 0.5%* (5 mg/mL) (equivalent to 100 mg of ropivacaine hydrochloride) into an appropriate sterile container. Bring the preparation to a final volume of 100 mL with *Sodium Chloride for Injection (0.9%)*. Pass through a sterile filter of 0.22-µm pore size.

[NOTE—Do not use a cellulose ester filter membrane for sterilization due to potential for adsorption.]

ASSAY

- **PROCEDURE**

Solution A: Add 2.72 g of dibasic potassium phosphate to 800 mL of water. Adjust with phosphoric acid to a pH of 5.8. Add 200 mL of methanol and mix well.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Methanol (%)	Solution A (%)
0.0	20	80
3.0	20	80
28.0	50	50
28.5	55	45
34.0	55	45
34.5	20	80
40	20	80

Standard solution: 2 mcg/mL of fentanyl and 0.075 mg/mL of ropivacaine hydrochloride prepared from USP Fentanyl Citrate RS and USP Ropivacaine Hydrochloride RS in water

Ropivacaine hydrochloride sample solution: Transfer 0.75 mL of Injection into a 10-mL volumetric flask, and dilute with water to volume.

Fentanyl sample solution: 2 mcg/mL of fentanyl from Injection

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; 2.6-µm packing L1
Column temperature: 40°
Flow rate: 0.8 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention times for ropivacaine hydrochloride and fentanyl are about 14.4 and 24.6 min, respectively.]
Suitability requirements
Tailing factor: NMT 2.0 for both fentanyl and ropivacaine hydrochloride
Relative standard deviation: NMT 2.0% for both fentanyl and ropivacaine hydrochloride from replicate injections

Analysis
Samples: *Standard solution* and sample solution
Calculate the percentage of the labeled amount of fentanyl ($C_{22}H_{28}N_2O$) and ropivacaine hydrochloride ($C_{17}H_{26}N_2O \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fentanyl or ropivacaine hydrochloride from the sample solution
r_S = peak response of fentanyl or ropivacaine hydrochloride from the *Standard solution*
C_S = concentration of fentanyl or ropivacaine hydrochloride in the *Standard solution* (mcg/mL or mg/mL, respectively)
C_U = nominal concentration of fentanyl or ropivacaine hydrochloride in the sample solution (mcg/mL or mg/mL, respectively)

Acceptance criteria: 90.0%–110.0%

- **ENANTIOMERIC PURITY**

Mobile phase: Add 0.418 g of monobasic sodium phosphate and 0.941 g of dibasic sodium phosphate to 465 mL of water. Adjust with sodium hydroxide to a pH of 7.2 and add 35 mL of isopropanol. Mix well.

System suitability solution: Dissolve suitable quantities of USP Ropivacaine Hydrochloride RS and USP Ropivacaine Related Compound B RS in water. Dilute quantitatively, and stepwise, with water to obtain a solution containing about 75 mcg/mL and 0.75 mcg/mL, respectively.

www.webofpharma.com

Sample solution: Transfer 0.75 mL of Injection into a 10-mL volumetric flask, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4-mm × 10-cm; packing L41
Column temperature: 25°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for ropivacaine related compound B and ropivacaine hydrochloride are about 0.75 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between ropivacaine related compound B (*R*-enantiomer) and ropivacaine (*S*-enantiomer)
Analysis
Sample: *Sample solution*
Calculate the percentage of ropivacaine related compound B (*R*-enantiomer) in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of ropivacaine related compound B (*R*-enantiomer)
r_T = sum of the peak responses of ropivacaine (*S*-enantiomer) and ropivacaine related compound B (*R*-enantiomer)

Acceptance criteria: NMT 2.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 5.1–6.1
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 50.0 USP Endotoxin Units/mg of fentanyl and NMT 2.5 USP Endotoxin Units/mg of ropivacaine hydrochloride
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in a light-resistant, single-dose container. Store at controlled room temperature or in a refrigerator.

Change to read:

- **BEYOND-USE DATE:** ▲In the absence of passing a sterility and endotoxins test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test and endotoxins test is passed, NMT 90 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator.▲ (CN 1-May-2020)
- **LABELING:** Label it to indicate that it is for use in a single patient only, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Fentanyl Citrate RS
USP Ropivacaine Hydrochloride RS
USP Ropivacaine Related Compound B RS

Ferric Subsulfate Solution

$Fe_4(OH)_2(SO_4)_5$ 737.71

Basic ferric sulfate solution.
Monsel's Solution [1310-45-8].

» Ferric Subsulfate Solution contains, in each 100 mL, basic ferric sulfate equivalent to not less than 20 g and not more than 22 g of iron (Fe). Ferric Subsulfate Solution may be prepared as follows. Add 55 mL of Sulfuric Acid to 800 mL of water in a porcelain dish and heat to nearly 100°, then add 75 mL of nitric acid, and mix. Divide 1045 g of Ferrous Sulfate, coarsely powdered, into 4 portions, and add these portions one at a time to the hot liquid, stirring after each addition until effervescence ceases. If, after the Ferrous Sulfate has dissolved, the solution has a black color, add nitric acid, a few drops at a time, with heating and stirring, until red fumes cease to be evolved. Boil the solution until it assumes a red color and is free from nitrate, as indicated by the test for *Limit of nitrate* below, maintaining the volume at about 1000 mL by the addition of water as needed. Cool, and add enough water to make the solution measure 1000 mL. Filter, if necessary, until the Solution is clear.

Packaging and storage—Preserve in tight, light-resistant containers, and store at temperatures above 22°.
Labeling—The label indicates that crystallization may occur if the Solution is exposed to temperatures below 22°, and that warming will redissolve the crystals. Label it to indicate that it is intended for topical and vaginal use only.

Identification—
A: A 1 in 20 dilution of the Solution in water yields a brownish-red precipitate with ammonia TS.
B: A 1 in 20 dilution of the Solution in water yields a blue precipitate with potassium ferrocyanide TS.
C: A 1 in 20 dilution of the Solution in water yields a white precipitate with barium chloride TS that is insoluble in hydrochloric acid.
Limit of nitrate—Add a clear crystal of ferrous sulfate to a cooled mixture of equal volumes of sulfuric acid and a 1 in 10 dilution of the Solution in water: the crystal does not become brown, nor does a brownish-black color develop around it.
Limit of ferrous salts—Add a few drops of freshly prepared potassium ferricyanide TS to 2 mL of 1 in 20 dilution of the solution in water: a brown color is produced and the solution remains free from even a transient green or greenish-blue color.
Assay—Transfer 10.0 mL of the Solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a glass-stoppered conical flask, and add 5 mL of hydrochloric acid and 3 g of potassium iodide. Insert the stopper into the flask, and allow the mixture to stand for 15 minutes. Add 15 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, using starch TS as the indicator. Each mL of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of iron (Fe).

Ferric Sulfate

$Fe_2(SO_4)_3 \cdot xH_2O$ (anhydrous) 399.88

www.webofpharma.com

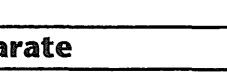
Ferric persulfate.
Ferric sesquisulfate.
Ferric tersulfate [10028-22-5].
Hydrate [142906-29-4].

» Ferric Sulfate is hydrated $Fe_2(SO_4)_3$. It contains not less than 73.0 percent and not more than 80.0 percent of $Fe_2(SO_4)_3$.

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.
Labeling—Label it to indicate that it is intended for use in compounding topical and periodontal dosage forms only.
Identification—Dissolve 0.5 g in a mixture of 50 mL of water and 3 mL of hydrochloric acid: this solution meets the requirements for the *Ferric Salts* test under *Iron* ⟨191⟩.
Limit of insoluble matter—Transfer 10 g in a covered beaker, and dissolve in a mixture of 100 mL of water and 5 mL of sulfuric acid. Heat to boiling, and warm on a steam bath for 1 hour. Filter the hot solution through a tared sintered-glass crucible of fine porosity. Wash the beaker and the filter with hot water, dry the crucible at 105°, cool in a desiccator, and weigh: not more than 2 mg of insoluble matter is found (0.02%).
Limit of chloride—Dissolve 1 g by warming with a mixture of 10 mL of water and 1 mL of nitric acid, add 4 mL of additional nitric acid, and dilute with water to 50 mL. To 25 mL of this solution add 1 mL of phosphoric acid and 1 mL of silver nitrate TS. Any turbidity does not exceed that produced in a control containing 0.01 mg of chloride ion (Cl), 1 mL of nitric acid, 1 mL of phosphoric acid, and 1 mL of silver nitrate TS (0.002%).
Limit of ferrous iron—Dissolve 4 g by warming with 50 mL of dilute sulfuric acid (1 in 10), cool, and titrate with 0.1 N potassium permanganate: not more than 0.16 mL is required to produce a permanent pink color (0.02% as Fe^{++}).

Limit of copper and zinc—
[NOTE—If the reagents used in the tests for *Copper* and *Zinc* contain excessive amounts of copper and zinc, then they should first be purified by extracting with *Dithizone Extraction Solution* (see *Lead* ⟨251⟩).]
Copper—Dissolve 1.2 g in 100 mL of water. To 10 mL of this test solution add 50 mL of a solution containing 5 g of ammonium tartrate and 5 mL of ammonium hydroxide. Add 10 mL of *Standard Dithizone Solution* (see *Lead* ⟨251⟩), shake for 2 minutes, draw off the dithizone layer, and compare the pink color with that in a control containing 6 µg of copper ion (Cu) and treated exactly as the 10-mL portion of test solution. If the color in the test solution is less than that in the control, then less than 0.005% of Cu and of Zn is found and the test for *Zinc* is not necessary. If the color in the test solution is more than that in the control, add 15 mL of dilute hydrochloric acid (1 in 250), and shake for 2 minutes. Draw off the dithizone solution, and shake with a second 15 mL of dilute hydrochloric acid (1 in 250) for 2 minutes. [NOTE—Draw off the dithizone, combine the two acid extracts, and reserve for the test for *Zinc*.] Any pink color in the dithizone solution is not darker than that in the control solution treated exactly as the test solution (0.005%).
Zinc—To the combined acid extracts saved from the test for *Copper*, add sufficient 0.5 M sodium acetate to bring the pH to between 5.0 and 5.5, and then add 1 mL of 0.1 N sodium thiosulfate. Add 10 mL of *Standard Dithizone Solution* (see *Lead* ⟨251⟩), shake for 2 minutes, and allow the layers to separate. Draw off the dithizone layer, and discard the water layer. Any pink color in the dithizone layer is not greater than that in a control prepared by adding 0.006 mg of zinc ion (Zn) to the combined acid extracts from the control used in the test for *Copper* (0.005%).
Limit of nitrate—Dissolve 10 g in 100 mL of dilute sulfuric acid (1 in 100), heat to boiling, and pour, slowly, into a mixture of 140 mL of water and 50 mL of ammonium hydroxide. Pass through a folded filter while still hot, wash the filter with hot water until the volume of filtrate is 300 mL, mix, and cool. To 15 mL of the diluted filtrate add 1 mL of sodium chloride solution (1 in 200), 0.10 mL of indigo carmine TS, and 15 mL of sulfuric acid. The blue color is not completely discharged at the end of 5 minutes (0.01%).
Substances not precipitated by ammonia—Evaporate to dryness 30 mL of the diluted filtrate obtained in the test for *Limit of nitrate*, and ignite gently: the weight of residue does not exceed 1 mg (0.1%).
Assay—Transfer about 700 mg of Ferric Sulfate, accurately weighed, to a glass-stoppered conical flask. Add a mixture of 50 mL of water and 3 mL of hydrochloric acid, and swirl to dissolve. Add 3 g of potassium iodide, insert the stopper into the flask, and allow to stand in the dark for 30 minutes. Then add 100 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 19.994 mg of ferric sulfate $[Fe_2(SO_4)_3]$.

Ferrous Fumarate



$C_4H_2FeO_4$ 169.90
2-Butenedioic acid, (*E*)-, iron(2+) salt;
Iron(2+) fumarate [141-01-5].

DEFINITION
Ferrous Fumarate contains NLT 97.0% and NMT 101.0% of ferrous fumarate ($C_4H_2FeO_4$), calculated on the dried basis.

IDENTIFICATION
• **A. INFRARED ABSORPTION**
Sample: To 1.5 g of Ferrous Fumarate, add 25 mL of dilute hydrochloric acid (1 in 2). Dilute with water to 50 mL, heat to dissolve, then cool, and filter on a fine-pore size, sintered-glass crucible. Wash the precipitate with dilute hydrochloric acid (3 in 100), saving the filtrate for *Identification* test *B*, and dry the precipitate at 105°.
Acceptance criteria: The IR absorption of a potassium bromide dispersion of the dried precipitate exhibits maxima only at the same wavelengths as that of a similar preparation of USP Fumaric Acid RS.
• **B. IDENTIFICATION TESTS—GENERAL,** *Iron* ⟨191⟩**:** A portion of the filtrate obtained in *Identification* test *A* meets the requirements.

ASSAY
• **PROCEDURE**
Sample: 500 mg of Ferrous Fumarate
Blank: Proceed as directed in the *Analysis* without the *Sample*.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N ceric sulfate VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 500-mL conical flask, and add 25 mL of dilute hydrochloric acid (2 in 5). Heat to boiling, and add, dropwise, a solution of 112 mg/mL of

www.webofpharma.com

stannous chloride in dilute hydrochloric acid (3 in 10) until the yellow color disappears, then add 2 drops in excess. Cool the solution in an ice bath to room temperature, add 10 mL of 50 mg/mL of mercuric chloride solution, and allow to stand for 5 min. Add 200 mL of water, 25 mL of dilute sulfuric acid (1 in 2), and 4 mL of phosphoric acid. Then add 2 drops of orthophenanthroline TS, and titrate with *Titrant*. Perform a blank determination.

Calculate the percentage of ferrous fumarate ($C_4H_2FeO_4$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual *Titrant* normality (mEq/mL)
F = equivalency factor, 169.9 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 97.0%–101.0% on the dried basis

IMPURITIES

• **SULFATE**

Sample solution: Transfer 1.0 g of Ferrous Fumarate to a 250-mL beaker, add 100 mL of water, and heat on a steam bath, adding hydrochloric acid dropwise until completely dissolved. [NOTE—About 2 mL of the acid will be required.] Filter the solution if necessary, and dilute the filtrate with water to 100 mL. Heat the filtrate to boiling, add 10 mL of barium chloride TS, warm on a steam bath for 2 h, cover, and allow to stand for 16 h. [NOTE—If crystals of ferrous fumarate form, warm the solution on the steam bath to dissolve them.]

Analysis: Pass the *Sample solution* through ashless filter paper, wash the residue with hot water until, with the addition of ammonium sulfide TS, a black precipitate is no longer formed in the filtrate. Transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents at 600° to constant weight.

Acceptance criteria: NMT 0.2%: Each mg of residue is equivalent to 0.412 mg of sulfate (SO_4).

• **ARSENIC,** *Method I* ⟨211⟩

Test preparation: To 2.0 g in 10 mL of water add 10 mL of sulfuric acid. Warm to precipitate the fumaric acid completely, cool, add 30 mL of water, and filter into a 100-mL volumetric flask. Wash the precipitate with water, adding the washings to the flask, then add water to volume, and mix. Transfer 50.0 mL of this solution into the arsine generator flask, and dilute with water to 55 mL.

Analysis: Proceed as directed in the chapter, except omit the addition of 20 mL of 7 N sulfuric acid specified for the *Procedure*.

Acceptance criteria: NMT 3 ppm

• **LIMIT OF FERRIC IRON**

Sample: 2 g of Ferrous Fumarate

Blank: Proceed as directed in the *Analysis* without the *Sample*.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a glass-stoppered, 250-mL conical flask, add 25 mL of water and 4 mL of hydrochloric acid, and heat on a hot plate until solution is complete. Insert the stopper in the flask, and cool to room temperature. Add 3 g of potassium iodide, insert the stopper in the flask, swirl to mix, and allow to stand in the dark for 5 min. Remove the stopper, add 75 mL of water, and titrate with the *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination.

Calculate the percentage of ferric iron in the portion of Ferrous Fumarate taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = *Titrant* normality (mEq/mL)
F = equivalency factor, 55.85 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 2.0%

• **LIMIT OF LEAD**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm nitric acid (1 in 2) for 30 min and by rinsing with deionized water.]

Ascorbic acid–sodium iodide solution: 100 mg/mL of ascorbic acid and 192.5 mg/mL of sodium iodide

Trioctylphosphine oxide solution: 50 mg/mL of trioctylphosphine oxide in 4-methyl-2-pentanone. [**CAUTION**—This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.]

Standard solution: Transfer 5.0 mL of lead nitrate stock solution TS to a 100-mL volumetric flask. Dilute with water to volume, and mix. Transfer 2.0 mL of the resulting solution to a 50-mL beaker. To this beaker add 6 mL of nitric acid and 10 mL of perchloric acid, and evaporate in a hood to dryness. [**CAUTION**—Use perchloric acid in a well-ventilated fume hood with proper precautions.] Cool, dissolve the residues in 10 mL of 9 N hydrochloric acid, and transfer with the aid of about 10 mL of water to a 50-mL volumetric flask. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*, shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic solvent layer is the *Standard solution*, and it contains 2.0 µg/mL of lead.

Sample solution: Transfer 1.0 g of Ferrous Fumarate to a 50-mL beaker, and add 6 mL of nitric acid and 10 mL of perchloric acid. [**CAUTION**—Use perchloric acid in a well-ventilated fume hood with proper precautions.] Cover with a ribbed watch glass, and heat in a hood until completely dry. Cool, dissolve the residue in 10 mL of 9 N hydrochloric acid, and transfer with the aid of about 10 mL of water to a 50-mL volumetric flask. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*, shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Sample solution*.

Blank: To a 50-mL beaker add 6 mL of nitric acid and 10 mL of perchloric acid, and evaporate in a hood to dryness. [**CAUTION**—Use perchloric acid in a well-ventilated fume hood with proper precautions.] Cool, dissolve the residue in 10 mL of 9 N hydrochloric acid, and transfer with the aid of about 10 mL of water to a 50-mL volumetric flask. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*, shake for 30 s, and allow to separate. Add water

USP Monographs

www.webofpharma.com

to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Blank*, and it contains 0 µg/mL of lead.

Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 283.3 nm
Lamp: Lead hollow-cathode
Flame: Air–acetylene

System suitability
Samples: *Standard solution* and *Blank*
Suitability requirements: The absorbance of the *Standard solution* and the absorbance of the *Blank* are significantly different.

Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Concomitantly determine the absorbances of the *Blank*, *Standard solution*, and the *Sample solution*. Use the *Blank* to set the instrument to zero.

Acceptance criteria: NMT 10 ppm: The absorbance of the *Sample solution* does not exceed that of the *Standard solution*.

• **MERCURY**
[NOTE—Carry out this test in subdued light, because mercuric dithizonate is light sensitive.]

Hydroxylamine hydrochloride solution, Standard mercury solution, Dithizone extraction solution, and **Diluted dithizone extraction solution:** Prepare as directed in *Mercury* ⟨261⟩, *Method I*.

Control solution: Mix 3.0 mL of *Standard mercury solution*, 30 mL of dilute nitric acid (1 in 10), 5 mL of 250 mg/mL of sodium citrate solution, and 1 mL of *Hydroxylamine hydrochloride solution*.

Test preparation: Dissolve 1 g of Ferrous Fumarate in 30 mL of dilute nitric acid (1 in 10) with the aid of heat, on a steam bath. Cool quickly by immersing in an ice bath, and pass through a fine-porosity filter that previously has been washed with dilute nitric acid (1 in 10) and water. To the filtrate add 20 mL of 250 mg/mL of sodium citrate solution and 1 mL of *Hydroxylamine hydrochloride solution*.

Analysis: Adjust the *Control solution* to a pH of 1.8 with ammonium hydroxide, and the *Sample solution* to a pH of 1.8 with sulfuric acid. Separately transfer the solutions to separators. Treat the *Sample solution* and the *Control solution* in parallel as follows.
Extract with two 5-mL portions of *Dithizone extraction solution* and 5 mL of chloroform, pooling the chloroform extracts in a second separator. Add 10 mL of hydrochloric acid (1 in 2), shake, allow the layers to separate, and discard the chloroform layer. Wash the acid extract with 3 mL of chloroform, and discard the washing. Add 0.1 mL of 20 mg/mL edetate disodium solution and 2 mL of 6 N acetic acid, mix, and add slowly 5 mL of ammonium hydroxide. Close the separator, cool it under cold running water, and dry its outer surface. Remove the stopper, and pour the contents into a beaker. Adjust the *Sample solution* and the *Control solution* to a pH of 1.8 in the same manner as before, and return the solutions to their respective separators. Add 5.0 mL of *Diluted dithizone extraction solution*, shake vigorously, and allow the layers to separate. Using *Diluted dithizone extraction solution* as a color blank, compare the colors developed in the chloroform layers of the *Sample solution* and the *Control solution*.

Acceptance criteria: NMT 3 µg/g: The color developed by the *Sample solution* is not more intense than that developed by the *Control solution*.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 16 h.
Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fumaric Acid RS

Ferrous Fumarate Tablets

DEFINITION

Ferrous Fumarate Tablets contain NLT 95.0% and NMT 110.0% of the labeled amount of ferrous fumarate ($C_4H_2FeO_4$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Iron* ⟨191⟩
Sample solution: To a portion of powdered Tablets, equivalent to 1 g of ferrous fumarate, add 25 mL of dilute hydrochloric acid (1 in 2), mix, and add 25 mL of water. Boil the solution for a few min, cool, and filter.
Acceptance criteria: The filtrate meets the requirements.

ASSAY

• **PROCEDURE**
Sample: A portion of the powder from NLT 20 finely powdered Tablets equivalent to 500 mg of ferrous fumarate
Blank: Proceed as in the *Analysis* without the *Sample*.

Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Indirect titration
Titrant: 0.1 N sodium thiosulfate VS
Indicator: Starch TS
Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 250-mL beaker. Add 25 mL of water, 25 mL of nitric acid, and 7.5 mL of perchloric acid. Cover with a ribbed watch glass, and heat to the production of strong fumes. Cool, rinse the watch glass and the sides of the beaker with water, and evaporate in a hood to near-dryness. Wash down the watch glass and the sides of the beaker with 2 mL of hydrochloric acid and then with a small volume of water. Warm slightly, if necessary, to dissolve the residue. Transfer to a glass-stoppered, 250-mL conical flask. Repeat the washing with 2 mL of hydrochloric acid, and complete the transfer to the flask, using NMT 20–25 mL of water for the transfer. Add 4 g of potassium iodide to the flask, insert the stopper, and allow to stand in the dark for 5 min. Add 75 mL of water and titrate with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination.

Calculate the percentage of the labeled amount of ferrous fumarate ($C_4H_2FeO_4$) in the portion of Tablets taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 169.9 mg/mEq
W = nominal weight of ferrous fumarate in the *Sample* taken (mg)

Acceptance criteria: 95.0%–110.0%

www.webofpharma.com

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid in 0.5% sodium lauryl sulfate; 900 mL

Apparatus 2: 75 rpm

Time: 45 min

Standard solution: Solution having a known concentration of iron in the *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 248.3 nm

Lamp: Iron hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the concentration of iron (Fe) in the *Sample solution* in comparison with a *Standard solution.*

Calculate the percentage of the labeled amount of ferrous fumarate ($C_4H_2FeO_4$) dissolved:

$$\text{Result} = (M_r/A_r) \times (C \times D \times V/L) \times 100$$

M_r = molecular weight of ferrous fumarate, 169.9

A_r = atomic weight of iron, 55.85

C = measured concentration of iron in the *Sample solution* (mg/mL)

D = dilution factor for the *Sample solution*

V = volume of *Medium*, 900 mL

L = label amount of ferrous fumarate (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of ferrous fumarate is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label the Tablets in terms of ferrous fumarate ($C_4H_2FeO_4$) and in terms of elemental iron.

Ferrous Fumarate and Docusate Sodium Extended-Release Tablets

» Ferrous Fumarate and Docusate Sodium Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ferrous fumarate ($C_4H_2FeO_4$) and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of docusate sodium ($C_{20}H_{37}NaO_7S$).

Packaging and storage—Preserve in well-closed containers.

Labeling—Label the Tablets in terms of the content of ferrous fumarate ($C_4H_2FeO_4$) and in terms of the content of elemental iron.

USP Reference standards ⟨11⟩—

USP Docusate Sodium RS

Dissolution ⟨711⟩—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Times: 1 and 3 hours.

Determine the amount of Fe (II) dissolved, on filtered portions of the solution under test, employing the method described under *Assay for ferrous fumarate* with the following modification.

Standard solution—Transfer the appropriate amount of *Iron stock solution* to a volumetric flask, and dilute with 0.1 N hydrochloric acid in such a way that the final concentration is similar to that expected in the solution under test.

Tolerances—The percentages of the labeled amount of Fe (II) dissolved at the times specified conform to *Acceptance Table 2.*

Time (hours)	Amount dissolved
1	between 40% and 75%
3	not less than 80%

Uniformity of dosage units ⟨905⟩**:** meet the requirements with respect to iron.

Assay for ferrous fumarate—

6 N Hydrochloric acid—Slowly add 5 mL of hydrochloric acid to 5 mL of water, and mix.

Diluting solution—Add 1 mL of *6 N Hydrochloric acid* to 59 mL of water, and mix.

Phosphoric acid solution—Dilute 20 mL of phosphoric acid with *Diluting solution* to 200 mL, and mix.

Iron stock solution—Transfer about 350 mg of ferrous ammonium sulfate hexahydrate, accurately weighed, to a 1000-mL volumetric flask, dissolve in *Diluting solution,* dilute with *Diluting solution* to volume, and mix to obtain a solution having a known concentration of about 50 µg per mL.

Standard preparations—To separate 100-mL volumetric flasks transfer 2.0, 4.0, 6.0, 8.0, and 10.0 mL of *Iron stock solution.* To each flask add 6.0 mL of *Phosphoric acid solution,* dilute with *Diluting solution* to volume, and mix. The *Standard preparations* so obtained contain about 1.0, 2.0, 3.0, 4.0, and 5.0 µg of iron per mL, respectively.

Blank solution—Transfer 6.0 mL of *Phosphoric acid solution* to a 100-mL volumetric flask, dilute with *Diluting solution* to volume, and mix.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1.5 g of ferrous fumarate, to a 1000-mL volumetric flask, add 110 mL of *6 N Hydrochloric acid,* and boil for 30 minutes. Cool, dilute with water to volume, mix, and filter. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluting solution* to volume, and mix. Transfer 8.0 mL of this solution to a 100-mL volumetric flask, add 6.0 mL of *Phosphoric acid solution,* dilute with *Diluting solution* to volume, and mix to obtain a solution having a known concentration of about 4 µg of iron per mL.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the iron emission line at 248.3 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with an iron hollow-cathode lamp and an air–acetylene flame, using the *Blank solution* as the blank. Plot the absorbances of the *Standard preparations* versus their concentrations, in µg per mL, of iron, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, in µg per mL, of iron in the *Assay preparation.* Calculate the average quantity, in mg, of ferrous fumarate ($C_4H_2FeO_4$) in each Tablet taken by the formula:

$$(TC/D)(169.90/55.85)$$

USP Monographs

www.webofpharma.com

in which *T* is the labeled quantity, in mg, of ferrous fumarate in each Tablet; *C* is the concentration, in µg per mL, of iron in the *Assay preparation; D* is the concentration, in µg per mL, of ferrous fumarate in the *Assay preparation,* based on the labeled quantity per Tablet and the extent of dilution; and 169.90 and 55.85 are the molecular weight of ferrous fumarate and the atomic weight of iron, respectively.

Assay for docusate sodium—

Calcium acetate solution—Dissolve 4 g of calcium acetate in 2000 mL of water.

Diluting solution—Mix 450 mL of acetonitrile and 550 mL of *Calcium acetate solution.*

Mobile phase—Add 2 mL of phosphoric acid to 1000 mL of *Diluting solution,* and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). Filter, and degas.

Standard preparation—Dissolve an accurately weighed quantity of USP Docusate Sodium RS in *Diluting solution* to obtain a solution having a known concentration of about 1 mg per mL.

Sodium benzoate solution—Dissolve an accurately weighed quantity of sodium benzoate in *Diluting solution,* and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about 8 µg per mL.

Resolution solution—Dissolve a suitable quantity of USP Docusate Sodium RS in *Sodium benzoate solution* to obtain a solution containing about 1 mg per mL of docusate sodium.

Assay preparation—Transfer a number of Tablets, equivalent to about 2 g of docusate sodium, to a 2000-mL volumetric flask. Add about 1500 mL of *Diluting solution,* and sonicate with frequent shaking until the Tablets are completely disintegrated. Cool, dilute with *Diluting solution* to volume, and mix. Centrifuge, and use the clear supernatant. If the supernatant is not clear, pass through a membrane filter.

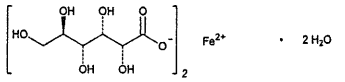
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 30-cm column that contains 3-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution* and the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between sodium benzoate and docusate sodium is not less than 7.0; the tailing factor is not less than 0.9 and not more than 3.5; the column efficiency is not less than 1000 theoretical plates; and the relative standard deviation for six replicate injections of the *Standard preparation* is not more than 2.0%. The relative retention times are 1.0 for docusate sodium and 0.25 for sodium benzoate.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the average quantity, in mg, of docusate sodium ($C_{20}H_{37}NaO_7S$) in each of the Tablets taken by the formula:

$$(2000C/N)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Docusate Sodium RS in the *Standard preparation; N* is the number of Tablets taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ferrous Gluconate



$C_{12}H_{22}FeO_{14} \cdot 2H_2O$ 482.17

D-Gluconic acid, iron(2+) salt (2:1), dihydrate; Iron(2+) gluconate (1:2) dihydrate [12389-15-0]. Anhydrous 446.15 [299-29-6].

DEFINITION

Ferrous Gluconate contains NLT 97.0% and NMT 102.0% of ferrous gluconate ($C_{12}H_{22}FeO_{14}$), calculated on the dried basis.

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: 10 mg/mL of Ferrous Gluconate, heating in a water bath at 60°, if necessary, to dissolve

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent.* Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution.*

• **B. Ferrous Ion**

Sample solution: 5 mg/mL in water

Analysis: Add potassium ferricyanide TS to the *Sample solution.*

Acceptance criteria: The solution yields a dark blue precipitate.

ASSAY

• **Procedure**

Sample: 1.5 g of Ferrous Gluconate

Blank: Proceed as directed in the *Analysis* without the *Sample.*

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N ceric sulfate VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in a mixture of 75 mL of water and 15 mL of 2 N sulfuric acid in a 300-mL conical flask. Add 250 mg of zinc dust, close the flask with a stopper containing a Bunsen valve, and allow to stand at room temperature for 20 min or until the solution becomes colorless. Pass the solution through a filtering crucible containing a thin layer of zinc dust, and wash the crucible and contents with 10 mL of 2 N sulfuric acid, followed by 10 mL of water. [Note—Prepare and use the filtering crucible in a well-ventilated hood.]

USP Monographs

www.webofpharma.com

Add orthophenanthroline TS, and immediately titrate the filtrate in the suction flask with *Titrant*. Perform a blank determination.

Calculate the percentage of ferrous gluconate ($C_{12}H_{22}FeO_{14}$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = *Titrant* normality (mEq/mL)
F = equivalency factor, 446.2 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Standard solution: 1.0 mL of 0.020 N hydrochloric acid
Sample: 1.0 g
Acceptance criteria: NMT 0.07%

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Standard solution: 1.0 mL of 0.020 N sulfuric acid
Sample: 1.0 g
Acceptance criteria: NMT 0.1%

• **MERCURY** ⟨261⟩**:** NMT 3 ppm

• **ARSENIC,** *Method I* ⟨211⟩
Test preparation: Transfer 1.0 g of Ferrous Gluconate to a 100-mL round-bottom flask fitted with a 24/40 standard-taper joint. Add 40 mL of 9 N sulfuric acid and 2 mL of potassium bromide solution (3 in 10). Immediately connect to a suitable distillation apparatus that has a reservoir with a water jacket, cooled with circulating ice water, and heat to dissolve the Ferrous Gluconate. Distill, collect 25 mL of distillate, and transfer the distillate to the arsine generator flask. Wash the condenser and reservoir several times with small portions of water, add the washings to the distillate in the generator flask, add bromine TS until the solution is slightly yellow, and dilute with water to 35 mL. Proceed as directed in the chapter.
Acceptance criteria: NMT 3 ppm

• **LIMIT OF LEAD**
[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]
Ascorbic acid–sodium iodide solution: 100 mg/mL of ascorbic acid and 192.5 mg/mL of sodium iodide
Trioctylphosphine oxide solution: 50 mg/mL of trioctylphosphine oxide in 4-methyl-2-pentanone. [**CAUTION**—This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.]
Standard solution: Transfer 5.0 mL of lead nitrate stock solution TS to a 100-mL volumetric flask, and dilute with water to volume. Transfer 2.0 mL of the resulting solution to a 50-mL volumetric flask, add 10 mL of 9 N hydrochloric acid and 10 mL of water. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*, shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Standard solution*, and it contains 2 µg/mL of lead.
Sample solution: To a 50-mL volumetric flask add 1.0 g of Ferrous Gluconate, 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Sample solution*.
Blank: To a 50-mL volumetric flask add 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Blank*, and it contains 0 µg/mL of lead.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 283.3 nm
Lamp: Lead hollow-cathode
Flame: Air–acetylene
System suitability
Samples: *Standard solution* and *Blank*
Suitability requirements: The absorbance of the *Standard solution* and the absorbance of the *Blank* are significantly different.
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Concomitantly determine the absorbances of the *Blank*, *Standard solution*, and *Sample solution*. Use the *Blank* to set the instrument to zero.
Acceptance criteria: The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 10 ppm).

• **LIMIT OF FERRIC IRON**
Sample: 5 g of Ferrous Gluconate
Blank: Proceed as directed in the *Analysis* without the *Sample*.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N sodium thiosulfate VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in a mixture of 100 mL of water and 10 mL of hydrochloric acid, and add 3 g of potassium iodide. Shake, and allow to stand in the dark for 5 min. Titrate any liberated iodine with the *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination.
Calculate the percentage of ferric iron in the portion of Ferrous Gluconate taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = *Titrant* normality (mEq/mL)
F = equivalency factor, 55.85 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 2.0%

• **OXALIC ACID**
Sample: 1.0 g
Analysis: Dissolve the *Sample* in 10 mL of water, add 2 mL of hydrochloric acid, and transfer to a separator. Extract successively with 50 mL and 20 mL of ether. Combine the ether extracts, add 10 mL of water, and evaporate the ether on a steam bath. Add 1 drop of 6 N acetic acid and 1 mL of a 50 mg/mL solution of calcium acetate.
Acceptance criteria: No turbidity is produced within 5 min.

USP Monographs

www.webofpharma.com

• **REDUCING SUGARS**
Sample: 500 mg
Analysis: Dissolve the *Sample* in 10 mL of water, warm, and render alkaline with 1 mL of 6 N ammonium hydroxide. Pass hydrogen sulfide gas into the solution to precipitate the iron, and allow the solution to stand for 30 min to coagulate the precipitate. Filter, and wash the precipitate with two successive 5-mL portions of water. Acidify the combined filtrate and washings with hydrochloric acid, and add 2 mL of 3 N hydrochloric acid in excess. Boil the solution until the vapors no longer darken lead acetate paper, and continue to boil, if necessary, until it has been concentrated to 10 mL. Cool, add 5 mL of sodium carbonate TS and 20 mL of water, filter, and adjust the volume of the filtrate to 100 mL. To 5 mL of the filtrate add 2 mL of alkaline cupric tartrate TS, and boil for 1 min.
Acceptance criteria: No red precipitate is formed within 1 min.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 16 h.
Acceptance criteria: 6.5%—10.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Potassium Gluconate RS

Ferrous Gluconate Capsules

DEFINITION
Ferrous Gluconate Capsules contain NLT 93.0% and NMT 107.0% of the labeled amount of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$).

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHY**
Standard solution: 10 mg/mL of USP Potassium Gluconate RS
Sample solution: A filtered solution in water, equivalent to 10 mg/mL of ferrous gluconate dihydrate from the contents of the Capsules
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 5 µL
Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)
Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.
Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.
• **B. FERROUS ION**
Sample solution: Equivalent to 5 mg/mL of ferrous gluconate dihydrate from a dilution of the *Sample solution* obtained in *Identification* test *A*
Analysis: Add potassium ferricyanide TS to the *Sample solution*.
Acceptance criteria: The solution yields a dark blue precipitate.

ASSAY
• **PROCEDURE**
Buffer: Dissolve 3.0 g of sodium acetate in 50 mL of water. Add 2.0 mL of glacial acetic acid, dilute with water to 200 mL, and mix. This *Buffer* has a pH of 4.6.
Color reagent solution: Dissolve 400 mg of 2,2′-bipyridine in 100 mL of water, using heat if necessary, to dissolve. Cool, and filter.
Standard stock solution: 7.022 mg/mL of ferrous ammonium sulfate hexahydrate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$] in water, equivalent to 1000 µg/mL of iron (Fe)
Standard solution: 100 µg/mL of iron (Fe) in 0.1 N sulfuric acid from *Standard stock solution*
Sample solution
For hard gelatin capsules: Transfer, as completely as possible, the contents of NLT 20 Capsules to a suitable tared container. Mix and finely powder the combined contents, and transfer a weighed portion of the powder, equivalent to 430 mg of ferrous gluconate dihydrate, to a 500-mL volumetric flask. Add 300 mL of water, heating on a steam bath if necessary to dissolve. Cool, and dilute with water to volume.
For soft gelatin capsules: Place a number of Capsules, equivalent to 430 mg of ferrous gluconate dihydrate, in a 500-mL volumetric flask. Add 300 mL of water, heating on a steam bath to dissolve the Capsules. Cool, and dilute with water to volume.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelength: 522 nm
Cell: 1 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Transfer 3.0 mL of the *Sample solution* to a 100-mL volumetric flask, and add, in the order named, 70 mL of *Buffer*, 10.0 mL of 100 mg/mL of sodium thiosulfate solution, and 5.0 mL of *Color reagent solution*, with mixing following each addition. Heat for 60 min on a steam bath, cool, dilute with *Buffer* to volume, and filter. Prepare reagent blanks for the *Standard solution* and the *Sample solution* by repeating the above procedure, omitting the addition of 5.0 mL of *Color reagent solution*.
Concomitantly determine the absorbances of the reacted solutions against the corresponding reagent blanks.
Calculate the percentage of the labeled amount of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$) in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_r/A_r) \times 100$$

A_U = absorbance of the *Sample solution* corrected by the absorbance of its reagent blank
A_S = absorbance of the *Standard solution* corrected by the absorbance of its reagent blank
C_S = concentration of iron in the *Standard solution* (µg/mL)
C_U = nominal concentration of ferrous gluconate dihydrate in the *Sample solution* (µg/mL)
M_r = molecular weight of ferrous gluconate dihydrate, 482.17
A_r = atomic weight of iron, 55.85

Acceptance criteria: 93.0%–107.0%

www.webofpharma.com

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: Solution having a known concentration of iron in the *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 248.3 nm

Lamp: Iron hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the concentration of iron (Fe) in the *Sample solution* in comparison with a *Standard solution.*

Calculate the percentage of the labeled amount of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$) dissolved:

$$\text{Result} = (M_r/A_r) \times (C \times D \times V/L) \times 100$$

M_r = molecular weight of ferrous gluconate dihydrate, 482.17

A_r = atomic weight of iron, 55.85

C = measured concentration of iron in the *Sample solution* (mg/mL)

D = dilution factor for the *Sample solution*

V = volume of *Medium*, 900 mL

L = label amount of ferrous gluconate dihydrate (mg/Capsule)

Tolerances: NLT 75% (*Q*) of the labeled amount of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label the Capsules in terms of the content of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$) and in terms of the content of elemental iron.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Potassium Gluconate RS

Ferrous Gluconate Oral Solution

DEFINITION

Ferrous Gluconate Oral Solution contains NLT 94.0% and NMT 106.0% of the labeled amount of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 10 mg/mL of USP Potassium Gluconate RS in water

Sample solution: A filtered solution in water, equivalent to 10 mg/mL of ferrous gluconate dihydrate from Oral Solution

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent.* Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution.*

• **B. FERROUS ION**

Sample solution: Equivalent to 5 mg/mL of ferrous gluconate dihydrate from a dilution in water of the *Sample solution* obtained in *Identification* test *A*

Analysis: Add potassium ferricyanide TS to the *Sample solution.*

Acceptance criteria: The solution yields a dark blue precipitate.

ASSAY

• **PROCEDURE**

Sample: An accurately measured volume of Oral Solution, equivalent to 1.2 g of ferrous gluconate dihydrate

Blank: Proceed as directed in the *Analysis* without the *Sample.*

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N ceric sulfate VS

Indicator: Orthophenanthroline TS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in a cooled mixture of 80 mL of recently boiled water and 80 mL of 2 N sulfuric acid. Add orthophenanthroline TS, and immediately titrate with *Titrant* until a change in color. Perform a *Blank* determination.

Calculate the percentage of the labeled amount of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$) in the portion of Oral Solution taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 482.2 mg/mEq

W = nominal amount of ferrous gluconate dihydrate in the *Sample* taken (mg)

Acceptance criteria: 94.0%–106.0%

OTHER COMPONENTS

• **ALCOHOL DETERMINATION** ⟨611⟩**:** 6.3%–7.7% of C_2H_5OH

SPECIFIC TESTS

• **PH** ⟨791⟩**:** 3.4–3.8

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **LABELING:** Label the Oral Solution in terms of the content of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$) and in terms of the content of elemental iron.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Potassium Gluconate RS

www.webofpharma.com

Ferrous Gluconate Tablets

DEFINITION

Ferrous Gluconate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$).

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: A filtered solution in water, equivalent to 10 mg/mL of ferrous gluconate dihydrate from powdered Tablets

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.

• **B. Ferrous Ion**

Sample solution: Equivalent to 5 mg/mL of ferrous gluconate dihydrate from a dilution of the *Sample solution* obtained in *Identification* test *A*

Analysis: Add potassium ferricyanide TS to the *Sample solution*.

Acceptance criteria: The solution yields a dark blue precipitate.

ASSAY

• **Procedure**

Sample: A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 1.5 g of ferrous gluconate dihydrate

Blank: Proceed as directed in the *Analysis* without the *Sample*.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N ceric sulfate VS

Indicator: Orthophenanthroline TS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in a mixture of 75 mL of water and 15 mL of 2 N sulfuric acid in a 300-mL conical flask. Add 250 mg of zinc dust, close the flask with a stopper containing a Bunsen valve, and allow to stand at room temperature for 20 min or until the solution becomes colorless. Pass the solution through a filtering crucible containing a thin layer of zinc dust, and wash the crucible and contents with 10 mL of 2 N sulfuric acid, followed by 10 mL of water.

[Note—Prepare and use the filtering crucible in a well-ventilated hood.]

Add orthophenanthroline TS, and immediately titrate the filtrate in the suction flask with *Titrant* until color change. Perform a *Blank* determination.

Calculate the percentage of the labeled amount of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$) in the portion of Tablets taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 482.2 mg/mEq
W = nominal amount of ferrous gluconate dihydrate in the *Sample* taken (mg)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: Simulated gastric fluid TS; 900 mL

Apparatus 2: 150 rpm

Time: 80 min

Standard solution: Solution having a known concentration of iron in the *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 248.3 nm

Lamp: Iron hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the concentration of iron (Fe) in the *Sample solution* in comparison with a *Standard solution*.

Calculate the percentage of the labeled amount of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$) dissolved:

$$\text{Result} = (M_r/A_r) \times (C \times D \times V/L) \times 100$$

M_r = molecular weight of ferrous gluconate dihydrate, 482.17
A_r = atomic weight of iron, 55.85
C = measured concentration of iron in the *Sample solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label amount of ferrous gluconate dihydrate (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **Labeling:** Label the Tablets in terms of the content of ferrous gluconate dihydrate ($C_{12}H_{22}FeO_{14} \cdot 2H_2O$) and in terms of the content of elemental iron.

• **USP Reference Standards** ⟨11⟩

USP Potassium Gluconate RS

www.webofpharma.com

Ferrous Sulfate

$FeSO_4 \cdot 7H_2O$ 278.01

$FeSO_4$ 151.91

Sulfuric acid, iron(2+) salt (1:1), heptahydrate; Iron(2+) sulfate (1:1) heptahydrate [7782-63-0]. Anhydrous [7720-78-7].

DEFINITION

Ferrous Sulfate contains an amount of anhydrous ferrous sulfate ($FeSO_4$) equivalent to NLT 99.5% and NMT 104.5% of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$).

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Iron, Ferrous Salts* ⟨191⟩ and *Sulfate* ⟨191⟩**:** Meets the requirements

ASSAY

• **PROCEDURE**

Sample: 1 g of Ferrous Sulfate

Blank: Proceed as in the *Analysis* without the *Sample.*

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N ceric sulfate VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in a mixture of 25 mL of 2 N sulfuric acid and 25 mL of freshly boiled and cooled water. Add orthophenanthroline TS, and immediately titrate with *Titrant.* Perform a blank determination.

Calculate the percentage of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = *Titrant* normality (mEq/mL)
F = equivalency factor, 278.0 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 99.5%–104.5% of ferrous sulfate heptahydrate

IMPURITIES

• **ARSENIC,** *Method I* ⟨211⟩

Test preparation: Transfer 1 g of Ferrous Sulfate to a round-bottomed, 100-mL flask fitted with a glass joint, and add 40 mL of sulfuric acid (1 in 4) and 2 mL of 300 mg/mL potassium bromide solution. Immediately connect the flask to a condenser having a matching glass joint and a reservoir with a water jacket that is cooled by ice water. Heat the flask gently over a low flame until the solid dissolves, then distill until 25 mL of distillate collects in the reservoir. Transfer this distillate to the arsine generator flask, and wash the condenser and the reservoir with several small portions of water, adding the washings to the generator flask. Swirl to mix, add bromine TS until the color of the solution is slightly yellow, and dilute with water to 35 mL.

Acceptance criteria: NMT 3 ppm

• **LEAD**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm nitric acid (1 in 2) for 30 min and by rinsing with deionized water.]

Ascorbic acid–sodium iodide solution: 100 mg/mL of ascorbic acid and 192.5 mg/mL of sodium iodide

Trioctylphosphine oxide solution: 50 mg/mL of trioctylphosphine oxide in 4-methyl-2-pentanone. [**CAUTION**—This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.]

Standard solution: Transfer 5.0 mL of lead nitrate stock solution TS to a 100-mL volumetric flask. Dilute with water to volume, and mix. Transfer 2.0 mL of the resulting solution to a 50-mL volumetric flask. Add 10 mL of 9 N hydrochloric acid and 10 mL of water. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution,* shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Standard solution,* and it contains 2 µg/mL of lead.

Sample solution: To a 50-mL volumetric flask add 1.0 g of Ferrous Sulfate, 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution,* and 5.0 mL of *Trioctylphosphine oxide solution.* Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Sample solution.*

Blank: To a 50-mL volumetric flask add 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution,* and 5.0 mL of *Trioctylphosphine oxide solution.* Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Blank,* and it contains 0 µg/mL of lead.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 283.3 nm

Lamp: Lead hollow-cathode

Flame: Air–acetylene

System suitability

Samples: *Standard solution* and *Blank*

Suitability requirements: The absorbance of the *Standard solution* and the absorbance of the *Blank* are significantly different.

Analysis

Samples: *Standard solution, Sample solution,* and *Blank*

Concomitantly determine the absorbances of the *Blank, Standard solution,* and the *Sample solution.* Use the *Blank* to set the instrument to zero.

Acceptance criteria: The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 10 ppm).

• **MERCURY**

[NOTE—Carry out this test in subdued light, because mercuric dithizonate is light sensitive.]

Hydroxylamine hydrochloride solution, Standard mercury solution, Dithizone extraction solution, and **Diluted dithizone extraction solution:** Prepare as directed in *Mercury* ⟨261⟩, *Method I.*

Control solution: Mix 3.0 mL of *Standard mercury solution,* 30 mL of dilute nitric acid (1 in 10), 5 mL of 250 mg/mL of sodium citrate solution, and 1 mL of *Hydroxylamine hydrochloride solution.*

Test preparation: Dissolve 1 g of Ferrous Sulfate in 30 mL of dilute nitric acid (1 in 10) with the aid of heat, on a steam bath. Cool quickly by immersing in an ice bath, and pass through a fine-porosity filter that previously has been washed with dilute nitric acid (1 in 10) and water. To the

www.webofpharma.com

filtrate add 20 mL of 250 mg/mL sodium citrate solution and 1 mL of *Hydroxylamine hydrochloride solution.*

Analysis: Adjust the *Control solution* to a pH of 1.8 with ammonium hydroxide and the *Sample solution* to a pH of 1.8 with sulfuric acid. Separately transfer the solutions to separators. Treat the *Sample solution* and the *Control solution* in parallel as follows.

Extract with two 5-mL portions of *Dithizone extraction solution* and 5 mL of chloroform, pooling the chloroform extracts in a second separator. Add 10 mL of dilute hydrochloric acid (1 in 2), shake, allow the layers to separate, and discard the chloroform layer. Wash the acid extract with 3 mL of chloroform, and discard the washing. Add 0.1 mL of 20 mg/mL of edetate disodium solution and 2 mL of 6 N acetic acid, mix, and add slowly 5 mL of ammonium hydroxide. Close the separator, cool it under cold running water, and dry its outer surface. Remove the stopper, and pour the contents into a beaker. Adjust the *Sample solution* and the *Control solution* to a pH of 1.8 in the same manner as before, and return the solutions to their respective separators. Add 5.0 mL of *Diluted dithizone extraction solution*, shake vigorously, and allow the layers to separate. Using *Diluted dithizone extraction solution* as a color blank, compare the colors developed in the chloroform layers of the *Sample solution* and the *Control solution.*

Acceptance criteria: The color developed by the *Sample solution* is not more intense than that developed by the *Control solution* (NMT 3 µg/g).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is not to be used if it is coated with brownish yellow basic ferric sulfate.

Ferrous Sulfate Oral Solution

DEFINITION

Ferrous Sulfate Oral Solution contains NLT 94.0% and NMT 106.0% of the labeled amount of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Iron, Ferrous Salts* ⟨191⟩ and *Sulfate* ⟨191⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Sample: A volume of Oral Solution equivalent to 625 mg of $FeSO_4 \cdot 7H_2O$ accurately measured

Blank: Proceed as in the *Analysis* without the *Sample.*

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N ceric sulfate VS

Indicator: Orthophenanthroline TS

Endpoint detection: Visual

Analysis: Add 25 mL of 2 N sulfuric acid and 75 mL of freshly boiled and cooled water to the *Sample,* and shake well. Then add orthophenanthroline TS, and immediately titrate with *Titrant* until the color changes. Perform a blank determination.

Calculate the percentage of the labeled amount of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 278.0 mg/mEq

W = nominal amount of ferrous sulfate heptahydrate in the *Sample* taken (mg)

Acceptance criteria: 94.0%–106.0% of the labeled amount of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$)

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 1.4–5.3

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label the Oral Solution in terms of the content of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and in terms of the content of elemental iron.

Ferrous Sulfate Syrup

DEFINITION

Ferrous Sulfate Syrup contains, in each 100 mL, NLT 3.75 g and NMT 4.25 g of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), equivalent to NLT 0.75 g and NMT 0.85 g of elemental iron.

Ferrous Sulfate Syrup is prepared as follows.

Ferrous Sulfate	40 g
Citric Acid, hydrous	2.1 g
Peppermint Spirit	2 mL
Sucrose	825 g
Purified Water, a sufficient quantity to make	1000 mL

Dissolve the *Ferrous Sulfate, Citric Acid, Peppermint Spirit,* and 200 g of *Sucrose* in 450 mL of *Purified Water,* and filter the solution until clear. Dissolve the remainder of the *Sucrose* in the clear filtrate, and add *Purified Water* to make 1000 mL. Mix, and filter if necessary, through a pledget of cotton.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Iron, Ferrous Salts* ⟨191⟩ and *Sulfate* ⟨191⟩

Sample solution: Dilute 1 mL of Syrup with water to 100 mL.

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Sample: 25 mL of Syrup

Blank: Proceed as in the *Analysis* without the *Sample.*

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N ceric sulfate VS

Indicator: Orthophenanthroline TS

Endpoint detection: Visual

Analysis: Add 15 mL of 2 N sulfuric acid and 100 mL of water to the *Sample,* and shake well. Add 3 drops of orthophenanthroline TS, and titrate with *Titrant* until the color changes. Perform a blank determination.

Calculate the amount of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) in g per each 100 mL of Syrup:

www.webofpharma.com

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 0.278 g/mEq
W = volume of Ferrous Sulfate Syrup for the *Sample* taken (mL)

Acceptance criteria: 3.75–4.25 g in each 100 mL

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label the Syrup in terms of the content of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and in terms of the content of elemental iron.

Ferrous Sulfate Tablets

DEFINITION
Ferrous Sulfate Tablets contain NLT 95.0% and NMT 110.0% of the labeled amount of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$). [NOTE—An equivalent amount of Dried Ferrous Sulfate may be used in place of $FeSO_4 \cdot 7H_2O$ in preparing Ferrous Sulfate Tablets.]

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Iron, Ferrous Salts* ⟨191⟩ and *Sulfate* ⟨191⟩
  Sample solution: Equivalent to 10 mg/mL of ferrous sulfate heptahydrate from powdered Tablets in water acidified with hydrochloric acid
  Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**
  Sample: Equivalent to 500 mg of ferrous sulfate heptahydrate from finely powdered Tablets (NLT 20)
  Blank: Proceed as in the *Analysis* without the *Sample*.
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N ceric sulfate VS
  Indicator: Orthophenanthroline TS
  Endpoint detection: Visual
  Analysis: In a beaker, dissolve the *Sample* in a mixture of 20 mL of 2 N sulfuric acid and 80 mL of freshly boiled and cooled water. Filter the solution rapidly as soon as all soluble ingredients in the Tablets are dissolved, and wash the container and the filter with small portions of a mixture of 20 mL of 2 N sulfuric acid and 80 mL of freshly boiled and cooled water.
  Add orthophenanthroline TS, and immediately titrate the filtrate in the suction flask with *Titrant* until the color changes. Perform a blank determination.
  Calculate the percentage of the labeled amount of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 278.0 mg/mEq
W = nominal weight of ferrous sulfate heptahydrate in the *Sample* taken (mg)

Acceptance criteria: 95.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
  Medium: 0.1 N hydrochloric acid; 900 mL
  Apparatus 2: 50 rpm
  Time: 45 min
  Standard solution: Solution having a known concentration of iron in the *Medium*
  Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary
  Instrumental conditions
  (See *Atomic Absorption Spectroscopy* ⟨852⟩.)
  Mode: Atomic absorption spectrophotometry
  Analytical wavelength: 248.3 nm
  Lamp: Iron hollow-cathode
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Determine the concentration of iron (Fe) in the *Sample solution* in comparison with a *Standard solution*.
  Calculate the percentage of the labeled amount of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) dissolved:

$$\text{Result} = (M_r/A_r) \times (C \times D \times V/L) \times 100$$

M_r = molecular weight of ferrous sulfate heptahydrate, 278.02
A_r = atomic weight of iron, 55.85
C = measured concentration of iron in the *Sample solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

  Tolerances: NLT 75% (Q) of the labeled amount of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label the Tablets in terms of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and in terms of elemental iron.

Dried Ferrous Sulfate

$FeSO_4 \cdot xH_2O$ 151.91
Sulfuric acid, iron(2+) salt (1:1), hydrate;
Iron(2+) sulfate (1:1) hydrate [13463-43-9].
Anhydrous [7720-78-7].

DEFINITION
Dried Ferrous Sulfate contains NLT 86.0% and NMT 89.0% of anhydrous ferrous sulfate ($FeSO_4$).

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Iron, Ferrous Salts* ⟨191⟩ and *Sulfate* ⟨191⟩: Meets the requirements

ASSAY
- **PROCEDURE**
  Sample: 800 mg of Dried Ferrous Sulfate
  Blank: Proceed as in the *Analysis* without the *Sample*.
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N ceric sulfate VS

USP Monographs

www.webofpharma.com

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in a mixture of 25 mL of 2 N sulfuric acid and 25 mL of freshly boiled and cooled water. Add orthophenanthroline TS, and immediately titrate with the *Titrant*. Perform a blank determination. Calculate the percentage of anhydrous ferrous sulfate ($FeSO_4$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = *Titrant* normality (mEq/mL)
F = equivalency factor, 151.9 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 86.0%–89.0%

IMPURITIES

• **ARSENIC,** *Method I* ⟨211⟩

Test preparation: Transfer 1 g of Dried Ferrous Sulfate to a round-bottomed, 100-mL flask fitted with a glass joint, and add 40 mL of sulfuric acid (1 in 2) and 2 mL of 300 mg/mL of potassium bromide solution. Immediately connect the flask to a condenser having a matching glass joint and a reservoir with a water jacket that is cooled by ice water. Heat the flask gently over a low flame until the solid dissolves, then distill until 25 mL of distillate collects in the reservoir. Transfer this distillate to the arsine generator flask, and wash the condenser and the reservoir with several small portions of water, adding the washings to the generator flask. Swirl to mix, add bromine TS until the color of the solution is slightly yellow, and dilute with water to 35 mL.

Acceptance criteria: NMT 3 ppm

• **LIMIT OF LEAD**

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm nitric acid (1 in 2) for 30 min and by rinsing with deionized water.]

Ascorbic acid–sodium iodide solution: 100 mg/mL of ascorbic acid and 192.5 mg/mL of sodium iodide

Trioctylphosphine oxide solution: 50 mg/mL of trioctylphosphine oxide in 4-methyl-2-pentanone. [**CAUTION**—This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.]

Standard solution: Transfer 5.0 mL of lead nitrate stock solution TS to a 100-mL volumetric flask. Dilute with water to volume. Transfer 2.0 mL of the resulting solution to a 50-mL volumetric flask. Add 10 mL of 9 N hydrochloric acid and 10 mL of water. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*, shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Standard solution*, and it contains 2 µg/mL of lead.

Sample solution: To a 50-mL volumetric flask add 1.0 g of Dried Ferrous Sulfate, 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Sample solution*.

Blank: To a 50-mL volumetric flask add 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Blank*, and it contains 0 µg/mL of lead.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 283.3 nm

Lamp: Lead hollow-cathode

Flame: Air–acetylene

System suitability

Samples: *Standard solution* and *Blank*

Suitability requirements: The absorbance of the *Standard solution* and the absorbance of the *Blank* are significantly different.

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Concomitantly determine the absorbances of the *Blank*, *Standard solution*, and the *Sample solution*. Use the *Blank* to set the instrument to zero.

Acceptance criteria: The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 10 ppm).

• **MERCURY**

[NOTE—Carry out this test in subdued light, because mercuric dithizonate is light sensitive.]

Hydroxylamine hydrochloride solution, Standard mercury solution, Dithizone extraction solution, and **Diluted dithizone extraction solution:** Prepare as directed in *Mercury* ⟨261⟩, *Method I*.

Control solution: Mix 3.0 mL of *Standard mercury solution*, 30 mL of nitric acid (1 in 10), 5 mL of 250 mg/mL of sodium citrate solution, and 1 mL of *Hydroxylamine hydrochloride solution*.

Test preparation: Dissolve 1 g of Dried Ferrous Sulfate in 30 mL of nitric acid (1 in 10) with the aid of heat, on a steam bath. Cool quickly by immersing in an ice bath, and pass through a fine-porosity filter that previously has been washed with nitric acid (1 in 10) and water. To the filtrate, add 20 mL of 250 mg/mL of sodium citrate solution and 1 mL of *Hydroxylamine hydrochloride solution*.

Analysis: Adjust the *Control solution* to a pH of 1.8 with ammonium hydroxide and the *Sample solution* to a pH of 1.8 with sulfuric acid. Separately transfer the solutions to separators. Treat the *Sample solution* and the *Control solution* in parallel as follows.

Extract with two 5-mL portions of *Dithizone extraction solution* and 5 mL of chloroform, pooling the chloroform extracts in a second separator. Add 10 mL of hydrochloric acid (1 in 2), shake, allow the layers to separate, and discard the chloroform layer. Wash the acid extract with 3 mL of chloroform, and discard the washing. Add 0.1 mL of 20 mg/mL of edetate disodium solution and 2 mL of 6 N acetic acid, mix, and add slowly 5 mL of ammonium hydroxide. Close the separator, cool it under cold running water, and dry its outer surface. Remove the stopper, and pour the contents into a beaker. Adjust the *Sample solution* and the *Control solution* to a pH of 1.8 in the same manner as before, and return the solutions to their respective separators. Add 5.0 mL of *Diluted dithizone extraction solution*, shake vigorously, and allow the layers to separate. Using *Diluted dithizone extraction solution* as a color blank, compare the colors developed in the chloroform layers of the *Sample solution* and the *Control solution*.

www.webofpharma.com

Acceptance criteria: The color developed by the *Sample solution* is not more intense than that developed by the *Control solution* (NMT 3 ppm).

• **INSOLUBLE SUBSTANCES**

Sample: 2.0 g of Dried Ferrous Sulfate

Analysis: Dissolve the *Sample* in 20 mL of freshly boiled dilute sulfuric acid (1 in 100), heat to boiling, and digest in a covered beaker on a steam bath for 1 h. Filter through a tared filtering crucible, wash thoroughly, and dry at 105°.

Acceptance criteria: The weight of the insoluble residue does not exceed 1 mg (NMT 0.05%).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Ferumoxides Injection

» Ferumoxides Injection is a sterile colloidal suspension of superparamagnetic iron oxide associated with dextran in Water for Injection. It contains not less than 95 percent and not more than 105 percent of the labeled amount of iron. It contains in each mL not less than 5.6 mg and not more than 9.1 mg of dextran. It contains in each mL not less than 0.25 mg and not more than 0.53 mg of citrate. It also contains mannitol. It contains no antimicrobial agents. Ferumoxides is a nonstoichiometric iron oxide magnetite of average formula $FeO_{1.44}$, with particles having a diameter between 100 and 250 nm.

Packaging and storage—Preserve in single-dose containers of Type I glass, and store at controlled room temperature. Avoid freezing.

Labeling—Label it to indicate that it is to be administered through a 5-µm filter and that it is not to be used if there are indications that the package has been exposed to freezing temperatures.

USP Reference standards ⟨11⟩—
USP Dextran T-10 RS
USP Dextrose RS

Identification—Transfer about 1 mL of the Injection to a test tube, and add 2 drops of ammonium hydroxide: no precipitate is formed. Add 2 mL of hydrochloric acid, mix, and add 2 mL of ammonium hydroxide: a brown precipitate is formed.

Specific gravity ⟨841⟩: between 1.031 and 1.041.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 12.5 USP Endotoxin Units per mL.

pH ⟨791⟩: between 5.0 and 9.0.

Colloidal particle size—

Apparatus—Use a submicron laser light-scattering instrument.[1]

Standards—Use 90- and 270-nm NIST-traceable polystyrene monospheres.

Standard dilutions—Transfer 3 mL of 0.2-µm filtered water to each of two clear acrylic cuvettes. Add a sufficient amount of the 90-nm monospheres to one of the cuvettes and a sufficient amount of the 270-nm monospheres to the other cuvette to make the dilutions slightly turbid, place in the *Apparatus*, and then measure the particle sizes: the particle size in the *Standards* is within 10% of the certified diameter.

Procedure—Transfer about 40 µL of the Injection to a clear acrylic cuvette, and add 3 mL of 0.2-µm filtered water. Cover, invert to mix (without shaking), place in the *Apparatus*, and measure the intensity-weighted effective diameter. [NOTE—Several minutes are necessary for the sample to reach equilibrium.] The intensity-weighted effective diameter is between 100 and 250 nm.

Magnetic susceptibility—

Apparatus: a calibrated magnetic susceptibility balance.

Standard solutions—Transfer 118.85 g of nickel chloride hexahydrate, accurately weighed, to a 500-mL volumetric flask, dilute with water to volume, and mix to obtain 1 M nickel chloride. Transfer 356.55 g of nickel chloride hexahydrate, accurately weighed, to a separate 500-mL volumetric flask, dilute with water to volume, and mix to obtain 3 M nickel chloride.

Balance constant— [NOTE—Each magnetic susceptibility balance has a *Balance constant, K*, that is determined each time the balance is moved to a new location. The *Balance constant* is referenced to the known magnetic susceptibility of nickel chloride hexahydrate.] Measure the length, *L*, in cm, of the large-bore susceptibility tube, from the bottom of the inside of the tube to the bottom of the black band. Tare the susceptibility tube on a balance, fill it with water to the bottom of the black band, and weigh again. Record the weight, *W*, in g, of the water in the tube, zero the susceptibility balance, and then place the tube containing water in the susceptibility balance. Zero the balance again. Rinse the tube with 1 M nickel chloride, and then fill the tube with fresh 1 M nickel chloride. Insert the tube into the susceptibility balance, and record the magnetic susceptibility, *R*, in cgs units, of 1 M nickel chloride. Calculate the *Balance constant, K*, by the formula:

$$(4.24 \times 10^{-6})(W \times 10^{9})/RL$$

in which 4.24×10^{-6} is the magnetic susceptibility, in cgs units, of nickel chloride hexahydrate; and the other terms are as defined above.

Tube constant— [NOTE—For each susceptibility tube, a tube constant, C_T, is determined.] Measure the length of the tube, *L*, in cm, from the bottom of the inside of the tube to the bottom of the black band. Tare the tube on a balance, fill it with water to the bottom of the black band, and weigh again. Record the weight, *W*, in g, of the water in the tube. Calculate the tube constant, C_T, by the formula:

$$KL/(W \times 10^{9})$$

in which *K* is the *Balance constant* as obtained above; and *L* and *W* are as defined above.

Diluted sample—Transfer about 0.2 g of the Injection, accurately weighed, to a 10-mL volumetric container, dilute with water to volume, mix, and accurately weigh the contents.

Procedure— [NOTE—Use the same susceptibility tube throughout the procedure.] Fill the susceptibility tube with water to the black band. Insert the tube into the susceptibility balance, and adjust to zero. Remove the tube from the balance, pour the water from the tube, rinse the tube with 1 M nickel chloride, and then fill the tube with fresh 1 M nickel chloride. Insert the tube into the balance, and record the magnetic susceptibility in cgs units. Similarly, measure the magnetic susceptibility of 3 M nickel chloride. The balance readings are within 5% of the expected values, which are $(4.24 \times 10^{-6})/C_T$ for 1 M nickel chloride and $(12.72 \times 10^{-6})/C_T$ for 3 M nickel chloride. Rinse the tube with well-mixed Injection, then fill the tube with the Injection to the black band, and weigh. Insert the tube into the susceptibility balance, and

[1] Brookhaven Instruments 90Plus or equivalent.

USP Monographs

www.webofpharma.com

proceed as directed for the *Standard solutions*. Calculate the magnetic susceptibility, in cgs units, per g of iron in the Injection by the formula:

$$1000C_T RT/WI$$

in which C_T is the *Tube constant* as obtained above; R is the balance reading, in cgs units; T is the weight, in g, of the *Diluted sample*; W is the weight, in g, of the ferumoxides in the *Diluted sample*; and I is the concentration of iron, in mg per g, in the Injection, obtained by using specific gravity to convert the concentration of iron, in mg per mL, as determined in the *Assay for iron*, to mg per g. The magnetic susceptibility is not less than $17{,}100 \times 10^{-6}$ in cgs units per g of iron.

Osmolality and Osmolarity ⟨785⟩, *Osmolality*: between 325 and 365 mOsmol per kg.

Other requirements—*Injections and Implanted Drug Products* ⟨1⟩; with the exception of *Foreign and Particulate Matter*

Assay for iron—

Iron standard solution—Use a NIST-traceable iron standard containing 1000 µg per mL (1000 ppm).

Standard preparations—Pipet 5.0, 10.0, 15.0, and 20.0 mL of the *Iron standard solution* into separate 1000-mL volumetric flasks, dilute each with water to volume, and mix to obtain solutions having known concentrations of 5 µg per mL, 10 µg per mL, 15 µg per mL, and 20 µg per mL, respectively.

Assay preparation—Accurately weigh 100 µL of the Injection, and transfer to a test tube. Add 2 mL of hydrochloric acid, and mix. [NOTE—The Injection dissolves to yield a medium yellow solution.] Add 2 mL of water, and then transfer the contents of the tube to a 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Using an atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with an iron hollow-cathode lamp and an air–acetylene flame, set the instrument to zero with water, and measure the absorbance, A_{20}, of the *Standard preparation* containing 20 µg per mL at the iron emission line of 296.7 nm. Concomitantly determine the absorbances of the *Standard preparations*. Calculate the iron concentration, in µg per mL, of each *Standard preparation* by the formula:

$$20(A_S/A_{20})$$

in which A_S is the absorbance of the relevant *Standard preparation*. The reading for each *Standard preparation* is within 0.3 µg per mL of its nominal concentration. Measure the absorbance of the *Assay preparation*, and calculate the content of iron, in mg per mL, in the Injection by the formula:

$$2(A_U/A_{20})(S/W)$$

in which A_U is the absorbance of the *Assay preparation*; A_{20} is the absorbance of the *Standard preparation* containing 20 µg per mL; S is the specific gravity of the Injection; and W is the weight, in g, of the volume of Injection taken to prepare the *Assay preparation*.

Assay for dextran—

Control preparation—Transfer about 50 mg of USP Dextrose RS, accurately weighed, to a 1000-mL volumetric flask, dilute with water to volume, mix, and filter.

Standard preparation—Transfer about 50 mg of USP Dextran T-10 RS, accurately weighed, to a 1000-mL volumetric flask, dilute with water to volume, mix, and filter.

Assay preparation—Transfer 0.25 g of the Injection, accurately weighed, to a test tube, add about 0.25 mL of hydrochloric acid, then add about 9 mL of water, and mix. Remove the free iron from this solution by passing it through a cation-exchange column into a 25-mL volumetric flask. Rinse the column with about 9 mL of water, collecting the washings in the 25-mL volumetric flask, dilute with water to volume, and mix.

Procedure—To each of four test tubes, separately add 0.2 mL of the *Assay preparation*, the *Standard preparation*, the *Control preparation*, and water (to be used as the blank). Add 0.2 mL of a 5% phenol solution to each test tube. Mix each tube briefly on a vortex mixer, rapidly add 1.0 mL of sulfuric acid to each test tube, and again mix briefly on a vortex mixer. [**CAUTION**—Reaction is exothermic.] Cover the test tubes, and allow to stand at room temperature for at least 15 minutes. [NOTE—The resultant solution is orange-yellow in color and free of any solid material.] Mix each tube on a vortex mixer. Using a suitable spectrophotometer, determine the absorbances of the solutions from the *Standard preparation*, the *Control preparation*, and the *Assay preparation* against the blank at the wavelength of maximum absorbance at about 490 nm. Calculate the percent recovery of dextran in the *Control preparation* by the formula:

$$100(1.11)(C/C_D)(A_C/A_S)$$

in which 1.11 is a correction factor (to account for dextrose being a monomer of dextran); C is the concentration, in mg per mL, of USP Dextran T-10 RS in the *Standard preparation*; C_D is the concentration, in mg per mL, of USP Dextrose RS in the *Control preparation*; and A_C and A_S are the absorbances of the solutions from the *Control preparation* and the *Standard preparation*, respectively: not less than 90% to 110% is found. Calculate the quantity, in mg per mL, of dextran in the volume of Injection taken by the formula:

$$25S(C/W)(A_U/A_S)$$

in which S is the specific gravity of the Injection; C is the concentration, in mg per mL, of USP Dextran T-10 RS in the *Standard preparation*; W is the weight, in g, of the portion of the Injection taken to prepare the *Assay preparation*; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for citrate—

Mobile phase—Prepare a filtered and degassed 0.0375 N sodium hydroxide solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock solution—Transfer 0.7776 g of trisodium citrate dihydrate to a 100-mL volumetric flask, dilute with water to volume, filter, and refrigerate. This solution contains 5000 µg of citrate per mL (5000 ppm).

Standard preparations—Transfer 10 mL of *Standard stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 1.0, 2.0, and 4.0 mL of this solution to separate 100-mL volumetric flasks, add 0.8 mL of hydrochloric acid to each flask, dilute with water to volume, and mix to obtain solutions having known concentrations of 5 µg per mL, 10 µg per mL, and 20 µg per mL.

System suitability solution—Use the filtered *Standard preparation* containing 5 µg per mL.

Assay preparation—Transfer about 0.5 mL of the Injection, accurately weighed, to a test tube, add 0.2 mL of hydrochloric acid and about 9 mL of water, and mix. Remove the free iron from this solution by passing the solution through a cation-exchange column[2] into a 25-mL volumetric flask. Rinse the column with about 9 mL of water, collecting the washings in the flask, dilute with water to volume, and mix.

[2] AG 50W-X8 (H+), available from Biorad.

www.webofpharma.com

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph[3] is equipped with an ion detector with suppressed conductivity at 30 µS, a 4-mm × 25-cm separator column[4] that contains 15-µm packing L48, a 4-mm × 50-mm guard column,[5] and integrators. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparations* and the *Assay preparation* into the chromatograph, record the chromatograms for about 16 minutes, and measure the responses for the major peaks. Prepare a standard curve by plotting the conductivities of the *Standard preparations* versus their concentrations, in µg per mL. Determine the concentration, *C,* in µg per mL, of citrate in the *Assay preparation* by extrapolation from the standard curve. Calculate the quantity, in mg per mL, of citrate in the volume of Injection taken by the formula:

$$0.025C(S/W)$$

in which *S* is the specific gravity of the Injection; and *W* is the weight, in g, of the volume of Injection taken to prepare the *Assay preparation.*

Ferumoxsil Oral Suspension

» Ferumoxsil Oral Suspension is an aqueous suspension of silicone-coated superparamagnetic iron oxide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of iron (Fe). It contains a preservative and a thickening agent. It may contain suitable colors, flavors, and sweetening agents. Ferumoxsil is poly[*N*-(2-aminoethyl)-3-aminopropyl] siloxane-coated nonstoichiometric magnetite $[FeO_X(C_5H_{13}N_2SiO_2)_Y]$.

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.

Labeling—Label it to indicate that it is to be well-shaken for 1 minute before use, and that it is not to be used if there are indications that the package has been exposed to freezing temperatures.

Viscosity—Capillary Methods ⟨911⟩ or ***Viscosity—Rotational Methods*** ⟨912⟩**:** between 11 and 60 centipoises.

Osmolality and Osmolarity ⟨785⟩, *Osmolarity***:** between 230 and 270 mOsmol per kg.

Microbial enumeration tests ⟨61⟩ and ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Escherichia coli.* The total aerobic microbial count does not exceed 100 cfu per mL.

pH ⟨791⟩**:** between 5.5 and 9.0.

Magnetic susceptibility—

Apparatus, Standard solutions, Balance constant, and *Tube constant*—Proceed as directed for *Magnetic susceptibility* under *Ferumoxides Injection.*

Procedure—Proceed as directed for *Magnetic susceptibility* under *Ferumoxides Injection* except to use undiluted Oral Suspension whenever Injection is indicated. Calculate the magnetic susceptibility, in cgs units, per g of iron in the Oral Suspension by the formula:

$$1000C_TR/I$$

in which C_T is the *Tube constant; R* is the balance reading, in cgs units; and *I* is the concentration, in mg per g, of iron in the Oral Suspension, as determined in the *Assay for iron.* The magnetic susceptibility is not less than $22{,}500 \times 10^{-6}$ in cgs units per g of iron.

Settling—Prepare a mixture of Oral Suspension and water (1:5). Mix by gentle inversion, and determine the absorbance at 500 nm in a suitable spectrophotometer, using water as the blank. Cover the cell, and allow to stand undisturbed for 4 hours at room temperature. Without mixing, determine the absorbance again. Calculate the percentage of iron remaining in solution after settling by the formula:

$$100(A_4/A_0)$$

in which A_4 is the absorbance of the solution after standing for 4 hours; and A_0 is the initial absorbance of the solution: not less than 80% is found.

Uniformity of dosage units ⟨905⟩**—**

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩**—**

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Assay for iron—

Iron standard solution and *Standard preparations*—Proceed as directed in the *Assay for iron* under *Ferumoxides Injection.*

Assay preparation—Transfer about 5 g of well-mixed Oral Suspension, accurately weighed, to a 100-mL volumetric flask. Add 2 mL of nitric acid, and mix. Transfer the flask to a boiling water bath, and boil for 30 minutes to obtain a yellowish solution. Allow to cool to room temperature, dilute with water to volume, and mix.

Procedure—Proceed as directed in the *Assay for iron* under *Ferumoxides Injection.* Calculate the iron concentrations, in µg per mL, of the three *Standard preparations* by the formula:

$$20(A_S/A_{20})$$

in which A_S is the absorbance of the relevant *Standard preparation;* and A_{20} is the absorbance of the *Standard preparation* containing 20 µg per mL. The reading for each *Standard preparation,* A_S, is within 0.5 µg per mL of its nominal concentration. Measure the absorbance of the *Assay preparation,* and calculate the content of iron, in µg per mL, in the Oral Suspension by the formula:

$$2000(A_U/A_{20})(1.01/W)$$

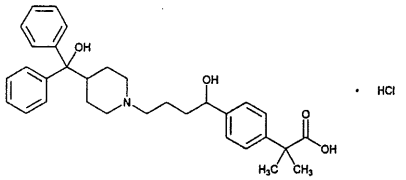
in which A_U is the absorbance of the *Assay preparation;* A_{20} is the absorbance as defined above; 1.01 is the specific gravity of the Oral Suspension; and *W* is the weight, in g, of Oral Suspension taken to prepare the *Assay preparation.*

[3] DX-500, available from Dionex Corporation or equivalent source.
[4] HPIC IonPac AS5 or equivalent
[5] HPIC IonPac AG5 or equivalent

USP Monographs

www.webofpharma.com

Fexofenadine Hydrochloride



$C_{32}H_{39}NO_4 \cdot HCl$ 538.12

Benzeneacetic acid, 4-[1-hydroxy-4-[4-(hydroxy diphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethyl-, hydrochloride, (±)-;

(±)-*p*-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)piperidino] butyl]-α-methylhydratropic acid, hydrochloride [153439-40-8].

DEFINITION

Fexofenadine Hydrochloride contains NLT 98.0% and NMT 102.0% of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the fexofenadine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.**

Analysis: Examine the precipitate formed in *Other Components* for the *Content of Chloride* test.

Acceptance criteria: A white precipitate is observed.

ASSAY

- **PROCEDURE**

Buffer: 6.64 g/L of monobasic sodium phosphate and 0.84 g/L of sodium perchlorate in water. Adjust with phosphoric acid to a pH of 2.0.

Diluent: Acetonitrile and *Buffer* (1:1)

Mobile phase: Acetonitrile and *Buffer* (7:13). Add 3 mL/L of triethylamine.

Standard solution: 0.06 mg/mL of USP Fexofenadine Hydrochloride RS and 0.005 mg/mL of USP Fexofenadine Related Compound A RS in *Mobile phase*

Sample stock solution: 1.0 mg/mL of Fexofenadine Hydrochloride in *Diluent*

Sample solution: 0.06 mg/mL of Fexofenadine Hydrochloride in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L11

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 10 between fexofenadine and fexofenadine related compound A

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for fexofenadine and NMT 3.0% for fexofenadine related compound A

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) in the portion of Fexofenadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Fexofenadine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

OTHER COMPONENTS

- **CONTENT OF CHLORIDE**

Sample: 300 mg of Fexofenadine Hydrochloride

Blank: Methanol

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Potentiometrically

Analysis: Dissolve the *Sample* in 50 mL of methanol. Each mL of 0.1 N silver nitrate VS is equivalent to 3.545 mg of chloride.

Acceptance criteria: 6.45%–6.75% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF FEXOFENADINE RELATED COMPOUND B**

Buffer: Glacial acetic acid and water (2.3: 2000). Adjust with 6 N ammonium hydroxide to a pH of 4.0 ± 0.1.

Mobile phase: Acetonitrile and *Buffer* (20:80)

System suitability solution: Add 1.2 mg of USP Fexofenadine Related Compound B RS to a 5-mL volumetric flask. Dilute with *Mobile phase* to volume. Transfer 2.0 mL of the solution into a 100-mL volumetric flask. Add 25 mg of USP Fexofenadine Hydrochloride RS, and dilute with *Mobile phase* to volume.

Standard solution: 2.5 µg/mL of USP Fexofenadine Hydrochloride RS in *Mobile phase* from the *System suitability solution*

Sample solution: 0.25 mg/mL of Fexofenadine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L45

Flow rate: 0.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for fexofenadine related compound B and fexofenadine are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between fexofenadine and fexofenadine related compound B

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fexofenadine related compound B in the portion of Fexofenadine Hydrochloride taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for fexofenadine related compound B from the *Sample solution*
r_S = peak response for fexofenadine from the *Standard solution*
C_S = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of fexofenadine in the *Sample solution* (mg/mL)
F = relative response factor for fexofenadine related compound B relative to fexofenadine, 0.8

Acceptance criteria: NMT 0.2%

• **OTHER ORGANIC IMPURITIES**

Buffer, Diluent, Mobile phase, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Sample solution: Use the *Sample stock solution* in the *Assay*.

Reference solution: Use the *Sample solution* in the *Assay*.

Samples: *Standard solution, Sample solution, Reference solution,* and *Mobile phase* (used as the blank)

Measure the peak areas, excluding the peaks corresponding to those from the *Mobile phase*.

Calculate the percentage of fexofenadine related compound A in the portion of Fexofenadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for fexofenadine related compound A from the *Sample solution*
r_S = peak response for fexofenadine related compound A from the *Standard solution*
C_S = concentration of USP Fexofenadine Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of fexofenadine in the *Sample solution* (mg/mL)

Calculate the percentage of decarboxylated degradant [(±)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene], with a relative retention time of 3.2, in the portion of Fexofenadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the decarboxylated degradant from the *Sample solution*
r_S = peak response of fexofenadine from the *Standard solution*
C_S = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of fexofenadine in the *Sample solution* (mg/mL)
F = relative response factor for the decarboxylated degradant relative to fexofenadine, 1.1

Calculate the percentage of other impurities in the portion of Fexofenadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for any other impurity from the *Sample solution*
r_S = peak response of fexofenadine from the *Reference solution*
C_S = concentration of fexofenadine in the *Reference solution* (mg/mL)
C_U = concentration of fexofenadine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Acceptance Criteria, NMT (%)
Fexofenadine related compound A[a]	0.2
Decarboxylated degradant[b]	0.15
Any other individual, unidentified impurity	0.1
Total impurities	0.5

[a] Benzeneacetic acid, 4-[1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl] butyl]-α,α-dimethyl.
[b] (±)-4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene.

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 2.0% for the anhydrous form; 6.0%–10.0% for the hydrate form. [NOTE—"Hydrate" refers to a mixture of dihydrate and trihydrate forms of fexofenadine hydrochloride.]

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.

• **LABELING:** Where it is the hydrate form, the label so indicates.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Fexofenadine Hydrochloride RS
USP Fexofenadine Related Compound A RS
Benzeneacetic acid, 4-[1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethyl.
$C_{32}H_{37}NO_4$ 499.65
USP Fexofenadine Related Compound B RS
3-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethyl benzeneacetic acid hydrochloride.
$C_{32}H_{39}NO_4 \cdot HCl$ 538.12

Fexofenadine Hydrochloride Capsules

DEFINITION

Fexofenadine Hydrochloride Capsules contain NLT 93.0% and NMT 105.0% of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample solution: Empty an equivalent of 60 mg of fexofenadine hydrochloride, from the contents of several Capsules, into a suitable capped tube. Add 10 mL of a

USP Monographs

www.webofpharma.com

mixture of acetonitrile and methanol (10:1), and shake until the sample is dispersed. Allow to settle. Decant, filter, and collect the supernatant in a suitable beaker. Evaporate the solvent to near dryness by using a stream of nitrogen and with gentle heating from an appropriate source (steam, low-temperature hot plate). While still warm, add 5 mL of water and 5 drops of diluted hydrochloric acid, and stir to induce precipitation. Chill in an ice bath for about 30 min. Pass through a 10- to 15-µm filtering crucible with fritted disk. Dry the precipitate in an air oven for 1 h at 105°.

Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**

Buffer: 6.64 g/L of monobasic sodium phosphate and 0.84 g/L of sodium perchlorate in water. Adjust with phosphoric acid to a pH of 2.0.

Diluent: Acetonitrile and *Buffer* (1:1)

Mobile phase: Acetonitrile and *Buffer* (7:13). Add 3 mL/L of triethylamine.

Standard solution: 0.06 mg/mL of USP Fexofenadine Hydrochloride RS and 0.005 mg/mL of USP Fexofenadine Related Compound A RS in *Mobile phase*

Sample stock solution: Remove, as completely as possible, the contents of NLT 20 Capsules, mix the combined contents, and finely powder by using a mortar and pestle. Transfer a portion of the powder, equivalent to about 50 mg of fexofenadine hydrochloride, to a 50-mL volumetric flask. Add 40 mL of *Diluent*, and shake by mechanical means for 60 min. Sonicate for about 2 min. Allow to cool to room temperature, and dilute with *Diluent* to volume.

Sample solution: Transfer 3.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L11

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 10 between fexofenadine and fexofenadine related compound A

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% and 3.0% for fexofenadine and fexofenadine related compound A, respectively

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response of fexofenadine from the *Standard solution*

C_S = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fexofenadine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 15 and 45 min

Buffer: 1.0 g of monobasic sodium phosphate, 0.5 g of sodium perchlorate, and 0.3 mL of phosphoric acid in 300 mL of water

Mobile phase: Acetonitrile and *Buffer* (7:3)

System suitability stock solution: 0.44 mg/mL of USP Fexofenadine Related Compound A RS in water. [NOTE—A small amount of glacial acetic acid, not to exceed 5% of the total volume, may be used if necessary to dissolve USP Fexofenadine Related Compound A RS.]

System suitability solution: Prepare a solution of USP Fexofenadine Hydrochloride RS in the *System suitability stock solution* containing 0.01 mg/mL of USP Fexofenadine Related Compound A RS and 0.06 mg/mL of USP Fexofenadine Hydrochloride RS.

Standard solution: 0.07 mg/mL of USP Fexofenadine Hydrochloride RS in water. [NOTE—A small amount of methanol, not to exceed 0.5% of the total volume, may be used if necessary to dissolve USP Fexofenadine Hydrochloride RS.]

Sample solution: Filtered portions of the solution under test

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between fexofenadine and fexofenadine related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) dissolved.

Tolerances: NLT 50% (*Q*) of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) is dissolved in 15 min; NLT 75% (*Q*) of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) is dissolved in 45 min.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium, Apparatus, Buffer, Mobile phase, System suitability stock solution, System suitability solution, Chromatographic system, and **Analysis:** Proceed as directed for *Test 1*.

Time: 45 min

Tolerances: NLT 75% (*Q*) of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

USP Monographs

www.webofpharma.com

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer: 6.64 mg/mL of monobasic sodium phosphate and 0.84 mg/mL of sodium perchlorate in water. Adjust with phosphoric acid to a pH of 2.0.

Diluent: Acetonitrile and *Buffer* (1:1)

Mobile phase: Acetonitrile and *Buffer* (7:13). Add 3 mL/L of triethylamine

Standard solution: 0.06 mg/mL of USP Fexofenadine Hydrochloride RS and 0.005 mg/mL of USP Fexofenadine Related Compound A RS in *Mobile phase*

Sample solution: Use the *Sample stock solution* as prepared in the *Assay*.

Reference solution: 0.06 mg/mL of fexofenadine hydrochloride in *Mobile phase* from the *Sample solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L11

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 10 between fexofenadine and fexofenadine related compound A

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% and 3.0% for fexofenadine and fexofenadine related compound A, respectively

Analysis

Samples: *Standard solution*, *Sample solution*, and *Reference solution*

Calculate the percentage of fexofenadine related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for fexofenadine related compound A from the *Sample solution*

r_S = peak response for fexofenadine related compound A from the *Standard solution*

C_S = concentration of USP Fexofenadine Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fexofenadine in the *Sample solution* (mg/mL)

Calculate the percentage of decarboxylated degradant [(±)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene], with a relative retention time of 3.2, in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the decarboxylated degradant from the *Sample solution*

r_S = peak response of fexofenadine from the *Standard solution*

C_S = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fexofenadine in the *Sample solution* (mg/mL)

F = response factor for the decarboxylated degradant relative to fexofenadine, 1.1

Calculate the percentage of other impurities in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for any other impurity from the *Sample solution*

r_S = peak response of fexofenadine from the *Reference solution*

C_S = concentration of fexofenadine in the *Reference solution* (mg/mL)

C_U = nominal concentration of fexofenadine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Acceptance Criteria, NMT (%)
Fexofenadine related compound A[a]	0.4
Decarboxylated degradant[b]	0.2
Any other individual, unidentified impurity	0.2
Total impurities	0.5

[a] Benzeneacetic acid, 4-[1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl] butyl]-α,α-dimethyl.

[b] (±)-4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Fexofenadine Hydrochloride RS

USP Fexofenadine Related Compound A RS

Benzeneacetic acid, 4-[1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethyl.

$C_{32}H_{37}NO_4$ 538.12

Fexofenadine Hydrochloride Tablets

DEFINITION

Fexofenadine Hydrochloride Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

Standard solution: Transfer 60 mg of USP Fexofenadine Hydrochloride RS to a suitable capped tube and add 10 mL of a mixture of acetonitrile and methanol (10:1).

Sample solution: Transfer an equivalent to 60 mg of fexofenadine hydrochloride, from a sufficient number of weighed and finely powdered Tablets, to a suitable capped tube, and add 10 mL of a mixture of acetonitrile and methanol (10:1).

Analysis: Shake or mix the *Standard solution* and *Sample solution* on a vortex mixer for 1–2 min to disperse the sample. Allow the solution to stand for 10 min, or centrifuge for 2–3 min. Pass the liquid into a 50-mL beaker using a

www.webofpharma.com

0.45-µm polytetrafluoroethlyene syringe filter. Evaporate the solvent until about 0.5 mL remains, using a stream of nitrogen with gentle heating (do not exceed 75°). Add 5 mL of water and 5 drops of dilute hydrochloric acid, and stir to induce precipitation. Chill in an ice bath for 30 min. Filter the solution through a 10- to 15-µm sintered-glass crucible. Dry the precipitate in an air oven for 1 h at 105° oven for 1 h at 105°. Prepare a bromide dispersion from the residue.

Acceptance criteria: The IR absorption spectrum of the potassium bromide dispersion of the residue from the sample exhibits maxima only at the same wavelengths as that of a potassium bromide dispersion from the Standard.

• **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Glacial acetic acid and water (17:983). Dilute 100 mL of this solution with water to 1 L.

Solution B: Dilute 15 mL of a solution containing acetonitrile and triethylamine (1:1) with *Solution A* to 1 L. Adjust with phosphoric acid to a pH of 5.25.

Diluent: Acetonitrile and *Solution A* (3:1)

Mobile phase: Acetonitrile and *Solution B* (9:16)

Standard stock solution: 0.25 mg/mL of USP Fexofenadine Hydrochloride RS in *Diluent*

Standard solution: 0.015 mg/mL from the *Standard stock solution* in *Mobile phase*

Sample stock solution: Transfer a sufficient number of whole Tablets (NLT 10) to a suitable volumetric flask, add *Solution A* (equivalent to 20% of the total flask volume), and shake by mechanical means at a high speed for 30 min or until the Tablets are fully disintegrated and finely dispersed. Add acetonitrile (sufficient to fill the flask to 80% of its volume), and shake by mechanical means for 60 min. Dilute with *Diluent* to volume. Pass a portion of this solution through a polytetrafluoroethylene filter having a 0.45-µm or finer pore size, and use the filtrate. Dilute, if necessary, with *Diluent* to obtain a solution containing an equivalent to 1.2 mg/mL of fexofenadine hydrochloride.

Sample solution: 0.018 mg/mL from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L11

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{32}H_{39}NO_4 \cdot HCl$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fexofenadine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.001 N hydrochloric acid; 900 mL, deaerated

Apparatus 2: 50 rpm

Time: 10 and 30 min

Determine the percentages of the labeled amount of $C_{32}H_{39}NO_4 \cdot HCl$ dissolved by using the following method.

Solution A: 1.0 g of monobasic sodium phosphate, 0.5 g of sodium perchlorate, and 0.3 mL of concentrated phosphoric acid in 300 mL of water

Mobile phase: Acetonitrile and *Solution A* (7:3)

Standard solution: USP Fexofenadine Hydrochloride RS in *Medium* to obtain a solution having a known concentration similar to that expected for the solution under test. [NOTE—A small amount of methanol, not exceeding 0.5% of the total volume, can be used to dissolve fexofenadine hydrochloride.]

System suitability solution: 0.44 mg/mL of USP Fexofenadine Related Compound A RS in water. Transfer 1.0 mL of this solution into a vial, and add 40 mL of the *Standard solution*. [NOTE—A small amount of glacial acetic acid, not exceeding 5% of the total volume, can be used to dissolve fexofenadine related compound A.]

Sample solution: Pass portions of the solution under test through a glass fiber filter having a 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection size: 2–3 µg column load of fexofenadine hydrochloride

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between fexofenadine and fexofenadine related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{32}H_{39}NO_4 \cdot HCl$ dissolved in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

L = Tablet label claim (mg)

D = dilution factor of the *Sample solution*

V = volume of *Medium*, 900 mL

Tolerances: NLT 60% (*Q*) of the labeled amount of $C_{32}H_{39}NO_4 \cdot HCl$ is dissolved in 10 min; NLT 80% (*Q*) of the labeled amount of $C_{32}H_{39}NO_4 \cdot HCl$ is dissolved in 30 min.

www.webofpharma.com

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: 0.001 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm. Use paddles and shafts coated with Teflon.
Time: 30 min
Determine the percentages of the labeled amount of $C_{32}H_{39}NO_4 \cdot HCl$ dissolved by using the following method.
Solution A: 7 mg/mL of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 4.0 ± 0.05.
Mobile phase: Acetonitrile and *Solution A* (2:3)
Standard solution 1: Transfer 20 mg of USP Fexofenadine Hydrochloride RS to a 100-mL volumetric flask. Add 3.0 mL of methanol, and mix. Dilute with *Medium* to volume.
Standard solution 2: Transfer 15.0 mL of *Standard solution 1* to a 50-mL volumetric flask. Dilute with *Medium* to volume.
Standard solution 3: Transfer 7.5 mL of *Standard solution 1* to a 50-mL volumetric flask. Dilute with *Medium* to volume.
Sample solution: Pass portions of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 259 nm
Column: 4.6-mm × 15-cm; packing L11
Flow rate: 1.5 mL/min
Injection size: 10 µL for *Standard solution 1* and 30 µL for *Standard solutions 2* and *3*
System suitability
Sample: Any of the *Standard solutions*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solutions 1*, *2*, and *3* and the *Sample solution*
Calculate the percentage of $C_{32}H_{39}NO_4 \cdot HCl$ dissolved in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of the appropriate *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = Tablet label claim (mg)

Tolerances: NLT 75% (Q) of the labeled amount of $C_{32}H_{39}NO_4 \cdot HCl$ is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
Medium: 0.001 N hydrochloric acid; 900 mL for Tablets labeled to contain 30 mg or 60 mg, and 1800 mL for Tablets labeled to contain 180 mg
Apparatus 2: 50 rpm
Time: 45 min
Buffer solution: 6.64 g/L of monobasic sodium phosphate monohydrate and 0.84 g/L of sodium perchlorate monohydrate in water. Add 4 mL/L of triethylamine. Adjust with phosphoric acid to a pH of 2.3 ± 0.1.
Mobile phase: *Buffer solution* and acetonitrile (65:35)
Standard stock solution: 0.5 mg/mL of USP Fexofenadine Hydrochloride RS in *Mobile phase*. This solution is stable for 3.5 months under refrigeration or for 18 days at room temperature.
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of 0.07 mg/mL of USP Fexofenadine Hydrochloride RS. This solution is stable for 8 days under refrigeration or for 24 h at room temperature.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 2.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Column efficiency: NLT 1000 theoretical plates
Relative standard deviation: NMT 2.0%
Calculate the percentage of fexofenadine hydrochloride dissolved in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = Tablet label claim (mg)
V = volume of *Medium*, 900 or 1800 mL

Tolerances: NLT 75% (Q) of the labeled amount of fexofenadine hydrochloride is dissolved.

▲**Test 4:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.
Medium: 0.001 N hydrochloric acid; 900 mL, degassed
Apparatus 2: 75 rpm
Time: 15 min
Buffer solution: 6.64 g/L of monobasic sodium phosphate monohydrate and 0.84 g/L of sodium perchlorate in water. Adjust with phosphoric acid to a pH of 2.0.
Mobile phase: Acetonitrile, *Buffer solution*, and triethylamine (50: 50: 0.3)
Standard stock solution: 0.55 mg/mL of USP Fexofenadine Hydrochloride RS in 0.01 N hydrochloric acid
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of 0.22 mg/mL of USP Fexofenadine Hydrochloride RS. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Column temperature: 25°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: NLT 2.7 times the retention time of fexofenadine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) dissolved in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) is dissolved. ▲ (RB 1-Nov-2018)

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

ORGANIC IMPURITIES

- **Procedure**

Solution A, Solution B, Diluent, Mobile phase, Standard stock solution, Sample stock solution, and **Sample solution:** Prepare as directed in the *Assay*.
Standard solution: 0.015 mg/mL of fexofenadine hydrochloride and 0.0045 mg/mL of fexofenadine related compound A from *Quantitative limit solution* and the *Standard stock solution* in *Mobile phase*
System suitability stock solution: Dilute 4.0 mL of the *Standard stock solution* with *Mobile phase* to 100 mL.
System suitability solution: Dilute 6.0 mL of the *System suitability stock solution* with *Mobile phase* to 100 mL.
Quantitative limit solution: 0.05 mg/mL of USP Fexofenadine Related Compound A RS in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—For the relative retention times, see *Impurity Table 1*.]
Suitability requirements
Resolution: NLT 7 between fexofenadine and fexofenadine related compound A, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 6%, *System suitability solution*; NMT 2.0% and NMT 3.0% for fexofenadine and fexofenadine related compound A, *Standard solution*
Analysis
Samples: *Standard solution*, *Sample stock solution*, and *Sample solution*
Calculate the percentage of fexofenadine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of fexofenadine related compound A in the *Sample stock solution*
r_S = peak area of fexofenadine related compound A in the *Standard solution*
C_S = concentration of fexofenadine related compound A in the *Standard solution* (mg/mL)
C_U = concentration of fexofenadine hydrochloride in the *Sample stock solution*

Calculate the percentage of the decarboxylated degradant [(±)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene] in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of the decarboxylated degradant in the *Sample stock solution*
r_S = peak area of fexofenadine in the *Standard solution*
C_S = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of fexofenadine hydrochloride in the *Sample stock solution*
F = relative response factor (see *Impurity Table 1*)

Calculate the percentage of any other impurities in the portion of Tablets taken:

$$\text{Result} = r_U/(F \times r_S + r_T) \times 100$$

r_U = peak area for each individual unknown impurity in the *Sample stock solution*
F = difference in concentration between the *Sample stock solution* and the *Sample solution*, 66.7
r_S = peak area response for fexofenadine in the *Sample solution*
r_T = sum of the peak areas of all unknown impurities in the *Sample stock solution*

[NOTE—Disregard any peak below 0.05%.]
Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fexofenadine related compound A	1.6	—	0.4
Decarboxylated degradant	6.7	1.1	0.15
Fexofenadine	1.0	—	—
Any individual other impurity	—	1.0	0.2

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Fexofenadine Hydrochloride RS
USP Fexofenadine Related Compound A RS
Benzeneacetic acid, 4-[1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethyl.
$C_{32}H_{37}NO_4$ 499.65

www.webofpharma.com

Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets

DEFINITION

Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets contain NLT 93.0% and NMT 107.0% of the labeled amounts of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution A: 6 mg/mL of USP Fexofenadine Hydrochloride RS in methanol

Standard solution B: 12 mg/mL of USP Pseudoephedrine Hydrochloride RS in methanol

Sample solution: Transfer the equivalent of 30 mg of fexofenadine hydrochloride and 60 mg of pseudoephedrine hydrochloride from finely powdered Tablets (NLT 4) into a suitable vessel, and add 5 mL of methanol. Cap the vessel, and shake vigorously for 2 min. Pass the resulting suspension through a suitable filter of 0.45-µm pore size. Use the filtrate.

Adsorbent: 0.2-mm layer of HPTLC silica gel mixture. Dry the plate at 105° for 1 h before use.

Application volume: 10 µL

Developing solvent system: Toluene, dehydrated alcohol, and ammonium hydroxide (50:45:5)

Analysis: Proceed as directed, using the *Developing solvent system*. After removal of the plate, mark the solvent front, and allow the plate to air-dry. Heat the plate at 105° until the odor of ammonia disappears (about 5 min). Allow the plate to cool, and examine under UV light at 254 nm. [NOTE—The R_F values for fexofenadine and pseudoephedrine are 0.17 and 0.39, respectively.]

Acceptance criteria: The R_F value of fexofenadine hydrochloride in the *Sample solution* is comparable to that of fexofenadine hydrochloride in *Standard solution A*. The R_F value of pseudoephedrine hydrochloride in the *Sample solution* is comparable to that of pseudoephedrine hydrochloride in *Standard solution B*.

ASSAY

Change to read:

- **PROCEDURE 1**

Buffer: Dissolve 6.8 g of sodium acetate and 16.22 g of sodium 1-octanesulfonate in water, and dilute with water to 1 L. Adjust with glacial acetic acid to a pH of 4.6.

Mobile phase: Methanol and *Buffer* (13:7)

Diluent: Methanol and *Buffer* (3:2)

System suitability solution: Transfer 40 mg of USP Pseudoephedrine Hydrochloride RS to a 50-mL volumetric flask. Add 5 mL of *tert*-butylhydroperoxide solution, and sonicate. Cover the flask opening with aluminum foil, and place the flask in an oven at 90° for 60 min. Remove from the oven, and allow to cool. Add 35 mL of *Mobile phase*, and cool to room temperature. Dilute with *Mobile phase* to volume. The degradation of pseudoephedrine hydrochloride by this process produces the related compound ephedrone.

Related compounds stock solution: Dissolve quantities of USP Fexofenadine Related Compound A RS and decarboxylated degradant[1] in a volume of methanol, and dilute with *Buffer* to obtain a ratio of methanol to *Buffer* of 3:2. Dilute with *Diluent* to obtain a solution having concentrations of 0.2 mg/mL for each component.

Related compounds solution: 0.02 mg/mL each of USP Fexofenadine Related Compound A RS and decarboxylated degradant from *Related compounds stock solution* diluted with *Mobile phase*

Standard stock solution: 0.4 mg/mL of fexofenadine hydrochloride and 0.8 mg/mL of pseudoephedrine hydrochloride from USP Fexofenadine Hydrochloride RS and USP Pseudoephedrine Hydrochloride RS, respectively, in *Mobile phase*

Standard solution: Dilute 6.0 mL of the *Standard stock solution* and 15.0 mL of the *Related compounds solution* with *Mobile phase* to 50 mL to obtain a solution having known concentrations of 0.096 mg/mL of pseudoephedrine hydrochloride, 0.048 mg/mL of fexofenadine hydrochloride, 0.006 mg/mL of fexofenadine related compound A, and 0.006 mg/mL of decarboxylated degradant.

Sample stock solution: Nominally equivalent to 1.2 mg/mL of fexofenadine hydrochloride and 2.4 mg/mL of pseudoephedrine hydrochloride. To prepare, transfer NLT 10 whole Tablets to a 500-mL volumetric flask. Add 300 mL of methanol, and shake by mechanical means at high speed for 60 min. Sonicate the flask for 60 min at 40°. Add 150 mL of *Buffer*, and sonicate for 60 min at 40°. Vent the flask, and vigorously shake the flask by hand at 15-min intervals during the mechanical shaking and sonication steps. Cool to room temperature, and dilute with *Buffer* to volume to obtain a final concentration. Pass a portion of this solution through a filter of 0.45-µm or finer pore size, and use the filtrate.

Sample solution: 0.048 mg/mL and 0.096 mg/mL of fexofenadine hydrochloride and pseudoephedrine hydrochloride, respectively, from the *Sample stock solution* diluted with *Mobile phase*. [NOTE—Alternatively, centrifuge the *Sample stock solution*, and use the supernatant to prepare the *Sample solution*. Filter the *Sample solution* before analysis.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 5-cm; 5-µm packing L6 connected in series to a 4.6-mm × 25-cm; 5-µm packing L11

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution* [NOTE—The relative retention times for pseudoephedrine and ephedrone are 1.0 and 1.2, respectively (*System suitability solution*); and for fexofenadine, fexofenadine related compound A, and decarboxylated degradant 1.0, 1.2, and 3.1, respectively (*Standard solution*).]

Suitability requirements

Resolution: NLT 1.5 between pseudoephedrine and ephedrone, *System suitability solution*; NLT 2.0 between fexofenadine and fexofenadine related compound A, *Standard solution*

[1] Available from USP as USP Fexofenadine Related Compound C ▲RS,▲ (RB 1-Jul-2018) Cat# 1270446.

www.webofpharma.com

Relative standard deviation: NMT 1.0% for replicate injections based on the pseudoephedrine peak, *System suitability solution*; NMT 1.0% for replicate injections based on the fexofenadine peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate separately the percentage of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of either fexofenadine or pseudoephedrine from the *Sample solution*

r_S = peak response of either fexofenadine or pseudoephedrine from the *Standard solution*

C_S = concentration of either USP Fexofenadine Hydrochloride RS or USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of either fexofenadine hydrochloride or pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

• **PROCEDURE 2:** Use this procedure for Tablets labeled to meet *Dissolution Test 5*.

Buffer: Dissolve 6.8 g of sodium acetate and 16.22 g of sodium 1-octanesulfonate in water, and dilute with water to 1 L. Adjust with glacial acetic acid to a pH of 4.0.

Mobile phase: Methanol and *Buffer* (13:7)

System suitability solution: Transfer 60 mg of USP Pseudoephedrine Hydrochloride RS to a 50-mL volumetric flask. Add 10 mL of hydrogen peroxide, and swirl the flask. Cover the flask opening with aluminum foil, and heat in an oven at 90° for 4 h. Add 35 mL of *Mobile phase*, and cool to room temperature. Dilute with *Mobile phase* to volume. The degradation of pseudoephedrine hydrochloride by this process produces the related compound ephedrone.

Related compounds stock solution: 0.225 mg/mL each of USP Fexofenadine Related Compound A RS and decarboxylated degradant, prepared as follows. Dissolve USP Fexofenadine Related Compound A RS and decarboxylated degradant in a volume of methanol, and dilute with *Buffer* to obtain a ratio of methanol to *Buffer* of 13:5. Dilute with *Buffer* to obtain the required concentrations of the components.

Related compounds solution: 0.0113 mg/mL each of USP Fexofenadine Related Compound A RS and decarboxylated degradant from *Related compounds stock solution* in *Mobile phase*

Standard stock solution: 0.36 mg/mL of USP Fexofenadine Hydrochloride RS and 0.48 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Mobile phase*

Standard solution: 0.096 mg/mL of USP Pseudoephedrine Hydrochloride RS, 0.072 mg/mL of USP Fexofenadine Hydrochloride RS, and 0.002 mg/mL each of USP Fexofenadine Related Compound A RS and decarboxylated degradant, prepared as follows. Transfer 10 mL of *Standard stock solution* and 8 mL of *Related compounds solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample stock solution: Nominally equivalent to 0.36 mg/mL of fexofenadine hydrochloride and 0.48 mg/mL of pseudoephedrine hydrochloride, prepared as follows. Crush NLT 10 Tablets into small pieces in a mortar, transfer the composite to a 500-mL volumetric flask, and add 325 mL of methanol. Shake by mechanical means for at least 30 min, and sonicate for at least an additional 35 min. Add 100 mL of *Buffer*, sonicate for 45 min, cool to room temperature, and allow to stand for 16 h without mechanical shaking. Dilute with *Buffer* to volume. Pass a portion of this solution through a suitable filter of 0.45-µm or finer pore size. Transfer 5 mL of the filtrate to a 50-mL volumetric flask, and dilute with *Buffer* to volume.

Sample solution: 0.072 mg/mL and 0.096 mg/mL of fexofenadine hydrochloride and pseudoephedrine hydrochloride, respectively, in *Mobile phase*, from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 5-cm; 5-µm packing L6 connected in series to a 4.6-mm × 25-cm; 5-µm packing L11

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between pseudoephedrine and ephedrone, *System suitability solution*; NLT 2.0 between fexofenadine and fexofenadine related compound A, *Standard solution*

Relative standard deviation: NMT 1.0% for replicate injections based on the pseudoephedrine peak, *System suitability solution*; NMT 1.0% for replicate injections based on the fexofenadine peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate separately the percentage of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of either fexofenadine or pseudoephedrine from the *Sample solution*

r_S = peak response of either fexofenadine or pseudoephedrine from the *Standard solution*

C_S = concentration of either USP Fexofenadine Hydrochloride RS or USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of either fexofenadine hydrochloride or pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: 0.001 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Times

Fexofenadine hydrochloride: 15 and 45 min

Pseudoephedrine hydrochloride: 45 min; 3, 5, and 12 h

Solution A: 7.0 mg/mL of monobasic sodium phosphate monohydrate in water. Adjust with 85% phosphoric acid to a pH of 2.00 ± 0.05.

Mobile phase: Acetonitrile and *Solution A* (9:11)

USP Monographs

www.webofpharma.com

Standard solution: Dissolve quantities of USP Fexofenadine Hydrochloride RS and USP Pseudoephedrine Hydrochloride RS in *Medium*, and dilute to obtain a solution containing known concentrations similar to those expected in the *Sample solution*. [NOTE—A small amount of methanol, NMT 0.5% of the total volume, can be used to dissolve the fexofenadine hydrochloride.]

Sample solution: Pass a portion of the solution under test through a suitable nylon filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; packing L6

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between fexofenadine and pseudoephedrine

Tailing factor: NMT 1.5 for fexofenadine and pseudoephedrine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of the labeled amounts of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved.

Tolerances

Fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$)**:** NLT 65% (*Q*) of the labeled amount is dissolved in 15 min, and NLT 80% (*Q*) of the labeled amount is dissolved in 45 min.

Pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$)**:** See *Table 1*.

Table 1

Time	Amount Dissolved (%)
45 min	NMT 36
3 h	45–69
5 h	61–80
12 h	NLT 80

The percentages of the labeled amount of pseudoephedrine hydrochloride, dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: 0.001 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Times

Fexofenadine hydrochloride: 45 min

Pseudoephedrine hydrochloride: 30 min; 2, 4, and 12 h

Solution A: 2.7 mg/mL of monobasic potassium phosphate and 2.2 mg/mL of sodium 1-octanesulfonate in water. Adjust with phosphoric acid to a pH of 2.50 ± 0.05.

Mobile phase: Methanol, acetonitrile, and *Solution A* (3:3:4)

Fexofenadine standard stock solution: Transfer 66 mg of USP Fexofenadine Hydrochloride RS to a 100-mL volumetric flask. Add 10 mL of methanol, and swirl until dissolved. Add 50 mL of *Medium*, and mix. Allow the solution to equilibrate to room temperature, and dilute with *Medium* to volume.

Pseudoephedrine standard stock solution: Transfer 66 mg of USP Pseudoephedrine Hydrochloride RS to a 100-mL volumetric flask. Add 10 mL of methanol, and swirl until dissolved. Add 50 mL of *Medium*, and mix. Allow the solution to equilibrate to room temperature, and dilute with *Medium* to volume.

Standard solution: 66 µg/mL of USP Fexofenadine Hydrochloride RS and 132 µg/mL of USP Pseudoephedrine Hydrochloride RS from a mixture of *Fexofenadine standard stock solution* and *Pseudoephedrine standard stock solution* diluted with *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between fexofenadine and pseudoephedrine

Tailing factor: NMT 2.0 for fexofenadine and NMT 2.5 for pseudoephedrine

Relative standard deviation: NMT 2.0% for both peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of the labeled amounts of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved.

Tolerances

Fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$)**:** NLT 80% (*Q*) of the labeled amount is dissolved in 45 min.

Pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$)**:** See *Table 2*.

Table 2

Time	Amount Dissolved (%)
30 min	NMT 35
2 h	38–58
4 h	56–76
12 h	NLT 80

The percentages of the labeled amount of pseudoephedrine hydrochloride, dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

Medium: 0.001 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Times

Fexofenadine hydrochloride: 30 min

Pseudoephedrine hydrochloride: 0.5, 2, 4, and 12 h

www.webofpharma.com

Buffer solution: 6.64 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 2.50 ± 0.05.
Mobile phase: *Buffer solution* and acetonitrile (3:2)
Standard solution: [NOTE—A small amount of methanol, not exceeding 0.5% of the final total volume, can be used to dissolve fexofenadine hydrochloride.] Prepare a solution in *Medium* containing known concentrations of USP Fexofenadine Hydrochloride RS and USP Pseudoephedrine Hydrochloride RS similar to those expected in the solution under test.
Sample solution: Pass a portion of the solution under test through a suitable PVDF or nylon filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 2.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for fexofenadine and pseudoephedrine
Relative standard deviation: NMT 2.0% for both peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of the labeled amounts of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved.
Tolerances
Fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$): NLT 80% (*Q*) of the labeled amount is dissolved in 30 min.
Pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$): See *Table 3*.

Table 3

Time (h)	Amount Dissolved (%)
0.5	13–33
2	35–55
4	50–70
12	NLT 80

The percentages of the labeled amount of pseudoephedrine hydrochloride, dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: For products labeled with a dosing interval of 24 h. If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.
Medium: 0.001 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Times
Fexofenadine hydrochloride: 30 min
Pseudoephedrine hydrochloride: 3, 7, and 23 h
Determine the percentages of the labeled amounts of fexofenadine hydrochloride and pseudoephedrine hydrochloride dissolved by using the chromatographic procedure described in *Test 1*.
Tolerances
Fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$): NLT 80% (*Q*) of the labeled amount is dissolved in 30 min.
Pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$): See *Table 4*.

Table 4

Time (h)	Amount Dissolved (%)
3	10–30
7	35–65
23	NLT 80

The percentages of the labeled amount of pseudoephedrine hydrochloride, dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: For products labeled with a dosing interval of 24 h. If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 5*.
Medium: 0.001 N hydrochloric acid; 900 mL deaerated
Apparatus 2: 50 rpm, with sinkers. [NOTE—A suitable sinker is available as catalog number CAPWST-31 from www.qla-llc.com.]
Times
Fexofenadine hydrochloride: 15 and 45 min
Pseudoephedrine hydrochloride: 3, 7, and 23 h
Buffer: 4.1 g/L of anhydrous sodium acetate in water. Adjust with glacial acetic acid to a pH of 3.6 ± 0.1.
Mobile phase: Methanol and *Buffer* (60:40)
Standard solution: Prepare a solution in *Medium* containing 0.20 mg/mL of USP Fexofenadine Hydrochloride RS and 0.27 mg/mL of USP Pseudoephedrine Hydrochloride RS. Sonicate to dissolve.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 10-cm; 5-µm packing L9
Column temperature: 40°
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for fexofenadine and pseudoephedrine are 0.45 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between fexofenadine and pseudoephedrine
Tailing factor: NMT 2.0 for fexofenadine and pseudoephedrine
Relative standard deviation: NMT 1.5% for fexofenadine and pseudoephedrine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) in the sample withdrawn from the vessel at each time point (*i*) shown in *Table 5*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

www.webofpharma.com

r_U = peak response of fexofenadine from the *Sample solution*
r_S = peak response of fexofenadine from the *Standard solution*
C_S = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount (Q_i) of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) dissolved at each time point (i) shown in *Table 5*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

C_i = concentration of fexofenadine hydrochloride in the portion of sample withdrawn at time point (i) (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim for fexofenadine hydrochloride (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Calculate the concentration (C_i) of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the sample withdrawn from the vessel at each time point (i) shown in *Table 6*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of pseudoephedrine from the *Sample solution*
r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount (Q_i) of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at each time point (i) shown in *Table 6*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of pseudoephedrine hydrochloride in the portion of sample withdrawn at time point (i) (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim for pseudoephedrine hydrochloride (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances

Fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$): See *Table 5*.

Table 5

Time Point (i)	Time (min)	Amount Dissolved (%)
1	15	NLT 60 (Q)
2	45	NLT 75 (Q)

Pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$): See *Table 6*.

Table 6

Time Point (i)	Time (h)	Amount Dissolved (%)
1	3	10–34
2	7	35–68
3	23	NLT 80

The percentages of the labeled amount of pseudoephedrine hydrochloride, dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 6*.
Medium: 0.001 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Times
Fexofenadine hydrochloride: 45 min
Pseudoephedrine hydrochloride: 30 min; 2, 4, and 12 h
Solution A: 7 g/L of monobasic sodium phosphate in water. Adjust with 85% phosphoric acid to a pH of 2.00.
Mobile phase: Acetonitrile and *Solution A* (45:55)
Standard solution: 0.07 mg/mL of USP Fexofenadine Hydrochloride RS and 0.13 mg/mL of USP Pseudoephedrine Hydrochloride RS, prepared as follows. Dissolve appropriate quantities of USP Fexofenadine Hydrochloride RS and USP Pseudoephedrine Hydrochloride RS in a small amount of methanol, NMT 0.8% of the final volume, and add 40% of the final volume of *Medium*. Sonicate to dissolve and dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 10-µm packing L6
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between fexofenadine and pseudoephedrine peaks
Tailing factor: NMT 2.0 for both fexofenadine and pseudoephedrine peaks
Relative standard deviation: NMT 2.0% for both peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of fexofenadine from the *Sample solution*
r_S = peak response of fexofenadine from the *Standard solution*
C_S = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL

USP Monographs

www.webofpharma.com

L = label claim for fexofenadine hydrochloride (mg/Tablet)

Calculate the concentration (C_i) of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the sample withdrawn from the vessel at each time point (i) shown in *Table 7*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of pseudoephedrine from the *Sample solution*
r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at each time point (i) shown in *Table 7*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of pseudoephedrine hydrochloride in the portion of sample withdrawn at time point (i) (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim for pseudoephedrine hydrochloride (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances

Fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$): NLT 80% ($Q$) of the labeled amount is dissolved.

Pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$): See *Table 7*.

Table 7

Time Point (i)	Time (h)	Amount Dissolved (%)
1	0.5	NMT 35
2	2	45–65
3	4	60–80
4	12	NLT 80

The percentages of the labeled amount of pseudoephedrine hydrochloride, dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 7:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 7*.

Medium: 0.001 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Times

Fexofenadine hydrochloride: 20 min

Pseudoephedrine hydrochloride: 45 min; ▲3, 5,▲ (RB 1-Aug-2018) and 12 h

Solution A: 7.0 g/L of monobasic sodium phosphate monohydrate in water. Adjust with phosphoric acid to a pH of 2.0.

Mobile phase: Acetonitrile and *Solution A* (45:55)

Standard stock solution A: 0.7 mg/mL of USP Fexofenadine Hydrochloride RS, prepared as follows. Transfer a quantity of USP Fexofenadine Hydrochloride RS to a suitable volumetric flask. Add methanol, NMT ▲5%▲ (RB 1-Aug-2018) of the total volume, and sonicate to dissolve. Dilute with *Medium* to volume.

Standard stock solution B: 1.3 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Medium*. Sonicate to dissolve if necessary.

Standard solution: 0.07 mg/mL of USP Fexofenadine Hydrochloride RS and 0.13 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Medium*, from *Standard stock solution A* and *Standard stock solution B*

Sample solution: Withdraw and pass a portion of the solution under test through a suitable nylon filter of 0.45-µm pore size. Replace the portion removed with the same volume of *Medium*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 10-µm packing L6

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 1.5 times the retention time of the pseudoephedrine peak

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between fexofenadine and pseudoephedrine

Tailing factor: NMT 1.5 for fexofenadine and pseudoephedrine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of fexofenadine from the *Sample solution*
r_S = peak response of fexofenadine from the *Standard solution*
C_S = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim for fexofenadine hydrochloride (mg/Tablet)

Calculate the concentration (C_i) of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in the sample withdrawn from the vessel at each time point (i) shown in *Table 8*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of pseudoephedrine from the *Sample solution*

USP Monographs

www.webofpharma.com

r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

[NOTE—Result$_1$ is used as calculation correction (C_1) for subsequent withdrawal time points.]
Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved at each time point (i) shown in *Table 8*:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_5 = \{(C_5 \times V) + [(C_4 + C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of pseudoephedrine hydrochloride in the portion of sample withdrawn at time point (i) (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim for pseudoephedrine hydrochloride (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances
Fexofenadine hydrochloride ($C_{32}H_{39}NO_4 \cdot HCl$): NLT 80% ($Q$) of the labeled amount is dissolved in 20 min.
Pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$): See *Table 8*.

Table 8

Time Point (i)	Time	Amount Dissolved (%)
1[a]	20 min	—
2	45 min	NMT 34
3	3 h	41–61
4	5 h	57–77
5	12 h	NLT 80

[a] The first time point is used as calculation correction (C_1) for subsequent withdrawal time points.

The percentages of the labeled amount of pseudoephedrine hydrochloride, dissolved at the times specified, conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 1-Jul-2018)

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

[NOTE—On the basis of knowledge of the product, perform either: (a) *Organic Impurities, Procedure 1* or (b) *Organic Impurities, Procedure 2*; *Organic Impurities, Procedure 3*; and *Organic Impurities, Procedure 4*.]

Change to read:

• **ORGANIC IMPURITIES, PROCEDURE 1**
Buffer, Mobile phase, Diluent, System suitability solution, Related compounds stock solution, Related compounds solution, Standard stock solution, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay, Procedure 1*.
Sample solution: Use the *Sample stock solution*, prepared as directed in the *Assay, Procedure 1*.
Reference solution: Use the *Sample solution*, prepared as directed in the *Assay, Procedure 1*.
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for pseudoephedrine and ephedrone are 1.0 and 1.2, respectively (*System suitability solution*); and for fexofenadine, fexofenadine related compound A, and decarboxylated degradant are 1.0, 1.2, and 3.1, respectively (*Standard solution*).]
Suitability requirements
Resolution: NLT 1.7 between pseudoephedrine and ephedrone, *System suitability solution*; NLT 2.0 between fexofenadine and fexofenadine related compound A, *Standard solution*
Relative standard deviation: NMT 1.0% for replicate injections based on the pseudoephedrine peak, *System suitability solution*; NMT 1.0% for replicate injections based on the fexofenadine peak and NMT 3.0% based on the individual peaks for fexofenadine related compound A and decarboxylated degradant, *Standard solution*
Analysis
Samples: *Sample solution* and *Reference solution*
Calculate the percentage of fexofenadine related compound A and decarboxylated degradant in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = individual peak area response of either fexofenadine related compound A or decarboxylated degradant from the *Sample solution*
r_S = peak area response of fexofenadine related compound A or decarboxylated degradant from the *Standard solution*
C_S = concentration of either USP Fexofenadine Related Compound A RS or decarboxylated degradant in the *Standard solution* (mg/mL)
C_U = nominal concentration of fexofenadine hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of ephedrone in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak height response of ephedrone from the *Sample solution*
r_S = peak height response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for ephedrone, 0.394

Calculate the percentage of any other impurities in the portion of Tablets taken:

$$\text{Result} = r_U/[(F \times r_S + r_T)] \times 100$$

r_U = individual peak area response of an individual unknown impurity from the *Sample solution*

USP Monographs

www.webofpharma.com

F = difference in concentration between the *Sample solution* and the *Reference solution*, 25
r_S = peak area response of fexofenadine hydrochloride from the *Reference solution*
r_T = sum of the peak area responses of all unknown impurities from the *Sample solution*

[NOTE—Disregard any peak below 0.05%.]
Acceptance criteria: See ▲*Table 9*.

Table 9▲ (RB 1-Jul-2018)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pseudoephedrine	1.0	—
Fexofenadine	1.0	—
Ephedrone	1.2[a]	0.2
Fexofenadine related compound A	1.2[b]	0.4
Tertiary dehydrated impurity[c]	1.8	0.2
Decarboxylated degradant[d]	3.1[b]	0.2
Any other individual impurity	—	0.2
Total impurities	—	0.8

[a] Relative to pseudoephedrine.
[b] Relative to fexofenadine.
[c] 4-[4{4-(Diphenylmethylene)-1-piperidinyl}-1-hydroxybutyl]-2,2-dimethyl phenyl acetic acid.
[d] (±)-4-(1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene.

Change to read:

• **ORGANIC IMPURITIES, PROCEDURE 2**
Solution A: Dissolve 2.7 g of monobasic potassium phosphate and 2.2 g of sodium 1-octanesulfonate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.50 ± 0.05.
Mobile phase: Methanol and *Solution A* (3:2)
Standard stock solution: 0.18 mg/mL of USP Fexofenadine Hydrochloride RS in *Mobile phase*
Standard solution: 0.0108 mg/mL of USP Fexofenadine Hydrochloride RS in *Mobile phase*, prepared from the *Standard stock solution*
Sensitivity solution: 0.54 µg/mL of USP Fexofenadine Hydrochloride RS in *Mobile phase*, prepared from the *Standard solution*
Sample solution: Weigh and finely powder 9 Tablets, and quantitatively transfer the ground powder to a 500-mL volumetric flask, with the aid of 200 mL of *Mobile phase*. Sonicate for 10 min, and add an additional 100 mL of *Mobile phase*. Shake by mechanical means for 30 min, and dilute with *Mobile phase* to volume. Pass a portion of the solution through a polypropylene or polysulfone membrane filter of 0.45-µm pore size, and discard at least the first 10 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
[NOTE—The run time is 6 times the retention time of fexofenadine.]
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of each impurity as a percentage of the label claim of fexofenadine hydrochloride in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of individual impurities from the *Sample solution*
r_S = peak response of fexofenadine from the *Standard solution*
C_S = concentration of USP Fexofenadine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fexofenadine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see ▲*Table 10*)▲ (RB 1-Jul-2018)

Acceptance criteria: See ▲*Table 10*.

Table 10▲ (RB 1-Jul-2018)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fexofenadine	1.0	1.0	—
Meta fexofenadine	1.14	1.0	0.2
Fexofenadine related compound A	1.38	0.83	0.4
Tertiary dehydrated impurity[a]	2.25	1.3	0.2
Individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	0.5

[a] 4-[4{4-(Diphenylmethylene)-1-piperidinyl}-1-hydroxybutyl]-2,2-dimethyl phenyl acetic acid.

• **ORGANIC IMPURITIES, PROCEDURE 3**
Solution A: 4 mg/mL of ammonium acetate
Mobile phase: Methanol and *Solution A* (19:1)
Diluent: Methanol and water (1:1)
Standard stock solution: 0.18 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*
Standard solution: 0.0216 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*, prepared from the *Standard stock solution*
Sensitivity solution: 1.08 µg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*, prepared from the *Standard solution*
Sample solution: Weigh and finely powder 9 Tablets, and quantitatively transfer the ground powder to a 500-mL volumetric flask, with the aid of 200 mL of *Diluent*. Sonicate for 10 min, and add an additional 100 mL of *Diluent*. Shake by mechanical means for 30 min, dilute with *Diluent* to volume, and mix. Pass a portion of the solution through a polypropylene or polysulfone membrane filter of 0.45-µm pore size, and discard at least the first 10 mL of the filtrate.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L3
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of each impurity as a percentage of the label claim of pseudoephedrine hydrochloride in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of individual impurities from the *Sample solution*
r_S = peak response of pseudoephedrine from the *Standard solution*
C_S = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor, equal to 0.52 for ephedrone (RRT, 0.85 relative to the pseudoephedrine peak) and 1 for all other impurities

Acceptance criteria
Individual impurities: NMT 0.2% for ephedrone; NMT 0.1% for any individual unspecified impurity

Change to read:

- **ORGANIC IMPURITIES, PROCEDURE 4**

Solution A: Dissolve 2.7 g of monobasic potassium phosphate and 2.2 g of sodium 1-octanesulfonate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.50 ± 0.05.
Solution B: Methanol and *Solution A* (2:3)
Solution C: Methanol and *Solution A* (7:3)
Mobile phase: See ▲*Table 11*.

Table 11▲ (RB 1-Jul-2018)

Time (min)	Solution B (%)	Solution C (%)
0	100	0
40	100	0
41	0	100
65	0	100
66	100	0
90	100	0

Diluent: Methanol and water (1:1)
Standard stock solution: 0.18 mg/mL of USP Benzoic Acid RS in *Diluent*
Standard solution: 0.0216 mg/mL of USP Benzoic Acid RS in *Diluent*, prepared from the *Standard stock solution*
Sensitivity solution: 1.08 µg/mL of USP Benzoic Acid RS in *Diluent*, prepared from the *Standard solution*
Sample solution: Weigh and finely powder 9 Tablets, and quantitatively transfer the ground powder to a 500-mL volumetric flask, with the aid of 200 mL of *Diluent*. Sonicate for 10 min, and add an additional 100 mL of *Diluent*. Shake by mechanical means for 30 min, dilute with *Diluent* to volume, and mix. Pass a portion of the solution through a polypropylene or polysulfone membrane filter of 0.45-µm pore size, and discard at least the first 10 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of each impurity as a percentage of the label claim of pseudoephedrine hydrochloride in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of individual impurities from the *Sample solution*
r_S = peak response of benzoic acid from the *Standard solution*
C_S = concentration of USP Benzoic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see ▲*Table 12*)▲ (RB 1-Jul-2018)

Acceptance criteria
Individual impurities: See ▲*Table 12*.▲ (RB 1-Jul-2018)
Total impurities: The combined total impurities from *Procedure 3* and *Procedure 4* is NMT 0.3%.

▲Table 12▲ (RB 1-Jul-2018)

Name	Relative Retention Time	Relative Response Factor[a]	Acceptance Criteria, NMT(%)
Benzaldehyde	0.43	0.40	0.1
Benzoic acid	0.55	1.0	0.1
Ephedrone[b]	0.97	—	—
Pseudoephedrine	1.0	0.52	—
Individual unspecified impurity	—	0.52[c]	0.1

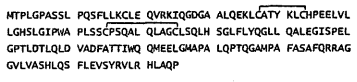
[a] Response factors relative to benzoic acid.
[b] Ephedrone is not quantitated in this method. A separate method is used for the quantitation of this impurity.
[c] The response factor of pseudoephedrine relative to that of benzoic acid is used in the calculation of individual unspecified impurities.

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used. If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states with which *Procedures* the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Benzoic Acid RS
  USP Fexofenadine Hydrochloride RS
  USP Fexofenadine Related Compound A RS
  Benzeneacetic acid, 4-[1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethyl.
  $C_{32}H_{37}NO_4$ 499.65
  USP Pseudoephedrine Hydrochloride RS

Filgrastim



$C_{845}H_{1339}N_{223}O_{243}S_9$ 18,799 daltons
[121181-53-1].

DEFINITION

Filgrastim is a recombinant form of human granulocyte colony-stimulating factor (r-metHuG-CSF). It is a single chain, 175 amino acid nonglycosylated polypeptide produced by *Escherichia coli* bacteria transfected with a gene encoding a methionyl human granulocyte colony-stimulating factor. When prepared as a drug substance, it contains NLT 0.9 mg/mL of Filgrastim. Formulation contains one or more suitable buffering and/or stabilizing agents. The presence of host cell DNA and protein in Filgrastim is process-specific. The capability of the process to clear host-derived DNA and protein requires validation and is determined by validated methods. It has a biological potency of NLT 80% and NMT 125% relative to standard on a mass-to-mass basis.

IDENTIFICATION

- **A.** It meets the requirements in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained as directed in the test for *Organic Impurities, Related Compounds*.
- **C. PEPTIDE MAPPING**
  (See *Biotechnology-Derived Articles—Peptide Mapping* ⟨1055⟩.)
  Solution A: Water and trifluoroacetic acid (1000:1)
  Solution B: Transfer 100 mL of water to a 1000-mL volumetric flask, add 1 mL of trifluoroacetic acid, and dilute with acetonitrile to volume.
  Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	98	2
2	98	2
30	70	30
75	40	60
90	2	98
100	2	98
101	98	2
120	98	2

Tris buffer: Prepare a 0.5 M solution of tris(hydroxymethyl) aminomethane solution, and adjust with hydrochloric acid to a pH of 8.1.
Methylamine solution: Dissolve 0.27 g of methylamine hydrochloride in 10 mL of water.
DTT solution: 30.9 mg/mL of dithiothreitol in water, freshly prepared
Digestion solution: Dissolve 0.30 g of urea in 200 µL of *Tris buffer*, 100 µL of *Methylamine solution*, 50 µL of *DTT solution*, and 420 µL of water. Overlay with nitrogen, and use immediately.
Endoproteinase Glu-C solution: 0.2 µg/µL of Endoproteinase Glu-C in water. Use immediately.
TFA solution: Water and trifluoroacetic acid (100:5)
Standard solution: Prepare a solution containing 80 µg of USP Filgrastim RS and 200 µL of *Digestion solution* in a suitable tube. Add water to a final volume of 390 µL. Add 10 µL of *Endoproteinase Glu-C solution*. Cap the tube, mix well, and incubate at approximately 25° for 18 h. Add 18 µL of *TFA solution*.
Sample solution: Proceed as directed for the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 2.1-mm × 25-cm; packing L26
Column temperature: 40°
Flow rate: 0.2 mL/min
Injection size: 70 µL
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Condition the *Chromatographic system* by running at least two blank gradient programs before injecting the digests. Separately inject the *Standard solution*, *Sample solution*, and *Standard solution* again, and record the responses of each peak.]
System suitability requirements: Eight major peaks should be present in each chromatogram as illustrated in the reference chromatogram provided with USP Filgrastim RS. The absolute difference in retention time of each of the eight major peaks between the two *Standard solution* chromatograms must be ≤0.5 min. The difference in retention time of each of the eight major peaks between the *Sample solution* chromatogram and the average of the *Standard solution* chromatograms must be ≤0.5 min. The relative difference in peak height of each of the eight major peaks between the two *Standard solution* chromatograms must be ≤15%.
Acceptance criteria: The chromatographic profile of the *Sample solution* is similar to that of the *Standard solution*. The relative difference in peak height between the normalized sample peak height (normalized by total peak height versus the average total peak height of the *Standard solution* chromatograms) and the average standard peak height of each of the eight major peaks must be ≤15%.

www.webofpharma.com

ASSAY

• **POTENCY**

Medium A:[1] RPMI 1640 modified to contain 2 mM L-glutamine, 2 g/L of sodium bicarbonate, 4.5 g/L of glucose, 10 mM HEPES, 1 mM sodium pyruvate, 0.05 mM 2-mercaptoethanol, and 5% heat-inactivated fetal bovine serum (FBS). The 2-mercaptoethanol is prepared fresh and added immediately before use.

Medium B: RPMI 1640 modified to contain 2 mM L-glutamine, 2 g/L of sodium bicarbonate, 4.5 g/L of glucose, 10 mM HEPES, 1 mM sodium pyruvate, 0.05 mM 2-mercaptoethanol, and 1% heat-inactivated FBS. The 2-mercaptoethanol is prepared fresh and added immediately before use.

Medium C: Prepare a mixture of *Medium A* with 20 ng/mL of interleukin 3 (IL-3).

Medium D: Prepare a mixture of *Medium A* with 1 ng/mL of granulocyte colony-stimulating factor (G-CSF).

Substrate solution: Reconstitute the luminescence substrate[2] with the supplied buffer. Aliquot the solution and store below −65° for up to 2 months.

Standard solution: 0.5 ng/mL of USP Filgrastim RS in *Medium B*. [NOTE—Do not use single step dilutions of more than 1:100 and smaller transfer volumes than 40 µL while performing serial dilutions. Mix gently and thoroughly (do not vortex).]

Positive control solution: 10 ng/mL of USP Filgrastim RS in *Medium B*

Sample solution: 0.5 ng/mL of Filgrastim in *Medium B*. [NOTE—Do not use single step dilutions of more than 1:100 and smaller transfer volumes than 40 µL while performing serial dilutions. Mix gently and thoroughly (do not vortex).]

Cell culture preparation: Adapt M-NFS-60 cells (ATCC CRL-1838) to growth in G-CSF. Culture the cells at 5% CO_2 and 37°. Cells should be passaged two times per week and reseeded at a density of 3×10^4 cells/mL for 3 days or 1×10^4 cells/mL for 4 days. Passage the cultures in *Medium C* until passage two. Transfer the cells into *Medium D*, and passage the cultures until passage 11. After passage 11, the cells are defined as G-CSF adapted and can be used in the *Assay* or can be banked. Bank the cells at a density of approximately 1.5×10^6 cells/mL in banking medium (90% FBS, 10% DMSO). Upon thawing the banked cells, culture the cells for 2–3 passages in *Medium D* before using in the *Assay*.

Cell suspension: Wash the cells twice with phosphate buffered saline (PBS), and adjust the cell concentration to 1×10^5 cells/mL in *Medium B*.

Preparation of cells for analysis: Use a 96-well flat-bottomed black microtiter plate (optically clear well bottoms) with wells arranged in eight rows (labeled A through H) with 12 wells (numbered 1 to 12) in each row. Place 50 µL of *Cell suspension* into all wells of the 96-well microtiter plate except the wells of column 1 (A1–H1, blank). Column 1 is filled with 50 µL of *Medium B*. Incubate the plate at 37° in the incubator until the *Standard solution* and the *Sample solution* are ready, for a maximum of 1 h.

Preparation of diluted Standard solution and Sample solution: Use a 96-well round-bottom microtiter plate with wells arranged in eight rows (labeled A through H) with 12 wells (numbered 1 to 12) in each row. Dispense 100 µL of *Medium B* into each well of the plate except column 12 (wells A12–H12, positive control) and A2–A10. Pipet 200 µL of *Standard solution* into wells A3, A6, and A9; and 200 µL of *Sample solution* into wells A2, A5, and A8. Two samples can be prepared on one plate. The second sample can be added to wells A4, A7, and A10. Pipet 100 µL of *Positive control solution* into all wells of the last column (A12–H12). Using a multi-channel pipet (12 channels), perform two-fold dilutions on the plate. Aspirate 100 µL from the first row (A2–A10), transfer to the second row, and mix three times. Then aspirate 100 µL from the second row, transfer to the third row, and mix three times. Repeat this procedure across the entire plate until row H. Discard the 100 µL aspirated from the last row.

Analysis: Using a multi-channel pipet, transfer 50 µL from each well of the round-bottom microtiter plate (containing the *Standard solution* and *Sample solution* serial dilutions, blanks, and positive and negative controls) into the same well of the black microtiter plate containing the cell suspension starting with the lowest concentration. Mix three times. Incubate the black microtiter plate in a humidified CO_2 incubator for 31 ± 2 h at 37° with 5% CO_2. Add 100 µL of reconstituted *Substrate solution* to all wells. Incubate the plate for 15 min at room temperature while gently agitating the plate on a plate shaker. Incubate the plate for an additional 15 min at room temperature without shaking. Read the plate in a microtiter plate luminescence reader.

System suitability criteria: The signal-to-noise ratio between the mean chemiluminescence signal of the positive control and the mean chemiluminescence signal of the negative control (A11–H11) should be ≥3. The number of technical outliers may not exceed four per curve.

Calculations: For each sample, first calculate the relative potency of Filgrastim (in percent) using statistical methods for parallel-line assays, then calculate potency in IU/mL. The statistical tests for linearity, slope, and parallelism for each sample compared to the standard have to be passed at the 95% level. The confidence limit must be within 75% and 133% of the estimated potency.

Acceptance criteria: The mean estimated potency is NLT 80% and NMT 125% of the stated potency.

IMPURITIES

ORGANIC IMPURITIES

• **RELATED COMPOUNDS**

Solution A: Water and trifluoroacetic acid (1000:1)

Solution B: Transfer 100 mL of water to a 1000-mL volumetric flask, add 1 mL of trifluoroacetic acid, and dilute with acetonitrile to volume.

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	60	40
30	20	80
35	20	80
45	60	40
55	60	40

Standard solution: 0.75 mg/mL of USP Filgrastim RS in water

Sample solution: 0.75 mg/mL of Filgrastim in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; packing L26

Column temperature: 60°

Flow rate: 0.8 mL/min

[1] A suitable RPMI 1640 medium is available from ATCC (catalog no.: 30-2001), or use an equivalent formulation.

[2] A suitable substrate is available from Promega (Cell Titer Glo-Luminescence Kit, catalog no.: G7572) or equivalent.

www.webofpharma.com

Injection size: 33 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative retention times: About 0.91 for oxidized Filgrastim 1, about 0.98 for oxidized Filgrastim 2, and about 1.04 for reduced Filgrastim relative to the major peak

Relative standard deviation: The relative standard deviation of the total area (main peak and minor product related peaks, excluding the void peak) and the relative standard deviation of the retention time of the major Filgrastim peak for replicate injections are NMT 5%.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxidized Filgrastim 1, oxidized Filgrastim 2, Filgrastim, and reduced Filgrastim in the portion of Filgrastim taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity

r_T = sum of the responses of all the peaks

Acceptance criteria

Individual impurities: NMT 1.0% of reduced Filgrastim is found, and NMT 2.0% of total impurities is found.

• **IMPURITIES WITH CHARGES DIFFERENT FROM FILGRASTIM**

(See *Biotechnology-Derived Articles—Isoelectric Focusing* ⟨1054⟩.)

1 M phosphoric acid solution: Dilute 6.8 mL of 85% phosphoric acid with water to a final volume of 100 mL.

1 M sodium hydroxide solution: Dilute 10 mL of 10 M sodium hydroxide with water to a final volume of 100 mL.

Anolyte solution: Add 10 mL of *1 M phosphoric acid solution* to 90 mL of water to obtain a 0.1 M phosphoric acid solution.

Catholyte solution: Add 10 mL of *1 M sodium hydroxide solution* to 90 mL of water to obtain a 0.1 M sodium hydroxide solution.

Initiator: Measure 0.072 g of potassium persulfate to 10 mL of water to obtain a 0.72% potassium persulfate solution.

Fixing solution: Mix 35 g of sulfosalicylic acid and 100 g of trichloroacetic acid in 1000 mL of water.

Gel wash: Prepare a solution containing, in each L, 400 mL of absolute methanol, 100 mL of glacial acetic acid, and water.

Coomassie staining solution: Measure 1.25 g of Coomassie Brilliant Blue R-250 into 1 L of *Gel wash*.

Coomassie destaining solution: Prepare a solution containing, in each L, 75 mL of absolute methanol, 100 mL of glacial acetic acid, and water.

Reference solution A: 1 mg/mL of USP Filgrastim RS in water

Reference solution B: Dilute *Reference solution A* with water to obtain a concentration of 20 µg/mL of USP Filgrastim RS.

Reference solution C: 3 mg/mL of USP Filgrastim RS in water

Reference solution D: Use an isoelectric point (pI) calibration solution, in the pI range of 2.5–6.5, prepared according to the manufacturer's instructions.

Sample solution: 1 mg/mL of Filgrastim in water

Gel: Prepare a 6% T (total acrylamide), 0.16% C (bisacrylamide) gel containing 0.3 g/mL of urea, 1.5% pH 3–10 ampholytes, 3.7% pH 5–7 ampholytes, and 0.05% *Initiator*.

Analysis: Gel electrophoresis is carried out on a horizontal gel apparatus at 10° and a constant power setting of 10 W (voltage and current are allowed to vary). Pre-focus the gel for 20–40 min. Apply 10 µL of *Sample solution* (10 µg), *Reference solution A* (10 µg), *Reference solution B* (0.2 µg), and *Reference solution C* (30 µg) to separate lanes on the gel. Apply about 10 µL of *Reference solution D* to each side of the gel. Focus the gel for about 2.5 h at 10° at a constant power setting of 10 W (voltage and current are allowed to vary). Remove the gel from the apparatus, place in a sufficient volume of *Fixing solution* to submerge the gel, and rock gently for NLT 15 min. Decant, and repeat wash in *Fixing solution* for an additional 15 min. Remove the *Fixing solution*, and submerge the gel in *Gel Wash* with gentle mixing for NLT 30 min. Decant the *Gel Wash* solution, and immerse the gel in *Coomassie staining solution* with gentle mixing for 15–60 min. Remove the *Coomassie staining solution*, rinse the gel with *Coomassie destaining solution*, and submerge the gel in fresh *Coomassie destaining solution*. Continue rocking the gel in *Coomassie destaining solution* until the background is clear and *Reference solution B* is still visible. Visually examine the gel.

System suitability requirements: The isoelectric point markers are distributed along the entire length of the gel; no artifacts obscure visualization of the bands; and *Reference solution B* must be visible.

Acceptance criteria: The major band in the *Sample solution* focuses at the same position as the major band in *Reference solution A*. Minor bands present in the *Sample solution* are also observed in *Reference solution C* and, based on visual estimates, are less intense or equal to *Reference solution B*. There are no bands observed in the *Sample solution* that are not present in *Reference solution C*.

• **IMPURITIES WITH MOLECULAR WEIGHT DIFFERENT FROM THAT OF FILGRASTIM**

(See *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* ⟨1056⟩.)

4X SDS sample buffer (nonreducing conditions): Prepare a solution containing, in each mL, 80 mg of sodium dodecyl sulfate and 30 mg of tris(hydroxymethyl)aminomethane. Adjust with hydrochloric acid to a pH of 6.8. Add 0.80 mg/mL of bromophenol blue and 0.4 mL/mL of glycerol.

4X SDS sample buffer (reducing conditions): Prepare a solution containing, in each mL, 80 mg of sodium dodecyl sulfate, 30 mg of tris(hydroxymethyl)aminomethane, and 38.5 mg of dithiothreitol. Adjust with hydrochloric acid to a pH of 6.8. Add 0.80 mg/mL of bromophenol blue and 0.4 mL/mL of glycerol.

1X SDS sample buffer (nonreducing conditions): Dilute 1 volume of the *4X SDS sample buffer (nonreducing conditions)* with 3 volumes of water.

1X SDS sample buffer (reducing conditions): Dilute 1 volume of the *4X SDS sample buffer (reducing conditions)* with 3 volumes of water.

Gel wash I: Prepare a solution containing, in each L, 400 mL of absolute methanol, 100 mL of glacial acetic acid, and water.

Gel wash II: Prepare a solution containing, in each L, 100 mL of 95% ethanol, 50 mL of glacial acetic acid, and water.

Reducer solution: Prepare a solution containing 2 mg of dithiothreitol in 400 mL of water. [NOTE—Prepare this solution immediately before use, and protect it from light. This amount of solution is sufficient for two slab gels.]

Silver nitrate solution: Measure 0.68 g of silver nitrate in 400 mL of water, and mix well. [NOTE—Prepare this solution immediately before use, and protect it from light. This amount of solution is sufficient for two slab gels.]

Developer: To 500 mL of water add 18.6 g of sodium carbonate monohydrate and 0.5 mL of formaldehyde. [NOTE—Prepare this solution fresh at the time of use. This amount of solution is sufficient for two slab gels.]

www.webofpharma.com

Acetic acid solution: Prepare a solution containing, in each L, 50 mL of glacial acetic acid and water.

Running buffer: Prepare a buffer solution containing 1 g of sodium dodecyl sulfate, 3.03 g of tris(hydroxymethyl) aminomethane, and 14.4 g of glycine per L.

Gel

Resolving gel: Use a 1.5-mm thick, 10%–20% polyacrylamide gradient gel [10%–20% T, 2.6% C (bisacrylamide)].

Stacking gel: 4% T (total acrylamide), 2.6% C (bisacrylamide)

Reference solution A: Dilute 25 µg of USP Filgrastim RS with 25 µL of *4X SDS sample buffer* and sufficient water to obtain 100 µL of a solution containing a 250-µg/mL preparation of USP Filgrastim RS in 1X SDS sample buffer. Prepare both a reduced and a nonreduced *Reference solution A* using the appropriate *4X SDS sample buffer*. Heat reduced sample at approximately 65° for 5–10 min. Do not heat the nonreduced sample.

Reference solution B: Prepare both a reduced and a nonreduced *Reference solution B* by diluting *Reference solution A* (1:100) with the appropriate 1X SDS sample buffer to obtain a 2.5-µg/mL preparation of USP Filgrastim RS. Heat reduced sample at approximately 65° for 5–10 min. Do not heat the nonreduced sample.

Reference solution C: Dilute 75 µg of USP Filgrastim RS with 25 µL of *4X SDS sample buffer* and sufficient water to obtain 100 µL of a solution containing a 750-µg/mL preparation of USP Filgrastim RS in 1X SDS sample buffer. Prepare both a reduced and a nonreduced *Reference solution C* using the appropriate *4X SDS sample buffer*. Heat reduced sample at approximately 65° for 5–10 min. Do not heat the nonreduced sample.

Reference solution D: Use a solution of molecular weight markers suitable for calibrating SDS-polyacrylamide gels in the range of 14.4–94 kDa.

Sample solution: Dilute 25 µg of the sample to be tested with 25 µL of *4X SDS sample buffer* and sufficient water to obtain 100 µL of a solution containing a 250-µg/mL preparation of the test article in 1X SDS sample buffer. Prepare both a reduced and a nonreduced *Sample solution* using the appropriate *4X SDS sample buffer*. Heat reduced sample at approximately 65° for 5–10 min. Do not heat the nonreduced sample.

Analysis: Separately apply 20 µL of the *Sample solution*, *Reference solution A*, *Reference solution B*, and *Reference solution C* into separate lanes of the gel. Apply about 20 µL of *Reference solution D* to each side of the gel. [NOTE—Reduced and nonreduced samples should either be run on separate gels or on the same gel if separated by at least three lanes containing *1X SDS sample buffer (nonreducing)*.] Perform the electrophoresis using a constant voltage of 125 V. [NOTE—Current and power are allowed to vary throughout the run.] Remove the gel from the apparatus after the tracking dye begins to approach the anode end of the gel, place the gel in a sufficient volume of *Gel Wash I* to submerge the gel, and mix gently for NLT 1 h. Decant the *Gel Wash I*, and immerse the gel in a similar volume of *Gel Wash II*. After 15 min of gentle mixing, decant, and wash the gel for an additional 15 min in *Gel Wash II*. Wash the gel twice for 15 min each with the *Reducer solution* followed by two washes of 15 min each in the *Silver nitrate solution*. Rinse twice with water, and decant. Transfer the gel to a clear container containing a sufficient volume of the *Developer* to immerse the gel, and rock the container changing the *Developer* frequently (10–15 s) until the molecular weight markers and *Reference standard B* become visible. When the gel is visibly stained, wash immediately with the *Acetic acid solution*. Rinse the gel repeatedly with *Acetic acid solution* to remove the *Developer*, then rock gently for approximately 30 min. Visually examine the gel.

System suitability requirements: No artifacts obscure visualization of the bands, and *Reference solution B* must be visible.

Acceptance criteria: The main band in the *Sample solution* migrates to the same position as the main band in *Reference solution A*. Minor bands present in the *Sample solution* are also observed in *Reference solution C* and, based on visual estimates, are less intense or equal to *Reference solution B*. There are no bands observed in the *Sample solution* that are not present in *Reference solution C*.

• **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**

Mobile phase: Add 11.5 g of concentrated phosphoric acid to 800 mL of water. Adjust with 10 N sodium hydroxide to a pH of 2.5, and dilute with water to 1000 mL. Filter, and degas.

Column conditioning solution: Dissolve 18 mg of bovine serum albumin (BSA) in 9 mL of water.

Resolution solution: Dissolve about 1 mg of USP Filgrastim RS in 0.33 mL of 0.25 M sucrose, mix gently, and transfer to a 1.5-mL polypropylene tube. Incubate at 55° for 20 min. Place the sample on ice for 5 min, then vortex at high speed for 30 s. Dilute to 0.3 mg/mL with 0.25 M sucrose, and inject within 1 h. The dimer can be regenerated from a previously heated 3-mg/mL sample. Store the previously heated sample overnight in a refrigerator, vortex at high speed for 30 s, dilute to 0.3 mg/mL with 0.25 M sucrose, and inject within 1 h.

Standard solution: 0.3 mg/mL of USP Filgrastim RS in water

Sample solution: Dilute Filgastim with water to 0.3 mg/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 7.8-mm × 30-cm; packing L59

Column temperature: Ambient

Flow rate: 1 mL/min

Injection size: 40 µL

System suitability

Samples: *Column conditioning solution*, *Resolution solution*, and *Standard solution*

Suitability requirements

Column solution peak response: Chromatograph the *Column conditioning solution* 3–10 times. The peak response of the final consecutive injections should be constant.

Relative retention times: Chromatograph the *Resolution solution* two times, and record the peak responses. The relative retention times are about 0.5 for Filgrastim aggregate, 0.9 for Filgrastim dimer, and 1.0 for Filgrastim monomer.

Relative standard deviation: The relative standard deviation of the total area (main peak and minor product related peaks, excluding the void peak) for replicate injections of the *Standard solution* is NMT 3%. The relative standard deviation of the retention time of the major Filgrastim peak for replicate injections of the *Standard solution* is NMT 3%.

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the areas of the main peak and of the peaks eluting prior to the main peak, excluding the solvent peaks. Calculate the percentage of aggregates (peaks eluting before dimer), dimer, and monomer in the portion of Filgrastim taken:

$$\text{Result} = (r_U/r_T) \times 100$$

www.webofpharma.com

r_U = individual peak response of aggregates, dimer, and monomer
r_T = sum of the responses of all the peaks

Acceptance criteria
Individual impurities: NMT 0.5% of aggregate is found, and NMT 2.0% of total high molecular weight impurities is found.

SPECIFIC TESTS
- **PROTEIN CONCENTRATION**

(See *Biotechnology-Derived Articles—Total Protein Assay* ⟨1057⟩.)
5% Sorbitol solution, pH 3.25: Prepare a 5% solution of sorbitol in water, and adjust with hydrochloric acid to a pH of 3.25.
Sample solution: Dilute the Filgrastim in *5% Sorbitol solution, pH 3.25* to obtain a solution with a concentration between 0.2 and 1.7 mg/mL.
Blank: *5% Sorbitol solution, pH 3.25*
Instrumental conditions
Mode: UV-Vis
Analytical wavelengths: 280, 320, and 350 nm
Cell: Quartz spectrophotometric cell of path length 1 cm
Analysis
Sample: *Sample solution*
Calculate the sample absorbance at 320 nm as a percentage of the absorbance at 280 nm:

$$\text{Result} = (A_{320}/A_{280}) \times 100$$

A_{320} = absorbance value of the *Sample solution* at 320 nm
A_{280} = absorbance value of the *Sample solution* at 280 nm

If the absorbance at 320 nm is less than 5.0% of the absorbance at 280 nm, calculate the protein concentration of the Filgrastim sample:

$$\text{Result} = A_{280} \times DF/0.86$$

A_{280} = absorbance value of the *Sample solution* at 280 nm
DF = dilution factor of the *Sample solution*

If the absorbance at 320 nm is more than 5.0% of the absorbance at 280 nm, calculate the protein concentration of the Filgrastim sample:

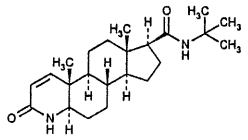
$$\text{Result} = \{A_{280} - [(3.3435 \times A_{320}) - (2.3435 \times A_{350})]\} \times DF/0.86$$

A_{280} = absorbance value of the *Sample solution* at 280 nm
A_{320} = absorbance value of the *Sample solution* at 320 nm
A_{350} = absorbance value of the *Sample solution* at 350 nm
DF = dilution factor of the *Sample solution*

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ **AND TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic count does not exceed 0 cfu/10 mL of the substance solution.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 2 USP Endotoxin Units per mg of drug substance.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 2° and 8°. Protect from light during long-term storage.
- **LABELING:** Label to indicate the content of the drug substance in g per container. The labeling states that the material is of recombinant DNA origin.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Filgrastim RS

Finasteride



$C_{23}H_{36}N_2O_2$ 372.54
4-Azaandrost-1-ene-17-carboxamide, *N*-(1,1-dimethylethyl)-3-oxo-, (5α,17β)-.
N-*tert*-Butyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide [98319-26-7].

» Finasteride contains not less than 98.5 percent and not more than 101.0 percent of $C_{23}H_{36}N_2O_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
USP Reference standards ⟨11⟩**—**
USP Finasteride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
Specific rotation ⟨781S⟩: between −56.0° and −60.0°, determined at 405 nm.
Test solution: 10 mg per mL, in methanol.
Water Determination, *Method I* ⟨921⟩: not more than 0.3%.
Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Mobile phase—Prepare a filtered and degassed mixture of water, tetrahydrofuran, and acetonitrile (8:1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluting solution—Prepare a solution of water and acetonitrile (1:1).
Standard solution—Dissolve an accurately weighed quantity of USP Finasteride RS in *Diluting solution,* and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 1.0 mg per mL.
Test solution—Transfer about 100 mg of Finasteride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 30-cm column that contains 4-µm packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 60°. Chromatograph the

USP Monographs

www.webofpharma.com

Standard solution, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 10,000 theoretical plates; and the tailing factor is not more than 1.3.

Procedure—Inject a volume (about 15 µL) of the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Finasteride taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity, and r_s is the sum of the responses of all peaks: not more than 0.5% of any individual impurity is found; and not more than 1.0% of total impurities is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and tetrahydrofuran (4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluting solution—Prepare a solution of water and acetonitrile (1:1).

Standard preparation—Dissolve an accurately weighed quantity of USP Finasteride RS in *Diluting solution,* and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 200 µg per mL.

Assay preparation—Transfer about 20 mg of Finasteride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 3.0-mm × 3.0-cm column that contains 3-µm packing L7. The flow rate is about 3 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 1800 theoretical plates; the tailing factor is not more than 1.3; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{23}H_{36}N_2O_2$ in the portion of Finasteride taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Finasteride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Finasteride Tablets

» Finasteride Tablets contain not less than 95.0 percent and not more than 105.0 percent of finasteride ($C_{23}H_{36}N_2O_2$).

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Finasteride RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

FOR PRODUCTS LABELED AS 5-MG TABLETS—

Time: 45 minutes.

Determine the amount of $C_{23}H_{36}N_2O_2$ dissolved by employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (29:21). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluting solution—Prepare a solution of acetonitrile and water (7:3).

Standard solution—Dissolve an accurately weighed quantity of USP Finasteride RS in *Diluting solution,* and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration approximately equivalent to the sample under test.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 5-cm column that contains packing L1. The column temperature is maintained at 45°. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the capacity factor, *k′,* is not less than 2.0; the column efficiency is greater than 1000 theoretical plates; the tailing factor is less than 2; and the relative standard deviation for replicate injections is less than 2.0%.

Procedure—Separately inject equal volumes (about 200 µL) of the solution under test and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of $C_{23}H_{36}N_2O_2$ dissolved.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{23}H_{36}N_2O_2$ is dissolved in 45 minutes.

FOR PRODUCTS LABELED AS 1-MG TABLETS—

Time: 30 minutes.

Mobile phase—Prepare a degassed mixture of acetonitrile and water (11:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluting solution—Prepare a solution of water and acetonitrile (7:3).

Standard solution—Dissolve an accurately weighed quantity of USP Finasteride RS in *Diluting solution,* to obtain a solution having a known concentration of 0.1 mg per mL. Dilute this solution quantitatively, and stepwise if necessary, in 0.5% sodium lauryl sulfate to obtain a solution containing 0.001 mg of finasteride per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L11. The column temperature is maintained at 45°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 5000 theoretical plates; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 100 µL) of the solution under test and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of finasteride ($C_{23}H_{36}N_2O_2$) dissolved.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{23}H_{36}N_2O_2$ is dissolved in 30 minutes.

USP Monographs

www.webofpharma.com

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 2.5 mM phosphoric acid and acetonitrile (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluting solution—Prepare a solution of acetonitrile and water (7:3).

Standard preparation—Dissolve an accurately weighed quantity of USP Finasteride RS in *Diluting solution*, and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 100 µg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an amount of powder equivalent to about 10 mg of finasteride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.

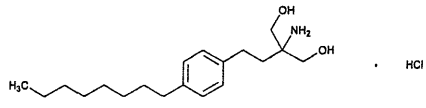
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 10.0-cm column that contains packing L1. The column temperature is maintained at 45°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the capacity factor, *k'*, is not less than 2.0; the column efficiency is not less than 1000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of finasteride ($C_{23}H_{36}N_2O_2$) in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Finasteride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Fingolimod Hydrochloride



$C_{19}H_{33}NO_2 \cdot HCl$ 343.93

1,3-Propanediol, 2-amino-2-[2-(4-octylphenyl)ethyl]-, hydrochloride;

2-Amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride [162359-56-0].

Fingolimod (free base)

$C_{19}H_{33}NO_2$ 307.48

[162359-55-9].

DEFINITION

Fingolimod Hydrochloride contains NLT 98.0% and NMT 102.0% of fingolimod hydrochloride ($C_{19}H_{33}NO_2 \cdot HCl$), calculated on an anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197M▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*

ASSAY

- **PROCEDURE**

Solution A: 0.1% phosphoric acid in water

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Acetonitrile (%)
0	80	20
20	5	95
23	5	95
23.1	80	20
33	80	20

Diluent: Acetonitrile and *Solution A* (1:1)

Standard solution: 0.6 mg/mL of USP Fingolimod Hydrochloride RS in *Diluent*

Sample solution: 0.6 mg/mL of Fingolimod Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 3-mm × 15-cm; 3-µm packing L1

Column temperature: 40°

Flow rate: 0.8 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 0.73%

Tailing factor: NMT 5

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fingolimod hydrochloride ($C_{19}H_{33}NO_2 \cdot HCl$) in the portion of Fingolimod Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fingolimod Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Fingolimod Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on an anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

USP Monographs

www.webofpharma.com

• **ORGANIC IMPURITIES**

Solution A, Mobile phase, Diluent, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 0.6 mg/mL of USP Fingolimod System Suitability Mixture RS in *Diluent*

Standard stock solution: Use the *Standard solution* from the *Assay*.

Standard solution: 0.003 mg/mL of USP Fingolimod Hydrochloride RS from the *Standard stock solution* in *Diluent*

Sensitivity solution: 0.3 µg/mL of USP Fingolimod Hydrochloride RS from the *Standard solution* in *Diluent*

System suitability

Samples: *System suitability solution, Standard solution,* and *Sensitivity solution*

[NOTE—The relative retention times of fingolimod and *O*-acetyl fingolimod are about 1.00 and 1.11, respectively.]

Suitability requirements

Resolution: NLT 1.2 between *O*-acetyl fingolimod and fingolimod nonyl homolog; NLT 0.8 between 2-phenethyl fingolimod analog and 3-phenethyl fingolimod analog, *System suitability solution*

Relative standard deviation: NMT 10%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each individual impurity in the portion of Fingolimod Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of fingolimod hydrochloride from the *Standard solution*

C_S = concentration of USP Fingolimod Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Fingolimod Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fingolimod hexyl homolog	0.82	1.1	0.5
Fingolimod heptyl homolog	0.93	1.0	0.2
Fingolimod	1.00	—	—
Fingolimod nonyl homolog	1.13	1.0	0.2
Fingolimod decyl homolog	1.23	1.0	0.5
3-Phenethyl fingolimod analog	1.97	1.3	0.2
2-Phenethyl fingolimod analog	2.00	1.4	0.2
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.5%

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count is NMT 10^3 cfu/g. The total combined molds and yeasts count is NMT 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store below 30° in a dry place.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Fingolimod Hydrochloride RS

USP Fingolimod System Suitability Mixture RS

It contains Fingolimod Hydrochloride and small amounts of the following impurities:

O-Acetyl fingolimod;
2-Amino-2-(hydroxymethyl)-4-(4-octylphenyl)butyl acetate.
$C_{21}H_{35}NO_3$ 349.51

Fingolimod decyl homolog;
2-Amino-2-(4-decylphenethyl)propane-1,3-diol.
$C_{21}H_{37}NO_2$ 335.53

Fingolimod heptyl homolog;
2-Amino-2-(4-heptylphenethyl)propane-1,3-diol.
$C_{18}H_{31}NO_2$ 293.45

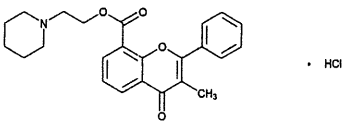
Fingolimod hexyl homolog;
2-Amino-2-(4-hexylphenethyl)propane-1,3-diol.
$C_{17}H_{29}NO_2$ 279.42

Fingolimod nonyl homolog;
2-Amino-2-(4-nonylphenethyl)propane-1,3-diol.
$C_{20}H_{35}NO_2$ 321.51

2-Phenethyl fingolimod analog;
2-Amino-2-[4-octyl-2-(4-octylphenethyl)phenethyl]propane-1,3-diol.
$C_{35}H_{57}NO_2$ 523.85

3-Phenethyl fingolimod analog;
2-Amino-2-[4-octyl-3-(4-octylphenethyl)phenethyl]propane-1,3-diol.
$C_{35}H_{57}NO_2$ 523.85

Flavoxate Hydrochloride



$C_{24}H_{25}NO_4 \cdot HCl$ 427.92

4*H*-1-Benzopyran-8-carboxylic acid, 3-methyl-4-oxo-2-phenyl-, 2-(1-piperidinyl)ethyl ester, hydrochloride;
2-Piperidinoethyl 3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxylate hydrochloride [3717-88-2].

DEFINITION

Flavoxate Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{24}H_{25}NO_4 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

• **C. Identification Tests—General** ⟨191⟩, *Chloride*: A solution of 10 mg/mL meets the requirements of test *A*.

ASSAY

• **Procedure**

Solution A: Mixture of 1.0 g of sodium hexanesulfonate in 650 mL of water, 1 mL of triethylamine, and 1.0 mL of phosphoric acid
Mobile phase: Add 350 mL of acetonitrile to *Solution A*.
Standard solution: 50 µg/mL of USP Flavoxate Hydrochloride RS in *Mobile phase*
Sample solution: 50 µg/mL of Flavoxate Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 293 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
[Note—The retention time of flavoxate is about 4.3 min.]
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{24}H_{25}NO_4 \cdot HCl$ in the portion of Flavoxate Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Flavoxate Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Flavoxate Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Inorganic Impurities

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

Organic Impurities

• **Procedure**

Solution A: 0.05% (v/v) trifluoroacetic acid in water
Solution B: Acetonitrile
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	68	32
15	68	32
25	20	80
35	20	80
36	68	32
41	68	32

Diluent: Methanol and 0.02% trifluoroacetic acid (1:1)
Standard stock solution 1: 2 mg/mL of USP Flavoxate Hydrochloride RS in *Diluent*
Standard stock solution 2: 0.05 mg/mL of USP Flavoxate Related Compound A RS in *Diluent*
Standard stock solution 3: 0.05 mg/mL of USP Flavoxate Related Compound B RS in methanol
Standard stock solution 4: 0.05 mg/mL of USP Flavoxate Related Compound C RS prepared as follows: Dissolve USP Flavoxate Related Compound C RS first in methanol using 10% of final volume, then fill 10% of final volume with 0.02% trifluoroacetic acid. Dilute with *Diluent* to volume.
Standard solution: 1 mg/mL of USP Flavoxate Hydrochloride RS and 5 µg/mL each of USP Flavoxate Related Compound A RS, USP Flavoxate Related Compound B RS, and USP Flavoxate Related Compound C RS in *Diluent* from *Standard stock solutions 1*, *2*, *3*, and *4*, respectively
Sample solution: 1 mg/mL of Flavoxate Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 0.8 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between the flavoxate related compound B and flavoxate related compound C peaks
Tailing factor: NMT 1.5 for the flavoxate related compounds A, B, and C peaks
Relative standard deviation: NMT 2.0% for the flavoxate peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of flavoxate related compounds A, B, and C in the portion of Flavoxate Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of flavoxate from the *Standard solution*
C_S = concentration of USP Flavoxate Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Flavoxate Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1*)

Calculate the percentage of any other unspecified degradation product in the portion of Flavoxate Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_T = sum of responses of all peaks from the *Sample solution*

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Flavoxate	1.0	—	—

USP Monographs

www.webofpharma.com

Impurity Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Flavoxate related compound A[a]	2.8	1.6	0.3
Flavoxate related compound B[b]	3.5	1.5	0.1
Flavoxate related compound C[c]	3.7	1.4	0.1
Any single unspecified degradation product	—	1.0	0.1

[a] 3-Methylflavone-8-carboxylic acid.
[b] 3-Methylflavone-8-carboxylic acid methyl ester.
[c] 3-Methylflavone-8-carboxylic acid ethyl ester.

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩: Dry a sample at 105° to constant weight: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light; and store at room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Flavoxate Hydrochloride RS
  USP Flavoxate Related Compound A RS
  3-Methylflavone-8-carboxylic acid.
  $C_{17}H_{12}O_4$ 280.27
  USP Flavoxate Related Compound B RS
  3-Methylflavone-8-carboxylic acid methyl ester.
  $C_{18}H_{14}O_4$ 294.30
  USP Flavoxate Related Compound C RS
  3-Methylflavone-8-carboxylic acid ethyl ester.
  $C_{19}H_{16}O_4$ 308.33

Flavoxate Hydrochloride Tablets

» Flavoxate Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of $C_{24}H_{25}NO_4 \cdot HCl$, based on the label claim.

Packaging and storage—Preserve in well-closed containers, protected from light.

USP Reference standards ⟨11⟩—
USP Flavoxate Hydrochloride RS
USP Flavoxate Related Compound A RS
3-Methylflavone-8-carboxylic acid.
$C_{17}H_{12}O_4$ 280.27

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—
Test specimen—Grind at least 4 Tablets into a fine powder. Use an amount of powder equivalent to about 100 mg of flavoxate hydrochloride. Add 30 mL of methanol, and stir for about 10 minutes. Pass through a Whatman #1 filter paper, and collect the filtrate. Evaporate the methanol to dryness on a steam bath with the help of nitrogen gas. Dry the residue in vacuum at 60° for about 30 minutes. Mix 1 to 2 mg of dried residue with 200 mg of potassium bromide, and grind thoroughly for 10 to 15 minutes.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to the major peak in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL, deaerated.
Apparatus 1: 100 rpm.
Time: 30 minutes.
Standard solution—Transfer an accurately weighed quantity of USP Flavoxate Hydrochloride RS to a suitable volumetric flask, dissolve in a small amount of methanol (not more than 2% of the final volume), and dilute with *Medium* to volume to obtain a solution having a known concentration of about 0.11 mg per mL.
Test solution—Pass a portion of the solution under test through a suitable filter having a porosity of 45 µm.
Procedure—Determine the amount of $C_{24}H_{25}NO_4 \cdot HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 294 nm, in portions of the *Test solution* in comparison with the *Standard solution,* using a cell with a path length of 0.2 cm and using *Medium* as the blank. Calculate the percentage of $C_{24}H_{25}NO_4 \cdot HCl$ dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times L}$$

in which A_U and A_S are the absorbances obtained from the *Test solution* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of the *Standard solution;* 900 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg.
Tolerances—Not less than 70% (Q) of the labeled amount of $C_{24}H_{25}NO_4 \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Related compounds—
Mobile phase—Prepare as directed in the *Assay.*
Standard stock solution—Dissolve an accurately weighed quantity of USP Flavoxate Hydrochloride RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.
USP Flavoxate Related Compound A RS stock solution—Dissolve an accurately weighed quantity of USP Flavoxate Related Compound A RS in acetonitrile to obtain a solution having a known concentration of about 0.3 mg per mL.
Standard solution—Quantitatively dilute suitable volumes of *Standard stock solution* and *USP Flavoxate Related Compound A RS stock solution* with *Mobile phase* to obtain a solution having a final known concentration of about 0.001 mg per mL of flavoxate hydrochloride and about 0.003 mg per mL of flavoxate related compound A.
Test solution—Filter the *Assay stock preparation,* prepared as directed in the *Assay,* through a 0.45-µm filter (polyvinylidene fluoride [PVDF] or equivalent). Use the filtrate.
Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay.* Chromatograph the *Standard solution,* and record the peak areas as directed for *Procedure:* the resolution, *R,* between flavoxate hydrochloride and flavoxate hydrochloride related compound A is not less than 10.0; the column efficiency is not less than 3000 plate counts; the tailing factor is not more than 2.0 for flavoxate hydrochloride; and the relative standard deviation for five replicate injections is not more than 2.0% for both peaks.

USP Monographs

www.webofpharma.com

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and record the chromatograms. Measure the peak areas of all the peaks in the *Test solution*. Identify the peaks using the relative retention times as given in *Table 1*.

Table 1

Component Name	Relative Retention Time	Relative Response Factor	Limit (%)
Flavoxate hydrochloride	1.0	—	—
Flavoxate related compound A*	2.5	1.5	0.3
Any individual unspecified degradation product	—	1.0	0.1
Total impurities	—	—	1.0

* 3-Methylflavone-8-carboxylic acid.

Calculate the percentage of each impurity relative to flavoxate hydrochloride in the portion of Tablets taken by the formula:

$$100(C_S/C_U)(r_i/r_S)(1/F)$$

in which C_S is the concentration, in mg per mL, of flavoxate hydrochloride in the *Standard solution*; C_U is the nominal concentration, in mg per mL, of flavoxate hydrochloride, based on label claim, in the *Test solution*; r_i is the peak response of each individual impurity; r_S is the response of the flavoxate hydrochloride peak obtained from the *Standard solution*; and F is the relative response factor for each of the impurities relative to flavoxate hydrochloride, as shown in *Table 1*.

Assay—

Mobile phase—Dissolve 1.0 g of sodium hexanesulfonate, 1.0 mL of triethylamine, and 1.0 mL of phosphoric acid in 650 mL of water. Filter and degas. To the filtrate, add 350 mL of acetonitrile. Mix well. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Flavoxate Hydrochloride RS, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

Assay stock preparation—Weigh and finely powder not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 100 mg of flavoxate hydrochloride based on label claim, and transfer to a 100-mL volumetric flask. Add about 80% volume of *Mobile phase*. Sonicate for 10 minutes, and stir for 15 minutes. Dilute with *Mobile phase* to volume.

Assay preparation—Dilute the *Assay stock preparation* quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution with a nominal concentration of about 0.05 mg per mL of flavoxate hydrochloride, based on label claim. Filter the solution through a 0.45-µm filter (polyvinylidene fluoride [PVDF] or equivalent). Use the filtrate.

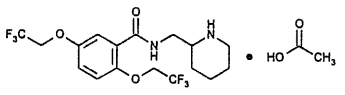
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 293-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 3000; the tailing factor is not more than 2.0; and the relative standard deviation for five replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the flavoxate hydrochloride peak whose retention time is about 4.0 minutes. Calculate the percentage of the label claim of flavoxate hydrochloride ($C_{24}H_{25}NO_4 \cdot HCl$) in the portion of the Tablets taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of flavoxate hydrochloride in the *Standard preparation*; C_U is the nominal concentration of flavoxate hydrochloride in the *Assay preparation*, based on the label claim; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Flecainide Acetate



$C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$ 474.39

Benzamide, *N*-(2-piperidinylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)-, monoacetate;
N-(2-Piperidylmethyl)-2,5-bis(2,2,2-trifluoroethoxy) benzamide monoacetate [54143-56-5].

DEFINITION

Change to read:

Flecainide Acetate contains NLT 98.0% and NMT ▲102.0%▲ 2S (USP41) of flecainide acetate ($C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*▲ (CN 1-May-2020) ▲⟨197⟩: [NOTE—Methods described in ⟨197K⟩ or ⟨197A⟩ may be used.]▲ 2S (USP41)

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Solution: 130 µg/mL in alcohol
Analytical wavelength: 298 nm
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

Add the following:

▲• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

• **PROCEDURE**
▲**Solution A:** 0.1 M ammonium acetate in water. Adjust with acetic acid to a pH of 5.4.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

USP Monographs

www.webofpharma.com

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
7.0	45	55
12.0	45	55
12.1	75	25
15.0	75	25

Diluent: Acetonitrile and water (25:75)
Standard solution: 0.2 mg/mL of USP Flecainide Acetate RS in *Diluent*. Sonication may be needed for complete dissolution.
Sample solution: 0.2 mg/mL of Flecainide Acetate in *Diluent*. Sonication may be needed for complete dissolution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm
Column: 3.0-mm × 15-cm; 3-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of flecainide acetate ($C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$) in the portion of Flecainide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of flecainide from the *Sample solution*
r_S = peak response of flecainide from the *Standard solution*
C_S = concentration of USP Flecainide Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Flecainide Acetate in the *Sample solution* (mg/mL)▲ 2S (USP41)

Acceptance criteria: 98.0%–▲102.0%▲ 2S (USP41) on the dried basis

OTHER COMPONENTS

• **CONTENT OF ACETATE**
Sample solution: Dissolve about 600 mg of Flecainide Acetate in about 100 mL of dimethylformamide, and stir by mechanical means until dissolved.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N tetrabutylammonium hydroxide VS
Endpoint detection: Potentiometric
Analysis: Perform a blank determination and make any necessary correction. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 6.005 mg of acetic acid ($C_2H_4O_2$).
Acceptance criteria: 12.4%–12.8% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%

Delete the following:

▲• **HEAVY METALS,** *Method II* ⟨231⟩: NMT 20 ppm▲ (Official 1-Jan-2018)

Change to read:

• **ORGANIC IMPURITIES**
▲**Solution A, Solution B, Mobile phase, Diluent,** and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.25 mg/mL of USP Flecainide Acetate RS and 0.1 mg/mL of flecainide acid in *Diluent*
Sensitivity solution: 0.001 mg/mL of USP Flecainide Acetate RS in *Diluent*
Standard solution: 0.01 mg/mL each of USP Flecainide Acetate RS and USP Flecainide Related Compound A RS in *Diluent*
Sample solution: 2.0 mg/mL of Flecainide Acetate in *Diluent*. Sonication may be needed for complete dissolution.
System suitability
Samples: *System suitability solution, Sensitivity solution,* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between flecainide acetate and flecainide acid, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 50, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of flecainide related compound A in the portion of Flecainide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of flecainide related compound A from the *Sample solution*
r_S = peak response of flecainide related compound A from the *Standard solution*
C_S = concentration of USP Flecainide Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Flecainide Acetate in the *Sample solution* (mg/mL)

Calculate the percentage of each specified or unspecified impurity in the portion of Flecainide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of flecainide acetate from the *Standard solution*
C_S = concentration of USP Flecainide Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Flecainide Acetate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

www.webofpharma.com

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Flecainide acid[a]	0.8	0.67	0.5
Flecainide	1.0	—	—
Flecainide related compound A	1.2	—	0.5
Flecainide pyridine analog[b]	1.6	1.0	0.5
Any individual unspecified impurity	—	—	0.10
Total impurities	—	—	2.0

[a] 2,5-Bis(2,2,2-trifluoroethoxy)benzoic acid.
[b] *N*-(Pyridin-2-ylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide. ▲2S (USP41)

SPECIFIC TESTS

Delete the following:

▲• **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 146°–152°, but the range between beginning and end of melting does not exceed 3°. ▲2S (USP41)

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 2 h.
Acceptance criteria: NMT 0.5%

Delete the following:

▲• **CLARITY OF SOLUTION**
Sample solution: Place 250 mg in a test tube about 18 mm in diameter. Add 10 mL of water and 1 drop of glacial acetic acid, shake to dissolve.
Acceptance criteria: The solution is colorless and is not less clear than an equal volume of water in a similar test tube. ▲2S (USP41)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Flecainide Acetate RS
USP Flecainide Related Compound A RS
3-[2,5-Bis(2,2,2-trifluoroethoxy)phenyl]-1,5,6,7,8,8a-hexahydroimidazo[1,5-*a*]pyridine hydrochloride.
$C_{17}H_{18}F_6N_2O_2 \cdot HCl$ 432.8

Flecainide Acetate Compounded Oral Suspension

DEFINITION

Flecainide Acetate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of flecainide acetate ($C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$).

Prepare Flecainide Acetate Compounded Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

Flecainide Acetate	2 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number in a suitable mortar, and comminute to a fine powder with a pestle, or use *Flecainide Acetate* powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a flecainide acetate liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the liquid *Vehicle* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**
Mobile phase: Acetonitrile and 0.06% phosphoric acid solution (40:60)
Standard stock solution: 1.0 mg/mL of USP Flecainide Acetate RS in *Mobile phase*
Standard solution: Transfer 2 mL of *Standard stock solution* to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution containing about 200 µg/mL of flecainide acetate.
Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution having a nominal concentration of about 200 µg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of flecainide acetate ($C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Flecainide Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of flecainide acetate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.8–4.8

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature or when stored in a refrigerator
- **LABELING:** Label it to indicate that it is to be well-shaken before use, protected from light, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Flecainide Acetate RS

Flecainide Acetate Tablets

DEFINITION

Flecainide Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of flecainide acetate ($C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$).

IDENTIFICATION

Delete the following:

▲• **A. ULTRAVIOLET ABSORPTION** ⟨197U⟩
Sample solution: Use the *Sample solution* under *Uniformity of Dosage Units*.
Analytical wavelength: 250–400 nm
Acceptance criteria: Meet the requirements ▲2S (USP41)

Add the following:

▲• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲2S (USP41)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲2S (USP41)

ASSAY

Change to read:

- **PROCEDURE**
  ▲Solution A: 0.1 M ammonium acetate in water. Adjust with acetic acid to a pH of 5.4.
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
7.0	45	55
12.0	45	55
12.1	75	25
15.0	75	25

Diluent: Lactic acid and water (2:98)

Standard solution: 0.2 mg/mL of USP Flecainide Acetate RS in *Diluent*. Sonication may be needed for complete dissolution.
Sample stock solution: Nominally 1 mg/mL of flecainide acetate in *Diluent* prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask, add *Diluent* to about 80% of the flask capacity, and sonicate for 30 min. Dilute with *Diluent* to volume.
Sample solution: Nominally 0.2 mg/mL of flecainide acetate from the *Sample stock solution* in *Diluent*. Centrifuge and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 300 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 3.0-mm × 15-cm; 3-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0% ▲2S (USP41)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of flecainide acetate ($C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of flecainide from the *Sample solution*
r_S = peak response of flecainide from the *Standard solution*
C_S = concentration of USP Flecainide Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of flecainide acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩
  Medium: 0.075 N hydrochloric acid; 900 mL
  Apparatus 2: 50 rpm
  Time: 30 min for Tablets each containing 50 mg of flecainide acetate; or 60 min for 100-, 150-, or 200-mg Tablets
  Standard solution: A known concentration of USP Flecainide Acetate RS in *Medium*
  Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.
  Instrumental conditions
  Mode: UV-Vis
  Analytical wavelength: 296 nm
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of flecainide acetate ($C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$) dissolved.
  Tolerances: NLT 70% (*Q*) of the labeled amount of flecainide acetate ($C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$) is dissolved from 50-mg Tablets in 30 min; or from 100-, 150-, or 200-mg Tablets in 60 min.

USP Monographs

www.webofpharma.com

Change to read:

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements
▲▲ 2S (USP41)

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability solution: 0.25 mg/mL of USP Flecainide Acetate RS and 0.1 mg/mL of flecainide acid in *Diluent*
Sensitivity solution: 0.002 mg/mL of USP Flecainide Acetate RS in *Diluent*
Standard solution: 0.01 mg/mL each of USP Flecainide Acetate RS and USP Flecainide Related Compound A RS in *Diluent*
Sample solution: Nominally 2 mg/mL of flecainide acetate in *Diluent* prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask, add *Diluent* to about 80% of the flask capacity, and sonicate for 30 min. Dilute with *Diluent* to volume. Centrifuge a portion of the solution and use the supernatant.
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 5.0 between flecainide acetate and flecainide acid, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 50, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of flecainide related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of flecainide related compound A from the *Sample solution*
r_S = peak response of flecainide related compound A from the *Standard solution*
C_S = concentration of USP Flecainide Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of flecainide acetate in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified degradation product from the *Sample solution*
r_S = peak response of flecainide acetate from the *Standard solution*
C_S = concentration of USP Flecainide Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of flecainide acetate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Flecainide acid[a, b]	0.8	—
Flecainide	1.0	—
Flecainide related compound A	1.2	0.5
Flecainide pyridine analog[b, c]	1.6	—
Each unspecified degradation product	—	0.2
Total impurities	—	2.0

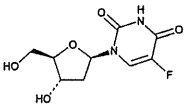
[a] 2,5-Bis(2,2,2-trifluoroethoxy)benzoic acid.
[b] Included for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and should not be included in the total impurities.
[c] *N*-(Pyridin-2-ylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide. ▲ 2S (USP41)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. ▲Store at controlled room temperature.▲ 2S (USP41)
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Flecainide Acetate RS
USP Flecainide Related Compound A RS
3-[2,5-Bis(2,2,2-trifluoroethoxy)phenyl]-1,5,6,7,8,8a-hexahydroimidazo[1,5-*a*]pyridine hydrochloride.
$C_{17}H_{18}F_6N_2O_2 \cdot HCl$ 432.8

Floxuridine



$C_9H_{11}FN_2O_5$ 246.19
Uridine, 2′-deoxy-5-fluoro-;
2′-Deoxy-5-fluorouridine [50-91-9].

DEFINITION
Floxuridine contains NLT 98.5% and NMT 101.0% of floxuridine ($C_9H_{11}FN_2O_5$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Analytical wavelength: 268 nm
Sample solution: 20 µg/mL
Medium: 0.1 N potassium hydroxide
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.
• **C.**
Sample solution: 20 mg/mL of Floxuridine in water

www.webofpharma.com

Analysis: Add a few drops of bromine TS to 10 mL of the *Sample solution*.
Acceptance criteria: The bromine color is discharged.

ASSAY
- **PROCEDURE**

Sample: 800 mg of Floxuridine
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N tetrabutylammonium hydroxide VS
Endpoint detection: Potentiometric
Analysis: Dissolve the *Sample* in 80 mL of dimethylformamide, and titrate with *Titrant*, determining the endpoint using a calomel–glass electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 24.62 mg of floxuridine ($C_9H_{11}FN_2O_5$).
Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF FLUORIDE IONS**

Use plasticware throughout this test.
Buffer: Dissolve 110 g of sodium chloride and 1 g of sodium citrate in 700 mL of water in a 2000-mL volumetric flask. Cautiously add 150 g of sodium hydroxide, and dissolve with shaking. Cool to room temperature, and while stirring, cautiously add 450 mL of glacial acetic acid to the cooled solution. Cool, add 600 mL of isopropyl alcohol, and dilute with water to volume. The pH of the *Buffer* is 5.0–5.5.
Standard stock solution: Transfer 221 mg of sodium fluoride, previously dried at 150° for 4 h, to a 100-mL volumetric flask, add 20 mL of water, and mix to dissolve. Add 1.0 mL of sodium hydroxide solution (1 in 2500), dilute with water to volume, and mix. Each mL of the *Standard stock solution* contains 1 mg of fluoride ions. Store in a tightly closed, plastic container.
Standard solutions: 1, 3, 5, and 10 µg/mL of fluoride ion; from *Standard stock solution* in *Buffer*
Sample solution: 10 mg/mL of Floxuridine in *Buffer*
Analysis: Concomitantly measure the potential (see *Titrimetry* ⟨541⟩), in mV, of the *Standard solutions* and the *Sample solution*, with a pH meter capable of a minimum reproducibility of ±0.2 mV, equipped with a glass-sleeved calomel–fluoride specific-ion electrode system. When taking measurements, immerse the electrodes in the solution, which has been transferred to a 150-mL beaker containing a polytef-coated stirring bar. Allow to stir on a magnetic stirrer having an insulated top until equilibrium is attained (1–2 min), and record the potential. Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the specific-ion electrode.
Plot the logarithm of the fluoride-ion concentrations, in µg/mL, of the *Standard solutions* versus potential, in mV. From the measured potential of the *Sample solution* and the standard curve, determine the concentration, in µg/mL, of fluoride ions in the *Sample solution*.
Acceptance criteria: NMT 0.05%

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩: 145°–153°, but the range between the beginning and end of melting does not exceed 2°.
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL of Floxuridine in water.
Acceptance criteria: +36° to +39°
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry under vacuum over silica gel at 60° for 4 h.
Acceptance criteria: NMT 0.2%
- **PYROGEN:** Where the label states that Floxuridine is sterile, it meets the requirements of *Pyrogen Test* ⟨151⟩, the test dose being 0.50 mL/kg of a solution prepared by diluting Floxuridine with Sodium Chloride Injection to a concentration of 100 mg/mL.
- **OTHER REQUIREMENTS:** Where the label states that Floxuridine is sterile, it meets the requirements in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*. Where the label states that Floxuridine must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for the *Pyrogen* test.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected for further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Floxuridine RS

Floxuridine for Injection

DEFINITION
Floxuridine for Injection is lyophilized Floxuridine suitable for intra-arterial infusion. It contains NLT 90.0% and NMT 110.0% of the labeled amount of floxuridine ($C_9H_{11}FN_2O_5$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample: Use the *Sample solution* prepared for the *Assay*.
Acceptance criteria: Meets the requirements
- **B.**

Sample solution: Nominally 20 mg/mL of floxuridine in water
Analysis: Add a few drops of bromine TS to 10 mL of the *Sample solution*.
Acceptance criteria: The bromine color is discharged.

ASSAY
- **PROCEDURE**

Diluent: Potassium hydroxide solution (1 in 180)
Standard solution: 20 µg/mL of USP Floxuridine RS in *Diluent*
Sample stock solution: Nominally 0.4 mg/mL of Floxuridine for Injection in *Diluent*
Sample solution: 20 µg/mL from the *Sample stock solution* in *Diluent*
Blank: *Diluent*
Instrumental conditions
Mode: UV
Analytical wavelength: 268 nm
Cell: 1 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of floxuridine ($C_9H_{11}FN_2O_5$) in the portion of Floxuridine for Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

USP Monographs

www.webofpharma.com

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Floxuridine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of floxuridine in the *Sample solution* (µg/mL)

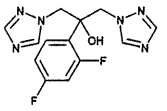
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

SPECIFIC TESTS
• **PH** ⟨791⟩
Sample solution: 1 in 50
Acceptance criteria: 4.0–5.5
• **CONSTITUTED SOLUTION:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*
• **PYROGEN:** Meets the requirements of *Pyrogen Test* ⟨151⟩, the test dose being 0.50 mL/kg of a solution prepared by diluting Floxuridine for Injection with Sodium Chloride Injection to a concentration of 100 mg/mL
• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*, and protect from light. Store containers of constituted Floxuridine for Injection under refrigeration for NMT 2 weeks.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Floxuridine RS

Fluconazole



$C_{13}H_{12}F_2N_6O$ 306.27
1*H*-1,2,4-Triazole-1-ethanol, 1-(2,4-difluorophenyl)-1-(1*H*-1,2,4-triazol-1-ylmethyl)-;
2,4-Difluoro-1′,1′-bis(1*H*-1,2,4-triazol-1-ylmethyl)benzyl alcohol [86386-73-4].

DEFINITION
Fluconazole contains NLT 98.0% and NMT 102.0% of fluconazole ($C_{13}H_{12}F_2N_6O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**
▲**Mobile phase:** Acetonitrile and water (20:80)
Standard solution: 0.5 mg/mL of USP Fluconazole RS in *Mobile phase*
Sample solution: 0.5 mg/mL of Fluconazole in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluconazole ($C_{13}H_{12}F_2N_6O$) in the portion of Fluconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluconazole from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluconazole in the *Sample solution* (mg/mL)▲ (USP 1-May-2019)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Change to read:

▲▲ (USP 1-May-2019)

Change to read:

• **ORGANIC IMPURITIES**▲▲ (USP 1-May-2019)
Mobile phase: Acetonitrile and water (20:80)
Standard solution: 10 µg/mL each of USP Fluconazole RS, USP Fluconazole Related Compound A RS, USP Fluconazole Related Compound B RS, and USP Fluconazole Related Compound C RS, dissolved in acetonitrile, and then diluted in *Mobile phase*
Sample solution: 3 mg/mL of Fluconazole in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
▲[NOTE— See *Table 1* for relative retention times.]▲ (USP 1-May-2019)

www.webofpharma.com

Suitability requirements
Resolution: NLT 1.5 between fluconazole related compound B and fluconazole related compound C
Relative standard deviation: NMT 5.0% for each peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluconazole related compound A, fluconazole related compound B, and fluconazole related compound C in the portion of Fluconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluconazole related compound A, fluconazole related compound B, or fluconazole related compound C from the *Sample solution*
r_S = peak response of fluconazole related compound A, fluconazole related compound B, and fluconazole related compound C from the *Standard solution*
C_S = concentration of USP Fluconazole Related Compound A RS, USP Fluconazole Related Compound B RS, and USP Fluconazole Related Compound C RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluconazole in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Fluconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluconazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fluconazole related compound A	0.5	0.2
Specified impurity	0.6	1.0
Fluconazole related compound B	0.81	0.1
Fluconazole related compound C	0.86	0.2
Fluconazole	1.0	—
Any individual unspecified impurity	—	0.1
Total unknown impurities	—	0.3
Total impurities	—	1.5

Delete the following:

▲• **ORGANIC IMPURITIES, PROCEDURE 2**
Solution A: 0.01 M anhydrous sodium acetate solution. Adjust with 1 N acetic acid to a pH of 5.0, filter, and degas.
Solution B: Acetonitrile
Solution C: Methanol
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	80	5	15
10	80	5	15
20	30	55	15
23	30	55	15
25	80	5	15
30	80	5	15

Diluent: Methanol and *Solution A* (16:84)
Standard solution: 0.01 mg/mL of USP Fluconazole RS in *Diluent*
System suitability solution: 0.02 mg/mL of USP Fluconazole RS and 6 µg/mL of USP Desacetyl Diltiazem Hydrochloride RS in *Diluent*
Sample solution: 2 mg/mL of Fluconazole in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 261 nm
Column: 4.0-mm × 10-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for fluconazole and desacetyl diltiazem are 1.0 and 1.2, respectively, *System suitability solution*.]
Suitability requirements
Resolution: NLT 10.0 between fluconazole and desacetyl diltiazem hydrochloride, *System suitability solution*
Column efficiency: NLT 30,000 theoretical plates for the fluconazole peak, *System suitability solution*
Tailing factor: NMT 1.4 for the fluconazole peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Fluconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluconazole in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

www.webofpharma.com

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Specified impurity	0.17—0.37	0.72	0.1
Specified impurity	0.48—0.60	0.85	0.1
Specified impurity	0.67—0.79	1.21	0.1
Specified impurity	1.14—1.18	0.96	0.1
Specified impurity	1.20—1.32	0.97	0.1
Any unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

▲ (USP 1-May-2019)

Delete the following:

▲• **ORGANIC IMPURITIES, PROCEDURE 3**
Standard solution A: 1 mg/mL of USP Fluconazole RS in methanol (2.0%)
Standard solution B: 0.1 mg/mL of USP Fluconazole RS from *Standard solution A* in methanol (0.2%)
Standard solution C: 0.05 mg/mL of USP Fluconazole RS from *Standard solution A* in methanol (0.1%)
Sample solution: 50 mg/mL of Fluconazole in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, methanol, and ammonium hydroxide (80:20:1)
Spray reagent A: 1.7 mg/mL of silver nitrate in water
Spray reagent B (potassium iodoplatinate solution): 375 mg of chloroplatinic acid in 5 mL of 1 N hydrochloric acid. Dissolve 5 g of potassium iodide in 50 mL of water, and store in a light-resistant container. Prepare a mixture of water, potassium iodide solution, and chloroplatinic acid solution (20:9:1).
Analysis
Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*
Spray the dry plate with *Spray reagent A*, and expose the plate to 365-nm UV light for 10–20 min. Dry the plate for 20 min between 80° and 90°, then spray the plate with *Spray reagent B*. Allow the plate to dry. Examine the plate and compare the intensities of any secondary spots observed in the *Sample solution* with those of the principal spots in the *Standard solutions*.
Acceptance criteria: No spot from the *Sample solution* with an R_F value between 0.10–0.25 and 0.27–0.41 is larger or more intense than that from *Standard solution B* (0.2%). ▲ (USP 1-May-2019)

• **RESIDUE ON IGNITION** ⟨281⟩
Sample: 0.5 g
Acceptance criteria: NMT 0.1%

Delete the following:

▲• **IRON** ⟨241⟩
Sample solution: Transfer 0.5 g of the sample into a test tube. Dissolve in 5 mL of alcohol, and add 5 mL of distilled water.
Acceptance criteria: NMT 20 ppm ▲ (USP 1-May-2019)

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.5%

Delete the following:

▲• **CLARITY AND COLOR OF SOLUTION**
Sample solution: Dissolve a sample in methanol to obtain a 5-in-100 solution (w/v).
Acceptance criteria: The solution is clear and colorless. ▲ (USP 1-May-2019)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store below 30°.

Delete the following:

▲• **LABELING:** If a procedure for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* procedure(s) the article complies. ▲ (USP 1-May-2019)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲ ▲ (USP 1-May-2019)
USP Fluconazole RS
USP Fluconazole Related Compound A RS
2-[2-Fluoro-4-(1*H*-1,2,4-triazol-1-yl)phenyl]-1,3-bis(1*H*-1,2,4-triazol-1-yl)-propan-2-ol.
▲$C_{15}H_{14}FN_9O$ 355.33 ▲ (USP 1-May-2019)
USP Fluconazole Related Compound B RS
2-(4-Fluorophenyl)-1,3-di(1*H*-1,2,4-triazol-1-yl)-propan-2-ol.
▲$C_{13}H_{13}FN_6O$ 288.28 ▲ (USP 1-May-2019)
USP Fluconazole Related Compound C RS
1,1′-(1,3-Phenylene)di(1*H*-1,2,4-triazole).
▲$C_{10}H_8N_6$ 212.21 ▲ (USP 1-May-2019)

Fluconazole Injection

(Title for this monograph—not to change until November 1, 2018)
(Prior to November 1, 2018, the current practice of labeling the article of commerce with the name Fluconazole Injection may be continued. Use of the names Fluconazole in Sodium Chloride Injection or Fluconazole in Dextrose Injection will be permitted as of May 1, 2016; however, the use of these names will not be mandatory until November 1, 2018. The 30-month extension will provide the time needed by manufacturers and users to make necessary changes.)

DEFINITION
Fluconazole Injection is a sterile solution of Fluconazole in a suitable vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: 0.82 g/L of sodium acetate in water. Adjust with 1 N acetic acid solution to a pH of 5.0.
Diluent: Methanol and *Buffer* (20:80)
Solution A: Methanol and *Buffer* (5:95)
Solution B: Acetonitrile and methanol (60:40)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
9	80	20
15	15	85
18	80	20
25	80	20

System suitability solution: 0.04 mg/mL each of benzyl alcohol and USP Fluconazole RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Fluconazole RS in *Diluent*
Sample solution: Equivalent to 0.2 mg/mL of fluconazole from Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 261 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection size: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for benzyl alcohol and fluconazole are about 0.8 and 1.0, respectively, from the *System suitability solution*.]
Suitability requirements
Resolution: NLT 1.8 between benzyl alcohol and fluconazole, *System suitability solution*
Column efficiency: NLT 4000 theoretical plates for the fluconazole peak, *System suitability solution*
Tailing factor: NMT 1.5 for the fluconazole peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

ORGANIC IMPURITIES

[NOTE—On the basis of the synthetic route, perform either (a) *Procedure 1* and *Procedure 2*, or (b) *Procedure 3*, or (c) *Procedure 4*. *Procedure 3* is recommended if bistriazole ketone and epoxyfluconazole (see *Table 4*) are potential impurities. *Procedure 4* is recommended if fluconazole bromohydrine and epoxyfluconazole (see *Table 5*) are potential impurities.]

• **Procedure 1: For Nonpolar Impurities**

Buffer and **Diluent:** Prepare as directed in the *Assay*.
Solution A: Methanol and *Buffer* (5:95)
Solution B: Acetonitrile and methanol (60:40)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	77	23
5	77	23
30	40	60
43	77	23
50	77	23

System suitability solution: 2.4 and 20 µg/mL, respectively of 1,4-benzoquinone and USP Fluconazole RS in *Diluent*
Standard solution: 2 µg/mL of USP Fluconazole RS in *Diluent*
Diluted standard solution: 0.2 µg/mL of USP Fluconazole RS in *Diluent* from the *Standard solution*
Sample solution: Equivalent to 1.0 mg/mL of fluconazole from Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 261 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection size: 100 µL
System suitability
Samples: *System suitability solution, Standard solution*, and *Diluted standard solution*
[NOTE—The relative retention times for 1,4-benzoquinone and fluconazole are about 0.5 and 1.0, respectively, *System suitability solution*.]
Suitability requirements
Resolution: NLT 5.0 between 1,4-benzoquinone and fluconazole, *System suitability solution*
Column efficiency: NLT 2500 theoretical plates for the fluconazole peak, *System suitability solution*
Tailing factor: NMT 1.5 for the fluconazole peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Ratio of average peak area: Between 8.0 and 12.0 for the ratio of the fluconazole peak from the *Standard solution* to that of the *Diluted standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—For the following calculations, do not include peaks eluting before fluconazole and do not include impurities at relative retention times of 2.00–2.12 and 3.14–3.26. The disregarded impurities at the specified relative retention times are process impurities monitored in the drug substance. Furthermore, disregard any peak due to an excipient or any peak less than 0.02%. This test is for determination of the late- eluting peaks, and hence the early-eluting peaks are not quantitated using this procedure.]
Calculate the percentage of the largest unknown nonpolar impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak response of the largest impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of fluconazole in the *Standard solution* (mg/mL)
C_U = concentration of fluconazole, based on the label claim and the extent of dilution, in the *Sample solution* (mg/mL)

Calculate the percentage of unknown nonpolar impurities in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak areas of the unknown peaks from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of fluconazole, based on the label claim and the extent of dilution, in the *Sample solution* (mg/mL)

Acceptance criteria
Largest unknown nonpolar impurity: NMT 0.1%
Total unknown nonpolar impurities: NMT 0.5%

• Procedure 2: For Polar Impurities

Buffer, Diluent, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 2 µg/mL of USP Fluconazole RS in *Diluent*
Diluted standard solution: 0.2 µg/mL of USP Fluconazole RS in *Diluent* from the *Standard solution*
Sample solution: Equivalent to 0.2 mg/mL of fluconazole from Injection in *Diluent*
System suitability
Samples: *System suitability solution*, *Standard solution*, and *Diluted standard solution*
[NOTE—The relative retention times for benzyl alcohol and fluconazole are about 0.8 and 1.0, respectively, *System suitability solution*.]
Suitability requirements
Resolution: NLT 1.8 between benzyl alcohol and fluconazole, *System suitability solution*
Column efficiency: NLT 4000 theoretical plates for the fluconazole peak, *System suitability solution*
Tailing factor: NMT 1.5 for the fluconazole peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Ratio of average peak area: Between 8.0 and 12.0 for the ratio of the fluconazole peak from the *Standard solution* to that of the *Diluted standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times of known related compounds versus fluconazole are included in *Table 3*.]
Calculate the percentage of the single largest unknown polar impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the largest impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of fluconazole in the *Standard solution* (mg/mL)
C_U = concentration of fluconazole, based on the label claim and the extent of dilution, in the *Sample solution* (mg/mL)

Calculate the quantity, as a percentage, of the unknown polar impurities in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = total peak area response of all the unknown peaks from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of fluconazole in the *Standard solution* (mg/mL)
C_U = concentration of fluconazole, based on the label claim and the extent of dilution, in the *Sample solution* (mg/mL)

Acceptance criteria
Largest unknown polar impurity: NMT 0.1%
Total unknown polar impurities: NMT 0.5%
Total unknown polar and nonpolar impurities: NMT 1.0% (sum of results from *Procedure 1* and *Procedure 2*)
[NOTE—Disregard any peak due to an excipient or any peak less than 0.03%.]

Table 3

Name	Relative Retention Time
Hydroxymethylfurfural (if dextrose is present)[a]	0.22–0.28
Aminofluconazole quaternary salt[b]	0.30–0.36
Unidentified impurity (if dextrose is present)[c]	0.37–0.43
Fluconazole isomer[d]	0.47–0.59
Fluconazole diol[e]	0.68–0.74
Cyclohexanone[f]	0.77–0.83
Fluconazole	1.0

[a] 5-Hydroxymethylfurfural.
[b] 4-Amino-1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl) propyl)-4*H*-1,2,4-triazolium bromide.
[c] Dextrose-related compound.
[d] 2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl) propan-2-ol.
[e] 2-(2,4-Difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propane-1,2-diol.
[f] A process impurity associated with drug product packaged in bags.

[NOTE—Disregard aminofluconazole quaternary salt, fluconazole isomer, and fluconazole diol impurities from *Table 3* in the following calculation because these are process impurities that are monitored in the drug substance.]

• Procedure 3

Buffer: 0.63 g/L of ammonium formate in water
Mobile phase: Acetonitrile and *Buffer* (14:86)
Standard solution: 2 mg/mL of USP Fluconazole RS in *Buffer*.
[NOTE—Use approximately 14% of the total volume as acetonitrile, and sonicate if necessary to facilitate dissolution.]
Sensitivity solution: 1 µg/mL of USP Fluconazole RS in *Mobile phase* from the *Standard solution*.
Sample solution: Equivalent to 2 mg/mL of fluconazole in 0.9% aqueous solution of sodium chloride
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm

www.webofpharma.com

Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection size: 20 µL

System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—The retention time for fluconazole is between 12 and 14 min.]
Suitability requirements
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Column efficiency: NLT 3000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of fluconazole in the *Standard solution* (mg/mL)
C_U = concentration of fluconazole, based on the label claim, in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Table 4*.
Total impurities: NMT 0.5%

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bistriazole ketone[a]	0.13	0.2
Fluconazole isomer[b]	0.5	0.2
Fluconazole	1.0	—
Epoxyfluconazole[c]	2.6	0.2
Any other individual impurity	—	0.2

[a] 1,3-Bis(1*H*-1,2,4-triazol-1-yl)propan-2-one.
[b] 2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl) propan-2-ol.
[c] 1-[2-(2,4-Difluorophenyl)-2,3-epoxypropyl]-1*H*-1,2,4-triazole.

• **Procedure 4**
Buffer: 13.4 g/L of dibasic sodium phosphate heptahydrate in water. Adust with phosphoric acid to a pH of 7.0.
Mobile phase: Acetonitrile and *Buffer* (26:74)
Standard solution: 2 mg/mL of USP Fluconazole RS in *Mobile phase*
Sample solution: Equivalent to 2 mg/mL of fluconazole in 0.9% aqueous solution of sodium chloride

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 0.5 mL/min
Injection size: 50 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of fluconazole in the *Standard solution* (mg/mL)
C_U = concentration of fluconazole, based on the label claim, in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Table 5*.
Total impurities: NMT 0.5%

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Aminofluconazole quaternary salt[a]	0.57	0.74	0.1
Fluconazole isomer[b]	0.68	0.93	0.1
Fluconazole diol[c]	0.91	1.3	0.1
Fluconazole	1.0	1.0	—
Fluconazole bromohydrine[d]	2.58	1.1	0.1
Epoxyfluconazole[e]	2.59	0.90	0.1
Any other individual impurity	—	1.0	0.1

[a] 4-Amino-1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl) propyl)-4*H*-1,2,4-triazol-1-ium bromide.
[b] 2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl) propan-2-ol.
[c] 2-(2,4-Difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propane-1,2-diol.
[d] 1-Bromo-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol.
[e] 1-[2-(2,4-Difluorophenyl)-2,3-epoxypropyl]-1*H*-1-2,4-triazole.

SPECIFIC TESTS
• **STERILITY TESTS** ⟨71⟩: Meets the requirements
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.416 USP Endotoxin Unit/mg of fluconazole
• **OTHER REQUIREMENTS:** Meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Store at controlled room temperature.
• **LABELING:** Label to indicate the vehicle used. If a test for *Organic Impurities* other than *Procedure 1* and *Procedure 2* is used, then the labeling states with which *Organic Impurities* test the article complies.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluconazole RS

www.webofpharma.com

Fluconazole in Dextrose Injection

(Title for this monograph–not to change until November 1, 2018)
(Prior to November 1, 2018, the current practice of labeling the article of commerce with the name Fluconazole Injection may be continued. Use of the name Fluconazole in Dextrose Injection will be permitted as of May 1, 2016; however, the use of this name will not be mandatory until November 1, 2018. The 30-month extension will provide the time needed by manufacturers and users to make necessary changes.)

DEFINITION

Fluconazole in Dextrose Injection is a sterile solution containing Fluconazole and Dextrose. It contains NLT 90.0% and NMT 110.0% of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. Test for Dextrose**

Analysis: Add a few drops of the Injection (1 in 20) to 5 mL of hot alkaline cupric tartrate TS.
Acceptance criteria: A red to orange precipitate of cuprous oxide is formed.

ASSAY

- **Procedure**

Buffer: 0.82 g/L of anhydrous sodium acetate in water. Adjust with 1 N acetic acid solution to a pH of 5.0.
Diluent: Methanol and *Buffer* (20:80)
Solution A: Methanol and *Buffer* (5:95)
Solution B: Acetonitrile and methanol (60:40)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
9	80	20
15	15	85
18	80	20
25	80	20

System suitability solution: 0.04 mg/mL each of benzyl alcohol and USP Fluconazole RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Fluconazole RS in *Diluent*
Sample solution: Nominally 0.2 mg/mL of fluconazole from Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 261 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for benzyl alcohol and fluconazole are about 0.8 and 1.0, respectively, from the *System suitability solution*.]
Suitability requirements
Resolution: NLT 1.8 between benzyl alcohol and fluconazole, *System suitability solution*
Tailing factor: NMT 1.5 for the fluconazole peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluconazole from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **Organic Impurities Procedure 1: For Nonpolar Impurities**

Buffer, Diluent, Solution A, and **Solution B:** Prepare as directed in the *Assay*.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	77	23
5	77	23
30	40	60
43	77	23
50	77	23

System suitability solution: 2.4 µg/mL of 1,4-benzoquinone and 20 µg/mL of USP Fluconazole RS in *Diluent*
Standard solution: 2 µg/mL of USP Fluconazole RS in *Diluent*
Sample solution: Nominally 1.0 mg/mL of fluconazole from Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 261 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for 1,4-benzoquinone and fluconazole are about 0.5 and 1.0, respectively, *System suitability solution*.]
Suitability requirements
Resolution: NLT 5.0 between 1,4-benzoquinone and fluconazole, *System suitability solution*
Tailing factor: NMT 1.5 for the fluconazole peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

[NOTE—For the following calculations, do not include peaks eluting before fluconazole and do not include impurities at relative retention times of 2.00–2.12 and 3.14–3.26. The disregarded impurities at the specified relative retention times are process impurities monitored in the drug substance. Furthermore, disregard any peak due to an excipient or any peak less than 0.02%. This test is for determination of the late-eluting peaks, and hence the early-eluting peaks are not quantitated using this procedure.]

Calculate the percentage of the largest unknown nonpolar impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the largest unknown nonpolar impurity from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Calculate the percentage of total unknown nonpolar impurities in the portion of Injection taken:

$$\text{Result} = (r_T/r_S) \times (C_S/C_U) \times 100$$

r_T = sum of the peak responses of all the unknown impurities from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Acceptance criteria
Largest unknown nonpolar impurity: NMT 0.1%
Total unknown nonpolar impurities: NMT 0.5%

• **ORGANIC IMPURITIES PROCEDURE 2: FOR POLAR IMPURITIES**

Buffer, Diluent, Solution A, Solution B, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 2 µg/mL of USP Fluconazole RS in *Diluent*

Sample solution: Nominally 0.2 mg/mL of fluconazole from Injection in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for benzyl alcohol and fluconazole are about 0.8 and 1.0, respectively, *System suitability solution*.]

Suitability requirements

Resolution: NLT 1.8 between benzyl alcohol and fluconazole, *System suitability solution*

Tailing factor: NMT 1.5 for the fluconazole peak, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—The relative retention times are listed in *Table 3*.]

Calculate the percentage of the single largest unknown polar impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the largest unknown polar impurity from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Calculate the percentage of total unknown polar impurities in the portion of Injection taken:

$$\text{Result} = (r_T/r_S) \times (C_S/C_U) \times 100$$

r_T = sum of the peak responses of all the unknown polar impurities from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard any peak due to an excipient or any peak less than 0.03%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Hydroxymethylfurfural[a]	0.22–0.28	—
Aminofluconazole quaternary salt[c]	0.30–0.36	—[b]
Unidentified dextrose-related impurity	0.37–0.43	—
Fluconazole isomer[d]	0.47–0.59	—[b]
Fluconazole diol[e]	0.68–0.74	—[b]
Cyclohexanone[f]	0.77–0.83	—
Fluconazole	1.0	—
Largest unknown polar impurity	—	0.1
Total unknown polar impurities	—	0.5
Total unknown polar and nonpolar impurities (sum of results from *Procedure 1* and *Procedure 2*)	—	1.0

[a] 5-Hydroxymethylfurfural.
[b] These are process impurities which are included in this table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and are not included in the total impurities.
[c] 4-Amino-1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl) propyl)-4*H*-1,2,4-triazolium bromide.
[d] 2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl) propan-2-ol.
[e] 2-(2,4-Difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propane-1,2-diol.
[f] Potential impurity associated with drug product packaged in bags.

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩: Meets the requirements
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.416 USP Endotoxin Units/mg of fluconazole
• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluconazole RS

www.webofpharma.com

Fluconazole in Sodium Chloride Injection

(Title for this monograph—not to change until November 1, 2018)
(Prior to November 1, 2018, the current practice of labeling the article of commerce with the name Fluconazole Injection may be continued. Use of the name Fluconazole in Sodium Chloride Injection will be permitted as of May 1, 2016; however, the use of this name will not be mandatory until November 1, 2018. The 30-month extension will provide the time needed by manufacturers and users to make necessary changes.)

DEFINITION

Fluconazole in Sodium Chloride Injection is a sterile solution containing Fluconazole and Sodium Chloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 0.82 g/L of anhydrous sodium acetate in water. Adjust with 1 N acetic acid solution to a pH of 5.0.
Diluent: Methanol and *Buffer* (20:80)
Solution A: Methanol and *Buffer* (5:95)
Solution B: Acetonitrile and methanol (60:40)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
9	80	20
15	15	85
18	80	20
25	80	20

System suitability solution: 0.04 mg/mL each of benzyl alcohol and USP Fluconazole RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Fluconazole RS in *Diluent*
Sample solution: Nominally 0.2 mg/mL of fluconazole from Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 261 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for benzyl alcohol and fluconazole are about 0.8 and 1.0, respectively, *System suitability solution*.]
Suitability requirements
Resolution: NLT 1.8 between benzyl alcohol and fluconazole, *System suitability solution*
Tailing factor: NMT 1.5 for the fluconazole peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **SODIUM CHLORIDE CONTENT**

Solution A: 2 g/L of polyvinyl alcohol in water
Solution B: Transfer 25 mL of 65% nitric acid to a 100-mL volumetric flask and dilute with water to volume.
Sample solution: Transfer 5 mL of Injection to a suitable container, and add 40 mL of water, 1 mL of *Solution A*, and 1 mL of *Solution B*.
Titrant: 0.1 N silver nitrate
Analysis: Perform potentiometric titration with *Titrant*. Each mL of *Titrant* is equivalent to 5.844 mg of sodium chloride (NaCl).
Acceptance criteria: 95%–105% of the labeled amount of sodium chloride (NaCl)

IMPURITIES

• **ORGANIC IMPURITIES, PROCEDURE 1: FOR NONPOLAR IMPURITIES**

[NOTE—On the basis of the synthetic route, perform either (a) *Procedure 1* and *Procedure 2*, or (b) *Procedure 3*, or (c) *Procedure 4*. *Procedure 3* is recommended if bistriazole ketone and epoxyfluconazole (see *Table 4*) are potential impurities. *Procedure 4* is recommended if fluconazole bromohydrine and epoxyfluconazole (see *Table 5*) are potential impurities.]
Buffer, Diluent, Solution A, and **Solution B:** Prepare as directed in the *Assay*.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	77	23
5	77	23
30	40	60
43	77	23
50	77	23

System suitability solution: 2.4 µg/mL of 1,4-benzoquinone and 20 µg/mL of USP Fluconazole RS in *Diluent*
Standard solution: 2 µg/mL of USP Fluconazole RS in *Diluent*
Sample solution: Nominally 1.0 mg/mL of fluconazole from Injection in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

www.webofpharma.com

Detector: UV 261 nm
Column: 4.0-mm × 10-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for 1,4-benzoquinone and fluconazole are about 0.5 and 1.0, respectively, *System suitability solution*.]
Suitability requirements
Resolution: NLT 5.0 between 1,4-benzoquinone and fluconazole, *System suitability solution*
Tailing factor: NMT 1.5 for the fluconazole peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—For the following calculations, do not include peaks eluting before fluconazole and do not include impurities at relative retention times of 2.00–2.12 and 3.14–3.26. The disregarded impurities at the specified relative retention times are process impurities monitored in the drug substance. Furthermore, disregard any peak due to an excipient or any peak less than 0.02%. This test is for determination of the late-eluting peaks, and hence the early-eluting peaks are not quantitated using this procedure.]
Calculate the percentage of the largest unknown nonpolar impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the largest unknown nonpolar impurity from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Calculate the percentage of total unknown nonpolar impurities in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses of all the unknown nonpolar impurities from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Acceptance criteria
Largest unknown nonpolar impurity: NMT 0.1%
Total unknown nonpolar impurities: NMT 0.5%

• **ORGANIC IMPURITIES, PROCEDURE 2: FOR POLAR IMPURITIES**

Buffer, Diluent, Solution A, Solution B, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 2 µg/mL of USP Fluconazole RS in *Diluent*
Sample solution: Nominally 0.2 mg/mL of fluconazole from Injection in *Diluent*
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for benzyl alcohol and fluconazole are about 0.8 and 1.0, respectively, *System suitability solution*.]
Suitability requirements
Resolution: NLT 1.8 between benzyl alcohol and fluconazole, *System suitability solution*
Tailing factor: NMT 1.5 for the fluconazole peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times of known related compounds are listed in *Table 3*.]
Calculate the percentage of the single largest unknown polar impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the largest unknown polar impurity from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Calculate percentage of the total unknown polar impurities in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses of all the unknown polar impurities from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard any peak due to an excipient or any peak less than 0.03%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminofluconazole quaternary salt[a]	0.30–0.36	—[b]
Fluconazole isomer[c]	0.47–0.59	—[b]
Fluconazole diol[d]	0.68–0.74	—[b]
Cyclohexanone[e]	0.77–0.83	—
Fluconazole	1.0	—
Largest unknown polar impurity	—	0.1
Total unknown polar impurities	—	0.5

www.webofpharma.com

Table 3 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total unknown polar and nonpolar impurities (sum of results from *Procedure 1* and *Procedure 2*)	—	1.0

[a] 4-Amino-1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl) propyl)-4*H*-1,2,4-triazolium bromide.
[b] These are process impurities which are included in this table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and are not included in the total impurities.
[c] 2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl) propan-2-ol.
[d] 2-(2,4-Difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propane-1,2-diol.
[e] Potential impurity associated with drug product packaged in bags.

• **Organic Impurities, Procedure 3**

Buffer: 0.63 g/L of ammonium formate in water
Mobile phase: Acetonitrile and *Buffer* (14:86)
Standard solution: 2 mg/mL of USP Fluconazole RS in *Buffer*. [Note—Use approximately 14% of the total volume of acetonitrile, and sonicate if necessary to facilitate dissolution.]
Sensitivity solution: 1 µg/mL of USP Fluconazole RS in *Mobile phase* from the *Standard solution*
Sample solution: Nominally 2 mg/mL of fluconazole in 0.9% aqueous solution of sodium chloride
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *Sensitivity solution*
[Note—The retention time for fluconazole is between 12 and 14 min.]
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bistriazole ketone[a]	0.13	0.2
Fluconazole isomer[b]	0.5	0.2
Fluconazole	1.0	—
Epoxyfluconazole[c]	2.6	0.2
Any other individual impurity	—	0.2
Total impurities	—	0.5

[a] 1,3-Bis(1*H*-1,2,4-triazol-1-yl)propan-2-one.
[b] 2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl) propan-2-ol.
[c] 1-[2-(2,4-Difluorophenyl)-2,3-epoxypropyl]-1*H*-1-2,4-triazole.

• **Organic Impurities, Procedure 4**

Buffer: 13.4 g/L of dibasic sodium phosphate heptahydrate in water. Adjust with phosphoric acid to a pH of 7.0.
Mobile phase: Acetonitrile and *Buffer* (26:74)
Standard solution: 2 mg/mL of USP Fluconazole RS in *Mobile phase*
Sample solution: Nominally 2 mg/mL of fluconazole in 0.9% aqueous solution of sodium chloride
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 0.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 5*)

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Aminofluconazole quaternary salt[a]	0.57	0.74	0.1
Fluconazole isomer[b]	0.68	0.93	0.1
Fluconazole diol[c]	0.91	1.3	0.1
Fluconazole	1.0	1.0	—
Fluconazole bromohydrine[d]	2.58	1.1	0.1
Epoxyfluconazole[e]	2.59	0.90	0.1
Any other individual impurity	—	1.0	0.1

USP Monographs

www.webofpharma.com

Table 5 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	0.5

[a] 4-Amino-1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl) propyl)-4*H*-1,2,4-triazol-1-ium bromide.
[b] 2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl) propan-2-ol.
[c] 2-(2,4-Difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propane-1,2-diol.
[d] 1-Bromo-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol.
[e] 1-[2-(2,4-Difluorophenyl)-2,3-epoxypropyl]-1*H*-1-2,4-triazole.

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.416 USP Endotoxin Unit/mg of fluconazole
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* and *Procedure 2* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fluconazole RS

Fluconazole for Oral Suspension

DEFINITION
Fluconazole for Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$). It may contain a suitable preservative.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Diluted phosphoric acid: Phosphoric acid (1 in 10)
Buffer: 2.72 g/L of monobasic potassium phosphate, adjusted with *Diluted phosphoric acid* to a pH of 2.5
Mobile phase: Acetonitrile and *Buffer* (20:80)
Diluent: Methanol and water (50:50)
System suitability solution: 0.1 mg/mL of USP Fluconazole RS and 0.024 mg/mL of USP Sodium Benzoate RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Fluconazole RS in *Diluent*
Sample solution: Reconstitute the sample as directed on the label. Transfer an accurately weighed quantity of the suspension to a suitable volumetric flask to obtain a nominal concentration of 0.1 mg/mL of fluconazole. Sonicate in 70% of the flask volume of *Diluent* for 20 min with intermittent shaking. Dilute with *Diluent* to final volume, mix, centrifuge, and pass through a suitable membrane filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 260 nm
Column: 4.6-mm × 15.0-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 5.0 between the fluconazole and benzoate peaks
Tailing factor: NMT 2.0 for the fluconazole peak
Relative standard deviation: NMT 2.0% for the fluconazole peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$) in the portion of Fluconazole for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution*
C_U = nominal concentration of the *Sample solution*

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Test 1
Medium: Water; 900 mL for 200 mg/5 mL suspension; 500 mL for 50 mg/5 mL suspension
Apparatus 2: 50 rpm
Time: 30 min
Standard stock solution: 1.1 mg/mL of USP Fluconazole RS in methanol
Standard solution
200 mg/5 mL suspension: 0.22 mg/mL of USP Fluconazole RS in *Medium* from the *Standard stock solution*
50 mg/5 mL suspension: 0.11 mg/mL of USP Fluconazole RS in *Medium* from the *Standard stock solution*
Sample solution: Reconstitute the suspension according to the label instructions. Weigh, and transfer an amount of the reconstituted suspension equivalent to 1 dose to the vessel. At the time specified withdraw 10 mL of the solution under test, and pass through a suitable filter of 0.45-µm pore size.
Mobile phase: Acetonitrile and water (20:80)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$) dissolved (Q):

$$\text{Result} = (r_U/r_S) \times C_S \times (V_1/V_2) \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

USP Monographs

www.webofpharma.com

C_S = concentration of the *Standard solution* (mg/mL)
V_1 = volume of *Medium* (900 mL or 500 mL)
V_2 = volume of the reconstituted suspension in the *Sample solution* (mL). [NOTE—This is equivalent to the weight (g) of the reconstituted suspension in the *Sample solution* divided by the density of the reconstituted solution (g/mL).]
L = label claim (mg/mL)

Tolerances: NLT 85% (Q) of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 900 mL for 200 mg/5 mL suspension; 500 mL for 50 mg/5 mL suspension
Apparatus 2: 75 rpm
Time: 30 min
Mobile phase: Methanol and water (40:60). Adjust with glacial acetic acid to a pH of 3.4.
Standard solution: 0.055 mg/mL of USP Fluconazole RS and 0.025 mg/mL of USP Sodium Benzoate RS in *Medium*
Sample solution: Reconstitute the suspension according to the label instructions. Weigh, and transfer an amount of the reconstituted suspension equivalent to one dose to the vessel. At the time specified withdraw an aliquot of the solution under test (5.0 mL for 50 mg/5 mL; 2.5 mL for 200 mg/5 mL), transfer to a 10-mL volumetric flask, and dilute with *Medium* to volume. Pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 261 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 40 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between fluconazole and sodium benzoate
Tailing factor: NMT 2.0 for the fluconazole peak
Relative standard deviation: NMT 2.0% for the fluconazole peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$) dissolved (Q):

$$\text{Result} = (r_U/r_S) \times C_S \times (V_1/V_2) \times (1/L) \times 100$$

r_U = peak response of fluconazole from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
V_1 = volume of *Medium* (900 mL or 500 mL)
V_2 = volume of the reconstituted suspension in the *Sample solution* (mL). [NOTE—This is equivalent to the weight (g) of the reconstituted suspension in the *Sample solution* divided by the density of the reconstituted solution (g/mL).]
L = label claim (mg/mL)

Tolerances: NLT 85% (Q) of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$) is dissolved.

• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements for oral suspension packaged in multiple-unit containers

IMPURITIES
• **PROCEDURE**
Solution A: 0.63 g/L of ammonium formate in water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	87	13
20	87	13
35	60	40
50	60	40
52	87	13
60	87	13

Diluent: Acetonitrile and *Solution A* (13:87)
System suitability solution: 0.3 mg/mL of USP Fluconazole RS, 3 µg/mL of USP Fluconazole Related Compound B RS, and 3 µg/mL of USP Fluconazole Related Compound C RS in *Diluent*. [NOTE—The use of sonication and stepwise dilutions may be appropriate.]
Standard solution: 6 µg/mL of USP Fluconazole RS in *Diluent*. [NOTE—The use of sonication and stepwise dilutions may be appropriate.]
Sample solution: Reconstitute the sample as directed on the label. Transfer an accurately weighed quantity of the suspension to a suitable volumetric flask to obtain a nominal concentration of 3 mg/mL of fluconazole. Sonicate in 40% of the flask volume of *Diluent* for 20 min with intermittent shaking. Dilute with *Diluent* to final volume, mix, centrifuge, and pass through a suitable membrane filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 260 nm
Column: 4.6-mm × 12.5-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between the fluconazole related compound B and fluconazole related compound C peaks and NLT 4.0 between the fluconazole related compound C and fluconazole peaks, *System suitability solution*
Tailing factor: NMT 2.0 for the fluconazole peak, *System suitability solution*
Relative standard deviation: NMT 5.0% for the fluconazole peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Fluconazole for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of fluconazole from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)

USP Monographs

www.webofpharma.com

C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fluconazole related compound A[a]	0.45	—[b]
Fluconazole isomer[c]	0.51	—[b]
Fluconazole related compound B	0.71	—[b]
Fluconazole related compound C	0.78	—[b]
Fluconazole	1.0	—
Any other individual, unspecified impurity	—	0.25
Total impurities	—	0.4

[a] 2-[2-Fluoro-4-(1*H*-1,2,4-triazol-1-yl)phenyl]-1,3-bis(1*H*-1,2,4-triazol-1-yl)-propan-2-ol.
[b] These are process impurities which are included in the table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and should not be included in the total impurities.
[c] 2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4*H*-1,2,4-triazol-4-yl) propan-2-ol.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/g, and the total combined molds and yeasts count does not exceed 5×10^1 cfu/g. It meets the requirements of the test for absence of *Escherichia coli*.
- **pH** ⟨791⟩**:** 3.0–5.0, in a suspension reconstituted as directed by the labeling

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store dry powder below 30°. Store reconstituted suspension at 5°–30°, and protect from freezing.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fluconazole RS
  USP Fluconazole Related Compound B RS
  2-(4-Fluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)propan-2-ol.
  $C_{13}H_{13}FN_6O$ 288.28
  USP Fluconazole Related Compound C RS
  1,1′-(1,3-Phenylene)di(1*H*-1,2,4-triazole).
  $C_{10}H_8N_6$ 212.21
  USP Sodium Benzoate RS

Fluconazole Tablets

DEFINITION

Fluconazole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.01 M anhydrous sodium acetate solution. Adjust with glacial acetic acid to a pH of 5.0.
Mobile phase: Methanol, acetonitrile, and *Buffer* (20:10:70)
Standard stock solution: 1.0 mg/mL of USP Fluconazole RS dissolved in water, and diluted with *Mobile phase* to volume. Sonicate the solution, if necessary. [NOTE—The target ratio is about 5% water to 95% *Mobile phase*.]
Standard solution: 0.2 mg/mL of USP Fluconazole RS in *Mobile phase* prepared from the *Standard stock solution*
Sample solution: Weigh NLT 5 Tablets, and disperse in a suitable quantity of water. Sonicate the solution, if necessary. Add a sufficient quantity of *Mobile phase*, sonicate for 5 min, and shake for 30 min. Dilute with *Mobile phase* to volume to obtain a concentration between about 1 and 4 mg/mL, and mix. [NOTE—The target ratio is about 5% water to 95% *Mobile phase*.] Centrifuge a suitable portion of the mixture. Filter and quantitatively dilute a portion of the supernatant with *Mobile phase* to obtain a solution of about 0.2 mg/mL of fluconazole.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 261 nm
Column: 3.9-mm × 150-mm; 4-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1100 theoretical plates
Tailing factor: NMT 3.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1
Medium: Water; 500 mL (900 mL for Tablets labeled to contain more than 100 mg)
Apparatus 2: 50 rpm
Time: 45 min
Buffer, Mobile phase, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 2 mg/mL of USP Fluconazole RS in *Medium*. Sonicate the solution to facilitate dissolution, if necessary. Quantitatively dilute a portion of this solution with *Medium* to obtain a final concentration similar to the one expected in the *Sample solution*.

USP Monographs

www.webofpharma.com

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system and **System suitability:** Proceed as directed in the *Assay*, making any necessary modifications.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluconazole RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 or 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of $C_{13}H_{12}F_2N_6O$ is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets *Dissolution Test 2*.
Medium: Water; 900 mL (for all Tablet strengths)
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase: Water and acetonitrile (4:1)
Standard stock solution: 1.1 mg/mL of USP Fluconazole RS in methanol
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (L/900) mg/mL, where L is the Tablet label claim in mg.
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Calculate the percentage of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$) dissolved:

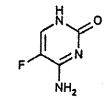
$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of fluconazole is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.
• **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP Reference Standards** ⟨11⟩
USP Fluconazole RS

Flucytosine



$C_4H_4FN_3O$ 129.09
Cytosine, 5-fluoro-.
5-Fluorocytosine [2022-85-7].

» Flucytosine contains not less than 98.5 percent and not more than 101.0 percent of $C_4H_4FN_3O$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Flucytosine RS
USP Fluorouracil RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 8 µg per mL.
Medium: dilute hydrochloric acid (1 in 100).
Absorptivities at 285 nm, calculated on the dried basis, do not differ by more than 2.0%.
B: The R_F value of the principal spot in the specimen chromatogram in the test for *Fluorouracil* corresponds to that obtained with the solution of USP Flucytosine RS.
Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 1.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Fluoride ions— [Note—All glassware and/or plasticware used in this test should be scrupulously clean and even free from trace amounts of fluoride. The use of plasticware to contain the solutions while the potential is measured is recommended.]
Buffer solution—To 110 g of sodium chloride in a 2-L volumetric flask add 1 g of sodium citrate and 700 mL of water, and dissolve with shaking. Carefully add 150 g of sodium hydroxide, and dissolve with shaking. Cool to room temperature, and while stirring, cautiously add 450 mL of glacial acetic acid. Cool to room temperature, add 600 mL of isopropyl alcohol, dilute with water to volume, and mix. The pH of this solution is between 5.0 and 5.5.
Standard stock solution—Accurately weigh 2.211 g of sodium fluoride, previously dried at 150° for 4 hours, into a 1-L volumetric flask, and dissolve in about 200 mL of water. Add 1.0 mL of sodium hydroxide solution (1 in 250), dilute with water to volume, and mix. Each mL of this solution contains 1 mg of fluoride ion. Store the solution in a closed plastic container.
Standard preparations—Dilute a portion of *Standard stock solution* quantitatively and stepwise with *Buffer solution* to obtain a *Standard preparation* having a fluoride concentration

USP Monographs

www.webofpharma.com

of 1 µg per mL. Prepare the final dilution in a 100-mL volumetric flask. In the same manner, prepare additional *Standard preparations* having fluoride concentrations of 3 µg per mL, 5 µg per mL, and 10 µg per mL, respectively.

Test preparation—Place 1 g of Flucytosine, accurately weighed, in a 100-mL volumetric flask, and dissolve in and dilute with *Buffer solution* to volume.

Procedure—Concomitantly measure the potential (see *Titrimetry* ⟨541⟩), in mV, of the *Standard preparations* and the *Test preparation*, with a suitable pH meter equipped with a fluoride-specific ion electrode and a glass-sleeved calomel reference electrode that has been modified in the following manner. Mix 70 mL of freshly prepared saturated potassium chloride solution with 30 mL of isopropyl alcohol, fill the electrode with the clear supernatant, and allow the electrode to remain in the mixture for not less than 2 hours prior to use, or preferably overnight.

When taking the measurements, transfer the solution to a 150-mL beaker, and immerse the electrodes. Insert a polytef-coated stirring bar into the beaker, place the beaker on a magnetic stirrer having an insulated top, and allow to stir until equilibrium is attained (about 1 to 2 minutes). Rinse and dry the electrodes between measurements, taking care not to scratch the crystal in the specific ion electrode.

Measure the potential of each *Standard preparation*, and plot the fluoride concentration, in mg per 100 mL, versus the potential, in mV, on semilogarithmic paper. Measure the potential of the *Test preparation*, and determine from the standard curve the fluoride concentration, in mg per 100 mL. Calculate the percentage of fluoride in the portion of Flucytosine taken by the formula:

$$C/10$$

in which C is the fluoride concentration, in mg per 100 mL, from the standard curve: not more than 0.05% of fluoride is found.

Fluorouracil—Dissolve 250 mg in 10 mL of a mixture of glacial acetic acid and water (4:1). Apply 20 µL of this solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.5-mm layer of chromatographic silica gel mixture. To the same plate apply 20 µL, in 10-µL increments, of a 0.025 mg per mL solution of USP Fluorouracil RS in a mixture of glacial acetic acid and water (4:1). Develop the chromatogram in a mixture of chloroform and glacial acetic acid (13:7) until the solvent front has moved not less than 14 cm from the origin. Remove the plate from the developing chamber, and allow the solvent to evaporate. Locate the spots on the plate by observing under short-wavelength UV radiation: any spot from the solution under test is not greater in size and intensity than the spot at the respective R_F produced by the Standard solution, corresponding to not more than 0.1% of fluorouracil.

Assay—Place about 400 mg of Flucytosine, accurately weighed, in a 250-mL beaker, add 150 mL of a mixture of 2 volumes of glacial acetic acid and 1 volume of acetic anhydride, and dissolve, warming gently if necessary. Titrate potentiometrically with 0.1 N perchloric acid VS, using a calomel-glass electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 12.91 mg of $C_4H_4FN_3O$.

Flucytosine Capsules

DEFINITION

Flucytosine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of flucytosine ($C_4H_4FN_3O$).

IDENTIFICATION

- **A.** The UV absorption spectrum of the *Sample solution* exhibits maximum absorption at the same wavelength as that of the *Standard solution*, as obtained in the *Assay*, and the two spectra are similar between 260 and 350 nm.
- **B.**

Solution A: 100 mg of sodium (tri)pentacyanoaminoferrate in 20 mL of sodium carbonate solution (1 in 100)

Sample solution: Equivalent of 500 mg of flucytosine from Capsules, in 10 mL of water

Analysis: Filter the *Sample solution*, and to 2 mL of the filtrate add 1 mL of *Solution A* and 1 mL of 3% hydrogen peroxide.

Acceptance criteria: On standing, a darker green is produced than that produced by a blank.

ASSAY

- **PROCEDURE**

Diluent: 0.1 N hydrochloric acid

Standard solution: 8 µg/mL of USP Flucytosine RS in *Diluent*

Sample solution: Nominally 250 mg of flucytosine from the contents of NLT 20 Capsules. Determine the average weight per Capsule, in 50 mL of *Diluent*. Shake by mechanical means for 30 min, dilute with *Diluent* to 250.0 mL, and filter, discarding the first 20 mL of the filtrate. Dilute 10 mL of the clear filtrate with *Diluent* to 250 mL. Dilute 10.0 mL of this solution with *Diluent* to 50 mL.

Blank: *Diluent*

Instrumental conditions

Mode: UV

Analytical wavelength: 285 nm

Cell: 1 cm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of the labeled amount of flucytosine ($C_4H_4FN_3O$) in the portion of Capsule contents taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Flucytosine RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of flucytosine in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 60 min

Detector: UV 276 nm

Standard solution: USP Flucytosine RS in *Medium*

Sample solution: Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: NLT 80% (Q) of the labeled amount of flucytosine ($C_4H_4FN_3O$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Flucytosine RS

Flucytosine Compounded Oral Suspension

DEFINITION
Flucytosine Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of flucytosine ($C_4H_4FN_3O$).
Prepare Flucytosine Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Flucytosine	1 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Empty the contents of the required number of capsules into a suitable mortar, or add *Flucytosine* powder to the mortar. Add 10 mL of *Vehicle*, and mix to a uniform paste. Add *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

ASSAY
- **PROCEDURE**

Solution A: 1 g of ammonium acetate and 1 mL of diisopropylamine in 1 L of water. Adjust with glacial acetic acid to a pH of 7.5.
Mobile phase: Methanol and *Solution A* (50:50). Filter, and degas.
Standard solution: 50 µg/mL of USP Flucytosine RS in *Mobile phase*
Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 0.5 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 200-mm; 5-µm packing L3
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for flucytosine is about 3 min.]
Suitability requirements
Relative standard deviation: NMT 1.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of flucytosine ($C_4H_4FN_3O$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

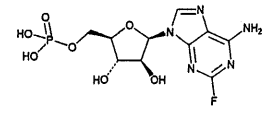
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Flucytosine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of flucytosine in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 4.0–5.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored in a refrigerator
- **LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Flucytosine RS

Fludarabine Phosphate



$C_{10}H_{13}FN_5O_7P$ 365.21
9*H*-Purin-6-amine, 2-fluoro-9-(5-*O*-phosphono-β-D-arabinofuranosyl)-;
9-β-D-Arabinofuranosyl-2-fluoroadenine 5′-(dihydrogen phosphate) [75607-67-9].

DEFINITION
Fludarabine Phosphate contains NLT 98.0% and NMT 102.0% of fludarabine phosphate ($C_{10}H_{13}FN_5O_7P$), calculated on the anhydrous, solvent-free basis.
[**CAUTION**—Fludarabine phosphate is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: 10 mM monobasic potassium phosphate
Mobile phase: Methanol and *Solution A* (6:94)
Standard solution: 0.02 mg/mL of USP Fludarabine Phosphate RS in *Mobile phase*
Sample solution: 0.02 mg/mL of Fludarabine Phosphate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1

USP Monographs

www.webofpharma.com

Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fludarabine phosphate ($C_{10}H_{13}FN_5O_7P$) in the portion of Fludarabine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fludarabine Phosphate RS in the *Standard solution* (mg/mL)
C_U = concentration of Fludarabine Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous, solvent-free basis

IMPURITIES

• **CHLORIDE**
Standard stock solution: 82.4 µg/mL of sodium chloride in water
Standard solution: Transfer 2.0 mL of *Standard stock solution* to a test tube, add 13.0 mL of water, and mix.
Sample solution: Transfer 50.0 mg of Fludarabine Phosphate to a test tube, add 15 mL of water to dissolve, and heat gently if necessary.
Analysis: Add 1.0 mL of nitric acid to the *Standard solution* and *Sample solution*, and place each in separate, colorless test tubes containing 1.0 mL of silver nitrate TS.
Acceptance criteria: NMT 0.2%; the *Sample solution* shows less turbidity than the *Standard solution*.

• **LIMIT OF FREE PHOSPHATE**
Reagent solution: Mix 4 g of finely powdered ammonium molybdate and 0.1 g of finely powdered ammonium vanadate in a 150-mL beaker. Add 70 mL of water, and grind the particles using a glass rod. A clear solution is obtained within a few minutes. Add 20 mL of nitric acid, adjust to room temperature, and dilute with water to 100 mL.
Standard solution: 7.16 µg/mL of potassium dihydrogen phosphate in water. Transfer 2.0 mL of this solution to a test tube.
Sample solution: Transfer 10 mg of Fludarabine Phosphate in 2.0 mL of water to a test tube and heat gently.
Blank: 2.0 mL of water in a test tube
Analysis: To each of the test tubes containing the *Standard solution*, *Sample solution*, and *Blank*, add 2.0 mL of *Reagent solution*.
Acceptance criteria: NMT 0.1%; the color of the *Standard solution* must be more intense than that of the *Blank*. Viewed downward in diffuse daylight against a white background, the yellow coloration of the *Sample solution* must not be more intense than that of the *Standard solution*.

• **LIMIT OF SODIUM**
Standard stock solution: 2.54 mg/mL of sodium chloride in water. Sodium chloride is previously dried at 105° for 2 h.
Standard solution: 1 µg/mL of sodium in water, from *Standard stock solution*
Sample solution: 0.5 mg/mL of Fludarabine Phosphate in water
Instrumental conditions
Mode: Flame photometry
Analytical wavelength: Sodium emission line at 589.0 nm
Blank: Water
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: NMT 0.2%; the emission response of the *Sample solution* is NMT that of the *Standard solution*.

• **ORGANIC IMPURITIES, PROCEDURE 1: EARLY-ELUTING IMPURITIES**
Mobile phase, Standard solution, and Chromatographic system: Proceed as directed in the *Assay*.
System suitability solution: 10 mg of Fludarabine Phosphate in 10 mL of 0.1 N hydrochloric acid. Heat the solution at 80° in a water bath for 15 min.
Sensitivity solution: 0.5 µg/mL of USP Fludarabine Phosphate RS in *Mobile phase*, from the *Standard solution*
Sample solution: 1 mg/mL of Fludarabine Phosphate in *Mobile phase*
System suitability
Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between the iso-ara-guanine monophosphate and isoguanine peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each early-eluting impurity in the portion of Fludarabine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (1/F_I) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of fludarabine phosphate from the *Sample solution*
F_I = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Iso-ara-guanine-monophosphate[a]	0.26	0.25	0.8
Isoguanine[b]	0.34	0.40	0.2
3′,5′-Diphosphate analog[c]	0.42	0.53	0.4
Any individual, unspecified impurity	<1.0	1.0	0.1
Fludarabine phosphate	1.0	—	—

[a] 6-Amino-9-β-D-arabinofuranosyl-2-oxo-1*H*-purine 5′-(dihydrogen phosphate).
[b] 6-Amino-1*H*-purin-2(9*H*)-one.
[c] 9-β-D-Arabinofuranosyl-2-fluoroadenine 3′,5′-bis(dihydrogen phosphate).

• **ORGANIC IMPURITIES, PROCEDURE 2: LATE-ELUTING IMPURITIES**
Solvent A: 10 mM monobasic potassium phosphate
Mobile phase: Methanol and *Solvent A* (1:4)

www.webofpharma.com

Standard solution and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution and **Sample solution:** Prepare as directed in *Organic Impurities, Procedure 1: Early-Eluting Impurities*.
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each late-eluting impurity in the portion of Fludarabine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (1/F_2) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of fludarabine phosphate from the *Sample solution*
F_2 = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fludarabine phosphate	1.0	—	—
2-Fluoroadenine[a]	1.5	2.0	0.1
2-Fluoro-ara-adenine[b]	1.9	1.7	0.2
2-Ethoxyphosphate analog[c]	2.5	0.56	0.2
Any individual, unspecified impurity	>1.0	1.0	0.1
Total unspecified impurities[d]	—	—	0.5
Total impurities[e]	—	—	1.5

[a] 2-Fluoro-9*H*-purin-6-amine.
[b] 9-β-D-Arabinofuranosyl-2-fluoroadenine.
[c] 2-Ethoxy-9-β-D-arabinofuranosyladenine 5′-(dihydrogen phosphate).
[d] The sum of all unspecified impurities found in *Organic Impurities, Procedure 1: Early-Eluting Impurities* and *Organic Impurities, Procedure 2: Late-Eluting Impurities*.
[e] The sum of all impurities found in *Organic Impurities, Procedure 1: Early-Eluting Impurities* and *Organic Impurities, Procedure 2: Late-Eluting Impurities*.

• **Limit of Alcohol**
Standard solution: 0.50 mg/mL of alcohol in dimethylformamide
Sample solution: 50 mg/mL of Fludarabine Phosphate in dimethylformamide
Blank: Dimethylformamide
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC equipped with a headspace injector
Detector: Flame ionization
Column: 0.25-mm × 30-m; 1.4-µm coating of phase G43
Temperatures
Injection port: 160°
Detector: 250°
Column: See *Table 3*.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	10
40	5	70	—
70	30	220	—

Carrier gas: Helium
Flow rate: 27 cm/s
Sample
Volume: 2 mL/vial. [NOTE—Seal the vials using a flanged cap so that the cap can no longer be turned.]
Conditioning temperature: 80°
Conditioning time: 60 min
Injection volume: 1.0 mL
System suitability
Samples: *Standard solution* and *Blank*
[NOTE—The retention time for alcohol is about 3 min.]
Suitability requirements
Relative standard deviation: NMT 4.0% for three injections, *Standard solution*
Peak interference: No peak at the retention time for alcohol, *Blank*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of alcohol in the portion of Fludarabine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of alcohol from the *Sample solution*
r_S = peak area of alcohol from the *Standard solution*
C_S = concentration of alcohol in the *Standard solution* (mg/mL)
C_U = concentration of Fludarabine Phosphate in the *Sample solution* (mg/mL)

[NOTE—Use the percentage obtained to calculate the *Assay* result on the solvent-free basis.]
Acceptance criteria: NMT 1.0%

SPECIFIC TESTS
• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count is NMT 10^3 cfu/g.
• **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 5 mg/mL in water
Acceptance criteria: +10° to +14°
• **Water Determination** ⟨921⟩, *Method I*: NMT 3.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed, light-resistant containers, and store in a refrigerator.
• **USP Reference Standards** ⟨11⟩
USP Fludarabine Phosphate RS

Fludarabine Phosphate Injection

DEFINITION
Fludarabine Phosphate Injection is a sterile solution of Fludarabine Phosphate in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of fludarabine phosphate ($C_{10}H_{13}FN_5O_7P$).

USP Monographs

www.webofpharma.com

[**CAUTION**—Fludarabine Phosphate is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

Solution: 27 µg/mL in 0.1 M hydrochloric acid

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 6.9 g/L of monobasic sodium phosphate monohydrate in water (50 mM). Adjust with 1.0 N sodium hydroxide to a pH of 4.5 ± 0.2.
Mobile phase: Methanol and *Solution A* (3:47)
Standard solution: 0.1 mg/mL of USP Fludarabine Phosphate RS in *Solution A*
Sample solution: Equivalent to 0.1 mg/mL of fludarabine phosphate from Injection diluted with *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8
Relative standard deviation: NMT 1%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of Fludarabine Phosphate ($C_{10}H_{13}FN_5O_7P$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fludarabine Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fludarabine phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

ORGANIC IMPURITIES

- **Procedure 1: Early-Eluting Impurities (Impurities Eluting Before Fludarabine)**

Solution A: 10 mM monobasic potassium phosphate in water
Mobile phase: *Solution A* and methanol (47:3)
System suitability solution: 1 mg/mL of fludarabine phosphate in 0.1 N hydrochloric acid. Heat the solution at 80° in a water bath for 15 min.
Standard solution: 0.02 mg/mL of USP Fludarabine Phosphate RS in *Mobile phase*
Quantitative limit solution: 0.5 µg/mL of USP Fludarabine Phosphate RS in *Mobile phase* from the *Standard solution*
Sample solution: Equivalent to 1 mg/mL of fludarabine phosphate from Injection diluted with *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *Standard solution*, *System suitability solution*, and *Quantitative limit solution*
Suitability requirements
Resolution: NLT 2.0 between the iso-ara-guanine monophosphate and isoguanine peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Quantitative limit solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each early-eluting impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of fludarabine phosphate from the *Sample solution*
F = relative response factor (see *Impurity Table 1*)

Acceptance criteria See *Impurity Table 1*

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Iso-ara-guanine-monophosphate[a]	0.26	0.25	1.0
Isoguanine[b]	0.34	0.40	0.2
3′,5′-Diphosphate analog[c]	0.42	—	—
Fludarabine phosphate	1.0	—	—
Any individual degradation product	<1.0	1.0	0.2

[a] 6-Amino-9-β-D-arabinofuranosyl-2-oxo-1*H*-purine 5′-(dihydrogen phosphate).
[b] 6-Amino-1*H*-purin-2(9*H*)-one.
[c] 9-β-D-Arabinofuranosyl-2-fluoroadenine 3′,5′-bis(dihydrogen phosphate). It is a process impurity and controlled in the drug substance monograph.

- **Procedure 2: Late-Eluting Impurities (Impurities Eluting After Fludarabine)**

Solution A, Standard solution, Quantitative limit solution, Sample solution, and **Chromatographic system:** Proceed as directed in *Procedure 1: Early-Eluting Impurities*
Mobile phase: *Solution A* and methanol (4:1)
System suitability
Samples: *Standard solution* and *Quantitative limit solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Quantitative limit solution*
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of each late-eluting impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of fludarabine phosphate from the *Sample solution*
F = relative response factor (see *Impurity Table 2*)

Acceptance criteria
Individual impurities: See *Impurity Table 2*.
Total impurities: The sum of all fludarabine phosphate degradation products found in *Procedure 1* and *Procedure 2* is NMT 2.0%.

Impurity Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fludarabine phosphate	1.0	—	—
2-Fluoroadenine[a]	1.5	2.0	0.2
2-Fluoro-ara-adenine[b]	1.9	1.7	0.2
2-Ethoxyphosphate analog[c]	2.5	—	—
Any individual degradation product	>1.0	1.0	0.2

[a] 2-Fluoro-9*H*-purin-6-amine.
[b] 9-β-D-Arabinofuranosyl-2-fluoroadenine.
[c] 2-Ethoxy-9-β-D-arabinofuranosyladenine 5′-(dihydrogen phosphate). It is a process impurity and controlled in the drug substance monograph.

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 7.7 USP Endotoxin Units/mg of fludarabine phosphate
- **STERILITY TESTS** ⟨71⟩: Meets the requirements when tested as directed under *Test for Sterility of the Product to be Examined, Membrane Filtration*
- **pH** ⟨791⟩: 6.0–7.1
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers, preferably of Type I glass, protected from light. Store in a refrigerator.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fludarabine Phosphate RS

Fludarabine Phosphate for Injection

DEFINITION
Fludarabine Phosphate for Injection contains NLT 95.0% and NMT 105.0% of the labeled amount of fludarabine phosphate ($C_{10}H_{13}FN_5O_7P$).
[**CAUTION**—Fludarabine Phosphate is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
  Sample solution: 27 µg/mL in 0.1 M hydrochloric acid
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 10 mM of monobasic potassium phosphate
  Mobile phase: Methanol and *Solution A* (6:94)
  Standard solution: 0.02 mg/mL of USP Fludarabine Phosphate RS in *Mobile phase*
  Sample stock solution: 1 mg/mL of fludarabine phosphate in *Mobile phase* prepared as follows. Inject 2.0 mL of *Mobile phase* into each of five vials of Fludarabine Phosphate for Injection. Transfer the contents of the vials into a 250-mL volumetric flask, using *Mobile phase* rinses. Dilute with *Mobile phase* to volume.
  Sample solution: 0.02 mg/mL of fludarabine phosphate in *Mobile phase*, from *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 260 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Flow rate: 1.0 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of fludarabine phosphate ($C_{10}H_{13}FN_5O_7P$) in the portion of Fludarabine Phosphate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fludarabine Phosphate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fludarabine phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meets the requirements

IMPURITIES
- **ORGANIC IMPURITIES PROCEDURE 1: EARLY-ELUTING IMPURITIES**
  Mobile phase, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
  System suitability solution: 10 mg of Fludarabine Phosphate in 10 mL of 0.1 N hydrochloric acid. Heat the solution at 80° in a water bath for 15 min.
  Sensitivity solution: 0.5 µg/mL of USP Fludarabine Phosphate RS in *Mobile phase*, from the *Standard solution*
  Sample solution: Use *Sample stock solution* as directed in the *Assay*.

www.webofpharma.com

System suitability
Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*
Suitability requirements
Resolution: NLT 2.0 between the iso-ara-guanine monophosphate and isoguanine peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each early-eluting impurity in the portion of Fludarabine Phosphate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (1/F_1) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of fludarabine phosphate from the *Sample solution*
F_1 = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Iso-ara-guanine-monophosphate	0.26	0.25	1.0
Isoguanine	0.34	0.40	0.2
3′,5′-Diphosphate analog[a]	0.42	—	—
Any other individual degradation product	<1.0	1.0	0.2
Fludarabine phosphate	1.0	—	—

[a] This is a process impurity and controlled in the drug substance monograph.

• **ORGANIC IMPURITIES PROCEDURE 2: LATE-ELUTING IMPURITIES**
Solvent A: 10 mM monobasic potassium phosphate
Mobile phase: Methanol and *Solvent A* (1:4)
Standard solution and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution and **Sample solution:** Prepare as directed in *Organic Impurities Procedure 1: Early-Eluting Impurities*.
System suitability
Samples: *Standard solution* and *Sensitivity solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Sample: *Sample solution*
Calculate the percentage of each late-eluting impurity in the portion of Fludarabine Phosphate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (1/F_2) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of fludarabine phosphate from the *Sample solution*
F_2 = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

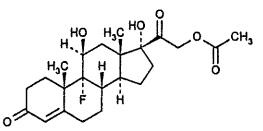
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fludarabine phosphate	1.0	—	—
2-Fluoroadenine	1.5	2.0	0.2
2-Fluoro-ara-adenine	1.9	1.7	0.2
2-Ethoxyphosphate analog[a]	2.5	—	—
Any other individual degradation product	>1.0	1.0	0.2
Total impurities[b]	—	—	2.0

[a] This is a process impurity and controlled in the drug substance monograph.
[b] The sum of all degradation products found in *Organic Impurities Procedure 1* and *Organic Impurities Procedure 2*.

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 7.7 USP Endotoxin Units/mg of fludarabine phosphate
• **pH** ⟨791⟩: 7.2–8.2
• **STERILITY TESTS** ⟨71⟩: Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 5.0%
• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, between 2° and 30°, or at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fludarabine Phosphate RS

Fludrocortisone Acetate



$C_{23}H_{31}FO_6$ 422.49
Pregn-4-ene-3,20-dione, 21-(acetyloxy)-9-fluoro-11,17-dihydroxy-, (11β)-.
9-Fluoro-11β,17,21-trihydroxypregn-4-ene-3,20-dione 21-acetate [514-36-3].

» Fludrocortisone Acetate contains not less than 97.0 percent and not more than 103.0 percent of $C_{23}H_{31}FO_6$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, protected from light.

www.webofpharma.com

USP Reference standards ⟨11⟩—
USP Fludrocortisone Acetate RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: ***197M*** ▲ (CN 1-May-2020).
Specific rotation ⟨781S⟩: between +126° and +138°.
Test solution: 5 mg per mL, in acetone.
Loss on drying ⟨731⟩—Dry it in vacuum at 100° for 2 hours over magnesium perchlorate: it loses not more than 3.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chromatographic purity—Dissolve about 100 mg of it in a mixture of 5 mL of chloroform and 1 mL of acetone in a 10-mL volumetric flask, and dilute with chloroform to volume. Dilute 1 mL of this solution with chloroform to 100 mL. Apply 10 µL, in 5-µL increments, of the test solution and of its dilution to a line parallel to and about 2.5 cm from the bottom of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the plate in a suitable chamber containing a mixture of chloroform, methanol, and water (85:14:1) until the solvent front has moved about 15 cm. Remove the plate, air-dry, and examine under short-wavelength UV light: no spot in the chromatogram of the more concentrated test solution, other than the principal spot, is larger or more intense than the spot from the diluted test solution (1.0%).

Assay—
Standard preparation—Dissolve about 25 mg of USP Fludrocortisone Acetate RS, accurately weighed, in chloroform to make 250 mL, and mix. Pipet 10 mL of this solution into a 50-mL volumetric flask, add chloroform to volume, and mix.
Assay preparation—Prepare as directed under *Standard preparation*, using Fludrocortisone Acetate instead of the USP Reference Standard.
Procedure—Pipet 10 mL of the *Assay preparation* and the *Standard preparation*, respectively, into separate 25-mL volumetric flasks, and pipet 10 mL of chloroform into a third flask to provide a blank. Treat each flask as follows. Add 1.0 mL of a solution prepared by dissolving 50 mg of blue tetrazolium in 10 mL of methanol, and mix. Add 1.0 mL of a mixture of 1 volume of tetramethylammonium hydroxide TS and 4 volumes of methanol, mix, and allow to stand for 10 minutes. Dilute with a 1 in 100 solution of hydrochloric acid in methanol to volume. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* in 1-cm cells at about 525 nm, with a suitable spectrophotometer, against the reagent blank. Calculate the quantity, in mg, of $C_{23}H_{31}FO_6$ in the portion of Fludrocortisone Acetate taken by the formula:

$$1.25C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Fludrocortisone Acetate RS in the *Standard preparation;* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Fludrocortisone Acetate Tablets

DEFINITION
Fludrocortisone Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of fludrocortisone acetate ($C_{23}H_{31}FO_6$).

IDENTIFICATION

Delete the following:

▲• **A. Thin-Layer Chromatography**
Standard solution: 100 µg/mL of USP Fludrocortisone Acetate RS in acetone
Sample solution: Nominally 0.1 mg/mL of fludrocortisone acetate from Tablets prepared as follows. Transfer a portion of powdered Tablets, equivalent to 1 mg of fludrocortisone acetate, to a glass stoppered centrifuge tube, add 10 mL of acetone, and shake by mechanical means for 3 min. Centrifuge the mixture. Use the clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volumes
Standard solution: 20 µL
Sample solution: 20 µL, in 5-µL increments
Developing solvent system: Chloroform, methanol, and water (85:14:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Apply the *Samples* in a line parallel to and about 2.5 cm from the bottom of the plate. Develop the plate in a chamber using the *Developing solvent system* until the solvent front has moved about 15 cm. Remove the plate, air-dry, and examine under short-wavelength UV light.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. ▲2S (USP41)

Add the following:

▲• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay*.▲2S (USP41)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay*.▲2S (USP41)

ASSAY

Change to read:

• **Procedure**
▲Throughout the following procedures protect the solutions containing samples or Reference Standards from light.
Mobile phase: Acetonitrile, water, and phosphoric acid (40: 60: 0.1)
Standard stock solution: 0.2 mg/mL of USP Fludrocortisone Acetate RS in acetonitrile
Standard solution: 4 µg/mL of USP Fludrocortisone Acetate RS from *Standard stock solution* in *Mobile phase*
Sample solution: Nominally 4 µg/mL of fludrocortisone acetate from Tablets prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask, and add 80% of the final flask volume of *Mobile phase.* Stir for 20 min, and dilute with *Mobile phase* to volume. Pass the resulting solution through a suitable filter, discarding the first 5 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

USP Monographs

www.webofpharma.com

Detector: UV 240 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: NLT 1.4 times the retention time of fludrocortisone acetate
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.7 for fludrocortisone acetate
Relative standard deviation: NMT 1.0% for fludrocortisone acetate
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fludrocortisone acetate ($C_{23}H_{31}FO_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fludrocortisone Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fludrocortisone acetate in the *Sample solution* (µg/mL)▲ 2S (USP41)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩
▲Throughout the following procedures protect the solutions containing samples or Reference Standards from light.▲ 2S (USP41)
Medium: ▲0.01 N hydrochloric acid TS;▲ 2S (USP41) 500 mL
Apparatus 2: 75 rpm
Time: 30 min
Mobile phase: Acetonitrile and water (45:55)
Standard stock solution: 0.025 mg/mL of USP Fludrocortisone Acetate RS prepared as follows. Transfer an appropriate amount of USP Fludrocortisone Acetate RS to a suitable volumetric flask. Add 5% of the flask volume of acetonitrile, sonicate for 5 min to promote dissolution, and dilute with *Medium* to volume.
Standard solution: ▲(*L*/500) mg/mL of USP Fludrocortisone Acetate RS from *Standard stock solution* in *Medium*, where *L* is the label claim in mg/Tablet▲ 2S (USP41)
Sample solution: Withdraw a portion of the solution under test with glass syringes, and pass through a membrane filter that has been checked for absorptive loss.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: ▲UV 254 nm▲ 2S (USP41)
Column: 4.6-mm × 15-cm; ▲5-µm▲ 2S (USP41) packing L1
Flow rate: 2 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
▲▲ 2S (USP41)
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fludrocortisone acetate ($C_{23}H_{31}FO_6$) dissolved:

$$\text{▲Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fludrocortisone Acetate RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)▲ 2S (USP41)

Tolerances: NLT 80% (*Q*) of the labeled amount of fludrocortisone acetate ($C_{23}H_{31}FO_6$) is dissolved.

Change to read:

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements
▲▲ 2S (USP41)

IMPURITIES

Change to read:

▲• **Organic Impurities**
Throughout the following procedures protect the solutions containing samples or Reference Standards from light.
Mobile phase: Acetonitrile, water, and phosphoric acid (40: 60: 0.1)
Standard stock solution: 0.40 mg/mL of USP Fludrocortisone Acetate RS in acetonitrile
Standard solution: 0.4 µg/mL of USP Fludrocortisone Acetate RS from *Standard stock solution* in *Mobile phase*
Sensitivity solution: 0.04 µg/mL of USP Fludrocortisone Acetate RS from *Standard solution* in *Mobile phase*
Sample solution: Nominally 80 µg/mL of fludrocortisone acetate from Tablets prepared as follows. Transfer NLT 20 Tablets to a suitable volumetric flask, add 80% of the final volume of *Mobile phase*, and stir for 20 min. Dilute with *Mobile phase* to volume. Centrifuge a portion of the solution and use the supernatant. [Note—The use of a centrifuge speed of 4000 rpm for 10 min may be suitable.]
Chromatographic system: Proceed as directed in the *Assay*, except for the *Run time*.
Run time: NLT 2.7 times the retention time of fludrocortisone acetate
System suitability
Samples: *Standard solution* and *Sensitivity solution*
[Note—See *Table 1* for the relative retention times.]
Suitability requirements
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10.0, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

www.webofpharma.com

r_S = peak response of fludrocortisone acetate from the *Standard solution*
C_S = concentration of USP Fludrocortisone Acetate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fludrocortisone acetate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fludrocortisone[a]	0.41	1.5
Fludrocortisone acetate	1.0	—
Any other individual degradation product	—	0.5
Total degradation products	—	4.0

▲[a] 9-Fluoro-11β,17,21-trihydroxypregn-4-ene-3,20-dione.▲ (ERR 1-Mar-2019)▲ 2S (USP41)

ADDITIONAL REQUIREMENTS

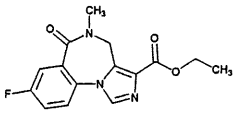
Change to read:

• **PACKAGING AND STORAGE:** ▲Preserve in tight, light-resistant containers. Store at controlled room temperature.▲ 2S (USP41)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fludrocortisone Acetate RS
▲▲ 2S (USP41)

Flumazenil



$C_{15}H_{14}FN_3O_3$ 303.29
4*H*-Imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid, 8-fluoro-5,6-dihydro-5-methyl-6-oxo-, ethyl ester;
Ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate [78755-81-4].

DEFINITION
Flumazenil contains NLT 98.0% and NMT 102.0% of flumazenil ($C_{15}H_{14}FN_3O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 800 mL of water, adjusted with phosphoric acid to a pH of 2.0 ± 0.05
Mobile phase: Methanol, tetrahydrofuran, and *Solution A* (13:7:80)
System suitability solution: 6.4 µg/mL each of USP Flumazenil RS and USP Flumazenil Related Compound B RS in *Mobile phase*
Standard solution: 1.0 mg/mL of USP Flumazenil RS in *Mobile phase*
Sample solution: 1.0 mg/mL of Flumazenil in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 5 µL
Run time: NLT 1.3 times the retention time of flumazenil
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between flumazenil related compound B and flumazenil, *System suitability solution*
Tailing factor: NMT 1.5 for flumazenil, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of flumazenil ($C_{15}H_{14}FN_3O_3$) in the portion of Flumazenil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Flumazenil RS in the *Standard solution* (mg/mL)
C_U = concentration of Flumazenil in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

• **LIMIT OF FLUMAZENIL RELATED COMPOUND C**
Diluent: Alcohol and chloroform (50:50)
Standard solution A: 0.5 mg/mL of USP Flumazenil RS and 0.6 µL/mL of USP Flumazenil Related Compound C RS in *Diluent*
Standard solution B: 0.1 mg/mL of USP Flumazenil RS and ▲0.12 µL/mL▲ (ERR 1-Jun-2018) of USP Flumazenil Related Compound C RS from *Standard solution A* in *Diluent*
Sample solution: 50 mg/mL of Flumazenil in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, glacial acetic acid, alcohol, and water (75: 15: 7.5: 2.5)
Spray reagent: Dissolve 0.5 g of ninhydrin in 90 mL of alcohol, and add 10 mL of glacial acetic acid.

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Proceed as directed in the chapter. Dry the plate for 10 min in a current of cold air, and view under short-wavelength UV light. Spray the plate with *Spray reagent*, and heat at 105° for 15 min. The R_F values of the analytes with their corresponding methods of detection are listed in *Table 1*.

Table 1

Compound	R_F	Detection
Flumazenil	About 0.8	UV
Flumazenil related compound C	About 0.04	Ninhydrin

Acceptance criteria: Any spot at an R_F value corresponding to flumazenil related compound C of the *Sample solution* is not more intense than the corresponding spot of *Standard solution B* (NMT 0.2%).

• **ORGANIC IMPURITIES**
Solution A, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay* except use a *Run time* of NLT 3 times the retention time of flumazenil.
Standard solution: 1 µg/mL of USP Flumazenil RS in *Mobile phase*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each impurity in the portion of Flumazenil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Flumazenil RS in the *Standard solution* (mg/mL)
C_U = concentration of Flumazenil in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Flumazenil related compound A[a]	0.4	1.1	0.2
Flumazenil dione[b]	0.5	1.5	0.2
Desfluoro flumazenil[c]	0.7	1.3	0.2
Flumazenil related compound B	0.8	1.1	0.2
Flumazenil	1.0	—	—
Chloroflumazenil[d]	2.2	1.1	0.2
Any individual unknown impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] 8-Fluoro-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylic acid; also known as 8-Fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol-[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid.
[b] 7-Fluoro-4-methyl-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione.
[c] Ethyl 5-methyl-6-oxo-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate.
[d] Ethyl 8-chloro-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate.

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 25.0 USP Endotoxin Units/mg of flumazenil
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Flumazenil RS
USP Flumazenil Related Compound B RS
Ethyl 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate; also known as Ethyl 8-hydroxy-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol-[1,5-*a*][1,4]benzodiazepine-3-carboxylate.
$C_{15}H_{15}N_3O_4$ 301.30
USP Flumazenil Related Compound C RS
1,1-Diethyoxy-*N*,*N*-dimethylmethanamine; also known as *N*,*N*-Dimethylformamide diethyl acetal.
$C_7H_{17}NO_2$ 147.22

Flumazenil Injection

DEFINITION
Flumazenil Injection is a sterile solution of Flumazenil. It contains NLT 90.0% and NMT 110.0% of the labeled amount of flumazenil ($C_{15}H_{14}FN_3O_3$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: 0.02 M monobasic potassium phosphate TS, adjusted with 0.02 M phosphoric acid to a pH of 2.7 ± 0.05
Mobile phase: Tetrahydrofuran, methanol, and *Buffer* (20:5:75)
Diluent: Tetrahydrofuran, methanol, and water (20:5:75)
System suitability solution: 0.01 mg/mL each of USP Flumazenil RS, USP Flumazenil Related Compound A RS, and USP Flumazenil Related Compound B RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Flumazenil RS in *Mobile phase*
Sample solution: Nominally 0.1 mg/mL of flumazenil from Injection prepared as follows. Dilute a portion of Injection with *Diluent*, if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L10
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of flumazenil
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 1.8 between flumazenil related compound B and flumazenil, *System suitability solution*
Tailing factor: NMT 2.0 for flumazenil related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of flumazenil ($C_{15}H_{14}FN_3O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Flumazenil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of flumazenil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Flumazenil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of flumazenil in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

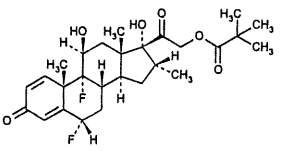
Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Flumazenil related compound A	0.71	1.1	1.0
Flumazenil related compound B	0.85	1.0	0.5
Flumazenil	1.0	—	—
Any individual unspecified impurity	—	1.0	0.5
Total impurities	—	—	2.0

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 100 USP Endotoxin Units/mg of flumazenil
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
• **pH** ⟨791⟩: 3.4–4.6
• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in multiple-dose containers, preferably of Type I glass, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Endotoxin RS
USP Flumazenil RS
USP Flumazenil Related Compound A RS
8-Fluoro-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylic acid;
Also known as 8-Fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol-[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid.
$C_{13}H_{10}FN_3O_3$ 275.24
USP Flumazenil Related Compound B RS
Ethyl 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate;
Also known as Ethyl 8-hydroxy-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol-[1,5-*a*][1,4]benzodiazepine-3-carboxylate.
$C_{15}H_{15}N_3O_4$ 301.30

Flumethasone Pivalate



$C_{27}H_{36}F_2O_6$ 494.57
Pregna-1,4-diene-3,20-dione, 21-(2,2-dimethyl-1-oxopropoxy)-6,9-difluoro-11,17-dihydroxy-16-methyl-, (6α,11β,16α)-.
6α,9-Difluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-pivalate [2002-29-1].

» Flumethasone Pivalate contains not less than 97.0 percent and not more than 103.0 percent of $C_{27}H_{36}F_2O_6$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Flumethasone Pivalate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 20 µg per mL.
Medium: methanol.
Absorptivities at 237 nm, calculated on the dried basis, do not differ by more than 3.0%.
Specific rotation ⟨781S⟩: between +71° and +82°.
Test solution: 10 mg per mL, in dioxane.
Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.

USP Monographs

www.webofpharma.com

Chromatographic purity—Prepare a solution in dioxane containing 20 mg per mL. Apply 5 µL of this solution and 5 µL each of three dioxane solutions containing in each mL, respectively, 200 (1%), 400 (2%), and 600 (3%) µg of USP Flumethasone Pivalate RS to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of toluene and ethyl acetate (7:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with dilute sulfuric acid (1 in 2), heating at 100° for 30 minutes, and inspecting under long-wavelength UV light: the total content of any impurities detected, when compared to the Standard solutions, does not exceed 3.0%.

Assay—

Standard preparation—Dissolve a suitable quantity of USP Flumethasone Pivalate RS, accurately weighed, in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a solution having a known concentration of about 20 µg per mL. Transfer 10.0 mL of this solution to a glass-stoppered, 20-mL conical flask.

Assay preparation—Transfer about 20 mg of Flumethasone Pivalate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with alcohol to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with alcohol to volume, and mix. Transfer 10.0 mL of this solution to a glass-stoppered, 20-mL conical flask.

Procedure—To each of the flasks containing the *Standard preparation* and the *Assay preparation*, and to a similar flask containing 10.0 mL of alcohol to provide the blank, add 1.0 mL of tetramethylammonium hydroxide TS. Mix, allow to stand for 20 minutes, accurately timed, add 1.0 mL of blue tetrazolium TS to each flask, and mix. Allow to stand for 40 minutes, add 1.0 mL of glacial acetic acid to each flask, mix, and concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 520 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_{27}H_{36}F_2O_6$ in the portion of Flumethasone Pivalate taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Flumethasone Pivalate RS in the *Standard preparation;* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Flumethasone Pivalate Cream

» Flumethasone Pivalate Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flumethasone pivalate ($C_{27}H_{36}F_2O_6$) in a suitable cream base.

Packaging and storage—Preserve in collapsible tubes.

USP Reference standards ⟨11⟩—
USP Flumethasone Pivalate RS

Identification—Place a quantity of Cream, equivalent to about 400 µg of flumethasone pivalate, in a 50-mL centrifuge tube, and treat as directed in the *Assay*, collecting the extracts in a 50-mL centrifuge tube. Evaporate the acetonitrile on a water bath (about 75°) with the aid of a stream of nitrogen. Dissolve the residue in 2.0 mL of acetonitrile with the aid of heat, allow to cool, and centrifuge. Apply 50 µL of this solution and 50 µL of a solution of chloroform and methanol (1:1) of USP Flumethasone Pivalate RS containing 200 µg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in toluene until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Redevelop the plate in the same manner in a solvent system consisting of toluene and ethyl acetate (7:3), remove the plate from the developing chamber, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Microbial enumeration tests ⟨61⟩ and ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Minimum fill ⟨755⟩: meets the requirements.

Assay—

Solvent acetonitrile—Saturate acetonitrile with isooctane.

Solvent isooctane—Saturate isooctane with acetonitrile.

4-Aminoantipyrine solution—Dissolve about 200 mg of 4-aminoantipyrine in a 1 in 100 solution of hydrochloric acid in methanol to make 50 mL of solution, and mix. Prepare this solution on the day of use.

Standard preparation—Dissolve a suitable quantity of USP Flumethasone Pivalate RS, accurately weighed, in *Solvent acetonitrile*, and dilute quantitatively and stepwise with *Solvent acetonitrile* to obtain a solution having a known concentration of about 40 µg per mL.

Assay preparation—Transfer an accurately weighed portion of Cream, equivalent to about 400 µg of flumethasone pivalate, to a tared glass-stoppered, 50-mL centrifuge tube. Place the tube in a vacuum desiccator, and dry the specimen in vacuum over silica gel until about 70% loss in weight is obtained.

Procedure— [NOTE—Perform the extractions in glass-stoppered, 50-mL centrifuge tubes with separation being effected by centrifugation, the portion to be retained being withdrawn into a hypodermic syringe fitted with a blunt-end, 14-gauge, 15-cm needle.] Add 10.0 mL of *Solvent acetonitrile* to the *Assay preparation*, and transfer 10.0 mL of the *Standard preparation* to a separate, glass-stoppered, 50-mL centrifuge tube. Add 25 mL of *Solvent isooctane* to each tube, shake by mechanical means until the cream is dispersed, and then shake both mixtures for 5 minutes. Separate and withdraw each acetonitrile layer, and filter through cotton pledgets, previously saturated with *Solvent acetonitrile*, into separate 25-mL volumetric flasks. Repeat the extraction, using a 10-mL portion of *Solvent acetonitrile* for each tube. Separate, withdraw, and filter each acetonitrile layer through the same respective filter, and combine the extracts with the main extracts. Dilute each with *Solvent acetonitrile* to volume, and mix. Transfer 10.0 mL of each solution to separate, glass-stoppered, 20-mL tubes, and to a third tube transfer 10.0 mL of *Solvent acetonitrile* to provide the blank. Evaporate the solvent on a water bath (about 75°) with the aid of a stream of nitrogen. Add 5.0 mL of *4-Aminoantipyrine solution* to each tube, insert the stopper, shake by mechanical means until the residue dissolves, and allow to stand for 1 hour. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 390 nm, with

USP Monographs

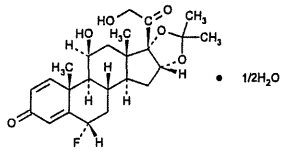
www.webofpharma.com

a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of flumethasone pivalate ($C_{27}H_{36}F_2O_6$) in the portion of Cream taken by the formula:

$$0.01C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Flumethasone Pivalate RS in the *Standard preparation;* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Flunisolide



$C_{24}H_{31}FO_6 \cdot ½H_2O$ 443.51
Pregna-1,4-diene-3,20-dione, 6-fluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, hemihydrate, (6α,11β,16α)-.
6α-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone, hemihydrate [77326-96-6].
Anhydrous 434.51
[3385-03-3].

» Flunisolide contains not less than 97.0 percent and not more than 102.0 percent of $C_{24}H_{31}FO_6$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Flunisolide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: methanol.
Specific rotation ⟨781S⟩: between +103° and +111°.
Test solution: 10 mg per mL, in chloroform.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.
Water Determination, *Method I* ⟨921⟩—The anhydrous form contains not more than 1.0%. The hemihydrate form contains between 1.8% and 2.5% (determined on a dried specimen).
Residue on ignition ⟨281⟩: not more than 0.1% from 250 mg.

Chromatographic purity—
Standard solutions—Prepare a solution of USP Flunisolide RS in acetone to contain 10 mg per mL (*Standard solution A*). Dilute 1 mL of *Standard solution A* with acetone to 100 mL (*Standard solution B*).
Test preparation—Prepare a solution of Flunisolide in acetone to contain 10 mg per mL.
Procedure—Apply 10-µL volumes of *Standard solution A*, *Standard solution B*, and the *Test preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a suitable chromatographic chamber previously equilibrated with a mixture of toluene and alcohol (90:10), seal the chamber, and develop the chromatogram until the solvent front has moved three-fourths of the length of the plate. Remove the plate, allow the solvent to evaporate, and examine the plate under short-wavelength UV light: the R_F value of the principal spot obtained from the *Test preparation* corresponds to that obtained from *Standard solution A*. No secondary spot exhibits an intensity greater than that of the principal spot from *Standard solution B*.

Assay—
Mobile phase—Prepare a suitable degassed solution of water and acetonitrile (3:2) such that at an approximate flow rate of 1.6 mL per minute, the retention time of Flunisolide is about 6 minutes.
Standard preparation—Dissolve an accurately weighed quantity of USP Flunisolide RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.
Assay preparation—Using 20 mg of Flunisolide, accurately weighed, proceed as directed for *Standard preparation*.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 25-cm column that contains 5- to 10-µm packing L7. The flow rate is about 1.6 mL per minute. Chromatograph the *Standard preparation*, and record the peak response as directed for *Procedure:* the column efficiency is not less than 2700 theoretical plates; the tailing factor for the flunisolide peak is not more than 1.7; and the relative standard deviation for replicate injections is not more than 1.0%.
Procedure—Separately inject equal volumes (between 15 µL and 30 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{24}H_{31}FO_6$ in the portion of Flunisolide taken by the formula:

$$(434.51/443.51)100C(r_U/r_S)$$

in which 434.51 and 443.51 are the molecular weights of $C_{24}H_{31}FO_6$ and $C_{24}H_{31}FO_6 \cdot ½H_2O$, respectively; C is the concentration, in mg per mL, of USP Flunisolide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Flunisolide Nasal Solution

» Flunisolide Nasal Solution is an aqueous, buffered solution of Flunisolide. It is supplied in a form suitable for nasal administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{24}H_{31}FO_6$.

www.webofpharma.com

Packaging and storage—Preserve in tight containers, protected from light, and store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Flunisolide RS

Identification—Proceed as directed in the *Assay*, except to inject 50 µL to 200 µL of a mixture of the *Assay preparation* and the *Standard preparation* (1:1) onto the column, adjusting the response to obtain a response that is between 50% and 90% full scale: a single peak is observed in the chromatogram for the mixed solution.

pH ⟨791⟩: between 4.5 and 6.0, a silver-silver chloride (internal element) electrode being used in conjunction with a fiber junction calomel electrode.

Quantity delivered per spray—Prime the spray pump by delivering 10 sprays into a fume hood. Accurately weigh the entire assembly, record the weight, and deliver 8 more sprays into the hood. Again weigh the assembly, and record the weight. Calculate the quantity, in µg, of $C_{24}H_{31}FO_6$ delivered per spray taken by the formula:

$$[(W_1 - W_2)/8][A/D]$$

in which W_1 and W_2 are the first and second weights, respectively, in g; A is the quantity, in µg per mL, of $C_{24}H_{31}FO_6$ found in the *Assay;* and D is the density of Nasal Solution, in g per mL. The quantity delivered is between 17 µg and 33 µg per spray.

Assay—

Mobile phase—Prepare a suitable degassed solution of water, acetonitrile, and glacial acetic acid (69:30:1 to 64:35:1). Adjust the ratio as necessary to obtain suitable chromatographic performance.

Internal standard solution—Dissolve norethindrone in acetonitrile to obtain a solution containing about 300 µg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Flunisolide RS in a mixture of acetonitrile and *Mobile phase* (1:1) to obtain a solution having a known concentration of about 250 µg per mL. Transfer 1.0 mL of this solution, and 1.0 mL of *Internal standard solution*, by means of "to contain" pipets, to a 50-mL volumetric flask. Rinse the pipets with *Mobile phase*, adding the rinsings to the flask, dilute with *Mobile phase* to volume, and mix. The final concentration of USP Flunisolide RS is about 5 µg per mL.

Assay preparation—Transfer an accurately measured volume of Nasal Solution, equivalent to about 250 µg of flunisolide, to a 50-mL volumetric flask, and add 1.0 mL of *Internal standard solution* by means of "to contain" pipets. Rinse the pipets with *Mobile phase*, adding the rinsing to the flask, dilute with *Mobile phase* to volume, and mix.

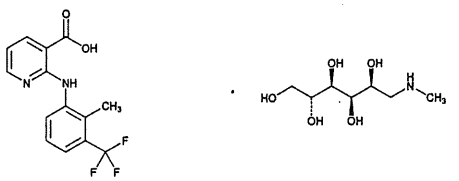
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 25-cm column that contains 5- to 10-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the analyte and the internal standard is not less than 5.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for flunisolide and 1.0 for norethindrone. Calculate the quantity, in mg, of $C_{24}H_{31}FO_6$ in each mL of the Nasal Solution taken by the formula:

$$(434.51/443.51)(50C/V)(R_U/R_S)$$

in which 434.51 and 443.51 are the molecular weights of $C_{24}H_{31}FO_6$ and $C_{24}H_{31}FO_6 \cdot ½H_2O$, respectively; C is the concentration, in mg per mL, of USP Flunisolide RS in the *Standard preparation;* V is the volume, in mL, of Nasal Solution taken; and R_U and R_S are the peak response ratios of the flunisolide peak and the norethindrone peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Flunixin Meglumine



$C_{14}H_{11}F_3N_2O_2 \cdot C_7H_{17}NO_5$ 491.46

3-Pyridinecarboxylic acid, 2-[[2-methyl-3-(trifluoromethyl) phenyl]amino]-, compd. with 1-deoxy-1-(methylamino)-D-glucitol (1:1);

2-(α^3,α^3,α^3-Trifluoro-2,3-xylidino)nicotinic acid compound with 1-deoxy-1-(methylamino)-D-glucitol (1:1)

1-Deoxy-1-(methylamino)-D-glucitol 2-{[2-methyl-3-(trifluoromethyl)phenyl]amino}nicotinate [42461-84-7].

DEFINITION

Flunixin Meglumine contains NLT 98.0% and NMT 102.0% of flunixin meglumine ($C_{14}H_{11}F_3N_2O_2 \cdot C_7H_{17}NO_5$).

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and water (70:30). Add 0.25 mL of phosphoric acid for each L prepared.

System suitability solution: 0.1 mg/mL each of USP Flunixin Meglumine RS and USP Flunixin Related Compound B RS in *Mobile phase*

Standard solution: 0.3 mg/mL of USP Flunixin Meglumine RS in *Mobile phase*

Sample solution: 0.3 mg/mL of Flunixin Meglumine in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between flunixin related compound B and flunixin meglumine, *System suitability solution*

www.webofpharma.com

Relative standard deviation: NMT 0.73, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of flunixin meglumine ($C_{14}H_{11}F_3N_2O_2 \cdot C_7H_{17}NO_5$) in the portion of Flunixin Meglumine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Flunixin Meglumine RS in the *Standard solution* (mg/mL)
C_U = concentration of Flunixin Meglumine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.01 mg/mL each of USP Flunixin Meglumine RS, USP Flunixin Related Compound A RS, USP Flunixin Related Compound B RS, and USP Flunixin Related Compound C RS in *Mobile phase*

Sample solution: 5.0 mg/mL of Flunixin Meglumine in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between flunixin related compound B and flunixin meglumine, *System suitability solution*

Relative standard deviation: NMT 1.0, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of flunixin related compounds A, B, and C in the portion of Flunixin Meglumine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of the corresponding related compound from the *Standard solution*
C_S = concentration of the corresponding related compound in the *Standard solution* (mg/mL)
C_U = concentration of Flunixin Meglumine in the *Sample solution* (mg/mL)

Calculate the percentage of flunixin ethyl ester and any other individual impurity in the portion of Flunixin Meglumine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of flunixin from the *Standard solution*
C_S = concentration of USP Flunixin Meglumine RS in the *Standard solution* (mg/mL)
C_U = concentration of Flunixin Meglumine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. The reporting level for impurities is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria NMT (%)
Flunixin related compound A[a]	0.5	0.2
Flunixin related compound C[b]	0.7	0.2
Flunixin related compound B[c]	0.8	0.2
Flunixin	1.0	—
Flunixin ethyl ester[d]	4.3	0.2
Any other individual impurity	—	0.2
Total impurities	—	0.5

[a] Chloronicotinic acid; 2-Chloronicotinic acid.
[b] Ethyl chloronicotinate; Ethyl 2-chloronicotinate.
[c] Trifluoromethyl toluidine; 2-Methyl-3-(trifluoromethyl)aniline.
[d] Ethyl 2-{[2-methyl-3-(trifluoromethyl)phenyl]amino}nicotinate.

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: 50 mg/mL in water

Acceptance criteria: 7.0–9.0

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 120 mg/mL in water

Acceptance criteria: –9° to –12°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Flunixin Meglumine RS
USP Flunixin Related Compound A RS
Chloronicotinic acid;
2-Chloronicotinic acid.
$C_6H_4ClNO_2$ 157.55
USP Flunixin Related Compound B RS
Trifluoromethyl toluidine;
2-Methyl-3-(trifluoromethyl)aniline.
$C_8H_8F_3N$ 175.15
USP Flunixin Related Compound C RS
Ethyl chloronicotinate;
Ethyl 2-chloronicotinate.
$C_8H_8ClNO_2$ 185.61

Flunixin Meglumine Granules

» Flunixin Meglumine Granules contain an amount of flunixin meglumine ($C_{14}H_{11}F_3N_2O_2 \cdot C_7H_{17}NO_5$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flunixin ($C_{14}H_{11}F_3N_2O_2$).

Packaging and storage—Preserve in well-closed containers.

Labeling—Label Granules to indicate that they are for veterinary use only.

www.webofpharma.com

USP Reference standards ⟨11⟩—
USP Flunixin Meglumine RS

Identification—

A: The UV absorption spectrum of the solution employed for measurement of absorbance in the *Assay* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Flunixin Meglumine RS, concomitantly measured.

B: Grind a quantity of Granules, equivalent to about 25 mg of flunixin, and transfer the powder to a 50-mL centrifuge tube. Add 20 mL of acetate buffer, prepared by dissolving 4.1 g of anhydrous sodium acetate in 500 mL of water, adding 2.9 mL of glacial acetic acid, and diluting with water to 1000 mL. Rotate the tube for 10 minutes. Extract with 25 mL of ethyl acetate, and use the upper phase as the test solution. Separately apply 10 µL of the test solution and 10 µL of a Standard solution of USP Flunixin Meglumine RS in methanol containing 1.5 mg per mL to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a mixture of toluene, ethyl acetate, glacial acetic acid, and water (65:30:10:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the spots to air-dry. Examine the plate under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the *Standard solution.*

Dissolution ⟨711⟩—
Medium: 0.1 N hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.
Procedure—Transfer a quantity of Granules, equivalent to about 12.5 mg of flunixin, to the dissolution flask. Determine the amount of flunixin ($C_{14}H_{11}F_3N_2O_2$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 252 nm on filtered portions of solution under test in comparison with a Standard solution having a known concentration of about 23.6 µg per mL of USP Flunixin Meglumine RS in the same *Medium.* Each µg of flunixin meglumine is equivalent to 0.6028 µg of flunixin.
Tolerances—Not less than 75% *(Q)* of the labeled amount of flunixin ($C_{14}H_{11}F_3N_2O_2$) is dissolved in 30 minutes.
Uniformity of dosage units ⟨905⟩—meet the requirements.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Flunixin Meglumine RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.82 mg per mL. Transfer 4.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.
Assay preparation—Dissolve an accurately weighed quantity of Granules in water by shaking for 30 minutes. Quantitatively dilute with water to obtain a solution containing about 0.5 mg of flunixin per mL, and mix. Centrifuge a portion of this solution. Transfer 4.0 mL of the supernatant layer to a 100-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.
Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 283 nm using 0.1 N sodium hydroxide to set the instrument. Calculate the quantity, in mg, of flunixin ($C_{14}H_{11}F_3N_2O_2$) in the portion of Granules taken by the formula:

$$(296.25/491.46)(12{,}500C)(A_U/A_S)$$

in which 296.25 and 491.46 are the molecular weights of flunixin and flunixin meglumine, respectively; C is the concentration, in mg per mL, of USP Flunixin Meglumine RS in the *Standard preparation*, and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation;* respectively.

Flunixin Meglumine Injection

DEFINITION

Flunixin Meglumine Injection is a sterile solution of Flunixin Meglumine in Water for Injection. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of flunixin ($C_{14}H_{11}F_3N_2O_2$). It may contain phenol or another suitable preservative.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Buffer: Dissolve 4.1 g of anhydrous sodium acetate in 500 mL of water. Add 2.9 mL of glacial acetic acid, and dilute with water to 1000 mL.
Standard solution: 3 mg/mL of USP Flunixin Meglumine RS in methanol
Sample solution: Transfer the equivalent to 50 mg of flunixin from Injection to a 50-mL centrifuge tube. Add 10 mL of *Buffer,* and extract with 25 mL of ethyl acetate. Use the upper phase as the *Sample solution.*
Chromatographic system
Adsorbent: Silica gel
Application volume: 10 µL
Developing solvent system: Toluene, ethyl acetate, glacial acetic acid, and water (75:25:10:1)
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, water, and acetic acid (70:30:1)
Diluent: Methanol and water (70:30)
System suitability solution: 0.33 mg/mL of USP Flunixin Meglumine RS and 0.02 mg/mL of phenol in *Diluent*
Standard solution: 0.33 mg/mL of USP Flunixin Meglumine RS in *Diluent*
Sample solution: Transfer an amount of Injection equivalent to 500 mg of flunixin to a 250-mL volumetric flask, and dilute with *Diluent* to volume. Further dilute this solution with *Diluent* to obtain a solution containing 0.2 mg/mL of flunixin. Pass a portion of this solution through a filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30° ± 2°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 17 between phenol and flunixin meglumine
Tailing factor: NMT 2.0 for flunixin meglumine

www.webofpharma.com

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of flunixin ($C_{14}H_{11}F_3N_2O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Flunixin Meglumine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of flunixin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of flunixin, 296.25
M_{r2} = molecular weight of flunixin meglumine, 491.46

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Sensitivity solution: 0.167 µg/mL of USP Flunixin Meglumine RS in *Diluent* from the *Standard solution*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 17 between phenol and flunixin meglumine, *System suitability solution*

Tailing factor: NMT 2.0, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Sensitivity solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Sample solution* and *Sensitivity solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*. Disregard any peak less than the response of the flunixin meglumine peak as obtained from the *Sensitivity solution* (0.05%). Disregard the peak due to phenol, if present, which elutes at a relative retention time of 0.25 with respect to flunixin.

Table 1

Name	Acceptance Criteria, NMT (%)
Any unspecified impurity	1.0
Total impurities	2.0

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 4.54 USP Endotoxin Units/mg of flunixin

• **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **pH** ⟨791⟩: 7.8–9.0

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets requirements if labeled for IV use

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in multiple-dose containers, and store between 2° and 30°.

• **LABELING:** Label Injection to indicate that it is for veterinary use only.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Flunixin Meglumine RS

Flunixin Meglumine Paste

» Flunixin Meglumine Paste contains an amount of flunixin meglumine ($C_{14}H_{11}F_3N_2O_2 \cdot C_7H_{17}NO_5$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flunixin ($C_{14}H_{11}F_3N_2O_2$).

Packaging and storage—Preserve in a well-closed container.

USP Reference standards ⟨11⟩—
USP Flunixin Meglumine RS

Labeling—Label the Paste to indicate that it is for veterinary use only.

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

Microbial enumeration tests ⟨61⟩ and ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of methanol, water, and glacial acetic acid (70:30:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve a quantity of sodium benzoate in water to obtain a solution containing 33 mg per mL.

Diluent—Prepare a mixture of methanol and water (7:3).

Standard preparation—Transfer about 83 mg of USP Flunixin Meglumine RS, accurately weighed, to a 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution*, 20.0 mL of water, and 10.0 mL of methanol to the tube, and mix to dissolve. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Assay preparation—Transfer an accurately weighed quantity of Paste, equivalent to about 50 mg of flunixin, to a 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution* and 20.0 mL of water to the tube, and rotate for 20 minutes. Add 10.0 mL of methanol, and mix. Heat the tube in a water bath at 60° for 5 minutes, with occasional shaking. Continue rotating the tube until cool, and centrifuge. Transfer 10.0 mL of the clear supernatant to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*:the relative retention times are about 0.5 for sodium benzoate and 1.0 for flunixin meglumine; the resolution, *R*, between sodium benzoate and flunixin meglumine is not less than 1.9; and the relative standard deviation for replicate injections is not more than 2.0%.

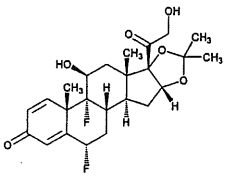
www.webofpharma.com

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of flunixin ($C_{14}H_{11}F_3N_2O_2$) in the portion of Paste taken by the formula:

$$(296.25/491.46)(87.5C)(R_U/R_S)$$

in which 296.25 and 491.46 are the molecular weights of flunixin and flunixin meglumine, respectively; C is the concentration, in mg per mL, of USP Flunixin Meglumine RS in the *Standard preparation;* and R_U and R_S are the ratios of the peak responses for flunixin and sodium benzoate obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Fluocinolone Acetonide



$C_{24}H_{30}F_2O_6$ (anhydrous) 452.49
Dihydrate 488.53

Pregna-1,4-diene-3,20-dione, 6,9-difluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (6α,11β,16α)-;
6α,9-Difluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione, cyclic 16,17-acetal with acetone [67-73-2].

DEFINITION

Fluocinolone Acetonide is anhydrous or contains two molecules of water of hydration. It contains NLT 97.0% and NMT 102.0% of fluocinolone acetonide ($C_{24}H_{30}F_2O_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020): If a difference appears, dissolve portions of both the sample and the USP Reference Standard in ethyl acetate, evaporate to dryness, and repeat the test on the residues.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, tetrahydrofuran, and water (13:10:77)
Diluent: Acetonitrile and tetrahydrofuran (13:10)
Standard solution: 0.2 mg/mL of USP Fluocinolone Acetonide RS, prepared as follows. Transfer a suitable amount of USP Fluocinolone Acetonide RS to a suitable volumetric flask, and dissolve in a volume of *Diluent* equal to 23% of the flask volume. Dilute with water to volume.
Sample solution: 0.2 mg/mL of Fluocinolone Acetonide, prepared as follows. Transfer a suitable amount of Fluocinolone Acetonide to a suitable volumetric flask, and dissolve in a volume of *Diluent* equal to 23% of the flask volume. Dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 2.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluocinolone acetonide ($C_{24}H_{30}F_2O_6$) in the portion of Fluocinolone Acetonide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluocinolone Acetonide RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluocinolone Acetonide in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

- **ORGANIC IMPURITIES**

Protect the solutions from light throughout the test.
Mobile phase: Acetonitrile and water (45:55), prepared as follows. Mix 450 mL of acetonitrile and 500 mL of water, and allow to equilibrate. Add water to make 1000 mL.
System suitability solution: 0.25 mg/mL each of USP Fluocinolone Acetonide RS and USP Triamcinolone Acetonide RS, prepared as follows. Transfer suitable amounts of USP Fluocinolone Acetonide RS and USP Triamcinolone Acetonide RS to a suitable volumetric flask. Dissolve in 45% of the flask volume of acetonitrile, and dilute with water to volume.
Standard solution: 0.025 mg/mL of USP Fluocinolone Acetonide RS in acetonitrile
Sample solution: 2.5 mg/mL of Fluocinolone Acetonide in acetonitrile
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 238 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: 4 times the retention time of fluocinolone acetonide
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for triamcinolone acetonide and fluocinolone acetonide are 0.85 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between triamcinolone acetonide and fluocinolone acetonide, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Fluocinolone Acetonide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

www.webofpharma.com

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of fluocinolone acetonide from the *Standard solution*
C_S = concentration of USP Fluocinolone Acetonide RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluocinolone Acetonide in the *Sample solution* (mg/mL)

Acceptance criteria
Any individual impurity: NMT 1%; NMT one such peak is greater than 0.5%.
Total impurities: NMT 2.5%. Disregard any peak below 0.05% of the peak area of fluocinolone acetonide from the *Standard solution*.

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in methanol
Acceptance criteria: +98° to +108°
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 105° for 3 h.
Acceptance criteria: NMT 1.0% for anhydrous Fluocinolone Acetonide; NMT 8.5% for hydrous Fluocinolone Acetonide

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate whether it is anhydrous or hydrous.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluocinolone Acetonide RS
USP Triamcinolone Acetonide RS

Fluocinolone Acetonide Cream

» Fluocinolone Acetonide Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{24}H_{30}F_2O_6$.

Packaging and storage—Preserve in collapsible tubes or tight containers.
USP Reference standards ⟨11⟩—
USP Fluocinolone Acetonide RS
USP Norethindrone RS
Identification—Transfer a quantity of the Cream, equivalent to about 0.5 mg of fluocinolone acetonide, to a centrifuge tube, disperse it in 5 mL of water, add 10 mL of chloroform, shake, and centrifuge. Remove and discard the aqueous layer, add 10 mL of water to the tube, shake, and centrifuge. Dry about 2 mL of the chloroform extract over about 200 mg of anhydrous sodium sulfate: the dried extract responds to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, 50 µL of the dried chloroform extract and 50 µL of a Standard solution containing about 50 µg per mL of USP Fluocinolone Acetonide RS being applied, and a mixture of chloroform and diethylamine (2:1) being used for development.
Microbial enumeration tests ⟨61⟩ and ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
Minimum fill ⟨755⟩: meets the requirements.

Assay—
Internal standard solution—Dissolve USP Norethindrone RS in acetonitrile to obtain a solution containing about 200 µg per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Fluocinolone Acetonide RS in acetonitrile to obtain a solution having a known concentration of about 300 µg per mL. Transfer 5.0 mL of this solution, 6.0 mL of *Internal standard solution*, and 15.0 mL of water to a 50-mL volumetric flask. Dilute with acetonitrile to volume, and mix. The *Standard preparation* contains 30 µg of USP Fluocinolone Acetonide RS per mL.
Mobile solvent—Prepare a mixture of water and acetonitrile (5:3). Adjust the ratio as necessary to obtain suitable chromatographic performance.
Assay preparation—Dissolve an accurately weighed portion of Cream, equivalent to about 0.75 mg of fluocinolone acetonide, in about 10 mL of acetonitrile by heating on a steam bath. Transfer the mixture to a 25-mL volumetric flask with the aid of three 2-mL portions of acetonitrile. Add 3.0 mL of *Internal standard solution* and 5.0 mL of water, cool, and mix. Dilute with acetonitrile to volume, mix, and cool in an ice bath. Centrifuge or filter the mixture to obtain a clear solution.
Apparatus—Use a high-pressure liquid chromatograph (see *Chromatography* ⟨621⟩) of the general type equipped with a detector for monitoring UV absorbance at about 254 nm, and capable of providing a flow rate of about 2 mL per minute for the *Mobile solvent*. Use a column that contains packing L1.
Procedure—Chromatograph equal volumes of the *Assay preparation* and the *Standard preparation*. Three replicate injections of the *Standard preparation* show a resolution factor of not less than 2.0 between the peaks for norethindrone and fluocinolone acetonide and a relative standard deviation of not more than 1.5%. Calculate the quantity, in mg, of $C_{24}H_{30}F_2O_6$ in the portion of Cream taken by the formula:

$$0.025C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Fluocinolone Acetonide RS in the *Standard preparation;* and R_U and R_S are the ratios of the peak areas of fluocinolone acetonide and norethindrone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Fluocinolone Acetonide Ointment

» Fluocinolone Acetonide Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{24}H_{30}F_2O_6$.

Packaging and storage—Preserve in collapsible tubes or tight containers.
USP Reference standards ⟨11⟩—
USP Fluocinolone Acetonide RS
USP Norethindrone RS
Identification—Evaporate 10.0 mL of the *Assay preparation* obtained in the *Assay* to dryness, and dissolve the residue in 1 mL of chloroform: it responds to the *Thin-layer Chromatographic Identification Test* ⟨201⟩, 50 µL of the test solution and 50 µL of the Standard solution, containing about 50 µg per mL of USP Fluocinolone Acetonide RS, being applied and a mixture of chloroform and diethylamine (2:1) being used for development.
Microbial enumeration tests ⟨61⟩ and ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests

www.webofpharma.com

for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
Minimum fill ⟨755⟩: meets the requirements.

Assay—
Internal standard solution—Dissolve a suitable quantity of USP Norethindrone RS in methanol to obtain a solution containing about 850 µg per mL.
Diluted internal standard solution—Transfer 5.0 mL of *Internal standard solution* to a 250-mL flask. Dilute with methanol to volume, and mix.
Standard preparation—Dissolve an accurately weighed quantity of USP Fluocinolone Acetonide RS in acetonitrile to obtain a solution having a known concentration of about 200 µg per mL. Transfer 10.0 mL of this solution and 2.0 mL of *Internal standard solution* to a 100-mL volumetric flask. Dilute with methanol to volume, and mix. The concentration of USP Fluocinolone Acetonide RS in the *Standard preparation* is 20 µg per mL.
Mobile solvent—Prepare a mixture of acetonitrile and water (1:1). Adjust the ratio as necessary to obtain suitable chromatographic performance.
Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 0.7 mg of fluocinolone acetonide, to a 50-mL, round-bottom centrifuge tube. Add 35.0 mL of *Diluted internal standard solution*, emulsify using an ultrasonic probe, and centrifuge to bring the insoluble matter to the bottom. The clear supernatant is the *Assay preparation.*
Apparatus—Use a suitable high-pressure liquid chromatograph (see *Chromatography* ⟨621⟩) of the general type equipped with a detector for monitoring UV absorbance at about 254 nm, and capable of providing a flow rate of about 2 mL per minute for the *Mobile solvent.* Use a 50-cm × 4-mm column that contains packing L1 so as to provide a resolution factor, *R* (see *Chromatography* ⟨621⟩), of at least 2.0 between peaks for norethindrone and fluocinolone acetonide. Three replicate injections of the *Standard preparation* show a relative standard deviation of not more than 1.5%.
Procedure—Chromatograph equal volumes of the *Assay preparation* and the *Standard preparation*, adjusting the system as necessary to obtain peaks of between about 50% and 90% of full-scale. Calculate the quantity, in mg, of $C_{24}H_{30}F_2O_6$ in the portion of Ointment taken by the formula:

$$0.035C(R_U/R_S)$$

in which *C* is the concentration, in µg per mL, of USP Fluocinolone Acetonide RS in the *Standard preparation;* and R_U and R_S are the ratios of the peak areas of fluocinolone acetonide and the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Fluocinolone Acetonide Topical Solution

» Fluocinolone Acetonide Topical Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{24}H_{30}F_2O_6$.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Fluocinolone Acetonide RS
Identification—Transfer a quantity of Topical Solution, equivalent to about 0.5 mg of fluocinolone acetonide, to a separator, add 5 mL of water, and extract with 10 mL of chloroform. Withdraw the chloroform layer into a second separator, wash with 10 mL of water, and dry about 2 mL of the chloroform extract over about 200 mg of anhydrous sodium sulfate: the dried extract responds to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, 50 µL of the dried chloroform extract and 50 µL of the Standard solution being applied, and a mixture of chloroform and diethylamine (2:1) being used for development.
Microbial enumeration tests ⟨61⟩ and ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Assay—
Internal standard solution—Dissolve norethindrone in acetonitrile to obtain a solution containing about 200 µg per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Fluocinolone Acetonide RS in acetonitrile to obtain a solution having a known concentration of about 200 µg per mL. Transfer 5.0 mL of this solution, 4.0 mL of *Internal standard solution*, 10 mL of propylene glycol, and about 25 mL of acetonitrile to a 50-mL volumetric flask. Mix, cool to room temperature, dilute with acetonitrile to volume, and mix. The final concentration of USP Fluocinolone Acetonide RS is 20 µg per mL.
Mobile solvent—Prepare a mixture of water and acetonitrile (3:2). Adjust the ratio as necessary to obtain suitable chromatographic performance.
Assay preparation—Transfer an accurately measured volume of Topical Solution, equivalent to about 0.5 mg of fluocinolone acetonide, to a 25-mL volumetric flask. Add 2.0 mL of *Internal standard solution* and 10 mL of acetonitrile. Mix, cool to room temperature, dilute with acetonitrile to volume, and mix.
Apparatus—Use a suitable high-pressure liquid chromatograph (see *Chromatography* ⟨621⟩) of the general type equipped with a detector for monitoring UV absorbance at about 254 nm, and capable of providing a flow rate of about 2 mL per minute for the *Mobile solvent.* Use a column containing packing L1 so as to provide a resolution factor, *R,* of at least 2.0 between peaks for norethindrone and fluocinolone acetonide.
Procedure—Chromatograph equal volumes of the *Assay preparation* and the *Standard preparation*, adjusting the system as necessary to obtain peaks of between about 50% and 90% full-scale. Calculate the quantity, in mg, of $C_{24}H_{30}F_2O_6$ in each mL of the Topical Solution taken by the formula:

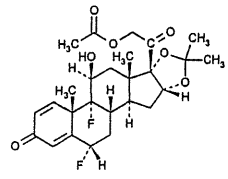
$$0.025(C/V)(R_U/R_S)$$

in which *C* is the concentration, in µg per mL, of USP Fluocinolone Acetonide RS in the *Standard preparation;* *V* is the volume, in mL, of Solution taken; and R_U and R_S are the ratios of the areas of the fluocinolone acetonide peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

USP Monographs

www.webofpharma.com

Fluocinonide



$C_{26}H_{32}F_2O_7$ 494.52

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-6,9-difluoro-11-hydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (6α,11β,16α)-.

6α,9-Difluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione, cyclic 16,17-acetal with acetone, 21-acetate [356-12-7].

» Fluocinonide contains not less than 97.0 percent and not more than 103.0 percent of $C_{26}H_{32}F_2O_7$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Fluocinonide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: methanol.
Absorptivities at 238 nm, calculated on the dried basis, do not differ by more than 3.0%.

Specific rotation ⟨781S⟩: between +81° and +89°.
Test solution: 10 mg per mL, in chloroform.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: negligible, from 100 mg.

Chromatographic purity—
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*.
Test preparation—Transfer about 25 mg of fluocinonide, accurately weighed, to a 10-mL volumetric flask, add acetonitrile to volume, and mix.
Procedure—Inject 30 µL of the *Test preparation* into the chromatograph, record the chromatogram, and measure the area responses of all peaks. Calculate the area percentage of each peak observed in the chromatogram. The largest secondary peak is not more than 1.0% of the total area, and no other secondary peak is more than 0.5% of the total area. The sum of the areas of all peaks, other than the main peak, does not constitute more than 2.0% of the total area.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer about 25 mg of USP Fluocinonide RS, accurately weighed, to a 100-mL volumetric flask, add acetonitrile to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.025 mg of USP Fluocinonide RS per mL.
Assay preparation—Using about 25 mg of Fluocinonide, accurately weighed, proceed as directed for *Standard preparation*.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.5%.
Procedure—Separately inject equal volumes (about 30 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{26}H_{32}F_2O_7$ in the portion of Fluocinonide taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Fluocinonide RS in the *Standard preparation;* and r_U and r_S are the peak responses due to the fluocinonide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Fluocinonide Cream

» Fluocinonide Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluocinonide ($C_{26}H_{32}F_2O_7$).

Packaging and storage—Preserve in collapsible tubes or tight containers.

USP Reference standards ⟨11⟩—
USP Fluocinonide RS

Identification—Weigh an amount of Cream, equivalent to about 2.5 mg of fluocinonide, into a glass-stoppered, 100-mL centrifuge tube containing 5 mL of water and 10 mL of methanol. Add 20 mL of cyclohexane, shake vigorously, centrifuge, and discard the upper phase. Add 20 mL of water and 5 mL of chloroform, shake vigorously, centrifuge until the lower phase is clear, and discard the upper phase. The clear chloroform extract is the *Test solution.* Separately apply 10 µL of the *Test solution* and 10 µL of a Standard solution having a concentration of 0.5 mg per mL of USP Fluocinonide RS in chloroform to equidistant points about 2 cm from one end of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel. Allow the applications to dry, and develop the chromatogram in a suitable chromatographic chamber using a mixture of chloroform and acetone (4:1). Air-dry, and view under short-wavelength UV light: the principal spot from the *Test solution* corresponds to that obtained from the Standard solution.

Microbial enumeration tests ⟨61⟩ and ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Minimum fill ⟨755⟩: meets the requirements.

www.webofpharma.com

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Fluocinonide RS in acetonitrile to obtain a solution having a known concentration of about 200 µg per mL. Transfer 10.0 mL of this solution and 10.0 mL of water to a 100-mL volumetric flask. Dilute with acetonitrile to volume, and mix. The final concentration of USP Fluocinonide RS is 20 µg per mL.

Assay preparation—Transfer an accurately weighed quantity of Cream, containing about 2 mg of fluocinonide, to a 100-mL volumetric flask. Add about 60 mL of acetonitrile, and dissolve the cream by heating on a steam bath. Add 10.0 mL of water, and allow to cool. Dilute with acetonitrile to volume, and mix. Filter the mixture through a fine-sintered glass funnel, using vacuum, and use the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 4500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Chromatograph equal volumes (about 20 µL) of the *Assay preparation* and the *Standard preparation*, record the chromatograms, and measure the peak responses due to fluocinonide. Calculate the quantity, in mg, of fluocinonide ($C_{26}H_{32}F_2O_7$) in the portion of Cream taken by the formula:

$$0.1C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Fluocinonide RS in the *Standard preparation;* and r_U and r_S are the peak responses due to fluocinonide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Fluocinonide Gel

» Fluocinonide Gel contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluocinonide ($C_{26}H_{32}F_2O_7$).

Packaging and storage—Preserve in collapsible tubes or tight containers.

USP Reference standards ⟨11⟩—
USP Fluocinonide RS

Identification—Weigh an amount of Gel, equivalent to about 2.5 mg of fluocinonide, into a glass-stoppered, 50-mL centrifuge tube containing 20 mL of sodium chloride solution (1 in 10). Add 5 mL of chloroform and 15 mL of methanol, and shake vigorously. Centrifuge to clarify the chloroform layer, and remove the solid material present at the interphase. Discard the upper phase. Dry a portion of the chloroform layer over anhydrous sodium sulfate. Using the dried extract as the *Test preparation*, proceed as directed in the *Identification* test under *Fluocinonide Cream*, beginning with "Apply 10 µL of the *Test solution.*"

Minimum fill ⟨755⟩: meets the requirements.

Assay—

Mobile phase—Prepare a mixture of acetonitrile and water (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Fluocinonide RS in acetonitrile to obtain a solution having a known concentration of about 200 µg per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with acetonitrile to volume, and mix. The final concentration is 20 µg per mL.

Assay preparation—Transfer an accurately weighed quantity of Gel, containing about 2 mg of fluocinonide, to a 100-mL volumetric flask. Add about 60 mL of acetonitrile, and dissolve the gel by heating on a steam bath. Cool to room temperature, dilute with acetonitrile to volume, and mix. Centrifuge a portion at about 2500 rpm for about 5 minutes. Filter a portion of the centrifugate through an acetonitrile-insoluble membrane filter. The filtrate is the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{26}H_{32}F_2O_7$ in the portion of Gel taken by the formula:

$$0.1C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Fluocinonide RS in the *Standard preparation;* and r_U and r_S are the peak responses due to fluocinonide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Fluocinonide Ointment

» Fluocinonide Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluocinonide ($C_{26}H_{32}F_2O_7$).

Packaging and storage—Preserve in collapsible tubes or tight containers.

USP Reference standards ⟨11⟩—
USP Fluocinonide RS

Identification—Weigh an amount of Ointment, equivalent to about 2.5 mg of fluocinonide, into a glass-stoppered, 50-mL centrifuge tube containing 20 mL of cyclohexane. Gently disperse to form a suspension. Add 5 mL of water and 10 mL of methanol. Shake vigorously, allow the phases to separate, and discard the upper phase. Add 20 mL of water and 5 mL of chloroform, shake vigorously, centrifuge, and transfer a portion of the chloroform layer to a small test tube containing about 200 mg of anhydrous sodium sulfate. Mix, and allow to stand until the extract is clear. Using the clear chloroform extract as the *Test preparation*, proceed as directed in the *Identification*— under *Fluocinonide Cream*, beginning with "Apply 10 µL of the *Test solution.*"

Minimum fill ⟨755⟩: meets the requirements.

www.webofpharma.com

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (1:1). Adjust the ratio as necessary to obtain suitable chromatographic performance.

Standard preparation—Dissolve an accurately weighed quantity of USP Fluocinonide RS in acetonitrile to obtain a solution having a known concentration of about 400 µg per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask. Dilute with methanol to volume, and mix. The final concentration of USP Fluocinonide RS is about 40 µg per mL.

Assay preparation—Transfer an accurately weighed quantity of Ointment, containing about 1.35 mg of fluocinonide, to a round-bottom, 50-mL centrifuge tube. Add 35.0 mL of methanol. Emulsify, using an ultrasonic probe, and centrifuge to bring the insoluble matter to the bottom. The clear supernatant is the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed under *Fluocinonide Cream*, except that the flow rate for the *Mobile phase* is about 1 mL per minute.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of fluocinonide ($C_{26}H_{32}F_2O_7$) in the portion of Ointment taken by the formula:

$$0.035C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Fluocinonide RS in the *Standard preparation;* and r_U and r_S are the peak responses due to fluocinonide obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Fluocinonide Topical Solution

» Fluocinonide Topical Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{26}H_{32}F_2O_7$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Fluocinonide RS

Identification—Transfer an amount of Topical Solution, equivalent to about 2.5 mg of fluocinonide, to a glass-stoppered, 50-mL centrifuge tube containing 5 mL of water and 10 mL of methanol, add 20 mL of cyclohexane, shake vigorously, centrifuge, and discard the upper phase. Add 20 mL of water and 5 mL of chloroform, shake vigorously, centrifuge until the lower phase is clear, and discard the upper phase. The clear chloroform extract is the test solution. Proceed as directed in the *Identification*— test under *Fluocinonide Cream* beginning with "Apply 10 µL of the *Test solution.*"

Minimum fill ⟨755⟩: meets the requirements.

Alcohol content—

Standard solution—Dilute 20.0 mL of alcohol with methanol to volume in a 200-mL volumetric flask.

Internal standard solution—Dilute 20.0 mL of isopropyl alcohol with methanol to volume in a 100-mL volumetric flask.

Test preparation—Using a "to contain" pipet, transfer 2 mL of Topical Solution to a 100-mL volumetric flask, rinsing the pipet 3 times with methanol and collecting the rinsings in the volumetric flask. Add 5.0 mL of *Internal standard solution,* dilute with methanol to volume, and mix.

Standard preparation—Pipet 6 mL of the *Standard solution* and 5 mL of the *Internal standard solution* into a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 1.8-m glass column that is packed with 80- to 100-mesh packing S3. The carrier gas is nitrogen or helium, flowing at a rate of about 40 mL per minute. The injection port and detector temperatures are maintained at about 225°. The column temperature is maintained at about 130°. Chromatograph the *Standard preparation,* record the chromatogram, and determine the peak response ratio as directed for *Procedure.* Adjust the carrier gas flow rate so that the resolution, *R,* of alcohol and isopropyl alcohol is not less than 1.5; the tailing factor of the alcohol peak is not more than 1.25; and the relative standard deviation for peak response ratios from replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (2 µL to 3 µL) of the *Test preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage (v/v) of C_2H_5OH in the Topical Solution taken by the formula:

$$(0.3)(95.45)(R_U/R_S)$$

in which 95.45 is the percentage (v/v) of C_2H_5OH in USP Alcohol; and R_U and R_S are the peak response ratios obtained from the *Test preparation* and the *Standard preparation,* respectively: between 28.4% and 39.0% of C_2H_5OH is present.

Assay—

Mobile phase—Use a mixture of acetonitrile and water (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Fluocinonide RS in acetonitrile to obtain a solution containing about 500 µg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 40 µg of USP Fluocinonide RS per mL.

Assay preparation—Using a "to contain" pipet, transfer a volume of Topical Solution, equivalent to about 1 mg of fluocinonide, to a 25-mL volumetric flask, rinsing the pipet with about 5 mL of *Mobile phase,* and adding the rinsings to the volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor for the analyte peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.5%.

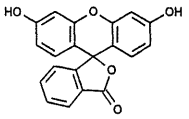
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{26}H_{32}F_2O_7$ in each mL of the Topical Solution taken by the formula:

$$0.025(C/V)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Fluocinonide RS in the *Standard preparation; V* is the volume, in mL, of Topical Solution taken; and r_U and r_S are the peak responses due to fluocinonide obtained from the *Assay preparation* and the *Standard preparation,* respectively.

www.webofpharma.com

Fluorescein



$C_{20}H_{12}O_5$ 332.31
Spiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen-3-one, 3′,6′-dihydroxy-.
Fluorescein [2321-07-5].

» Fluorescein contains not less than 97.0 percent and not more than 102.0 percent of $C_{20}H_{12}O_5$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Diacetylfluorescein RS
$C_{24}H_{16}O_7$ 416.39
USP Fluorescein RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy*: ***197K*** ▲(CN 1-May-2020)
previously dried over silica gel for 16 hours.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%.
Zinc—Suspend 100 mg in 10 mL of a saturated solution of sodium chloride, add 2 mL of 3 N hydrochloric acid, mix, filter, and add 1 mL of potassium ferrocyanide TS to the filtrate: no turbidity is produced.
Acriflavine—Suspend 10 mg in 5 mL of water, swirl the mixture, and filter. To the filtrate add a few drops of sodium salicylate solution (1 in 10): no precipitate is formed.

Assay—
Standard preparation—Dissolve about 110 mg of USP Diacetylfluorescein RS, accurately weighed, in 10 mL of alcohol contained in a 100-mL volumetric flask. Add 2 mL of 2.5 N sodium hydroxide, and heat on a steam bath at about the boiling temperature for 20 minutes, with frequent swirling. Cool, dilute with water to volume, and mix. Dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 1.1 µg of diacetylfluorescein per mL. Transfer 3.0 mL of this solution to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), dilute with water to volume, and mix.
Assay preparation—Dissolve about 90 mg of Fluorescein, accurately weighed, in 10 mL of alcohol contained in a 100-mL volumetric flask. Add 2 mL of 2.5 N sodium hydroxide, and heat on a steam bath at about the boiling temperature for 20 minutes, with frequent swirling. Cool, dilute with water to volume, and mix. Dilute quantitatively and stepwise with water to obtain a solution having a concentration of 0.9 µg per mL. Transfer 3.0 mL of this solution to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), dilute with water to volume, and mix.
Procedure—Concomitantly determine the fluorescence intensities, *I*, of the *Standard preparation* and the *Assay preparation* in a fluorometer at an excitation wavelength of 485 nm and an emission wavelength of 515 nm. Calculate the quantity, in mg, of $C_{20}H_{12}O_5$ in the Fluorescein taken by the formula:

$$(332.31/416.39)(3333C)(I_U/I_S)$$

in which 332.31 and 416.39 are the molecular weights of fluorescein and diacetylfluorescein, respectively; *C* is the concentration, in µg per mL, of USP Diacetylfluorescein RS in the *Standard preparation;* and I_U and I_S are the fluorescence values observed for the *Assay preparation* and the *Standard preparation*, respectively.

Fluorescein Injection

DEFINITION
Fluorescein Injection is a sterile solution, in Water for Injection, of Fluorescein prepared with the aid of Sodium Hydroxide. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of fluorescein sodium ($C_{20}H_{10}Na_2O_5$). It may contain Sodium Bicarbonate.

IDENTIFICATION
- **A.** A solution of it is strongly fluorescent, even in extreme dilution. The fluorescence disappears when the solution is made acid and reappears when the solution is again made alkaline.

Change to read:

- **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲(USP 1-May-2019)

ASSAY

Change to read:

- **PROCEDURE**

▲**Buffer:** 0.61 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.0.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	85	15
20	20	80
29	20	80
30	85	15
35	85	15

Diluent: Acetonitrile and *Buffer* (30:70)
Standard stock solution: [NOTE—1.1 mg of USP Diacetylfluorescein RS is equivalent to 1 mg of fluorescein sodium.] 1.0 mg/mL of fluorescein sodium prepared as follows. Transfer USP Diacetylfluorescein RS to a suitable volumetric flask. Add 2.5 N sodium hydroxide to fill 2% of the final volume and add alcohol to fill 10% of the final volume. Heat on a water bath for 20 min, mixing frequently. Cool and dilute with water to volume.
Standard solution: 0.02 mg/mL of fluorescein sodium in *Diluent* from *Standard stock solution*
Sample stock solution: Nominally 1.0 mg/mL of fluorescein sodium in *Diluent* prepared as follows. Transfer a portion of Injection equivalent to 10.0 mg of fluorescein sodium into a 10-mL volumetric flask. Add 1 mL of alcohol. Dilute with *Diluent* to volume and mix well.

USP Monographs

www.webofpharma.com

Sample solution: Nominally 0.02 mg/mL of fluorescein sodium in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluorescein sodium ($C_{20}H_{10}Na_2O_5$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of fluorescein sodium in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluorescein sodium in the *Sample solution* (mg/mL) ▲(USP 1-May-2019)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**
Buffer, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution A: Use the *Standard stock solution* from the *Assay*.
Standard stock solution B: 0.05 mg/mL of fluorescein sodium in *Diluent* from *Standard stock solution A*
Standard stock solution C: 0.05 mg/mL each of USP Resorcinol RS, USP Phthalic Acid RS, and USP Fluorescein Related Compound C RS in *Diluent*
Standard solution: 0.001 mg/mL each of fluorescein sodium, USP Resorcinol RS, USP Phthalic Acid RS, and USP Fluorescein Related Compound C RS in *Diluent* from *Standard stock solution B* and *Standard stock solution C*
Sample solution: Use the *Sample stock solution* from the *Assay*.
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between resorcinol and phthalic acid
Relative standard deviation: NMT 5.0% for the resorcinol, phthalic acid, fluorescein related compound C, and fluorescein peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of resorcinol, phthalic acid, and fluorescein related compound C in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of resorcinol, phthalic acid, or fluorescein related compound C from the *Sample solution*
r_S = peak response of the corresponding Reference Standard from the *Standard solution*
C_S = concentration of USP Resorcinol RS, USP Phthalic Acid RS, or USP Fluorescein Related Compound C RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluorescein sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of fluorescein sodium from the *Standard solution*
C_S = concentration of fluorescein sodium in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluorescein sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Resorcinol	0.4	0.5
Phthalic acid	0.5	0.5
Fluorescein related compound C	0.9	0.5
Fluorescein	1.0	—
Any individual unspecified impurity	—	0.2
Total unspecified impurities	—	0.5 ▲(USP 1-May-2019)

SPECIFIC TESTS

- **PYROGEN TEST** ⟨151⟩: Meets the requirements, the test dose being the equivalent of 250 mg/kg of fluorescein sodium
- **pH** ⟨791⟩: 8.0–9.8
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩: Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass.

Change to read:

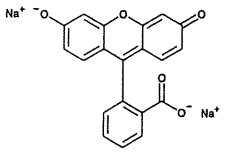
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Diacetylfluorescein RS
Spiro[isobenzofuran-1(3*H*),9′-(9*H*)xanthen]-3-one,3′,6′-bis(acetyloxy)-.
$C_{24}H_{16}O_7$ 416.39
▲USP Fluorescein Related Compound C RS
2-(2,4-Dihydroxybenzoyl)benzoic acid.
$C_{14}H_{10}O_5$ 258.23
USP Phthalic Acid RS
Phthalic acid.
$C_8H_6O_4$ 166.13

USP Monographs

www.webofpharma.com

USP Resorcinol RS
Resorcinol.
$C_6H_6O_2$ 110.11 ▲ (USP 1-May-2019)

Fluorescein Sodium



$C_{20}H_{10}Na_2O_5$ 376.27
Benzoic acid, 2-(6-hydroxy-3-oxo-3*H*-xanthene-9-yl), sodium salt (1:2);
Fluorescein disodium salt [518-47-8].

DEFINITION
Fluorescein Sodium contains NLT 90.0% and NMT 102.0% of fluorescein sodium ($C_{20}H_{10}Na_2O_5$), calculated on the anhydrous basis.

IDENTIFICATION
- **A.** A solution is strongly fluorescent, even in extreme dilution. The fluorescence disappears when the solution is made acid, and reappears when the solution is again made alkaline.
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩**:** The residue remaining after incineration meets the requirements.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Buffer: 0.61 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.0.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	85	15
20	20	80
29	20	80
30	85	15
35	85	15

Diluent: Acetonitrile and *Buffer* (30:70)
Standard stock solution: [NOTE—1.1 mg of USP Diacetylfluorescein RS is equivalent to 1 mg of fluorescein sodium.] 1.0 mg/mL of fluorescein sodium is prepared as follows. Transfer USP Diacetylfluorescein RS to a suitable volumetric flask. Add 2.5 N sodium hydroxide to fill 2% of the final volume and alcohol to fill 10% of the final volume. Heat on a water bath for 20 min, mixing frequently. Cool and dilute with water to volume.
Standard solution: 0.02 mg/mL of fluorescein sodium in *Diluent* from *Standard stock solution*
Sample stock solution: 1.0 mg/mL of Fluorescein Sodium in *Diluent*
Sample solution: 0.02 mg/mL of Fluorescein Sodium in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluorescein sodium ($C_{20}H_{10}Na_2O_5$) in the portion of Fluorescein Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of fluorescein sodium in the *Standard solution* (mg/mL)
C_U = concentration of Fluorescein Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–102.0% on the anhydrous basis

IMPURITIES
- **ZINC**

Sample solution: 10 mg/mL of Fluorescein Sodium in a saturated solution of sodium chloride
Analysis: To 10 mL of *Sample solution* add 2 mL of 3 N hydrochloric acid. Shake well, filter, and add 1 mL of potassium ferrocyanide TS to the filtrate.
Acceptance criteria: No turbidity is produced.

- **ACRIFLAVINE**

Sample solution: 2 mg/mL of Fluorescein Sodium in water. Swirl the mixture, and filter.
Analysis: To the *Sample solution* add a few drops of 100-mg/mL sodium salicylate solution.
Acceptance criteria: No precipitate is formed.

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: Proceed as directed in the *Assay* for *Standard solution*.
Standard solution A: 0.005 mg/mL of fluorescein sodium in *Diluent* from *Standard stock solution*
Standard solution B: 0.005 mg/mL each of USP Resorcinol RS, USP Phthalic Acid RS, and USP Fluorescein Related Compound C RS in *Diluent*
Sample solution: Use *Sample stock solution* from the *Assay*.
System suitability
Sample: *Standard solution B*
Suitability requirements
Resolution: NLT 1.5 between resorcinol and phthalic acid
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Calculate the percentage of resorcinol, phthalic acid, and fluorescein related compound C in the portion of Fluorescein Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

www.webofpharma.com

r_U = peak response of resorcinol or phthalic acid or fluorescein related compound C from the *Sample solution*
r_S = peak response of resorcinol or phthalic acid or fluorescein related compound C from *Standard solution B*
C_S = concentration of USP Resorcinol RS or USP Phthalic Acid RS or USP Fluorescein Related Compound C RS in *Standard solution B* (mg/mL)
C_U = concentration of Fluorescein Sodium in the *Sample solution* (mg/mL)

Calculate the percentage of unspecified impurity in the portion of Fluorescein Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of unspecified impurity from the *Sample solution*
r_S = peak response from *Standard solution A*
C_S = concentration of fluorescein sodium in *Standard solution A* (mg/mL)
C_U = concentration of Fluorescein Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Resorcinol	0.42	0.5
Phthalic acid	0.48	0.5
Fluorescein related compound C	0.86	0.5
Fluorescein	1.0	—
Unspecified impurity	—	0.10
Total unspecified impurities	—	0.5

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 17.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Diacetylfluorescein RS
$C_{24}H_{16}O_7$ 416.39
USP Fluorescein Related Compound C RS
2-(2,4-Dihydroxybenzoyl)benzoic acid.
$C_{14}H_{10}O_5$ 258.23
USP Phthalic Acid RS
$C_8H_6O_4$ 166.13
USP Resorcinol RS
$C_6H_6O_2$ 110.11

Fluorescein Sodium Ophthalmic Strips

DEFINITION
Fluorescein Sodium Ophthalmic Strips contain NLT 100.0% and NMT 160.0% of the labeled amount of fluorescein sodium ($C_{20}H_{10}Na_2O_5$).

IDENTIFICATION
• **A.**
Sample solution: Cut the colored tip from 1 Ophthalmic Strip, place it in a small test tube containing 1 mL of water, and agitate for 1 min.
Acceptance criteria: *Sample solution* is strongly fluorescent, even in extreme dilution. The fluorescence disappears when the *Sample solution* is made acid and reappears when it is again made alkaline.
• **B.**
Sample solution: Cut the colored tip from 1 Ophthalmic Strip, place it in a small test tube containing 1 mL of water, and agitate for 1 min. Place 1 drop of solution upon a piece of filter paper.
Acceptance criteria: A yellow spot is produced, and when exposed while moist to the vapor of bromine for 1 min and then to ammonia vapor, it becomes deep pink in color.

ASSAY
• **PROCEDURE**
Standard stock solution: 1 µg/mL of fluorescein sodium from USP Diacetylfluorescein RS in water prepared as follows. Dissolve USP Diacetylfluorescein RS in 10 mL of alcohol contained in a 100-mL volumetric flask, add 2 mL of 2.5 N sodium hydroxide, and heat on a steam bath at the boiling temperature for 20 min, with frequent swirling. Cool, and dilute with water to volume. Transfer a suitable aliquot to a volumetric flask, and dilute with water to volume. [NOTE—110.7 mg of anhydrous USP Diacetylfluorescein RS is equivalent to 100.0 mg of fluorescein sodium.]
Standard solution: 0.03 µg/mL of fluorescein sodium in water prepared as follows. Transfer 3 mL of *Standard stock solution* to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*), and dilute with water to volume.
Sample stock solution: Remove 1 Ophthalmic Strip from its package, taking care not to allow any portion of the tip to adhere to the packaging material. Transfer to a 100-mL volumetric flask, add 50 mL of water, shake the flask vigorously, and dilute with water to volume. Shake occasionally, and after 1 h, mix the contents of the flask. Transfer an aliquot (V) of this solution, equivalent to 100 µg of fluorescein sodium, to a 100-mL volumetric flask, and dilute with water to volume.
Sample solution: Transfer 3 mL of the *Sample stock solution* to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see *Reagents, Indicators, and Solutions —Buffer Solutions*), and dilute with water to volume.
Instrumental conditions
Mode: Fluorescence
Excitation wavelength: 485 nm
Emission wavelength: 515 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Concomitantly determine the fluorescence intensities, *I*, of the *Standard solution* and the *Sample solution*.
Calculate the percentage of the labeled amount of fluorescein sodium ($C_{20}H_{10}Na_2O_5$) in the Ophthalmic Strip taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence value of the *Sample solution*
I_S = fluorescence value of the *Standard solution*
C_S = concentration of fluorescein sodium in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluorescein sodium in the *Sample solution* (µg/mL)

USP Monographs

www.webofpharma.com

Acceptance criteria: 100.0%–160.0% on the average content calculated from the individual assays of NLT 10 Ophthalmic Strips

PERFORMANCE TESTS

• **CONTENT UNIFORMITY:** The content of fluorescein sodium ($C_{20}H_{10}Na_2O_5$) in each of NLT 10 Ophthalmic Strips, determined as directed in the *Assay*, is 85.0%–175.0% of the labeled amount.

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package NMT 2 Ophthalmic Strips in a single-unit container in such a manner as to maintain sterility until the package is opened. Package individual packages in a second protective container.

• **LABELING:** The label of the second protective container bears a statement that the contents may not be sterile if the individual package has been damaged or opened previously. The label states the amount of fluorescein sodium in each Ophthalmic Strip.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Diacetylfluorescein RS

Spiro(isobenzofuran-1(3*H*), 9′-(9*H*)xanthen)-3-one, 3′,6′-bis(acetyloxy)-.

$C_{24}H_{16}O_7$ 416.39

Fluorescein Sodium and Benoxinate Hydrochloride Ophthalmic Solution

DEFINITION

Fluorescein Sodium and Benoxinate Hydrochloride Ophthalmic Solution is a sterile aqueous solution of fluorescein sodium and benoxinate hydrochloride. It contains NLT 90.0% and NMT 120.0% of the labeled amounts of fluorescein sodium ($C_{20}H_{10}Na_2O_5$) and benoxinate hydrochloride ($C_{17}H_{28}N_2O_3 \cdot HCl$). It contains a suitable preservative.

IDENTIFICATION

• **A.** A solution of it is strongly fluorescent, even in extreme dilution. The fluorescence disappears when the solution is made acid, and reappears when the solution is again made alkaline.

• **B.** The relative retention times of the major peaks of the *Sample solution* correspond to those of *Standard solution A* and *Standard solution B*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Dissolve 100 mg of sodium 1-pentanesulfonate in 40 mL of glacial acetic acid in a 2000-mL volumetric flask. Add 600 mL of acetonitrile and 10 mL of triethanolamine, and dilute with water to volume. Adjust with phosphoric acid to a pH of 3.

Standard stock solution A: Transfer 55 mg of USP Diacetylfluorescein RS to a 50-mL volumetric flask containing 5 mL of alcohol. Add 1 mL of 2.5 N sodium hydroxide, and heat on a steam bath at the boiling temperature for 20 min, with frequent swirling. Cool, and dilute with water to volume.

Standard solution A: 0.1 mg/mL of fluorescein sodium in *Mobile phase* prepared as follows. Transfer 10.0 mL of *Standard stock solution A* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Standard solution B: 0.1*J* mg/mL of USP Benoxinate Hydrochloride RS in *Mobile phase*, where *J* is the ratio of the labeled amount, in mg, of benoxinate hydrochloride to the labeled amount, in mg, of fluorescein sodium in each mL of Ophthalmic Solution

Sample solution: Nominally equivalent to 0.1 mg/mL of fluorescein sodium in *Mobile phase* from Ophthalmic Solution

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Tailing factor: NMT 2.0 for each analyte peak, *Standard solution A* and *Standard solution B*

Relative standard deviation: NMT 2.0% for each analyte peak, *Standard solution A* and *Standard solution B*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of the labeled amount of fluorescein sodium ($C_{20}H_{10}Na_2O_5$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of fluorescein from the *Sample solution*

r_S = peak area of fluorescein from *Standard solution A*

C_S = concentration of USP Diacetylfluorescein RS in *Standard solution A* (mg/mL)

C_U = nominal concentration of fluorescein sodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of fluorescein sodium, 376.28

M_{r2} = molecular weight of diacetylfluorescein, 416.39

Calculate the percentage of the labeled amount of benoxinate hydrochloride ($C_{17}H_{28}N_2O_3 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of benoxinate from the *Sample solution*

r_S = peak area of benoxinate from *Standard solution B*

C_S = concentration of USP Benoxinate Hydrochloride RS in *Standard solution B* (mg/mL)

C_U = nominal concentration of benoxinate hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria

Fluorescein sodium: 90.0%–120.0%

Benoxinate hydrochloride: 90.0%–120.0%

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **pH** ⟨791⟩: 4.3–5.3

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Benoxinate Hydrochloride RS

USP Monographs

www.webofpharma.com

USP Diacetylfluorescein RS
Spiro(isobenzofuran-1(3*H*), 9′-(9*H*)xanthen)-3-one, 3′,6′-bis(acetyloxy)-.
$C_{24}H_{16}O_7$ 416.39

Fluorescein Sodium and Proparacaine Hydrochloride Ophthalmic Solution

DEFINITION
Fluorescein Sodium and Proparacaine Hydrochloride Ophthalmic Solution is a sterile aqueous solution of Fluorescein Sodium and Proparacaine Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of fluorescein sodium ($C_{20}H_{10}Na_2O_5$) and proparacaine hydrochloride ($C_{16}H_{26}N_2O_3 \cdot HCl$). It contains a suitable preservative.

IDENTIFICATION
- **A.** A solution of it is strongly fluorescent, even in extreme dilution. The fluorescence disappears when the solution is made acid and reappears when the solution is again made alkaline.
- **B.**

Solution A: 20 mg/mL of 2-naphthol in 1 N sodium hydroxide

Analysis: To 1 mL of Ophthalmic Solution in a test tube add 5 mL of diluted hydrochloric acid (1 in 100), and cool in an ice bath for 2 min. Add 1 mL of *Solution A*.

Acceptance criteria: A scarlet-red precipitate is formed. Add 5 mL of acetone. The precipitate does not dissolve.

ASSAY
- **FLUORESCEIN SODIUM**

Standard stock solution: 1 µg/mL of fluorescein sodium from USP Diacetylfluorescein RS in water prepared as follows. Dissolve USP Diacetylfluorescein RS in 10 mL of alcohol contained in a 100-mL volumetric flask, add 2 mL of 2.5 N sodium hydroxide, and heat on a steam bath at the boiling temperature for 20 min, with frequent swirling. Cool, and dilute with water to volume. Transfer a suitable aliquot to a volumetric flask, and dilute with water to volume. [NOTE—110.7 mg of anhydrous USP Diacetylfluorescein RS is equivalent to 100.0 mg of fluorescein sodium.]

Standard solution: 0.03 µg/mL of fluorescein sodium in water prepared as follows. Transfer 3 mL of *Standard stock solution* to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*), and dilute with water to volume.

Sample stock solution: Nominally equivalent to 1 µg/mL of fluorescein sodium from Ophthalmic Solution in water

Sample solution: Nominally equivalent to 0.03 µg/mL of fluorescein sodium in water prepared as follows. Transfer 3.0 mL of *Sample stock solution* to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*), and dilute with water to volume.

Instrumental conditions
Mode: Fluorescence
Excitation wavelength: 485 nm
Emission wavelength: 515 nm

Analysis
Samples: *Standard solution* and *Sample solution*
Concomitantly determine the fluorescence intensities, *I*, of the *Standard solution* and the *Sample solution*.

Calculate the percentage of the labeled amount of fluorescein sodium ($C_{20}H_{10}Na_2O_5$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence value of the *Sample solution*
I_S = fluorescence value of the *Standard solution*
C_S = concentration of fluorescein sodium in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluorescein sodium in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

- **PROPARACAINE HYDROCHLORIDE**

Buffer: 6.8 g/L of monobasic potassium phosphate in 1000 mL of water. Add 5 mL of triethylamine, and adjust with 5 N potassium hydroxide to a pH of 7.5.

Mobile phase: Acetonitrile and *Buffer* (40:60)

Standard solution: 0.1 mg/mL of proparacaine in *Mobile phase* prepared as follows. Prepare 1 mg/mL of USP Proparacaine Hydrochloride RS in water, and dilute it with *Mobile phase* to 0.1 mg/mL. Use this solution within 6 h.

Sample solution: Nominally equivalent to 0.1 mg/mL of proparacaine hydrochloride in *Mobile phase* from Ophthalmic Solution. Use this solution within 6 h.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 15-cm; 5-µm packing L10
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 3000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of proparacaine hydrochloride ($C_{16}H_{26}N_2O_3 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Proparacaine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of proparacaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **pH** ⟨791⟩: 4.0–5.2

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, preferably of Type I amber glass, and store in a refrigerator.
- **LABELING:** Label it to state that it is to be stored in a refrigerator before and after the container is opened.
- **USP REFERENCE STANDARDS** ⟨11⟩

www.webofpharma.com

USP Diacetylfluorescein RS
Spiro(isobenzofuran-1(3*H*), 9′-(9*H*)xanthen)-3-one, 3′,6′-bis(acetyloxy)-.
$C_{24}H_{16}O_7$ 416.39
USP Proparacaine Hydrochloride RS

Fludeoxyglucose F 18 Injection

DEFINITION

Change to read:

▲Fludeoxyglucose F 18 Injection is a sterile, aqueous solution of 2-deoxy-2-[^{18}F]fluoro-D-glucose. It contains NLT 90% and NMT 110% of the labeled amount of 2-deoxy-2-[^{18}F]fluoro-D-glucose expressed in MBq (or mCi)/mL at the time indicated in the labeling. It may contain buffering agents, preservatives, stabilizing agents or sodium chloride. It does not contain any added carrier. ▲ 2S (USP41)

IDENTIFICATION

Change to read:

• **A. RADIONUCLIDIC IDENTITY**
▲(See *Radioactivity* ⟨821⟩, *Identification of Radionuclides, Half-Life Determination.*) ▲ 2S (USP41)
Acceptance criteria: The half-life is 105–115 min.

Change to read:

• **B. RADIOCHEMICAL IDENTITY**
▲**Spray reagent:** 10 mg/mL of *p*-anisidine in a mixture of methanol and butanol (10:90)
Procedure: After completing the *Analysis* in the test for *Radiochemical Purity*, spray the TLC strip with *Spray reagent* and heat strip on a hot plate until the spot for USP Fludeoxyglucose RS is visible.
Acceptance criteria: The R_F value of fludeoxyglucose F 18 in the *Sample solution* is 90%–110% of the R_F value of USP Fludeoxyglucose RS in the *Standard solution* as determined in the test for *Radiochemical Purity*. ▲ 2S (USP41)

ASSAY

Delete the following:

▲• **PROCEDURE**
Radioactivity: Using a suitable calibrated system as directed under *Radioactivity* ⟨821⟩, determine the radioactivity of the Injection in MBq (or mCi)/mL.
Acceptance criteria: 90.0%–110.0% ▲ 2S (USP41)

Add the following:

▲• **RADIOACTIVE CONCENTRATION (STRENGTH)**
(See *Radioactivity* ⟨821⟩, *Assay of Radionuclides.*)
Analysis: Using a dose calibrator or other suitable instrument for radiation measurements, measure the quantity of radioactivity in a known volume of the Injection. Determine the radioactivity, in MBq (or mCi)/mL.
Acceptance criteria: 90.0%–110.0% at the time indicated on the label ▲ 2S (USP41)

PURITY

Change to read:

• **RADIONUCLIDIC PURITY**
▲(See *Radioactivity* ⟨821⟩, *Identification of Radionuclides, Gamma-Ray Spectrometry.*)
[NOTE—This may be a periodic quality indicator test. The Injection may be distributed and dispensed prior to completion of this test.]
Analysis: Determine the purity of fludeoxyglucose F 18 in the portion of Injection taken for the *Radionuclidic Impurities* test:

$$\text{Result} = [1 - (C_I/C_T)] \times 100$$

C_I = sum of the concentrations of all longer-lived radionuclides, decay corrected to the expiration time from the *Radionuclidic Impurities* test (Bq/mL or µCi/mL)
C_T = sum of the concentrations of all long-lived radionuclides and fludeoxyglucose F 18, all decay corrected to the expiration time from the *Radionuclidic Impurities* test (Bq/mL or µCi/mL)

Acceptance criteria: At the time of expiration, NLT 99.5% of radionuclides in the Injection corresponds to ^{18}F. ▲ 2S (USP41)

Change to read:

• **RADIOCHEMICAL PURITY**
▲**Diluent:** Acetonitrile and water (95:5)
Standard solution: 0.1 mg/mL of USP Fludeoxyglucose RS in *Diluent*
Sample solution: A volume of Injection, appropriately diluted to a concentration of 185–1110 MBq/mL (5–30 mCi/mL) or to a concentration suitable for the detector to be used
Chromatographic system
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel strip
Application volume: 0.5 µL
Developing solvent system: *Diluent*
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram in the *Developing solvent system* until the solvent has moved about 90% of the length of the strip. Remove the strip and allow to dry in the air or under a stream of a compressed gas such as nitrogen or air. Determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. After completing the test for *Radiochemical purity*, use the TLC strip for the *Radiochemical Identity* test.
Calculate the percentage of fludeoxyglucose F 18 in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = area of the peak due to fludeoxyglucose F 18 in the radio-TLC chromatogram
r_T = sum of areas of all the peaks including the peak due to fludeoxyglucose F 18 in the radio-TLC chromatogram

USP Monographs

www.webofpharma.com

Acceptance criteria: NLT 90% of the total radioactivity is due to fludeoxyglucose F 18. ▲ 2S (USP41)

IMPURITIES

Delete the following:

▲• **ORGANIC IMPURITIES**

[NOTE—The methods and limits described in this section relate to potential impurities associated with the acid-hydrolysis method of synthesis for the Injection. Specific examples include aminopolyether (Kryptofix®) and 2-chloro-2-deoxy-D-glucose. If methods of synthesis that may result in different impurities are used, the presence of unlabeled ingredients, reagents, and by-products specific to the process must be controlled, and their potential for physiological or pharmacological effects must be considered (see *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* ⟨823⟩). Any ingredients with toxic potential must be within appropriate limits, and conformance with these limits is to be demonstrated by the use of one or more validated limit tests.]

▲ 2S (USP41)

Add the following:

▲• **RADIONUCLIDIC IMPURITIES**

[NOTE—This may be a periodic quality indicator test. The Injection may be distributed or dispensed prior to completion of the test.]

Sample: A suitable volume of Injection, decayed for a suitable length of time to eliminate interference due to ^{18}F emissions

Analysis: Using a suitable gamma-ray spectrometer count an appropriate aliquot of the sample solution for a period of time sufficient to collect a gamma spectrum. The resultant gamma spectrum should be analyzed for the presence of identifiable photopeaks, which are characteristic of radionuclidic impurities.

Determine the concentration of radionuclidic impurities in Bq (or µCi)/mL, decay corrected to the expiration time of the Injection:

$$\text{Result} = (C_i/C_T) \times 100$$

C_i = sum of the concentrations of all longer-lived radionuclides, decay corrected to the expiration time (Bq/mL or µCi/mL)

C_T = sum of the concentrations of all long-lived radionuclides and fludeoxyglucose F 18, all decay corrected to the expiration time (Bq/mL or µCi/mL)

Acceptance criteria: Total radionuclidic impurities are NMT 0.5% of the radioactivity of the Injection (see the *Assay*), decay corrected to the time of expiration. ▲ 2S (USP41)

Add the following:

▲• **RADIOCHEMICAL IMPURITIES**

Analysis: From the radio-TLC chromatogram obtained in the *Radiochemical Purity* test, calculate the radiochemical impurities in the portion of Injection taken:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = sum of areas of all the peaks other than fludeoxyglucose F 18 in the radio-TLC chromatogram

r_T = sum of areas of all the peaks including the peak due to fludeoxyglucose F18 in the radio-TLC chromatogram

Acceptance criteria: NMT 10% ▲ 2S (USP41)

Delete the following:

▲• **LIMIT OF AMINOPOLYETHER**

[NOTE—This test must be performed for fludeoxyglucose F 18 produced by any route of synthesis that uses this reagent.]

Adsorbent: 0.25-mm layer of chromatographic silica gel

Sample solution: Use the Injection.

Standard solution: 50 µg/mL in saline TS

Application volume: 5 µL

Developing solvent system: Methanol and 30% ammonium hydroxide (9:1)

Chromatographic system

(See *Chromatography* ⟨621⟩, *General Procedures, Thin-layer Chromatography*.)

Mode: TLC

Analysis

Samples: *Sample solution* and *Standard solution*

Proceed as directed for *Chromatography* ⟨621⟩, *General Procedures, Thin-layer Chromatography*. Place the plate in a chamber containing iodine crystals. Develop the plate until a spot is visible on the chromatogram of the *Standard solution*.

Acceptance criteria: The size and intensity of the spot from the *Sample solution* is NMT that from the *Standard solution*. ▲ 2S (USP41)

Add the following:

▲• **LIMIT OF FLUDEOXYGLUCOSE RELATED COMPOUND A**

[NOTE—Perform this test if fludeoxyglucose related compound A (also known as aminopolyether) is used in the synthesis of fludeoxyglucose F 18.]

Adsorbent: Aluminum oxide strips

Standard solution: 50 µg/mL of USP Fludeoxyglucose Related Compound A RS in saline TS

Sample solution: Use the Injection.

Application volume: 4 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Spot the *Standard solution* and *Sample solution* on the strip. Allow to dry and place the strip in a chamber containing fresh iodine crystals. Develop the plate until a spot is visible on the strip for the *Standard solution*.

Acceptance criteria: The size and intensity of the spot from the *Sample solution* is NMT that from the *Standard solution*. ▲ 2S (USP41)

Delete the following:

▲• **LIMIT OF 2-CHLORO-2-DEOXY-D-GLUCOSE**

[NOTE—This test is performed when the nucleophilic synthesis includes hydrolysis with hydrochloric acid or the use of anionic exchange resins in the chloride form to trap fluoride ^{18}F released from the target prior to its use in the synthesis of fludeoxyglucose F 18.]

Mobile phase: 16 g of 50% sodium hydroxide solution in 1000 mL of water

USP Monographs

www.webofpharma.com

System suitability solution: 1.0 mg/mL of USP Fludeoxyglucose RS and 0.1 mg/mL of USP Fludeoxyglucose Related Compound B RS in *Mobile phase*
Standard solution: 0.1 mg/mL of USP Fludeoxyglucose Related Compound B RS
Sample solution: Use the Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Pulsed amperometric
Column: 4.0-mm × 25-cm; 10-µm packing L46
Flow rate: 0.5 mL/min
Injection volume: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between fludeoxyglucose and fludeoxyglucose related compound B
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the areas of the major peaks. Calculate the quantity, in mg, of 2-chloro-2-deoxy-D-glucose in each mL of the Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak area for 2-chloro-2-deoxy-D-glucose from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Fludeoxyglucose Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1 mg/dose of Injection is found. ▲ 2S (USP41)

Add the following:

▲• **LIMIT OF FLUDEOXYGLUCOSE RELATED COMPOUND B**
[NOTE—This may be a periodic quality indicator test. Perform this test if fludeoxyglucose related compound B is a potential process impurity in the synthesis of fludeoxyglucose F 18. The Injection may be distributed or dispensed prior to completion of the test.]
Mobile phase: 0.1 M sodium hydroxide
System suitability solution: 0.01 mg/mL each of USP Fludeoxyglucose RS and USP Fludeoxyglucose Related Compound B RS in water
Standard solution: 0.01 mg/mL of USP Fludeoxyglucose Related Compound B RS in water
Sample solution: Use the Injection.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Pulsed amperometric detector with suitable pulse sequence
Columns
Guard: 4.0-mm × 5-cm; 10-µm packing L46
Analytical: 4.0-mm × 25-cm; 10-µm packing L46
Flow rate: 0.4 mL/min
Injection volume: 10 µL
Run time: NLT 1.5 times the retention of fludeoxyglucose
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between fludeoxyglucose and fludeoxyglucose related compound B, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Signal-to-noise ratio: NLT 50, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the areas of the major peaks.
Calculate the amount of fludeoxyglucose related compound B in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak area of fludeoxyglucose related compound B from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Fludeoxyglucose Related Compound B RS in the *Standard solution* (mg/mL)

Acceptance criteria: NMT 1.0 mg/*V* of fludeoxyglucose related compound B is found, where *V* is the volume (mL) of dose injected. ▲ 2S (USP41)

Delete the following:

▲• **RESIDUAL SOLVENTS**
Standard solution A: 0.1% ether in water
Standard solution B: 0.01% acetonitrile in water
Standard solution C: 0.1% dehydrated alcohol in water
Sample solution: Use the Injection.
Chromatographic system
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused-silica column coated with a 0.25-µm, chemically cross-linked G16 stationary phase
Temperature:
Detector: 300°
Injection port: 250°
Column: See *Table 1*

Table 1

Initial Temperature°	Temperature Ramp(°/min)	Final Temperature °	Hold Time at Final Temperature
40	0	40	2
130	20	130	5.5

Carrier gas: Helium.
[NOTE—Nitrogen may be used as a makeup gas.]
Flow rate: 10 mL/min
Injection volume: 1 µL
Injection type: Splitless injector system
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between any two components
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solutions* and *Sample solution*
Calculate the percentage of acetonitrile, ether, and alcohol in the Injection:

$$\text{Result} = (C_S) \times (r_U/r_S)$$

C_S = percentage of the relevant analyte in the *Standard solution*

www.webofpharma.com

r_U = peak response of the relevant analyte from the *Sample solution*, if any
r_S = peak response of the relevant analyte from the *Standard solution*

Acceptance criteria: NMT 0.04% of acetonitrile is found; NMT 0.5% of ether is found; and NMT 0.5% of alcohol is found. ▲ 2S (USP41)

Add the following:

▲• **LIMIT OF ACETONITRILE AND ETHANOL**
[NOTE—Perform this test if ethanol and acetonitrile are known to be present in the article.]
Standard solution: 0.04% (v/v) acetonitrile and 0.5% (v/v) ethanol from USP Alcohol Determination—Alcohol RS in water
Sample solution: Use the Injection. [NOTE—Injection may be appropriately diluted if necessary.]
Chromatographic system
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused-silica; coated with a 1.0–1.2-µm film of phase G16
Temperatures
Injection port: 150°
Detector: 250°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	0	60	3.5
60	15	160	0

Carrier gas: Helium
[NOTE—Nitrogen may be used as a makeup gas.]
Flow rate: 9 mL/min
Injection volume: 1 µL
Injection type: Splitless injector system
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 7.0 between acetonitrile and ethanol
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of acetonitrile and ethanol in the Injection:

$$\text{Result} = (r_U/r_S) \times (C_S)$$

r_U = peak response of acetonitrile or ethanol from the *Sample solution*, if any
r_S = peak response of acetonitrile or ethanol from the *Standard solution*
C_S = percentage of the acetonitrile or ethanol in the *Standard solution*

Acceptance criteria: NMT 0.041% of acetonitrile is found; NMT 0.5% of ethanol is found. ▲ 2S (USP41)

SPECIFIC TESTS

Delete the following:

▲• **SPECIFIC ACTIVITY:** No carrier added ▲ 2S (USP41)

Add the following:

▲• **APPEARANCE:** Clear, colorless solution, free from visible particulates ▲ 2S (USP41)

Change to read:

• **pH**
▲**Analysis:** Apply a suitable volume of the Injection on a suitable short-range pH indicator paper.
Acceptance criteria: 4.5–7.5 ▲ 2S (USP41)

Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: ▲▲ 2S (USP41) Meets the requirements

Add the following:

▲• **STERILITY TESTS** ⟨71⟩
[NOTE—The Injection may be distributed or dispensed prior to completion of the test, the latter test being started within 36 h of final manufacture.]
Sample: Use NLT 0.25 mL of the Injection.
Acceptance criteria: Meets the requirements ▲ 2S (USP41)

Delete the following:

▲• **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the Injection may be distributed or dispensed prior to completion of the test under ⟨71⟩, the latter test being started within 24 h of final manufacture, and except that it is not subject to the recommendation of *Container content*. ▲ 2S (USP41)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers that are adequately shielded.
▲Store at controlled room temperature. ▲ 2S (USP41)

Change to read:

• **LABELING:** ▲The label indicates the time and date of calibration; the radioactivity concentration of fludeoxyglucose F 18 expressed in MBq (or mCi)/mL at the time of calibration; the total volume or total radioactivity at the time of calibration the expiration time and date; and the name and quantity of any added preservative or stabilizer. The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay. The radioactive half-life of ^{18}F is 109.8 min. The label also indicates the following:
[**CAUTION**—Radioactive Material. Do not use if cloudy or if it contains visible particulate matter. ▲ 2S (USP41)]

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲▲ (CN 1-May-2018)
USP Alcohol Determination—Alcohol RS

www.webofpharma.com

USP Fludeoxyglucose RS
USP Fludeoxyglucose Related Compound A RS
4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8] hexacosane.
$C_{18}H_{36}N_2O_6$ 376.49
USP Fludeoxyglucose Related Compound B RS
2-Chloro-2-deoxy-D-glucose.
$C_6H_{11}ClO_5$ 198.60

Sodium Fluoride F 18 Injection

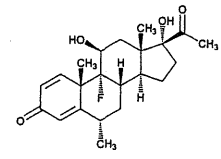
» Sodium Fluoride F 18 Injection is a sterile solution, suitable for intravenous administration, of sodium fluoride in Sodium Chloride Injection in which a portion of the molecules are labeled with radioactive ^{18}F (see *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* ⟨823⟩). It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{18}F expressed in megabecquerels (or in millicuries) per mL at the time indicated in the labeling.

Specific activity: no carrier added.
Packaging and storage—Preserve in single-dose or multiple-dose containers that are adequately shielded.
Labeling—Label container to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{18}F as fluoride anion expressed as MBq (or mCi) per mL, at time of calibration; total activity at time of calibration; the expiration time and date; and the statements "Caution—Radioactive Material" and "Do not use if cloudy or if it contains particulate matter." Label it to indicate that the radioactive half-life of ^{18}F is 109.7 minutes, and that in making dosage calculations, correction is to be made for radioactive decay.
USP Reference standards ⟨11⟩—
USP Sodium Fluoride RS

Radionuclide identification (see *Radioactivity* ⟨821⟩)—
A: Its half-life, determined using a suitable detector system, is between 105 and 115 minutes.
B: Radiochemical identity—The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the test for *Radiochemical purity*.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 175/*V* USP Endotoxin Unit per mL of the Injection, in which *V* is the maximum administered total dose, in mL, at the expiration time. It also meets the requirements of *Quality Control* under *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* ⟨823⟩.
Sterility Tests ⟨71⟩: meets the requirements.
pH ⟨791⟩: between 4.5 and 8.0.
Chemical purity—This article may be synthesized by different methods and processes and may, therefore, may contain different impurities. The presence of unlabeled ingredients, reagents, and by-products specific to the process must be controlled, and their potential for physiological or pharmacological effects must be considered.

Radiochemical purity—
Mobile phase—Prepare a filtered and degassed solution of 0.003 N sulfuric acid in water.
Standard solution—Dissolve an accurately weighed quantity of USP Sodium Fluoride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg per mL.
Test solution—Use the Injection.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a flow-through gamma ray detector, a conductivity detector, and a 10-mm × 25-cm column that contains 10-µm packing L31. The flow rate is about 0.8 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5%.
Procedure—Prepare a solution of the *Test solution* and the *Standard solution*, inject about 50 µL of the combined solution into the chromatograph, record the chromatograms, and measure the areas for both the radioactive and nonradioactive peaks (the injection volume may be adjusted to obtain suitable detection system sensitivity): the radioactivity of the major peak is not less than 95% of the total radioactivity measured; and the retention time of the *Test solution* corresponds to the retention time (about 8 minutes) of the *Standard solution*.
Radionuclide purity (see *Radioactivity* ⟨821⟩)—Using a suitable gamma-ray spectrometer, count an appropriate aliquot of Injection for a period of time sufficient to obtain a gamma spectrum. The resultant gamma spectrum should be analyzed for the presence of identifiable photopeaks which are not characteristic of ^{18}F emission: not less than 99.5% of the gamma emissions correspond to the 0.511 MeV, 1.022 MeV, or Compton scatter peaks of ^{18}F.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the Injection may be distributed or dispensed prior to completion of the test for *Sterility* ⟨71⟩, the latter test started within 24 hours of final manufacture, and except that it is not subject to the recommendation on *Container Content*.
Assay for radioactivity—Using a suitable calibrated system as directed under *Radioactivity* ⟨821⟩, determine the radioactivity, in MBq (or MCi) per mL, of Injection.

Fluorometholone



$C_{22}H_{29}FO_4$ 376.46
Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-6-methyl-, (6α,11β)-;
9-Fluoro-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione [426-13-1].

DEFINITION
Fluorometholone contains NLT 97.0% and NMT 103.0% of fluorometholone ($C_{22}H_{29}FO_4$), calculated on the dried basis.

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Mix 425 g (538 mL) of methanol and 400 g (400 mL) of water. Adjust with phosphoric acid to a pH of 2.4.

Standard stock solution: 0.25 mg/mL of USP Fluorometholone RS prepared as follows. Transfer a suitable amount of USP Fluorometholone RS to a suitable volumetric flask and dissolve in 2% of the final volume of tetrahydrofuran. Dilute with *Mobile phase* to volume.

Standard solution: 50 µg/mL of USP Fluorometholone RS in *Mobile phase* from *Standard stock solution*

Sample stock solution: 0.25 mg/mL of Fluorometholone prepared as follows. Transfer a suitable amount of Fluorometholone to a suitable volumetric flask and dissolve in 2% of the final volume of tetrahydrofuran. Dilute with *Mobile phase* to volume.

Sample solution: 50 µg/mL of Fluorometholone in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 0.6 mL/min

Injection volume: 20 µL

Run time: NLT 1.6 times the retention time of fluorometholone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.3

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fluorometholone ($C_{22}H_{29}FO_4$) in the portion of Fluorometholone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluorometholone from the *Sample solution*

r_S = peak response of fluorometholone from the *Standard solution*

C_S = concentration of USP Fluorometholone RS in the *Standard solution* (µg/mL)

C_U = concentration of Fluorometholone in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.2%
- **ORGANIC IMPURITIES**

Mobile phase: Proceed as directed in the *Assay*.

System suitability stock solution: 0.5 mg/mL each of USP Fluorometholone RS and USP Fluorometholone Related Compound A RS prepared as follows. Transfer a suitable amount of USP Fluorometholone RS and USP Fluorometholone Related Compound A RS to a suitable volumetric flask and dissolve in 4% of the final volume of tetrahydrofuran. Dilute with *Mobile phase* to volume.

System suitability solution: 0.005 mg/mL each of USP Fluorometholone RS and USP Fluorometholone Related Compound A RS in *Mobile phase* from *System suitability stock solution*

Standard stock solution: 0.5 mg/mL of USP Fluorometholone RS prepared as follows. Transfer a suitable amount of USP Fluorometholone RS to a suitable volumetric flask and dissolve in 4% of the final volume of tetrahydrofuran. Dilute with *Mobile phase* to volume.

Standard solution: 0.5 µg/mL of USP Fluorometholone RS in *Mobile phase* from *Standard stock solution*

Sample solution: 0.5 mg/mL of Fluorometholone prepared as follows. Transfer a suitable amount of Fluorometholone to a suitable volumetric flask and dissolve in 4% of the final volume of tetrahydrofuran. Dilute with *Mobile phase* to volume.

Chromatographic system: Proceed as directed in the *Assay* except for the *Run time*.

Run time: NLT 2.1 times the retention time of fluorometholone

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3 between fluorometholone and fluorometholone related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Fluorometholone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of fluorometholone from the *Standard solution*

C_S = concentration of USP Fluorometholone RS in the *Standard solution* (mg/mL)

C_U = concentration of Fluorometholone in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peaks below 0.02%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fluorometholone	1.0	—
Fluorometholone related compound A	1.2	0.5
Individual unspecified impurities	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL in dimethyl sulfoxide

Acceptance criteria: +62° to +70°

- **LOSS ON DRYING ⟨731⟩**

Analysis: Dry under vacuum at 60° for 3 h.

Acceptance criteria: NMT 1.0%

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fluorometholone RS
  USP Fluorometholone Related Compound A RS
  11β,17α-Dihydroxy-6α-methylpregna-1,4-diene-3,20-dione.
  $C_{22}H_{30}O_4$ 358.47

Fluorometholone Cream

» Fluorometholone Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluorometholone ($C_{22}H_{29}FO_4$).

Packaging and storage—Preserve in collapsible tubes.
USP Reference standards ⟨11⟩—
USP Fluorometholone RS
Identification—The retention ratios of the main peak to the internal standard peak obtained with the *Standard preparation* and the *Assay preparation* as directed in the *Assay* do not differ by more than 2.0%.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
Minimum fill ⟨755⟩: meets the requirements.

Assay—

Internal standard solution—Dissolve fluoxymesterone in acetonitrile to obtain a solution containing about 100 µg per mL.

Mobile solvent—Prepare a solution containing butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6).

Standard preparation—Dissolve a suitable quantity of USP Fluorometholone RS, accurately weighed, in *Internal standard solution* to obtain a solution having a known concentration of about 50 µg per mL.

Assay preparation—Transfer an accurately weighed quantity of Cream, equivalent to about 1 mg of fluorometholone, into a suitable container, add 20.0 mL of *Internal standard solution*, and mix.

Procedure—Treat 20.0 mL each of the *Standard preparation* and the *Assay preparation* in the following manner. To each add 10.0 mL of hexane, and shake for about 15 minutes, then allow the layers to separate, and centrifuge, if necessary. Using a suitable microsyringe or sampling valve, inject equal volumes of lower (acetonitrile) layers obtained from the *Standard preparation* and the *Assay preparation* into a suitable high-pressure liquid chromatograph (see *Chromatography* ⟨621⟩) of the general type equipped with a detector for monitoring UV light absorption at about 254 nm, equipped with a suitable recorder, capable of providing column pressure up to about 1000 psi. The instrument contains a 4-mm × 30-cm stainless steel column that contains packing L3. In a suitable chromatogram, the resolution factor, *R* (see *Chromatography* ⟨621⟩), is not less than 2.4 between peaks for fluorometholone and the internal standard, and the lowest and highest peak area ratios (R_S) of three replicate injections of the *Standard preparation* agree within 2.0%. Calculate the quantity, in mg, of fluorometholone ($C_{22}H_{29}FO_4$) in the portion of Cream taken by the formula:

$$20C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Fluorometholone RS in the *Standard preparation;* and R_U and R_S are the peak area ratios of the fluorometholone peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Fluorometholone Ophthalmic Suspension

DEFINITION

Fluorometholone Ophthalmic Suspension is a sterile suspension of Fluorometholone in a suitable aqueous medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of fluorometholone ($C_{22}H_{29}FO_4$). It may contain suitable stabilizers, buffers, and antimicrobial agents.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Shake a quantity of Ophthalmic Suspension, nominally equivalent to 5 mg of fluorometholone, with 20 mL of acetone. Filter, and evaporate the filtrate to dryness. Dissolve the residue in 10 mL of acetone, filter, and evaporate the filtrate to dryness.
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: Methanol and water (60:40)
  Diluent: Methanol and water (1:1)
  System suitability solution: 0.5 µg/mL each of USP Fluorometholone RS and USP Fluorometholone Related Compound A RS in *Diluent*
  Standard stock solution: 0.5 mg/mL of USP Fluorometholone RS in methanol
  Standard solution: 40 µg/mL of USP Fluorometholone RS in *Diluent*, from *Standard stock solution*
  Sample solution: Nominally equivalent to 40 µg/mL of fluorometholone in *Diluent* prepared from a suitable quantity of well-shaken Ophthalmic Suspension
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 3.9-mm × 30-cm; 10-µm packing L1
  Flow rate: 2 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Suitability requirements
  Resolution: NLT 1.5 between fluorometholone and fluorometholone related compound A, *System suitability solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the percentage of the labeled amount of fluorometholone ($C_{22}H_{29}FO_4$) in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluorometholone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluorometholone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.5 µg/mL of USP Fluorometholone RS in *Diluent*

Sample solution: Nominally equivalent to 100 µg/mL of fluorometholone in *Diluent* prepared from a suitable quantity of Ophthalmic Suspension

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between fluorometholone and fluorometholone related compound A, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of fluorometholone from the *Standard solution*
C_S = concentration of USP Fluorometholone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluorometholone in the *Sample solution* (µg/mL)

Acceptance criteria

Each impurity: NMT 0.5%

Total impurities: NMT 1%

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩: Meets the requirements

• **pH** ⟨791⟩: 6.0–7.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

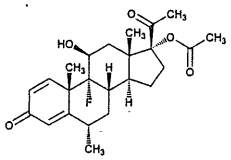
USP Fluorometholone RS

USP Fluorometholone Related Compound A RS

11β,17α-Dihydroxy-6α-methylpregna-1,4-diene-3,20-dione.

$C_{22}H_{30}O_4$ 358.47

Fluorometholone Acetate



$C_{24}H_{31}FO_5$ 418.50

Pregna-1,4-diene-3,20-dione, 17-(acetyloxy)-9-fluoro-11-hydroxy-6-methyl-, (6α,11β)-.

9-Fluoro-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione, 17 acetate [3801-06-7].

» Fluorometholone Acetate contains not less than 98.0 percent and not more than 101.0 percent of $C_{24}H_{31}FO_5$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—

USP Fluorometholone RS

USP Fluorometholone Acetate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)

Solution: 10 µg per mL.

Medium: methanol.

Specific rotation ⟨781S⟩: between +25.0° and +31.0°.

Test solution: 20 mg per mL, in chloroform.

Loss on drying ⟨731⟩—Dry in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

Related compounds—

Mobile phase, System suitability solution, and *Chromatographic system*—Prepare as directed in the *Assay*.

Standard solution—Dissolve an accurately weighed quantity of USP Fluorometholone RS in methanol, and dilute quantitatively and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 0.03 mg per mL.

Test solution—Use the *Assay preparation*.

Blank—Use acetonitrile.

Procedure—Separately inject equal volumes (about 20 µL) of the *Blank*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses.

[NOTE—Allow the elution to continue for about two and a half times the elution time of the fluorometholone acetate peak before making the next injection.]

Calculate the percentage of fluorometholone or fluorometholone diacetate in the portion of Fluorometholone Acetate taken by the formula:

$$100 \times 50 \times (1/F)(C/W)(r_U/r_S)$$

in which F is the relative response factor (see values in the accompanying *Table*); C is the concentration, in mg per mL,

USP Monographs

www.webofpharma.com

of USP Fluorometholone RS in the *Standard solution; W* is the weight, in mg, taken to prepare the *Test solution;* r_U is the fluorometholone or fluorometholone diacetate peak height response obtained from the *Test solution;* and r_S is the fluorometholone peak height response obtained from the *Standard solution.*

Calculate the percentage of all other fluorometholone acetate impurities in the portion of Fluorometholone Acetate taken by the formula:

$$100(1/F)(r_i/r_T)$$

in which *F* is the relative response factor (see values in the accompanying *Table*); r_i is the peak area for each impurity (except fluorometholone and fluorometholone diacetate) obtained from the *Test solution;* and r_T is the sum of the peak areas of all impurity peaks plus the fluorometholone acetate peak obtained from the *Test solution.*

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Fluorometholone Acetate RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 1.0 mg per mL.

System suitability solution—Prepare a solution of USP Fluorometholone RS by dissolving a quantity in methanol and diluting in acetonitrile to a final concentration of about 1 mg per mL. Mix equal volumes of this solution and the *Standard preparation,* and dilute with acetonitrile to a final concentration of about 0.03 mg per mL each for fluorometholone and fluorometholone acetate.

Assay preparation—Transfer about 50 mg of Fluorometholone Acetate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between fluorometholone and fluorometholone acetate is not less than 10. The column efficiency for fluorometholone acetate is not less than 10,000 theoretical plates, and the tailing factor for fluorometholone acetate is not more than 2.0. Chromatograph the *Standard preparation* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{24}H_{31}FO_5$ in the portion of Fluorometholone Acetate taken by the formula:

$$50C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Fluorometholone Acetate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Fluorouracil



$C_4H_3FN_2O_2$ 130.08

2,4(1*H*,3*H*)-Pyrimidinedione, 5-fluoro-;
5-Fluorouracil [51-21-8].

DEFINITION

Fluorouracil contains NLT 98.0% and NMT 102.0% of fluorouracil ($C_4H_3FN_2O_2$), calculated on the dried basis.

[**CAUTION**—Great care should be taken to prevent inhaling particles of Fluorouracil and exposing the skin to it.]

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197M**▲ (CN 1-May-2020)

Impurity	Relative Retention Time	Relative Response Factor	Limit (%)
Fluorometholone	0.6	1.0[a]	1.0
Compound 5OX[1]	0.89	1.0	0.5
Fluorometholone Acetate	1.0	—	—
Fluorometholone Diacetate	1.39	0.45[a]	1.0
Fluorometholone Acetate, epoxy analog[2]	1.58	1.0	0.5
Fluorometholone Acetate, Delta 9(11)[3]	1.82	1.0	0.2
Fluorometholone Acetate 7, 9(11) Diene[4]	1.77	1.8	0.3
Individual unknown	—	1.0	0.1
Total impurities	—	—	1.5

[1] 19b,11b-Epoxy-17a-hydroxy-6a-methylpregna-1,4-dien-3,20-dione.
[2] 17a-Acetoxy-9b,11b-epoxy-6a-methylpregna-1,4-dien-3,20-dione.
[3] 17a-Acetoxy-6a-methylpregna-1,4,9(11)-trien-3,20-dione.
[4] 17a-Acetoxy-6a-methylpregna-1,4,7,9(11)-tetraen-3,20-dione.
[a] Relative to fluorometholone.

USP Monographs

www.webofpharma.com

Change to read:

• **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Medium: pH 4.7 acetate buffer prepared from 8.4 g of sodium acetate and 3.35 mL of glacial acetic acid mixed with water to make 1000 mL
Sample solution: 10 µg/mL in *Medium*
Acceptance criteria: Meets the requirements
• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 5 M potassium hydroxide to a pH of 5.7 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (5:95)
Standard solution: 10 µg/mL of USP Fluorouracil RS in water
Sample solution: 10 µg/mL of Fluorouracil in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.73%, *Standard solution*
Tailing factor: NMT 1.5, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluorouracil ($C_4H_3FN_2O_2$) in the portion of Fluorouracil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluorouracil RS in the *Standard solution* (µg/mL)
C_U = concentration of Fluorouracil in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **ORGANIC IMPURITIES**
Protect the *Standard solution* and *Sample solution* from light.
Mobile phase: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 5 M potassium hydroxide to a pH of 5.7 ± 0.1.
Standard solution: 0.1 µg/mL each of USP Fluorouracil RS, USP Fluorouracil Related Compound A RS, USP Fluorouracil Related Compound B RS, and USP Uracil RS, and 0.2 µg/mL of USP Fluorouracil Related Compound E RS in *Mobile phase*
Sample solution: 0.1 mg/mL of Fluorouracil in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 266 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Run time: NLT 3 times the retention time of the fluorouracil peak
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2 between the uracil and fluorouracil peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluorouracil related compound A, fluorouracil related compound B, and uracil in the portion of Fluorouracil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of relevant fluorouracil related compound from the *Sample solution*
r_S = peak response of relevant fluorouracil related compound from the *Standard solution*
C_S = concentration of relevant fluorouracil related compound in the *Standard solution* (mg/mL)
C_U = concentration of Fluorouracil in the *Sample solution* (mg/mL)

Calculate the percentage of 5-methoxyuracil, fluorouracil related compound E, and any unspecified impurity in the portion of Fluorouracil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of fluorouracil from the *Standard solution*
C_S = concentration of USP Fluorouracil RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluorouracil in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fluorouracil related compound A[a]	0.5	—	0.15
Fluorouracil related compound B[b]	0.7	—	0.15
Uracil	0.9	—	0.15
Fluorouracil	1.0	—	—
5-Methoxyuracil[c]	1.6	0.67	0.15
Fluorouracil related compound E[d]	1.9	0.77	0.15
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] Pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione.
[b] Dihydropyrimidine-2,4,5(3*H*)-trione.
[c] 5-Methoxypyrimidine-2,4(1*H*,3*H*)-dione.
[d] 5-Chloropyrimidine-2,4(1*H*,3*H*)-dione.

www.webofpharma.com

• **Limits of Fluorouracil Related Compound F and Urea**
Diluent: Methanol and water (1:1)
Standard solution A: 0.025 mg/mL of USP Fluorouracil Related Compound F RS in *Diluent*
Standard solution B: 0.02 mg/mL of USP Urea RS in methanol
Sample solution: 10 mg/mL of Fluorouracil in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: Chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Ethyl acetate, methanol, and water (70:15:15)
Reagent: Prepare a 10-mg/mL solution of *p*-dimethylaminobenzaldehyde in anhydrous alcohol. Prepare a mixture of this solution and hydrochloric acid (10:1).
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Procedure: Develop with *Developing solvent system*, followed by air drying. Examine the plate under UV light at 254 nm for fluorouracil related compound F. Spray the plate at least twice with *Reagent*, and dry the plate in an oven at 80° for 3–4 min. Examine the plate under daylight for urea. [Note—The urea produces a yellow spot, and fluorouracil is not detected by the spray.]
Acceptance criteria: The spot of fluorouracil related compound F in the *Sample solution* is not more intense than the spot of fluorouracil related compound F from *Standard solution A* (NMT 0.25%). The spot of urea in the *Sample solution* is not more intense than the spot of urea from *Standard solution B* (NMT 0.2%).

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry under vacuum over phosphorus pentoxide at 80° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Fluorouracil RS
USP Fluorouracil Related Compound A RS
Pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione.
$C_4H_4N_2O_3$ 128.09
USP Fluorouracil Related Compound B RS
Dihydropyrimidine-2,4,5(3*H*)-trione.
$C_4H_4N_2O_3$ 128.09
USP Fluorouracil Related Compound E RS
5-Chloropyrimidine-2,4(1*H*,3*H*)-dione.
$C_4H_3ClN_2O_2$ 146.53
USP Fluorouracil Related Compound F RS
2-Ethoxy-5-fluoropyrimidin-4(1*H*)-one.
$C_6H_7FN_2O_2$ 158.13
USP Uracil RS
Uracil.
$C_4H_4N_2O_2$ 112.09
USP Urea RS
Urea.
CH_4N_2O 60.06

Fluorouracil Cream

DEFINITION
Fluorouracil Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of fluorouracil ($C_4H_3FN_2O_2$). It may contain Sodium Hydroxide to adjust the pH.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
Sample: Dry a portion of Cream nominally equivalent to 50 mg of fluorouracil at a pressure NMT 0.7 kPa. Mix with 100 mL of ether. Decant, wash the residue with 50 mL of ether, and allow to air dry.
Acceptance criteria: Meets the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 5 M potassium hydroxide to a pH of 5.7 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (5:95)
Standard solution: 10 µg/mL of USP Fluorouracil RS in water
Sample stock solution: Nominally equivalent to 0.1 mg/mL of fluorouracil prepared as follows. Transfer a portion of Cream, nominally equivalent to 10 mg of fluorouracil, into a 100-mL volumetric flask. Add 20 mL of methanol, and mix on a vortex mixer to dissolve. Dilute with water to volume.
Sample solution: 10 µg/mL of fluorouracil in water, from *Sample stock solution*. Mix, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.73%, *Standard solution*
Tailing factor: NMT 1.5, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluorouracil ($C_4H_3FN_2O_2$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluorouracil RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluorouracil in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **Limit of Urea**
Standard solution: 0.05 mg/mL of USP Urea RS in water
Sample solution: Nominally equivalent to 5 mg/mL of fluorouracil prepared as follows. Shake a portion of Cream

www.webofpharma.com

containing 100 mg of fluorouracil with 10 mL of water for 5 min. Add 10 mL of alcohol, and mix. Pass through a glass-fiber filter, and use the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: Chromatographic silica gel
Application volume: 20 µL
Developing solvent system: Ethyl acetate, acetone, and water (70:40:10)
Reagent solution: Prepare a 10-mg/mL solution of *p*-dimethylaminobenzaldehyde in alcohol. Prepare a mixture of this solution and hydrochloric acid (10:1).

Analysis
Samples: *Standard solution* and *Sample solution*
Procedure: Develop with *Developing solvent system*, followed by air drying. Spray the plate at least twice with *Reagent solution*, and dry the plate in an oven at 100° until the maximum intensity of the spots is obtained. Examine the plate under daylight.

Acceptance criteria: NMT 1.0%; the spot of urea in the *Sample solution* is not more intense than the spot of urea from the *Standard solution*.

PERFORMANCE TESTS
• **Minimum Fill** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS
• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Fluorouracil RS
USP Urea RS
Urea.
CH_4N_2O 60.06

Fluorouracil Injection

DEFINITION
Fluorouracil Injection is a sterile solution of Fluorouracil in Water for Injection, prepared with the aid of Sodium Hydroxide. It contains NLT 90.0% and NMT 110.0% of the labeled amount of fluorouracil ($C_4H_3FN_2O_2$).
[Note—If a precipitate is formed as a result of exposure to low temperatures, redissolve it by heating to 60° with vigorous shaking, and allow to cool to body temperature prior to use.]

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

• **B.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
Sample: Carefully acidify a portion of Injection, equivalent to 100 mg of fluorouracil, with glacial acetic acid. Stir, and slightly chill the solution to precipitate the fluorouracil. Collect the precipitate, wash with 1 mL of water, and then dry under vacuum over phosphorus pentoxide at 80° for 4 h.
Acceptance criteria: Meets the requirements

ASSAY
• **Procedure**
Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 5 M potassium hydroxide to a pH of 5.7 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (5:95)
Standard solution: 10 µg/mL of USP Fluorouracil RS in water
Sample solution: Nominally equivalent to 10 µg/mL of fluorouracil, from Injection in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.73%, *Standard solution*
Tailing factor: NMT 1.5, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluorouracil ($C_4H_3FN_2O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluorouracil RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluorouracil in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 8.6–9.4
• **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 0.33 USP Endotoxin Unit/mg of fluorouracil
• **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass, and store at controlled room temperature. Avoid freezing and exposure to light.
• **Labeling:** Label it to indicate the expiration date, which is NMT 24 months after date of manufacture.
• **USP Reference Standards** ⟨11⟩
USP Fluorouracil RS

Fluorouracil Topical Solution

DEFINITION
Fluorouracil Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of fluorouracil ($C_4H_3FN_2O_2$).

www.webofpharma.com

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 5 M potassium hydroxide to a pH of 5.7 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (5:95)
Standard solution: 10 µg/mL of USP Fluorouracil RS in water
Sample stock solution: Nominally equivalent to 0.1 mg/mL of fluorouracil prepared as follows. Transfer a portion of Topical Solution, nominally equivalent to 10 mg of fluorouracil, into a 100-mL volumetric flask. Add 20 mL of methanol, and mix on a vortex mixer to dissolve. Dilute with water to volume.
Sample solution: 10 µg/mL of fluorouracil in water, from *Sample stock solution*. Mix, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm or diode array. [NOTE—Use the diode array detector to perform *Identification* test *B*.]
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.73%, *Standard solution*
Tailing factor: NMT 1.5, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluorouracil ($C_4H_3FN_2O_2$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluorouracil RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluorouracil in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluorouracil RS

Fluoxetine Capsules

DEFINITION

Fluoxetine Capsules contain an amount of Fluoxetine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Nominally 10 mg of fluoxetine from Capsules prepared as follows. Transfer a portion of Capsule contents, equivalent to 10 mg of fluoxetine, to a suitable container. Dissolve in 10 mL of methanol and pass through a suitable filter. Rinse the container and filter with 5 mL of methanol, and evaporate with the aid of a current of air and mild heat to dryness.
Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**

Buffer: Triethylamine and water (1:98), adjusted with phosphoric acid to a pH of 6.0
Mobile phase: Stabilizer-free tetrahydrofuran, methanol, and *Buffer* (30:10:60)
Standard solution: 0.11 mg/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*
Sample solution: Nominally 0.1 mg/mL of fluoxetine from Capsules prepared as follows. Remove, as completely as possible, the contents of NLT 20 Capsules and mix. Transfer a suitable portion of the contents to an appropriate volumetric flask and dissolve in *Mobile phase*. Dilute with *Mobile phase* to volume. Pass the resulting solution through a suitable filter, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 227 nm
Column: 4.6-mm × 25-cm; 5-µm base-deactivated packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluoxetine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of fluoxetine, 309.33
M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79

Acceptance criteria: 90.0%–110.0%

www.webofpharma.com

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Solution A: Transfer 250 mL of acetonitrile to a suitable container, add 1.0 mL of diethylamine, mix, and adjust with phosphoric acid to a pH of 3.5. [NOTE—Diethylamine phosphate will precipitate; therefore, keep it well-mixed.]
Mobile phase: Acetonitrile, diethylamine, and water (400:4:600), adjusted with phosphoric acid to a pH of 3.5
Standard stock solution: USP Fluoxetine Hydrochloride RS having a concentration similar to that of the *Sample stock solution* passed through a suitable filter. Use the filtrate.
Standard solution: Transfer 5.0 mL of *Standard stock solution* to a suitable container, add 2.0 mL of *Solution A*, and mix.
Sample stock solution: Pass 20 mL of the solution under test through a suitable filter. Use the filtrate.
Sample solution: Transfer 5.0 mL of *Sample stock solution* to a suitable container, add 2.0 mL of *Solution A*, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 15-cm; packing L10
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of fluoxetine, 309.33
M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer: Triethylamine and water (1:98), adjusted with phosphoric acid to a pH of 6.0
Mobile phase: Acetonitrile and *Buffer* (35:65)
System suitability solution: 0.01 mg/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*
Sample solution: Nominally 2 mg/mL of fluoxetine from Capsules prepared as follows. Remove, as completely as possible, the contents of NLT 20 Capsules and mix. Transfer a suitable portion of the contents to an appropriate volumetric flask and dissolve in *Mobile phase*. Dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L10
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 22 min
System suitability
Sample: *System suitability solution*
Suitability requirements
Column efficiency: NLT 1100 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria
Any individual impurity: NMT 0.25%
Total impurities: NMT 0.80%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluoxetine Hydrochloride RS

Fluoxetine Delayed-Release Capsules

DEFINITION

Fluoxetine Delayed-Release Capsules contain an amount of Fluoxetine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)
Sample: Transfer the contents of 3 Capsules to a suitable container and grind to a fine powder. Transfer a portion of the powder, equivalent to 40 mg of fluoxetine, to another suitable container, and dissolve in 25 mL of 0.1 N hydrochloric acid. Pass the resulting solution through a suitable filter. Transfer 10 mL of the filtrate to a separatory funnel, add 20 mL of methylene chloride, and mix. Allow the phases to separate, and transfer the organic layer to a small glass container. Evaporate to dryness with the aid of a current of air and mild heat. Redissolve the residue with a few drops of methylene chloride, and transfer to a potassium bromide plate. Dry or evaporate to a thin film with the aid of a stream of nitrogen.
Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: Dissolve about 2.9 mL of glacial acetic acid and about 7.1 g of sodium 1-pentanesulfonate in 1 L of water. Adjust with 5 N sodium hydroxide to a pH of 5.0.

Mobile phase: Methanol and *Solution A* (67:33)

System suitability solution: 110 µg/mL of USP Fluoxetine Hydrochloride RS and 20 µg/mL of 4-trifluoromethylphenol in *Mobile phase*

Standard solution: 110 µg/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*

Sample stock solution: Nominally 0.2 mg/mL of fluoxetine from Capsules prepared as follows. Remove, as completely as possible, the contents of NLT 20 Capsules. Transfer a suitable portion of the contents to an appropriate volumetric flask and dissolve in 50% of the final flask volume of *Mobile phase*. Shake by mechanical means for 10 min and then sonicate for 5 min. Allow the solution to cool to room temperature and dilute with *Mobile phase* to volume.

Sample solution: Nominally 100 µg/mL of fluoxetine from *Sample stock solution* in *Mobile phase*. Pass through a suitable filter. Use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm × 7.5-cm; 3.5-µm packing L7

Column temperature: 38°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for 4-trifluoromethylphenol and fluoxetine are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between 4-trifluoromethylphenol and fluoxetine

Tailing factor: NMT 1.7 for the fluoxetine peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluoxetine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of fluoxetine, 309.33
M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Acid stage

Acid stage medium: 0.1 N hydrochloric acid; 250 mL, deaerated

Apparatus 3: 12 dips/min (dpm), using a polypropylene 40-mesh screen on the top and bottom of the reciprocating cylinder

Time: 2 h; Operate the apparatus for 2 h at 12 dpm, withdraw an aliquot of the *Acid stage medium*, and allow the apparatus to proceed to the *Buffer stage*.

Standard solution: 0.036 mg/mL of USP Fluoxetine Hydrochloride RS in *Acid stage medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Use the filtrate.

Instrumental conditions

Mode: UV

Analytical wavelength: About 278 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

A_U = minimum of the first derivative of the absorbance of the *Sample solution*
A_S = minimum of the first derivative of the absorbance of the *Standard solution*
C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of fluoxetine, 309.33
M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79
V = volume of *Acid stage medium*, 250 mL
L = label claim (mg/Capsule)

Tolerances: NMT 10% of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) is dissolved.

Buffer stage

Buffer stage medium: pH 6.8 phosphate buffer (prepared by mixing 3 L of 0.1 N hydrochloric acid and 1 L of 0.2 M tribasic sodium phosphate, and adjusting, if necessary, with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 6.8 ± 0.05); 250 mL, deaerated

Apparatus 3: 12 dips/min (dpm), using a polypropylene 40-mesh screen on the top and bottom of the reciprocating cylinder

Time: 45 min; Operate the apparatus for 45 min at 12 dpm, and withdraw an aliquot of the *Buffer stage medium*.

Standard solution: 0.36 mg/mL of USP Fluoxetine Hydrochloride RS in *Buffer stage medium*

Sample solution: Pass a portion of the solution under test through a filter of 0.45-µm pore size. Use the filtrate.

Instrumental conditions

Mode: UV

Analytical wavelengths: Maximum absorbance at about 264 and 290 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

A_U = absorbance at 264 nm – absorbance at 290 nm for the *Sample solution*
A_S = absorbance at 264 nm – absorbance at 290 nm for the *Standard solution*
C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of fluoxetine, 309.33
M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79
V = volume of *Buffer stage medium*, 250 mL
L = label claim (mg/Capsule)

www.webofpharma.com

Tolerances: NLT 75% (*Q*) of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**

Solution A: 6.5 g/L of sodium 1-octanesulfonate and 2.9 g/L of anhydrous sodium acetate in water adjusted with glacial acetic acid to a pH of 5.0

Mobile phase: Acetonitrile and *Solution A* (42:58)

Impurity identification solution A: 0.5 mg/mL of USP Fluoxetine Related Compound C RS in *Mobile phase*. [Note—The resulting solution contains 4-trifluoromethylphenol.]

Impurity identification solution B: Nominally 2.2 mg/mL of fluoxetine hydrochloride from USP Fluoxetine Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Fluoxetine Hydrochloride RS to an appropriate volumetric flask and dilute with 1 N sulfuric acid to volume. Heat the flask to 85° for 3 h, and allow to cool to room temperature. [Note—The resulting solution contains aminomethyl-1-phenylpropanol, which is also known as 3-methylamino-1-phenylpropan-1-ol and α-[2-(methylamino)ethyl]benzenemethanol.]

System suitability stock solution: 0.135 mg/mL of USP Fluoxetine Hydrochloride RS, 0.01 mg/mL of USP Fluoxetine Related Compound C RS from *Impurity identification solution A*, and nominally 0.044 mg/mL of fluoxetine from *Impurity identification solution B* prepared as follows. Transfer 13.5 mg of USP Fluoxetine Hydrochloride RS to a 100-mL volumetric flask and add 2 mL of *Impurity identification solution A* and 2 mL of *Impurity identification solution B*. Dilute with *Mobile phase* to volume.

System suitability solution: 5.4 µg/mL of USP Fluoxetine Hydrochloride RS and 0.4 µg/mL of USP Fluoxetine Related Compound C RS from *System suitability stock solution* in *Mobile phase*

Sensitivity solution: 0.43 µg/mL of USP Fluoxetine Hydrochloride RS from *System suitability solution* in *Mobile phase*

Sample solution: Nominally 0.4 mg/mL of fluoxetine from Capsules prepared as follows. Finely powder NLT 20 Capsules and transfer a suitable portion of the powder to an appropriate volumetric flask. Dissolve in and dilute with *Mobile phase* to volume. Pass the resulting solution through a suitable filter and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time: NLT 3 times the retention time of fluoxetine

System suitability

Samples: *System suitability solution* and *Sensitivity solution* [Note—The relative retention times for 4-trifluoromethylphenol, fluoxetine related compound C, and fluoxetine are 0.49, 0.70, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between 4-trifluoromethylphenol and fluoxetine related compound C; NLT 6.0 between fluoxetine related compound C and fluoxetine, *System suitability solution*

Signal-to-noise ratio: NLT 10 for fluoxetine, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = peak response of each impurity from the *Sample solution*

r_T = sum of all of the peak responses from the *Sample solution*

Acceptance criteria

Any individual impurity: NMT 0.2%

Total impurities: NMT 0.7%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩

USP Fluoxetine Hydrochloride RS

USP Fluoxetine Related Compound C RS

4-(Methyl{3-phenyl-3-[4-(trifluoromethyl)phenoxy] propyl}amino)-4-oxobutanoic acid;

also known as *N*-Methyl-*N*-[3-phenyl-3-(4-trifluoromethyl-phenoxy)-propyl]-succinamic acid.

$C_{21}H_{22}F_3NO_4$ 409.40

Fluoxetine Oral Solution

DEFINITION

Fluoxetine Oral Solution contains an amount of Fluoxetine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$). It may contain one or more preservatives.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197S▲** (CN 1-May-2020)

Sample solution: Transfer a volume of Oral Solution, equivalent to 20 mg of fluoxetine, to a separatory funnel. Add 5.0 mL of water and 0.5 mL of 1 N sodium hydroxide, extract with 5 mL of chloroform, and discard the aqueous layer. Evaporate the remaining layer to dryness, and dissolve the residue in 0.4 mL of chloroform.

Acceptance criteria: Meets the requirements

ASSAY

• **Procedure**

Buffer: Triethylamine and water (1:98), adjusted with phosphoric acid to a pH of 6.0

Mobile phase: Acetonitrile and *Buffer* (50:50)

Standard solution: 45 µg/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*

Sample solution: Nominally 40 µg/mL of fluoxetine from Oral Solution in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; packing L10

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

www.webofpharma.com

Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluoxetine in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of fluoxetine, 309.33
M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DELIVERABLE VOLUME** ⟨698⟩
For multiple-unit containers
Acceptance criteria: Meets the requirements
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
For single-unit containers
Acceptance criteria: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: 4.3 g/L of sodium 1-octanesulfonate and 13.8 g/L of monobasic sodium phosphate in water adjusted with phosphoric acid to a pH of 3.0
Solution B: Methanol, acetonitrile, and *Solution A* (26:21:53)
Solution C: Methanol, acetonitrile, and *Solution A* (22:35:43)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
13	100	0
15	0	100
29	0	100
30	100	0
End	100	0

Diluent: Methanol, acetonitrile, and *Solution A* (30:10:60)
Impurity identification solution: Nominally 2.0 mg/mL of USP Fluoxetine Hydrochloride RS prepared as follows. Transfer a suitable quantity of USP Fluoxetine Hydrochloride RS to a container, dissolve in 1 N sulfuric acid, and heat at 85° for 1 h.
System suitability solution: 0.1 mg/mL of USP Fluoxetine Hydrochloride RS prepared as follows. Transfer 10 mg of USP Fluoxetine Hydrochloride RS to a 100-mL volumetric flask. Add 1.0 mL of *Impurity identification solution*. Dissolve in and dilute with *Diluent* to volume.
Sample solution: Nominally 1.9 mg/mL of fluoxetine from Oral Solution in *Diluent*
Dilute sample solution: Nominally 76 µg/mL of fluoxetine from *Sample solution* in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements: The retention time of any peak, except the fluoxetine peak, is less than 13 min.
Analysis
Samples: *Sample solution* and *Dilute sample solution*
Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = \{r_i/[r_T + (D \times r_{UD})]\} \times 100$$

r_i = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses excluding fluoxetine from the *Sample solution*
D = dilution factor between the *Sample solution* and the *Dilute sample solution*, 25
r_{UD} = peak response of fluoxetine from the *Dilute sample solution*

Acceptance criteria
Any individual impurity: NMT 0.4%
Total impurities: NMT 0.8%

SPECIFIC TESTS

• **pH** ⟨791⟩: 2.5–4.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluoxetine Hydrochloride RS

Fluoxetine Tablets

DEFINITION

Fluoxetine Tablets contain an amount of Fluoxetine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Transfer 1 Tablet to a suitable container, dissolve in 10 mL of chloroform, and pass through a suitable filter. Rinse the container with 5 mL of chloroform, and pass the rinsings through a suitable filter. Evaporate the combined filtrate in a hood with the aid of a current of air and mild heat to dryness.
Acceptance criteria: Meet the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: 7.1 g/L of sodium 1-pentane-sulfonate in water. To each L add 2.9 mL of glacial acetic acid, and adjust with 5 N sodium hydroxide solution to a pH of 5.0.

Mobile phase: Methanol and *Solution A* (67:33)

System suitability stock solution: 0.2 mg/mL of 4-trifluoromethylphenol in *Mobile phase*

System suitability solution: 0.02 mg/mL of 4-trifluoromethylphenol from *System suitability stock solution* and 0.11 mg/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*

Standard solution: 0.1 mg/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*

Sample stock solution: Transfer 10 Tablets to a 1000-mL volumetric flask. Add 500 mL of *Mobile phase*, and shake to disintegrate the Tablets. Dilute with *Mobile phase* to volume, and sonicate for 10 min.

Sample solution: Nominally 0.1 mg/mL of fluoxetine from *Sample stock solution* in *Mobile phase*. Pass through a suitable filter. Use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm × 7.5-cm; 3.5-µm packing L7

Column temperature: 38°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 4.0 between fluoxetine and 4-trifluoromethylphenol

Tailing factor: NMT 1.7 for fluoxetine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fluoxetine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of fluoxetine, 309.33

M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 1000 mL

Apparatus 1: 100 rpm

Time: 15 min

Solution A, Mobile phase, and **System suitability solution:** Prepare as directed in the *Assay*.

Sample solution: Pass 20 mL of the solution under test through a suitable filter.

Standard solution: USP Fluoxetine Hydrochloride RS in *Medium* having a known concentration similar to that of the *Sample solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm × 7.5-cm; 3.5-µm packing L7

Column temperature: 38°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between fluoxetine and 4-trifluoromethylphenol

Tailing factor: NMT 1.7 for fluoxetine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fluoxetine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of fluoxetine, 309.33

M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79

Tolerances: NLT 80% (Q) of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: 6.5 g/L of sodium 1-octanesulfonate in water. To each L add 2.9 mL of phosphoric acid, and adjust with 5 N sodium hydroxide solution to a pH of 3.0.

Mobile phase: Acetonitrile and *Solution A* (43:57)

Impurity identification solution: Nominally 2.2 mg/mL of fluoxetine hydrochloride from USP Fluoxetine Hydrochloride RS prepared as follows. Transfer 22 mg of USP Fluoxetine Hydrochloride RS to a 10-mL volumetric flask and dilute with 1 N sulfuric acid to volume. Heat the flask to 85° for 3 h, and allow to cool to room temperature. [NOTE—The resulting solution contains aminomethyl-1-phenylpropanol, which is also known as 3-methylamino-1-phenylpropan-1-ol or α-[2-(methylamino)ethyl] benzenemethanol.]

System suitability solution: 0.001 mg/mL of USP Fluoxetine Related Compound B RS and 0.015 mg/mL of USP Fluoxetine Hydrochloride RS prepared as follows. Transfer suitable quantities of USP Fluoxetine Related Compound B RS and USP Fluoxetine Hydrochloride RS to a 10-mL volumetric flask. Add 0.2 mL of *Impurity identification solution* and dilute with *Mobile phase* to volume.

Standard solution: 0.015 mg/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*

Sensitivity solution: 0.2 µg/mL of USP Fluoxetine Hydrochloride RS from *Standard solution* in *Mobile phase*

Sample solution: 2 mg/mL of fluoxetine from Tablets prepared as follows. Transfer 10 Tablets to a suitable volumetric flask and add 50% of the final flask volume of *Mobile phase*. Shake to disintegrate and dilute with *Mobile phase* to volume. Sonicate the resulting solution for 10 min, pass a portion through a suitable filter, and use the filtrate.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 3 times the retention time of fluoxetine
System suitability
Samples: *Mobile phase, System suitability solution, Standard solution,* and *Sensitivity solution*
Injection order: *Mobile phase, Sensitivity solution, System suitability solution,* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 4.5 between aminomethyl-1-phenylpropanol and fluoxetine related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of fluoxetine from the *Standard solution*
C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluoxetine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of fluoxetine, 309.33
M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79

Acceptance criteria: See *Table 1*.

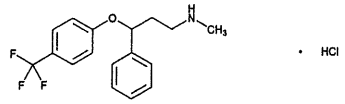
Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminomethyl-1-phenylpropanol[a]	0.19	0.25
Fluoxetine related compound B	0.26	0.25
Fluoxetine	1.0	—
Any individual unspecified impurity	—	0.25
Total impurities	—	0.80

[a] 3-Methylamino-1-phenylpropan-1-ol; also known as α-[2-(Methylamino)ethyl] benzenemethanol.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fluoxetine Hydrochloride RS
  USP Fluoxetine Related Compound B RS
  N-Methyl-3-phenylpropan-1-amine;
  also known as *N*-Methyl-3-phenylpropylamine.
  $C_{10}H_{15}N$ 149.24

Fluoxetine Hydrochloride



$C_{17}H_{18}F_3NO \cdot HCl$ 345.79
Benzenepropanamine, *N*-methyl-γ-[4-(trifluoromethyl) phenoxy]-, hydrochloride, (±);
(±)-*N*-Methyl-3-phenyl-3-[(α,α,α-trifluoro-*p*-tolyl)oxy] propylamine, hydrochloride;
N-Methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine hydrochloride [56296-78-7].

DEFINITION
Fluoxetine Hydrochloride contains NLT 98.0% and NMT 102.0% of fluoxetine hydrochloride ($C_{17}H_{18}F_3NO \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*: Meets the requirements

Add the following:

- **▲C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Dec-2019)

ASSAY

Change to read:

- **PROCEDURE**
Buffer: Triethylamine and water (1:98), adjusted with phosphoric acid to a pH of 6.0
Mobile phase: Stabilizer-free tetrahydrofuran, methanol, and *Buffer* (30:10:60)
Standard solution: 0.11 mg/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*
Sample solution: 0.11 mg/mL of Fluoxetine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 227 nm
Column: 4.6-mm × 25-cm; 5-µm base-deactivated packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
▲Run time: NLT 1.2 times the retention time of fluoxetine▲ (USP 1-Dec-2019)
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT ▲0.73%▲ (USP 1-Dec-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluoxetine hydrochloride ($C_{17}H_{18}F_3NO \cdot HCl$) in the portion of Fluoxetine Hydrochloride taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluoxetine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Buffer and **Mobile phase:** Prepare as directed in the *Assay*.

System suitability solution: Dissolve about 22 mg of USP Fluoxetine Hydrochloride RS in 10 mL of 1 N sulfuric acid ▲VS .▲ (USP 1-Dec-2019) Heat at 85° for 3 h. Cool, and transfer 0.4 mL of this solution to a 25-mL volumetric flask. Add 28 mg of USP Fluoxetine Hydrochloride RS, 1 mg of USP Fluoxetine Related Compound A RS, and 1 mg of USP Fluoxetine Related Compound B RS. Dilute with *Mobile phase* to volume.

▲Sensitivity solution: 0.0028 mg/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*▲ (USP 1-Dec-2019)

Sample solution A: 5.6 mg/mL of Fluoxetine Hydrochloride in *Mobile phase*

Sample solution B: 1.1 mg/mL of Fluoxetine Hydrochloride from *Sample solution A* in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm base-deactivated packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of fluoxetine

System suitability
Samples: *System suitability solution* ▲and *Sensitivity solution*▲ (USP 1-Dec-2019)
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NMT 1.1 for the ratio of the height of the fluoxetine related compound A peak to the depth of the valley between the fluoxetine and fluoxetine related compound A peaks (measured from the fluoxetine related compound A peak height), ▲*System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*▲ (USP 1-Dec-2019)

Analysis
Samples: *Sample solution A* and *Sample solution B*
Calculate the percentage of fluoxetine related compound A in the portion of Fluoxetine Hydrochloride taken:

$$\text{Result} = [r_{iB}/(r_{iB} + r_{UB})] \times 100$$

r_{iB} = peak response of fluoxetine related compound A from *Sample solution B*
r_{UB} = peak response of fluoxetine from *Sample solution B*

Calculate the percentage of any other impurity in the portion of Fluoxetine Hydrochloride taken:

$$\text{Result} = \{r_{iA}/[r_{TA} + (D \times r_{UB})]\} \times 100$$

r_{iA} = peak response of any other impurity from *Sample solution A*
r_{TA} = sum of all the peak responses excluding fluoxetine from *Sample solution A*
D = dilution factor between *Sample solution A* and *Sample solution B*, 5
r_{UB} = peak response of fluoxetine from *Sample solution B*

Acceptance criteria: See *Table 1*. ▲The reporting threshold is 0.05%.▲ (USP 1-Dec-2019)

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminomethyl-1-phenylpropanol[a, b]	0.24	0.25
Fluoxetine related compound B	0.27	0.25
Fluoxetine related compound A	0.94	0.15
Fluoxetine	1.0	—
4-Trifluoromethylphenol	2.17	▲0.10▲ (USP 1-Dec-2019)
Any individual unspecified impurity	—	▲0.10▲ (USP 1-Dec-2019)
Total impurities	—	0.5

[a] 3-Methylamino-1-phenylpropan-1-ol; also known as α-[2-(methylamino)ethyl] benzenemethanol.
[b] This impurity may not be present.

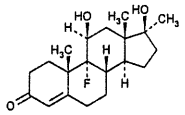
SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluoxetine Hydrochloride RS
USP Fluoxetine Related Compound A RS
N-Methyl-3-phenyl-3-[3-(trifluoromethyl)phenoxy] propan-1-amine hydrochloride;
Also known as *N*-Methyl-3-phenyl-3-[(α,α,α-(trifluoro-*m*-tolyl)oxy]propylamine hydrochloride.
$C_{17}H_{18}F_3NO \cdot HCl$ 345.79
USP Fluoxetine Related Compound B RS
N-Methyl-3-phenylpropan-1-amine;
Also known as *N*-Methyl-3-phenylpropylamine.
$C_{10}H_{15}N$ 149.24

Fluoxymesterone



$C_{20}H_{29}FO_3$ 336.44
Androst-4-en-3-one, 9-fluoro-11,17-dihydroxy-17-methyl-, (11β,17β)-.
9-Fluoro-11β,17β-dihydroxy-17-methylandrost-4-en-3-one [76-43-7].

USP Monographs

www.webofpharma.com

» Fluoxymesterone contains not less than 97.0 percent and not more than 102.0 percent of $C_{20}H_{29}FO_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, protected from light.
USP Reference standards ⟨11⟩—
USP Fluoxymesterone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020)—If a difference appears, dissolve portions of both the test specimen and the USP Reference Standard in dehydrated alcohol, evaporate the solutions to dryness, and repeat the test on the residues.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: alcohol.
Absorptivities at 242 nm do not differ by more than 2.5%.
Specific rotation ⟨781S⟩: between +104° and +112°.
Test solution: 10 mg per mL, in alcohol.
Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Chromatographic purity—
Solution A—Prepare a filtered and degassed mixture of methanol and water (55:45).
Solution B—Use filtered and degassed methanol.
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.*
Blank solution—Use *Solution B.*
System suitability solution—Dilute a volume of the *Test solution* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a concentration of about 5 µg of fluoxymesterone per mL.
Test solution—Prepare a solution of Fluoxymesterone in *Solution B* containing about 0.5 mg per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The column temperature is maintained at 40°. The flow rate is 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–20	100→60	0→40	linear gradient
20–40	60→0	40→100	linear gradient
40–45.0	0	100	isocratic
45.0–45.1	0→100	100→0	linear gradient
45.1–60	100	0	isocratic

Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 15,000 theoretical plates. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the signal-to-noise ratio for the fluoxymesterone peak is not less than 100.

Procedure—Separately inject equal volumes (about 5 µL) of the *Blank solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for any peaks that do not appear in the *Blank solution* that have an area equal to or greater than 0.1% of the fluoxymesterone peak. Calculate the percentage of each impurity in the portion of Fluoxymesterone taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

Assay—
Internal standard solution—Dissolve methylprednisolone in a mixture of chloroform and methanol (95:5) to obtain a solution containing about 200 µg per mL.
Mobile phase—Prepare a solution containing butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30).
Standard preparation—Dissolve an accurately weighed quantity of USP Fluoxymesterone RS in *Internal standard solution* to obtain a solution having a known concentration of about 0.25 mg per mL.
Assay preparation—Dissolve about 25 mg of Fluoxymesterone, accurately weighed, in 100.0 mL of *Internal standard solution* to obtain a solution having a concentration of about 0.25 mg per mL.
Procedure—Inject equal volumes of the *Assay preparation* and the *Standard preparation* into a suitable high-pressure liquid chromatograph (see *Chromatography* ⟨621⟩) of the general type equipped with a detector for monitoring UV light at 254 nm, equipped with a suitable recorder, and capable of providing column pressure up to about 2000 psi. The instrument contains a 4-mm × 30-cm stainless steel column that contains packing L3. In a suitable chromatogram, the resolution, *R,* between fluoxymesterone and the internal standard is not less than 3.0; and the relative standard deviation of the peak response ratios of four replicate injections of the *Standard preparation* is not more than 2.0%. Calculate the quantity, in mg, of $C_{20}H_{29}FO_3$ in the portion of Fluoxymesterone taken by the formula:

$$100C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Fluoxymesterone RS in the *Standard preparation;* and R_U and R_S are the peak response ratios of fluoxymesterone to the internal standard obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Fluoxymesterone Tablets

» Fluoxymesterone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluoxymesterone ($C_{20}H_{29}FO_3$).

Packaging and storage—Preserve in well-closed containers, protected from light.

USP Monographs

www.webofpharma.com

USP Reference standards ⟨11⟩—
USP Fluoxymesterone RS
USP Norethindrone RS

Identification—Triturate a quantity of powdered Tablets, equivalent to about 20 mg of fluoxymesterone, with 20 mL of hot chloroform, and decant the supernatant through a filter. Repeat the extraction with two 20-mL portions of hot chloroform. Evaporate the combined chloroform solutions on a water bath to dryness, digest the residue with 5 mL of acetone, decant the supernatant, add to it 20 mL of water, and filter off the precipitate. Dissolve the precipitate in 5 mL of acetone, add 20 mL of water, and filter: the precipitate, after being dried at 105° for 3 hours, meets the requirements for *Identification* test *A:* under *Fluoxymesterone.*

Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 2: 75 rpm.
Time: 60 minutes.

Determine the amount of $C_{20}H_{29}FO_3$ dissolved by employing the following method.

Mobile phase—Prepare a degassed and filtered solution of water and acetonitrile (58:42). Make adjustments if necessary (see *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve a quantity of USP Norethindrone RS in alcohol to obtain a solution having a final concentration of about 46 µg per mL.

Standard solution—Transfer about 28 mg of USP Fluoxymesterone RS, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with alcohol to volume, and mix. Pipet 5 mL of the resulting solution into a 250-mL volumetric flask, dilute with *Dissolution Medium* to volume, and mix. Pipet 5 mL of this solution and 2 mL of *Internal standard solution* into a 25-mL volumetric flask, dilute with *Dissolution Medium* to volume, and mix.

Test solution—Pipet a filtered 20-mL aliquot of the solution under test and 2 mL of *Internal standard solution* into a 25-mL volumetric flask, dilute with *Dissolution Medium* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 3 mL per minute. Chromatograph replicate injections of the *Standard solution,* and measure the peak responses as directed for *Procedure:* the relative retention times are 0.5 for fluoxymesterone and 1.0 for norethindrone; the resolution, *R,* between fluoxymesterone and norethindrone is not less than 2; and the relative standard deviation is not more than 2.0%.

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Calculate the amount of $C_{20}H_{29}FO_3$ dissolved by comparison with the *Standard solution,* similarly chromatographed.

Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{20}H_{29}FO_3$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—
Internal standard solution, Mobile phase, and *Standard preparation*—Prepare as directed in the *Assay* under *Fluoxymesterone.*

Test preparation—Transfer 1 Tablet to a suitable container, add 2 mL of water, and sonicate for 30 minutes or until the Tablet completely disintegrates. Add an accurately measured volume of *Internal standard solution* (5.0 mL for each mg of fluoxymesterone in the Tablet), and shake the mixture for 15 minutes. Filter a portion of the chloroform layer, and use the clear filtrate.

Procedure—Proceed as directed in the *Assay* under *Fluoxymesterone* using the *Test preparation* in place of the *Assay preparation.* Calculate the quantity, in mg, of fluoxymesterone ($C_{20}H_{29}FO_3$) in the Tablet taken by the formula:

$$(TC/D)(R_U/R_S)$$

in which T is the labeled quantity, in mg, of fluoxymesterone in the Tablet; D is the concentration, in mg per mL, of fluoxymesterone in the *Test preparation,* based on the labeled quantity per Tablet and the extent of dilution; and the other terms are as defined therein.

Assay—
Internal standard solution, Mobile phase, and *Standard preparation*—Prepare as directed in the *Assay* under *Fluoxymesterone.*

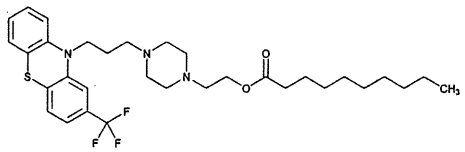
Assay preparation—Accurately weigh 20 Tablets, and grind to a fine powder in a mortar and pestle. Accurately weigh a portion of the powder, equivalent to about 5 mg of fluoxymesterone, and transfer to a suitable container. Add 20.0 mL of *Internal standard solution,* sonicate for 10 minutes, and shake for 15 minutes. Filter a portion of the liquid, and analyze the clear filtrate as directed for *Procedure.*

Procedure—Proceed as directed in the *Assay* under *Fluoxymesterone.* Calculate the quantity, in mg, of fluoxymesterone ($C_{20}H_{29}FO_3$) in the portion of Tablets taken by the formula:

$$20C(R_U/R_S)$$

in which the terms are as defined therein.

Fluphenazine Decanoate



$C_{32}H_{44}F_3N_3O_2S$ 591.77

Decanoic acid, 2-[4-[3-[2-(trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]-1-piperazinyl]ethyl ester;
2-[4-[3-[2-(Trifluoromethyl)phenothiazin-10-yl]propyl]-1-piperazinyl]ethyl decanoate [5002-47-1].

DEFINITION

Fluphenazine Decanoate contains NLT 98.0% and NMT 102.0% of fluphenazine decanoate ($C_{32}H_{44}F_3N_3O_2S$), calculated on the dried basis.

[NOTE—Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them by conducting the procedures without delay under subdued light, or using low-actinic glassware.]

IDENTIFICATION

• **A. INFRARED ABSORPTION**

Solution A: Sodium hydroxide solution (1 in 250)

Standard solution: Transfer 50 mg of USP Fluphenazine Decanoate Dihydrochloride RS to a glass-stoppered, small centrifuge tube, and add 1.5 mL of *Solution A.* Add 2 mL of carbon disulfide, shake vigorously for 2 min, and centrifuge. Dry the lower, clear layer by filtering through 2 g of anhydrous sodium sulfate. Use the filtrate.

www.webofpharma.com

Sample solution: Transfer 50 mg of Fluphenazine Decanoate to a glass-stoppered, small centrifuge tube, and add 1.5 mL of *Solution A*. Add 2 mL of carbon disulfide, shake vigorously for 2 min, and centrifuge. Dry the lower, clear layer by filtering through 2 g of anhydrous sodium sulfate. Use the filtrate.
Instrumental conditions
Mode: IR
Cell: 0.1 mm
Acceptance criteria: The absorption spectrum of the *Sample solution* exhibits maxima only at the same wavelengths as that of the *Standard solution*.

• **B. Thin-Layer Chromatography**
Standard solution: 22.5 mg/mL of USP Fluphenazine Decanoate Dihydrochloride RS (equivalent to 20 mg/mL of fluphenazine decanoate) in alcohol
Sample solution: 20 mg/mL of Fluphenazine Decanoate in alcohol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture. Impregnate the plate with a 1-in-20 solution of tetradecane in hexane, and allow to air-dry.
Application volume: 1 µL
Developing solvent system: Methanol and water (90:10)
Analysis
Samples: *Standard solution* and *Sample solution*
Apply the *Standard solution* and the *Sample solution* to the plate. Allow the spots to dry. Apply 1.0 µL of 0.1 N sodium hydroxide to the spot from the *Standard solution*, and allow it to dry. Develop the plate in an equilibrated chamber using the *Developing solvent system*. Examine the plate under short-wavelength UV light at 254 nm.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **Procedure**
Sample: 500 mg of Fluphenazine Decanoate
Blank: 50 mL of glacial acetic acid
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 50 mL of glacial acetic acid, add one drop of crystal violet TS, and titrate with *Titrant* to a blue-green endpoint. Perform the *Blank* determination, and make any necessary correction.
Calculate the percentage of fluphenazine decanoate ($C_{32}H_{44}F_3N_3O_2S$) in the portion of Fluphenazine Decanoate taken. Each mL of 0.1 N perchloric acid is equivalent to 29.59 mg of fluphenazine decanoate ($C_{32}H_{44}F_3N_3O_2S$).
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.2%
• **Ordinary Impurities** ⟨466⟩
Test solution and **Standard solution:** Methanol
Eluant: Acetone, cyclohexane, and ammonium hydroxide (16:6:1)
Visualization: 1; then spray the plate with 50% sulfuric acid.
Acceptance criteria
Individual impurity: NMT 1.0%
Total impurities: NMT 2.0%

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩
Analysis: Dry under vacuum at 60° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Fluphenazine Decanoate Dihydrochloride RS

Fluphenazine Decanoate Injection

DEFINITION

Fluphenazine Decanoate Injection is a sterile solution of Fluphenazine Decanoate in a suitable vegetable oil. It contains NLT 90.0% and NMT 115.0% of the labeled amount of fluphenazine decanoate ($C_{32}H_{44}F_3N_3O_2S$).
[Note—Throughout the following procedures, protect samples, the Reference Standards, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

• **A.**
Solution A: Palladium chloride solution (1 in 1000)
Sample: Nominally 50 mg of fluphenazine decanoate from Injection
Analysis
Part 1: Add 2 mL of methanol and 3 mL of *Solution A* to the *Sample*.
Part 2: Add an excess of *Solution A* to the resulting mixture from *Part 1*.
Acceptance criteria: The criteria for *Part 1* and *Part 2* must both be met.
Part 1: A rust-red color is produced.
Part 2: The color is intensified to a brownish red.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**
Mobile phase: Acetonitrile, methanol, and 0.05 M ammonium acetate (2:2:1)
Diluent: Acetonitrile and methanol (50:50)
System suitability stock solution: USP Fluphenazine Decanoate Dihydrochloride RS and USP Fluphenazine Hydrochloride RS (5:4) in *Diluent*
System suitability solution: 0.1 mg/mL of anhydrous fluphenazine decanoate dihydrochloride and 0.08 mg/mL of fluphenazine hydrochloride from *System suitability stock solution* in *Diluent*
Standard stock solution A: 0.1 mg/mL of USP Fluphenazine Hydrochloride RS in isopropyl alcohol
Standard stock solution B: 0.5 mg/mL of USP Fluphenazine Decanoate Dihydrochloride RS and 0.02 mg/mL of fluphenazine hydrochloride from *Standard stock solution A* prepared as follows. Transfer 25 mg of USP Fluphenazine Decanoate Dihydrochloride RS to a 50-mL volumetric flask, and add 10 mL of *Standard stock solution A*. Add 20 mL of isopropyl alcohol. Sonication may be used to aid in dissolution. Dilute with isopropyl alcohol to volume.
Standard solution: 0.1 mg/mL of fluphenazine decanoate and 0.004 mg/mL of fluphenazine hydrochloride from *Standard stock solution B* in acetonitrile

USP Monographs

www.webofpharma.com

Sample stock solution: Nominally 0.5 mg/mL of fluphenazine decanoate in isopropyl alcohol prepared as follows. Transfer a quantity of Injection, equivalent to 25 mg of fluphenazine decanoate, to a 50-mL volumetric flask. Add 20 mL of isopropyl alcohol, and shake vigorously for at least 1 min. Add 20 mL more of isopropyl alcohol, and repeat the vigorous shaking. Dilute with isopropyl alcohol to volume.

Sample solution: Nominally 0.1 mg/mL of fluphenazine decanoate from *Sample stock solution* in acetonitrile

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Columns
Guard: 4.6-mm × 5-cm; 30- to 40-µm packing L1
Analytical: 4.6-mm × 25-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
Run time: 2.5 times the retention time of fluphenazine decanoate

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for fluphenazine and fluphenazine decanoate are about 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between fluphenazine and fluphenazine decanoate, *System suitability solution*
Relative standard deviation: NMT 2.0% for fluphenazine decanoate, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluphenazine decanoate ($C_{32}H_{44}F_3N_3O_2S$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of fluphenazine decanoate from the *Sample solution*
r_S = peak response of fluphenazine decanoate from the *Standard solution*
C_S = concentration of USP Fluphenazine Decanoate Dihydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluphenazine decanoate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of fluphenazine decanoate, 591.77
M_{r2} = molecular weight of fluphenazine decanoate dihydrochloride, 664.70

Acceptance criteria: 90.0%–115.0%

IMPURITIES
• **LIMIT OF FLUPHENAZINE AND LATE-ELUTING IMPURITIES**
Analysis: Using the chromatograms from the *Assay*, calculate the percentage of fluphenazine in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak height of fluphenazine from the *Sample solution*
r_S = peak height of fluphenazine from the *Standard solution*
C_S = concentration of USP Fluphenazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluphenazine decanoate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of fluphenazine, 437.52
M_{r2} = molecular weight of fluphenazine hydrochloride, 510.44

Calculate the percentage of each impurity eluting after fluphenazine decanoate in the portion of Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

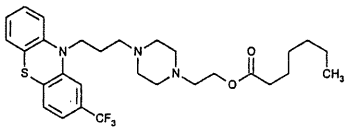
r_U = peak height of each impurity eluting after fluphenazine decanoate from the *Sample solution*
r_T = sum of the peak heights of fluphenazine decanoate and all the impurities eluting after fluphenazine decanoate from the *Sample solution*

Acceptance criteria
Fluphenazine: NMT 4%
Total impurities eluting after fluphenazine decanoate: NMT 2%

SPECIFIC TESTS
• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, of Type I glass, protected from light. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluphenazine Decanoate Dihydrochloride RS
USP Fluphenazine Hydrochloride RS

Fluphenazine Enanthate



$C_{29}H_{38}F_3N_3O_2S$ 549.69
Heptanoic acid, 2-[4-[3-[2-(trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]-1-piperazinyl]ethyl ester.
2-[4-[3-[2-(Trifluoromethyl)phenothiazin-10-yl]propyl]-1-piperazinyl]ethyl heptanoate [2746-81-8].

» Fluphenazine Enanthate contains not less than 97.0 percent and not more than 103.0 percent of $C_{29}H_{38}F_3N_3O_2S$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Fluphenazine Enanthate Dihydrochloride RS
$C_{29}H_{38}F_3N_3O_2S \cdot 2HCl$ 622.63
[NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Identification—
A: Place about 50 mg of Fluphenazine Enanthate and about 50 mg of USP Fluphenazine Enanthate Dihydrochloride RS in

www.webofpharma.com

separate, glass-stoppered, small centrifuge tubes, and treat each tube as follows. Add 1.5 mL of sodium hydroxide solution (1 in 250), and mix. Add 2 mL of carbon disulfide, shake vigorously for 2 minutes, and centrifuge. Dry the lower, clear layer by filtering it through 2 g of anhydrous sodium sulfate: the IR absorption spectrum of the test preparation, determined in a 0.1-mm cell, exhibits maxima only at the same wavelengths as that of the Standard preparation, similarly measured.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: methanolic hydrochloric acid (8.5 in 1000).
Absorptivities at 258 nm, calculated on the dried basis, do not differ by more than 2.5%.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.

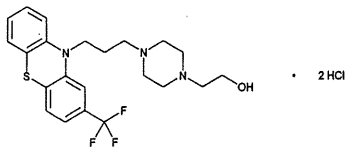
Ordinary impurities ⟨466⟩—
Test solution: alcohol.
Standard solution: alcohol.
Eluant: a mixture of alcohol, glacial acetic acid, and water (3:1:1).
Visualization: 1.
Assay—Dissolve about 500 mg of Fluphenazine Enanthate, accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.49 mg of $C_{29}H_{38}F_3N_3O_2S$.

Fluphenazine Enanthate Injection

» Fluphenazine Enanthate Injection is a sterile solution of Fluphenazine Enanthate in a suitable vegetable oil. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluphenazine enanthate ($C_{29}H_{38}F_3N_3O_2S$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass, protected from light.
[NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]
Identification—To a volume of Injection, equivalent to about 50 mg of fluphenazine enanthate, add 2 mL of methanol and 3 mL of palladium chloride solution (1 in 1000): a rust-red color is produced. Add an excess of the palladium chloride solution: the color is intensified to a brownish red.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Dissolve an accurately measured volume of Injection, equivalent to about 150 mg of fluphenazine enanthate, in 75 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.49 mg of fluphenazine enanthate ($C_{29}H_{38}F_3N_3O_2S$).

Fluphenazine Hydrochloride



$C_{22}H_{26}F_3N_3OS \cdot 2HCl$ 510.44
1-Piperazineethanol, 4-[3-[2-(trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]-, dihydrochloride;
4-[3-[2-(Trifluoromethyl)phenothiazin-10-yl]propyl]-1-piperazineethanol dihydrochloride [146-56-5].

DEFINITION
Fluphenazine Hydrochloride contains NLT 97.0% and NMT 103.0% of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$), calculated on the dried basis.
[NOTE—Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Analytical wavelength: 259 nm
Sample solution: 10 µg/mL of Fluphenazine Hydrochloride in methanol
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 2.5%.
- **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY
- **PROCEDURE**
Buffer: 0.05 M monobasic potassium phosphate adjusted with phosphoric acid to a pH of 2.5
Diluent: Acetonitrile, methanol, and *Buffer* (30:30:40)
Mobile phase: 0.2% triethylamine in *Diluent*
Standard solution: 0.06 mg/mL of USP Fluphenazine Hydrochloride RS in *Diluent*
Sample stock solution: 1.2 mg/mL of Fluphenazine Hydrochloride in *Diluent*
Sample solution: 0.06 mg/mL of Fluphenazine Hydrochloride from *Sample stock solution* in *Diluent*. Filter, and discard the first 5 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 12.5-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 25 µL

USP Monographs

www.webofpharma.com

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$) in the portion of Fluphenazine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluphenazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluphenazine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.5%
• **Ordinary Impurities** ⟨466⟩
Standard solution and **Test solution:** 0.1 M methanolic sodium hydroxide
Eluant: Acetone, cyclohexane, and diethylamine (40:15:1)
Visualization: 1
Acceptance criteria: Meets the requirements

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩
Analysis: Dry at 65° for 3 h.
Acceptance criteria: NMT 1%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Fluphenazine Hydrochloride RS

Fluphenazine Hydrochloride Elixir

DEFINITION

Fluphenazine Hydrochloride Elixir contains NLT 90.0% and NMT 110.0% of the labeled amount of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$).
[Note—Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

• **A. Thin-Layer Chromatography**
Diluent: Methanol and water (80:20)
Standard solution: 20 mg/mL of USP Fluphenazine Hydrochloride RS in *Diluent* prepared as follows. Transfer 10 mg of USP Fluphenazine Hydrochloride RS to a separator, and add 20 mL of 6 N sodium hydroxide. Extract the resulting mixture with 20 mL of isooctane. Evaporate the isooctane solution to dryness, and dissolve the residue in 0.5 mL of *Diluent*.
Sample solution: Nominally 20 mg/mL of fluphenazine hydrochloride from Elixir in *Diluent* prepared as follows. Transfer a volume of Elixir, equivalent to 10 mg of fluphenazine hydrochloride, to a separator, and add 20 mL of 6 N sodium hydroxide. Extract the resulting mixture with 20 mL of isooctane. Evaporate the isooctane solution to dryness, and dissolve the residue in 0.5 mL of *Diluent*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Acetone, cyclohexane, and diethylamine (40:15:1)
Spray reagent: Sulfuric acid in methanol (2 in 5)
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Locate the spots on the plate by lightly spraying it with *Spray reagent*.
Acceptance criteria: The R_F value and color of the principal spot of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY

• **Procedure**
Buffer: 0.05 M monobasic potassium phosphate adjusted with phosphoric acid to a pH of 2.5
Diluent: Acetonitrile, methanol, and *Buffer* (30:30:40)
Mobile phase: 0.2% triethylamine in *Diluent*
Standard solution: 0.06 mg/mL of USP Fluphenazine Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.06 mg/mL of fluphenazine hydrochloride from Elixir in *Diluent* prepared as follows. Transfer a suitable volume of Elixir, equivalent to 6 mg of fluphenazine hydrochloride, to a 100-mL volumetric flask using a "to contain" pipet. Rinse the pipet with *Diluent* to complete the transfer, and dilute with *Diluent* to volume. Filter, and use the filtrate after discarding the first 5 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 12.5-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$) in the portion of Elixir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluphenazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluphenazine hydrochloride in the *Sample solution* (mg/mL)

www.webofpharma.com

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

- **ALCOHOL DETERMINATION** ⟨611⟩**:** 90.0%–110.0% of the labeled amount, the labeled amount being NMT 15.0% of alcohol (C_2H_5OH)

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
  For Elixir packaged in single-unit containers: Meets the requirements
- **DELIVERABLE VOLUME** ⟨698⟩
  For Elixir packaged in multiple-unit containers: Meets the requirements

SPECIFIC TESTS

- **PH** ⟨791⟩**:** 5.3–5.8

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fluphenazine Hydrochloride RS

Fluphenazine Hydrochloride Injection

DEFINITION

Fluphenazine Hydrochloride Injection is a sterile solution of Fluphenazine Hydrochloride in Water for Injection. It contains NLT 95.0% and NMT 110.0% of the labeled amount of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$).

[NOTE—Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Methanol and water (80:20)

Standard solution: 20 mg/mL of USP Fluphenazine Hydrochloride RS in *Diluent* prepared as follows. Transfer 10 mg of USP Fluphenazine Hydrochloride RS to a separator, and add 20 mL of 6 N sodium hydroxide. Extract the resulting mixture with 20 mL of isooctane. Evaporate the isooctane solution to dryness, and dissolve the residue in 0.5 mL of *Diluent*.

Sample solution: Nominally 20 mg/mL of fluphenazine hydrochloride from Injection in *Diluent* prepared as follows. Transfer a volume of Injection, equivalent to 10 mg of fluphenazine hydrochloride, to a separator, and add 20 mL of 6 N sodium hydroxide. Extract the resulting mixture with 20 mL of isooctane. Evaporate the isooctane solution to dryness, and dissolve the residue in 0.5 mL of *Diluent*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Acetone, cyclohexane, and diethylamine (40:15:1)

Spray reagent: Sulfuric acid in methanol (2 in 5)

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Locate the spots on the plate by lightly spraying it with *Spray reagent*.

Acceptance criteria: The R_F value and color of the principal spot of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY

- **PROCEDURE**

Buffer: 0.05 M monobasic potassium phosphate adjusted with phosphoric acid to a pH of 2.5

Diluent: Acetonitrile, methanol, and *Buffer* (30:30:40)

Mobile phase: 0.2% triethylamine in *Diluent*

Standard solution: 0.06 mg/mL of USP Fluphenazine Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.06 mg/mL of fluphenazine hydrochloride from Injection in *Diluent* prepared as follows. Transfer a suitable volume of Injection, equivalent to 6 mg of fluphenazine hydrochloride, to a 100-mL volumetric flask using a "to contain" pipet. Rinse the pipet with *Diluent* to complete the transfer, and dilute with *Diluent* to volume. Filter, and use the filtrate after discarding the first 5 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 12.5-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluphenazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluphenazine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–110.0%

SPECIFIC TESTS

- **PH** ⟨791⟩**:** 4.8–5.2
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 166.7 USP Endotoxin Units/mg of fluphenazine hydrochloride
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and protect from light.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fluphenazine Hydrochloride RS

USP Monographs

www.webofpharma.com

Fluphenazine Hydrochloride Oral Solution

DEFINITION

Fluphenazine Hydrochloride Oral Solution is an aqueous solution of Fluphenazine Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$).

[NOTE—Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Methanol and water (80:20)

Standard solution: 20 mg/mL of USP Fluphenazine Hydrochloride RS in *Diluent* prepared as follows. Transfer 10 mg of USP Fluphenazine Hydrochloride RS to a separator, and add 20 mL of sodium hydroxide solution (1 in 4). Extract the resulting mixture with 20 mL of isooctane. Evaporate the isooctane solution to dryness, and dissolve the residue in 0.5 mL of *Diluent*.

Sample solution: Nominally 20 mg/mL of fluphenazine hydrochloride from Oral Solution in *Diluent* prepared as follows. Transfer a volume of Oral Solution, equivalent to 10 mg of fluphenazine hydrochloride, to a separator, and add 20 mL of sodium hydroxide solution (1 in 4). Extract the resulting mixture with 20 mL of isooctane. Evaporate the isooctane solution to dryness, and dissolve the residue in 0.5 mL of *Diluent*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Acetone, cyclohexane, and diethylamine (40:15:1)

Spray reagent: Sulfuric acid in methanol (2 in 5)

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Locate the spots on the plate by lightly spraying it with *Spray reagent*.

Acceptance criteria: The R_F value and color of the principal spot of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY

• **PROCEDURE**

Buffer: 0.05 M monobasic potassium phosphate adjusted with phosphoric acid to a pH of 2.5

Diluent: Acetonitrile, methanol, and *Buffer* (30:30:40)

Mobile phase: 0.2% triethylamine in *Diluent*

Standard solution: 0.06 mg/mL of USP Fluphenazine Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.06 mg/mL of fluphenazine hydrochloride from Oral Solution in *Diluent* prepared as follows. Transfer a suitable volume of Oral Solution, equivalent to 6 mg of fluphenazine hydrochloride, to a 100-mL volumetric flask using a "to contain" pipet. Rinse the pipet with *Diluent* to complete the transfer, and dilute with *Diluent* to volume. Filter, and use the filtrate after discarding the first 5 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 12.5-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluphenazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fluphenazine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **ALCOHOL DETERMINATION** ⟨611⟩**:** 90.0%–110.0% of the labeled amount, the labeled amount being NMT 15.0% of alcohol (C_2H_5OH)

SPECIFIC TESTS

• **PH** ⟨791⟩**:** 4.0–5.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.

• **LABELING:** Label it to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Fluphenazine Hydrochloride RS

Fluphenazine Hydrochloride Tablets

DEFINITION

Fluphenazine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$).

[NOTE—Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Methanol and water (80:20)

Standard solution: 20 mg/mL of USP Fluphenazine Hydrochloride RS in *Diluent* prepared as follows. Transfer 10 mg of USP Fluphenazine Hydrochloride RS to a separator. Add 5 mL of water and 20 mL of dilute hydrochloric acid (1 in 120) to the separator, shake for 10 min, and add 20 mL of chloroform-saturated sodium carbonate solution (1 in 10). Extract the resulting mixture with five 20-mL portions of chloroform, shaking gently to avoid emulsion formation, and pass the extract through a

www.webofpharma.com

chloroform-washed cotton filter into a 150-mL beaker. Evaporate the extract on a steam bath to dryness, and dissolve the residue in 0.5 mL of *Diluent*.

Sample solution: Nominally 20 mg/mL of fluphenazine hydrochloride from Tablets in *Diluent* prepared as follows. Transfer a portion of finely powdered Tablets, equivalent to 10 mg of fluphenazine hydrochloride, to a separator. Add 5 mL of water and 20 mL of dilute hydrochloric acid (1 in 120) to the separator, shake for 10 min, and add 20 mL of chloroform-saturated sodium carbonate solution (1 in 10). Extract the resulting mixture with five 20-mL portions of chloroform, shaking gently to avoid emulsion formation, and pass the extract through a chloroform-washed cotton filter into a 150-mL beaker. Evaporate the extract on a steam bath to dryness, and dissolve the residue in 0.5 mL of *Diluent*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Acetone, cyclohexane, and diethylamine (40:15:1)
Spray reagent: Sulfuric acid in methanol (2 in 5)

Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Locate the spots on the plate by lightly spraying it with *Spray reagent*.

Acceptance criteria: The R_F value and color of the principal spot of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY

• **PROCEDURE**

Buffer: 0.05 M monobasic potassium phosphate adjusted with phosphoric acid to a pH of 2.5
Diluent: Acetonitrile, methanol, and *Buffer* (30:30:40)
Mobile phase: 0.2% triethylamine in *Diluent*
Standard solution: 0.06 mg/mL of USP Fluphenazine Hydrochloride RS in *Diluent*
Sample stock solution: Transfer 6 Tablets to a suitable volumetric flask, add *Diluent*, shake for 1 h, and sonicate for 10 min or until a fine suspension is obtained.
Sample solution: Nominally 0.06 mg/mL of fluphenazine hydrochloride from *Sample stock solution* in *Diluent*. Filter, and use the filtrate after discarding the first 5 mL of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 12.5-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 25 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluphenazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluphenazine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Buffer: 0.05 M monobasic potassium phosphate adjusted with phosphoric acid to a pH of 2.5
Diluent: Acetonitrile, methanol, and *Buffer* (30:30:40)
Mobile phase: 0.3% triethylamine in *Diluent*
Sample solution: Dilute a portion of the solution under test with an equal volume of *Mobile phase*.
Standard solution: USP Fluphenazine Hydrochloride RS at a concentration and composition similar to that of the *Sample solution*
Chromatographic system and **System suitability:** Proceed as directed in the *Assay*, except use a flow rate of 2 mL/min and an injection volume of 100 µL.

Analysis
Samples: *Sample solution* and *Standard solution*
Determine the amount of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$) dissolved.

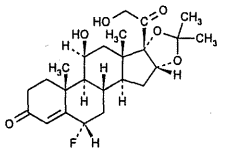
Tolerances: NLT 75% (*Q*) of the labeled amount of fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS \cdot 2HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluphenazine Hydrochloride RS

Flurandrenolide



$C_{24}H_{33}FO_6$ 436.51
Pregn-4-ene-3,20-dione, 6-fluoro-11,21-dihydroxy-16,17-[(1- methylethylidene)bis(oxy)]-, 6α11β,16α)-.
6α-Fluoro-11β,16α,17,21-tetrahydroxypregn-4-ene-3,20-dione, cyclic 16,17-acetal with acetone [1524-88-5].

» Flurandrenolide contains not less than 97.0 percent and not more than 102.0 percent of $C_{24}H_{33}FO_6$, calculated on the dried basis.

USP Monographs

www.webofpharma.com

Packaging and storage—Preserve in tight containers in a cold place, protected from light.
USP Reference standards ⟨11⟩—
USP Flurandrenolide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 20 µg per mL.
Medium: methanol.
Absorptivities at 237 nm, calculated on the dried basis, do not differ by more than 3.0%.
Specific rotation ⟨781S⟩: between +145° and +153°.
Test solution: 10 mg per mL, in chloroform.
Loss on drying ⟨731⟩—Dry it in vacuum at 105° for 4 hours: it loses not more than 1.0% of its weight.

Ordinary impurities ⟨466⟩—
Test solution: methanol.
Standard solution: methanol.
Application volume: 10 µL.
Eluant: a mixture of toluene and isopropyl alcohol (90:10), in a nonequilibrated chamber.
Visualization: 1.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of methanol and water (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Dissolve Prednisone in *Mobile phase*, with the aid of sonication, to obtain a solution containing about 1 mg per mL.
Standard preparation—Transfer about 5 mg of USP Flurandrenolide RS, accurately weighed, to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, sonicate to aid solution, and mix to obtain a solution having a known concentration of about 0.5 mg of USP Flurandrenolide RS per mL.
Assay preparation—Transfer about 5 mg of Flurandrenolide, accurately weighed, to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, sonicate to aid solution, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*:the order of elution is prednisone followed by flurandrenolide, the resolution; *R*, between the analyte and internal standard is not less than 2.0; and the relative standard deviation for replicate injections is not more than 3.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for prednisone and 1.0 for flurandrenolide. Calculate the quantity, in mg, of $C_{24}H_{33}FO_6$ in the portion of Flurandrenolide taken by the formula:

$$10C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Flurandrenolide Cream

» Flurandrenolide Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide ($C_{24}H_{33}FO_6$).

Packaging and storage—Preserve in tight containers, protected from light.
USP Reference standards ⟨11⟩—
USP Flurandrenolide RS

Thin-layer chromatographic identification test ⟨201⟩—
Test solution—Extract a quantity of weighed Cream, equivalent to about 500 µg of flurandrenolide, as directed for the *Assay preparation*. Omit the addition of the internal standard, and evaporate the chloroform extracts on a steam bath under a stream of nitrogen to about 3 mL. Transfer the chloroform solution to a 10-mL flask, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 2 mL of chloroform.
Application volume: 4.0 µL.
Developing solvent system: a mixture of ethyl acetate and ethyl ether (70:30).
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
Minimum fill ⟨755⟩: meets the requirements.

Assay—
Methanolic sodium chloride—Transfer 100 mL of sodium chloride solution (1 in 10) to a 500-mL volumetric flask. Dilute with methanol to volume, and mix.
Mobile phase—Prepare a filtered and degassed mixture of methanol and water (70:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Transfer about 10 mg of testosterone to a 100-mL volumetric flask, add methanol to volume, and mix.
Standard preparation—Transfer about 16 mg of USP Flurandrenolide RS, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 3.0 mL of this solution to a 10-mL volumetric flask, add 4.0 mL of *Internal standard solution*, dilute with water to volume, and mix to obtain a solution having a known concentration of about 48 µg of USP Flurandrenolide RS per mL.
Assay preparation—Transfer an accurately weighed quantity of Cream, equivalent to about 500 µg of flurandrenolide, to a 125-mL separator. Add 50 mL of hexanes and 25 mL of *Methanolic sodium chloride*, and shake until the Cream is thoroughly dispersed. Allow the phases to separate, and drain the lower aqueous phase into a second 125-mL separator containing 15 mL of hexanes. Shake vigorously, allow the phases to separate, and drain the lower aqueous phase into a 250-mL separator containing 75 mL of water. Serially extract the hexane phases remaining in the two 125-mL separators with two additional 25-mL portions of *Methanolic sodium chloride*, adding each aqueous phase to the 250-mL separator. Discard the hexane phases. Extract the combined aqueous phases with four 25-mL portions of chloroform. Filter each chloroform extract through 10 g of

www.webofpharma.com

anhydrous sodium sulfate into a 125-mL conical beaker. Rinse the sodium sulfate with water-washed chloroform, and add the wash to the beaker. Add 4.0 mL of *Internal standard solution* to the beaker containing the chloroform extract. Evaporate the solution on a steam bath under a stream of nitrogen nearly to dryness. Remove the beaker from the steam bath, and evaporate the remaining solution with the aid of nitrogen to dryness. Add 10 mL of *Mobile phase* to the beaker, and place it in an ultrasonic bath to dissolve the residue. Pass the solution through a suitable filter having a 0.5-µm porosity and a prefilter above the membrane filter to prevent clogging.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 2 for testosterone and 1.0 for flurandrenolide; the resolution, *R,* between the analyte and internal standard is not less than 2.0; and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of flurandrenolide ($C_{24}H_{33}FO_6$) in the portion of Cream taken by the formula:

$$10C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Flurandrenolide Lotion

» Flurandrenolide Lotion contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide ($C_{24}H_{33}FO_6$).

Packaging and storage—Preserve in tight containers, protected from heat, light, and freezing.
USP Reference standards ⟨11⟩—
USP Flurandrenolide RS
Identification—It responds to the *Identification* test under *Flurandrenolide Cream.*
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
Minimum fill ⟨755⟩: meets the requirements.
pH ⟨791⟩: between 3.5 and 6.0, determined in a 1 in 10 dilution of the Lotion in water containing 0.30 mL of saturated potassium chloride solution per 100 mL.

Assay—

Methanolic sodium chloride , Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Flurandrenolide Cream.*

Assay preparation—Transfer an accurately weighed portion of Lotion, calculated from the density to contain about 500 µg of flurandrenolide, to a separator. (Determine the density by taring a 100-mL volumetric flask containing 50.0 mL of water, adding approximately 25 g of well-shaken Lotion, and again weighing, then carefully adjusting the contents of the volumetric flask with water from a buret to volume, and finally calculating the density taken by the formula:

$$A/B$$

in which *A* is the weight, in g, of the Lotion taken; and *B* is 50.0 mL minus the volume, in mL, of water necessary to adjust the contents of the volumetric flask to volume.) Proceed as directed for *Assay preparation* in the *Assay* under *Flurandrenolide Cream,* beginning with "Add 50 mL of hexane and 25 mL of *Methanolic sodium chloride.*"

Procedure—Proceed as directed in the *Assay* under *Flurandrenolide Cream.* Calculate the quantity, in mg, of flurandrenolide ($C_{24}H_{33}FO_6$) in each mL of the Lotion taken by the formula:

$$10C(D/W)(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation; D* is the density of the Lotion; *W* is the weight, in g, of Lotion taken; and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Flurandrenolide Ointment

» Flurandrenolide Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide ($C_{24}H_{33}FO_6$).

Packaging and storage—Preserve in tight containers, protected from light.
USP Reference standards ⟨11⟩—
USP Flurandrenolide RS
Identification—It meets the requirements for *Thin-layer chromatographic identification test* under *Flurandrenolide Cream*
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
Minimum fill ⟨755⟩: meets the requirements.
Assay—Proceed as directed in the *Assay* under *Flurandrenolide Cream,* using Ointment in place of Cream.

Flurandrenolide Tape

» Flurandrenolide Tape is a nonporous, pliable, adhesive-type tape having Flurandrenolide impregnated in the adhesive material, the adhesive material on one side being transported on a removable, protective slit-paper liner. Flurandrenolide Tape contains not less than 80.0 percent and not more than 125.0 percent of the labeled amount of $C_{24}H_{33}FO_6$.

Packaging and storage—Preserve at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Flurandrenolide RS

USP Monographs

www.webofpharma.com

Identification—Extract a portion of Tape, equivalent to about 200 µg of flurandrenolide, as directed for the *Assay preparation* in the *Assay*. Omit the addition of the internal standard, and evaporate the chloroform extracts on a steam bath under a stream of nitrogen to about 3 mL. Transfer the chloroform solution to a 10-mL flask, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 1.0 mL of a mixture of equal volumes of chloroform and methanol, warming gently to effect solution: it meets the requirements of the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, 5 µL each of the test solution and Standard solution being applied, and the solvent mixture consisting of ethyl acetate and ether (70:30).

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Assay—

Methanolic sodium chloride, *Mobile phase*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Flurandrenolide Cream*.

Flurandrenolide standard solution—Dissolve about 7 mg of USP Flurandrenolide RS, accurately weighed, in 50 mL of methanol in a 100-mL volumetric flask. Dilute with methanol to volume, and mix.

Internal standard solution—Dissolve about 4 mg of Testosterone in 50 mL of methanol in a 100-mL volumetric flask. Dilute with methanol to volume, and mix.

Standard preparation—Pipet 3.0 mL of *Flurandrenolide standard solution* and 4.0 mL of *Internal standard solution* into a 10-mL volumetric flask. Dilute with water to volume, and mix.

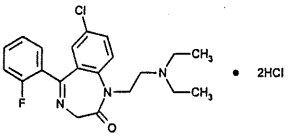
Assay preparation—Accurately measure and cut a portion of Tape, equivalent to about 200 µg of flurandrenolide. Remove and discard the paper liner from the portion of Tape. Touch the flattened end of a glass rod to the adhesive side of the Tape, and carefully transfer the tape to the bottom of a 600-mL beaker containing 15 mL of anhydrous methanol, taking care that the adhesive side of the tape does not adhere to the wall of the beaker. Remove the glass rod from the tape, and wash it with 5 mL of anhydrous methanol, adding the wash to the beaker. Place the beaker containing the Tape and the methanol in an ultrasonic bath for 3 minutes, rotating the beaker in such manner that the methanol is in contact with all portions of the Tape. Transfer the methanol to a 250-mL separator. Extract the Tape, using sonication, with two additional 20-mL portions of anhydrous methanol, adding each portion to the separator. To the combined methanol extract add 15 mL of sodium chloride solution (1 in 10) and 50 mL of hexane, and shake vigorously. Allow the phases to separate, and drain the lower aqueous phase into a second separator containing 15 mL of hexane. Shake vigorously, allow the phases to separate, and drain the lower phase into a third 250-mL separator containing 100 mL of water. Serially extract the hexane phases remaining in the two separators with one 25-mL portion of *Methanolic sodium chloride*, adding the extract to the third separator. Discard the hexane phases. Extract the combined aqueous phases with four 25-mL portions of chloroform. Filter each chloroform extract through 10 g of anhydrous sodium sulfate into a 125-mL conical beaker. Rinse the sodium sulfate with water-washed chloroform, and add the wash to the beaker. Add 4.0 mL of *Internal standard solution* to the beaker. Evaporate the solution on a steam bath under a stream of nitrogen to near dryness. Remove the beaker from the steam bath and evaporate the remaining solution with the aid of nitrogen to dryness. Add 10 mL of *Mobile solvent* to the beaker, and place it in an ultrasonic bath to dissolve the residue. Pass the solution through a suitable porosity filter having a 0.5-µm filter with a prefilter above the membrane filter to prevent clogging.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 2 for testosterone and 1.0 for flurandrenolide. Calculate the quantity, in µg, of $C_{24}H_{33}FO_6$ in the portion of Tape taken by the formula:

$$10C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Flurandrenolide RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Flurazepam Hydrochloride



$C_{21}H_{23}ClFN_3O \cdot 2HCl$ 460.80

2*H*-1,4-Benzodiazepin-2-one, 7-chloro-1-[2-(diethylamino) ethyl]-5-(2-fluorophenyl)-1,3-dihydro-, dihydrochloride.

7-Chloro-1-[2-(diethylamino)ethyl]-5-(*o*-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one dihydrochloride [1172-18-5].

» Flurazepam Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of $C_{21}H_{23}ClFN_3O \cdot 2HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Flurazepam Hydrochloride RS

USP Flurazepam Related Compound C RS
5-Chloro-2-(2-diethylaminoethyl(amino)-2′-fluorobenzophenone hydrochloride.
$C_{19}H_{22}ClFN_2O \cdot HCl$ 385.31

USP Flurazepam Related Compound F RS
7-Chloro-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.
$C_{15}H_{10}ClFN_2O$ 288.71

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020). [NOTE—Do not grind excessively, as decomposition may occur.]

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: sulfuric acid in methanol (1 in 36).

www.webofpharma.com

Absorptivities at 239 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

C: Prepare a solution of it in methanol containing 3 mg per mL. Apply 10 µL of this solution and 10 µL of a methanol solution of USP Flurazepam Hydrochloride RS containing 3 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate and ammonium hydroxide (200:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the R_f value of the principal spot in the chromatogram of the test solution corresponds to that obtained from the solution of the Reference Standard.

D: To 2 mL of a solution (1 in 20) add 1 mL of 2 N nitric acid: the solution responds to the tests for *Chloride* ⟨191⟩, 5 drops of silver nitrate TS being used.

Water Determination, *Method Ia* ⟨921⟩ **:** not more than 0.5%.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Limit of fluoride ion— [NOTE—Use plasticware throughout the procedure.]

pH 5.25 Buffer—Dissolve 110 g of sodium chloride and 1 g of sodium citrate in 700 mL of water in a 2000-mL volumetric flask. Cautiously add 150 g of sodium hydroxide, and dissolve with shaking. Cool to room temperature, and, while stirring, cautiously add 450 mL of glacial acetic acid to the cooled solution. Cool, add 600 mL of isopropyl alcohol, dilute with water to volume, and mix: the pH of this solution is between 5.0 and 5.5.

Standard stock solution—Transfer 221 mg of sodium fluoride to a 100-mL volumetric flask, add about 20 mL of water, and mix to dissolve. Add 1.0 mL of sodium hydroxide solution (1 in 2500), dilute with water to volume, and mix. Each mL of this solution contains 1 mg of fluoride ions. Store in a tightly closed, plastic container.

Standard preparations—Dilute portions of the *Standard stock solution* quantitatively and stepwise with *pH 5.25 Buffer* to obtain 100-mL solutions having concentrations of 1, 3, 5, and 10 µg per mL.

Test preparation—Transfer 1.0 g of Flurazepam Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *pH 5.25 Buffer* to volume, and mix.

Procedure—Concomitantly measure the potential (see *Titrimetry* ⟨541⟩), in mV, of the *Standard preparations* and of the *Test preparation,* with a pH meter capable of a minimum reproducibility of ±0.2 mV, equipped with a glass-sleeved calomel-fluoride specific-ion electrode system. [NOTE—When taking measurements, immerse the electrodes in the solution, which has been transferred to a 150-mL beaker containing a polytetrafluoroethylene-coated stirring bar. Allow to stir on a magnetic stirrer having an insulated top until equilibrium is attained (1 to 2 minutes), and record the potential. Rinse and dry the electrodes between measurements, being careful to avoid damaging the crystal of the specific-ion electrode.] Plot the logarithm of the fluoride-ion concentrations, in µg per mL, of the *Standard preparations* versus the potential in mV. From the measured potential of the *Test preparation* and the standard curve determine the concentration, in µg per mL, of fluoride ion in the *Test preparation:* not more than 0.05% is found.

Related compounds—

Mobile phase—Prepare a filtered and degassed mixture of methanol and 1% ammonium acetate (80:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve accurately weighed quantities of USP Flurazepam Related Compound C RS and USP Flurazepam Related Compound F RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 2 µg per mL for each component.

[NOTE—Prepare fresh daily.]

Test solution—Transfer about 50 mg of Flurazepam Hydrochloride, accurately weighed, to a 25-mL volumetric flask, add methanol to volume, and mix. [NOTE—Prepare this solution just prior to use.]

System suitability solution—Dissolve accurately weighed quantities of USP Flurazepam Hydrochloride RS and 2-amino-5-chlorobenzophenone in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration in each mL of about 150 µg of USP Flurazepam Hydrochloride RS and about 60 µg of 2-amino-5-chlorobenzophenone.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 239-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between 2-amino-5-chlorobenzophenone and flurazepam is not less than 2. Chromatograph replicate injections of the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in percentage, of flurazepam related compound C and flurazepam related compound F in the portion of Flurazepam Hydrochloride taken by the formula:

$$2.5(C/W)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Flurazepam Related Compound C RS or USP Flurazepam Related Compound F RS in the *Standard solution; W* is the weight, in mg, of Flurazepam Hydrochloride taken; and r_U and r_S are the peak responses for the related compounds obtained from the *Test solution* and the *Standard solution,* respectively. The limit is not more than 0.1% of flurazepam related compound C and not more than 0.1% of flurazepam related compound F.

Assay—Transfer about 600 mg of Flurazepam Hydrochloride, accurately weighed, to a 250-mL beaker, dissolve in 80 mL of glacial acetic acid, and add 20 mL of mercuric acetate TS. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a calomel-glass electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 23.04 mg of $C_{21}H_{23}ClFN_3O \cdot 2HCl$.

Flurazepam Hydrochloride Capsules

» Flurazepam Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurazepam hydrochloride ($C_{21}H_{23}ClFN_3O \cdot 2HCl$).

www.webofpharma.com

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Flurazepam Hydrochloride RS

Identification—
A: Dissolve a portion of Capsules, equivalent to about 30 mg of flurazepam hydrochloride, in 10 mL of methanol, filter, and proceed as directed for *Identification* test *C:* under *Flurazepam Hydrochloride.*
B: The retention time of the major peak for flurazepam hydrochloride in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*
C: Capsules meet the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

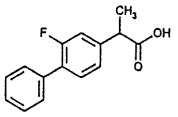
Dissolution ⟨711⟩—
Medium: 0.01 N hydrochloric acid; 900 mL.
Apparatus 1: 100 rpm.
Time: 20 minutes.
Standard solution—Prepare a solution of USP Flurazepam Hydrochloride RS having an accurately known concentration similar to the concentration of the solution under test. Pipet 5 mL of this solution to a 10-mL volumetric flask, and dilute with 1% ammonium acetate to volume.
Test solution—Pipet 5 mL of a filtered portion of the solution under test into a 10-mL volumetric flask, and dilute with 1% ammonium acetate to volume.
Procedure—Determine the amount of $C_{21}H_{23}ClFN_3O \cdot 2HCl$ dissolved, using the *Chromatographic system* as set forth in the *Related compounds* test under *Flurazepam Hydrochloride.*
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{21}H_{23}ClFN_3O \cdot 2HCl$ is dissolved in 20 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase, System suitability solution, and Chromatographic system —Prepare as directed for *Related compounds*— under *Flurazepam Hydrochloride.*
Standard preparation—Dissolve an accurately weighed quantity of USP Flurazepam Hydrochloride RS in methanol, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.15 mg per mL. Prepare fresh daily.
Assay preparation— [NOTE—Prepare fresh daily.] Weigh and mix the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the Capsule contents, equivalent to about 30 mg of flurazepam hydrochloride, to a 200-mL volumetric flask. Add 40 mL of methanol, and shake by mechanical means for 10 minutes. Add 10 mL of 1% ammonium acetate, and shake by mechanical means for 5 minutes. Dilute with *Mobile phase* to volume, mix and sonicate for 2 minutes, and filter.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of flurazepam hydrochloride ($C_{21}H_{23}ClFN_3O \cdot 2HCl$) in the portion of Capsules taken by the formula:

$$200C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Flurazepam Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Flurbiprofen



$C_{15}H_{13}FO_2$ 244.26
[1,1′-Biphenyl]-4-acetic acid, 2-fluoro-α-methyl-, (±)-.
(±)-2-(2-Fluoro-4-biphenylyl)propionic acid [5104-49-4].

» Flurbiprofen contains not less than 99.0 percent and not more than 100.5 percent of $C_{15}H_{13}FO_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Flurbiprofen RS
USP Flurbiprofen Related Compound A RS
2-(4-Biphenylyl)propionic acid.
$C_{15}H_{14}O_2$ 226.28

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B:▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: 0.1 N sodium hydroxide.
Absorbance maximum at 247 nm is about 0.8.
Melting range ⟨741⟩: between 114° and 117°.
Loss on drying ⟨731⟩—Dry it in vacuum at 60° to constant weight: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—
Diluent—Prepare a mixture of water and acetonitrile (11:9).
Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (12:7:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard stock solution—Dissolve an accurately weighed quantity of USP Flurbiprofen Related Compound A RS in *Diluent* to obtain a solution having a concentration of about 50 µg per mL.
System suitability solution—Pipet 2.0 mL of *Standard stock solution* into a 10-mL volumetric flask, add about 20 mg of USP Flurbiprofen RS, dilute with *Diluent* to volume, and mix.
Standard solution—Transfer 2.0 mL of *Standard stock solution* to a 10.0-mL volumetric flask, dilute with *Diluent* to volume, and mix.
Test solution—Prepare a solution of Flurbiprofen in *Diluent* containing 2.0 mg per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 15-cm column that contains 4-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.9 for flurbiprofen related compound A and 1.0 for

www.webofpharma.com

flurbiprofen; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of flurbiprofen related compound A in the portion of Flurbiprofen taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in µg per mL, of USP Flurbiprofen Related Compound A RS in the *Standard solution;* C_U is the concentration, in µg per mL, of Flurbiprofen in the *Test solution;* and r_U and r_S are the peak responses for flurbiprofen related compound A obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.5% of flurbiprofen related compound A is found. Calculate the percentage of each impurity in the portion of Flurbiprofen taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity obtained from the *Test solution;* and r_s is the sum of the responses of all the peaks obtained from the *Test solution:* the sum of all impurities is not more than 1.0%.

Assay—Dissolve about 0.5 g of Flurbiprofen, accurately weighed, in 100 mL of alcohol, previously neutralized with 0.1 N sodium hydroxide VS to the phenolphthalein endpoint, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS to the first appearance of a faint pink color that persists for not less than 30 seconds. Each mL of 0.1 N sodium hydroxide is equivalent to 24.43 mg of $C_{15}H_{13}FO_2$.

Flurbiprofen Tablets

DEFINITION

Flurbiprofen Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of flurbiprofen ($C_{15}H_{13}FO_2$).

IDENTIFICATION

• **A. Infrared Absorption**

Sample: Place 100 mg of flurbiprofen from a number of Tablets in a flask. Add 10 mL of 0.1 N hydrochloric acid, and sonicate until the Tablets disintegrate. Extract with two 15-mL portions of ether, and combine the ether extracts in a flask containing 1 g of anhydrous sodium sulfate. Decant the ether, and evaporate to dryness.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the residue exhibits maxima only at the same wavelengths as those of a similar solution of USP Flurbiprofen RS.

• **B.** The retention time of the major peak for flurbiprofen of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Mobile phase: Dissolve 1.4 g of monobasic sodium phosphate in 570 mL of water, add 430 mL of acetonitrile, and adjust with phosphoric acid to a pH of 3.0.

Internal standard solution: 0.8 µL/mL of acetophenone in *Mobile phase*

Standard stock solution: 3 mg/mL of USP Flurbiprofen RS in *Internal standard solution*

Standard solution: 0.15 mg/mL of USP Flurbiprofen RS from *Standard stock solution* in *Mobile phase*

Sample stock solution: Nominally 3 mg/mL of flurbiprofen in *Internal standard solution* prepared as follows. Place 3 Tablets in a stoppered container. Based on the labeled amount, in mg, of flurbiprofen in each Tablet, add 25.0 mL of *Internal standard solution* for each 75 mg of flurbiprofen in the 3 Tablets. Shake by mechanical means for 15 min, and centrifuge.

Sample solution: Nominally 0.15 mg/mL of flurbiprofen in *Mobile phase* from *Sample stock solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 25-cm; packing L7

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for acetophenone and flurbiprofen are 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8 between the acetophenone and flurbiprofen peaks

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of flurbiprofen ($C_{15}H_{13}FO_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of flurbiprofen to acetophenone from the *Sample solution*

R_S = peak response ratio of flurbiprofen to acetophenone from the *Standard solution*

C_S = concentration of USP Flurbiprofen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of flurbiprofen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: 122.5 g/L of monobasic potassium phosphate and 25 g/L of sodium hydroxide in water. Dilute 166.5 mL of this stock solution with water to 3000 mL. If necessary, adjust with 5 N sodium hydroxide or with phosphoric acid to a pH of 7.20 ± 0.05; 900 mL.

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: A known concentration of USP Flurbiprofen RS at in *Medium*

Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium* to obtain a concentration similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at 247 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Analysis: Calculate the percentage of the labeled amount of flurbiprofen ($C_{15}H_{13}FO_2$) dissolved.

Tolerances: NLT 75% (*Q*) of the labeled amount of flurbiprofen ($C_{15}H_{13}FO_2$) is dissolved.

USP Monographs

www.webofpharma.com

• **UNIFORMITY OF DOSAGE UNITS,** *Procedure for Content Uniformity* ⟨905⟩

Analysis: Proceed as directed in the *Assay*, except when preparing the *Sample solution*, use 1 Tablet and 10.0 mL of *Internal standard solution* for each 25 mg of flurbiprofen in the Tablet, based on the labeled amount.

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile: Acetonitrile, glacial acetic acid, and water (35:5:60)

Diluent: Acetonitrile and water (45:55)

Standard solution A: 0.004 mg/mL of USP Flurbiprofen RS in *Diluent*

Standard solution B: 0.01 mg/mL of USP Flurbiprofen Related Compound A RS in *Diluent*

Sample solution: Nominally 2 mg/mL of flurbiprofen prepared as follows. Weigh, and finely powder NLT 20 Tablets. Dissolve 500 mg of flurbiprofen from a portion of powdered Tablets in 50 mL of water, add 200 mL of acetonitrile, centrifuge, and use the supernatant.

System suitability solution: 0.01 mg/mL each of USP Flurbiprofen RS and USP Flurbiprofen Related Compound A RS. Dilute 1 mL of the *Sample solution* with 200 mL of *Standard solution B*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution A* and *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between flurbiprofen and flurbiprofen related compound A, *System suitability solution*

Relative standard deviation: NMT 5% for flurbiprofen, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of flurbiprofen related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of flurbiprofen related compound A from the *Sample solution*

r_S = peak response of flurbiprofen related compound A from *Standard solution B*

C_S = concentration of USP Flurbiprofen Related Compound A RS in *Standard solution B* (mg/mL)

C_U = nominal concentration of flurbiprofen in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for any unspecified impurity from the *Sample solution*

r_S = peak response of flurbiprofen from *Standard solution A*

C_S = concentration of USP Flurbiprofen RS in *Standard solution A* (mg/mL)

C_U = nominal concentration of flurbiprofen in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Flurbiprofen related compound A	0.87	0.5
Flurbiprofen	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities	—	1.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

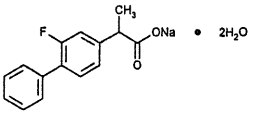
USP Flurbiprofen RS

USP Flurbiprofen Related Compound A RS

2-(4-Biphenyl)propionic acid.

$C_{15}H_{14}O_2$ 226.28

Flurbiprofen Sodium



$C_{15}H_{12}FNaO_2 \cdot 2H_2O$ 302.27

[1,1′-Biphenyl]-4-acetic acid, 2-fluoro-α-methyl, sodium salt dihydrate, (±)-.

Sodium (±)-2-(2-fluoro-4-biphenylyl)propionate dihydrate.

Anhydrous 266.25

» Flurbiprofen Sodium contains not less than 97.0 percent and not more than 103.0 percent of $C_{15}H_{12}FNaO_2 \cdot 2H_2O$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—

USP Flurbiprofen RS

USP Flurbiprofen Sodium RS

USP Flurbiprofen Related Compound A RS

2-(4-Biphenylyl)propionic acid.

$C_{15}H_{14}O_2$ 226.28

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020)—

Test specimen: previously dried.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)

Solution: 10 µg per mL.

www.webofpharma.com

Medium: pH 6.0 buffer consisting of 2.42 g of monobasic sodium phosphate and 0.66 g of dibasic sodium phosphate dissolved in water to make 1000 mL.

Absorptivities at 246 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: The residue obtained by igniting it meets the requirements of the tests for *Sodium* ⟨191⟩.

Specific rotation ⟨781S⟩: between –0.45° and +0.45°.

Test solution: 50 mg per mL, in methanol.

Loss on drying ⟨731⟩—Dry about 0.3 g of it in vacuum at a pressure not exceeding 1 mm of mercury over phosphorus pentoxide in a suitable drying tube at 60° for 18 hours: it loses not less than 11.3% and not more than 12.5% of its weight.

Limit of flurbiprofen related compound A—

Diluent, Mobile phase, and *System suitability preparation*—Proceed as directed in the *Assay.*

Standard solution—Use *Standard flurbiprofen related compound A preparation,* prepared as directed in the *Assay.*

Test solution—Use the *Assay preparation.*

Chromatographic system—Proceed as directed in the *Assay,* except to chromatograph the *Standard solution* instead of the *Standard preparation.*

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of flurbiprofen related compound A in the portion of Flurbiprofen Sodium taken by the formula:

$$200(C/W)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Flurbiprofen Related Compound A RS in the *Standard solution;* W is the weight, in mg, of the portion of Flurbiprofen Sodium taken to prepare the *Test solution;* and r_U and r_S are the peak areas for flurbiprofen related compound A obtained from the *Test solution* and the *Standard solution,* respectively: not more than 1.5% is found.

Assay—

Diluent—Mix 500 mL of methanol and 250 mL of water.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and glacial acetic acid (50:49:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard flurbiprofen related compound A preparation—Dissolve an accurately weighed quantity of USP Flurbiprofen Related Compound A RS in methanol to obtain a stock solution having a known concentration of about 150 µg per mL. Transfer 1.0 mL of this solution to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Flurbiprofen RS in methanol to obtain a stock solution having a known concentration of about 1 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

System suitability preparation—Transfer 5 mL of the stock solution used to prepare the *Standard preparation* and 2 mL of the stock solution used to prepare the *Standard flurbiprofen related compound A preparation* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Assay preparation—Transfer about 100 mg of Flurbiprofen Sodium, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 25-cm column that contains 10-µm packing L7. The flow rate is about 2 mL per minute. Chromatograph the *System suitability preparation,* and record the peak responses as directed for *Procedure:* the resolution, R, between flurbiprofen related compound A and flurbiprofen is not less than 1.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of $C_{15}H_{12}FNaO_2 \cdot 2H_2O$ in the portion of Flurbiprofen Sodium taken by the formula:

$$200(302.27/244.27)(C/W)(r_U/r_S)$$

in which 302.27 and 244.27 are the molecular weights of flurbiprofen sodium dihydrate and anhydrous flurbiprofen, respectively; C is the concentration, in µg per mL, of USP Flurbiprofen RS in the *Standard preparation;* W is the weight, in mg, of the portion of Flurbiprofen Sodium taken to prepare the *Assay preparation;* and r_U and r_S are the flurbiprofen peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Flurbiprofen Sodium Ophthalmic Solution

» Flurbiprofen Sodium Ophthalmic Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurbiprofen sodium ($C_{15}H_{12}FNaO_2 \cdot 2H_2O$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Flurbiprofen RS

USP Flurbiprofen Related Compound A RS

2-(4-Biphenylyl)propionic acid.

$C_{15}H_{14}O_2$ 226.28

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

pH ⟨791⟩: between 6.0 and 7.0.

Antimicrobial effectiveness ⟨51⟩: meets the requirements.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

Assay—

Diluent, Mobile phase, Standard flurbiprofen related compound A preparation, Standard preparation, and *System suitability preparation*—Proceed as directed in the *Assay*— under *Flurbiprofen Sodium.*

Assay preparation—Use the undiluted Ophthalmic Solution.

Chromatographic system—Proceed as directed in the *Assay* — under *Flurbiprofen Sodium,* using a 4.0-mm × 5-cm guard column that contains 5-µm packing L1.

Procedure—Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity of flurbiprofen sodium ($C_{15}H_{12}FNaO_2 \cdot 2H_2O$) in each mL of the Ophthalmic Solution taken by the formula:

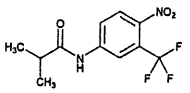
USP Monographs

www.webofpharma.com

$$(302.27/244.27)C(r_U/r_S)$$

in which 302.27 and 244.27 are the molecular weights of flurbiprofen sodium dihydrate and anhydrous flurbiprofen, respectively; C is the concentration, in mg per mL, of USP Flurbiprofen RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Flutamide



$C_{11}H_{11}F_3N_2O_3$ 276.21

Propanamide, 2-methyl-*N*-[4-nitro-3-trifluoromethyl)phenyl]-; α,α,α-Trifluoro-2-methyl-4′-nitro-*m*-propionotoluidide [13311-84-7].

DEFINITION

Flutamide contains NLT 98.0% and NMT 101.0% of flutamide ($C_{11}H_{11}F_3N_2O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197M▲** (CN 1-May-2020)
- **B.** The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Mobile phase: Acetonitrile and water (45:55)

Diluent: Acetonitrile and water (20:80)

System suitability stock solution: 1 mg/mL of USP *o*-Flutamide RS, prepared as follows. Dissolve the Standard in acetonitrile using 20% of the final volume. Sonicate to dissolve. Dilute with water to volume. Mix, and allow to warm to room temperature.

Standard solution: 0.2 mg/mL of USP Flutamide RS, prepared as follows. Dissolve the Standard in acetonitrile using 20% of the final volume. Sonicate to dissolve. Dilute with water to volume. Mix, and allow to warm to room temperature.

System suitability solution: 10 µg/mL each of USP *o*-Flutamide RS and USP Flutamide RS in *Diluent* prepared from the *System suitability stock solution* and the *Standard solution*

Sample solution: 0.2 mg/mL of Flutamide, prepared as follows. Dissolve a previously dried sample in acetonitrile using 20% of the final volume. Sonicate to dissolve. Dilute with water to volume. Mix, and allow to warm to room temperature.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 25 ± 5°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—For the relative retention times, see *Table 1*.]

Suitability requirements

Resolution: NLT 6.0 between flutamide and *o*-flutamide, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of flutamide ($C_{11}H_{11}F_3N_2O_3$) in the portion of Flutamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Flutamide RS in the *Standard solution* (mg/mL)

C_U = concentration of Flutamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.1%
- **Organic Impurities**

Mobile phase, Diluent, Standard solution, Sample solution, and Chromatographic system: Prepare as directed in the *Assay*.

System suitability stock solution: 1 mg/mL each of USP *o*-Flutamide RS, USP Flutamide Related Compound A RS, and USP Flutamide Related Compound B RS, prepared as follows. Dissolve the Standards in acetonitrile using 20% of the final volume. Sonicate to dissolve. Dilute with water to volume. Mix, and allow to warm to room temperature.

System suitability solution: 10 µg/mL each of USP *o*-Flutamide RS, USP Flutamide Related Compound A RS, USP Flutamide Related Compound B RS, and USP Flutamide RS in *Diluent*, prepared from the *System suitability stock solution* and *Standard solution*

Sensitivity solution: 0.1 µg/mL of USP Flutamide RS from the *Standard solution* in *Diluent*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—For the relative retention times, see *Table 1*.]

Suitability requirements

Resolution: NLT 6.0 between flutamide and *o*-flutamide; NLT 2.0 between flutamide related compound A and flutamide related compound B, *System suitability solution*

Relative standard deviation: NMT 10.0% for flutamide, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Flutamide taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area for each impurity

r_T = sum of all the peak responses

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

USP Monographs

www.webofpharma.com

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Flutamide related compound B[a]	0.42	1.06	0.2
Flutamide related compound A[b]	0.45	1.10	0.15
3-(Trifluoromethyl) aniline	0.63	1.10	0.2
Propionyl analog[c]	0.66	1.02	0.3
Desnitroflutamide[d]	0.80	1.95	0.2
o-Flutamide[e]	1.40	1.78	0.2
Flutamide	1.0	—	—
Any unknown impurity	—	1.0	0.05
Total impurities	—	—	0.4

[a] *N*-[4-Nitro-3-(trifluoromethyl)phenyl]acetamide.
[b] 4-Nitro-3-(trifluoromethyl)aniline.
[c] *N*-[4-Nitro-3-(trifluoromethyl) phenyl]propionamide.
[d] *N*-[3-(Trifluoromethyl)phenyl]isobutyramide.
[e] *N*-[2-Nitro-3-(trifluoromethyl)phenyl]isobutyramide.

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample in vacuum at 60° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Flutamide RS
USP *o*-Flutamide RS
N-[2-Nitro-3-(trifluoromethyl)phenyl]isobutyramide.
$C_{11}H_{11}F_3N_2O_3$ 276.21
USP Flutamide Related Compound A RS
4-Nitro-3-(trifluoromethyl)aniline.
$C_7H_5F_3N_2O_2$ 206.12
USP Flutamide Related Compound B RS
N-[4-Nitro-3-(trifluoromethyl)phenyl]acetamide.
$C_9H_7F_3N_2O_3$ 248.16

Flutamide Capsules

DEFINITION
Flutamide Capsules contain NLT 93.0% and NMT 107.0% of the labeled amount of flutamide ($C_{11}H_{11}F_3N_2O_3$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Diluent: Acetonitrile and water (50:50)
Mobile phase: Acetonitrile and water (45:55)
Standard stock solution: 0.5 mg/mL of USP Flutamide RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Flutamide RS from the *Standard stock solution* in water
Sample stock solution: Nominally 0.5 mg/mL of flutamide prepared as follows. Remove the contents of NLT 20 Capsules, and mix. Transfer a portion of the powder, equivalent to 125 mg of flutamide, into a 250-mL volumetric flask. Add 180 mL of *Diluent* and shake the flask for 15 min. Dilute with *Diluent* to volume, and mix. Allow the insoluble material to settle. The concentration is equivalent to 0.5 mg/mL of flutamide.
Sample solution: Nominally 0.2 mg/mL of flutamide prepared as follows. Transfer 20.0 mL of the supernatant from the *Sample stock solution* into a 50-mL volumetric flask, dilute with water to volume, mix, and pass through a polytef membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 25 ± 5°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Run time: About two times the retention time of flutamide
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of flutamide ($C_{11}H_{11}F_3N_2O_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Flutamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of flutamide in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: 2% sodium lauryl sulfate solution; 1000 mL
Apparatus 2: 75 rpm
Time: 60 min
Standard solution: A known concentration of USP Flutamide RS in *Medium*
Sample solution: Filter portions of the solution under test. Dilute with *Medium* as needed.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 306 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of flutamide ($C_{11}H_{11}F_3N_2O_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)

USP Monographs

www.webofpharma.com

D = dilution factor for the *Sample solution*
V = volume of *Medium*, 1000 mL
L = label claim (mg/Capsule)

Tolerances: NLT 75% (Q) of the labeled amount of flutamide ($C_{11}H_{11}F_3N_2O_3$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

• **Organic Impurities**

Diluent, Mobile phase, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Detector sensitivity solution: 0.2 µg/mL from the *Standard solution* in a mixture of acetonitrile and water (1:4)

System suitability

Sample: *Detector sensitivity solution*

Suitability requirements

Relative standard deviation: NMT 10.0% for flutamide

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each impurity from the *Sample solution*, excluding those where peak areas are less than those of the *Detector sensitivity solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria

Individual impurities: NMT 0.2% for any impurity having a relative retention time of 0.45; NMT 0.1% for any other impurity

Total impurities: NMT 0.3%

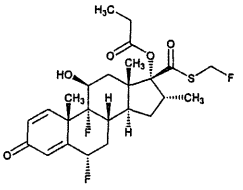
ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, light-resistant containers.

• **USP Reference Standards** ⟨11⟩

USP Flutamide RS

Fluticasone Propionate



$C_{25}H_{31}F_3O_5S$ 500.57

Androsta-1,4-diene-17-carbothioic acid, 6,9-difluoro-11-hydroxy-16-methyl-3-oxo-17-(1-oxopropoxy)-, (6α,11β,16α,17α)-*S*-(fluoromethyl) ester;

S-Fluoromethyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate [80474-14-2].

DEFINITION

Fluticasone Propionate contains NLT 98.0% and NMT 101.0% of $C_{25}H_{31}F_3O_5S$, calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Buffer: 1.15 g/L of monobasic ammonium phosphate, adjusted with phosphoric acid to a pH of 3.5 ± 0.05

Mobile phase: Methanol, acetonitrile, and *Buffer* (50:15:35)

System suitability solution: 0.05 mg/mL of USP Fluticasone Propionate Resolution Mixture RS in *Mobile phase*

Standard solution: 0.04 mg/mL of USP Fluticasone Propionate RS in *Mobile phase*

Sample solution: 0.04 mg/mL of Fluticasone Propionate in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for fluticasone propionate and fluticasone propionate related compound D are about 1.0 and 1.10, respectively.]

Suitability requirements

Resolution: NLT 1.5 between fluticasone propionate and fluticasone propionate related compound D, *System suitability solution*

Relative standard deviation: NMT 2%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the portion of Fluticasone Propionate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluticasone propionate from the *Sample solution*
r_S = peak response of fluticasone propionate from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluticasone Propionate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the anhydrous, solvent-free basis

IMPURITIES

• **Organic Impurities**

Solution A: 0.5 mL of phosphoric acid in 1000 mL of acetonitrile

Solution B: 0.5 mL of phosphoric acid in 1000 mL of methanol

Solution C: 0.5 mL of phosphoric acid in 1000 mL of water

Mobile phase: See *Table 1*.

USP Monographs

www.webofpharma.com

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	42	3	55
40	53	3	44
60	87	3	10
70	87	3	10
75	42	3	55

System suitability solution: Dissolve 2.0 mg of USP Fluticasone Propionate System Suitability Mixture RS in 5 mL of *Solution A* using sonication. Add 5 mL of *Solution C*.
Sample solution: 0.2 mg/mL prepared as follows: Dissolve 2.0 mg of Fluticasone Propionate in 5 mL of *Solution A* using sonication. Add 5 mL of *Solution C*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 50 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 0.6 between fluticasone propionate related compound B and fluticasone propionate related compound C; NLT 1.5 between fluticasone propionate related compound D and fluticasone propionate
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Fluticasone Propionate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of the responses of all the peaks

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fluticasone propionate related compound A	0.5	0.2
Fluticasone propionate related compound B	0.75	0.1
Fluticasone propionate related compound C	0.8	0.1
Fluticasone propionate related compound D	0.95	0.3
Fluticasone propionate	1.0	—
Fluticasone propionate related compound E	1.3	0.3
Any individual unspecified impurity	—	0.1
Total impurities*	—	1.0

* Include all impurity peaks greater than or equal to 0.05%.

• **LIMIT OF ACETONE**
Internal standard solution: 0.05% (v/v) solution of tetrahydrofuran in dimethylformamide
Standard solution: 0.05% (v/v) of acetone in *Internal standard solution*
Sample solution: 50 mg/mL of Fluticasone Propionate in the *Internal standard solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 25-m; 2-µm film of phase G16
Carrier gas: Nitrogen or helium
Temperature
Detector: 250°
Splitless injector: 150°
Column: See *Table 3*.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	0	60	3.5
60	30	180	3.0

Flow rate: 5.5 mL/min
Injection size: 0.1 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetone (w/w) in the portion of Fluticasone Propionate taken:

$$\text{Result} = (R_U/R_S) \times D \times (C_S/C_U)$$

R_U = peak response ratio of acetone to tetrahydrofuran from the *Sample solution*
R_S = peak response ratio of acetone to tetrahydrofuran from the *Standard solution*
D = density of acetone at 20° (g/mL)
C_S = concentration of acetone in the *Standard solution* (%v/v)
C_U = concentration of Fluticasone Propionate in the *Sample solution* (g/mL)

Acceptance criteria: NMT 1.0% (w/w)

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩**:** +32° to +36° at 20°, calculated on the anhydrous, solvent-free basis
Sample solution: 0.5% (w/v) of Fluticasone Propionate in dichloromethane (0.5 g in 100 mL)
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.2% (w/w)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at a temperature not exceeding 30°.
• **LABELING:** Fluticasone Propionate in the form of microcrystals is so labeled.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluticasone Propionate RS
USP Fluticasone Propionate Resolution Mixture RS

www.webofpharma.com

USP Fluticasone Propionate System Suitability Mixture RS
It is a mixture of USP Fluticasone Propionate RS and fluticasone propionate related compounds B, C, and D.
Fluticasone propionate related compound A: 6α,9α-Difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbonylsulfenic acid.
Fluticasone propionate related compound B: 6α,9α-Difluoro-11β-hydroxy-16α-methyl-2′,3,4′-trioxo-17α-spiro(androsta-1,4-diene-17,5′-(1,3)oxathiolane).
Fluticasone propionate related compound C: *S*-Fluoromethyl 17α-acetyloxy-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioate.
Fluticasone propionate related compound D: *S*-Methyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxy-androsta-1,4-diene-17β-carbothioate.
Fluticasone propionate related compound E: 6α,9α-Difluoro-11β,17α-dihydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carboxylic acid 6α,9α-difluoro-17β-(fluoromethylthio) carbonyl-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-dien-17α-yl ester.

Fluticasone Propionate Cream

DEFINITION

Fluticasone Propionate Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 0.4 mg/mL of USP Fluticasone Propionate RS in acetonitrile

Test solution: Transfer a quantity of Cream, equivalent to 1000 µg of fluticasone propionate, to a 125-mL separatory funnel. Add 25 mL of acetonitrile and 25 mL of hexane to the separatory funnel. Stopper and shake the funnel until the Cream is completely dispersed. Shake the separatory funnel for an additional 3 min, and allow the phases to separate. Filter the lower layer through a 20-mL syringe containing a cotton plug into a 50-mL volumetric flask. Repeat the extraction with one 7-mL aliquot of acetonitrile, and filter the lower layer into the volumetric flask. Wash the cotton plug with 2 mL of acetonitrile, and collect the washings into the volumetric flask. Dilute the sample extract with acetonitrile to volume. Transfer 12 mL of the sample extract to a glass tube suitable for evaporation, and evaporate to dryness at about 40°. Dissolve the residue in 0.6 mL of acetonitrile. [NOTE—The *Test solution* may be cloudy because of the presence of undissolved excipients.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.2-mm layer of chromatographic silica gel mixture on a high-performance thin-layer chromatographic plate, 5-µm particle size
Application volume: 40 µL
Developing solvent system: Dichloromethane, ethyl acetate, and glacial acetic acid (30:8:1)
Analysis
Samples: *Standard solution* and *Test solution*
Separately apply the *Standard solution* and the *Test solution* to the plate. On the same plate, apply 20 µL of the *Standard solution,* allow the application to dry, and apply 20 µL of the *Test solution* on top of the dried 20-µL *Standard solution* spot. Allow each of the applications to dry thoroughly. Place the plate in a tank equilibrated with the developing solvent, and allow the developing solvent to travel about 8 cm from the point of application. Remove the plate and allow to air-dry. Examine the plate under ultraviolet light at 254 nm.
Acceptance criteria: The R_F value of the principal spot from the *Test solution* corresponds to that of the *Standard solution*. [NOTE—If the excipients in the Cream interfere with the appearance of the principal spot obtained for the *Test solution*, use the *Standard solution* and the *Test solution* overspot to confirm identity.]

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

[NOTE—Protect the *Standard solution* and the *Sample solution* from direct light by using a light-protective volumetric flask and autosampler vials.]

Buffer: 1.2 g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of 3.50 ± 0.03.
Mobile phase: Methanol, acetonitrile, and *Buffer* (46:14:40)
Diluent: Alcohol and water (65:35)
System suitability stock solution: 0.5 mg/mL of USP Fluticasone Propionate Nasal Spray Resolution Mixture RS in methanol. [NOTE—USP Fluticasone Propionate Nasal Spray Resolution Mixture RS is a mixture of fluticasone propionate and fluticasone propionate related compound D.]
System suitability solution: 10 µg/mL of USP Fluticasone Propionate Nasal Spray Resolution Mixture RS in *Diluent* from *System suitability stock solution*
Standard stock solution: 0.5 mg/mL USP Fluticasone Propionate RS. Dissolve the standard first in a volume of methanol, equivalent to 80% of the final volume, and dilute with water to volume.
Standard solution: 20 µg/mL USP Fluticasone Propionate RS in *Diluent,* from *Standard stock solution*
Sample solution: Equivalent to 20 µg/mL of fluticasone propionate. Transfer a quantity of Cream, equivalent to 1000 µg of fluticasone propionate, to a 125-mL separatory funnel. Add to the separatory funnel 25 mL of *Diluent*. Stopper and shake vigorously until the Cream is completely dispersed. Add 25 mL of hexane, shake for an additional 3 min, and allow the phases to separate. Filter the lower layer through a 20-mL syringe containing a cotton plug into a 50-mL volumetric flask. Repeat the extraction with one 5-mL and one 2-mL aliquot of *Diluent*, filtering the lower layers into the volumetric flask. Wash the cotton plug with 1 mL of *Diluent*, and collect the washings into the volumetric flask. Dilute the sample with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.4 between fluticasone propionate and fluticasone propionate related compound D, *System suitability solution*
Tailing factor: NMT 1.4, *Standard solution* (calculated using the width of the peak at 10% of the height)

www.webofpharma.com

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluticasone propionate from the *Sample solution*

r_S = peak response of fluticasone propionate from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS

• **pH** ⟨791⟩: 4.5–6.5

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The total aerobic microbial count is NMT 100 cfu/g, and the total combined molds and yeasts count is NMT 10 cfu/g.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, protected from light. Store between 2° and 30°.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Fluticasone Propionate RS

USP Fluticasone Propionate Nasal Spray Resolution Mixture RS

This Reference Standard is a mixture of fluticasone propionate and fluticasone propionate related compound D, and the chemical names for both are given below:

Fluticasone propionate: *S*-Fluoromethyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate.

Fluticasone propionate related compound D: *S*-Methyl-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxy-androsta-1,4-diene-17β-carbothioate.

Fluticasone Propionate Inhalation Aerosol

DEFINITION

Fluticasone Propionate Inhalation Aerosol is a suspension of Fluticasone Propionate with suitable propellants in a pressurized container. The mean content per actuation contains NLT 85% and NMT 115% of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*:**197A**▲ (CN 1-May-2020)

Spectral range: 4000 cm^{-1} to 600 cm^{-1}

Samples: Deposit between 5 and 20 actuations onto the zinc selenide plate of the HATR (Horizontal ATR) accessory. Dry the zinc selenide plate.

Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 0.01 M sodium dodecyl sulfate containing 0.1% glacial acetic acid

Solution A: Methanol and *Buffer* (20:80)

Mobile phase: Acetonitrile and *Solution A* (1:1)

Standard solution: 50 µg/mL of USP Fluticasone Propionate RS in acetonitrile and water (1:1)

Sample solution: Nominally 50 µg/mL of fluticasone propionate prepared as follows. Shake the canister vigorously, and cool for 10 min in a dry ice–methanol bath. Remove the canister from the bath, and shake vigorously. Using a suitable device, carefully remove and keep the valve, and pour the contents into a suitable container. Allow the propellant to evaporate. Transfer the canister content to a suitable volumetric flask fitted with a suitable funnel using about 12% of the flask volume of acetonitrile. Add 50% of the flask volume of water. Swab the outer part of the valve with damp cotton wool soaked in acetonitrile. Place the swab in the neck of the funnel in the volumetric flask. Allow the valve to dry, then dismantle, and wash the components and the canister wall with acetonitrile. Collect the washings, and place in the same volumetric flask as the sample. Allow the flask to come to room temperature, and dilute with acetonitrile to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 5-cm; 3.5-µm packing L1

Flow rate: 2 mL/min

Column temperature: 40°

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for the peak due to fluticasone propionate must be within the range of 1.0–1.6 min.]

Suitability requirements

Tailing factor: NMT 1.3 for the fluticasone propionate peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the portion of the Inhalation Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response from the *Sample solution*

r_S = response from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)

Acceptance criteria: 85%–115%

USP Monographs

www.webofpharma.com

PERFORMANCE TESTS

• **PARTICLE SIZE DISTRIBUTION BY CASCADE IMPACTION**

Sampling apparatus: *Apparatus 1* in *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* ⟨601⟩ with a modified induction port (*Figure 1. Expanded view of the modified induction port.*) and entrance cone (*Figure 2. Expanded view of the modified entrance cone.*) are to be used.

Buffer, Solution A, and **Mobile phase:** Proceed as directed in the *Assay.*

Diluent: Methanol and water (60:40)

Standard solution: 0.75 µg/mL of USP Fluticasone Propionate RS in *Diluent*

Sample solutions: Prime the valve by discharging a predetermined number of actuations to waste. Discharge the number of actuations given in *Table 1* into the cascade impaction sampling apparatus described in *Figure 1. Expanded view of the modified induction port.* and *Figure 2. Expanded view of the modified entrance cone.*

Table 1

Product Strength (µg/actuation)	Number of Actuations
44	10
110	8
220	4

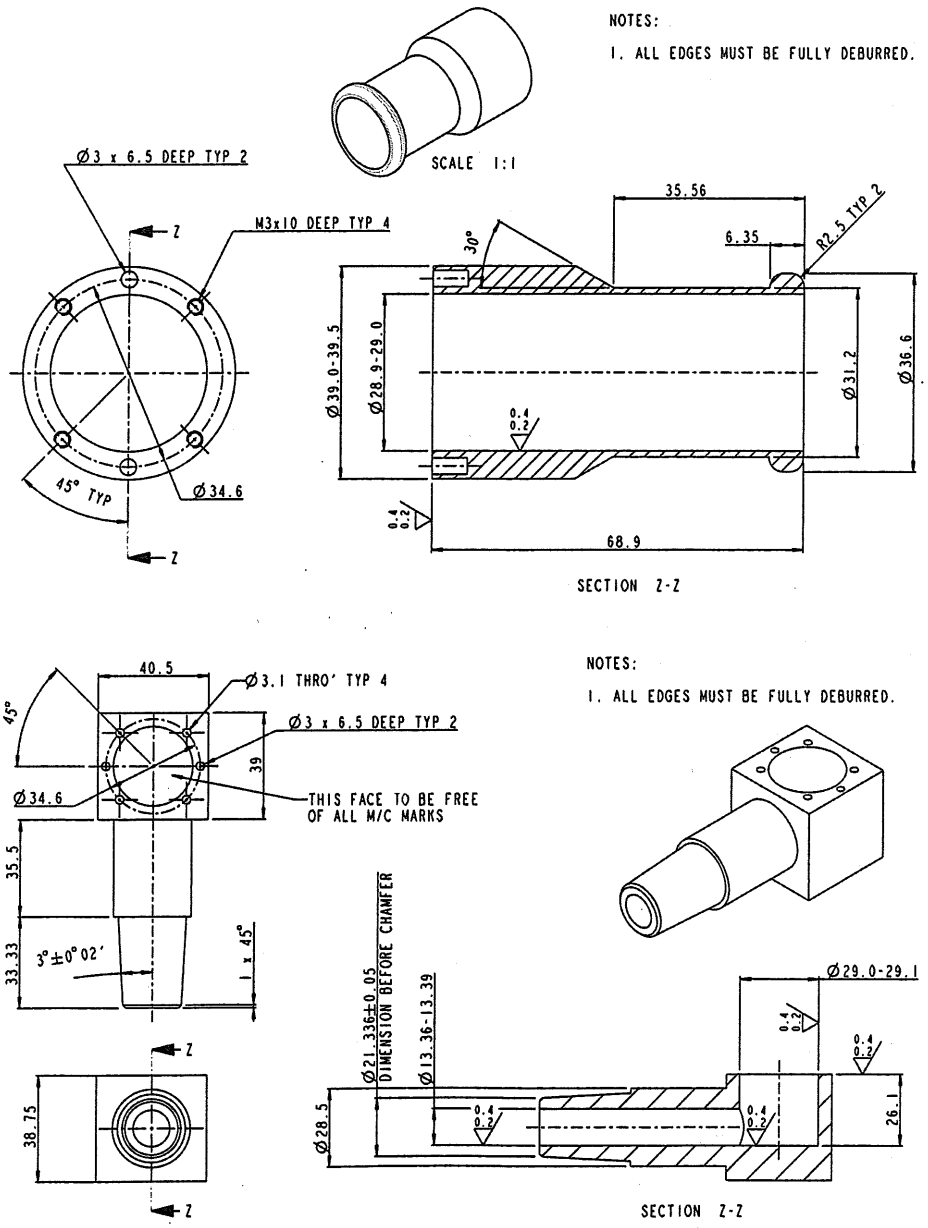


Figure 1. Expanded view of the modified induction port. (Fluticasone Propionate Inhalation Aerosol)

www.webofpharma.com

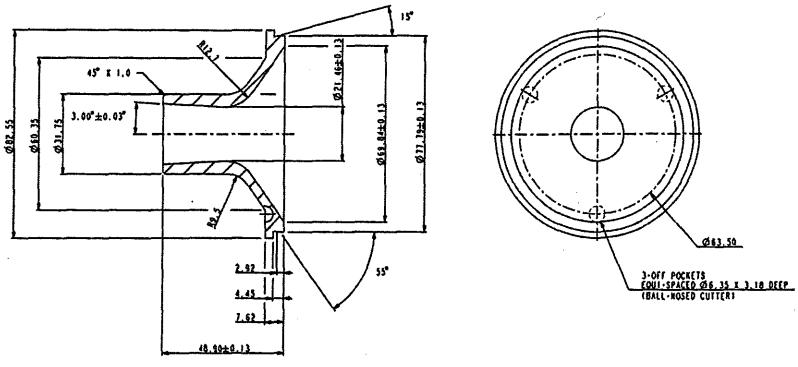


Figure 2. Expanded view of the modified entrance cone. (Fluticasone Propionate Inhalation Aerosol)

Detach the inhaler, and rinse each piece of the apparatus with methanol into a suitable volumetric flask containing 40% of the flask volume of water. The final concentration of the expected amount of fluticasone propionate should be in the range of 0.02–3.5 µg/mL. Allow the solutions to equilibrate, and dilute with methanol to volume. Repeat these steps on an additional four Inhalation Aerosols for a total of five *Sample solutions*.

Chromatographic system and **System suitability:** Proceed as directed in the *Assay*, except use 50 µL for *Injection volume*.

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the quantity, in µg/actuation, of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the *Sample solutions*:

$$\text{Result} = [(r_U/r_S) \times C_S] \times V/N$$

r_U = response from the *Sample solution*
r_S = response from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)
V = total volume of the *Sample solution* (mL)
N = number of actuations discharged into the apparatus

Acceptance criteria: The mass of fluticasone propionate recovered from the cascade impactor is given in *Table 2*.

Table 2

Product Strength (µg/actuation)	Amount of Fluticasone Propionate Recovered (µg/actuation)
44	35.2–48.4
110	90–124
220	176–242

The mass of fluticasone propionate deposited in each grouping of the *Sampling apparatus* for each inhaler is given in *Table 3*.

Table 3

Apparatus Component	Amount of Fluticasone Propionate Deposited (µg)		
	44 µg/actuation	110 µg/actuation	220 µg/actuation
Induction port	9–22	26–56	66–112
Sum of Stages 0, 1, and 2	NMT 8	NMT 21	NMT 46
Sum of Stages 3, 4, and 5	14–27	35–60	67–110
Sum of Stages 6, 7, and filter	NMT 2	NMT 3	NMT 3

The mean mass of fluticasone propionate deposited in each grouping of the *Sampling apparatus* for the five inhalers is given in *Table 4*.

Table 4

Apparatus Component	Amount of Fluticasone Propionate Deposited (µg)		
	44 µg/actuation	110 µg/actuation	220 µg/actuation
Induction port	10–21	28–54	69–109
Sum of Stages 0, 1 and 2	NMT 7	NMT 19	NMT 44
Sum of Stages 3, 4 and 5	15–26	37–58	71–106
Sum of Stages 6, 7, and filter	NMT 2	NMT 3	NMT 3

• **INHALATION AND NASAL DRUG PRODUCTS: AEROSOLS, SPRAYS, AND POWDERS—PERFORMANCE QUALITY TESTS,** ⟨601⟩, *Delivered-Dose Uniformity*

Sampling apparatus: Use the apparatus (*Figure 3A. Sampling apparatus.*) with modified glass sampling device (*Figure 3B. Expanded view of the modified glass sample collection apparatus.*).

USP Monographs

www.webofpharma.com

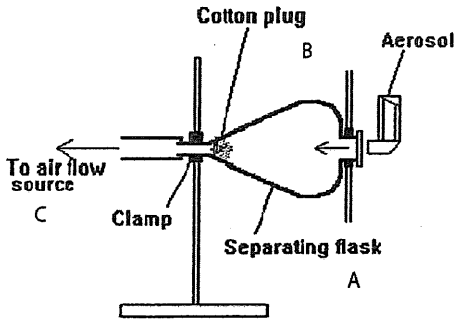


Figure 3A. Sampling apparatus.

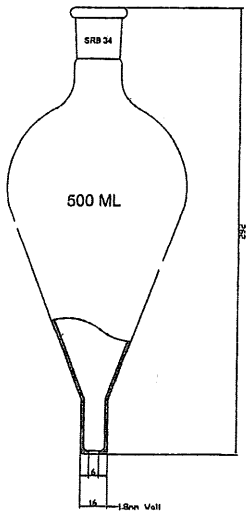


Figure 3B. Expanded view of the modified glass sample collection apparatus.

Buffer, Solution A, and **Mobile phase:** Proceed as directed in the *Assay*.

Diluent: Methanol and water (60:40)

Standard solution: 0.75 µg/mL of USP Fluticasone Propionate RS in *Diluent*

Sample solutions: Prime the valve by discharging a predetermined number of actuations to waste. Discharge the recommended dose at the beginning of use (BOU) into the *Sampling apparatus*, and detach the inhaler. Rinse the apparatus with methanol, and quantitatively transfer the solutions to a 200-mL volumetric flask containing 80 mL of water. Allow the solution to equilibrate, and dilute with methanol to volume. Discharge the requisite number of actuations to waste, shaking the inhaler before each discharge, until the last recommended dose remains. Discharge the remaining recommended dose at end of use (EOU) corresponding to the labeled number of actuations into the *Sampling apparatus*, and detach the inhaler. Rinse the apparatus with methanol, and quantitatively transfer the solution to a 200-mL volumetric flask containing 80 mL of water. Allow the solution to equilibrate, and dilute with methanol to volume. Repeat these steps on an additional nine inhalers for a total of 20 *Sample solutions*.

Chromatographic system and **System suitability:** Proceed as directed in the *Assay*, except use 50 µL for *Injection volume*.

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) delivered by the inhaler in each *Sample solution*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response from the *Sample solution*

r_S = response from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 5* and *Table 6*.

Table 5

Parameter	Acceptance Criteria		
Label Claim	44 µg/ actuation	110 µg/ actuation	220 µg/ actuation
Mean of 10 BOU contents	85–115% of label claim	85–115% of label claim	85–115% of label claim
Mean of 10 EOU contents	85–115% of label claim	85–115% of label claim	85–115% of label claim
Number of individual contents outside 80–120% of label claim	NMT 2 of 20	NMT 2 of 20	NMT 2 of 20
Each individual content	70–130% of label claim	75–125% of label claim	75–125% of label claim

Test an additional 20 inhalers (BOU and EOU) if up to 6 of the 20 individual results are outside of 80%–120% of label claim, but none are outside 70%–130% of the label claim for the 44 µg/actuation product strength, or none are outside 75%–125% of the label claim for the 110 µg/actuation and 220 µg/actuation.

Table 6

Parameter	Acceptance Criteria		
Label Claim	44 µg/ actuation	110 µg/ actuation	220 µg/ actuation
Mean of 30 BOU contents	85–115% of label claim	85–115% of label claim	85–115% of label claim
Mean of 30 EOU contents	85–115% of label claim	85–115% of label claim	85–115% of label claim
Number of individual contents outside 80–120% of label claim	NMT 6 of 60	NMT 6 of 60	NMT 6 of 60
Each individual content	70–130% of label claim	75–125% of label claim	75–125% of label claim

IMPURITIES

• **ORGANIC IMPURITIES:** Protect the *Sample solutions* and *Standard solutions* from light.

Solution A: Prepare a solution of 0.05% v/v phosphoric acid in acetonitrile.

Solution B: Prepare a solution of 0.05% v/v phosphoric acid in methanol.

Solution C: Prepare a solution of 0.05% v/v phosphoric acid in water.

Mobile phase: See *Table 7*.

USP Monographs

www.webofpharma.com

Table 7

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	42	3	55
40	53	3	44
60	87	3	10
70	87	3	10

System suitability solution: Dissolve 2 mg of USP Fluticasone Propionate System Suitability Mixture RS in 5 mL of *Solution A*. Add 5 mL of *Solution C*, and mix.

Sample solution: Nominally 200 µg/mL of fluticasone propionate in *Solution A* prepared as follows. Place a canister into a freezing mixture of dry ice and methanol, and cool for approximately 5 min. Carefully remove the valve from the canister, and pass the contents through a filter of 0.22-µm pore size. When the filter bed is dry, wash it with two 5-mL aliquots of cyclohexane. Allow the paper to dry, then carefully transfer to an appropriate container. Dissolve the drug present on the filter paper in 50% of the final required volume of *Solution A*. Dilute with *Solution C* to volume to obtain the nominal concentration.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Column temperature: 40°

Injection volume: 50 µL

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 8* for relative retention times.]

Suitability requirements

Resolution: NLT 0.6 between the fluticasone propionate related compound B and fluticasone propionate related compound C peaks and NLT 1.5 between the fluticasone propionate related compound D and fluticasone propionate peaks

Tailing factor: NMT 1.1 for the fluticasone propionate peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Inhalation Aerosol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each impurity from the *Sample solution*

r_T = total peak area for all peaks ≥0.05% by area of the peak due to fluticasone propionate from the *Sample solution*

Acceptance criteria: See *Table 8*.

Table 8

Name	Relative Retention Time	Acceptance Criteria (%)
Fluticasone sulfenic acid[a]	0.47	<0.2
Fluticasone propionate related compound B[b]	0.74	—
Fluticasone propionate related compound C[b]	0.76	—
Fluticasone propionate related compound D	0.95	<0.3
Fluticasone propionate	1.0	—
Chlorofluticasone propionate[c]	1.19	<0.3
Fluticasone propionate dimer[d]	1.33	<0.3
Any unspecified degradation product	—	<0.1
Total impurities[e]	—	NMT 1.0

[a] 6α,9α-Difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbonylsulfenic acid.

[b] These are process impurities that are included in the table for identification only. These impurities are controlled in the drug substance and are not included in the total impurities.

[c] *S*-Chloromethyl-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate.

[d] 6α,9α-Difluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid 6α,9α-difluoro-17β-(fluoromethylthio)carbonyl-11β-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-yl ester.

[e] Sum of all impurity peaks ≥0.05%.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^1 cfu/g of formulation. The total aerobic yeasts and molds count does not exceed 10^1 cfu/g of formulation. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species in 10 g of the formulation.
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩

The test described below and the specification is only applicable to a microscopic particle count test methodology. *Particulate Matter in Injections* ⟨788⟩ describes details of the test apparatus to be used for the determination of particulate matter using a microscopic particle count test methodology. Samples should be carefully prepared to avoid environmental contamination, and testing should be performed with suitable controls, including the appropriate use of blank determinations.

Filter: Mixed cellulose and ester filter; 25-mm diameter and 0.45-µm pore size

Sample solution: Perform testing on two previously unused inhalers. Prime each inhaler by discharging a predetermined number of actuations to waste. Discharge, and dissolve 16 actuations, 8 from each of two canisters in 50 mL of methanol.

Analysis

Sample: *Sample solutions*

Pass the *Sample solution* through the *Filter*, and allow the *Filter* to dry under conditions that will limit particulate contamination. Using a microscopic particle count test method, enumerate the number of particles present in the *Sample solution*. Calculate the number of particles per actuation by the formula:

$$\text{Result} = (N_{<10} + N_{10\text{–}100} + N_{>100})/16$$

$N_{<10}$ = total number of particles <10 µm present in the *Sample solution*

$N_{10\text{–}100}$ = total number of particles between 10 and 100 µm present in the *Sample solution*

www.webofpharma.com

$N_{>100}$ = total number of particles >100 µm present in the *Sample solution*

Acceptance criteria: See *Table 9*.

Table 9

Particle Size Range (µm)	Number of Particles per Actuation (NMT)
<10	140
10–100	50
>100	5
Total	185

- **OTHER REQUIREMENTS:** It meets the requirements for *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* ⟨601⟩, *Number of Discharges per Container* and *Leakage Test*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in nonreactive, light-resistant aerosol containers with metered valves fitted with a dose counter and provided with oral inhalation actuators. Avoid exposure to heat. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fluticasone Propionate RS
  USP Fluticasone Propionate System Suitability Mixture RS
  It is a mixture of:
  Fluticasone propionate;
  Fluticasone propionate related compound B: [6α, 9α-difluoro-11β-hydroxy-16α-methyl-2′,3,4′-trioxo-17α-spiro(androsta-1,4-diene-17,5′-(1,3)oxathiolane];
  Fluticasone propionate related compound C: [*S*-fluoromethyl 17α-acetyloxy-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carbothioate];
  Fluticasone propionate related compound D: [*S*-methyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate].

Fluticasone Propionate Inhalation Powder

DEFINITION

Fluticasone Propionate Inhalation Powder contains NLT 90% and NMT 110% of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Delivered Dose Uniformity*.

ASSAY

- **PROCEDURE**

Buffer: 0.01 M sodium dodecyl sulfate containing 0.1% glacial acetic acid
Solution A: Methanol and *Buffer* (20:80)
Mobile phase: Acetonitrile and *Solution A* (49:51)
Diluent: Methanol and water (70:30)
Standard solution: 6–12 µg/mL of USP Fluticasone Propionate RS in *Diluent*
Sample solution: Nominally 6–12 µg/mL of fluticasone propionate from NLT 12 unit doses in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 5-cm; 3.5-µm packing L1
Flow rate: 2 mL/min
Column temperature: 40°
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for the peak due to fluticasone propionate will be within the range of 1.0–1.6 min.]
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the portion of Inhalation Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response from the *Sample solution*
r_S = response from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)

Acceptance criteria: NLT 90% and NMT 110%

PERFORMANCE TESTS

- **PARTICLE SIZE DISTRIBUTION BY CASCADE IMPACTION**

Sampling apparatus: Modified *Apparatus 3* (*Figure 1. Cascade impaction sampling apparatus (modified Apparatus 3 in 601) including Induction Port and Preseparator Lid.*) in *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* ⟨601⟩ with a modified induction port (*Figure 2. Expanded view of the modified induction port.*), and preseparator lid (*Figure 3. Expanded view of the preseparator lid.*) are to be used.
Buffer, Solution A, Mobile phase, Diluent, Chromatographic system, and **System suitability:** Proceed as directed in the test for *Assay*.
Standard solution: 0.5–1.5 µg/mL of USP Fluticasone Propionate RS in *Diluent*
Sample solutions: Discharge the required number of unit doses given in *Table 1* into the cascade impaction sampling apparatus described in *Figure 1. Cascade impaction sampling apparatus (modified Apparatus 3 in 601) including Induction Port and Preseparator Lid.*

Table 1

Product Strength (µg/dose)	Number of Unit Doses
50	15
100	10
250	10

Operate the pump for 3 s at an airflow rate of 60 L/min for each dose discharged. Detach the inhaler, and rinse each piece of the apparatus with methanol. Transfer the washings to a suitable volumetric flask containing 30% of the flask volume of water. Allow the solutions to equilibrate to room temperature, and dilute with methanol to volume. The concentration of the expected amount of fluticasone propionate should be in the concentration range of 0.1–5

www.webofpharma.com

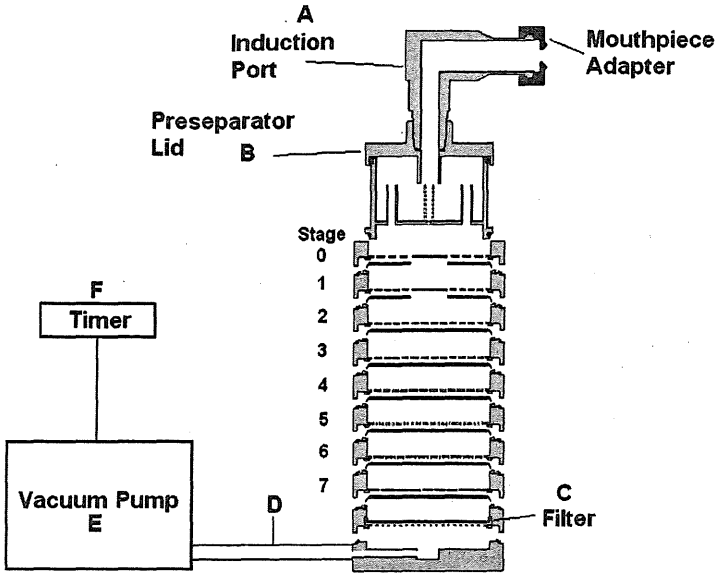


Figure 1. Cascade impaction sampling apparatus (modified *Apparatus 3* in ⟨601⟩) including *Induction Port* and *Preseparator Lid*.

(Fluticasone Propionate Inhalation Powder)

µg/mL. Repeat these steps for three additional sample preparations, for a total of four sample solutions.

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the quantity, in µg/unit dose, of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in each *Sample solution*:

$$\text{Result} = [(r_U/r_S) \times C_S] \times V/N$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

V = total volume of the *Sample solution* (mL)

N = number of unit doses discharged into the apparatus

Acceptance criteria

The total mass of drug recovered from the cascade impactor must be within ±15% of the target emitted dose in *Table 2. Fluticasone Propionate Target Emitted Dose*.

Table 2. Fluticasone Propionate Target Emitted Dose

Product Strength (µg/dose)	Fluticasone Propionate Target Emitted Dose (µg/unit dose)
50	46
100	94
250	229

The mass of fluticasone propionate deposited in each grouping of the cascade impactor *Sampling apparatus* for each unit dose is given in *Table 3. Amount of Fluticasone Propionate in Cascade Impactor Stage Groupings*.

Table 3. Amount of Fluticasone Propionate in Cascade Impactor Stage Groupings

Apparatus Component	Amount of Fluticasone Propionate Deposited (µg)		
	50 µg/dose	100 µg/dose	250 µg/dose
Mouthpiece adapter, induction port, preseparator, and Stage 0	30–45	55–90	140–210
Sum of Stages 1–5	8–14	15–30	40–71
Sum of Stages 3 and 4	3–8	6–18	19–42
Sum of Stages 6, 7, and filter	<1	NMT 1	NMT 3

All the groupings for each sample preparation must meet the criteria in *Table 3. Amount of Fluticasone Propionate in Cascade Impactor Stage Groupings*.

If NMT one of the four sample preparations fails to meet the requirements in *Table 3. Amount of Fluticasone Propionate in Cascade Impactor Stage Groupings*, but is within 25% of either the lower or upper specification limit being tested, analyze two additional samples. The batch meets the requirements if five of the six sample preparations meet the limits in *Table 3. Amount of Fluticasone Propionate in Cascade Impactor Stage Groupings* for the individual sample preparations.

• **INHALATION AND NASAL DRUG PRODUCTS: AEROSOLS, SPRAYS, AND POWDERS—PERFORMANCE QUALITY TESTS** ⟨601⟩, *Delivered-Dose Uniformity*

Sampling apparatus: Use the apparatus in *Figure 4A. Sampling apparatus.* with the modified glass sampling device (*Figure 4B. Expanded view of the modified glass sample collection apparatus.*).

USP Monographs

www.webofpharma.com

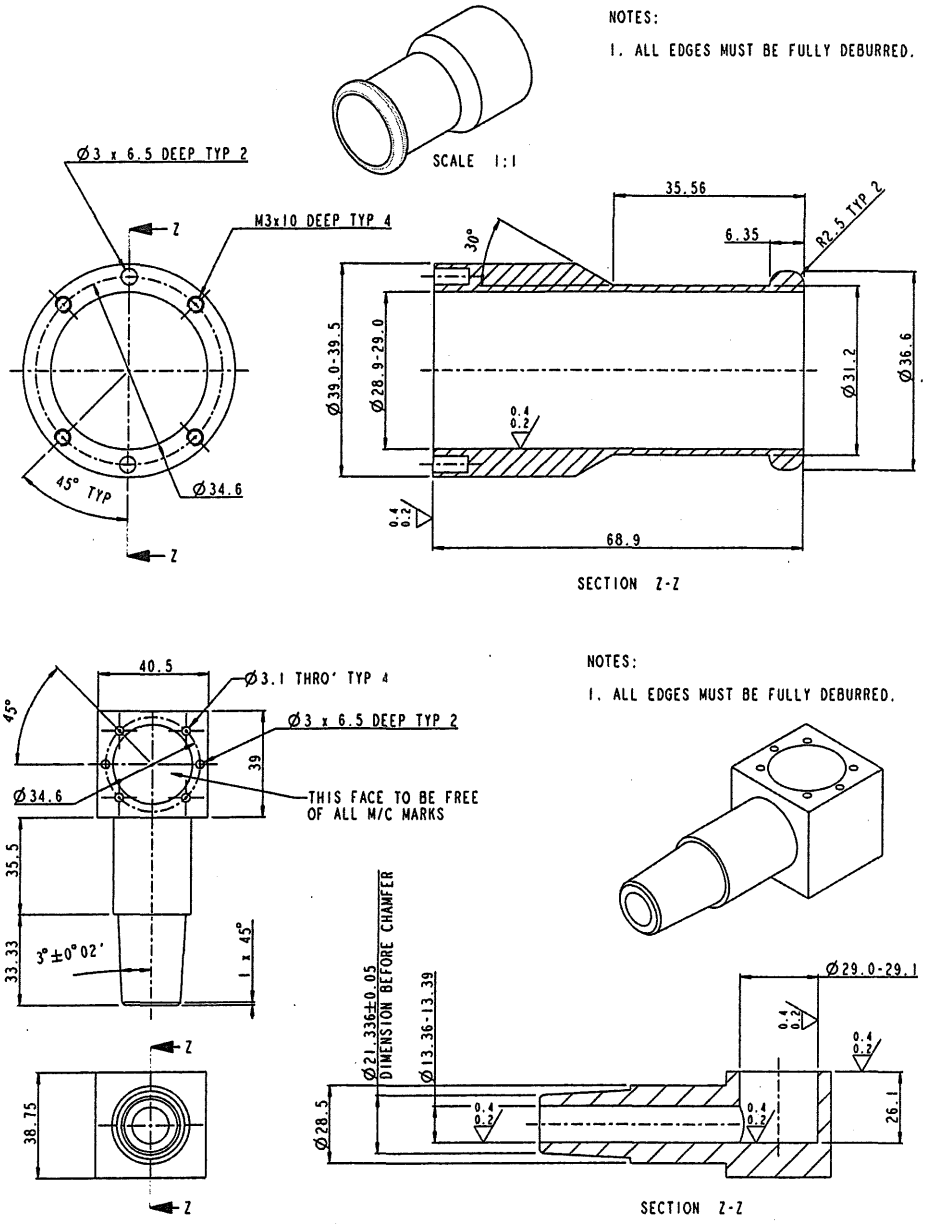


Figure 2. Expanded view of the modified induction port. (Fluticasone Propionate Inhalation Powder)

www.webofpharma.com

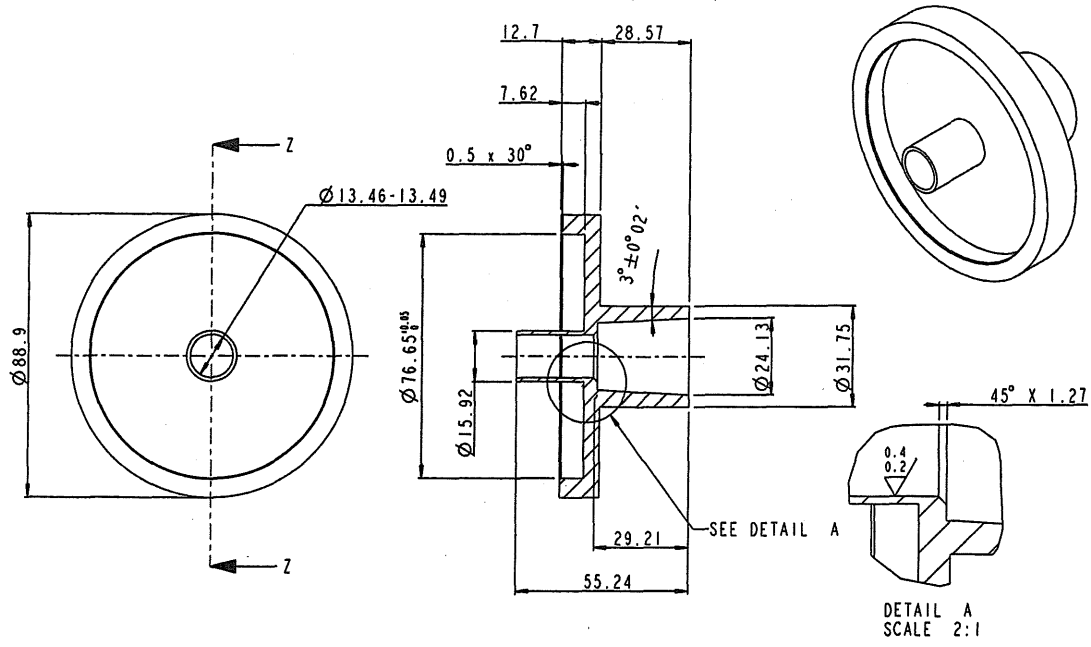


Figure 3. Expanded view of the preseparator lid. (Fluticasone Propionate Inhalation Powder)

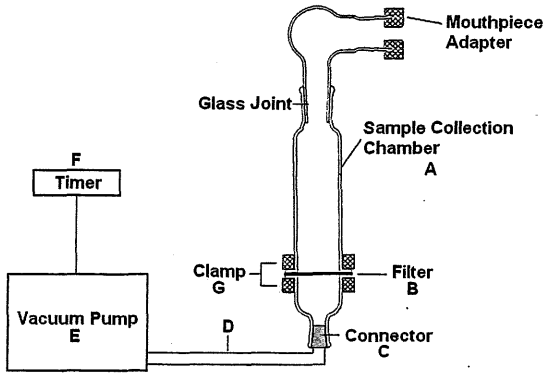


Figure 4A. Sampling apparatus.

Buffer, Solution A, Mobile phase, Diluent, Chromatographic system, and **System suitability:** Proceed as directed in *Assay*.

Standard solution: (*L*/200) µg/mL of USP Fluticasone Propionate RS in *Diluent*, where *L* is the labeled amount of fluticasone propionate in µg/dose

Sample solutions: Discharge a single unit dose into the apparatus shown in *Figure 4A. Sampling apparatus.*. Operate the pump for 2 s at an airflow of 60 L/min to collect the dose. Detach the inhaler, and rinse the mouthpiece adapter and each piece of the sample collection chamber with methanol. Place the filter and washings into a container, and sonicate for 5 min. Quantitatively transfer the contents to a 200-mL volumetric flask containing 60 mL of water. Allow the solution to equilibrate, and dilute with methanol to volume. Prepare nine additional samples from stratified unit doses. For multi-dose inhalers, collect one dose from each of 10 inhalers with the 10 doses collected across the

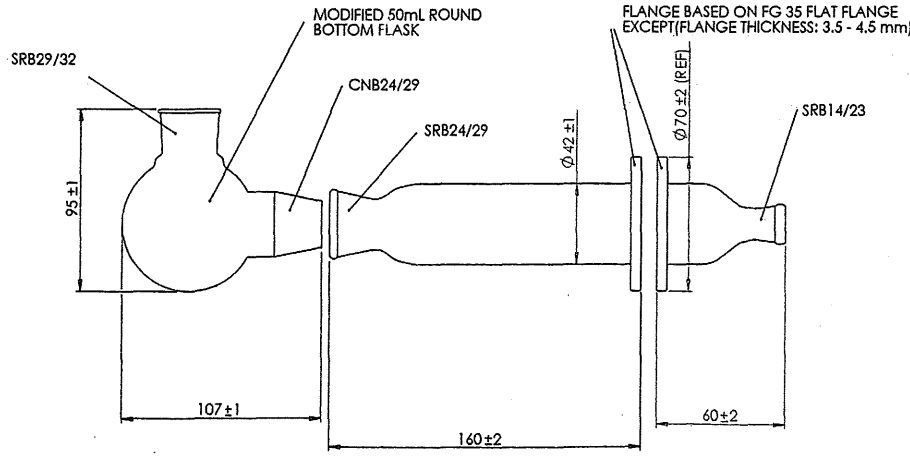


Figure 4B. Expanded view of the modified glass sample collection apparatus. (Fluticasone Propionate Inhalation Powder)

www.webofpharma.com

minimum number of recommended doses on the label of the inhaler.

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the percentage of the target emitted dose of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) delivered by the Inhalation Powder in each of the *Sample solutions*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL), based on target emitted dose from *Table 2. Fluticasone Propionate Target Emitted Dose*

Acceptance criteria

1. The mean content of fluticasone propionate of 10 doses is NLT 85% and NMT 115% of the target emitted dose.
2. NMT 1 emitted dose is outside 80%–120% of the target emitted dose.
3. No dose is outside 75%–125% of the target emitted dose.

If requirements 1 and 2 described above are not met, test an additional 20 unit doses. The mean dose of fluticasone propionate of 30 doses is:

- NLT 85% and NMT 115% of the target emitted dose.
- NMT 3 doses are outside 80%–120% of the target emitted dose.
- No dose is outside 75%–125% of the target emitted dose.

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES:** Protect sample and standard solutions from light.

Solution A: Prepare a solution of 0.05% (v/v) phosphoric acid in acetonitrile.

Solution B: Prepare a solution of 0.05% (v/v) phosphoric acid in methanol.

Solution C: Prepare a solution of 0.05% (v/v) phosphoric acid in water.

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	42	3	55
40	53	3	44
60	87	3	10
70	87	3	10
70.1	42	3	55
90	42	3	55

System suitability solution: Dissolve 2 mg of USP Fluticasone Propionate System Suitability Mixture RS in 12 mL of *Solution A*. Add 8 mL of *Solution C*, and mix.

Sensitivity solution: 0.5 µg/mL of USP Fluticasone Propionate RS in *Solution A*

Sample solution: Nominally 100 µg/mL of fluticasone propionate from NLT 10 unit doses prepared as follows. Transfer the contents of the appropriate number of unit doses into suitable volumetric flask. Add 60% of the flask volume of *Solvent A*, and sonicate for 2 min. Add 40% of the flask volume of *Solvent C*, mix, and allow the solution to equilibrate. Dilute with *Solvent A* to volume if necessary. Mix, allow any undissolved excipient material to settle, and inject the supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Column temperature: 40°

Injection volume: 100 µL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 0.6 between fluticasone propionate related compound B and fluticasone propionate related compound C and NLT 1.5 between fluticasone propionate related compound D and fluticasone propionate, *System suitability solution*

Tailing factor: ▲NMT 1.3▲ (ERR 1-Dec-2018) for fluticasone propionate, *System suitability solution*

Signal-to-noise ratio: NLT 10 for fluticasone propionate, *Sensitivity solution*

Analysis

Samples: *Sample solution*

Calculate the percentage of each fluticasone propionate related impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = total peak response for all peaks ≥0.05% by area of the peak due to fluticasone propionate from the *Sample solution*

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria (%)
Fluticasone sulfenic acid[a]	0.47	<0.2
Fluticasone propionate related compound B[b]	0.75	—
Fluticasone propionate related compound C[b]	0.77	—
Fluticasone propionate related compound D	0.95	<0.3
Fluticasone propionate	1.0	—
Fluticasone propionate dimer[c]	1.31	<0.3
Any unspecified degradation product	—	<0.1

www.webofpharma.com

Table 5 *(continued)*

Name	Relative Retention Time	Acceptance Criteria (%)
Total[d]	—	NMT 1.1

[a] 6α,9α-Difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbonylsulfenic acid.
[b] These are process impurities that are included in the table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and are not included in the total impurities.
[c] 6α,9α-Difluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid 6α,9α-difluoro-17β-(fluoromethylthio)carbonyl-11β-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-yl ester.
[d] Sum of all impurity peaks ≥0.05%.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^1 cfu/g of bulked powder, the total aerobic yeasts and molds count does not exceed 10^1 cfu/g of bulked powder. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella* species, and Enterobacteriaceae in 1 g of bulked powder.
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩

The test described below and the specification are applicable only to a microscopic particle count test methodology. *Particulate Matter in Injections* ⟨788⟩ describes details of the test apparatus to be used for the determination of particulate matter using a microscopic particle count test methodology. Samples should be carefully prepared to avoid environmental contamination and testing should be performed with suitable controls, including the appropriate use of blank determinations.

Diluent: Methanol and water (65:35) filtered through a filter of 0.45-µm pore size

Filter: Mixed cellulose and ester; 25-mm diameter and 0.45-µm pore size

Sample solution: Transfer the contents of 4 unit doses from each of two inhalers into a suitable receptacle, and dissolve the drug and excipient particles in about 75 mL of *Diluent*.

Analysis

Samples: *Sample solutions*

Pass the *Sample solution* through the *Filter* and allow the *Filter* to dry under conditions that will limit particulate contamination. Using a microscopic particle count test method, enumerate the number of particles present in the *Sample solution*.

Calculate the number of particles per dose:

$$\text{Number of particles/dose} = (N_{<10} + N_{10\text{–}100} + N_{>100})/8$$

$N_{<10}$ = total number of particles <10 µm in the *Sample solution*
$N_{10\text{–}100}$ = total number of particles between 10 and 100 µm in the *Sample solution*
$N_{>100}$ = total number of particles >100 µm present in the *Sample solution*

Acceptance criteria: See *Table 6*.

Table 6

Particle Size Range (µm)	Number of Particles/Dose (NMT)
<10	200
10–100	100
>100	10
Total	300

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature, in a dry place away from direct heat or sunlight.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Fluticasone Propionate RS

USP Fluticasone Propionate System Suitability Mixture RS

It is a mixture of:

Fluticasone propionate;

Fluticasone propionate related compound B: [6α, 9α-difluoro-11β-hydroxy-16α-methyl-2′,3,4′-trioxo-17α-spiro(androsta-1,4-diene-17,5′-(1,3)oxathiolane];

Fluticasone propionate related compound C: [*S*-fluoromethyl 17α-acetyloxy-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carbothioate];

Fluticasone propionate related compound D: [*S*-methyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate].

Fluticasone Propionate Lotion

DEFINITION

Fluticasone Propionate Lotion is fluticasone propionate in a suitable lotion base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 1.15 g/L of monobasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 3.5. Pass the solution through a suitable filter of 0.45-µm pore size.

Mobile phase: Acetonitrile, methanol, and *Buffer* (13:50:37)

Standard stock solution: 0.8 mg/mL of USP Fluticasone Propionate RS, prepared as follows. Transfer 40 mg of USP Fluticasone Propionate RS to a 50-mL volumetric flask. Add 10 mL of tetrahydrofuran and sonicate to dissolve. Dilute with methanol to volume.

Standard solution: 0.04 mg/mL of USP Fluticasone Propionate RS in methanol from *Standard stock solution*

Sample solution: Nominally 0.04 mg/mL of fluticasone propionate from Lotion, prepared as follows. Transfer a portion of Lotion to a suitable volumetric flask. Add tetrahydrofuran equivalent to about 20% of the flask volume and vortex until the Lotion is dispersed. Add methanol equivalent to 60% of the flask volume and sonicate for 10 min to dissolve. Dilute with methanol to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 239 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.25 times the retention time of fluticasone propionate
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Column efficiency: NLT 5000 theoretical plates
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the portion of Lotion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluticasone propionate from the *Sample solution*
r_S = peak response of fluticasone propionate from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluticasone propionate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Minimum Fill** ⟨755⟩**:** Meets the requirements

IMPURITIES

• **Organic Impurities**
Solution A: Methanol, phosphoric acid, and water (30: 0.5: 970)
Solution B: Acetonitrile, methanol, and phosphoric acid (950: 50: 0.5)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	56	44
45	56	44
49	10	90
70	10	90
71	56	44
80	56	44

System suitability solution: 0.13 mg/mL of USP Fluticasone Propionate RS and 0.38 µg/mL of USP Fluticasone Propionate Related Compound D RS in methanol
Standard stock solution: 0.25 mg/mL of USP Fluticasone Propionate RS, prepared as follows. Transfer 25 mg of USP Fluticasone Propionate RS to a 100-mL volumetric flask. Add 25 mL of tetrahydrofuran and sonicate to dissolve. Dilute with methanol to volume.
Standard solution: 0.63 µg/mL of USP Fluticasone Propionate RS in methanol from *Standard stock solution*
Sample solution: Nominally 0.13 mg/mL of fluticasone propionate from Lotion, prepared as follows. Transfer a portion of Lotion, equivalent to 1.25 mg of fluticasone propionate, to a suitable volumetric flask. Add tetrahydrofuran equivalent to 20% of the flask volume and vortex until the Lotion is dispersed. Add methanol equivalent to 40% of the flask volume and sonicate for 10 min to dissolve. Dilute with methanol to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 239 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 40 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between fluticasone propionate related compound D and fluticasone propionate, *System suitability solution*
Relative standard deviation: NMT 5.0% from six replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluticasone propionate related compound C, fluticasone propionate related compound D, or any individual unspecified degradation product in the portion of Lotion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of fluticasone propionate related compound C, fluticasone propionate related compound D, or each unspecified degradation product from the *Sample solution*
r_S = peak response of fluticasone propionate from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fluticasone propionate related compound C[a]	0.67	0.82	0.10
Fluticasone propionate related compound D	0.91	1.1	0.30
Fluticasone propionate	1.0	1.0	—
Any unspecified degradation product	—	1.0	0.10
Total degradation products	—	—	1.0

[a] *S*-Fluoromethyl 17α-acetyloxy-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carbothioate.

www.webofpharma.com

SPECIFIC TESTS

- **pH** ⟨791⟩
  Sample: 50 mg/mL of Lotion in water
  Acceptance criteria: 4.0–6.0
- **Microbial Enumeration Tests** ⟨61⟩ and **Tests For Specified Microorganisms** ⟨62⟩**:** Meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The total aerobic microbial count is NMT 10^2 cfu/g, and the total combined molds and yeasts count is NMT 10 cfu/g.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature. Do not refrigerate.
- **USP Reference Standards** ⟨11⟩
  USP Fluticasone Propionate RS
  USP Fluticasone Propionate Related Compound D RS
  S-Methyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate.
  $C_{25}H_{32}F_2O_5S$ 482.58

Fluticasone Propionate Nasal Spray

DEFINITION

Fluticasone Propionate Nasal Spray is a white, opaque suspension of Fluticasone Propionate in water. It is supplied in a form suitable for nasal administration. It contains NLT 95.0% and NMT 115.0% of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$).

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M▲** (CN 1-May-2020)
  Sample: Transfer 30 g of Nasal Spray equally into two 50-mL centrifuge tubes. Add 10 mL of water to each tube, insert the stopper, and shake vigorously for 2 min. Centrifuge at about 3500 rpm for about 10 min, and discard the supernatant. Add 10 mL of water to each tube, insert the stopper, and shake vigorously for 2 min. Centrifuge at about 3500 rpm for about 10 min, and discard the supernatant. Add 10 mL of water to each tube, insert the stopper, and shake vigorously for about 2 min. Centrifuge at 3500 rpm for 10 min, and discard the supernatant. To one tube add 10 mL of methanol. Shake to disperse the residue, and transfer to the other tube. Shake the other tube for 1 min. Centrifuge at about 3500 rpm for about 10 min. Decant the supernatant into an agate mortar. Evaporate the methanol either by carefully blowing dry with compressed air or nitrogen, or by allowing the methanol to evaporate naturally. If using an air or nitrogen line, use a suitable in-line filter to avoid contamination. Allow the residue to dry overnight in a desiccator over silica gel.
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**
  Diluent: Acetonitrile and 0.001 M hydrochloric acid (60:40)
  Buffer: 1.2 g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of 3.5.
  Mobile phase: Methanol, acetonitrile, and *Buffer* (50:15:35)
  System suitability solution: 50 µg/mL of USP Phenylethyl Alcohol RS, 10 µg/mL of USP Fluticasone Propionate RS, and 1 µg/mL of USP Fluticasone Propionate Related Compound D RS in *Diluent*
  Standard solution: 10 µg/mL of USP Fluticasone Propionate RS in *Diluent*
  Sample solution: Nominally 10 µg/mL of fluticasone propionate prepared as follows. Transfer an amount of the Nasal Spray containing 0.5 mg of fluticasone propionate to a 50-mL volumetric flask. Add about 40 mL of *Diluent*, and sonicate the flask for 10 min. Dilute with *Diluent* to volume, and shake. Allow to stand for 10 min until the supernatant is a clear solution. Inject the clear supernatant into the chromatograph.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 and 239 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 40°
  Flow rate: 1.5 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Record the chromatogram at 210 nm for 5 min and then change the wavelength to 239 nm.
  [Note—The relative retention times for phenylethyl alcohol, fluticasone propionate, and fluticasone propionate related compound D are about 0.42, 1.0, and 1.10, respectively.]
  Suitability requirements
  Resolution: NLT 1.5 between fluticasone propionate and fluticasone propionate related compound D, *System suitability solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–115.0%

OTHER COMPONENTS

- **Content of Phenylethyl Alcohol**
  Diluent, Mobile phase, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
  Standard solution: 0.05 mg/mL of USP Phenylethyl Alcohol RS in *Diluent*
  Sample solution: Transfer 1.0 g of the Nasal Spray to a 50-mL volumetric flask. Add about 40 mL of *Diluent*, and sonicate for 10 min until the supernatant is clear. Use the clear supernatant for analysis.
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the quantity, in mg/g, of phenylethyl alcohol in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/W)$$

www.webofpharma.com

r_U = peak response of phenylethyl alcohol from the *Sample solution*
r_S = peak response of phenylethyl alcohol from the *Standard solution*
C_S = concentration of USP Phenylethyl Alcohol RS in the *Standard solution* (mg/mL)
V = volume of the *Sample solution*, 50 mL
W = weight of the Nasal Spray in the *Sample solution* (g)

Acceptance criteria
For 50 sprays: 1.75–2.63 mg/g
For 120 sprays: 1.88–2.63 mg/g

• **CONTENT OF BENZALKONIUM CHLORIDE**
Buffer: Dissolve 10.8 g of monobasic sodium phosphate dihydrate in 90 mL of water, and adjust with phosphoric acid to a pH of 2.5. Dilute with water to 100 mL.
Solution A: Mix 50 mL of *Buffer*, 750 mL of water, and 200 mL of methanol. Add 5 mL of triethylamine. Mix and adjust with phosphoric acid to a pH of 2.5.
Solution B: Mix 1 L of methanol with 50 mL of phosphoric acid.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	55	45
3.0	5	95
3.2	55	45
5.0	55	45

Diluent: Methanol, water, and hydrochloric acid (693:297:10)
System suitability solution: 0.04 mg/mL of USP Benzalkonium Chloride RS in *Diluent* prepared as follows. Transfer a suitable volume of USP Benzalkonium Chloride RS to a suitable volumetric flask and dilute to volume with *Diluent*.
Standard stock solution: 200 µg/mL of USP Benzalkonium Bromide RS in water. [NOTE—Sonication for about 2 min may be used to ensure complete dissolution. A few drops of methanol may be used to resolve the formation of foam prior to dilution.]
Standard solution: 20 µg/mL of USP Benzalkonium Bromide RS. Transfer an aliquot of *Standard stock solution* to a suitable volumetric flask. Dilute with *Diluent* to volume.
Sample solution: Transfer 1.0 g of the Nasal Spray to a 10-mL volumetric flask. Dilute with *Diluent* to volume. Centrifuge if necessary. [NOTE—Centrifuging at about 4000 rpm for about 15 min may be suitable. Supernatant may be passed through a suitable filter with a pore size of NMT 0.2 µm with a glass syringe.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 3.0-cm; 2.6-µm packing L1
Column temperature: 50°
Flow rate: 2 mL/min
Injection volume: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times. The C_{10} benzalkonium homolog may not be present.]
Suitability requirements
Resolution: NLT 2.5 between the pairs of C_{12} and C_{14} benzalkonium homologs and the C_{14} and C_{16} benzalkonium homologs, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the sum of the corrected benzalkonium peak responses (r_{CU}) in the portion of *Sample solution* taken:

$$\text{Result} = \Sigma[r_U \times (1/F)]$$

r_U = peak response of each C_{10}, C_{12}, C_{14}, and C_{16} benzalkonium homolog from the *Sample solution*
F = relative response factor for the corresponding benzalkonium homolog relative to benzalkonium bromide (see *Table 2*)

Table 2

Benzalkonium Chloride Homolog	Relative Retention Time	Relative Response Factor
C_{10}	0.65	1.2
C_{12}	1.0	1.1
C_{14}	1.35	1.0
C_{16}	1.59	0.98

Calculate the amount of benzalkonium chloride in the portion of Nasal Spray taken:

$$\text{Result} = (r_{CU}/r_S) \times C_S \times (V/W)$$

r_{CU} = sum of the corrected peak responses of the benzalkonium homologs from the *Sample solution*
r_S = peak response of benzalkonium from the *Standard solution*
C_S = concentration of USP Benzalkonium Bromide RS in the *Standard solution* (µg/mL)
V = volume of the *Sample solution* (mL)
W = weight of Nasal Spray in the *Sample solution* (g)

Acceptance criteria: 140–220 µg/g

PERFORMANCE TESTS

• **DELIVERED DOSE UNIFORMITY** (within container)
Diluent: Acetonitrile and 0.001 M hydrochloric acid (60:40)
Buffer: 1.2 g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of 3.5.
Mobile phase: Methanol, acetonitrile, and *Buffer* (50:15:35)
System suitability solution: 5 µg/mL of USP Fluticasone Propionate RS and 0.5 µg/mL of USP Fluticasone Propionate Related Compound D RS in *Diluent*
Standard solution: 4 µg/mL of USP Fluticasone Propionate RS in *Diluent*
Sample solution: Wipe the pump clean. Shake the bottle for 30 s, and mechanically prime the bottle. Hold a 25-mL volumetric flask in an inverted position, and discharge the first two actuations (1 dose) into the flask. Turn the flask to the upright position immediately after each actuation. Insert the stopper into the flask after collecting two actuations. Discharge actuations 3–48 (50-spray pack) or 3–118 (120-spray pack) to waste. Wipe the bottle clean,

www.webofpharma.com

and collect the last two actuations (49 and 50 or 119 and 120) in a second 25-mL volumetric flask. Turn the flask to the upright position immediately after each actuation, and insert the stopper into the flask. Add 20 mL of *Diluent* to each flask, and shake well for 10 min to disperse the suspension. Dilute with *Diluent* to volume, and mix thoroughly. Allow the flask to stand until the excipients have settled. Inject the clear supernatant. Repeat this procedure with four additional bottles.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 50 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for fluticasone propionate and fluticasone propionate related compound D are about 1.0 and 1.10, respectively.]
Suitability requirements
Resolution: NLT 1.5 between fluticasone propionate and fluticasone propionate related compound D, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in each dose taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)

Acceptance criteria
Tier 1
1. Mean dose delivered from 10 doses is within 85%–115% of the labeled amount of fluticasone propionate.
2. NMT 1 dose is outside 80%–120% of the labeled amount of fluticasone propionate.
3. No doses are outside 75%–125% of the labeled amount of fluticasone propionate.

If the criteria in *Tier 1* cannot be met, proceed to *Tier 2*.

Tier 2
If 2 or 3 doses are outside 80%–120% of the label claim, test an additional 10 bottles.
1. Mean dose delivered from 30 doses is within 85%–115% of the labeled amount of fluticasone propionate.
2. NMT 3 doses are outside 80%–120% of the labeled amount of fluticasone propionate.
3. No doses are outside 75%–125% of the labeled amount of fluticasone propionate.

• **DELIVERED DOSE UNIFORMITY** (within batch)
Diluent, Buffer, Mobile phase, System suitability solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the test for *Delivered Dose Uniformity*.

Sample solution: Wipe the pump clean. Shake the bottle for 30 s, and mechanically prime the bottle. Hold a 25-mL volumetric flask in an inverted position, and discharge the first two actuations into the flask. Turn the flask to the upright position immediately after each actuation. Insert the stopper into the flask after collecting two actuations (1 dose). Repeat this procedure with nine additional bottles.

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in each dose taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)

Acceptance criteria
Tier 1
1. Mean dose delivered from 10 doses is within 85%–115% of the labeled amount of fluticasone propionate.
2. NMT 1 dose is outside 80%–120% of the labeled amount of fluticasone propionate.
3. No doses are outside 75%–125% of the labeled amount of fluticasone propionate.

If the criteria in *Tier 1* cannot be met, proceed to *Tier 2*.

Tier 2
If 2 or 3 doses are outside 80%–120% of the label claim, test an additional 20 bottles.
1. Mean dose delivered from 30 doses is within 85%–115% of the labeled amount of fluticasone propionate.
2. NMT 3 doses are outside 80%–120% of the labeled amount of fluticasone propionate.
3. No doses are outside 75%–125% of the labeled amount of fluticasone propionate.

IMPURITIES

• **ORGANIC IMPURITIES**
Diluent: Acetonitrile and 0.001 M hydrochloric acid (60:40)
Solution A: Methanol and acetonitrile (77:23)
Buffer: 1.2 g/L of monobasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 3.4.
Mobile phase: *Solution A* and *Buffer* (60:40)
System suitability solution: 0.1 mg/mL of USP Fluticasone Propionate RS and 0.05 mg/mL of USP Fluticasone Propionate Related Compound D RS in *Diluent*
Identification solution: 0.5 mg/mL of USP Phenylethyl Alcohol RS and 0.08 mg/mL of USP Benzalkonium Chloride RS in a mixture of *Diluent* and water (4:1)
Sample solution: 0.2 g/mL of Nasal Spray in *Diluent*. Shake the flask vigorously to dissolve. Pass through a filter of 0.5-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 239 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*

www.webofpharma.com

[NOTE—See *Table 3* for the relative retention times.]

Suitability requirements

Resolution: NLT 2 between fluticasone propionate related compound D and fluticasone propionate

Analysis

Samples: *System suitability solution, Identification solution,* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*, excluding the peaks from the *Identification solution*

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fluticasone acetate[a]		
6-Ketofluticasone propionate[b]	0.7	0.3
Fluticasone propionate	1.0	—
Fluticasone propionate related compound D	1.1	0.3
Fluticasone propionate dimer[c]	2.1	0.3
Any individual unspecified degradation product	—	0.2
Total degradation products	—	1.5

[a] *S*-Fluoromethyl 17α-acetyloxy-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carbothioate.

[b] *S*-Fluoromethyl 9α-fluoro-11β-hydroxy-16α-methyl-3,6-dioxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate.

[c] 6α,9α-Difluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid-6α,9α-difluoro-17β-(fluoromethylthio)carbonyl-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17β-yl ester.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: Meets the requirements of the tests for absence of *Staphylococcus aureus, Escherichia coli, Salmonella* species, and *Pseudomonas aeruginosa*. The total aerobic microbial count does not exceed 25 cfu/mL, and the total combined molds and yeasts count does not exceed 25 cfu/mL.
- **pH** ⟨791⟩: 5.0–7.0
- **PARTICLE SIZE**

Analysis: Remove the pump system after shaking the test bottle to ensure product uniformity. Transfer 1 drop of the Nasal Spray onto a clean microscope slide. Examine 10 random fields of view on the slide using 400× magnification. Drug substance particles are irregular in shape, whereas the excipient particles are elongated and angular. Record the number of individual drug substance particles that are less than 5 µm in diameter, greater than 5 µm but less than 15 µm in diameter, and greater than 15 µm in diameter. Calculate the percentage of each category by number.

Acceptance criteria: See *Table 4*.

Table 4

Particle Size (µm)	Acceptance Criteria (%)
<5	NLT 98
>5 to <15	NMT 1.8
>15	NMT 0.2

- **FOREIGN PARTICULATES**

Analysis: Shake the required number of bottles to ensure uniformity. Remove the pump system carefully to minimize contamination of the sample. Collect about 100 g of Nasal Spray, and pass it through a wetted 250-µm screen. Rinse each bottle with a portion of water equal to twice the volume of each bottle. Pass the rinse through the 250-µm screen. Visually observe the screen and filtrate for any foreign particulates. Also examine the screen under a microscope using transmitted light.

Acceptance criteria: No foreign particulates greater than 250 µm are visible.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 4° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Benzalkonium Bromide RS
USP Benzalkonium Chloride RS
USP Fluticasone Propionate RS
USP Fluticasone Propionate Related Compound D RS
S-Methyl-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate.
USP Phenylethyl Alcohol RS

Fluticasone Propionate Ointment

DEFINITION

Fluticasone Propionate Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 0.17 mg/mL of USP Fluticasone Propionate RS in acetonitrile

Test solution: Transfer a quantity of Ointment, equivalent to 100 µg of fluticasone propionate, to a 125-mL separatory funnel. Add 50 mL of hexane and 10 mL of acetonitrile to the separatory funnel. Stopper and shake the funnel until the Ointment is completely dispersed. Shake the separatory funnel for an additional min, and allow the phases to separate. Filter the lower layer through a 10-mL syringe containing a cotton plug into a 25-mL volumetric flask. Repeat the extraction with one 10-mL aliquot of acetonitrile, and filter the lower layer into the volumetric flask. Wash the cotton plug with 2 mL of acetonitrile, and collect the washings into the volumetric flask. Dilute the sample extract with acetonitrile to volume. Transfer one-half of the sample extract to a glass tube suitable for evaporation, and evaporate to dryness at about 40°. Transfer the remainder of the sample extract into the same tube, and continue evaporating to dryness. Dissolve the residue in 0.6 mL of acetonitrile. [NOTE—The *Test solution* may be cloudy because of the presence of undissolved excipients.]

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.2-mm layer of chromatographic silica gel mixture on a high-performance thin-layer chromatographic plate, 5-µm particle size
Application volume: 40 µL
Developing solvent system: Dichloromethane, ethyl acetate, and glacial acetic acid (30:8:1)
Analysis
Samples: *Standard solution* and *Test solution*
Separately apply the *Standard solution* and the *Test solution* to the plate. On the same plate, apply 20 µL of the *Standard solution*, allow the application to dry, and apply 20 µL of the *Test solution* on top of the dried 20-µL *Standard solution* spot. Allow each of the applications to dry thoroughly. Place the plate in a tank equilibrated with the developing solvent, and allow the developing solvent to travel 8 cm from the point of application. Remove the plate and allow to air-dry. Examine the plate under ultraviolet light at 254 nm.
Acceptance criteria: The R_F value of the principal spot from the *Test solution* corresponds to that of the *Standard solution*. [NOTE—If the excipients in the Ointment interfere with the appearance of the principal spot obtained for the *Test solution*, use the *Standard solution* and the *Test solution* overspot to confirm identity.]

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
[NOTE—Protect the *Standard solution* and the *Sample solution* from direct light by using a light-protective volumetric flask and autosampler vials.]
Buffer: 1.20 g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of 3.50 ± 0.03.
Mobile phase: Methanol, acetonitrile, and *Buffer* (46:14:40)
Diluent: Methanol and water (80:20)
System suitability stock solution: 0.5 mg/mL of USP Fluticasone Propionate Nasal Spray Resolution Mixture RS in methanol. [NOTE—USP Fluticasone Propionate Nasal Spray Resolution Mixture RS is a mixture of fluticasone propionate and fluticasone propionate related compound D.]
System suitability solution: 5 µg/mL of USP Fluticasone Propionate Nasal Spray Resolution Mixture RS in *Diluent* from *System suitability stock solution*
Standard stock solution: 0.25 mg/mL USP Fluticasone Propionate RS. Dissolve the standard first in a volume of methanol, equivalent to 80% of the final volume, and dilute with water to volume.
Standard solution: 4 µg/mL USP Fluticasone Propionate RS in *Diluent*, from *Standard stock solution*
Sample solution: Equivalent to 4 µg/mL of fluticasone propionate. Transfer a quantity of Ointment, equivalent to about 100 µg of fluticasone propionate, to a 125-mL separatory funnel. Add to the separatory funnel 45 mL of hexane previously heated to about 60° in a water bath. [NOTE—Hexane is highly flammable.] Stopper and shake vigorously until the Ointment is completely dispersed. Wash the stopper and neck of the separatory funnel with 5 mL of hexane heated to 60°, and allow the funnel to cool to room temperature. Transfer 10 mL of *Diluent* into the separatory funnel, and shake the contents for 1 min. Allow the phases to separate, and filter the lower aqueous layer via a cotton wool plug, previously washed with *Diluent*, into a 25-mL volumetric flask. Repeat the extraction with two 5-mL aliquots of *Diluent*, filtering the lower layers into the volumetric flask. Wash the cotton plug with 2 mL of *Diluent*, and collect the washings into the volumetric flask. Dilute the sample with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1.5 mL/min
Injection size: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.4 between fluticasone propionate and fluticasone propionate related compound D, *System suitability solution*
Tailing factor: NMT 1.4, *Standard solution* (calculated using the width of the peak at 10% of the height)
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluticasone propionate from the *Sample solution*
r_S = peak response of fluticasone propionate from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The total aerobic microbial count is NMT 100 cfu/g, and the total combined molds and yeasts count is NMT 10 cfu/g.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, protected from light. Store between 2° and 30°.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluticasone Propionate RS
USP Fluticasone Propionate Nasal Spray Resolution Mixture RS
This Reference Standard is a mixture of fluticasone propionate and fluticasone propionate related compound D, and the chemical names for both are given below:
Fluticasone propionate: S-Fluoromethyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate.
Fluticasone propionate related compound D: S-Methyl- 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxy-androsta-1,4-diene-17β-carbothioate.

www.webofpharma.com

Fluticasone Propionate and Salmeterol Inhalation Aerosol

DEFINITION

Fluticasone Propionate and Salmeterol Inhalation Aerosol is a suspension of Fluticasone Propionate and Salmeterol with suitable propellants in a pressurized container. The mean content per actuation contains NLT 88% and NMT 112% of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$). The mean content per actuation contains NLT 88% and NMT 112% of the labeled amount of salmeterol ($C_{25}H_{37}NO_4$) as salmeterol xinafoate.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A▲** (CN 1-May-2020)
Wavenumber range: 4000 –600 cm^{-1}
Sample: Discharge an appropriate number of actuations from two containers into an agate mortar. Allow the propellant to evaporate and dry if necessary. Transfer the residue to the sample window.
Acceptance criteria: Meets the requirements. In addition, the ratio of the fluticasone propionate band at 833 cm^{-1} to that of the salmeterol band at 744 cm^{-1} meets the requirements in *Table 1*.

Table 1

Fluticasone Propionate/Salmeterol (µg/µg for each dose)	Ratio (band at 833 cm^{-1}/band at 744 cm^{-1})
45/21	NMT 2.5
115/21	2.5–4.0
230/21	NLT 4.0

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Delivered-Dose Uniformity*.

ASSAY

• **PROCEDURE**
Buffer: 0.01 M sodium dodecyl sulfate containing 0.1% glacial acetic acid
Solution A: Methanol and *Buffer* (20:80)
Mobile phase: Acetonitrile and *Solution A* (50:50)
Diluent: Methanol and water (70:30)
Standard solution: 50 µg/mL of USP Fluticasone Propionate RS and 15 µg/mL of USP Salmeterol Xinafoate RS in *Diluent*
Sample solution: Nominally 20–110 µg/mL of fluticasone propionate and 10 µg/mL of salmeterol prepared as follows. Shake the canister vigorously, and cool for 10 min in a dry ice–methanol bath. Remove the canister from the bath, and shake vigorously. Using a suitable device, carefully remove and keep the valve, and pour the contents into a suitable container. Allow the propellant to evaporate. Dissolve the canister contents in a minimum amount of methanol and quantitatively transfer to a suitable volumetric flask containing 30% of the flask volume of water. Rinse the canister and valve with methanol into the same volumetric flask. Allow the flask to come to room temperature and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Fluticasone propionate: UV 239 nm
Salmeterol: Fluorescence with excitation at 225 nm and emission at 305 nm. Use emission response for quantification.
Column: 4.6-mm × 5-cm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 2 mL/min
Injection volume: 5 µL
Run time: NLT 1.5 times the retention time of salmeterol
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for fluticasone propionate and salmeterol are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.5 between fluticasone propionate and salmeterol
Tailing factor: NMT 1.5 for fluticasone propionate and salmeterol
Relative standard deviation: NMT 2.0% for fluticasone propionate and salmeterol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the portion of Inhalation Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluticasone propionate from the *Sample solution*
r_S = peak response of fluticasone propionate from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)

Calculate the percentage of the labeled amount of salmeterol ($C_{25}H_{37}NO_4$) in the portion of Inhalation Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of salmeterol from the *Sample solution*
r_S = peak response of salmeterol from the *Standard solution*
C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of salmeterol free base in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of salmeterol free base, 415.57
M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria: 88%–112% each for fluticasone propionate and salmeterol

PERFORMANCE TESTS

• **AERODYNAMIC SIZE DISTRIBUTION**
(See *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* ⟨601⟩, *Aerodynamic Size Distribution—Inhalation Aerosols, Sprays, and Powders*.)
Sampling apparatus: Use *Apparatus 1* in ⟨601⟩ with a modified *Induction port* (*Figure 1. Expanded view of the modified Induction port.*) and *Entrance cone* (*Figure 2. Expanded view of the modified Entrance cone.*).

www.webofpharma.com

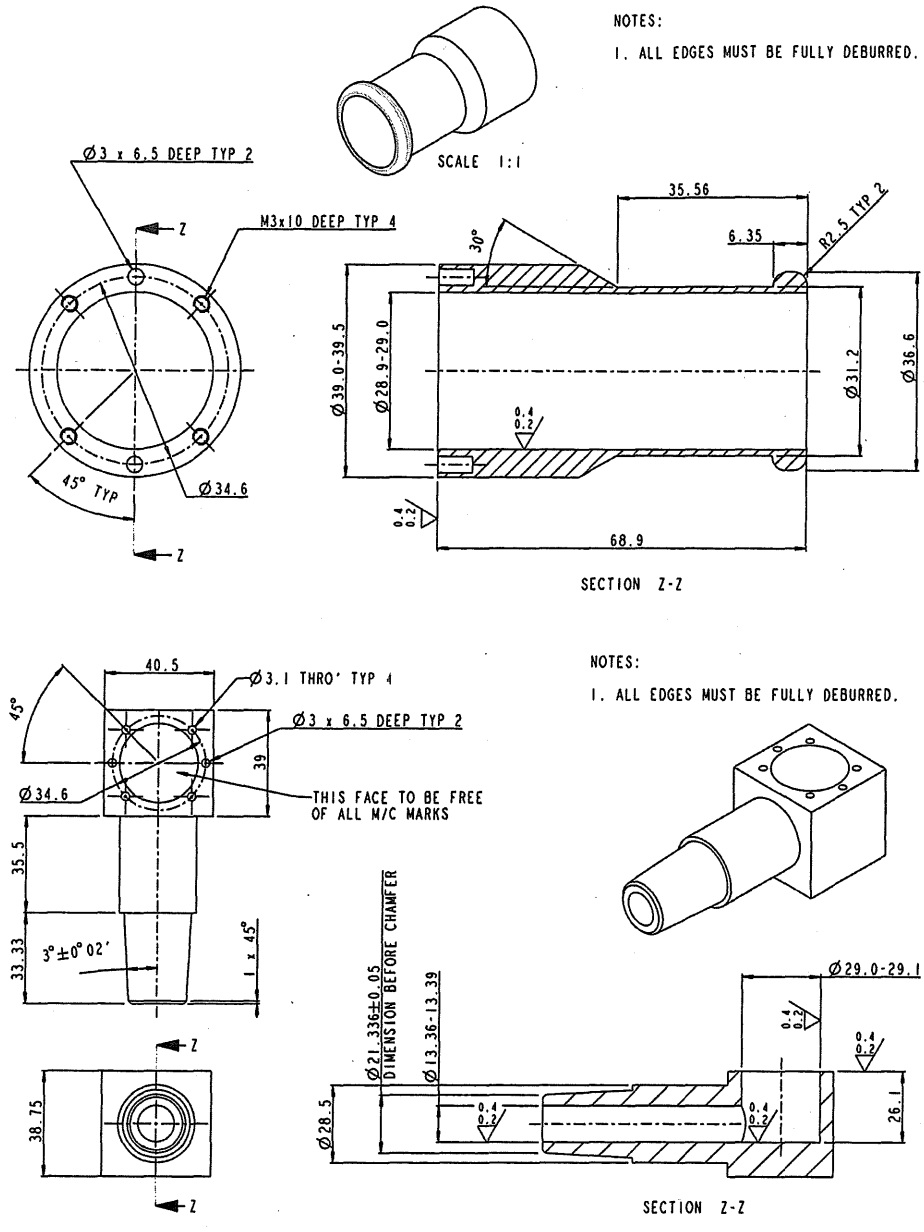


Figure 1. Expanded view of the modified *Induction port.* (Fluticasone Propionate and Salmeterol Inhalation Aerosol)

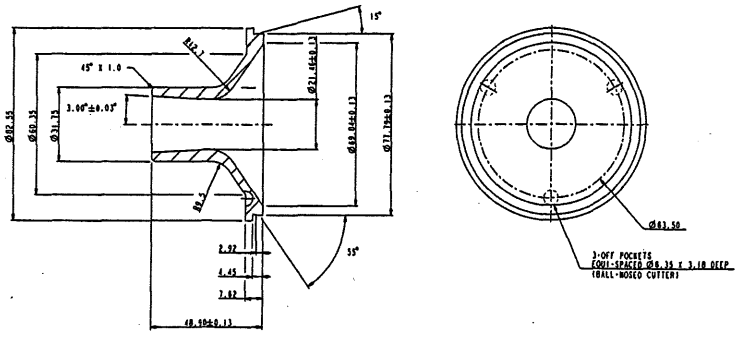


Figure 2. Expanded view of the modified *Entrance cone.* (Fluticasone Propionate and Salmeterol Inhalation Aerosol)

www.webofpharma.com

Buffer, Solution A, and **Mobile phase:** Proceed as directed in the *Assay*.
Diluent: Methanol and water (70:30)
Standard solution: 1.25 µg/mL of USP Fluticasone Propionate RS and 0.38 µg/mL of USP Salmeterol Xinafoate RS in *Diluent*
Sample solutions: Prime the valve by discharging a predetermined number of actuations to waste. Discharge the 10 actuations into the cascade impaction *Sampling apparatus* described in *Figure 1. Expanded view of the modified Induction port.* and *Figure 2. Expanded view of the modified Entrance cone.*. Detach the inhaler, and rinse each piece of the apparatus with methanol into a suitable volumetric flask containing 30% of the flask volume of water. The final concentration of the expected amount of fluticasone propionate should be in the range of 0.05–3.5 µg/mL. Allow the solutions to equilibrate, and dilute with methanol to volume. Repeat these steps on an additional four Inhalation Aerosols for a total of five *Sample solutions*.
Chromatographic system and **System suitability:** Proceed as directed in the *Assay*, except for the *Injection volume*.
Injection volume: 50 µL
Analysis
Samples: *Standard solution* and *Sample solutions*
Calculate the quantity, in µg/actuation, of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the *Sample solutions*:

$$\text{Result} = [(r_U/r_S) \times C_S] \times (V/N)$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)
V = total volume of the *Sample solution* (mL)
N = number of actuations discharged into the apparatus

Calculate the quantity, in µg/actuation, of salmeterol ($C_{25}H_{37}NO_4$) in the *Sample solutions*:

$$\text{Result} = [(r_U/r_S) \times C_S] \times (V/N) \times (M_{r1}/M_{r2})$$

r_U = peak response of salmeterol from the *Sample solution*
r_S = peak response of salmeterol from the *Standard solution*
C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (µg/mL)
V = total volume of the *Sample solution* (mL)
N = number of actuations discharged into the apparatus
M_{r1} = molecular weight of salmeterol free base, 415.57
M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria: The mass of fluticasone propionate and salmeterol deposited in each grouping of the *Sampling apparatus* for each individual inhaler is given in *Table 2*. The mass of fluticasone propionate and salmeterol deposited in each grouping of the *Sampling apparatus* for the mean of the five inhalers is given in *Table 3*.

• **DELIVERED-DOSE UNIFORMITY**
(See *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders–Performance Quality Tests* ⟨601⟩, *Delivered-Dose Uniformity—Inhalation Aerosols and Inhalation Sprays*.)
Sampling apparatus: Use the apparatus (*Figure 3A. Sampling apparatus.*) with modified glass sampling device (*Figure 3B. Expanded view of the modified glass sample collection apparatus.*).

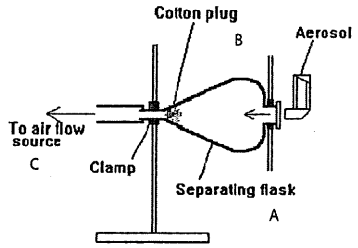


Figure 3A. Sampling apparatus.

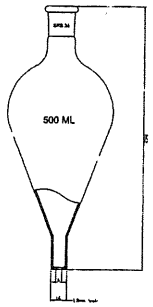


Figure 3B. Expanded view of the modified glass sample collection apparatus.

Table 2 (Fluticasone Propionate and Salmeterol Inhalation Aerosol)

Parameter	Amount of Fluticasone Propionate Deposited (µg/actuation)			Amount of Salmeterol Deposited (µg/actuation)		
Label claim of fluticasone propionate/ salmeterol (µg/actuation)	45/21	115/21	230/21	45/21	115/21	230/21
Mass of mouthpiece adapter, and induction port	10–21	24–54	59–109	2–10	3–11	4–12
Sum of Stages 0–2	NMT 8	NMT 25	NMT 59	NMT 5	NMT 6	NMT 7
Sum of Stages 3, 4, and 5	15–27	39–67	77–124	7–13	7–13	7–13
Sum of Stages 6, 7, and filter	NMT 2	NMT 3	NMT 4	NMT 1	NMT 1	NMT 1

www.webofpharma.com

Table 3 (Fluticasone Propionate and Salmeterol Inhalation Aerosol)

Parameter	Amount of Fluticasone Propionate Deposited (µg/actuation)			Amount of Salmeterol Deposited (µg/actuation)		
Label claim of fluticasone propionate/ salmeterol (µg/actuation)	45/21	115/21	230/21	45/21	115/21	230/21
Mass of mouthpiece adapter, and induction port	11–20	26–52	64–104	3–9	4–10	5–11
Sum of Stages 0, 1, and 2	NMT 7	NMT 22	NMT 52	NMT 4	NMT 5	NMT 6
Sum of Stages 3, 4, and 5	16–26	41–65	81–120	7–12	7–12	7–12
Sum of Stages 6, 7, and filter	NMT 2	NMT 3	NMT 4	NMT 1	NMT 1	NMT 1

Buffer, Solution A, Mobile phase, and **Diluent:** Prepare as directed in the *Assay*.

Standard solution: 1.25 µg/mL of USP Fluticasone Propionate RS and 0.38 µg/mL of USP Salmeterol Xinafoate RS in *Diluent*

Sample solutions: Prime the valve by discharging a predetermined number of actuations to waste. Discharge the recommended dose at the beginning of use (BOU) into the *Sampling apparatus*, and detach the inhaler. Rinse the apparatus with methanol, and quantitatively transfer the solutions to a 100-mL volumetric flask containing 30 mL of water. Allow the solution to equilibrate, and dilute with methanol to volume. Discharge the requisite number of actuations to waste, shaking the inhaler before each discharge, until the last recommended dose remains. Discharge the remaining recommended dose at end of use (EOU) corresponding to the labeled number of actuations into the *Sampling apparatus*, and detach the inhaler. Rinse the apparatus with methanol, and quantitatively transfer the solution to a 100-mL volumetric flask containing 30 mL of water. Allow the solution to equilibrate, and dilute with methanol to volume. Repeat these steps on an additional nine inhalers for a total of 20 *Sample solutions*.

Chromatographic system and **System suitability:** Proceed as directed in the *Assay*, except for the *Injection volume*.

Injection volume: 50 µL

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) delivered by the inhaler in each *Sample solution*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)

Calculate the percentage of the labeled amount of salmeterol ($C_{25}H_{37}NO_4$) delivered by the inhaler in each *Sample solution*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of salmeterol from the *Sample solution*
r_S = peak response of salmeterol from the *Standard solution*
C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of salmeterol free base in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of salmeterol free base, 415.57
M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria: See *Table 4* and *Table 5*.

Table 4

Parameter	Acceptance Criteria		
Label claim of fluticasone propionate/ salmeterol (µg/actuation)	**45 /21**	**115/21**	**230/21**
Mean of 10 BOU contents	80%–120% of label claim	80%–120% of label claim	80%–120% of label claim
Mean of 10 EOU contents	80%–120% of label claim	80%–120% of label claim	80%–120% of label claim
Number of individual contents outside 80%–120% of label claim	NMT 2 of 20	NMT 2 of 20	NMT 2 of 20
Mean of 20 contents/ actuations	88%–112% of label claim	88%–112% of label claim	88%–112% of label claim
Each individual content	70%–130% of label claim	75%–125% of label claim	75%–125% of label claim

Test an additional 20 inhalers (BOU and EOU) if up to 6 of the 20 individual results are outside of 80%–120% of label claim, but none are outside 70%–130% of the label claim for the 45/21 µg/actuation product strength, or none are outside 75%–125% of the label claim for the 115/21 µg/actuation and 230/21 µg/actuation.

USP Monographs

www.webofpharma.com

Table 5

Parameter	Acceptance Criteria		
Label claim of fluticasone propionate/ salmeterol (µg/actuation)	**45 /21**	**115/21**	**230/21**
Mean of 30 BOU contents	80%–120% of label claim	80%–120% of label claim	80%–120% of label claim
Mean of 30 EOU contents	80%–120% of label claim	80%–120% of label claim	80%–120% of label claim
Number of individual contents outside 80%–120% of label claim	NMT 6 of 60	NMT 6 of 60	NMT 6 of 60
Mean of 60 contents/ actuations	88%–112% of label claim	88%–112% of label claim	88%–112% of label claim
Each individual content	70%–130% of label claim	75%–125% of label claim	75%–125% of label claim

IMPURITIES

• **ORGANIC IMPURITIES**

[NOTE—Protect all solutions containing fluticasone propionate or salmeterol from light.]

Buffer: 0.05 M monobasic ammonium phosphate adjusted with 10% (v/v) of phosphoric acid to a pH of 2.9

Solution A: Acetonitrile and *Buffer* (30:70)

Solution B: Acetonitrile and *Buffer* (78:22)

Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	100	0
60	0	100
61	100	0
70	100	0

Diluent 1: Methanol, water, and phosphoric acid (70: 30: 0.05)

Diluent 2: Methanol and water (70:30)

Diluent 3: 0.05% Phosphoric acid in methanol

System suitability solution: 0.075 mg/mL of USP Salmeterol Xinafoate RS, 0.025 mg/mL of USP Fluticasone Propionate RS, and 0.2 µg/mL each of USP Fluticasone Propionate Related Compound D RS and USP Fluticasone Propionate Related Compound J RS in *Diluent 1*

Sensitivity solution: 0.05 µg/mL of USP Salmeterol Xinafoate RS and 0.1 µg/mL of USP Fluticasone Propionate RS in *Diluent 2*

Sample solution: Nominally 50–100 µg/mL of salmeterol prepared as follows. Place a canister into a freezing mixture of dry ice and methanol, and cool for approximately 5 min. Carefully remove the valve from the canister. Pour the contents into a suitable container and allow the propellant to evaporate. Dissolve the residue in *Diluent 3* and dilute with water to the required volume to obtain the nominal concentration.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 228 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—See *Table 7* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between salmeterol and fluticasone propionate related compound J; NLT 1.5 between fluticasone propionate related compound D and fluticasone propionate, *System suitability solution*

Tailing factor: NMT 1.5 for fluticasone propionate and salmeterol, *System suitability solution*

Signal-to-noise ratio: NLT 10 for both fluticasone propionate and salmeterol, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each degradation product in the portion of Inhalation Aerosol taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of either salmeterol or fluticasone propionate from the *Sample solution*

Acceptance criteria: See *Table 7*. Disregard any peak less than 0.05%. [NOTE—Any unspecified degradation product eluting before salmeterol is related to salmeterol. Any unspecified degradation product eluting after salmeterol is related to fluticasone propionate.]

Table 7

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salmeterol-*N*-phenylbutyl aminoalcohol[a, b]	0.14	—
Salmeterol-phenylethoxy[a, c]	0.25	—
Salmeterol-phenylpropoxy [a, d]	0.32	—
Salmeterol-phenyl-2-butoxy[a, e]	0.37	—
Fluticasone propionate related compound J[a]	0.38	—
Salmeterol	0.41	—
Hydroxynaphthoic acid[f]	0.50	—
Salmeterol-deoxy[a, g]	0.55	—
Fluticasone propionate dithioacid[a, h]	0.67	—
Salmeterol-*N*-alkyl[i]	0.71	0.2
Fluticasone propionate related compound D	0.97	—
Fluticasone propionate	1.0	—
Fluticasone dimer[a, j]	1.09	—
Any unspecified degradation product	—	0.10

USP Monographs

www.webofpharma.com

Table 7 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total degradation products	—	0.2

[a] This is a process impurity that is included in this table for identification purposes only. This impurity is controlled in drug substance. This impurity is not to be reported for the drug product or to be included in total degradation products.
[b] 4-[1-Hydroxy-2-(4-phenylbutylamino)ethyl]-2-(hydroxymethyl)phenol.
[c] 4-[1-Hydroxy-2-(6-phenethoxyhexylamino)ethyl]-2-(hydroxymethyl)phenol.
[d] 4-{1-Hydroxy-2-[6-(3-phenylpropoxy)hexylamino]ethyl}-2-(hydroxymethyl) phenol.
[e] 4-{1-Hydroxy-2-[6-(4-phenylbutan-2-yloxy)hexylamino]ethyl}-2-(hydroxymethyl)phenol.
[f] This is a counter ion of salmeterol that is included in this table for identification purposes only. It is not to be reported for the drug product or to be included in the total degradation products.
[g] 4-{1-Hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}-2-methylphenol.
[h] 6α,9α-Difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbodithioic acid.
[i] 4-{1-Hydroxy-2-[(2-hydroxy-5-{1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino] ethyl}benzyl)[6-(4-phenylbutoxy)hexyl]amino]ethyl}-2-(hydroxymethyl) phenol.
[j] 6α, 9α-Difluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid 6α, 9α-difluoro-17β-(fluoromethylthio)carbonyl-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-yl ester.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^1 cfu/g of formulation. The total aerobic yeasts and molds count does not exceed 10^1 cfu/g of formulation. It meets the requirements of the tests for absence of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Salmonella* species.
- **FOREIGN PARTICULATE MATTER**

The test described below and the specification is only applicable to a microscopic particle count test methodology. *Particulate Matter in Injections* ⟨788⟩ describes details of the test apparatus to be used for the determination of particulate matter using a microscopic particle count test methodology. Samples should be carefully prepared to avoid environmental contamination, and testing should be performed with suitable controls, including the appropriate use of blank determinations.

Filter: Mixed cellulose and ester; 25-mm diameter and 0.45-µm pore size

Sample solution: Perform testing on two previously unused inhalers. Prime each inhaler by discharging a predetermined number of actuations to waste. Discharge, and dissolve 16 actuations, eight from each of two canisters in 50 mL of methanol.

Analysis

Sample: *Sample solution*

Pass the *Sample solution* through the *Filter,* and allow the *Filter* to dry under conditions that will limit particulate contamination. Using a microscopic particle count test method, enumerate the number of particles present in the *Sample solution.*

Calculate the number of particles per actuation:

$$\text{Result} = (N_{<10} + N_{10\text{–}100} + N_{>100})/16$$

$N_{<10}$ = total number of particles <10 µm present in the *Sample solution*

$N_{10\text{–}100}$ = total number of particles between 10 and 100 µm present in the *Sample solution*

$N_{>100}$ = total number of particles >100 µm present in the *Sample solution*

Acceptance criteria: See *Table 8.*

Table 8

Particle Size Range (µm)	Number of Particles per Actuation
<10	140
10–100	50
>100	5
Total	185

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in nonreactive, light-resistant aerosol containers with metered valves fitted with a dose counter and provided with oral inhalation actuators. Avoid exposure to heat. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Fluticasone Propionate RS

USP Fluticasone Propionate Related Compound D RS
S-Methyl 6α, 9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate.
$C_{25}H_{32}F_2O_5S$ 482.58

USP Fluticasone Propionate Related Compound J RS
6α, 9α-Difluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid.
$C_{21}H_{26}F_2O_5$ 396.42

USP Salmeterol Xinafoate RS

Fluticasone Propionate and Salmeterol Inhalation Powder

DEFINITION

Fluticasone Propionate and Salmeterol Inhalation Powder is a mixture of fluticasone propionate and salmeterol xinafoate for use in dry powder inhalers. The Inhalation Powder contains NLT 90% and NMT 110% of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) and NLT 90% and NMT 110% of the labeled amount of salmeterol ($C_{25}H_{37}NO_4$) as salmeterol xinafoate.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Diluent: Methanol and water (70:30)

Standard solution: A mixture of USP Fluticasone Propionate RS and USP Salmeterol Xinafoate RS according to the individual product strengths in the Inhalation Powder under test in *Diluent*

Sample solution: Dissolve a suitable number of unit doses of the Inhalation Powder under test in a suitable volume of *Diluent.*

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the test for *Delivered-Dose Uniformity.*

USP Monographs

www.webofpharma.com

ASSAY

Change to read:

• **PROCEDURE**

Buffer: ▲To each liter of 2.9 g/L of sodium dodecyl sulfate in water, add 1 mL of glacial acetic acid.▲ (RB 8-Feb-2019)

Solution A: Methanol and *Buffer* (20:80)

Mobile phase: Acetonitrile and *Solution A* (50:50)

Diluent: Methanol and water (70:30)

Standard solution: 10 µg/mL of USP Fluticasone Propionate RS and 3 µg/mL of USP Salmeterol Xinafoate RS in *Diluent*

Sample solution: Nominally 5–25 µg/mL of fluticasone propionate and 2.4 µg/mL of salmeterol from NLT 12 unit doses in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detectors

Fluticasone propionate: UV 239 nm

Salmeterol: Fluorescence with excitation at 225 nm and emission at 305 nm. Use emission response for quantification.

Column: 4.6-mm × 5-cm; 3.5-µm packing L1

Flow rate: 2 mL/min

Column temperature: 40°

Injection volume: 10 µL

Run time: NLT 1.5 times the retention time of salmeterol

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for fluticasone propionate and salmeterol are 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.5 between salmeterol and fluticasone propionate

Tailing factor: NMT 1.5 for salmeterol and fluticasone propionate

Relative standard deviation: NMT 2.0% for salmeterol and fluticasone propionate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the portion of Inhalation Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fluticasone propionate from the *Sample solution*

r_S = peak response of fluticasone propionate from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL)

Calculate the percentage of the labeled amount of salmeterol ($C_{25}H_{37}NO_4$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of salmeterol from the *Sample solution*

r_S = peak response of salmeterol from the *Standard solution*

C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of salmeterol free base in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of salmeterol free base, 415.57

M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria: 90%–110% each for fluticasone propionate and salmeterol

PERFORMANCE TESTS

Change to read:

• **AERODYNAMIC SIZE DISTRIBUTION**

(See *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* ⟨601⟩, *Aerodynamic Size Distribution—Inhalation Aerosols, Sprays, and Powders*.)

▲Test 1▲ (RB 8-Feb-2019)

Sampling apparatus: Modified *Apparatus 3* (*Figure 1. Cascade impaction sampling apparatus (modified Apparatus 3 in 601) including Induction Port and Preseparator Lid.*) in ⟨601⟩ with a modified induction port (*Figure 2. Expanded view of the modified induction port.*), and preseparator lid (*Figure 3. Expanded view of the preseparator lid.*) are to be used.

Buffer, Solution A, and **Mobile phase:** Proceed as directed in the *Assay*.

Diluent: Methanol and water (70:30)

Standard solution: 2.5 µg/mL of USP Fluticasone Propionate RS and 0.75 µg/mL of USP Salmeterol Xinafoate RS in *Diluent*

Sample solutions: Discharge 10 unit doses given into the cascade impaction sampling apparatus described in *Figure 1. Cascade impaction sampling apparatus (modified Apparatus 3 in 601) including Induction Port and Preseparator Lid.*.

Operate the pump for 3 s at an airflow rate of 60 L/min for each dose discharged. Detach the inhaler, and rinse each piece of the apparatus with methanol into a separate suitable volumetric flask containing 30% of the flask volume of water. The final expected amount of fluticasone propionate should be in the concentration range of 0.1–5 µg/mL. Allow the solutions to equilibrate, and dilute with methanol to volume. Repeat these steps for three additional sample preparations, for a total of four *Sample solutions*.

Chromatographic system and **System suitability:** Proceed as directed in the *Assay*, except for *Injection volume*.

Injection volume: 50 µL

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the quantity, in µg/actuation, of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in the *Sample solutions*:

$$\text{Result} = [(r_U/r_S) \times C_S] \times (V/N)$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

V = total volume of the *Sample solution* (mL)

N = number of unit doses discharged into the apparatus

Calculate the quantity, in µg/actuation, of salmeterol ($C_{25}H_{37}NO_4$) in the *Sample solutions*:

$$\text{Result} = [(r_U/r_S) \times C_S] \times (V/N) \times (M_{r1}/M_{r2})$$

USP Monographs

www.webofpharma.com

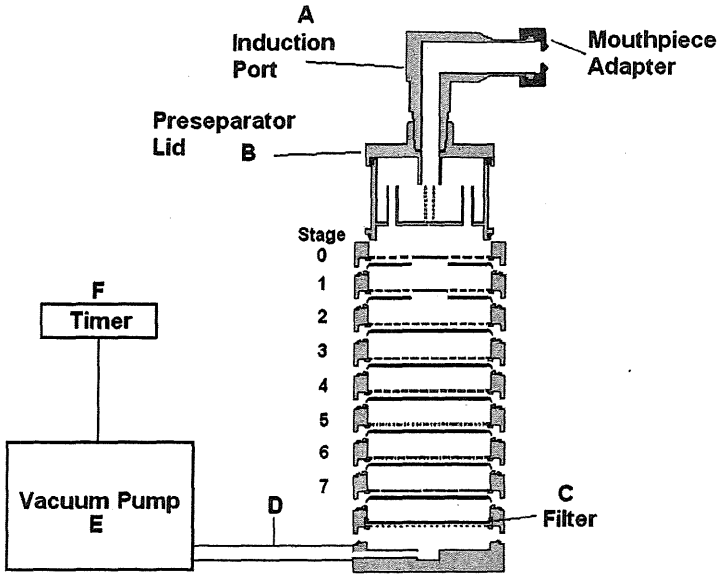


Figure 1. Cascade impaction sampling apparatus (modified *Apparatus 3* in ⟨601⟩) including *Induction Port* and *Preseparator Lid*.

(Fluticasone Propionate and Salmeterol Inhalation Powder)

r_U = peak response of salmeterol from the *Sample solution*
r_S = peak response of salmeterol from the *Standard solution*
C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (µg/mL)
V = total volume of the *Sample solution* (mL)
N = number of unit doses discharged into the apparatus
M_{r1} = molecular weight of salmeterol free base, 415.57
M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria

The mass of fluticasone propionate and salmeterol deposited in each grouping of the *Sampling apparatus* for each inhaler is given in *Table 1*.

All the groupings for each sample preparation must meet the criteria in *Table 1*.

If NMT one of the four sample preparations fails to meet the requirements in *Table 1*, but is within 25% of either the lower or upper specification limit being tested, analyze two additional samples. The batch meets the requirements if five of the six sample preparations meet the limits in *Table 1* for the individual sample preparations.

▲**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Aerodynamic Size Distribution Test 2*.

Sampling apparatus: *Apparatus 5*

Buffer: To each liter of 3.1 g/L of dihydrate monobasic sodium phosphate and 5 g/L of sodium dodecyl sulfate in water, add 4 mL of 1 M phosphoric acid TS.

Solution A: 1% silicone prepared as follows. To an appropriate volumetric flask, transfer 1% of the flask volume of silicone oil and dilute with cyclohexane. [NOTE—Silicone oil (poly[dimethylsiloxane-co-methylphenylsiloxane]; 63148-52-7) with a viscosity of 125 centistokes may be suitable.[1]]

Mobile phase: Methanol and *Buffer* (60:40)

Standard stock solution A: 20 µg/mL of USP Fluticasone Propionate RS prepared as follows. Transfer a suitable quantity of USP Fluticasone Propionate RS to an appropriate volumetric flask and dissolve in 2% of the flask volume of methanol. Dilute with *Mobile phase* to volume.

Standard stock solution B: 29 µg/mL of USP Salmeterol Xinafoate RS (20 µg/mL of salmeterol) prepared as follows. Transfer a suitable quantity of USP Salmeterol Xinafoate RS to an appropriate volumetric flask and dissolve in 2% of the flask volume of methanol. Dilute with *Mobile phase* to volume.

Standard solution: 2 µg/mL of USP Fluticasone Propionate RS from *Standard stock solution A* and 0.58 µg/mL of USP Salmeterol Xinafoate RS (0.4 µg/mL of salmeterol) from *Standard stock solution B* in *Mobile phase*

Sensitivity solution: 0.2 µg/mL of USP Fluticasone Propionate RS from *Standard stock solution A* and 0.15 µg/mL of USP Salmeterol Xinafoate RS (0.1 µg/mL of salmeterol) from *Standard stock solution B* in *Mobile phase*

Sample solutions: Proceed as directed in the chapter using *Solution A* to coat the particle collection surface. Discard waste solution and then allow *Solution A* to evaporate. Add 15 mL of *Mobile phase* to the central cup of the preseparator insert as the solvent used for sample recovery. Discharge a single actuation of an inhaler by operating the pump for 4 s at a flow rate of 60 L/min. Dismantle the apparatus and prepare the *Sample solutions*. Repeat these steps for 5 additional inhalers for a total of 6 sets of *Sample solutions*. See *Table 2*.

[1] A suitable grade is available as catalog #378488 from www.sigmaaldrich.com.

USP Monographs

www.webofpharma.com

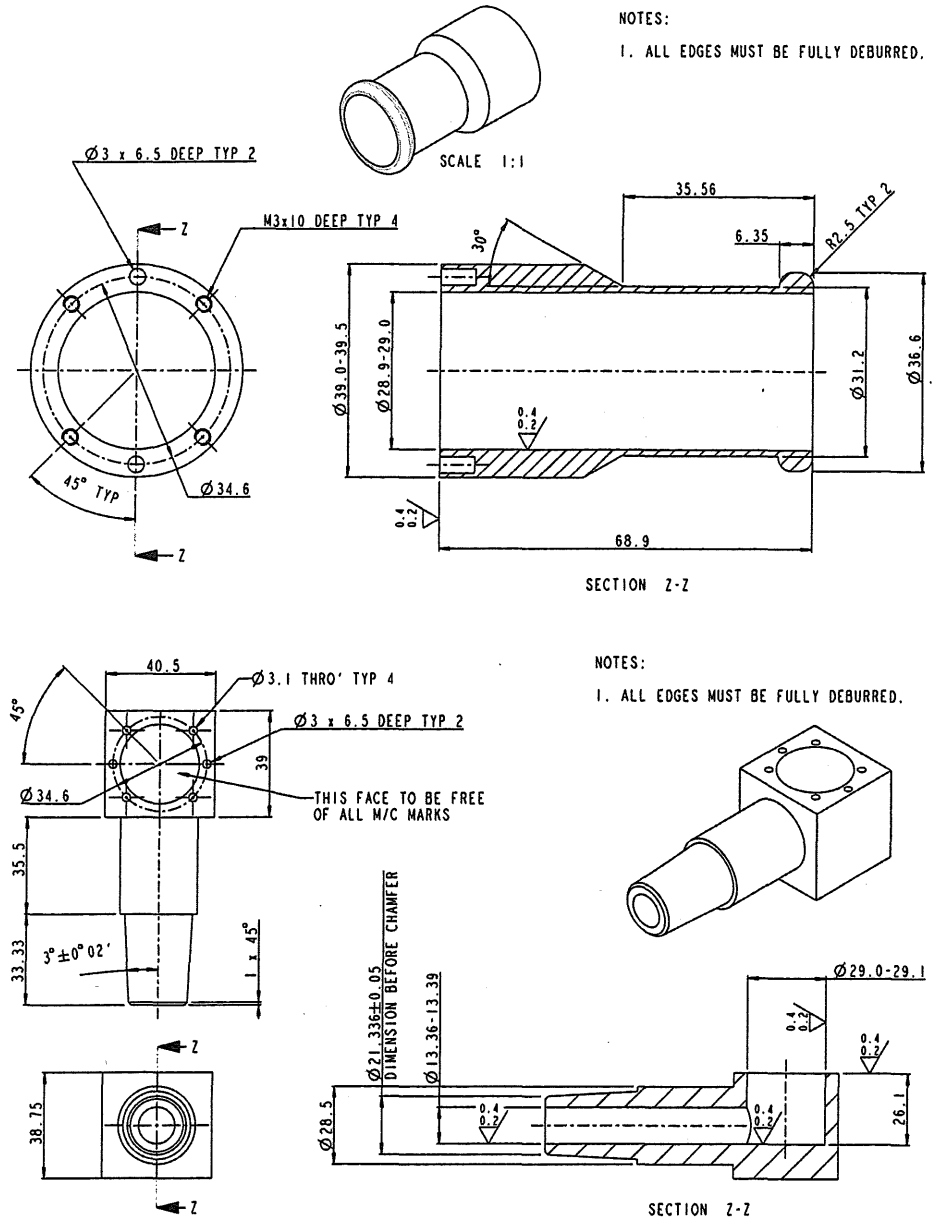


Figure 2. Expanded view of the modified induction port. (Fluticasone Propionate and Salmeterol Inhalation Powder)

Table 2

Parameter	Description	Final Volume (mL)
Mouthpiece adapter and induction port	Add 80 mL of *Buffer* to a volumetric flask. Transfer any powder from the mouthpiece adapter and induction port to the volumetric flask using methanol. Dilute with methanol to volume.	200
Preseparator	Stopper the preseparator and add 85 mL of *Mobile phase*.	100
Stages 1–5 and MOC[a]	Transfer 10 mL of *Mobile phase* to each cup.	10

Table 2 *(continued)*

Parameter	Description	Final Volume (mL)
Stages 6 and 7[a]	Transfer 5 mL of *Mobile phase* to each cup.	5

[a] Agitation using a gentle rocker may be used to promote dissolution. [NOTE—The use of a gentle rocker for 10–15 min may be suitable.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors: The wavelength may be switched from 240 to 220 nm after the elution of fluticasone propionate and before the elution of salmeterol.
Fluticasone propionate: UV 240 nm

www.webofpharma.com

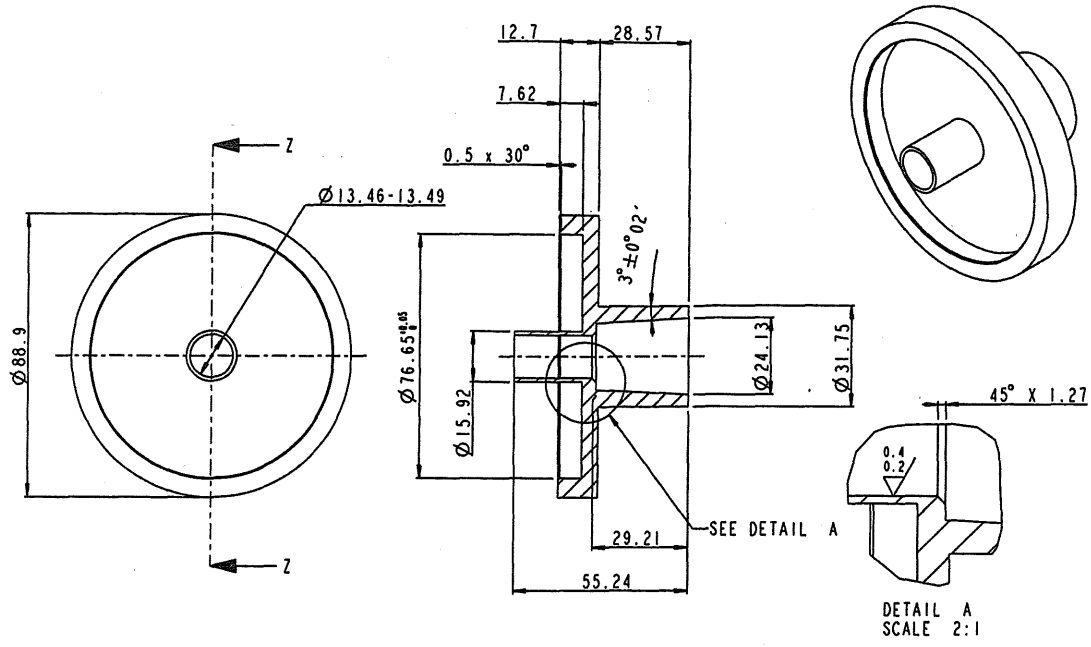


Figure 3. Expanded view of the preseparator lid. (Fluticasone Propionate and Salmeterol Inhalation Powder)

Table 1 (Fluticasone Propionate and Salmeterol Inhalation Powder)

Parameter	Amount of Fluticasone Propionate Deposited (µg▲/actuation)▲ (RB 8-Feb-2019)			Amount of Salmeterol Deposited (µg▲/actuation)▲ (RB 8-Feb-2019)		
Label claim of fluticasone propionate/salmeterol (µg/actuation)	100/50 ▲▲ (RB 8-Feb-2019)	250/50 ▲▲ (RB 8-Feb-2019)	500/50 ▲▲ (RB 8-Feb-2019)	100/50 ▲▲ (RB 8-Feb-2019)	250/50 ▲▲ (RB 8-Feb-2019)	500/50 ▲▲ (RB 8-Feb-2019)
Mass of mouthpiece adapter, induction port, preseparator, and Stage 0	55–80	140–200	290–400	28–42	28–42	28–42
Sum of Stages 1–5	15–30	42–73	96–150	7–13	7–13	7–13
Sum of Stages 3 and 4	6–18	19–45	43–92	3–8	3–8	4–8
Sum of Stages 6, 7, and filter	NMT 1	NMT 2	NMT 2	NMT 0.5	NMT 0.5	NMT 0.5

Salmeterol: UV 220 nm
Column: 2.1-mm × 5-cm; 1.7-µm packing L1
Flow rate: 1 mL/min
Temperatures
Autosampler temperature: 5°
Column temperature: 75°
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of salmeterol

System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—The relative retention times for fluticasone propionate and salmeterol are 0.28 and 1.0, respectively.]
Suitability requirements
Tailing factor: NMT 2.0 for fluticasone propionate; NMT 1.7 for salmeterol, *Standard solution*
Relative standard deviation: NMT 1.0% for fluticasone propionate and salmeterol, *Standard solution*; NMT 10.0% for fluticasone propionate and salmeterol, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solutions*
Calculate the quantity, in µg/actuation, of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) in each of the *Sample solutions*:

$$\text{Result} = [(r_U/r_S) \times C_S] \times (V/N)$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)
V = total volume of the *Sample solution* (mL)
N = number of unit doses discharged into the apparatus, 1

Calculate the quantity, in µg/actuation, of salmeterol ($C_{25}H_{37}NO_4$) in each of the *Sample solutions*:

$$\text{Result} = [(r_U/r_S) \times C_S] \times (V/N) \times (M_{r1}/M_{r2})$$

r_U = peak response of salmeterol from the *Sample solution*
r_S = peak response of salmeterol from the *Standard solution*
C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (µg/mL)
V = total volume of the *Sample solution* (mL)
N = number of unit doses discharged into the apparatus, 1
M_{r1} = molecular weight of salmeterol free base, 415.57

www.webofpharma.com

M_{r2} = molecular weight of salmeterol xinafoate, 603.75

Acceptance criteria: The requirements for the masses of fluticasone propionate and salmeterol deposited in each grouping of the *Sampling apparatus* for each inhaler are given in *Table 3*. The article meets the requirements if NMT 1 of the 6 inhalers fails to meet the requirements in *Table 3* but meets the requirements in *Table 4*.

Table 3

Parameter	Amount of Fluticasone Propionate Deposited (µg/actuation)			Amount of Salmeterol Deposited (µg/actuation)		
Label claim of fluticasone propionate/salmeterol (µg/actuation)	100/50	250/50	500/50	100/50	250/50	500/50
Sum of mouthpiece adapter, induction port, preseparator, Stage 1 and Stage 2	63–91	158–232	285–454	34–47	36–48	32–46
Sum of Stages 3–7 and MOC	12–27	27–64	64–140	5–11	4–10	6–11
Sum of Stages 4 and 5	6–15	13–38	30–78	2–6	2–6	2–6
Sum of Stage 6, Stage 7, and MOC	NMT 2	NMT 3	NMT 7	NMT 1	NMT 0.5	NMT 0.5

Table 4

Parameter	Amount of Fluticasone Propionate Deposited (µg/actuation)			Amount of Salmeterol Deposited (µg/actuation)		
Label claim of fluticasone propionate/salmeterol (µg/actuation)	100/50	250/50	500/50	100/50	250/50	500/50
Sum of mouthpiece adapter, induction port, preseparator, Stage 1 and Stage 2	57–100	142–255	256–499	31–52	32–53	29–51
Sum of Stages 3–7 and MOC	11–30	24–70	58–154	4–12	4–11	5–12
Sum of Stages 4 and 5	5–17	12–42	27–86	2–7	2–7	2–7
Sum of Stage 6, Stage 7, and MOC	NMT 2	NMT 3	NMT 8	NMT 1	NMT 0.6	NMT 0.6▲ (RB 8-Feb-2019)

Change to read:

- **DELIVERED-DOSE UNIFORMITY**

(See *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* ⟨601⟩, *Delivered-Dose Uniformity, Inhalation Powders*.)

Sampling apparatus: Use the apparatus in *Figure 4A. Sampling apparatus.* with modified glass sampling device (*Figure 4B. Expanded view of the modified glass sample collection apparatus.*).

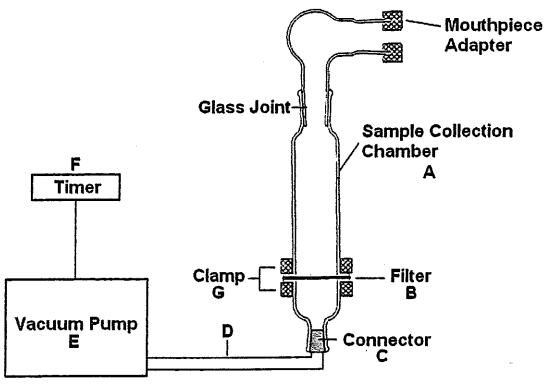


Figure 4A. Sampling apparatus.

Buffer, Solution A, Mobile phase, and **Diluent:** Proceed as directed in the *Assay*.

Standard solution: 2.5 µg/mL of USP Fluticasone Propionate RS and 0.75 µg/mL of USP Salmeterol Xinafoate RS in *Diluent*

Sample solutions: Discharge a single unit dose into the apparatus shown in *Figure 4A. Sampling apparatus.*. Operate the pump for 2 s at an airflow of 60 L/min to collect the dose. Detach the inhaler. Rinse the mouthpiece adapter and each piece of the sample collection chamber with methanol. Place the filter and washings into a container. Sonicate for 5 min. Quantitatively transfer the contents to a 200-mL volumetric flask containing 60 mL of water. Allow the solution to equilibrate, and dilute with methanol to volume. Prepare nine additional *Sample solutions* from nine additional unit doses. For multi-dose inhalers, collect one dose from each of 10 inhalers with the 10 doses collected across the minimum number of recommended doses on the label of the inhaler.

Chromatographic system and **System suitability:** Proceed as directed in the *Assay*, except for the *Injection volume*.

Injection volume: 50 µL

Analysis

Samples: *Standard solution* and *Sample solutions*

Calculate the percentage of the labeled amount of fluticasone propionate ($C_{25}H_{31}F_3O_5S$) delivered by the inhaler in each *Sample solution*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of fluticasone propionate in the *Sample solution* (µg/mL), based on target

www.webofpharma.com

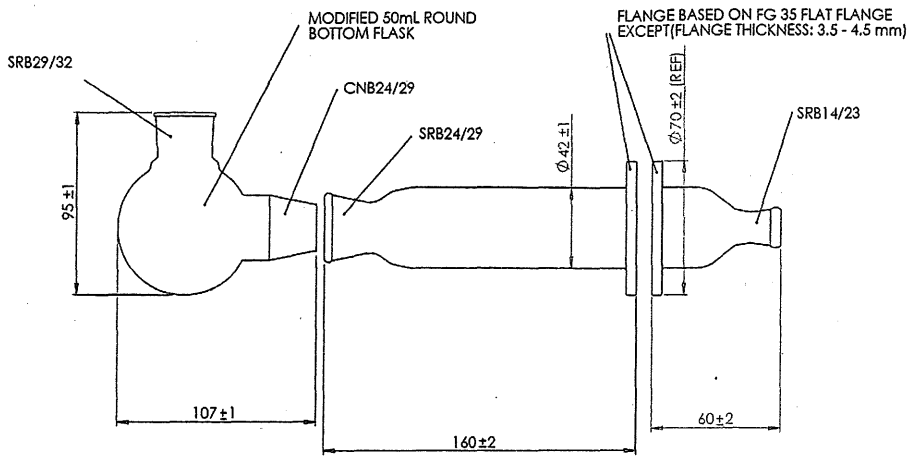


Figure 4B. Expanded view of the modified glass sample collection apparatus. (Fluticasone Propionate and Salmeterol Inhalation Powder)

emitted dose from ▲*Table 5*▲ (RB 8-Feb-2019)

Calculate the percentage of the labeled amount of salmeterol ($C_{25}H_{37}NO_4$) delivered by the inhaler in each *Sample solution*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of salmeterol from the *Sample solution*
r_S = peak response of salmeterol from the *Standard solution*
C_S = concentration of USP Salmeterol Xinafoate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of salmeterol free base in the *Sample solution* (µg/mL), based on target emitted dose from ▲*Table 5*▲ (RB 8-Feb-2019)

M_{r1} = molecular weight of salmeterol free base, 415.57
M_{r2} = molecular weight of salmeterol xinafoate, 603.75

▲Table 5▲ (RB 8-Feb-2019) Target Emitted Dose

Label Claim of Fluticasone Propionate/ Salmeterol (µg/unit dose)	Fluticasone Propionate Target Emitted Dose (µg/unit dose)	Salmeterol Target Emitted Dose (µg/unit dose)
100/50	93	45
250/50	233	45
500/50	465	45

Acceptance criteria

1. The mean content of fluticasone propionate and salmeterol from 10 doses is NLT 85% and NMT 115% of the target emitted dose.
2. NMT 1 emitted dose is outside 80%–120% of the target emitted dose.
3. No dose is outside 75%–125% of the target emitted dose.

If requirements 1 and 2 described above are not met, test an additional 20 unit doses. The mean dose of fluticasone propionate and salmeterol from 30 doses is:

- NLT 85% and NMT 115% of the target emitted dose.
- NMT 3 doses are outside 80%–120% of the target emitted dose.
- No dose is outside 75%–125% of the target emitted dose.

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

[NOTE—Protect all solutions containing fluticasone propionate or salmeterol from light.]

Solution A: ▲5.7 g/L of monobasic ammonium phosphate in water adjusted with 10% phosphoric acid TS▲ (RB 8-Feb-2019) to a pH of 2.9

Solution B: Acetonitrile

Mobile phase: See ▲*Table 6*.

Table 6▲ (RB 8-Feb-2019)

Time (min)	Solution A (%)	Solution B (%)
0	70	30
60	22	78
61	70	30
70	70	30

Diluent: Methanol, water, and phosphoric acid (70: 30: 0.05)

Acidified methanol: ▲To each liter of methanol, add 0.5 mL of phosphoric acid.▲ (RB 8-Feb-2019)

System suitability solution: 0.15 mg/mL of USP Salmeterol Xinafoate RS, 0.05 mg/mL of USP Fluticasone Propionate RS, and 0.4 µg/mL each of USP Fluticasone Propionate Related Compound D RS and USP Fluticasone Propionate Related Compound J RS in *Diluent*

Standard solution: 2 µg/mL of USP Salmeterol Related Compound H RS and 4 µg/mL of USP Fluticasone Propionate RS in *Diluent*

Sensitivity solution: 0.05 µg/mL of USP Salmeterol Related Compound H RS and 0.1 µg/mL of USP Fluticasone Propionate RS from *Standard solution* in *Diluent*

Sample solution: Nominally 200–500 µg/mL of fluticasone propionate prepared as follows. Transfer the contents of NLT 10 unit doses to a 10-mL volumetric flask. Add 6 mL

www.webofpharma.com

of acidified methanol and sonicate for 10 min. Add 3 mL of water, mix, and allow the solution to equilibrate. Dilute with acidified methanol to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 228 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Column temperature: 35°
Injection volume: 50 µL

System suitability
Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*
[NOTE—See ▲*Table 7*▲ (RB 8-Feb-2019) for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between fluticasone propionate related compound J and salmeterol; NLT 1.5 between fluticasone propionate related compound D and fluticasone propionate, *System suitability solution*
Tailing factor: NMT 2.0 for salmeterol related compound H and fluticasone propionate, *Standard solution*
Relative standard deviation: NMT 5.0% for salmeterol related compound H and fluticasone propionate, *Standard solution*
Signal-to-noise ratio: NLT 10 for both fluticasone propionate and salmeterol related compound H, *Sensitivity solution*

Analysis
Samples: *Standard solution*, *Sensitivity solution*, and *Sample solution*
Calculate the percentage of each fluticasone propionate related degradation product in the portion of Inhalation Powder taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (W_N/W_U) \times (1/L) \times 100$$

r_U = peak response of each fluticasone propionate related degradation product from the *Sample solution*
r_S = peak response of fluticasone propionate from the *Standard solution*
C_S = concentration of USP Fluticasone Propionate RS in the *Standard solution* (µg/mL)
V = volume of the *Sample solution* (mL)
W_N = nominal weight of each unit dose (mg)
W_U = weight of the unit doses in the *Sample solution* (mg)
L = label claim of fluticasone propionate (µg/unit dose)

Disregard any fluticasone propionate related degradation product peak less than the area of fluticasone propionate in the *Sensitivity solution*.
Calculate the percentage of each salmeterol related degradation product in the portion of Inhalation Powder taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (W_N/W_U) \times (1/L) \times 100$$

r_U = response of each salmeterol related degradation product from the *Sample solution*
r_S = response of salmeterol related compound H from the *Standard solution*
C_S = concentration of USP Salmeterol Related compound H RS in the *Standard solution* (µg/mL)
V = volume of the *Sample solution* (mL)
W_N = nominal weight of each unit dose (mg)
W_U = weight of the unit doses in the *Sample solution* (mg)
L = label claim of salmeterol free base (µg/unit dose)

Acceptance criteria: See ▲*Table 7*.▲ (RB 8-Feb-2019) Disregard any salmeterol related degradation product peak less than the area of salmeterol related compound H in the *Sensitivity solution*. [NOTE—Any unspecified degradation product eluting before salmeterol is related to salmeterol. Any unspecified degradation product eluting after salmeterol is related to fluticasone propionate.]

▲Table 7▲ (RB 8-Feb-2019)

Name	Relative Retention Time	Acceptance Criteria (NMT %)
Salmeterol-*N*-phenylbutyl aminoalcohol[a, b]	0.14	—
Salmeterol-phenylethoxy[a, c]	0.25	—
Salmeterol-phenylpropoxy[a, d]	0.32	—
Salmeterol-phenyl-2-butoxy[a, e]	0.37	—
Fluticasone propionate related compound J[a]	0.38	—
Salmeterol[a]	0.41	N/A
Hydroxynapthoic acid[f]	0.5	—
Salmeterol-deoxy[a, g]	0.55	—
Fluticasone propionate dithioacid[a, h]	0.67	—
Salmeterol-*N*-alkyl[i]	0.71	0.2
Salmeterol related compound H	0.74	0.9
Fluticasone propionate related compound D[a]	0.97	—
Fluticasone propionate	1.0	N/A
Fluticasone dimer[a, i]	1.09	—
Any fluticasone propionate related unspecified degradation product	—	0.1
Any salmeterol related unspecified degradation product	—	0.1

www.webofpharma.com

▲**Table 7**▲ (RB 8-Feb-2019) *(continued)*

Name	Relative Retention Time	Acceptance Criteria (NMT %)
Total degradation products	—	1.3

[a] This is a process impurity that is included in this table for identification only. This impurity is controlled in the drug substance. This impurity is not to be reported for the drug product or to be included in the total degradation products.
[b] 4-[1-Hydroxy-2-(4-phenylbutylamino)ethyl]-2-(hydroxymethyl)phenol.
[c] 4-[1-Hydroxy-2-(6-phenethoxyhexylamino)ethyl]-2-(hydroxymethyl)phenol.
[d] 4-{1-Hydroxy-2-[6-(3-phenylpropoxy)hexylamino]ethyl}-2-(hydroxymethyl) phenol.
[e] 4-{1-Hydroxy-2-[6-(4-phenylbutan-2-yloxy)hexylamino]ethyl}-2-(hydroxymethyl)phenol.
[f] This is a counter ion of salmeterol that is included in this table for identification only. It is not to be reported for the drug product or to be included in the total degradation products.
[g] 4-{1-Hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl}-2-methylphenol.
[h] 6α,9α-Difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbodithioic acid.
[i] 4-(1-Hydroxy-2-[(2-hydroxy-5-{1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino] ethyl}benzyl)[6-(4-phenylbutoxy)hexyl]amino]ethyl}-2-(hydroxymethyl) phenol.
[j] 6α,9α-Difluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid 6α,9α-difluoro-17β-(fluoromethylthio)carbonyl-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-yl ester.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^1 cfu/g of powder. The total aerobic yeasts and molds count does not exceed 10^1 cfu/g of formulation. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.

Change to read:

- **FOREIGN PARTICULATE MATTER**

Particulate Matter in Injections ⟨788⟩ describes details of the test apparatus to be used for the determination of particulate matter using a microscopic particle count test methodology. Samples should be carefully prepared to avoid environmental contamination, and testing should be performed with suitable controls, including the appropriate use of blank determinations.

Diluent: Methanol and water (65:35) passed through a filter of 0.45-µm pore size

Filter: Mixed cellulose and ester filter; 25-mm diameter and 0.45-µm pore size

Sample solution: Transfer contents of NLT 8 unit doses to a suitable container. Dissolve in 75 mL of *Diluent*.

Analysis

Sample: *Sample solution*

Pass the *Sample solution* through the filter and allow the filter to dry under conditions that will limit particulate contamination. Using a microscopic particle count test method, enumerate the number of particles present in the *Sample solution*.

Calculate the total number of particles per actuation by the formula:

$$\text{Result} = (N_{<10} + N_{10\text{–}100} + N_{>100})/8$$

$N_{<10}$ = total number of particles <10 µm present in the *Sample solution*

$N_{10\text{–}100}$ = total number of particles between 10 and 100 µm present in the *Sample solution*

$N_{>100}$ = total number of particles >100 µm present in the *Sample solution*

Acceptance criteria: See ▲*Table 8*.

Table 8▲ (RB 8-Feb-2019)

Particle Size Range (µm)	Number of Particles/Dose (NMT)
<10	200
10–100	100
>100	10
Total	300

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature, in a dry place away from direct heat or sunlight.

Add the following:

▲• **LABELING:** The labeling states the *Aerodynamic Size Distribution* test used only if *Test 1* is not used.▲ (RB 8-Feb-2019)

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Fluticasone Propionate RS

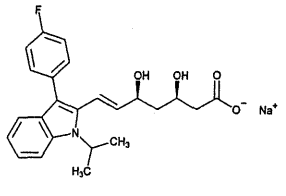
USP Fluticasone Propionate Related Compound D RS
S-Methyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17▲α▲ (RB 8-Feb-2019)-propionyloxyandrosta-1,4-diene-17▲β▲ (RB 8-Feb-2019)-carbothioate.
$C_{25}H_{32}F_2O_5S$ 482.58

USP Fluticasone Propionate Related Compound J RS
6α,9α-Difluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid.
$C_{21}H_{26}F_2O_5$ 396.42

USP Salmeterol Related Compound H RS
1-Hydroxy-4-[2-hydroxy-5-(1-hydroxy-2-{[6-(4-phenylbutoxy)hexyl]amino}ethyl)benzyl]-2-naphthoic acid, monohydrate.
$C_{36}H_{43}NO_6 \cdot H_2O$ 603.76

USP Salmeterol Xinafoate RS

Fluvastatin Sodium



$C_{24}H_{25}FNNaO_4$ 433.45

6-Heptenoic acid, 7-[3-(4-fluorophenyl)-1-(1-methyl ethyl)-1*H*-indol-2-yl]-3,5-dihydroxy-, monosodium salt, [*R**,*S**-(*E*)]-(±)-;

Sodium (±)-(3*R**,5*S**,6*E*)-7-[3-(*p*-fluorophenyl)-1-iso propylindol-2-yl]-3,5-dihydroxy-6-heptenoate [93957-55-2].

DEFINITION

Fluvastatin Sodium contains NLT 98.0% and NMT 102.0% of fluvastatin sodium ($C_{24}H_{25}FNNaO_4$), calculated on the anhydrous basis.

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

If a difference appears in the IR spectra of the analyte and the Standard, dissolve equal portions of the sample specimen and the USP Reference Standard in equal volumes of methanol. Evaporate the solutions to dryness on a steam bath, protecting the solutions from light, and dry at 105° for 30 min. Repeat the test on the residues.

- **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sodium*: Meets the requirements of test *A*
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Add 20 mL of 25% aqueous tetramethylammonium hydroxide solution to 880 mL of water. Adjust with about 2.3 mL of phosphoric acid to a pH of 7.2 ± 0.2. Add 100 mL of a mixture of methanol and acetonitrile (60:40).

Solution B: Add 20 mL of 25% aqueous tetramethylammonium hydroxide solution and 80 mL of water to 900 mL of a mixture of methanol and acetonitrile (60:40). Adjust with about 2.3 mL of phosphoric acid to a pH of 7.2 ± 0.2.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
6	60	40
20	18	82
20.1	60	40
25.1	60	40

System suitability solution: 0.5 mg/mL of fluvastatin sodium from USP Fluvastatin for System Suitability RS, dissolved first in *Solution B*, using 40% of the final volume, then diluted with *Solution A* to volume

Standard solution: 0.5 mg/mL of USP Fluvastatin Sodium RS, dissolved first in *Solution B*, using 40% of the final volume, then diluted with *Solution A* to volume

Sample solution: 0.5 mg/mL of Fluvastatin Sodium, dissolved first in *Solution B*, using 40% of the final volume, then diluted with *Solution A* to volume

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 305 nm

Column: 4.6-mm × 5-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 3 mL/min

Injection volume: 20 µL

[NOTE—Adjust the start time of the gradient step and the equilibration time for each instrument.]

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for fluvastatin and fluvastatin *anti*-isomer are about 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 1.6 between fluvastatin *anti*-isomer and fluvastatin, *System suitability solution*

Tailing factor: NMT 3.0 for the fluvastatin peak, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fluvastatin sodium ($C_{24}H_{25}FNNaO_4$) in the portion of Fluvastatin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Fluvastatin Sodium RS in the *Standard solution* (mg/mL)

C_U = concentration of Fluvastatin Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **ORGANIC IMPURITIES**

[NOTE—Protect all solutions from light, and use amber autosampler vials and low-actinic glassware.]

Solution A, Solution B, Mobile phase, Standard solution, and Sample solution: Prepare as directed in the *Assay*.

System suitability solution A: Prepare as directed for the *System suitability solution* in the *Assay*.

System suitability solution B: 0.1 mg/mL of USP Fluvastatin Related Compound B RS in a mixture of methanol and acetonitrile (60:40). Transfer about 0.5 mL of this solution to a 10-mL volumetric flask, and dilute with *System suitability solution A* to volume. [NOTE—*System suitability solution B* is stable for up to 6 months if stored in a refrigerator.]

Chromatographic system: Proceed as directed in the *Assay*, except for the *Detector*.

Detector

UV 365 nm: 3-Hydroxy-5-keto fluvastatin

UV 305 nm: For all other peaks

System suitability

Samples: *Standard solution* and *System suitability solution B*

Suitability requirements

Resolution: NLT 1.6 between fluvastatin *anti*-isomer and fluvastatin, *System suitability solution B*

Tailing factor: NMT 3.0 for the fluvastatin peak, *System suitability solution B*

Relative standard deviation: NMT 1.0% at 305 nm, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms at 305 and 365 nm, identify the impurities listed in *Table 2*, and measure the peak responses.

Calculate the percentage of each impurity, except for 3-hydroxy-5-keto fluvastatin, in the portion of Fluvastatin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response at 305 nm of each impurity from the *Sample solution*

r_S = peak response at 305 nm of fluvastatin from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Fluvastatin Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluvastatin Sodium in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Calculate the percentage of 3-hydroxy-5-keto fluvastatin in the portion of Fluvastatin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response at 365 nm of 3-hydroxy-5-keto fluvastatin from the *Sample solution*
r_S = peak response at 365 nm of fluvastatin from the *Standard solution*
C_S = concentration of USP Fluvastatin Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluvastatin Sodium in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fluvastatin *N*-ethyl analog[a]	0.7	1.2	0.1
Fluvastatin *anti*-isomer[b]	1.2	1.0	0.8
3-Hydroxy-5-keto fluvastatin[c]	1.5	27.0[d]	0.1
Fluvastatin hydroxydiene[e]	2.0	0.92	0.1
Fluvastatin short-chain aldehyde[f]	3.0	1.4	0.1
Fluvastatin related compound B	3.4	0.94	0.2
Any unspecified impurity	—	1.0	0.1
Total impurities	—	—	1.0

[a] Sodium (3*R*,5*S*,*E*)-7-[1-ethyl-3-(4-fluorophenyl)-1*H*-indol-2-yl]-3,5-dihydroxyhept-6-enoate.
[b] [*R**,*R**-*E*]-(±)-7-[3-(4-Fluorophenyl)-1-(methylethyl)-1*H*-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid monosodium salt; also known as Sodium (3*RS*,5*RS*,*E*)-7-(3-(4-fluorophenyl)-1-isopropyl-1*H*-indol-2-yl)-3,5-dihydroxyhept-6-enoate.
[c] Sodium (*E*)-7-[3-(4-fluorophenyl)-1-isopropyl-1*H*-indol-2-yl]-3-hydroxy-5-oxohept-6-enoate.
[d] At 365 nm.
[e] Sodium (4*E*,6*E*)-7-[3-(4-fluorophenyl)-1-isopropyl-1*H*-indol-2-yl]-3-hydroxyhepta-4,6-dienoate.
[f] 3-(4-Fluorophenyl)-1-isopropyl-1*H*-indole-2-carbaldehyde.

SPECIFIC TESTS

- **pH** ⟨791⟩: 8.0–10.0, in a 10-mg/mL solution. Perform the test immediately after preparation.
- **Water Determination** ⟨921⟩, *Method I*: NMT 4.0%; if labeled as a hydrated form, NMT 12.0%. [Note—For this monograph, the term "hydrated form" refers to several known forms of fluvastatin sodium that are not stoichiometric hydrates.]

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, protected from moisture. Store at a temperature not exceeding 40°.
- **Labeling:** Where it is a hydrated form, the label so indicates.
- **USP Reference Standards** ⟨11⟩
  USP Fluvastatin Sodium RS
  USP Fluvastatin Related Compound B RS
  Fluvastatin *t*-butyl ester.
  [*R**,*S**-*E*]-(±)-7-[3-(4-Fluorophenyl)-1-methylethyl-1*H*-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid 1,1-dimethylethyl ester; also known as *tert*-Butyl (3*RS*,5*SR*,*E*)-7-[3-(4-fluorophenyl)-1-isopropyl-1*H*-indol-2-yl]-3,5-dihydroxyhept-6-enoate.
  $C_{28}H_{34}FNO_4$ 467.58
  USP Fluvastatin for System Suitability RS
  Fluvastatin sodium, containing 1%–2% of the fluvastatin sodium *anti*-isomer.

Fluvastatin Capsules

DEFINITION

Fluvastatin Capsules contain fluvastatin sodium ($C_{24}H_{25}FNNaO_4$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of fluvastatin ($C_{24}H_{26}FNO_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

- **Procedure**

▲[Note—Protect all solutions from light.]▲ (USP 1-Aug-2019)

Buffer: Prepare a solution containing 40 mL of 25% aqueous tetramethylammonium hydroxide in 1 L of water. Adjust with approximately 4.5 mL of phosphoric acid to a pH of 7.2.
Solvent mixture: Methanol and acetonitrile (60:40)
Solution A: *Solvent mixture* and *Buffer* (12.5: 87.5)
Solution B: *Solvent mixture* and *Buffer* (87.5: 12.5)
Mobile phase: See *Table 1*. ▲▲ (USP 1-Aug-2019)

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	54	46
6	54	46
17	17	83
20	17	83
20.1	54	46
26.1	54	46

Diluent: *Solvent mixture* and *Buffer* (46:54)
System suitability solution: 0.42 mg/mL of fluvastatin sodium from USP Fluvastatin for System Suitability RS in *Diluent*

www.webofpharma.com

Standard solution: 0.42 mg/mL of USP Fluvastatin Sodium RS, equivalent to 0.4 mg/mL of fluvastatin, in *Diluent*
Sample stock solution: Nominally 1.0 mg/mL of fluvastatin from Capsules prepared as follows. Transfer the contents and the empty shells of ▲a suitable number of▲ (USP 1-Aug-2019) Capsules, equivalent to 200 mg of fluvastatin, to a 200-mL volumetric flask. Add 100 mL of methanol, and stir with a magnetic or mechanical stirrer for 45 min. Centrifuge a portion of this solution at 4000 rpm for 20 min.
Sample solution: Nominally 0.4 mg/mL of fluvastatin from *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 305 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Aug-2019)
Column: 4.6-mm × 5-cm; 5-µm packing L1
Flow rate: 2.0 mL/min
Injection volume: 25 µL
▲▲ (USP 1-Aug-2019)
System suitability
Samples: *System suitability solution* ▲and *Standard solution*▲ (USP 1-Aug-2019)
[NOTE—The relative retention times for fluvastatin and fluvastatin anti-isomer are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 1.4 between fluvastatin anti-isomer and fluvastatin, *System suitability solution*
▲▲ (USP 1-Aug-2019)
Relative standard deviation: NMT 1.5%, ▲*Standard solution*▲ (USP 1-Aug-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fluvastatin ($C_{24}H_{26}FNO_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of fluvastatin from the *Sample solution*
r_S = peak response of fluvastatin from the *Standard solution*
C_S = concentration of USP Fluvastatin Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluvastatin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of fluvastatin, ▲411.47▲ (USP 1-Aug-2019)
M_{r2} = molecular weight of fluvastatin sodium, 433.45

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Medium: Water; 500 mL
Apparatus 2: 50 rpm, sinkers not used
Time: 30 min
▲▲ (USP 1-Aug-2019)
Buffer: 1.534 g of monobasic ammonium phosphate in 800 mL of water. Adjust with phosphoric acid or ammonium hydroxide to a pH of 3.5.
Mobile phase: Methanol and *Buffer* (7:3)
Standard solution: ▲(0.002 × *L*) mg/mL of USP Fluvastatin Sodium RS in *Medium*, where *L* is the label claim in mg/Capsule▲ (USP 1-Aug-2019)
[NOTE—A volume of methanol, not exceeding 2% of the final volume of solution, may be used to aid in dissolving USP Fluvastatin Sodium RS.]
Sample solution: Pass a portion of the solution under test through a suitable filter, discarding the first 2 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Columns
Guard: ▲3.2-mm × 1.5-cm;▲ (USP 1-Aug-2019) 7-µm packing L1
Analytical: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 2.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
▲Calculate the percentage of the labeled amount of fluvastatin ($C_{24}H_{26}FNO_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of fluvastatin from the *Sample solution*
r_S = peak response of fluvastatin from the *Standard solution*
C_S = concentration of USP Fluvastatin Sodium RS in the *Standard solution* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Capsule)
M_{r1} = molecular weight of fluvastatin, 411.47
M_{r2} = molecular weight of fluvastatin sodium, 433.45▲ (USP 1-Aug-2019)

Tolerances: NLT 80% (*Q*) of the labeled amount of fluvastatin ($C_{24}H_{26}FNO_4$) is dissolved.

Change to read:

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements▲▲ (USP 1-Aug-2019)

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
▲▲ (USP 1-Aug-2019)
Buffer, Solvent mixture, Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Standard solution, and **Sample solution:** Prepare as directed in the *Assay*.
▲**Sensitivity solution:** 0.21 µg/mL of USP Fluvastatin Sodium RS in *Diluent*, from *Standard solution*▲ (USP 1-Aug-2019)
Chromatographic system: Proceed as directed in the *Assay* except for the *Detector*.
Detector
UV 365 nm: For 3-hydroxy-5-keto fluvastatin
UV 305 nm: For all the other impurities
System suitability
Samples: *System suitability solution*, ▲*Standard solution*, and *Sensitivity solution*▲ (USP 1-Aug-2019)

www.webofpharma.com

Suitability requirements
Resolution: NLT 1.4 between fluvastatin anti-isomer and fluvastatin, *System suitability solution*
Relative standard deviation: NMT 1.5%, ▲*Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*▲ (USP 1-Aug-2019)

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each ▲degradation product▲ (USP 1-Aug-2019) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each ▲degradation product▲ (USP 1-Aug-2019) from the *Sample solution*
r_S = peak response of fluvastatin from the *Standard solution*
C_S = concentration of USP Fluvastatin Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluvastatin in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of fluvastatin, ▲411.47▲ (USP 1-Aug-2019)
M_{r2} = molecular weight of fluvastatin sodium, 433.45
F = relative response factor (see *Table 2*)

▲▲ (USP 1-Aug-2019)
Acceptance criteria: ▲See *Table 2*. The reporting threshold is 0.05%.▲ (USP 1-Aug-2019)

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
▲Fluvastatin	1.0	—	—▲ (USP 1-Aug-2019)
Fluvastatin *anti*-isomer[▲a]▲ (USP 1-Aug-2019)	1.2	1.0	1.5
3-Hydroxy-5-keto fluvastatin[▲b]▲ (USP 1-Aug-2019)	1.6	27.0[c]	1.0
Fluvastatin hydroxydiene[▲d]▲ (USP 1-Aug-2019)	2.2	0.92	1.0
Fluvastatin short-chain aldehyde[▲e]▲ (USP 1-Aug-2019)	3.2	1.4	0.5
▲Any unspecified degradation product	—	1.0	0.5▲ (USP 1-Aug-2019)
▲Total degradation products	—	—	4.0▲ (USP 1-Aug-2019)

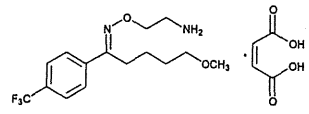
[a] Sodium (3*RS*,5*RS*,*E*)-7-(3-(4-fluorophenyl)-1-isopropyl-1*H*-indol-2-yl)-3,5-dihydroxyhept-6-enoate.
[b] Sodium (*E*)-7-[3-(4-fluorophenyl)-1-isopropyl-1*H*-indol-2-yl]-3-hydroxy-5-oxohept-6-enoate.
[c] At 365 nm.
[d] Sodium (4*E*,6*E*)-7-[3-(4-fluorophenyl)-1-isopropyl-1*H*-indol-2-yl]-3-hydroxyhepta-4,6-dienoate.
[e] 3-(4-Fluorophenyl)-1-(methylethyl)-1*H*-indole-2-carboxaldehyde.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, protected from moisture and from light. Store in a cool place or at controlled room temperature.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluvastatin Sodium RS
USP Fluvastatin for System Suitability RS
▲Fluvastatin sodium, containing 1%–2% of the fluvastatin sodium *anti*-isomer.▲ (USP 1-Aug-2019)

Fluvoxamine Maleate



$C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$ 434.41
1-Pentanone, 5-methoxy-1-[4-(trifluoromethyl)phenyl]-, *O*-(2-aminoethyl)oxime, (*E*)-, (*Z*)-2-butenedioate (1:1); (*E*)-5-Methoxy-4′-(trifluoromethyl)valerophenone *O*-(2-aminoethyl)oxime, maleate (1:1) [61718-82-9].

DEFINITION
Fluvoxamine Maleate contains NLT 98.0% and NMT 102.0% of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: 8 g/L of sodium 1-pentanesulfonate and 1.1 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.00 ± 0.05.
Mobile phase: Acetonitrile and *Solution A* (38:62)
System suitability solution: Transfer 6 mg of Fluvoxamine Maleate to a 50-mL volumetric flask. Heat the sample at 120° for 10 min. Cool to room temperature, and add 3.0 mL of 0.1 N hydrochloric acid. Heat the solution in a water bath for 10 min. Cool to room temperature, add 50 mg of Fluvoxamine Maleate, and dissolve in 25 mL of *Mobile phase*. Dilute with *Mobile phase* to volume.
Standard solution: 0.05 mg/mL of USP Fluvoxamine Maleate RS in *Mobile phase*
Sample solution: 0.05 mg/mL of Fluvoxamine Maleate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 234 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.7 mL/min
Injection volume: 20 µL
Run time: NLT 2.5 times the retention of fluvoxamine
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 3.0 between the *Z*-isomer and fluvoxamine maleate; NLT 5.0 between succinyl fluvoxamine and the *Z*-isomer, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$) in the portion of Fluvoxamine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Fluvoxamine Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluvoxamine Maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Solution A, Mobile phase, System suitability solution, Standard solution, and **System suitability:** Proceed as directed in the *Assay*.
Identification solution: 0.35 mg/mL of maleic acid in *Mobile phase*
Sample solution: 1 mg/mL of Fluvoxamine Maleate in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay* except for the *Run time*.
Run time: NLT 6 times the retention of fluvoxamine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Fluvoxamine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of fluvoxamine from the *Standard solution*
C_S = concentration of USP Fluvoxamine Maleate RS in the *Standard solution* (mg/mL)
C_U = concentration of Fluvoxamine Maleate in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Maleic acid[a]	0.19	—	—
Succinyl fluvoxamine[b]	0.50	1.0	0.3
Aminoethyl fluvoxamine[c]	0.67	0.71	0.2
Z-isomer[d]	0.79	1.0	0.5
Fluvoxamine	1.0	—	—
Aminoethyl desmethoxy fluvoxamine[e]	1.2	1.0	0.2
Dealkyl benzyl fluvoxamine[f]	1.7	1.0	0.2
Desmethoxy fluvoxamine[g]	2.0	1.0	0.2
Fluvoxamine oxime[h]	3.5	1.7	0.2
Valerophenone analog[i]	4.2	3.3	0.2
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	1.5

[a] This is the counterion. It is not to be reported or included in the total impurities.
[b] (*E*)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone *O*-[2-[(2-succinyl)amino]ethyl]oxime.
[c] (*E*)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one *O*-{2-[(2-aminoethyl)amino]ethyl}oxime.
[d] (*Z*)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one *O*-(2-aminoethyl)oxime.
[e] (*E*)-1-[4-(Trifluoromethyl)phenyl]pentan-1-one *O*-{2-[(2-aminoethyl)amino]ethyl}oxime.
[f] (*E*)-2-Phenyl-1-[4-(trifluoromethyl)phenyl]ethan-1-one *O*-(2-aminoethyl)oxime.
[g] (*E*)-1-[4-(Trifluoromethyl)phenyl]pentan-1-one *O*-(2-aminoethyl)oxime.
[h] (*E*)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one oxime.
[i] 5-Methoxy-1-(4-(trifluoromethyl)phenyl)pentan-1-one.

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 80° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Fluvoxamine Maleate RS

Fluvoxamine Maleate Tablets

DEFINITION
Fluvoxamine Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

- **PROCEDURE**

▲**Solution A:** 8 g/L of 1-pentanesulfonic acid sodium salt and 1 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.00 ± 0.05.▲ (USP 1-Aug-2019)
Mobile phase: Acetonitrile and ▲*Solution A* (38:62)▲ (USP 1-Aug-2019)

www.webofpharma.com

System suitability solution: ▲Transfer▲ (USP 1-Aug-2019) 6 mg of fluvoxamine maleate to a 50-mL volumetric flask. Heat the sample at 120° for 10 min. Cool to room temperature, and add 3.0 mL of 0.1 N hydrochloric acid. Heat the solution in a water bath for 10 min. Cool to room temperature, add 50 mg of fluvoxamine maleate, and dissolve in 25 mL of *Mobile phase*. Dilute with *Mobile phase* to volume.

Standard solution: 0.05 mg/mL of USP Fluvoxamine Maleate RS in *Mobile phase*

Sample stock solution: ▲Nominally 1 mg/mL of fluvoxamine maleate from Tablets prepared as follows. Finely powder NLT 20 Tablets and transfer a portion of the powder to a suitable volumetric flask.▲ (USP 1-Aug-2019) Add 50% of the flask volume of *Mobile phase*. Sonicate for 15 min followed by mechanical shaking for 15 min. Dilute with *Mobile phase* to volume. Centrifuge a portion of this solution for 10 min.

Sample solution: Nominally 0.05 mg/mL from *Sample stock solution* diluted with *Mobile phase*. ▲Pass through a filter of 0.45-µm or finer pore size.▲ (USP 1-Aug-2019)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 234 nm. ▲For *Identification B*, use a diode array detector in the range of 210–400 nm.▲ (USP 1-Aug-2019)
Column: 4.6-mm × 25-cm; ▲5-µm ▲ (USP 1-Aug-2019) packing L7
Column temperature: 40°
Flow rate: 1.7 mL/min
Injection volume: 20 µL
▲Run time: NLT 2.5 times the retention time of fluvoxamine▲ (USP 1-Aug-2019)

System suitability
Samples: *System suitability solution* and *Standard solution*
▲[NOTE—See *Table 1* for relative retention times.]▲ (USP 1-Aug-2019)
Suitability requirements
Resolution: NLT 2.0 between *Z*-isomer and fluvoxamine maleate; NLT 5.0 between ▲succinyl fluvoxamine▲ (USP 1-Aug-2019) and the *Z*-isomer, *System suitability solution*
▲▲ (USP 1-Aug-2019)
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage ▲of the labeled amount of fluvoxamine maleate▲ (USP 1-Aug-2019) ($C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Fluvoxamine Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fluvoxamine maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL, degassed
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: USP Fluvoxamine Maleate RS▲▲ (USP 1-Aug-2019) in *Medium*
Sample solution: Centrifuge a portion of the solution under test, ▲▲ (USP 1-Aug-2019) and dilute with *Medium*, if necessary. ▲[NOTE—The use of a centrifuge speed of NLT 2000 rpm for NLT 10 min may be suitable.]
Instrumental▲ (USP 1-Aug-2019) conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 246 nm
Analysis
Samples: *Standard solution* and *Sample solution*
▲When there are known interferences due to excipients, excipient interference corrections may be applied, as necessary.
Calculate the percentage of the labeled amount of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)▲ (USP 1-Aug-2019)

Tolerances: NLT 80% (*Q*) of the labeled amount of ▲fluvoxamine maleate▲ (USP 1-Aug-2019) ($C_{15}H_{21}F_3N_2O_2 \cdot C_4H_4O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES, ▲PROCEDURE 1**
Use *Procedure 1* when the impurity profile includes aminoethyl desmethoxy fluvoxamine, dealkyl benzyl fluvoxamine, or fluvoxamine maleamide.
Solution A,▲ (USP 1-Aug-2019) Mobile phase, System suitability solution, Standard solution, and **▲System suitability:▲ (USP 1-Aug-2019)** Proceed as directed in the *Assay*.
▲▲ (USP 1-Aug-2019)
Sample solution: Use the *Sample stock solution*, prepared as directed in the *Assay*.
▲Chromatographic system: Proceed as directed in the *Assay*, except for the *Run time*.
Run time: NLT 6 times the retention of fluvoxamine▲ (USP 1-Aug-2019)

Analysis
Samples: ▲▲ (USP 1-Aug-2019) *Standard solution* and *Sample solution*
▲▲ (USP 1-Aug-2019)
Calculate the percentage of ▲each degradation product▲ (USP 1-Aug-2019) in the portion of Tablets taken:

▲$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$▲ (USP 1-Aug-2019)

www.webofpharma.com

r_U = ▲▲ (USP 1-Aug-2019) peak area of each ▲degradation product▲ (USP 1-Aug-2019) from the *Sample solution*

r_S = peak area of fluvoxamine ▲▲ (USP 1-Aug-2019) from the *Standard solution*

C_S = concentration of USP Fluvoxamine Maleate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fluvoxamine maleate in the *Sample solution* (mg/mL)

F = ▲relative▲ (USP 1-Aug-2019) response factor for each ▲degradation product▲ (USP 1-Aug-2019) (see ▲*Table 1*▲ (USP 1-Aug-2019))

Acceptance criteria: ▲See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Maleic acid[a]	0.19	—	—
Succinyl fluvoxamine[b]	0.50	1.0	0.8
Aminoethyl fluvoxamine[c]	0.67	0.71	0.2
Z-isomer[d]	0.79	1.0	0.5
Fluvoxamine	1.0	—	—
Aminoethyl desmethoxy fluvoxamine[e]	1.18	1.0	0.2
Dealkyl benzyl fluvoxamine[f]	1.74	1.0	0.2
Desmethoxy fluvoxamine[g]	2.00	1.0	0.2
Fluvoxamine oxime[h]	3.45	1.66	0.2
Valerophenone analog[i]	4.2	3.3	0.2
Fluvoxamine maleamide[j]	4.3	1.0	0.2
Any individual unspecified degradation product	—	1.0	0.1
Total degradation products	—	—	1.8

[a] This is the counterion. It is not to be reported or included in the total degradation products for the drug product.
[b] 5-Methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone (*E*)-*O*-[2-[(2-succinyl)amino]ethyl]oxime.
[c] (*E*)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one-*O*-[2-[(2-aminoethyl)amino]ethyl] oxime.
[d] (*Z*)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one *O*-(2-aminoethyl) oxime.
[e] (*E*)-1-[4-(Trifluoromethyl)phenyl]pentan-1-one *O*-[2-[(2-aminoethyl)amino] ethyl] oxime.
[f] (*E*)-2-Phenyl-1-[4-(trifluoromethyl)phenyl]ethan-1-one *O*-(2-aminoethyl) oxime.
[g] (*E*)-1-[4-(Trifluoromethyl)phenyl]pentan-1-one *O*-(2-aminoethyl) oxime.
[h] (*E*)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one oxime.
[i] 5-Methoxy-1-(4-(trifluoromethyl)phenyl)pentan-1-one.
[j] (2*Z*,9*E*)-4-Oxo-10-[4-(trifluoromethyl)phenyl]-8,15-dioxa-5,9-diazahexadeca-2,9-dienoic acid. ▲ (USP 1-Aug-2019)

Change to read:

• **▲ORGANIC IMPURITIES, PROCEDURE 2**

Use *Organic Impurities, Procedure 2* when the impurity profile includes desfluoro fluvoxamine.

Solution A: 13.6 g/L of sodium acetate in water

Mobile phase: Acetonitrile, methanol, and *Solution A* (30:15:55). To each L add 2 mL of triethylamine, and adjust with glacial acetic acid to a pH of 4.5.

Diluent: Methanol and water (60:40)▲ (USP 1-Aug-2019)

System suitability solution: Prepare as directed in the *Assay*.

Standard solution: 0.001 mg/mL of fluvoxamine maleate in *Diluent* prepared by dilution of the *Standard solution* in the *Assay*

Sample stock solution: Prepare as directed in the *Assay*.

Sample solution: Nominally 0.1 mg/mL of fluvoxamine maleate from *Sample stock solution*▲in▲ (USP 1-Aug-2019)*Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm;▲5-µm ▲ (USP 1-Aug-2019) packing L7

Column temperature: 40°

Flow rate: 2 mL/min

▲Injection volumes

System suitability solution: 20 µL

Other solutions: 100 µL

Run time: NLT 2.5 times the retention time of fluvoxamine▲ (USP 1-Aug-2019)

System suitability

Samples: *System suitability solution* and *Standard solution*

▲[NOTE—See *Table 2* for the relative retention times.]▲ (USP 1-Aug-2019)

Suitability requirements

Resolution: NLT 1.0 between *Z*-isomer and fluvoxamine, ▲▲ (USP 1-Aug-2019)*System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

▲▲ (USP 1-Aug-2019)

Calculate the percentage of ▲each degradation product▲ (USP 1-Aug-2019) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = ▲▲ (USP 1-Aug-2019) peak area of each ▲degradation product▲ (USP 1-Aug-2019) from the *Sample solution*

r_S = peak area of fluvoxamine ▲▲ (USP 1-Aug-2019) from the *Standard solution*

C_S = concentration of USP Fluvoxamine Maleate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fluvoxamine maleate in the *Sample solution* (mg/mL)

F = relative response factor for each impurity (see ▲*Table 2*▲ (USP 1-Aug-2019))

Acceptance criteria: ▲See *Table 2*.

USP Monographs

www.webofpharma.com

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desfluoro fluvoxamine[a]	0.58	1.0	0.2
Succinyl fluvoxamine[b]	0.70	1.0	1.2
Aminoethyl fluvoxamine[c]	0.75	1.0	0.2
Z-isomer[d]	0.85	0.5	0.5
Fluvoxamine	1.0	—	—
Desmethoxy fluvoxamine[e]	1.86	1.0	0.2
Fluvoxamine oxime[f]	1.99	1.0	0.2
Valerophenone analog[g]	2.17	1.0	0.2
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.5

[a] (*E*)-1-[4-(Difluoromethyl)phenyl]-5-methoxypentan-1-one *O*-(2-aminoethyl) oxime.
[b] 5-Methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone (*E*)-*O*-[2-[(2-succinyl) amino]ethyl]oxime.
[c] (*E*)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one-*O*-{2-[(2-aminoethyl)amino]ethyl} oxime.
[d] (*Z*)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one *O*-(2-aminoethyl) oxime.
[e] (*E*)-1-[4-(Trifluoromethyl)phenyl]pentan-1-one *O*-(2-aminoethyl) oxime.
[f] (*E*)-5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one oxime.
[g] 5-Methoxy-1-(4-(trifluoromethyl)phenyl)pentan-1-one. ▲(USP 1-Aug-2019)

ADDITIONAL REQUIREMENTS

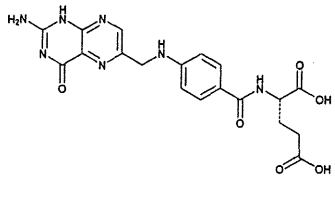
Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at ▲controlled▲ (USP 1-Aug-2019) room temperature.

Change to read:

- **LABELING:** ▲The labeling indicates which test for *Organic Impurities* is used only if *Procedure 1* is not used.▲ (USP 1-Aug-2019)
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fluvoxamine Maleate RS

Folic Acid



$C_{19}H_{19}N_7O_6$ 441.40

L-Glutamic acid, *N*-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-;
N-[*p*-[[(2-Amino-4-hydroxy-6-pteridinyl)methyl]amino] benzoyl]-L-glutamic acid [59-30-3].

DEFINITION

Folic Acid contains NLT 97.0% and NMT 102.0% of folic acid ($C_{19}H_{19}N_7O_6$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Sample solution: 10 µg/mL in 0.1 N sodium hydroxide solution
  Acceptance criteria: Meets the requirements. The ratio A_{256}/A_{365} is 2.80–3.00.

ASSAY

- **PROCEDURE**
  [NOTE—Use low-actinic glassware throughout the following procedure.]
  3 N phosphoric acid: 98 g/L of phosphoric acid in water
  6 N ammonium hydroxide: Dilute 40 mL of ammonium hydroxide with water to 100 mL.
  Mobile phase: Transfer 2.0 g of monobasic potassium phosphate into a 1000-mL volumetric flask, and dissolve in 650 mL of water. Add 15.0 mL of a solution of 0.5 M tetrabutylammonium hydroxide in methanol, 7.0 mL of *3 N phosphoric acid*, and 270 mL of methanol. Cool to room temperature, adjust with *3 N phosphoric acid* or *6 N ammonium hydroxide* to a pH of 5.0, and dilute with water to volume. Recheck the pH before use.
  Internal standard solution: 2 mg/mL of methylparaben in *Mobile phase*. Dissolve the methylparaben first with methanol (about 4% of the final volume), and dilute with *Mobile phase* to volume.
  Standard stock solution: 1 mg/mL of USP Folic Acid RS in *Mobile phase*. Dissolve the folic acid with the aid of 10% ammonium hydroxide (about 1% of the final volume), and dilute with *Mobile phase* to volume.
  Standard solution: Transfer 4.0 mL of *Standard stock solution* and 4.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Sample stock solution: Transfer 100 mg of Folic Acid to a 100-mL volumetric flask, and dissolve in 40 mL of *Mobile phase* and 1 mL of 10% ammonium hydroxide. Dilute with *Mobile phase* to volume.
  Sample solution: Transfer 4.0 mL of *Sample stock solution* and 4.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 4.0-mm × 25-cm; packing L1
  Flow rate: 1.2 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 3.6 between methylparaben and folic acid
  Relative standard deviation: NMT 2.0% for the ratios of the folic acid peak area to the internal standard peak area
  Analysis
  Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of folic acid ($C_{19}H_{19}N_7O_6$) in the sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = internal standard ratio (peak response of folic acid/peak response of the internal standard) from the *Sample solution*

R_S = internal standard ratio (peak response of folic acid/peak response of the internal standard) from the *Standard solution*

C_S = concentration of USP Folic Acid RS in the *Standard stock solution* (mg/mL)

C_U = concentration of Folic Acid in the *Sample stock solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%
- **RELATED COMPOUNDS**

3 N phosphoric acid, 6 N ammonium hydroxide, Internal standard solution, Standard stock solution, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sample solution: Use the *Sample stock solution*, prepared as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Allow the *Sample solution* to elute for NLT 2 times the retention time of folic acid. Record the chromatogram, and measure the areas of all the peaks.

Calculate the percentage of total secondary peaks in the portion of Folic Acid taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = sum of the areas of all the peaks except that of the folic acid peak

r_T = sum of the areas of all the peaks

Acceptance criteria: NMT 2.0%

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩

Analysis: Proceed as directed in the chapter, except stir the methanol solvent before and during the addition of the test specimen and during the titration.

Acceptance criteria: NMT 8.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Folic Acid RS

Folic Acid Injection

» Folic Acid Injection is a sterile solution of Folic Acid in Water for Injection prepared with the aid of Sodium Hydroxide or Sodium Carbonate. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

Change to read:

USP Reference standards ⟨11⟩—

USP Folic Acid RS

▲ (ERR 1-Aug-2018)

Identification—To a volume of the Injection equivalent to about 100 mg of folic acid add water to make about 25 mL. Adjust with hydrochloric acid to a pH of 3.0, cool to 5°, then filter, and wash the precipitate of folic acid with cold water until the last washing shows an absence of chloride. Then wash with acetone, and dry at 80° for 1 hour: the UV absorption spectrum of a 1 in 100,000 solution of the folic acid so obtained in sodium hydroxide solution (1 in 250) exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Folic Acid RS, concomitantly measured. The ratio A_{256}/A_{365} is between 2.80 and 3.00.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 357.1 USP Endotoxin Units per mg of folic acid.

pH ⟨791⟩: between 8.0 and 11.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *ASSAY* under *Folic Acid Tablets*.

Assay preparation—Dilute an accurately measured volume of Injection, quantitatively and stepwise, with an aqueous solvent containing 2 mL of ammonium hydroxide and 1 g of sodium perchlorate per 100 mL, to obtain a solution having a concentration close to that of the *Standard preparation* and between 0.20 and 0.80 mg per mL.

Procedure—Proceed as directed in the *Assay* under *Folic Acid Tablets*, and calculate the quantity, in mg, of folic acid ($C_{19}H_{19}N_7O_6$) in each mL of the Injection.

Folic Acid Compounded Oral Solution

DEFINITION

Folic Acid Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$).

Prepare Folic Acid Compounded Oral Solution, 1 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Folic Acid	100 mg
Sodium Bicarbonate	1000 mg
Glycerin	35 mL
Purified Water, a sufficient quantity to make	100 mL

Dissolve the *Sodium Bicarbonate* in 60 mL of *Purified Water* followed by the *Folic Acid*. Add the *Glycerin* to the mixture. Mix well. Add sufficient *Purified Water* to bring to final volume.

ASSAY

- **PROCEDURE**

Solution A: 50 mM monobasic potassium phosphate adjusted with phosphoric acid to a pH of 2.5

www.webofpharma.com

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Methanol (%)
0.0	95	5
2.0	95	5
10.0	90	10
25.0	85	15
26.0	65	35
26.1	95	5
36.0	95	5

Diluent: 0.01 N sodium hydroxide
Standard solution: 0.2 mg/mL of USP Folic Acid RS in *Diluent*
Sample solution: Transfer 1.0 mL of Oral Solution to a 5-mL volumetric flask and add *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 10-cm; 2.6-µm packing L1
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for folic acid is about 21.4 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of folic acid from the *Sample solution*
r_S = peak response of folic acid from the *Standard solution*
C_S = concentration of USP Folic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of folic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **PH** ⟨791⟩: 7.8–8.8

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant plastic containers. Store in a refrigerator or at controlled room temperature.
• **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored in a refrigerator or at controlled room temperature.
• **LABELING:** Label it to state the *Beyond-Use Date*.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Folic Acid RS

Folic Acid Tablets

DEFINITION
Folic Acid Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$).

IDENTIFICATION
• **A. ULTRAVIOLET ABSORPTION**
Sample solution: Digest the quantity of powdered Tablets, equivalent to 100 mg of folic acid, with 100 mL of 0.1 N sodium hydroxide, and filter. Adjust with hydrochloric acid to a pH of 3.0. Cool to 5°, filter, and wash the precipitate of folic acid with cold water until the last washing shows an absence of chloride. Then wash with acetone, and dry at 80° for 1 h. Dissolve the residue in 0.1 N sodium hydroxide to obtain a 10-µg/mL solution.
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Folic Acid RS, concomitantly measured. The absorbance ratio A_{256}/A_{365} is 2.80–3.00.

ASSAY
• **PROCEDURE**
Mobile phase: Transfer 35.1 g of sodium perchlorate and 1.40 g of monobasic potassium phosphate to a 1-L volumetric flask. Add 7.0 mL of 1 N potassium hydroxide and 40 mL of methanol, dilute with water to volume, and mix. Adjust with 1 N potassium hydroxide or phosphoric acid to a pH of 7.2.
Diluent: Aqueous solution containing 2 mL of ammonium hydroxide and 1 g of sodium perchlorate per 100 mL
System suitability solution: 0.2 mg/mL each of USP Folic Acid RS and USP Folic Acid Related Compound A RS in *Diluent*. [NOTE—Before use, pass through a filter of 1-µm or finer pore size.]
Standard solution: 0.20 mg/mL of USP Folic Acid RS, corrected for water content in *Diluent*
Sample solution: Equivalent to 0.2 mg/mL of folic acid, from NLT 20 powdered Tablets in *Diluent*; shake gently to aid dissolution, and filter, discarding the first portion.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection size: 25 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.6 between folic acid related compound A (calcium formyltetrahydrofolate) and folic acid, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of folic acid from the *Sample solution*
r_S = peak area of folic acid from the *Standard solution*
C_S = concentration of USP Folic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of folic acid in the *Sample solution* (mg/mL)

www.webofpharma.com

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: Solution having a known concentration of USP Folic Acid RS, corrected for water content, in *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the *Assay*, making any necessary modifications.

Calculate the percentage of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

r_U = peak area of folic acid from the *Sample solution*

r_S = peak area of folic acid from the *Standard solution*

C_S = concentration of USP Folic Acid RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

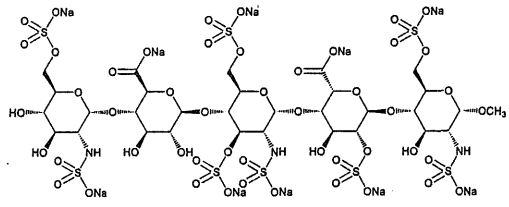
• **USP REFERENCE STANDARDS** ⟨11⟩

USP Folic Acid RS

USP Folic Acid Related Compound A RS

Calcium formyltetrahydrofolate.

Fondaparinux Sodium



$C_{31}H_{43}N_3O_{49}S_8Na_{10}$ 1728.08

α-D-Glucopyranoside, methyl *O*-2-deoxy-6-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranuronosyl-(1→4)-*O*-2-deoxy-3,6-di-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranosyl-(1→4)-*O*-2-*O*-sulfo-α-L-idopyranuronosyl-(1→4)-2-deoxy-2-(sulfoamino)-, 6-(hydrogen sulfate), decasodium salt;

Methyl *O*-2-deoxy-6-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranuronosyl-(1→4)-*O*-2-deoxy-3,6-di-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranosyl-(1→4)-*O*-2-*O*-sulfo-α-L-idopyranuronosyl-(1→4)-2-deoxy-6-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranoside, decasodium salt [114870-03-0].

DEFINITION

Fondaparinux Sodium is the sodium salt of a synthetic sulfated pentasaccharide anticoagulant based on the antithrombin binding moiety of heparin. It is synthesized from a natural source of chirally pure sugars (mono- and di-saccharides). A range of coupling, (de)protection and functionalization reactions leads to crude fondaparinux sodium, which is further purified to yield the drug substance. Fondaparinux Sodium contains NLT 95.0% and NMT 103.0% of fondaparinux sodium, calculated on the anhydrous and solvent-free basis. Fondaparinux Sodium is a white to off-white powder.

IDENTIFICATION

• **A. ^{13}C NMR SPECTRUM**

NMR reference: Dissolve in 1 mL of deuterium oxide 20 mg of (2,2,3,3-(d4)-3-(trimethylsilyl)propionic acid sodium salt (TSP), enriched to 98% deuterated or equivalent, as a chemical shift reference.

Standard solution: NLT 40 mg/mL of USP Fondaparinux Sodium Identification RS in deuterium oxide

Sample solution: NLT 40 mg/mL of Fondaparinux Sodium in deuterium oxide

Instrumental conditions

(See *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩.)

Mode: NMR, pulsed (Fourier transform)

Frequency: NLT 100 MHz (for ^{13}C)

Temperature: 40°

System suitability

Sample: *Standard solution*

Using a pulsed (Fourier transform) NMR spectrometer operating at NLT 100 MHz for ^{13}C, acquire a free induction decay (FID) using a 90° pulse and a 5-s delay. Record the ^{13}C NMR spectra of the *NMR reference* at 40°, and set the trimethylsilyl resonance to 0.0 ppm. Collect the ^{13}C NMR spectrum with a spectral window of at least 235 to −10 ppm with spinning at 20 Hz, using line broadening of NLT 1. The number of transients should be adjusted until the signal-to-noise ratio of the signal for the C-1 in the β-D-glucopyranosyluronic acid ring of fondaparinux sodium in the *Standard solution* meets the suitability requirements. The *Standard solution* shall be run at least daily when the *Sample solution* is being run. The chemical shift for the C-1 resonance of the β-D-glucopyranosyluronic acid ring of fondaparinux sodium in the *Standard solution* should be observed at 103.9 ± 0.1 ppm. Record the ^{13}C NMR spectrum of the *Sample solution* at 40° using the same conditions.

Suitability requirements

Number of transients: The signal-to-noise of the β-D-glucopyranosyluronic acid ring of fondaparinux sodium in the *Standard solution* is at least 20/1 in the region near 103.9 ppm.

Chemical shift: The trimethylsilyl resonance for the *NMR reference* should be set to 0.0 ppm, which acts as an external calibration for all samples.

Chemical shifts for system suitability: The *O*-methyl and two carbonyl carbons of fondaparinux sodium should be observed at 58.2, 176.7, and 178.0 ppm, all ± 0.3 ppm, respectively, in the *Standard solution*.

Analysis

Sample: *Sample solution*

Acceptance criteria: Resonances for fondaparinux sodium should be observed at 58.2, 59.5, 60.5, 60.8, 68.9, 69.2, 69.6, 98.9, 100.4, 101.1, 102.4, 103.9, 176.7, and 178.0 ppm. The chemical shifts of these signals do not differ by more than ±0.3 ppm. Other signals of variable heights and chemical shifts, attributable to fondaparinux sodium, may be seen between 58.0–80.5 ppm and 98.7–104.5 ppm.

www.webofpharma.com

• **B. CHROMATOGRAPHIC IDENTITY**

Analysis: Proceed as directed in the *Assay*.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to ±5% of that of the *Standard solution*.

• **C. SODIUM DETERMINATION**

Analysis: Proceed as directed in *Sodium Determination*.

Acceptance criteria: It meets the requirements.

ASSAY

• **PROCEDURE**

5 mM phosphate solution: Dissolve 0.210 g of monobasic sodium phosphate dihydrate and 0.650 g of dibasic sodium phosphate dihydrate in water, and dilute with water to 1000 mL. pH of solution is approximately 7.3.

Solution A: 15 ± 10 ppm dimethylsulfoxide (DMSO) in *5 mM phosphate solution* (1 in 67000, v/v). Filter before use.

Solution B: 2.0 M sodium chloride solution with *5 mM phosphate solution*

Mobile phase: See *Table 1*.

[NOTE—Make adjustments to *Solution A* as necessary, and degas the *Mobile phase* and the sample before use. Dissolved gas in the injected solution may lead to baseline interference. Degassing of the *Mobile phase* is critical to obtain a suitable signal-to-noise ratio and higher sensitivity. An eluant generator[1] installed between the injector and the column may reduce the baseline interference.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	50	50
5	50	50
25	5	95
30	5	95
35	50	50
50	50	50

System suitability solution: 5.0 mg/mL of USP Fondaparinux Sodium System Suitability Mixture A RS

Standard solution: 5.0 mg/mL of USP Fondaparinux Sodium for Assay RS in water. Prepare in duplicate.

Sensitivity check solution: 0.01 mg/mL of USP Fondaparinux Sodium for Assay RS in water from the *Standard solution*

Sample solution: 5.0 mg/mL of fondaparinux sodium in water. Prepare in at least duplicate.

Blank: Water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm × 25-cm; packing L46

Column temperature: 25°

Flow rate: 1.0 mL/min

Injection volume: 100 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, *Sensitivity check solution*, and *Blank*

Inject the *Blank* in duplicate, the *Sensitivity check solution*, and the *System suitability solution*. Inject the *Standard solution* at least six times consecutively.

Suitability requirements

Specificity and baseline drift

The chromatogram of the second *Blank* injection shows a baseline drift between 0.00 and 0.02 AU over 30 min. If necessary, adjust the DMSO content of the *Mobile phase* until an acceptable baseline is achieved.

The chromatogram of the second *Blank* injection does not contain peaks between 3.00 and 30.00 min.

Signal-to-noise ratio: NLT 10 for the fondaparinux peak in the chromatogram of the *Sensitivity check solution*

Chromatogram similarity: The chromatogram of the *System suitability solution* corresponds to that of the chromatogram provided with USP Fondaparinux Sodium System Suitability Mixture A RS.

Relative standard deviation: For six consecutive injections of the *Standard solution*, the calculated % RSD of the area of the fondaparinux peak is NMT 2.0%. The retention time of the fondaparinux peak should be ±5% of the mean value. The calculated % RSD of the response factors for all replicate injections of the *Standard solution* is NMT 2.0%. The calculated % RSD of the pooled response factors for all injections of the *Standard solution* is NMT 2.0%. The % RSD of the mean response factors for each duplicate *Standard solution* is NMT 2.0%.

Analysis

Samples: *Standard solution* and *Sample solution*

Inject the *Standard solution* at least six times consecutively and the *Sample solution* in duplicate. Record the chromatograms and measure the retention times and areas for the major peaks (excluding peaks before 3.00 and after 30.00 min).

For each injection of the *Standard solution*, calculate a response factor (F_R):

$$F_R = (C_S/r_S)$$

C_S = concentration of fondaparinux sodium in the *Standard solution* (mg/mL)

r_S = peak response of fondaparinux sodium from the *Standard solution*

Calculate the mean response factor (F_M) for each duplicate injection, and determine the % RSD for the peak areas of fondaparinux sodium (r_S) for six consecutive injections of the *Standard solution*.

Using the mean response factor, calculate the percentage of fondaparinux in the portion of sample taken:

$$\text{Result (\% w/w)} = (F_M \times r_U \times D \times 100)/W$$

F_M = mean response factor for each duplicate injection

r_U = peak response of fondaparinux sodium in the *Sample solution*

D = dilution factor for the sample (mL)

W = weight of fondaparinux sodium taken to prepare the *Sample solution* (mg)

Acceptance criteria: 95.0%–103.0% on an anhydrous and solvent-free basis

OTHER COMPONENTS

• **SODIUM DETERMINATION**

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

2% Nitric acid solution: 21 mL nitric acid diluted with water to 1000 mL

Sodium solution: 1000 ppm sodium in *2% Nitric acid solution*

Standard solutions: Prepare *Standard solutions* containing 20, 30, 40, 50, and 60 ppm of sodium ion from the *Sodium solution*, diluting with *2% Nitric acid solution*.

[1] One suitable eluant generator is Dionex DEGAS EG40/50 (12 × 17 cm, thickness 2.2 cm).

www.webofpharma.com

Sample solution: 0.3 mg/mL of Fondaparinux Sodium in *2% Nitric acid solution*
Analysis: Concomitantly determine the absorbances of the *Sample solution* and *Standard solutions* at 330.2 nm by using a sodium hollow-cathode lamp and an air–acetylene flame. Using the absorbances of the *Standard solutions*, determine the sodium content in the *Sample solution* after appropriate blank correction.
Acceptance criteria: 11.5%–15.0% on the anhydrous and solvent-free basis

IMPURITIES

• **FREE SULFATE AND RESIDUAL CHLORIDE DETERMINATION**

Mobile phase: 3 mM carbonate solution containing 0.106 g of sodium carbonate and 0.168 g of sodium hydrogen carbonate in 1000 mL of water
Standard solution 1: Dissolve 164.9 mg of sodium chloride in 80 mL of water, and dilute with water to 100.0 mL.
Standard solution 2: Dissolve 147.9 mg of anhydrous sodium sulfate in 80 mL of water, and dilute with water to 100.0 mL.
Standard solution 3: Dilute 1.0 mL of *Standard solution 1* with water to 100.0 mL.
Standard solution 4: Dilute 1.0 mL of *Standard solution 2* with water to 100.0 mL.
Calibration standard solutions: Using appropriate volumes of the *Standard solutions*, prepare calibration standards as shown in *Table 2*.

Table 2

Concentration	Volume of Sulfate Solution (mL)	Volume of Chloride Solution (mL)	Final Volume (mL)
0.5 ppm SO_4^{-2}/1 ppm Cl^-	5.0, *Standard solution 4*	10.0, *Standard solution 3*	100.0
2.5 ppm SO_4^{-2}/2.5 ppm Cl^-	0.50, *Standard solution 2*	0.50, *Standard solution 1*	200.0
5.0 ppm SO_4^{-2}/5.0 ppm Cl^-	0.50, *Standard solution 2*	0.50, *Standard solution 1*	100.0
20.0 ppm SO_4^{-2}/20 ppm Cl^-	2.0, *Standard solution 2*	2.0, *Standard solution 1*	100.0
50.0 ppm SO_4^{-2}/50 ppm Cl^-	5.0, *Standard solution 2*	5.0, *Standard solution 1*	100.0

Resolution solution: Dissolve 150 mg of sodium nitrite in 100.0 mL of water. Combine 2.0 mL of this solution and 2.0 mL of *Standard solution 1* in 80 mL of water, and dilute with water to 100.0 mL.
Sample solution: 3 mg/mL of Fondaparinux Sodium in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductivity (range 200 µS, suppressor current 300 mA)
Column: 4.6-mm × 5-cm; packing L23, coupled with a neutralization micromembrane suppressor[2]
Regenerating solvent for the suppressor: Ultrapurified water in a counter current direction
Column temperature: Ambient
Flow rate: 1.0 mL/min
Injection volume: 50 µL
Run time: 24 min
System suitability
Samples: *Calibration standard solutions* and *Resolution solution*
Suitability requirements
Resolution: NLT 2 between the chloride and nitrite ion peaks, *Resolution solution*
Response stability: ±5% between injections of 5 ppm of each of the *Calibration standard solutions* before and after the *Sample solution*
Relative standard deviation: NMT 3% for NLT 5 injections of 5-ppm *Calibration standard solutions*
Analysis
Sample: *Sample solution*
[NOTE—Regenerate the anion-exchange column for 15 min with 0.1 M sodium hydroxide after each injection of fondaparinux sample, followed by equilibration with *Mobile phase* for 21 min.]
Inject 50 µL of each of the *Calibration standard solutions* and 50 µL of the *Sample solution* in triplicate. The peak area responses for the chloride and sulfate ion peaks in the chromatograms obtained with the *Calibration standard solutions* show two peaks corresponding respectively to chloride ions at a retention time of approximately 3.6 min and to sulfate ions at a retention time of approximately 14.1 min. The *Calibration standard solutions* and the corresponding standard concentrations are used to construct five-point calibration curves for both chloride and sulfate ions. The concentrations of sulfate and chloride ions in the *Sample solutions* are calculated using the standard curves.
Calculations: Calculate the free sulfate and residual chloride ion contents in % w/w of fondaparinux sodium in the solution to be examined:

$$\text{Result} = C_S \times F \times (1/C_U) \times 100$$

C_S = concentration of the ion calculated from the quadratic calibration equation (µg/mL)
F = conversion factor (µg/mL to mg/mL)
C_U = concentration of Fondaparinux Sodium in the *Sample solution* (mg/mL)

Report the average of the triplicate determinations.
Acceptance criteria: NMT 0.30% free sulfate; NMT 1.0% chloride

• **ORGANIC IMPURITIES**

Analysis: Proceed as directed in the *Assay*.
Samples: *System suitability solution*, *Standard solution*, *Sensitivity check solution*, *Sample solution*, and *Blank*
Calculate the percentage of each individual impurity in the portion of Fondaparinux Sodium taken:

$$\text{Result (\% area/area)} = [r_U/(r_S + r_T)] \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of fondaparinux sodium from the *Sample solution*
r_T = sum of all peak responses for impurities from the *Sample solution*

The total impurities content (% area/area) is the sum of all mean unrounded contents of an individual impurity that are NLT 0.200%.
Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Impurity peak A[a]	0.93	0.8 (a/a)
Impurity peak B[a]	1.2[b]	0.6 (a/a)

[2] One suitable suppressor is Dionex ASRS 300 4 mm.

USP Monographs

www.webofpharma.com

Table 3 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any unspecified impurity	—	0.3
Total impurities	—	NMT 2.0%

[a] Impurity peak A contains two structures: Methyl (2-deoxy-2-sodium sulfoamino-6-*O*-sodium sulfonato-α-D-glucopyranosyl)-(1→4)-(sodium β-D-glucopyranosyluronate)-(1→4)-(2-deoxy-2-sodium sulfoamino-3,6-di-*O*-sodium sulfonato-α-D-glucopyranosyl)-(1→4)-(sodium 2,3-di-*O*-sodium sulfonato-α-L-idopyranosyluronate)-2-deoxy-2-sodium sulfoamino-6-*O*-sodium sulfonato-α-D-glucopyranoside; and Methyl (2-deoxy-2-formylamino-6-*O*-sodium sulfonato-α-D-glucopyranosyl)-(1→4)-(sodium-β-D-glucopyranosyluronate)-(1→4)-(2-deoxy-2-sodium sulfoamino-3,6-di-*O*-sodium sulfonato-α-D-glucopyranosyl)-(1→4)-(sodium 2-*O*-sodium sulfonato-α-L-idopyranosyluronate)-2-deoxy-2-sodium sulfoamino-6-*O*-sodium sulfonato-α-D-glucopyranoside.

[b] Impurity peak B can appear as a complex set of peaks and not fully resolved. In such a case, the integration should be performed such that all such peaks are combined.

• **PYRIDINE AND ETHANOL DETERMINATION**

(See *Residual Solvents* ⟨467⟩.)

Pyridine standard solution: In a 100-mL volumetric flask containing about 20 mL of water, transfer 101.8 µL of pyridine accurately. Dilute with water to 100 mL.

Internal standard solution: 500-µg/mL solution of 1-butanol in water

Standard solution 1: In a 100-mL volumetric flask containing about 20 mL of water, transfer accurately 1.27 mL of ethanol and 1.0 mL of *Pyridine standard solution*. Dilute with water to 100.0 mL.

Standard solution 2: *Standard solution 1* and water (1:100). Prepare in duplicate (*A* and *B*).

Sample stock solution: 10 mg/mL of Fondaparinux Sodium in water in triplicate

Sample solution: 2 mg/mL of Fondaparinux Sodium in water from the *Sample stock solution*

Blank: Water

Sample preparation: For the *Blank*, transfer 5.0 mL of water and 5 g of potassium carbonate to an appropriate headspace vial, apply stopper, cap, and mix. For samples and standards, add 5.0 mL of the *Sample solution* or *Standard solution 2* with 5 g of potassium carbonate and 0.1 mL of the *Internal standard solution* to an appropriate headspace vial, apply stopper, cap, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC with headspace sampler

Detector: Flame ionization

Column: 0.32-mm × 30-m fused silica, 1.8-µm film thickness; support G43

Temperatures

[NOTE—At initial temperature NLT 3 min between injections.]

Injector: 140°

Detector: 250°

Column: See *Table 4*.

Table 4

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	20
40	10	240	0
240	—	240	5

Carrier gas: Helium with a linear velocity of 20–30 cm/s

Injection type: Split ratio, 1:7

Head space autosampler

Sample equilibration temperature: 80°

Sample equilibration time: 60 min

Transfer line temperature: 110°

System suitability

Samples: *Standard solution 2* (*A* and *B*) and *Blank*

Assay a water *Blank* followed by six consecutive samples of *Standard solution 2(A)*, followed by a single injection of *Standard solution 2(B)*.

Suitability requirements

Blank: The chromatogram of the water *Blank* should not present a peak corresponding to ethanol or pyridine.

Signal-to-noise ratio: NLT 40 of the pyridine peak in the chromatogram of *Standard solution 2(A)*

Relative standard deviation: NMT 5% for the average areas of the chromatographic peaks for ethanol and pyridine in six consecutive assays

Analysis

Samples: *Internal standard solution*, *Standard solution 2(A)*, and *Sample solution*

Calculations: Calculate the ethanol and pyridine content in ppm (µg/g) in the portion of Fondaparinux Sodium taken:

$$\text{Result} = C_S \times (R_U/R_S) \times (V/M) \times D$$

C_S = concentration of *Standard solution 2* (µg/mL)

R_U = peak response ratio of solvent "s" in the *Sample solution* to solvent "s" in the *Internal standard solution*

R_S = peak response ratio of solvent "s" in *Standard solution 2* to solvent "s" in the *Internal standard solution*

V = volume of solution used to prepare the *Sample solution* (mL)

M = mass of sample dissolved to prepare the *Sample solution* (g)

D = dilution factor of the *Sample solution*

The average of three independent assays constitutes the results.

Acceptance criteria: NMT 5×10^4 ppm for ethanol and 50 ppm for pyridine

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 25 USP Endotoxin Units/mg.

• **PH** ⟨791⟩**:** 6.0–8.0, in a solution, at 20°–25° (2.5% w/v)

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩**:** NMT 350 cfu/g

• **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** It contains NMT 20.0% (w/w).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at or below 25° in a dry environment.

• **LABELING:** Label to indicate mass of active drug substance per container.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Fondaparinux Sodium for Assay RS

USP Fondaparinux Sodium Identification RS

USP Fondaparinux Sodium System Suitability Mixture A RS

USP Monographs

www.webofpharma.com

Fondaparinux Sodium Injection

DEFINITION

Fondaparinux Sodium Injection is a sterile solution of Fondaparinux Sodium in Water for Injection with sodium chloride added for isotonicity. It is a clear, colorless to slightly yellow solution.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Proceed as directed in the chapter. Meets the requirements of the *Chloride and Sulfate* ⟨221⟩ test.

ASSAY

- **PROCEDURE**

5 mM phosphate solution: 0.210 g of monobasic sodium phosphate dihydrate and 0.650 g of dibasic sodium phosphate dihydrate. Dissolve in and dilute with water to 1000 mL. pH is approximately 7.3.

Solution A: 15 ± 10 ppm of dimethylsulfoxide (DMSO) in *5 mM phosphate solution* (1 in 67000, v/v)

Solution B: 2.0 M sodium chloride solution in *5 mM phosphate solution*

Mobile phase: See *Table 1*. [NOTE—Make adjustments to *Solution A* as necessary, and degas the *Mobile phase* before use. Dissolved gas in the injected solution may lead to baseline interference. Degassing of the *Mobile phase* is critical to obtain a suitable signal-to-noise ratio and higher sensitivity. An eluant generator[1] installed between the injector and the column may reduce the baseline interference.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	50	50
5	50	50
25	5	95
30	5	95
35	50	50
50	50	50

System suitability solution: 5.0 mg/mL of USP Fondaparinux Sodium System Suitability Mixture B RS

Standard solution: 5.0 mg/mL of USP Fondaparinux Sodium for Assay RS in water. Prepare in duplicate.

Sensitivity check solution: 0.01 mg/mL of USP Fondaparinux Sodium for Assay RS in water from the *Standard solution*

Sample solution: Transfer the contents of prefilled syringes to a suitable container, and mix well. Dilute with water, if needed, to obtain a 5.0-mg/mL solution of fondaparinux sodium.

Blank: Water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm × 25-cm; packing L46

Column temperature: 25°

Flow rate: 1.0 mL/min

Injection volume: 100 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, *Sensitivity check solution*, and *Blank*

Inject the *Blank* in duplicate, the *Sensitivity check solution*, and the *System suitability solution*. Inject the *Standard solution* at least six times consecutively.

Suitability requirements

Specificity and baseline drift: The chromatogram of a second *Blank* injection shows a baseline drift between 0.00 and 0.02 AU over 30 min. If necessary, adjust the DMSO content of the *Mobile phase* until an acceptable baseline is achieved. The chromatogram of a second *Blank* injection does not contain peaks between 3.00 and 30.00 min.

Chromatogram similarity: The chromatogram of the *System suitability solution* corresponds to that of the reference chromatogram provided with USP Fondaparinux Sodium System Suitability Mixture B RS.

Signal-to-noise ratio: NLT 10 for the fondaparinux peak in the chromatogram of the *Sensitivity check solution*

Resolution: NLT 1.2 between fondaparinux related compound C and fondaparinux related compound D, *System suitability solution*; NLT 1.1 between fondaparinux related compound F and fondaparinux related compound G (see *Table 2*), *System suitability solution*

Standard agreement: The difference in the mean response factors for each *Standard solution* is NMT 2.0%.

Relative standard deviation: For six consecutive injections of the *Standard solution* the calculated % RSD of the area of the fondaparinux peak is NMT 2.0%. The retention time of the fondaparinux peak should be ±5% of the mean value. The calculated % RSD of the response factors for six consecutive injections of the *Standard solution* is NMT 2.0%. The calculated % RSD of the pooled response factors for all injections of the *Standard solution* is NMT 2.0%. The % RSD of the mean response factors for the duplicate preparations of the duplicate *Standard solutions* is NMT 2.0%.

Analysis

Samples: *Standard solution* and *Sample solution*

Inject the *Standard solution* at least six times consecutively. Inject duplicate preparations of the *Sample solution*. Record the chromatograms, and measure the retention times and areas for the major peaks (excluding peaks before 3.00 and after 30.00 min).

Calculations: For each injection of the *Standard solution* calculate a response factor (F_R):

$$F_R = (C_S / r_S)$$

C_S = concentration of fondaparinux sodium in the *Standard solution* (mg/mL)

r_S = peak response of fondaparinux sodium from the *Standard solution*

Relative retention times (RRT) are calculated by dividing the retention time of the peak by the retention time of fondaparinux established by the *Standard solution*. Using the mean response factor (F_M), calculate the concentration (mg/mL) of fondaparinux sodium in each injection of the *Sample solution*:

$$\text{Result} = F_M \times r_U \times D_U$$

F_M = mean response factor from the *Standard solution*

r_U = peak response of fondaparinux sodium in the *Sample solution*

[1] One suitable eluant generator is Dionex DEGAS EG40/50 (12 × 17 cm, thickness 2.2 cm).

www.webofpharma.com

D_U = dilution factor for the *Sample solution*, if needed

Acceptance criteria: 90%–105% (for the 2.5-mg/0.5-mL injection) or 95%–105% (for the 5.0-mg/0.4-mL, 7.5-mg/0.6-mL, and 10-mg/0.8-mL injections)

IMPURITIES

• **FREE SULFATE DETERMINATION**

[NOTE—Regenerate the anion-exchange column for 15 min with 0.1 M sodium hydroxide after each injection of fondaparinux sample, followed by equilibration with *Mobile phase* for 21 min.]

Mobile phase: 3 mM carbonate solution using 0.106 g of sodium carbonate and 0.168 g of sodium hydrogen carbonate in 1000 mL of water

Standard solution 1: Prepare a 1000-ppm sulfate solution, using anhydrous sodium sulfate in water.

Standard solution 2: Prepare a 10-ppm sulfate solution by diluting *Standard solution 1* in water.

Sensitivity check solution: Dilute 1.0 mL of *Standard solution 2* with water to 5.0 mL.

Resolution solution: 0.100 g of anhydrous sodium sulfate and 0.100 g of sodium chloride. Dissolve in and dilute with water to 100.0 mL. Dilute 1.0 mL with water to 100.0 mL.

Sample solution: In triplicate, combine and mix the contents of a suitable number of syringes. Dilute 0.8 mL (strengths of 5.0 mg/0.4 mL, 7.5 mg/0.6 mL, and 10.0 mg/0.8 mL) or 2.0 mL (strengths of 1.5 mg/0.3 mL and 2.5 mg/0.5 mL) with water to 5.0 mL.

Blank: A sample of the water used to prepare other solutions

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*).

Mode: LC

Detector: Conductivity; range 200 µS, suppressor current 300 mA

Column: 4.6-mm × 5-cm; packing L23, coupled with a neutralization micromembrane suppressor[2]

Column temperature: Ambient

Regenerating solvent for the suppressor: Ultrapurified water in a counter current direction

Flow rate: 1.0 mL/min

Injection volume: 50 µL

Run time: 24 min

System suitability

Samples: *Standard solution 2*, *Sensitivity check solution*, *Resolution solution*, and *Blank*

Suitability requirements

Specificity: The chromatogram of a second *Blank* injection does not contain a peak corresponding to the sulfate ion.

Signal-to-noise-ratio: NLT 10, *Sensitivity check solution*

Resolution: NLT 10 between the sulfate and chloride peaks, *Resolution solution*

Relative standard deviation: NMT 5% of the response factors for six consecutive injections of *Standard solution 2*

Standard agreement: NMT 5% difference in the mean response factors for each *Standard solution 2* injection

Analysis: Inject the *Blank* in duplicate, the *Sensitivity check solution*, and the *Resolution solution*. Inject *Standard solution 2* at least six times consecutively. Inject triplicate preparations of the *Sample solution*. Record the chromatograms, and measure the retention times and areas for the sulfate peaks found.

Calculations: For each injection of *Standard solution 2*, calculate a response factor (F):

$$F = (C_S/r_S)$$

C_S = concentration of sodium sulfate in *Standard solution 2* (mg/mL)

r_S = peak response of the sulfate peak from *Standard solution 2*

Using the mean response factor (F_M), calculate the concentration (% w/w) of free sulfate in each injection of the *Sample solution*:

$$\text{Result} = F_M \times r_U \times D_U \times (M_{r1}/M_{r2}) \times (100/C)$$

F_M = mean response factor from *Standard solution 2*

r_U = peak response of the sulfate ion in the *Sample solution*

D_U = dilution factor for the *Sample solution*

M_{r1} = molecular weight of the sulfate ion, 96.1

M_{r2} = molecular weight of sodium sulfate, 142.0

C = nominal concentration of fondaparinux sodium in the content of the syringe

Acceptance criteria: NMT 0.50% (w/w)

• **ORGANIC IMPURITIES**

System suitability solution, Standard solution, Sensitivity check solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Samples: *System suitability solution*, *Standard solution*, *Sensitivity check solution*, *Sample solution*, and *Blank*

Calculate the percentage (area/area) of each individual unspecified impurity for each injection of the *Sample solution*:

$$\text{Result} = [r_U/(r_T + r_S)] \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses for degradation impurities from the *Sample solution*

r_S = peak response of fondaparinux sodium from the *Sample solution*

Taking into account the response factors for specified impurities (see *Table 2*), calculate the individual content (% w/w) of specified fondaparinux related compounds B, C, and G:

$$\text{Result} = (r_U \times F_i \times 100)/\{[\Sigma(r_U \times F_i)] + r_S\}$$

r_U = peak response of each impurity from the *Sample solution*

F_i = relative response factor for the individual impurity peak (response factor of fondaparinux sodium/response factor of individual impurity [see *Table 2*])

r_S = peak response of fondaparinux sodium from the *Sample solution*

Calculate the total degradation product content by summing the mean unrounded content values for the following peaks: fondaparinux related compounds A, B, C, D, F, and G and any unspecified impurities that are not synthetic impurities. Exclude peaks below the LOQ (0.003% w/w for fondaparinux related compound B, 0.002% w/w for fondaparinux related compound G, and 0.200% for all other degradation products and fondaparinux related compound E).

Individual impurities: See *Table 2*.

[2] One suitable suppressor is Dionex ASRS 300 4 mm.

USP Monographs

www.webofpharma.com

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fondaparinux related compound A	0.35	1.0	1.0 (a/a)
Fondaparinux related compound B[a]	0.48	70	0.150 (w/w)
Fondaparinux related compound C[b]	0.76	1.0	0.8 (w/w)/0.4 (w/w)[c]
Fondaparinux related compound D	0.80	1.0	0.8 (a/a)
Fondaparinux related compound E[d]	0.93	—	—
Fondaparinux related compound F[e]	1.29	1.0	2.0 (a/a)
Fondaparinux related compound G[f]	1.34	100	0.10 (w/w)
Fondaparinux sodium	—	1.0	—
Total impurities	—	—	5.0

[a] Methyl-*O*-(4-deoxy-2-*O*-sulfo-α-L-threo-hex-4-enopyranosyluronate)-(1→4)-*O*-(2-deoxy-6-*O*-sulfo-2-sulfamino-α-D-glucopyranoside), tetrasodium salt.
[b] Methyl *O*-(2-deoxy-6-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyluronate)-(1→4)-*O*-(2-deoxy-3,6-di-*O*-sulfo-2-amino-α-D-glucopyranosyl-(1→4)-*O*-2-*O*-sulfo-α-L-idopyranosyluronate)-(1→4)-(2-deoxy-6-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranoside), nonasodium salt.
[c] 0.8 (w/w) for Injection at 12.5 mg/mL and 0.4% (w/w) for Injection at 5 mg/mL.
[d] Synthetic impurity included for identification purposes only and excluded from impurities calculations.
[e] The fondaparinux related compound F peak can appear as a complex set of peaks in the region RRT 1.2 to RRT 1.24. These peaks, which may not be fully resolved from each other, appear before the fondaparinux related compound G peak. In such a case, the integration should be performed so that all such peaks are combined. Specified degradation products can be assigned by reference to the specimen chromatogram of the *System suitability solution* associated with USP Fondaparinux Sodium System Suitability Mixture B RS.
[f] 2-Deoxy-6-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranosyl-(1→4)-*O*-(β-D-glucopyranosyluronate)-(1→4)-*O*-(2-deoxy-3,6-di-*O*-sulfo-2-(sulfoamino)-α-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-sulfo-α-L-idopyranosyluronate)-(1→4)-(1,2-dideoxy-6-*O*-sulfo-2-(sulfoamino)-D-enoglucopyranoside), decasodium salt.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 3.3 USP Endotoxin Units/mg of fondaparinux sodium
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **STERILITY TESTS** ⟨71⟩: Where it is labeled as sterile, it meets the requirements.
- **pH** ⟨791⟩: 5.0–8.0, in a solution, at 20°–25°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers in Type I glass or other validated container-closure system. Store at or below 25°.
- **LABELING:** Label it to indicate the amount, in mg, of fondaparinux sodium in the total volume of contents.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fondaparinux Sodium for Assay RS
  USP Fondaparinux Sodium System Suitability Mixture B RS

Formaldehyde Solution

CH_2O 30.03
Formaldehyde.
Formaldehyde [50-00-0].

» Formaldehyde Solution contains not less than 34.5 percent, by weight, of formaldehyde (CH_2O), with methanol added (9.0% to 15.0%) to prevent polymerization.

Packaging and storage—Preserve in tight containers, and preferably store at a temperature not below 15°.

Identification—
A: Dilute 2 mL with 10 mL of water in a test tube, and add 1 mL of silver-ammonia-nitrate TS: metallic silver is produced either in the form of a finely divided, gray precipitate, or as a bright, metallic mirror on the sides of the test tube.
B: Add 2 drops to 5 mL of sulfuric acid in which about 20 mg of salicylic acid has been dissolved, and warm the liquid very gently: a permanent, deep-red color appears.
Acidity—Measure 20.0 mL into a flask containing 20 mL of water, add 2 drops of bromothymol blue TS, and titrate with 0.1 N sodium hydroxide VS: not more than 10.0 mL of 0.1 N sodium hydroxide is consumed.

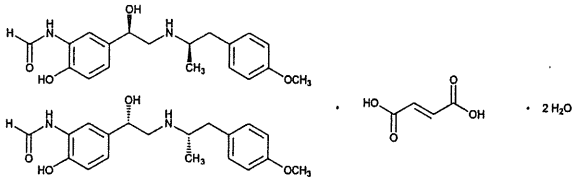
Content of methanol—
Internal standard solution—Dilute 10 mL of dehydrated alcohol with water to 100 mL.
Test solution—To 10.0 mL of Solution add 10.0 mL of the *Internal standard solution,* and dilute with water to 100.0 mL.
Standard solution—To 1.0 mL of methanol add 10.0 mL of the *Internal standard solution,* and dilute with water to 100.0 mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 2- to 4-mm × 1.5- to 2.0-m column containing packing S3. The carrier gas is nitrogen or helium, flowing at a rate of 30 to 40 mL per minute. The column temperature is maintained at 120°. The injection port temperature and the detector temperature are maintained at 150°.
Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the peaks corresponding to methanol and alcohol is not less than 2.0.
Procedure—Separately inject equal volumes (1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage (v/v) of methanol in the portion of Solution taken by the formula:

$$100 \times (V_M/V)(R_U/R_S)$$

in which V_M is the volume, in mL, of methanol taken to prepare the *Standard solution; V* is the volume, in mL, of Solution taken to prepare the *Test solution;* and R_U and R_S are the peak response ratios of methanol to that of the internal standard obtained from the *Test solution* and the *Standard solution,* respectively: between 9.0% and 15.0% (v/v) is found.
Assay—Into a 100-mL volumetric flask containing 2.5 mL of water and 1 mL of sodium hydroxide TS 2, introduce 1.0 g of the Solution to be examined, shake, and dilute with water to 100.0 mL. To 10.0 mL of the solution add 30.0 mL of 0.1 N iodine VS. Mix, and add 10 mL of sodium hydroxide TS 2. After 15 minutes, add 25 mL of diluted sulfuric acid and 4 mL of starch TS. Titrate with 0.1 N sodium thiosulphate VS. Each 1 mL of 0.05 M iodine is equivalent to 1.501 mg of CH_2O.

www.webofpharma.com

Formoterol Fumarate



$(C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4 \cdot 2H_2O$ 840.91

(±)-2′-Hydroxy-5′-[(*R**)-1-hydroxy-2-[[(*R**)-*p*-methoxy-α-methylphenethyl]amino]ethyl]formanilide fumarate (2:1) (salt), dihydrate [183814-30-4].

DEFINITION

Formoterol Fumarate contains NLT 98.5% and NMT 101.5% of formoterol fumarate $[(C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4]$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the formoterol peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: 6.1 g/L of monobasic sodium phosphate and 1.0 g/L of dibasic sodium phosphate dihydrate in water. [Note—The pH is 6.0 ± 0.1.]
Solution A: 3.7 g/L of monobasic sodium phosphate and 0.35 g/L of phosphoric acid in water. [Note—The pH is 3.1 ± 0.1.]
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	84	16
10	84	16
12.7	30	70
12.8	84	16
18	84	16

Diluent: Acetonitrile and *Buffer* (16:84)
Standard solution: 0.2 mg/mL of USP Formoterol Fumarate RS in *Diluent*
Sample solution: 0.2 mg/mL of Formoterol Fumarate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of formoterol fumarate $[(C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4]$ in the portion of Formoterol Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of formoterol from the *Sample solution*
r_S = peak response of formoterol from the *Standard solution*
C_S = concentration of USP Formoterol Fumarate RS in the *Standard solution* (mg/mL)
C_U = concentration of Formoterol Fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the anhydrous basis

IMPURITIES

- **Residue on Ignition ⟨281⟩**

Sample: 1 g
Acceptance criteria: NMT 0.1%

- **Organic Impurities**

Buffer, Solution A, Solution B, and **Diluent:** Proceed as directed in the *Assay*.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	84	16
10	84	16
37	30	70
40	84	16
55	84	16

System suitability solution: 0.2 mg/mL of USP Formoterol Fumarate System Suitability Mixture RS in *Diluent*. Sonicate to dissolve prior to final dilution.
Sample solution: 0.20 mg/mL of Formoterol Fumarate in *Diluent*. Sonicate to dissolve before final dilution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[Note—The relative retention times for the peaks are given in *Table 3*.]
Suitability requirements
Resolution: NLT 1.5 between formoterol related compound G and formoterol related compound A
Peak-to-valley ratio: NLT 2.5 for formoterol related compound C and formoterol
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Formoterol Fumarate taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

USP Monographs

www.webofpharma.com

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of the responses of all peaks from the *Sample solution*
F = relative response factor for each peak (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any peak less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Formoterol related compound G	0.4	1.0	0.1
Formoterol related compound A	0.5	0.57	0.3
Formoterol related compound B	0.7	1.0	0.2
Formoterol	1.0	—	—
Formoterol related compound C	1.2	1.0	0.2
Formoterol related compound D	1.3	1.0	0.2
Formoterol related compound E	1.8	1.0	0.1
Formoterol related compound F	2.0	1.0	0.2
Formoterol related compound H	2.2	1.0	0.1
Any individual unspecified impurity	—	1.0	0.10
Total	—	—	0.5

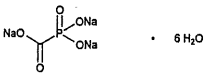
- **LIMIT OF FORMOTEROL RELATED COMPOUND I**

Buffer: 4.2 g/L of tribasic potassium phosphate. Adjust with potassium hydroxide or phosphoric acid to a pH of 12.0 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (12:88)
System suitability solution: 0.1 mg/mL of USP Formoterol Fumarate Resolution Mixture RS
Sample solution: 0.1 mg/mL of Formoterol Fumarate
Diluted sample solution: 0.2 µg/mL of Formoterol Fumarate from the *Sample solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; packing L67
Flow rate: 0.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for formoterol and formoterol related compound I are 1.0 and 1.17, respectively.]
Suitability requirements
Peak-to-valley ratio: NLT 2.5 for formoterol related compound I and formoterol
Analysis
Samples: *Sample solution* and *Diluted sample solution*
Acceptance criteria: NMT 0.3%; the peak area of formoterol diastereomer in the *Sample solution* is NMT 1.5 times the peak area of formoterol fumarate in the *Diluted sample solution*.

SPECIFIC TESTS
- **pH** ⟨791⟩
Sample solution: 1 mg/mL in water
Acceptance criteria: 5.5–6.5
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** 4.0%–5.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Formoterol Fumarate RS
USP Formoterol Fumarate Resolution Mixture RS
Mixture of formoterol and formoterol related compound I.
Formoterol related compound I: *N*-[2-hydroxy-5-[(1*RS*)-1-hydroxy-2-[[(1*SR*)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide fumarate salt (2:1) (diastereoisomer).
USP Formoterol Fumarate System Suitability Mixture RS
Mixture of formoterol fumarate and formoterol related compounds A, B, C, D, E, F, G, and H.
Formoterol related compound A: 1-(3-Amino-4-hydroxyphenyl)-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethanol.
Formoterol related compound B: *N*-[2-Hydroxy-5-[(1*RS*)-1-hydroxy-2-[[2-(4-methoxyphenyl)ethyl]amino]ethyl]phenyl]formamide.
Formoterol related compound C: *N*-[2-Hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]acetamide.
Formoterol related compound D: *N*-[2-Hydroxy-5-[1-hydroxy-2-[methyl[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide.
Formoterol related compound E: *N*-[2-Hydroxy-5-[1-hydroxy-2-[[2-(4-methoxy-3-methylphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide.
Formoterol related compound F: *N*-[2-Hydroxy-5-[1-[[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]amino]-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide.
Formoterol related compound G: (2*RS*)-1-(4-Methoxyphenyl)propan-2-amine.
Formoterol related compound H: *N*-[5-[(1*RS*)-2-[Benzyl[(1*RS*)-2-(4-methoxyphenyl)-1-methylethyl]amino]-1-hydroxyethyl]-2-hydroxyphenyl]formamide (monobenzyl analog).

Foscarnet Sodium



$CNa_3O_5P \cdot 6H_2O$ 300.04
Phosphinecarboxylic acid, dihydroxy-, oxide, trisodium salt, hexahydrate;
Phosphonoformic acid, trisodium salt, hexahydrate [34156-56-4].

DEFINITION
Foscarnet Sodium contains NLT 98.5% and NMT 101.0% of foscarnet sodium (CNa_3O_5P), calculated on the dried basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Sample solution: 0.2 g of Foscarnet Sodium in 50 mL of water

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M sulfuric acid

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with *Titrant,* determining the endpoint at the first inflection point. Each mL of 0.05 M sulfuric acid is equivalent to 19.20 mg of foscarnet sodium (CNa_3O_5P).

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

- **LIMIT OF PHOSPHATE AND PHOSPHITE**

Mobile phase: Dissolve 0.1 g of monobasic potassium phthalate in water. Add 2.5 mL of 1 M nitric acid, and dilute with water to 1000 mL.

Standard stock solution 1: 0.28 mg/mL of sodium dihydrogen phosphate monohydrate in water

Standard stock solution 2: 0.43 mg/mL of sodium phosphite pentahydrate in water

Standard solution: Transfer 1 mL each of *Standard stock solution 1* and *Standard stock solution 2* to a 25-mL volumetric flask, and dilute with water to volume.

Sample solution: 2.4 mg/mL of Foscarnet Sodium in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 290 nm

Column: 4.6-mm × 5-cm; packing L23

Flow rate: 1.4 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between phosphate and phosphite

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The peak area of phosphate and phosphite from the *Sample solution* is NMT the area of the corresponding peak of the *Standard solution* (0.3% of phosphate and 0.3% of phosphite).

- **LIMIT OF FOSCARNET RELATED COMPOUND B, UNKNOWN IMPURITIES, AND TOTAL IMPURITIES**

Solution A: Dissolve 3.2 g of sodium sulfate decahydrate in water. Add 3 mL of glacial acetic acid and 6 mL of 0.1 M sodium pyrophosphate, and dilute with water to 1000 mL.

Solution B: Dissolve 3.2 g of sodium sulfate decahydrate in water. Add 6.8 g of sodium acetate and 6 mL of 0.1 M sodium pyrophosphate, and dilute with water to 1000 mL.

Mobile phase: *Solution A* and *Solution B* (70:30). [NOTE—The pH of this mixture is about 4.4.] To 1000 mL of this solution add 0.25 g of tetrahexylammonium hydrogen sulfate and 100 mL of methanol.

Sample solution: 2.5 mg/mL of Foscarnet Sodium in *Mobile phase*

System suitability solution: Transfer 2.0 mL of the *Sample solution* into a 50-mL volumetric flask. Add 5.0 mg of USP Foscarnet Related Compound B RS, and dilute with *Mobile phase* to volume.

Standard solution: 5.0 µg/mL of Foscarnet Sodium from the *Sample solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 2.5 times the retention time of foscarnet

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 7 between foscarnet and foscarnet related compound B

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The peak area of any individual impurity from the *Sample solution* is NMT the area of the major peak of the *Standard solution* (0.2%); the sum of all the peak areas, apart from the major peak, of the *Sample solution* is NMT twice the area of the major peak of the *Standard solution* (0.4%). Disregard any peak with a relative retention time less than 0.6 and any peak with an area less than 0.2 times that of the peak of the *Standard solution.*

- **Limit of Foscarnet Related Compound D**

Standard solution: 25 µg/mL of USP Foscarnet Related Compound D RS in alcohol

Sample solution: Dissolve 250 mg of Foscarnet Sodium in 9 mL of 0.1 M acetic acid, and add 1 mL of alcohol.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: GC

Detector: Flame ionization

Column: 0.31-mm × 25-m; 0.5-µm coating of phase G27

Temperatures

Injector: 200°

Detector: 250°

Column: See *Table 1.*

Table 1

Time (min)	Temperature Ramp (°/min)	Temperature (°)
0	0	100
8	10	180

Carrier gas: Helium

Flow rate: 1 mL/min

Injection volume: 3 µL

Injection type: Split (1:20)

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria

Individual impurities: The peak area of foscarnet related compound D from the *Sample solution* is NMT the peak area of the *Standard solution* (0.1%).

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: 20 mg/mL of Foscarnet Sodium in a carbon dioxide-free aqueous solution

Acceptance criteria: 9.0–11.0

- **LOSS ON DRYING** ⟨731⟩

Sample: 0.1 g

Analysis: Dry the *Sample* at 150° for at least 15 min.

Acceptance criteria: 35.0%–37.0%

www.webofpharma.com

ADDITIONAL REQUIREMENTS

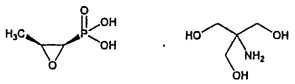
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Foscarnet Related Compound B RS
Disodium (ethoxyoxidophosphanyl)formate.
$C_3H_5Na_2O_5P$ 198.02

USP Foscarnet Related Compound D RS
O,O-Diethyl ethoxycarbonylphosphonate.
$C_7H_{15}O_5P$ 210.16

USP Foscarnet Sodium RS

Fosfomycin Tromethamine



$C_3H_7O_4P \cdot C_4H_{11}NO_3$ 259.19

Phosphonic acid, (3-methyloxiranyl)-, (2*R*-cis)-, compd. with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1); (1*R*,2*S*)-(1,2-Epoxypropyl)phosphonic acid, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1) [78964-85-9].

DEFINITION

Fosfomycin Tromethamine contains NLT 98.0% and NMT 102.0% of $C_3H_7O_4P \cdot C_4H_{11}NO_3$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the fosfomycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.** The retention time of the tromethamine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Prepare the solutions immediately before use.]

Mobile phase: 10.89 g/L of potassium dihydrogen phosphate in water

Standard solution: 120 mg/mL of USP Fosfomycin Tromethamine RS in *Mobile phase*

Sample solution: 120 mg/mL of Fosfomycin Tromethamine in *Mobile phase*

Solution A: Wet 300 mg of fosfomycin tromethamine with 60 µL of water, and heat in an oven at 60° for 24 h. Dissolve the residue, and dilute with *Mobile phase* to 20.0 mL.

System suitability solution: Dissolve 600 mg of fosfomycin tromethamine, and dilute with *Solution A* to 5.0 mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Differential refractometer at 35°
Column: 4.6-mm × 25-cm; 5-µm packing L8
Flow rate: 1 mL/min
Injection size: 5 µL
Run time: Twice the retention time of fosfomycin

System suitability
Sample: *System suitability solution*

[NOTE—The relative retention times are listed in *Impurity Table 1*.]

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tromethamine[a]	0.30	—
Fosfomycin trometha-mine adduct[b]	0.48	0.3
Tromethamine phos-phate[c]	0.54	0.1
Fosfomycin open ring[d]	0.88	0.3
Fosfomycin	1.00	—
Fosfomycin dimer tro-methamine adduct[e]	1.27	0.1
Any other individual im-purity	—	0.1

[a] 1,3-Propanediol, 2-amino-2-(hydroxymethyl)-; 2-Amino-2-(hydroxymethyl)-1,3-propanediol. [NOTE—Disregard two peaks due to tromethamine.]
[b] 2-[2-Amino-3-hydroxy-2-(hydroxymethyl)propoxy]-1-hydroxy-propylphosphonic acid.
[c] 2-Amino-3-hydroxy-2-(hydroxymethyl)propyl dihydrogen phosphate.
[d] (1,2-Dihydroxypropyl)phosphonic acid.
[e] 2-({2-[2-Amino-3-hydroxy-2-(hydroxymethyl)propoxy]-1-hydroxypropyl} hydroxyphosphoryloxy)-1-hydroxypropylphosphonic acid.

Suitability requirements

Resolution: NLT 1.5 between fosfomycin open ring and fosfomycin

Peak-to-valley ratio: NLT 1.5. [NOTE—Ratio is based on the height above the baseline due to the tromethamine phosphate peak and to the height above the baseline of the lowest point of the curve separating this peak from the peak due to the fosfomycin tromethamine adduct.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_3H_7O_4P \cdot C_4H_{11}NO_3$ in the portion of Fosfomycin Tromethamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fosfomycin from the *Sample solution*
r_S = peak response of fosfomycin from the *Standard solution*
C_S = concentration of USP Fosfomycin Tromethamine RS in the *Standard solution* (mg/mL)
C_U = concentration of Fosfomycin Tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

INORGANIC IMPURITIES

- **LIMIT OF INORGANIC PHOSPHATES**

Solution A: Dissolve 4 g of finely powdered ammonium molybdate and 0.1 g of finely powdered ammonium vanadate in 70 mL of water. Add 20 mL of nitric acid, and dilute with water to 100 mL.

Standard solution: Dissolve 7.16 mg of potassium dihydrogen phosphate in 1000 mL of water (5 ppm PO_4). [NOTE—Prepare immediately before use.]

Sample solution: Dissolve 0.1 g of Fosfomycin Tromethamine in 3 mL of 2 N nitric acid, and dilute with water to 10 mL.

USP Monographs

www.webofpharma.com

Analysis: In separate containers, transfer 5 mL of *Standard solution* and 5 mL of *Sample solution*. Add 5 mL of water and 5 mL of *Solution A* to both solutions, and shake vigorously. After 5 min, any color in the *Sample solution* is not more intense than the *Standard solution*.
Acceptance criteria: NMT 500 ppm

ORGANIC IMPURITIES
• **Procedure**
[NOTE—Prepare the solutions immediately before use.]
Mobile phase, Sample solution, Solution A, System suitability solution, and System suitability: Proceed as directed in the *Assay*.
Standard solution: 0.36 mg/mL of USP Fosfomycin Tromethamine RS in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Differential refractometer at 35°
Column: 4.6-mm × 25-cm; 5-µm packing L8
Flow rate: 1 mL/min
Injection size: 10 µL
Run time: Twice the retention time of fosfomycin
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of impurities in the portion of Fosfomycin Tromethamine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

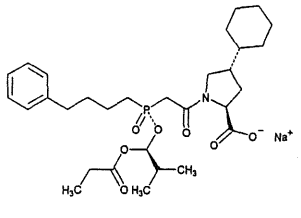
r_U = peak response of any individual impurity in the *Sample solution*
r_T = peak responses of fosfomycin in the *Standard solution*

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
[NOTE—Disregard any peak less than 0.05%.]
Total impurities: NMT 0.5%

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 3.5–5.5, in a carbon dioxide-free aqueous solution containing 50 mg/mL of fosfomycin tromethamine
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩**:** −13.5° to −12.5°
Sample solution: 50 mg/mL in a carbon dioxide-free water
Detection: Mercury lamp at 365 nm
• **WATER DETERMINATION,** *Method Ic* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fosfomycin Tromethamine RS

Fosinopril Sodium



$C_{30}H_{45}NNaO_7P$ 585.64

L-Proline, 4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy) propoxy](4-phenylbutyl)phosphinyl]acetyl]-, sodium salt, [1[*S**(*R**)],2α,4β]-;
(4*S*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy] (4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt [88889-14-9].

DEFINITION
Fosinopril Sodium contains NLT 97.5% and NMT 102.0% of fosinopril sodium ($C_{30}H_{45}NNaO_7P$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197M▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, phosphoric acid, and water (2000:1:10)
System suitability solution: 0.1 mg/mL of USP Fosinopril Sodium RS and 0.01 mg/mL of USP Fosinopril Related Compound B RS in *Mobile phase*
Standard solution: 0.10 mg/mL of USP Fosinopril Sodium RS in *Mobile phase*
Sample solution: 0.10 mg/mL of Fosinopril Sodium in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 3.9-mm × 15-cm; 5-µm packing L3
Column temperature: 33°
Flow rate: 1.2 mL/min
Injection volume: 20 µL
Run time: NLT 4 times the retention time of the fosinopril peak
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between fosinopril related compound B and fosinopril
Relative standard deviation: NMT 0.73% for the fosinopril peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fosinopril sodium ($C_{30}H_{45}NNaO_7P$) in the portion of Fosinopril Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fosinopril from the *Sample solution*
r_S = peak response of fosinopril from the *Standard solution*
C_S = concentration of USP Fosinopril Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Fosinopril Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.0% on the anhydrous basis

USP Monographs

www.webofpharma.com

IMPURITIES

• **ORGANIC IMPURITIES, PROCEDURE 1**

Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*

System suitability solution: 0.1 mg/mL of USP Fosinopril Sodium RS and 0.01 mg/mL each of USP Fosinopril Related Compound A RS and USP Fosinopril Related Compound B RS in *Mobile phase*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual related compound in the portion of Fosinopril Sodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = individual peak response, other than the fosinopril peak

r_T = sum of all the peak responses

Acceptance criteria See *Table 1.*

Table 1

Name	Relative Retention Time	Procedure	Acceptance Criteria, NMT (%)
Impurity 3 (if present)[a]	0.37	1	0.15
Impurity 1[b]	0.53	1	0.3
Impurity 2[c]	0.67	1	0.2
Fosinopril related compound B[d]	0.7	1	1.0
Fosinopril related compound E[e]	0.8	3	0.3
Fosinopril related compound F[f]	0.9	3	0.3
Fosinopril related compound C[g]	1.2	2	0.3
Fosinopril related compound D[h]	1.3	2	0.3
Fosinopril related compound A[i]	2.0	1	0.75
Any individual unspecified impurity	—	1	0.1
Total impurities[j]	—	1, 2, 3	1.5

[a] (*S*)-4-Cyclohexyl-1-(3-oxopentanoyl)-L-proline.

[b] (2*S*,4*S*)-4-Cyclohexyl-1-pivaloylpyrrolidine-2-carboxylic acid.

[c] 2-((*RS*)-((*SR*)-2-Methyl-1-(propionyloxy)propoxy)(4-phenylbutyl)phosphinyl) acetic acid.

[d] (4*S*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl) phosphinyl]acetyl-D-proline propionate (ester). If present, two more diastereomers may not be resolved from fosinopril related compound B by this method. These peaks, appearing at a relative retention time of 0.7, should be integrated together to determine conformance with the limit.

[e] (4*S*)-4-Phenyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl) phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

[f] (4*S*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxypropoxy](4-phenylbutyl)phosphinyl] acetyl-L-proline propionate (ester), sodium salt.

[g] Mixture of (4*S*)-4-cyclohexyl-1-[(*S*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt and (4*S*)-4-cyclohexyl-1-[[(*R*)-[(*R*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl) phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

[h] (4*R*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl) phosphinyl]acetyl-L-proline propionate (ester), sodium salt.

[i] (4*S*)-4-Cyclohexyl-[(4-phenylbutyl)phosphinyl]acetyl-L-proline.

[j] Sum of all impurities from *Organic Impurities, Procedure 1; Organic Impurities, Procedure 2;* and *Organic Impurities, Procedure 3.*

• **ORGANIC IMPURITIES, PROCEDURE 2**

Mobile phase: Acetonitrile, phosphoric acid, and water (2000:1:7.5)

System suitability solution: 0.1 mg/mL of USP Fosinopril Sodium RS and 0.01 mg/mL each of USP Fosinopril Related Compound C RS and USP Fosinopril Related Compound D RS in *Mobile phase*

Sample solution: Prepare as directed in the *Assay.*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; 5-µm packing L14

Column temperature: 45°

Flow rate: 0.9 mL/min

Injection volume: 20 µL

Run time: NLT 2 times the retention time of the fosinopril peak

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between fosinopril and fosinopril related compound C

Analysis

Sample: *Sample solution*

Calculate the percentages of fosinopril related compound C and fosinopril related compound D only in the portion of Fosinopril Sodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of fosinopril related compound C or fosinopril related compound D

r_T = sum of all the peak responses

Acceptance criteria: See *Table 1.*

• **ORGANIC IMPURITIES, PROCEDURE 3**

Solution A: Phosphoric acid (1 in 500)

Mobile phase: Acetonitrile and *Solution A* (56:44)

System suitability solution: 0.01 mg/mL each of USP Fosinopril Sodium RS, USP Fosinopril Related Compound E RS, and USP Fosinopril Related Compound F RS in *Mobile phase*

www.webofpharma.com

Sample solution: 0.2 mg/mL of Fosinopril Sodium in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 205 nm
Column: 4.6-mm × 25-cm; 5-µm packing L11
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 4 times the retention time of the fosinopril peak
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between fosinopril related compound F and fosinopril; NLT 1.5 between fosinopril related compound E and fosinopril related compound F
Analysis
Sample: *Sample solution*
Calculate the percentages of fosinopril related compound E and fosinopril related compound F in the portion of Fosinopril Sodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of fosinopril related compound E or fosinopril related compound F
r_T = sum of all the peak responses

Acceptance criteria: See *Table 1*.

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.2%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fosinopril Sodium RS
USP Fosinopril Related Compound A RS
(4*S*)-4-Cyclohexyl-[(4-phenylbutyl)phosphinyl]acetyl-L-proline.
$C_{23}H_{34}NO_5P$ 435.49
USP Fosinopril Related Compound B RS
(4*S*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-D-proline propionate (ester), hemibarium salt, sesquihydrate.
$C_{30}H_{45}NO_7P \cdot \frac{1}{2}Ba \cdot 1\frac{1}{2}H_2O$ 658.34
USP Fosinopril Related Compound C RS
(4*S*)-4-Cyclohexyl-1-[(*RS*)-[(*RS*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.
$C_{30}H_{45}NNaO_7P$ 585.64
USP Fosinopril Related Compound D RS
(4*R*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.
$C_{30}H_{45}NNaO_7P$ 585.64
USP Fosinopril Related Compound E RS
(4*S*)-4-Phenyl-1-[(*R*)-[(*S*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.
$C_{30}H_{39}NNaO_7P$ 579.60
USP Fosinopril Related Compound F RS
(4*S*)-4-Cyclohexyl-1-[(*R*)-[(*S*)-1-hydroxypropoxy](4-phenylbutyl)phosphinyl]acetyl-L-proline propionate (ester), sodium salt.
$C_{29}H_{43}NNaO_7P$ 571.62

Fosinopril Sodium Tablets

DEFINITION
Fosinopril Sodium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of fosinopril sodium ($C_{30}H_{45}NNaO_7P$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F▲** (CN 1-May-2020)
Sample: Transfer a portion of finely powdered Tablets, equivalent to 25 mg of fosinopril sodium, to a 100-mL beaker containing 40 mL of water. Heat at 25° for 5 min with stirring, and pass through a fritted-disc funnel of medium pore size. Centrifuge the filtrate at 2500 rpm for 30 min. Adjust the filtrate with phosphoric acid to a pH of 3 to precipitate the fosinopril, and pass through a fritted-disc funnel. Dissolve the precipitate by passing chloroform through the filter, and evaporate the chloroform solution to dryness under a current of air. Proceed as directed, using the oily residue so obtained.
Standard: Transfer 25 mg of USP Fosinopril Sodium RS to a 100-mL beaker containing 40 mL of water. Proceed as directed for the *Sample*, beginning with "Heat at 25° for 5 min with stirring". Use the residue.
Acceptance criteria: Meet the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol and 0.2% phosphoric acid (780:220)
Diluent: Acetonitrile and 0.2 M urea solution (20:80)
System suitability solution: 30 µg/mL of USP Fosinopril Related Compound A RS and 70 µg/mL of USP Fosinopril Sodium RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Fosinopril Sodium RS in *Diluent*
Sample stock solution: Transfer NLT 10 Tablets to a 500-mL volumetric flask, add 400 mL of *Diluent*, and stir for 40 min. Dilute with *Diluent* to volume, mix, and centrifuge. Use the clear supernatant.
Sample solution: Nominally 0.1 mg/mL of fosinopril sodium in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 215 nm
Column: 4.0-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
Run time: NLT 1.5 times the retention time of the fosinopril peak
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for fosinopril related compound A and fosinopril are about 0.4 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the fosinopril and fosinopril related compound A peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fosinopril sodium ($C_{30}H_{45}NNaO_7P$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fosinopril from the *Sample solution*
r_S = peak response of fosinopril from the *Standard solution*
C_S = concentration of USP Fosinopril Sodium RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of fosinopril sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Mobile phase: Acetonitrile and 0.2% phosphoric acid (640:360)
System suitability solution: 0.02 mg/mL each of USP Fosinopril Sodium RS and USP Fosinopril Related Compound G RS in *Mobile phase*
Standard stock solution: 0.1 mg/ml of USP Fosinopril Sodium RS prepared as follows. Add methanol, about 3% of the total volume, to a suitable quantity of USP Fosinopril Sodium RS in a suitable volumetric flask, sonicate briefly, and dilute with *Medium* to volume.
Standard solution: Dilute the *Standard stock solution* with *Medium* as directed in *Table 1*.

Table 1

Label Claim (mg)	Standard Stock Solution (mL)	Final Volume (flask size)
5	5.0	100
10	10	100
20	20	100
40	40	100

Sample solution: Pass portions of the solution under test through a 1.2-µm acrylic filter. [NOTE—Do not use glass filters.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 3 mL/min
Injection volume: 50 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.7 between fosinopril and fosinopril related compound G, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of fosinopril sodium ($C_{30}H_{45}NNaO_7P$) dissolved.

Tolerances: NLT 80% (*Q*) of the labeled amount of fosinopril sodium ($C_{30}H_{45}NNaO_7P$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **LIMIT OF FOSINOPRIL RELATED COMPOUND A**
Mobile phase, Diluent, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard stock solution: 0.1 mg/mL of USP Fosinopril Related Compound A RS in methanol
Standard solution: 0.0025 mg/mL of USP Fosinopril Related Compound A RS in *Diluent* from *Standard stock solution*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of fosinopril related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fosinopril related compound A from the *Sample solution*
r_S = peak response of fosinopril related compound A from the *Standard solution*
C_S = concentration of fosinopril related compound A in the *Standard solution* (mg/mL)
C_U = nominal concentration of fosinopril sodium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 4%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fosinopril Related Compound A RS
(4*S*)-4-Cyclohexyl-[(4-phenylbutyl)phosphinyl]acetyl-L-proline.
$C_{23}H_{34}NO_5P$ 435.49
USP Fosinopril Related Compound G RS
(4-Phenylbutyl)phosphinylacetic acid, disodium salt.
$C_{12}H_{15}Na_2O_4P$ 300.20
USP Fosinopril Sodium RS

Fosinopril Sodium and Hydrochlorothiazide Tablets

DEFINITION

Fosinopril Sodium and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of fosinopril sodium ($C_{30}H_{45}NNaO_7P$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F▲** (CN 1-May-2020)
Fosinopril Sodium: Transfer an amount equivalent to 25 mg of fosinopril sodium from finely powdered Tablets to a 100-mL beaker containing 40 mL of water. Heat at 30° for 5 min with stirring, and filter through a fritted disk of medium pore size. Centrifuge the filtrate at 2500 rpm for 30 min. Adjust the filtrate with hydrochloric acid to a pH of

USP Monographs

www.webofpharma.com

1 to precipitate the fosinopril, and filter through a fritted-disk funnel. Dissolve the precipitate by passing methyl isobutyl ketone through the filter, and evaporate the filtrate to dryness under a stream of nitrogen. Proceed as directed, using the oily residue so obtained and a similarly prepared residue from 25 mg of USP Fosinopril Sodium RS.

Hydrochlorothiazide: Transfer an amount equivalent to 37.5 mg of hydrochlorothiazide from finely powdered Tablets to a 250-mL beaker containing 120 mL of water, heat at 30° for 5 min with stirring, and filter through a a fritted disk of medium pore size. Wash the precipitate with 60 mL of methylene chloride and glacial acetic acid (90:10) mixture, and discard the filtrate. Dissolve the precipitate by passing 10 mL of methyl isobutyl ketone through the filter, and evaporate the filtrate to dryness under a stream of nitrogen. Proceed as directed, using the waxy residue so obtained and a similarly prepared residue from 37 mg of USP Hydrochlorothiazide RS.

Acceptance criteria: Meet the requirements

• **B.** The retention times of fosinopril and hydrochlorothiazide peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 0.01 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.0.

Solution A: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Solution A (%)
0	88	12
2	88	12
20	10	90
28	10	90
37	88	12

Diluent A: Acetonitrile and water (1:2)

Diluent B: Acetonitrile and 0.001 N hydrochloric acid (1:2)

System suitability solution: 0.08 mg/mL each of USP Hydrochlorothiazide RS and USP Fosinopril Sodium RS, and 0.025 mg/mL of USP Chlorothiazide RS in *Diluent B*

Standard stock solution A: 2.0 mg/mL of USP Fosinopril Sodium RS in *Diluent A*

Standard stock solution B: 2.0 mg/mL of USP Hydrochlorothiazide RS in *Diluent B*

Standard solution: 0.08 mg/mL each of USP Fosinopril Sodium RS and USP Hydrochlorothiazide RS from *Standard stock solution A* and *Standard stock solution B*, respectively, in *Diluent B*

Sample stock solution: Nominally equivalent to 0.1 mg/mL of fosinopril sodium and 0.125 mg/mL of hydrochlorothiazide in *Diluent B* from NLT 20 finely powdered Tablets. Initially add *Diluent B* to fill 75% of the final volume, and sonicate for about 10 min. Shake by mechanical means for 15 min, and dilute with *Diluent B* to volume. Pass a portion of this solution through a filter (PTFE or PVDF) of 0.45-µm pore size, and collect the rest of the filtrate.

Sample solution: 0.075 mg/mL of fosinopril sodium in *Diluent B* from *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 206 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.8 between chlorothiazide and hydrochlorothiazide, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fosinopril sodium ($C_{30}H_{45}NNaO_7P$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fosinopril from the *Sample solution*

r_S = peak response of fosinopril from the *Standard solution*

C_S = concentration of USP Fosinopril Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fosinopril sodium in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydrochlorothiazide from the *Sample solution*

r_S = peak response of hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of fosinopril sodium ($C_{30}H_{45}NNaO_7P$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Buffer: 0.01 M monobasic potassium phosphate; pH 3.0

Mobile phase: Methanol, acetonitrile, and *Buffer* (350:200:450)

Standard stock solution A: 0.1 mg/mL of USP Fosinopril Sodium RS. Initially add 0.3 mL of methanol per mg of USP Fosinopril Sodium RS, and dilute with water to final concentration.

Standard stock solution B: 0.1 mg/mL of USP Hydrochlorothiazide RS. Initially add 0.3 mL of methanol per mg of USP Fosinopril Sodium RS, and dilute with water to final concentration.

Standard solution: Mix 25 mL of *Standard stock solution B* and *x*25 mL of *Standard stock solution A*, *x* being the ratio of the respective labeled amounts, in mg, of fosinopril sodium to that of hydrochlorothiazide per Tablet, and dilute with water to 200 mL.

www.webofpharma.com

System suitability solution: 0.05 mg/mL of USP Fosinopril Related Compound H RS and 0.002 mg/mL of USP Hydrochlorothiazide RS. Initially add 1 mL of methanol per mg of USP Fosinopril Related Compound H RS to dissolve, add *Standard stock solution B*, and dilute with water to final concentration.

Sample solution: Pass portions of the solution under test through an acrylic filter of 1.2-µm pore size. Do not use glass filters.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detectors

UV 215 nm: For *Standard solution* and *System suitability solution*

UV 272 nm: For hydrochlorothiazide from 0 to 5 min

UV 210 nm: For fosinopril sodium from 5 to 9 min

Column: 4.6-mm × 25-cm; 5-µm packing L10

Column temperature: 40°

Flow rate: 1.3 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between fosinopril related compound H and hydrochlorothiazide, *System suitability solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of fosinopril sodium ($C_{30}H_{45}NNaO_7P$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved.

Tolerances: NLT 80% (*Q*) of the labeled amount of fosinopril sodium ($C_{30}H_{45}NNaO_7P$) and NLT 75% (*Q*) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**

Solution A, Solution B, Diluent B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 4 µg/mL each of USP Fosinopril Sodium RS and USP Hydrochlorothiazide RS in *Diluent B*

System suitability solution: 5 µg/mL each of USP Chlorothiazide RS, USP Fosinopril Related Compound A RS, and USP Benzothiadiazine Related Compound A RS; and 0.5 mg/mL each of USP Hydrochlorothiazide RS and USP Fosinopril Sodium RS in *Diluent B*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.8 between chlorothiazide and hydrochlorothiazide, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the peak responses eluting between 2.7 min and 27 min.

Calculate the percentage of any impurity or degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of fosinopril or hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Fosinopril Sodium RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fosinopril sodium or hydrochlorothiazide in the *Sample solution* (mg/mL)

F = relative response factor of each impurity relative to fosinopril sodium or hydrochlorothiazide (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A[a]	0.81 (relative to hydrochlorothiazide)	1.0	0.5
Fosinopril related compound A[b]	0.72 (relative to fosinopril)	1.2	4
Chlorothiazide[c]	0.90 (relative to hydrochlorothiazide)	1.7	0.3
Any other individual impurity	—	—	0.2[d]
Total impurities	—	—	5.0

[a] 4-Amino-6-chloro-1,3-benzenedisulfonamide.
[b] (4*S*)-4-Cyclohexyl-[(4-phenylbutyl)phosphinyl]acetyl-L-proline.
[c] 6-Chloro-2(*H*)-1,2,4-benzothiazine-7-sulfonamide.
[d] Relative to hydrochlorothiazide.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩

USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73

USP Chlorothiazide RS

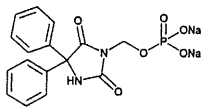
USP Fosinopril Sodium RS

USP Fosinopril Related Compound A RS
(4*S*)-4-Cyclohexyl-[(4-phenylbutyl)phosphinyl]acetyl-L-proline.
$C_{23}H_{34}NO_5P$ 435.49

USP Fosinopril Related Compound H RS
4-Phenylbutyl phosphonic acid.
$C_{10}H_{15}O_3P$ 214.20

USP Hydrochlorothiazide RS

Fosphenytoin Sodium



$C_{16}H_{13}N_2Na_2O_6P$ 406.24

2,4-Imidazolidinedione, 5,5-diphenyl-3- [(phosphonooxy) methyl]-, disodium salt;

www.webofpharma.com

3-(Hydroxymethyl)-5,5-diphenylhydantoin, disodium phosphate (ester) [92134-98-0].

DEFINITION

Fosphenytoin Sodium contains NLT 98.0% and NMT 102.0% of fosphenytoin sodium ($C_{16}H_{13}N_2Na_2O_6P$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩: Meets the requirements

ASSAY

- **PROCEDURE**

Buffer: Dissolve 6.80 g of monobasic potassium phosphate and 30 mL of 0.5 M dodecyltriethylammonium phosphate in 900 mL of water. Adjust with 1.5 M phosphoric acid to a pH of 5.0, and dilute with water to 1000 mL.
Mobile phase: Acetonitrile and *Buffer* (35:65)
Standard solution: 0.15 mg/mL of USP Fosphenytoin Sodium RS in *Mobile phase*
Sample solution: 0.15 mg/mL of Fosphenytoin Sodium in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5000 theoretical plates
Tailing factor: NMT 1.6
Relative standard deviation: NMT 0.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of fosphenytoin sodium ($C_{16}H_{13}N_2Na_2O_6P$) in the portion of Fosphenytoin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Fosphenytoin Sodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Fosphenytoin Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
Standard solution: 0.003 mg/mL of USP Phenytoin Related Compound A RS, 0.003 mg/mL of USP Phenytoin Related Compound B RS, and 0.0015 mg/mL of USP Phenytoin RS in *Mobile phase*
Sample solution: 3 mg/mL of Fosphenytoin Sodium in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*, except use a run time of NLT 6 times the retention time of the major peak.
System suitability
Sample: *Standard solution*
[NOTE—The order of elution is phenytoin related compound A, phenytoin, phenytoin related compound B, followed by the major peak due to fosphenytoin.]
Suitability requirements
Resolution: NLT 2.0 between phenytoin and phenytoin related compound B
Relative standard deviation: NMT 5.0% for each compound
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phenytoin, phenytoin related compound A, and phenytoin related compound B, if present, in the portion of Fosphenytoin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenytoin, phenytoin related compound A, or phenytoin related compound B from the *Sample solution*
r_S = peak response of phenytoin, phenytoin related compound A, or phenytoin related compound B from the *Standard solution*
C_S = concentration of the corresponding analyte in the *Standard solution* (mg/mL)
C_U = concentration of Fosphenytoin Sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified individual impurity in the portion of Fosphenytoin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of phenytoin from the *Standard solution*
C_S = concentration of the USP Phenytoin RS in the *Standard solution* (mg/mL)
C_U = concentration of Fosphenytoin Sodium in the *Sample solution* (mg/mL)

Acceptance criteria
Phenytoin: NMT 0.1%
Any other impurity: NMT 0.1%
Total impurities: NMT 0.5%

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: 75 mg/mL of Fosphenytoin Sodium in water
Acceptance criteria: 8.5–9.5

- **WATER DETERMINATION,** *Method I* ⟨921⟩: 21.7%–25.7%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Fosphenytoin Sodium RS
USP Phenytoin RS
USP Phenytoin Related Compound A RS
Diphenylglycine.
$C_{14}H_{13}NO_2$ 227.26
USP Phenytoin Related Compound B RS
Diphenylhydantoic acid.
$C_{15}H_{14}N_2O_3$ 270.29

USP Monographs

www.webofpharma.com

Fosphenytoin Sodium Injection

DEFINITION

Fosphenytoin Sodium Injection is a sterile solution of Fosphenytoin Sodium in Water for Injection. Fosphenytoin Sodium is a prodrug. Injection containing 1 mg/mL of Fosphenytoin Sodium is equivalent to 0.667 mg/mL of Phenytoin Sodium after injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of fosphenytoin sodium ($C_{16}H_{13}N_2Na_2O_6P$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)

Sample: Transfer a 5-mL aliquot of Injection to a 100-mL beaker. Add 30 mL of acetone to form a white precipitate, and stir for 20 min using a magnetic stirrer. Filter under vacuum, and collect the precipitate using suitable filter paper. Allow to dry under vacuum for 15 min.

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 8.2 g/L of monobasic potassium phosphate in water. Adjust with 6 N potassium hydroxide solution to a pH of 6.5 ± 0.05.

Mobile phase: Methanol, acetonitrile, and *Buffer* (25:2:73)

Standard stock solution A: 0.75 mg/mL of USP Fosphenytoin Sodium RS prepared as follows. Transfer a suitable amount of the standard in a suitable volumetric flask. Dissolve in a minimum amount of methanol. Dilute with *Buffer* to volume.

Standard stock solution B: 7.5 µg/mL of USP Phenytoin RS, 7.5 µg/mL of USP Phenytoin Related Compound A RS, and 15 µg/mL of USP Phenytoin Related Compound B RS in methanol

Standard solution: 150 µg/mL of USP Fosphenytoin Sodium RS from *Standard stock solution A*, 0.75 µg/mL each of USP Phenytoin RS and USP Phenytoin Related Compound A RS, and 1.5 µg/mL of USP Phenytoin Related Compound B RS from *Standard stock solution B* in *Buffer*

Sample stock solution: Nominally 1.5 mg/mL of fosphenytoin sodium from a volume of Injection prepared in methanol

Sample solution: Nominally 150 µg/mL of fosphenytoin sodium from *Sample stock solution* in *Buffer*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; packing L11

Flow rate: 1.25 mL/min

Injection volume: 40 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 1* for the approximate relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between phenytoin related compound B and phenytoin related compound A

Column efficiency: NLT 2250 theoretical plates for fosphenytoin

Tailing factor: NMT 1.8 for fosphenytoin

Relative standard deviation: NMT 1.0% for fosphenytoin and NMT 5.0% for phenytoin, phenytoin related compound A, and phenytoin related compound B

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fosphenytoin sodium ($C_{16}H_{13}N_2Na_2O_6P$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fosphenytoin sodium from the *Sample solution*

r_S = peak response of fosphenytoin sodium from the *Standard solution*

C_S = concentration of USP Fosphenytoin Sodium RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of fosphenytoin sodium in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of phenytoin, phenytoin related compound A, and phenytoin related compound B in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenytoin, phenytoin related compound A, or phenytoin related compound B from the *Sample solution*

r_S = peak response of phenytoin, phenytoin related compound A, or phenytoin related compound B from the *Standard solution*

C_S = concentration of the corresponding analyte in the *Standard solution* (µg/mL)

C_U = nominal concentration of fosphenytoin sodium in the *Sample solution* (µg/mL)

Calculate the percentages of unspecified degradation products in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*

r_S = peak response of phenytoin from the *Standard solution*

C_S = concentration of USP Phenytoin RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of fosphenytoin sodium in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phenytoin related compound B	0.3	1.5
Phenytoin related compound A	0.5	0.2

www.webofpharma.com

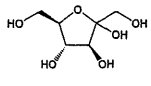
Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fosphenytoin	1.0	—
Phenytoin	3.8	0.2
Any other individual unspecified degradation product	—	0.1
Total impurities	—	2.0

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 14 USP Endotoxin Units/mL
- **pH** ⟨791⟩: 8.3–9.3
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store between 2° and 8°. Do not store at room temperature for more than 48 h.
- **LABELING:** Both the actual content of fosphenytoin sodium and the content of phenytoin sodium, expressed in terms of phenytoin sodium equivalents, are stated on the label.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fosphenytoin Sodium RS
  USP Phenytoin RS
  USP Phenytoin Related Compound A RS
  Diphenylglycine.
  $C_{14}H_{13}NO_2$ 227.26
  USP Phenytoin Related Compound B RS
  Diphenylhydantoic acid.
  $C_{15}H_{14}N_2O_3$ 270.29

Fructose



$C_6H_{12}O_6$ 180.16
D-Fructose [57-48-7].

DEFINITION
Fructose, dried in vacuum at 70° for 4 h, contains NLT 98.0% and NMT 102.0% of fructose ($C_6H_{12}O_6$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Previously dried
  Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**
  Sample: 10 g, previously dried
  Optical rotation
  (See *Optical Rotation, Angular Rotation* ⟨781A⟩.)
  Tube length: 100 mm
  Temperature: 25°
  Analysis: Transfer the *Sample* to a 100-mL volumetric flask, and dissolve in 50 mL of water. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume. After 30 min, determine the angular rotation. The observed rotation, in degrees, represents the weight, in g. Calculate the percentage of fructose ($C_6H_{12}O_6$) in the Fructose taken.

$$\text{Result} = [(a \times F)/W] \times 100$$

a = observed rotation of the sample (°)
F = correction factor, −1.124
W = weight of the *Sample* (g)

Acceptance criteria: 98.0%–102.0% on a previously dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
  Sample: 2.0 g
  Control: 0.50 mL of 0.020 N hydrochloric acid
  Acceptance criteria: NMT 0.018%; the *Sample* shows no more chloride than corresponds to the *Control.*
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
  Sample: 2.0 g
  Control: 0.50 mL of 0.020 N sulfuric acid
  Acceptance criteria: NMT 0.025%; the *Sample* shows no more sulfate than corresponds to the *Control.*
- **ARSENIC,** *Method II* ⟨211⟩
  Test preparation: Prepare by heating the acidified mixture nearly to dryness. Cool, and add 30% hydrogen peroxide.
  Acceptance criteria: NMT 1 µg/g
- **CALCIUM AND MAGNESIUM (AS CALCIUM)**
  Sample: 20 g
  Titrimetric system
  Mode: Direct titration
  Titrant: 0.005 M edetate disodium VS
  Endpoint detection: Visual
  Analysis: Dissolve the *Sample* in 200 mL of water. Add 2 drops of hydrochloric acid, 5 mL of ammonia–ammonium chloride buffer TS, and 8 drops of eriochrome black TS, and mix. Titrate with *Titrant* to a blue endpoint. Each mL of *Titrant* is equivalent to 200.4 µg of Ca.
  Acceptance criteria: 0.005% of Ca; NMT 5.0 mL of *Titrant* is consumed.
- **LIMIT OF HYDROXYMETHYLFURFURAL**
  Resorcinol solution: 10 mg/mL of resorcinol in hydrochloric acid
  Sample solution: 100 mg/mL
  Analysis: To 10 mL of *Sample solution* add 5 mL of ether, and shake vigorously. Transfer 2 mL of the ether layer to a test tube, and add 1 mL of *Resorcinol solution.*
  Acceptance criteria: A slight pink color may develop, but no cherry-red color appears immediately.

SPECIFIC TESTS
- **COLOR OF SOLUTION**
  Sample solution: 25 g of Fructose in water to make 50 mL
  Comparison solution: Mix 1.0 mL of cobaltous chloride CS, 3.0 mL of ferric chloride CS, and 2.0 mL of cupric sulfate CS with water to make 10 mL, and dilute 3 mL of this solution with water to make 50 mL.
  Analysis: View the *Sample solution* and *Compariosn solution* downward in matched color-comparison tubes against a white surface.
  Acceptance criteria: The *Sample solution* has no more color than the *Comparison solution.*
- **ACIDITY**
  Sample: 5.0 g
  Titrimetric system
  Mode: Direct titration

www.webofpharma.com

Titrant: 0.02 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 50 mL of carbon dioxide-free water. Add phenolphthalein TS, and titrate with *Titrant* to a distinct pink color.
Acceptance criteria: NMT 0.50 mL of *Titrant* is needed for neutralization.

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 70° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fructose RS

Fructose Injection

DEFINITION

Fructose Injection is a sterile solution of Fructose in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of fructose ($C_6H_{12}O_6$). Fructose Injection contains no antimicrobial agents.

IDENTIFICATION

• **A.**
Silver nitrate solution: Dissolve 0.6 g of silver nitrate in 2.0 mL of water in a 100-mL volumetric flask, dilute with acetone to volume.
Sodium hydroxide solution: Dissolve 2.0 g of sodium hydroxide in 5.0 mL of water in a 100-mL volumetric flask, dilute with alcohol to volume.
Alcoholic monobasic sodium phosphate: Dissolve 4.0 g of monobasic sodium phosphate in 150 mL of water. Add, with mixing, 500 mL of alcohol. Use the entire mixture, even if there are two phases. Prepare the solution fresh daily.
Standard solution: 2.5 mg/mL of USP Fructose RS
Sample solution: Nominally 2.5 mg/mL of fructose from a volume of Injection diluted with water
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 5 µL
Developing solvent system: Acetone, *n*-butyl alcohol, and water (50:40:10)
Analysis
Samples: *Standard solution* and *Sample solution*
Immerse the TLC plate coated side down in *Alcoholic monobasic sodium phosphate* for 2.0 min. Remove the plate from the solution, place it coated side up on a clean, absorbent towel, dry it in a current of warm air, and activate it at 105° for 30 min. Cool to room temperature, and apply the *Standard solution* and the *Sample solution*. Allow the spots to dry, and develop the chromatogram in the *Developing solvent system*. Remove the plate, allow the solvent to evaporate, and spray the plate with *Silver nitrate solution*. Allow the plate to dry for 30 s, then spray with *Sodium hydroxide solution*.
Acceptance criteria: The R_F value of the spot appearing within 3 min from the *Sample solution* corresponds to that from the *Standard solution*.

ASSAY

• **PROCEDURE**
Sample solution: Transfer an accurately measured volume of Injection, equivalent to 5 g of fructose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix.
Analysis: After 30 min, determine the angular rotation (see *Optical Rotation* ⟨781⟩), and record the observed rotation, α, as an absolute number.
Calculate the percentage of the labeled amount of fructose ($C_6H_{12}O_6$) in the portion of Injection taken:

$$\text{Result} = [(F \times \alpha)/(l \times V \times L)] \times 100$$

F = correction factor, 1124
l = length of the polarimeter tube (dm)
V = volume of Injection taken (mL)
L = labeled amount of fructose in Injection (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **LIMIT OF HYDROXYMETHYLFURFURAL**
Resorcinol solution: 10 mg/mL of resorcinol in hydrochloric acid
Sample solution: Nominally 100 mg/mL of fructose from a volume of Injection diluted with water
Analysis: To 10 mL of *Sample solution* add 5 mL of ether, and shake vigorously. Transfer 2 mL of the ether layer to a test tube, and add 1 mL of *Resorcinol solution*.
Acceptance criteria: A slight pink color may develop, but no cherry-red color appears immediately.

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.5 USP Endotoxin Units/mL

• **pH** ⟨791⟩
Analysis: Dilute a portion with water, if necessary, to NMT 5% of fructose. Add 0.3 mL of a saturated potassium chloride solution for each 100 mL.
Acceptance criteria: 3.0–6.0

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I or Type II glass.

• **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection, but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol/mL.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Fructose RS

Fructose and Sodium Chloride Injection

» Fructose and Sodium Chloride Injection is a sterile solution of Fructose and Sodium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of $C_6H_{12}O_6$ (fructose) and of NaCl (sodium chloride). Fructose and Sodium Chloride Injection contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose containers, preferably of Type I or Type II glass.
Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct

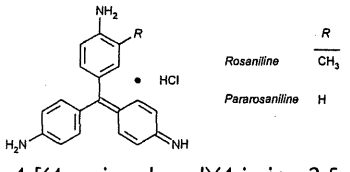
www.webofpharma.com

injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.
USP Reference standards ⟨11⟩—
USP Fructose RS
Identification—It responds to the *IDENTIFICATION* tests under *Fructose Injection* and to the tests for *Sodium* ⟨191⟩ and for *Chloride* ⟨191⟩.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.5 USP Endotoxin Unit per mL.
pH ⟨791⟩: between 3.0 and 6.0.
Limit of hydroxymethylfurfural—It meets the requirements of the test for Limit of Hydroxymethylfurfural under *Fructose*.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay for fructose—Transfer an accurately measured volume of Injection, containing about 5 g of fructose, to a 100-mL volumetric flask, add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix. After 30 minutes determine the angular rotation (see *Optical Rotation* ⟨781⟩), and record the observed rotation, *a*, as an absolute number. Calculate the quantity, in mg, of fructose ($C_6H_{12}O_6$) in each mL of the Injection taken by the formula:

$$1124a/lV$$

in which *l* is the length, in dm, of the polarimeter tube; and *V* is the volume, in mL, of Injection taken.
Assay for sodium chloride—Transfer an accurately measured volume of Injection, equivalent to about 90 mg of sodium chloride, to a conical flask, evaporate to a volume of about 10 mL, and add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride (NaCl).

Basic Fuchsin



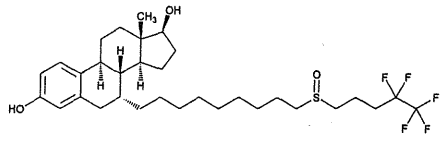
Benzenamine, 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl-2-methyl]-, monohydrochloride.
C.I. Basic Violet 14 monohydrochloride [632-99-5].

» Basic Fuchsin is a mixture of rosaniline and pararosaniline hydrochlorides. It contains the equivalent of not less than 88.0 percent of rosaniline hydrochloride ($C_{20}H_{19}N_3 \cdot HCl$), calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

Identification—
A: To 5 mL of a solution (1 in 1000) add a few drops of hydrochloric acid: a yellow color is produced (*distinction from acid fuchsin*).
B: To 5 mL of a solution (1 in 500) add a few drops of tannic acid TS: a red precipitate is formed.
C: To 10 mL of a solution (1 in 500) add 10 mL of ammonia TS and 500 mg of zinc dust, and agitate the mixture: the solution becomes decolorized. Place a few drops of the decolorized solution on filter paper, and nearby on the same paper place a few drops of 3 N hydrochloric acid: a red color develops at the zone of contact.
Loss on drying ⟨731⟩—Dry it at 105° to constant weight: it loses not more than 5.0% of its weight.
Residue on ignition ⟨281⟩—Ignite 1 g with 0.5 mL of sulfuric acid: the weight of the residue is not more than 0.3%.
Alcohol-insoluble substances—Boil 1 g, accurately weighed, with 50 mL of alcohol under a reflux condenser for 15 minutes, filter through a tared filtering crucible, wash the residue on the filter with hot alcohol until the washings cease to be colored violet, and dry the crucible at 105° for 1 hour: the amount of insoluble residue is not more than 1.0%.
Arsenic, *Method II* ⟨211⟩: 8 ppm.
Lead ⟨251⟩: Place 1 g in a small Kjeldahl flask, add 5 mL of sulfuric acid, and insert a small funnel into the flask. Gently rotate the flask until the sulfuric acid has completely wetted the Basic Fuchsin, then heat with a small flame until carbonization is complete. Allow to cool, and add, in small quantities, 5 mL of nitric acid. Again heat gently until fumes of sulfur trioxide are evolved. Allow to cool, add another 5 mL of nitric acid, and heat to the evolution of sulfur trioxide. Allow to cool, add about 25 mL of water, and boil for a few minutes. Cool, neutralize with stronger ammonia water, using litmus paper as the indicator, and add 5 mL of nitric acid. Transfer the solution to a 100-mL volumetric flask, dilute to volume, and mix. A 20-mL portion of this solution contains not more than 30 ppm of lead.
Assay—Dissolve about 100 mg of Basic Fuchsin, accurately weighed, in 175 mL of water in a 500-mL closed system titration vessel fitted with a gas inlet tube, a gas outlet tube, an upright reflux condenser, and a buret. Add about 25 mL of sodium tartrate solution (30 in 100) and a polytef-coated magnetic stirring bar, and heat to boiling. Flush this titration vessel for 15 minutes with nitrogen that has been passed through two successive gas washing bottles each containing 500 mL of a mixture of water, titanium trichloride solution (20 in 100), and hydrochloric acid (400:40:40) to which about 10 mg of safranin O has been added. Continue the heating and nitrogen flow, and while stirring titrate with 0.05 N titanium trichloride VS to a yellow endpoint. Each mL of 0.05 N titanium trichloride is equivalent to 3.379 mg of $C_{20}H_{19}N_3 \cdot HCl$.

Fulvestrant



$C_{32}H_{47}F_5O_3S$ 606.77
Estra-1,3,5(10)-triene-3,17-diol, 7-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]-, (7α,17β)-;
7α-[9-[(4,4,5,5,5,-Pentafluoropentyl)sulfinyl]non yl]estra-1,3,5(10)-triene-3,17β-diol [129453-61-8].

» Fulvestrant is a mixture of the diastereoisomers A and B. It contains not less than 97.0 percent and

USP Monographs

www.webofpharma.com

not more than 102.0 percent of $C_{32}H_{47}F_5O_3S$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed, light-resistant containers. Store refrigerated at 2° to 8°.

USP Reference standards ⟨11⟩—
USP Fulvestrant RS
USP Fulvestrant System Suitability Mixture RS
Contains fulvestrant isomer A, fulvestrant isomer B, and fulvestrant β-isomer.

Identification—

Change to read:

A: ▲Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197K▲ (CN 1-May-2020)—
Spectral range: 4000 to 400 cm^{-1}.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Specific rotation ⟨781S⟩: between +108° and +115° measured at 365 nm.
Test solution: 20 mg per mL, in methanol.

Water Determination, *Method Ic* ⟨921⟩ : not more than 0.5%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—
Mobile phase and *System suitability solution*—Prepare as directed in the *Assay.*
Standard solution—Prepare as directed for the *Standard preparation* in the *Assay.*
Test solution—Use the *Assay preparation.*
Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.*
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Fulvestrant taken by the formula:

$$100(CV/W)(1/F)(r_i/r_S)$$

in which C is the concentration, in mg per mL, of USP Fulvestrant RS in the *Standard solution; V* is the volume, in mL, of the *Test solution; W* is the weight, in mg, of Fulvestrant taken to prepare the *Test solution; F* is the relative response factor as listed in the accompanying table; r_i is the individual peak response for each impurity obtained from the *Test solution;* and r_S is the fulvestrant peak response obtained from the *Standard solution.* Disregard impurity peaks less than 0.05%. The limits are as shown in the accompanying table.

Compound	Relative Retention Time	Relative Response Factor	Limit (%)
6-Keto-fulvestrant[1]	0.5	2.9	0.1
Δ6,7-Fulvestrant[2]	0.9	3.3	0.1
Fulvestrant	1.0	1.0	—
Fulvestrant sulfone[3]	1.2	1.0	0.2
Fulvestrant extended[4]	1.7	1.0	0.3
Fulvestrant sterol dimer[5]	1.9	1.0	0.8
Fulvestrant β-isomer[6]	1.1	—	*
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	1.0

* Fulvestrant β-isomer, a component of USP Fulvestrant System Suitability Mixture RS, is not a specified impurity.
[1] Estra-1,3,5(10)-triene-6-one-3,17-diol,7-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]-(7α,17β)
[2] Estra-1,3,5(10),6-tetraene-3,17-diol,7-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]-(7α,17β)
[3] Estra-1,3,5(10)-triene-3,17-diol,7-[9-[(4,4,5,5,5-pentafluoropentyl)sulfonyl]nonyl]-(7α,17β)
[4] Estra-1,3,5(10)-triene-3,17-diol,7-[9-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonylsulfinyl]nonyl)-(7α,17β)
[5] 7,7-Nonamethylene-bis(estra-1,3,5(10)-triene-3,17-diol-(7α,17β)
[6] Estra-1,3,5(10)-triene-3,17-diol,7-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]-(7β,17β)

Diastereoisomer ratio—
Mobile phase—Prepare a filtered and degassed mixture of 2-methylpentane and dehydrated alcohol (880:120). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve a suitable quantity of USP Fulvestrant System Suitability Mixture RS in *Mobile phase* to obtain a solution containing 1 mg of USP Fulvestrant System Suitability Mixture RS per mL.
Test solution—Transfer about 20 mg of Fulvestrant, accurately weighed, to a 20-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 10-µm packing L51. The flow rate is about 1 mL per minute. The column temperature is maintained at 40°. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between fulvestrant isomer A and fulvestrant isomer B is not less than 1.3; and the tailing factor for fulvestrant isomer B is not more than 1.5. [NOTE—For the purpose of peak identification, the retention times are about 20 minutes for fulvestrant isomer B and 23 minutes for fulvestrant isomer A.]
Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for the two fulvestrant isomer peaks. Calculate the content of fulvestrant isomer A or fulvestrant isomer B, as a percentage, by the formula:

$$100(r_U/r_S)$$

in which r_U is the peak response of either fulvestrant isomer A or fulvestrant isomer B; and r_S is the total peak response of both fulvestrant isomer A and fulvestrant isomer B: between 42% and 48% of fulvestrant isomer A and between 52% and 58% of fulvestrant isomer B is obtained.

Assay—
Solution A—Prepare a filtered and degassed mixture of water, acetonitrile, and methanol (410:320:270).
Solution B—Prepare a filtered and degassed mixture of acetonitrile, methanol, and water (490:410:100).
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve suitable quantities of USP Fulvestrant System Suitability Mixture RS in methanol to

www.webofpharma.com

obtain a solution containing about 10 mg of USP Fulvestrant System Suitability Mixture RS per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Fulvestrant RS in methanol to obtain a solution having a known concentration of about 10 mg per mL.

Assay preparation—Transfer about 100 mg of Fulvestrant, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 15-cm column that contains 3.5-µm packing L7. The flow rate is about 2 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–25	100	0	isocratic
25–55	100→0	0→100	linear gradient
55–65	0	100	isocratic
65–66	0→100	100→0	linear gradient
66–70	100	0	equilibration

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.1 for fulvestrant β-isomer and 1.0 for fulvestrant; the resolution, *R,* between fulvestrant and fulvestrant β-isomer is not less than 1.5; and the tailing factor for the fulvestrant peak is not more than 1.5.

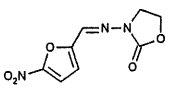
Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the fulvestrant peaks. Calculate the quantity, in mg, of $C_{32}H_{47}F_5O_3S$ in the portion of Fulvestrant taken by the formula:

$$CV(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Fulvestrant RS in the *Standard preparation; V* is the volume, in mL, of the *Assay preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Furazolidone



$C_8H_7N_3O_5$ 225.16

2-Oxazolidinone, 3-[[(5-nitro-2-furanyl)methylene]amino]-.

3-[(5-Nitrofurfurylidene)amino]-2-oxazolidinone [67-45-8].

» Furazolidone contains not less than 97.0 percent and not more than 103.0 percent of $C_8H_7N_3O_5$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight.

USP Reference standards ⟨11⟩—

USP Furazolidone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy:* ⟨197K⟩▲ (CN 1-May-2020): previously dried.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)

Solution: 10 µg per mL.

C: Add about 50 mg to 10 mL of a freshly prepared mixture of dimethylformamide and alcoholic potassium hydroxide TS (9:1): the solution becomes purple, immediately changes to deep blue, and, upon standing for 10 minutes, again turns purple.

Loss on drying ⟨731⟩—Dry it at 100° for 1 hour: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.25%.

Assay—Transfer about 100 mg of Furazolidone, accurately weighed, to a 250-mL volumetric flask, dilute with dimethylformamide to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with water to volume, and mix (assay solution). Similarly, dissolve a suitable quantity of USP Furazolidone RS, accurately weighed, in dimethylformamide to obtain a Standard stock solution having a known concentration of about 400 µg per mL. Transfer 5.0 mL of this stock solution to a 250-mL volumetric flask, dilute with water to volume, and mix (Standard solution). Concomitantly determine the absorbances of the assay solution and the Standard solution at the wavelength of maximum absorbance at about 367 nm, with a suitable spectrophotometer, using dimethylformamide solution (1 in 50) as the blank. Calculate the quantity, in mg, of $C_8H_7N_3O_5$ in the Furazolidone taken by the formula:

$$12.5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Furazolidone RS in the Standard solution; and A_U and A_S are the absorbances of the assay solution and the Standard solution, respectively.

Furazolidone Oral Suspension

» Furazolidone Oral Suspension is a suspension of Furazolidone in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of furazolidone ($C_8H_7N_3O_5$).

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

USP Reference standards ⟨11⟩—

USP Furazolidone RS

Identification—Add a quantity of Oral Suspension, equivalent to about 50 mg of furazolidone, to 10 mL of a freshly prepared mixture of dimethylformamide and alcoholic potassium hydroxide TS (9:1): the solution turns purple,

USP Monographs

www.webofpharma.com

immediately changes to deep blue, and, upon standing for 10 minutes, again turns purple.
pH ⟨791⟩: between 6.0 and 8.5.
Assay—Transfer an accurately measured volume of Oral Suspension, equivalent to about 160 mg of furazolidone, to a suitable flask. Add 5 mL of water, and mix. Transfer the mixture with the aid of dimethylformamide to a 1000-mL volumetric flask. Add about 500 mL of dimethylformamide, shake by mechanical means for 10 minutes, dilute with dimethylformamide to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix (assay solution). Similarly, dissolve a suitable quantity of USP Furazolidone RS, accurately weighed, in dimethylformamide to obtain a Standard stock solution having a known concentration of about 160 µg per mL. Transfer 5.0 mL of this stock solution to a 100-mL volumetric flask, dilute with water to volume, and mix (Standard solution). Concomitantly determine the absorbances of the assay solution and the Standard solution at the wavelength of maximum absorbance at about 367 nm, with a suitable spectrophotometer, using dimethylformamide solution (1 in 20) as the blank. Calculate the quantity, in mg, of furazolidone ($C_8H_7N_3O_5$) in each mL of the Oral Suspension taken by the formula:

$$20(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Furazolidone RS in the Standard solution; V is the volume, in mL, of Oral Suspension taken; and A_U and A_S are the absorbances of the assay solution and the Standard solution, respectively.

Furazolidone Tablets

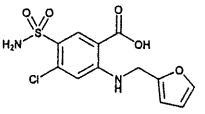
» Furazolidone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_8H_7N_3O_5$.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
USP Reference standards ⟨11⟩—
USP Furazolidone RS
Identification—Add a quantity of powdered Tablets, equivalent to about 50 mg of furazolidone, to 10 mL of a freshly prepared mixture of dimethylformamide and alcoholic potassium hydroxide TS (9:1): the solution turns purple, immediately changes to deep blue, and, upon standing for 10 minutes, again turns purple.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Assay—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of furazolidone, to a 250-mL volumetric flask. Add about 150 mL of dimethylformamide, warm to about 50°, and sonicate to aid in dissolving the furazolidone. Cool, dilute with dimethylformamide to volume, mix, and centrifuge a portion of the mixture. Transfer 5.0 mL of the clear solution so obtained to a 250-mL volumetric flask, dilute with water to volume, and mix (assay solution). Similarly, dissolve a suitable quantity of USP Furazolidone RS, accurately weighed, in dimethylformamide to obtain a Standard stock solution having a known concentration of about 400 µg per mL. Transfer 5.0 mL of this stock solution to a 250-mL volumetric flask, dilute with water to volume, and mix (Standard solution). Concomitantly determine the absorbances of the assay solution and the Standard solution at the wavelength of maximum absorbance at about 367 nm, with a suitable spectrophotometer, using dimethylformamide solution (1 in 50) as the blank. Calculate the quantity, in mg, of $C_8H_7N_3O_5$ in the portion of Tablets taken by the formula:

$$12.5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Furazolidone RS in the Standard solution; and A_U and A_S are the absorbances of the assay solution and the Standard solution, respectively.

Furosemide



$C_{12}H_{11}ClN_2O_5S$ 330.74
Benzoic acid, 5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl) amino]-;
4-Chloro-*N*-furfuryl-5-sulfamoylanthranilic acid [54-31-9].

DEFINITION
Furosemide contains NLT 98.0% and NMT 102.0 of furosemide ($C_{12}H_{11}ClN_2O_5S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Protect Furosemide solutions from exposure to light.
Mobile phase: Tetrahydrofuran, glacial acetic acid, and water (30:1:70)
Solution A: Acetonitrile and water (50:50)
Diluent: *Solution A* and glacial acetic acid (978:22)
System suitability solution: 20 µg/mL of USP Furosemide RS and 12 µg/mL of USP Furosemide Related Compound A RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Furosemide RS in *Diluent*
Sample solution: 0.2 mg/mL of Furosemide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 272 nm. For *Identification* test *C*, use a diode-array detector in the range of 200–400 nm.
Column: 4.6-mm × 25.0-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*

www.webofpharma.com

Suitability requirements
Resolution: NLT 1.5 between furosemide related compound A and furosemide, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of furosemide ($C_{12}H_{11}ClN_2O_5S$) in the portion of Furosemide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Furosemide RS in the *Standard solution* (mg/mL)
C_U = concentration of Furosemide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **ORGANIC IMPURITIES**
Protect Furosemide solutions from exposure to light.
Mobile phase, Solution A, Diluent, and **System suitability solution:** Proceed as directed in the *Assay*.
Standard solution: 5.0 µg/mL each of USP Furosemide Related Compound A RS and USP Furosemide Related Compound B RS in *Diluent*
Sample solution: 1.0 mg/mL of Furosemide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors: 254 and 272 nm
[NOTE—The 2,4-dichloro-5-sulfamoylbenzoic acid impurity does not respond at 272 nm, and the 2,4-bis(furfurylamino)-5-sulfamoylbenzoic acid impurity has a very intense absorbance at 254 nm. The response for furosemide is at 254 nm.]
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 2.5 times the retention time of the furosemide peak
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.5 between furosemide and furosemide related compound A
Relative standard deviation: NMT 2.0% for furosemide
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The sum of the peak areas of peaks eluting before furosemide at 254 nm from the *Sample solution* is NMT the area of the furosemide related compound B peak at 254 nm from the *Standard solution* (0.5%). The sum of the peak areas of peaks eluting after furosemide at 272 nm from the *Sample solution* is NMT the area of the furosemide related compound A peak at 272 nm from the *Standard solution* (0.5%).

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Furosemide RS
USP Furosemide Related Compound A RS
2-Chloro-4-*N*-furfurylamino-5-sulfamoylbenzoic acid.
$C_{12}H_{11}ClN_2O_5S$ 330.74
USP Furosemide Related Compound B RS
4-Chloro-5-sulfamoylanthranilic acid.
$C_7H_7ClN_2O_4S$ 250.66

Furosemide Injection

» Furosemide Injection is a sterile solution of Furosemide in Water for Injection prepared with the aid of Sodium Hydroxide or, where intended solely for veterinary use, Diethanolamine or Monoethanolamine. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of furosemide ($C_{12}H_{11}ClN_2O_5S$).

Packaging and storage—Store in single-dose or multiple-dose, light-resistant containers, of Type I glass.
Labeling—Injection intended solely for veterinary use is so labeled.
USP Reference standards ⟨11⟩—
USP Furosemide RS
USP Furosemide Related Compound A RS
2-Chloro-4-*N*-furfurylamino-5-sulfamoylbenzoic acid.
$C_{12}H_{11}ClN_2O_5S$ 330.74
USP Furosemide Related Compound B RS
4-Chloro-5-sulfamoylanthranilic acid.
$C_7H_7ClN_2O_4S$ 250.66
Identification—Transfer to a 100-mL volumetric flask a volume of Injection, equivalent to about 40 mg of furosemide, dilute with water to volume, and mix. Dilute 2.0 mL of this solution with 0.02 N sodium hydroxide in a second 100-mL volumetric flask to volume, and mix. Dissolve about 10 mg of USP Furosemide RS in 6.0 mL of 0.1 N sodium hydroxide in a 25-mL volumetric flask, and dilute with water to volume. Dilute 2.0 mL of the resulting solution quantitatively with 0.02 N sodium hydroxide to obtain a Standard solution having a concentration of about 8 µg per mL. Concomitantly determine the UV absorption spectra of both solutions: the UV absorption spectra so obtained exhibit maxima and minima at the same wavelengths.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 3.6 USP Endotoxin Units per mg of furosemide.
pH ⟨791⟩**:** between 8.0 and 9.3 or, where labeled as intended solely for veterinary use, between 7.0 and 7.8 if it contains diethanolamine, or between 8.0 and 9.3 if it contains monoethanolamine.
Particulate Matter in Injections ⟨788⟩ **:** meets the requirements for small-volume injections.
Limit of furosemide related compound B—
[NOTE—Protect furosemide solutions from exposure to light.]
Mobile phase, Diluting solution, System suitability solution and *Chromatographic system* —Prepare as directed in the test for *SPECIFIC TESTS* under *Furosemide*.
Standard solution—Prepare a solution in *Diluting solution* containing 10.0 µg of USP Furosemide Related Compound B RS per mL.

USP Monographs

www.webofpharma.com

Test solution—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of furosemide, to a 10-mL volumetric flask, add *Diluting solution* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. The response at 254 nm obtained for any peak observed in the chromatogram of the *Test solution* at a retention time corresponding to that of the Reference Standard in the *Standard solution* is not greater than the response at 254 nm obtained for the peak in the chromatogram of the *Standard solution*, corresponding to not more than 1.0% of furosemide related compound B. Where the Injection is labeled as intended solely for veterinary use, the response at 254 nm obtained in the chromatogram of the *Test solution* at a retention time corresponding to that of the Reference Standard in the *Standard solution* is not greater than 2.5 times the response at 254 nm obtained for the peak in the chromatogram of the *Standard solution*, corresponding to not more than 2.5% of furosemide related compound B.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay— [NOTE—Protect furosemide solutions from exposure to light.]

Mobile phase, Diluting solution, System suitability solution, and *Chromatographic system*—Prepare as directed in the test for *SPECIFIC TESTS* under *Furosemide.*

Standard preparation—Dissolve an accurately weighed quantity of USP Furosemide RS in *Diluting solution* to obtain a solution having a known concentration of about 1.0 mg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of furosemide, to a 10-mL volumetric flask, add *Diluting solution* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Using the response at 254 nm, calculate the quantity, in mg, of furosemide ($C_{12}H_{11}ClN_2O_5S$) in each mL of the Injection taken by the formula:

$$10(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Furosemide RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Furosemide Oral Solution

» Furosemide Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of furosemide ($C_{12}H_{11}ClN_2O_5S$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Furosemide RS
USP Furosemide Related Compound A RS
2-Chloro-4-*N*-furfurylamino-5-sulfamoylbenzoic acid.
$C_{12}H_{11}ClN_2O_5S$ 330.74

USP Furosemide Related Compound B RS
4-Chloro-5-sulfamoylanthranilic acid.
$C_7H_7ClN_2O_4S$ 250.66

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 6 µg per mL.
Medium: 0.01 N sodium hydroxide.
Absorptivities are not significantly different.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Minimum fill ⟨755⟩**:** meets the requirements.

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

pH ⟨791⟩**:** between 7.0 and 10.0.

[NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standards, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

Limit of furosemide related compound B—

Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay.*

Standard solution—Dissolve an accurately weighed quantity of USP Furosemide Related Compound B RS in *Diluting solution,* and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 15.0 µg per mL.

Test solution—Transfer an accurately measured portion of Oral Solution, equivalent to about 10 mg of furosemide, to a 10-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks: the peak response of furosemide related compound B obtained from the *Test solution* is not greater than the corresponding peak response obtained from the *Standard solution.*

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (165:35:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluting solution—Prepare a mixture consisting of water, acetonitrile, and glacial acetic acid (22:22:1).

System suitability solution—Dissolve suitable quantities of USP Furosemide RS, USP Furosemide Related Compound A RS, and USP Furosemide Related Compound B RS in *Diluting solution* to obtain a solution containing about 0.1 mg of each per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Furosemide RS in *Diluting solution,* and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 1 mg per mL.

www.webofpharma.com

Assay preparation—Transfer an accurately measured portion of Oral Solution, equivalent to about 10 mg of furosemide, to a 10-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.2 for furosemide related compound B, 1.0 for furosemide, and 1.1 for furosemide related compound A; the resolution, *R,* between furosemide and furosemide related compound A is not less than 1.5; and the tailing factor for the furosemide peak is not more than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of furosemide ($C_{12}H_{11}ClN_2O_5S$) in the portion of Oral Solution taken by the formula:

$$10C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Furosemide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Furosemide Tablets

» Furosemide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of furosemide ($C_{12}H_{11}ClN_2O_5S$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

Labeling—The labeling indicates with which *Dissolution* test the product complies. Tablets intended solely for veterinary use are so labeled.

USP Reference standards ⟨11⟩—
USP Furosemide RS
USP Furosemide Related Compound A RS
2-Chloro-4-*N*-furfurylamino-5-sulfamoylbenzoic acid.
$C_{12}H_{11}ClN_2O_5S$ 330.74
USP Furosemide Related Compound B RS
4-Chloro-5-sulfamoylanthranilic acid.
$C_7H_7ClN_2O_4S$ 250.66

Identification—Transfer a portion of finely powdered Tablets, equivalent to about 40 mg of furosemide, to a 100-mL volumetric flask. Add 25 mL of 0.1 N sodium hydroxide, and allow to stand for 30 minutes with occasional shaking. Dilute with water to volume, and mix. Filter the solution, discarding the first 10 mL of the filtrate, and transfer 2.0 mL to a second 100-mL volumetric flask. Add 0.02 N sodium hydroxide to volume, and mix. Proceed as directed in the *Identification* test under *Furosemide Injection*, beginning with "Dissolve about 10 mg of USP Furosemide RS."

Dissolution ⟨711⟩—
Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1.*
Medium: pH 5.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.
Apparatus 2: 50 rpm.
Time: 60 minutes.
Procedure—Determine the amount of $C_{12}H_{11}ClN_2O_5S$ dissolved from UV absorbances at the isosbestic point at 274 nm on filtered portions of the solution under test, suitably diluted with pH 5.8 phosphate buffer, in comparison with a Standard solution having a known concentration of USP Furosemide RS in the same *Medium.*
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{12}H_{11}ClN_2O_5S$ is dissolved in 60 minutes.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.* Tablets labeled as intended for veterinary use comply with this test.
Apparatus 2: 65 rpm.
Medium, Time, and *Procedure*—Proceed as directed under *Test 1.*
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{12}H_{11}ClN_2O_5S$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Limit of furosemide related compound B—
[NOTE—Protect furosemide solutions from exposure to light.]
Mobile phase, Diluting solution, System suitability solution, and *Chromatographic system*—Prepare as directed in the test for *SPECIFIC TESTS* under *Furosemide.*
Standard solution—Prepare a solution in *Diluting solution* containing 8.0 µg of USP Furosemide Related Compound B RS per mL.
Test solution—Transfer an accurately weighed portion of finely powdered Tablets, equivalent to about 10 mg of furosemide, to a 10-mL volumetric flask, add *Diluting solution* to volume, and mix.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. The response at 254 nm obtained for any peak observed in the chromatogram of the *Test solution* at a retention time corresponding to that of the Reference Standard in the *Standard solution* is not greater than the response at 254 nm obtained for the peak in the chromatogram of the *Standard solution,* corresponding to not more than 0.8% of furosemide related compound B.

Assay— [NOTE—Protect furosemide solutions from exposure to light.]
Mobile phase, Diluting solution, System suitability solution, and *Chromatographic system*—Prepare as directed in the test for *SPECIFIC TESTS* under *Furosemide.*
Standard preparation—Dissolve an accurately weighed quantity of USP Furosemide RS in *Diluting solution* to obtain a solution having a known concentration of about 1.0 mg per mL.
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of furosemide, to a 50-mL volumetric flask, add 30 mL of *Diluting solution,* and sonicate for 10 minutes. Add *Diluting solution* to volume, mix, and filter, discarding the first 10 mL of the filtrate.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Using the response at 254 nm, calculate the quantity, in mg, of furosemide ($C_{12}H_{11}ClN_2O_5S$) in the portion of Tablets taken by the formula:

$$50C(r_U/r_S)$$

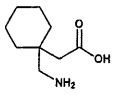
in which C is the concentration, in mg per mL, of USP Furosemide RS in the *Standard preparation;* and r_U and r_S are

USP Monographs

www.webofpharma.com

the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Gabapentin



$C_9H_{17}NO_2$ 171.24

Cyclohexaneacetic acid, 1-(aminomethyl)-;
1-(Aminomethyl)cyclohexaneacetic acid [60142-96-3].

DEFINITION

Gabapentin contains NLT 98.0% and NMT 102.0% of gabapentin ($C_9H_{17}NO_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: 2.32 g/L of monobasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 2.0.
Buffer: 0.58 g/L of monobasic ammonium phosphate and 1.83 g/L of sodium perchlorate in water. Adjust with perchloric acid to a pH of 1.8.
Mobile phase: Acetonitrile and *Buffer* (24:76)
Standard solution: 14.0 mg/mL of USP Gabapentin RS in *Diluent*
System suitability solution: 2.3 mg/mL of USP Gabapentin RS from the *Standard solution* in *Diluent*
Sample solution: 14 mg/mL of Gabapentin in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 5 times the retention time of gabapentin
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Column efficiency: NLT 1900 theoretical plates for the gabapentin peak, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of gabapentin ($C_9H_{17}NO_2$) in the portion of Gabapentin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Gabapentin RS in the *Standard solution* (mg/mL)
C_U = concentration of Gabapentin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **EARLY-ELUTING ORGANIC IMPURITIES**

Diluent, Buffer, and **Mobile phase:** Prepare as directed in the *Assay*.
System suitability stock solution: 1.4 mg/mL of USP Gabapentin Related Compound A RS and 0.84 mg/mL of USP Gabapentin Related Compound B RS in methanol
System suitability solution: Dissolve a suitable quantity of USP Gabapentin RS in *Diluent* in a suitable volumetric flask, and add an appropriate volume of *System suitability stock solution* to obtain a solution containing 14.0 mg/mL of USP Gabapentin RS, 14 µg/mL of USP Gabapentin Related Compound A RS, and 8.4 µg/mL of USP Gabapentin Related Compound B RS in *Diluent*. Inject within 24 h.
Standard solution: 0.0084 mg/mL of USP Gabapentin Related Compound E RS in *Diluent*
Sample solution: 14 mg/mL of Gabapentin in *Diluent*. Inject within 24 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 5 ± 2°
Column: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 5 times the retention time of gabapentin
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.3 between gabapentin related compound A and gabapentin related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0% for gabapentin related compound E, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any impurity in the portion of Gabapentin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of gabapentin related compound E from the *Standard solution*
C_S = concentration of USP Gabapentin Related Compound E RS in the *Standard solution* (mg/mL)
C_U = concentration of Gabapentin in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time[a]	Relative Response Factor[b]	Acceptance Criteria, NMT (%)
Gabapentin	1.0	—	—

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time[a]	Relative Response Factor[b]	Acceptance Criteria, NMT (%)
Gabapentin related compound E	2.9	1.0	0.10
Gabapentin related compound A	3.5	5.3	0.1
Gabapentin related compound B	3.8	0.35	0.06
Any other individual impurity	—	0.41	0.10

[a] The relative retention times are calculated based on the retention time of gabapentin.
[b] The relative response factors are calculated based on the response of gabapentin related compound E due to the low absorptivity of gabapentin at the monitoring wavelength (215 nm).

• **LATE-ELUTING ORGANIC IMPURITIES**
Diluent, Buffer, and **Sample solution:** Prepare as directed in the *Assay.*
Mobile phase: Acetonitrile, methanol, and *Buffer* (35:30:35)
Standard solution: 0.0028 mg/mL of USP Gabapentin Related Compound D RS in *Diluent.* Initially dissolve USP Gabapentin Related Compound D RS in a small amount of methanol, then dilute with *Diluent.*
Sample solution: 14 mg/mL of Gabapentin in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of gabapentin related compound D
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 13,600 theoretical plates
Relative standard deviation: NMT 7.0%
Analysis
Samples: *Sample solution* and *Standard solution*
[NOTE—Disregard peaks with a relative retention time of NMT 0.35, relative to gabapentin related compound D.]
Calculate the percentage of any impurity in the portion of Gabapentin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any impurity from the *Sample solution*
r_S = peak response of gabapentin related compound D from the *Standard solution*
C_S = concentration of USP Gabapentin Related Compound D RS in the *Standard solution* (mg/mL)
C_U = concentration of Gabapentin in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: NMT 0.10%
Total impurities: NMT 0.5% (including impurities found in the test for *Early-Eluting Organic Impurities*)

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 20 mg/mL in water
Acceptance criteria: 6.5–8.0
• **WATER DETERMINATION** ⟨921⟩, *Method I:* NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Gabapentin RS
USP Gabapentin Related Compound A RS
2-Azaspiro[4.5]decan-3-one.
$C_9H_{15}NO$ 153.22
USP Gabapentin Related Compound B RS
(1-Cyanocyclohexyl)acetic acid.
$C_9H_{13}NO_2$ 167.21
USP Gabapentin Related Compound D RS
[1-(3-Oxo-2-aza-spiro[4.5]dec-2-ylmethyl)cyclohexyl] acetic acid.
$C_{18}H_{29}NO_3$ 307.43
USP Gabapentin Related Compound E RS
Carboxymethylcyclohexanecarboxylic acid.
$C_9H_{14}O_4$ 186.21

Gabapentin Capsules

DEFINITION
Gabapentin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of gabapentin ($C_9H_{17}NO_2$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Empty the contents of NLT 10 Capsules, and grind to a fine powder. Use a quantity of the powder, equivalent to 2 mg of gabapentin, and 200 mg of potassium bromide.
Acceptance criteria: Meet the requirements
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Diluent: 1.2 g/L of monobasic potassium phosphate in water. Adjust with 5 N potassium hydroxide to a pH of 6.9.
Mobile phase: Dissolve 1.2 g of monobasic potassium phosphate in 940 mL water. Adjust with 5 N potassium hydroxide to a pH of 6.9. Add 60 mL of acetonitrile and stir.
Standard solution: 4.0 mg/mL of USP Gabapentin RS in *Diluent*
Sample solution: Nominally 4.0 mg/mL of gabapentin, from the contents of NLT 20 Capsules, equivalent to 100 mg of gabapentin, in *Diluent.* Sonication for about 60 s may be necessary to dissolve the contents.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*

www.webofpharma.com

Suitability requirements
Column efficiency: NLT 7000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of gabapentin ($C_9H_{17}NO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Gabapentin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of gabapentin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.06 N hydrochloric acid (prepared by adding 51 mL of hydrochloric acid to 10 L of water); 900 mL
Apparatus 2: 50 rpm
Time: 20 min
Sample solution: Filter a portion of the solution under test using a suitable filter of 0.45-µm pore size.
Mobile phase: Prepare as directed in the *Assay*.
Standard solution: 0.0011*L* mg/mL of USP Gabapentin RS in the *Medium*, where *L* is the label claim in mg/Capsule
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 7000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of gabapentin ($C_9H_{17}NO_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of the *Medium* in the dissolution vessel, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (*Q*) of the labeled amount of gabapentin ($C_9H_{17}NO_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Diluent: Prepare as directed in the *Assay*.
Solution A: Dissolve 1.2 g of monobasic potassium phosphate in 940 mL water. Adjust with 5 N potassium hydroxide to a pH of 6.9. Add 60 mL of acetonitrile and stir.
Solution B: Dissolve 1.2 g of monobasic potassium phosphate in 700 mL water. Adjust with 5 N potassium hydroxide to a pH of 6.9. Add 300 mL of acetonitrile and stir.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
4.0	100	0
45.0	0	100
45.1	100	0
50.0	100	0

Standard solution: 0.04 mg/mL each of USP Gabapentin RS and USP Gabapentin Related Compound A RS in *Diluent*
Sample solution: Nominally 20 mg/mL of gabapentin, from the contents of NLT 20 Capsules, equivalent to 500 mg of gabapentin, in *Diluent*. Sonication for about 30 s may be necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the gabapentin peak
Relative standard deviation: NMT 5.0% for gabapentin and gabapentin related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of gabapentin related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for gabapentin related compound A from the *Sample solution*
r_S = peak response for gabapentin related compound A from the *Standard solution*
C_S = concentration of USP Gabapentin Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of gabapentin in the *Sample solution* (mg/mL)

Calculate the percentage of any other unspecified degradation product, relative to gabapentin content, in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each unspecified impurity from the *Sample solution*
r_S = peak response for gabapentin from the *Standard solution*
C_S = concentration of USP Gabapentin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of gabapentin in the *Sample solution* (mg/mL)

www.webofpharma.com

Acceptance criteria
Gabapentin related compound A: NMT 0.4%
Any individual unspecified impurity: NMT 0.1%
Total impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Gabapentin RS
USP Gabapentin Related Compound A RS
2-Aza-spiro[4.5]decan-3-one.
$C_9H_{15}NO$ 153.22

Gabapentin Tablets

DEFINITION
Gabapentin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of gabapentin ($C_9H_{17}NO_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
Sample: Grind at least 20 Tablets to a fine powder. Use an amount of powder equivalent to 2 mg of gabapentin and 200 mg of dry potassium bromide.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Diluent: 1.2 g/L of monobasic potassium phosphate in water. Adjust with 5 N potassium hydroxide to a pH of 6.9.
Mobile phase: Dissolve 1.2 g of monobasic potassium phosphate in 940 mL of water. Adjust with 5 N potassium hydroxide to a pH of 6.9. Add 60 mL of acetonitrile, and stir. Filter and degas.
Standard solution: 4.0 mg/mL of USP Gabapentin RS in *Diluent*
Sample solution: 4.0 mg/mL of gabapentin from NLT 20 finely powdered Tablets in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 7000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% of gabapentin
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of $C_9H_{17}NO_2$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Gabapentin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of gabapentin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.06 N hydrochloric acid (51 mL of hydrochloric acid in 10 L of water); 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter.
Determine the amount of $C_9H_{17}NO_2$ dissolved by using the following method.
Mobile phase: Prepare as directed in the *Assay*.
Standard solution: 0.0011 × L mg/mL of USP Gabapentin RS in the *Medium*, where L is the label claim in mg/Tablet
Chromatographic system: Proceed as directed for the *Assay*.
Injection size: 100 µL for the Tablets labeled to contain 100, 300, or 400 mg; 50 µL for Tablets labeled to contain 600 or 800 mg
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_9H_{17}NO_2$ dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of the *Medium*, 900 mL
L = Tablet label claim in mg

Tolerances: NLT 80% (Q) of the labeled amount of $C_9H_{17}NO_2$ is dissolved.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium, Apparatus 2, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **Analysis:** Proceed as directed for *Test 1*.
Time: 30 min
Tolerances: NLT 80% (Q) of the labeled amount of $C_9H_{17}NO_2$ is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
ORGANIC IMPURITIES
- **Procedure**
Diluent: Prepare as directed in the *Assay*.
Solution A: Dissolve 1.2 g of monobasic potassium phosphate in 940 mL of water. Adjust with 5 N potassium hydroxide to a pH of 6.9. Add 60 mL of acetonitrile, and stir. Filter and degas.
Solution B: Dissolve 1.2 g of monobasic potassium phosphate in 700 mL of water. Adjust with 5 N potassium hydroxide to a pH of 6.9. Add 300 mL of acetonitrile and stir. Filter and degas.
Mobile phase: See the gradient table below.

USP Monographs

www.webofpharma.com

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
4.0	100	0
45.0	0	100
45.1	100	0
50.0	100	0

Standard solution: 0.04 mg/mL each of USP Gabapentin RS and USP Gabapentin Related Compound A RS in *Diluent*
Sample solution: Equivalent to 20 mg/mL of gabapentin, from NLT 20 powdered Tablets, in *Diluent*. [NOTE—Sonication for about 30 s may be necessary.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the gabapentin peak
Relative standard deviation: NMT 5.0% for gabapentin and gabapentin related compound A peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of gabapentin related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for gabapentin related compound A from the *Sample solution*
r_S = peak response for gabapentin related compound A from the *Standard solution*
C_S = concentration of USP Gabapentin Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of gabapentin in the *Sample solution* (mg/mL)

Calculate the percentage of any other unspecified degradation product relative to the gabapentin content in the portion of Tablets taken:

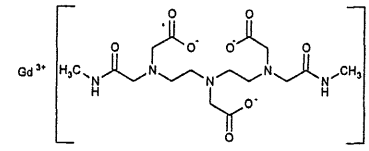
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each unspecified impurity from the *Sample solution*
r_S = peak response for gabapentin from the *Standard solution*
C_S = concentration of USP Gabapentin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of gabapentin in the *Sample solution* (mg/mL)

Acceptance criteria
Gabapentin related compound A: NMT 0.4%
Any individual unspecified impurity: NMT 0.1%
Total impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Gabapentin RS
USP Gabapentin Related Compound A RS
2-Aza-spiro[4.5]decan-3-one.
$C_9H_{15}NO$ 153.22

Gadodiamide



$C_{16}H_{26}GdN_5O_8$ 573.66
[5,8-Bis(carboxymethyl)-11-[2-(methylamino)-2-oxoethyl]-3-oxo-2,5,8,11-tetraazatridecan-13-oato(3-)]gadolinium.
[*N*,*N*-Bis[2-[(carboxymethyl)](methylcarbamoyl)methyl]-amino]ethyl]glycinato(3-)]gadolinium [131410-48-5].

» Gadodiamide contains not less than 97.0 percent and not more than 103.0 percent of $C_{16}H_{26}GdN_5O_8$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Gadodiamide RS
USP Gadodiamide Related Compound A RS
Gadolinium sodium diethylenetriamine pentaacetic acid monomethylamide.
$C_{15}H_{22}GdN_4NaO_9$ 582.60
USP Gadodiamide Related Compound B RS
Gadolinium disodium diethylenetriamine pentaacetic acid.
$C_{14}H_{18}GdN_3Na_2O_{10}$ 591.54

Clarity of solution—
Reference solution—
REFERENCE SOLUTION A—Transfer 1.0 g of hydrazine sulfate to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Allow to stand for 4 to 6 hours. [**CAUTION**—Hydrazine sulfate is highly toxic. Avoid skin contact.]
REFERENCE SOLUTION B—Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25 mL of water, and mix to dissolve.
PRIMARY OPALESCENT MIXTURE—To the flask containing *Reference solution B*, add 25.0 mL of *Reference solution A*, mix, and allow to stand for 24 hours. [NOTE—The suspension is stable for 2 months. Mix before use, and do not use if it adheres to the container.]
OPALESCENCE STANDARD—Dilute 15.0 mL of the *Primary opalescent mixture* with water to 1000.0 mL, and mix. This standard must be freshly prepared.
PROCEDURE—Transfer 10.0 mL of the *Opalescence standard* to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 40 mL of this solution (*Reference solution*) to a 50-mL color comparison tube.
Test solution—Transfer an accurately weighed quantity of Gadodiamide, equivalent to about 15 g of anhydrous

www.webofpharma.com

gadodiamide, to a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 40 mL of this solution to a 50-mL color comparison tube.

Blank—Transfer 40 mL of water to a 50-mL color comparison tube.

Procedure—Five minutes after preparation of the *Reference solution*, view the *Reference solution*, the *Test solution*, and the *Blank* against a black background: the *Test solution* is not more opalescent than the *Reference solution*. [NOTE—If the *Test solution* is more opalescent than the *Reference solution*, heat the *Test solution* to 60° to 70° for 2 to 3 minutes, cool to room temperature, and view again.]

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: It exhibits the maximum absorption at the relevant wavelength specified when tested as directed under the test for *Content of gadolinium*.

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—The total aerobic microbial count is not more than 500 cfu per g. The total combined molds and yeasts count is not more than 50 cfu per g.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 3.5 USP Endotoxin Units per g.

Water Determination, *Method I* ⟨921⟩: between 3.0% and 14.0%.

Limit of free gadolinium (III)—

Arsenazo III indicator—Transfer 150 mg of arsenazo III disodium to a 100-mL volumetric flask, dilute with water to volume, and mix.

MES buffer—Transfer 48.8 g of 2-(*N*-morpholino) ethanesulfonic acid (MES) to a 250-mL volumetric flask. Add 180 mL of water and 25 mL of 2 N sodium hydroxide, and mix. Adjust with 2 N sodium hydroxide to a pH of 6.0, dilute with water to volume, and mix.

Edetate disodium titrant—Pipet 100 mL of 0.02 M edetate disodium VS into a 1000-mL volumetric flask, dilute with water to volume, and mix.

Test solution—Transfer about 1 g of Gadodiamide, accurately weighed, to a 125-mL conical flask, add 25 mL of *MES buffer* and 0.1 mL of *Arsenazo III indicator*, and mix. A turquoise color indicates the presence of excess gadolinium.

Procedure—Titrate the *Test solution* with *Edetate disodium titrant* to a violet-pink endpoint. Each mL of *Edetate disodium titrant* is equivalent to 0.3145 mg of excess gadolinium: not more than 0.3% of free gadolinium is found, calculated on the anhydrous basis.

Limit of free diethylenetriamine pentaacetic acid bismethylamide—

Arsenazo III indicator and *MES buffer*—Proceed as directed for *Limit of free gadolinium (III)*.

0.002 M gadolinium (III) titrant—Transfer 18.6 g of gadolinium chloride to a 1000-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix. Pipet 10 mL of this solution into a conical flask, and add 25 mL of *MES buffer* and 0.1 mL of *Arsenazo III indicator*. Titrate with 0.02 M edetate disodium VS to a violet-pink endpoint, and determine the molarity. Pipet 40 mL of this solution into a 1000-mL volumetric flask, dilute with water to volume, and mix.

Blank solution—Transfer 25 mL of *MES buffer* and 0.1 mL of *Arsenazo III indicator* into a suitable flask, and mix.

Test solution—Transfer about 1 g of Gadodiamide, accurately weighed, to a 125-mL conical flask. Add 25 mL of *MES buffer* and 0.1 mL of *Arsenazo III indicator*, and mix. A violet-pink color indicates the presence of excess diethylenetriamine pentaacetic acid bismethylamide.

Procedure—Concomitantly titrate the *Blank solution* and the *Test solution* with *0.002 M gadolinium (III) titrant* to a turquoise endpoint. Calculate the percentage of free diethylenetriamine pentaacetic acid bismethylamide in the portion of Gadodiamide taken by the formula:

$$100(V_U - V_B)M_T(419.43)/W$$

in which V_U is the volume of gadolinium (III) in the *Test solution;* V_B is the volume of gadolinium (III) in the *Blank solution;* M_T is the molarity of the *0.002 M gadolinium (III) titrant;* 419.43 is the molecular weight of diethylenetriamine pentaacetic acid bismethylamide; and W is the weight, in mg, of Gadodiamide taken to prepare the *Test solution:* not more than 0.7% of diethylenetriamine pentaacetic acid bismethylamide is found, calculated on the anhydrous basis.

Limit of methylamine—

Borate buffer—Transfer 12.4 g of boric acid to a 500-mL volumetric flask, and suspend it in 300 mL of water. Add 100 mL of 1 N potassium hydroxide, and mix. Adjust with 1 N potassium hydroxide to a pH of 10.0, dilute with water to volume, and mix. Store in a closed plastic container.

OPA reagent—Transfer 100 mg of *o*-phthalaldehyde to an amber bottle, add 3 mL of methanol, and mix. Add 220 mL of *Borate buffer* and 0.1 mL of mercaptoethanol, and mix. [NOTE—This solution must be freshly prepared.]

Standard solutions—Transfer about 110 mg of methylamine hydrochloride, accurately weighed, to a 500-mL volumetric flask, and dilute with water to volume to obtain a solution having a concentration of about 100 µg of methylamine per mL. Pipet 1, 5, 10, and 20 mL of this solution into separate 100-mL volumetric flasks, dilute the contents of each flask with water to volume, and mix.

Test solution—Transfer about 1.5 g of Gadodiamide, accurately weighed, to a 10-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Proceed as follows for each of the *Standard solutions*. Add 3.0 mL of *OPA reagent*, mix, and within 1 minute measure the absorbance at 335 nm. Compare to a blank consisting of 3.0 mL of water and 3.0 mL of *OPA reagent*. Plot a calibration curve of absorbance versus standard concentration, in µg of methylamine per mL. Mix 3.0 mL of the *Test solution* with 3.0 mL of *OPA reagent*, and proceed as directed above. [NOTE—If the absorbance obtained with the *Test solution* exceeds the absorbance of the highest *Standard solution*, perform an additional quantitative dilution of the *Test solution*, and repeat the analysis.] Determine the concentration, in µg per mL, of methylamine in the *Test solution* by interpolation from the calibration curve. Calculate the amount of methylamine in the portion of Gadodiamide taken: not more than 0.05% of methylamine is found.

Limit of acetone, ethyl alcohol, and isopropyl alcohol—

Internal standard solution—Transfer about 500 mg of methyl ethyl ketone to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, dilute with water to volume, and mix.

Stock solution—Transfer about 1000 mg each of acetone, ethyl alcohol, and isopropyl alcohol, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix.

Diluted stock solution A—Pipet 5 mL of the *Stock solution* into a 100-mL volumetric flask, dilute with water to volume, and mix.

Diluted stock solution B—Pipet 10 mL of the *Stock solution* into a 100-mL volumetric flask, dilute with water to volume, and mix.

USP Monographs

www.webofpharma.com

System suitability solution—Pipet 10 mL of the *Internal standard solution* and 15 mL of *Diluted stock solution A* into a 100-mL volumetric flask, dilute with water to volume, and mix.

Test solution 1—Pipet 10 mL of the *Internal standard solution* into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this solution to a 10-mL gas chromatographic headspace vial, add about 1300 mg of Gadodiamide, accurately weighed, and cap immediately. Swirl to dissolve.

Test solution 2—Pipet 10 mL of the *Internal standard solution* and 2.5 mL of *Diluted stock solution A* into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this solution to a 10-mL gas chromatographic headspace vial, add about 1300 mg of Gadodiamide, accurately weighed, and cap immediately. Swirl to dissolve.

Test solution 3—Pipet 10 mL of the *Internal standard solution* and 15 mL of *Diluted stock solution A* into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this solution to a 10-mL gas chromatographic headspace vial, add about 1300 mg of Gadodiamide, accurately weighed, and cap immediately. Swirl to dissolve.

Test solution 4—Pipet 10 mL of the *Internal standard solution* and 25 mL of *Diluted stock solution B* into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this solution to a 10-mL gas chromatographic headspace vial, add about 1300 mg of Gadodiamide, accurately weighed, and cap immediately. Swirl to dissolve.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm × 30-m capillary column coated with a 1.8-µm phase G43. The column temperature is maintained at 40°. Helium is used as the carrier gas at a flow rate of about 1.5 mL per minute, and the split ratio is 1:15. The injection port and detector block are maintained at about 250°. Chromatograph the *System suitability solution* as directed for *Procedure:* the order of elution is ethyl alcohol, acetone, isopropyl alcohol, and methyl ethyl ketone; the resolution, *R,* between the ethyl alcohol and acetone peaks is not less than 1.0 and between the acetone and isopropyl alcohol peaks is not less than 1.0; and the relative standard deviation is not more than 3.0% for each of the three analytes.

Procedure—Separately inject equal volumes (about 1 µL) of each of the four *Test solutions* into the chromatograph, record the chromatograms, and measure the areas for the major peaks relative to the area for the internal standard peak. Plot the responses of the *Test solutions* versus the content, in µg per mL, of the relevant analyte in each vial, draw the straight line best fitting the four points, and calculate the correlation coefficient for the line. A suitable system is one that yields a line having a correlation coefficient of not less than 0.99. Calculate the percentage of each analyte in the portion of Gadodiamide taken by the formula:

$$a/(10{,}000b)$$

in which a is the intercept and b is the slope of the straight line, evaluated by linear regression analysis. [NOTE—If a is negative, report the result as none detected.] Not more than 0.2% of acetone, ethyl alcohol, and isopropyl alcohol is found, calculated on the anhydrous basis; and the sum of all three analytes is not more than 0.2%, calculated on the anhydrous basis.

Related compounds—

Mobile phase—Prepare as directed in the *Assay.*

Postcolumn reagent—Dissolve 120 mg of arsenazo III acid in 400 mL of water previously acidifed with 6.3 mL of nitric acid. Add 650 mg of urea, and mix to dissolve. Pass the solution through a 0.45-µm porosity filter, washing the filter with 600 mL of water. Dilute with water to 1000 mL, mix, and degas.

System suitability solution—Prepare an aqueous solution containing about 0.01 mg of USP Gadodiamide Related Compound A RS, 0.01 mg of USP Gadodiamide Related Compound B RS, and 2 mg of USP Gadodiamide RS in each mL.

Test solution—Transfer about 200 mg of Gadodiamide, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay,* except to use the *Postcolumn reagent* prepared as directed above. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the gadodiamide and gadodiamide related compound A peaks is not less than 1.0, and between gadodiamide related compound A and gadodiamide related compound B peaks is not less than 1.5; and the relative standard deviation for replicate injections is not more than 10%.

Procedure—Inject about 10 µL of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. [NOTE—The tail of the gadodiamide peak may contain a small shoulder due to an isomer; the area of the shoulder should be included in the gadodiamide peak area.] Calculate the percentage of each impurity in the portion of Gadodiamide taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the response of each impurity; and r_s is the sum of all peaks having a percentage greater than 0.10%: not more than 2.0% of gadodiamide related compounds A and B is found; no individual impurity is more than 0.2%; and the sum of all impurities, other than gadodiamide related compounds A and B, is not more than 0.5%.

Content of gadolinium—

Standard solutions—Prepare three separate solutions in 0.2 M nitric acid to obtain concentrations of 100, 150, and 200 µg of gadolinium per mL.

Test solution—Transfer about 600 mg of Gadodiamide, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute with 0.2 M nitric acid to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard solutions* and the *Test solution* at the gado linium resonance line of 342.3 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩), using 0.2 M nitric acid as the blank. Plot the absorbances of the *Standard solutions* versus concentration, in µg per mL, of gadolinium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, in µg per mL, of gadolinium in the *Test solution:* the content of gadolinium is between 26.0% and 29.0%, calculated on the anhydrous basis.

Assay—

Mobile phase—Transfer 14.0 mL of triethylamine, 5.7 mL of glacial acetic acid, and 5.7 mL of water to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 50 mL of this solution to a 1000-mL volumetric flask, add 900 mL of water, and mix. Adjust with 1 N acetic acid or 1 N sodium hydroxide to a pH between 6.5 and 7.0. Dilute with water to volume, mix, filter, and degas (see *System Suitability* under *Chromatography* ⟨621⟩).

Postcolumn reagent—Dissolve 325 mg of urea in a solution of 60 mg of arsenazo III acid in 550 mL of water previously

www.webofpharma.com

acidified with 3.2 mL of nitric acid. Pass the solution through a 0.45-µm porosity filter, wash the filter with 400 mL of water, dilute with water to 1000 mL, mix, and degas.

Standard preparation—Prepare an aqueous solution of USP Gadodiamide RS having a known concentration of about 0.6 mg per mL.

Assay preparation—Transfer about 60 mg of Gado diamide, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 658-nm detector and a 4.6-mm × 25-cm column that contains 5-µm base-deactivated packing L1. A second pump mixes the *Mobile phase* with the *Postcolumn reagent* prior to detection via a T-junction. The system is maintained at a constant temperature between 20° and 35°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{16}H_{26}GdN_5O_8$ in the portion of Gadodiamide taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Gadodiamide RS in the *Standard preparation;* and r_U and r_S are the gadodiamide peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Gadodiamide Injection

DEFINITION

Gadodiamide Injection is a sterile solution of Gadodiamide in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of gadodiamide ($C_{16}H_{26}GdN_5O_8$). It may contain stabilizers and buffers. Gadodiamide Injection intended for intravascular use contains no antimicrobial agents.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
  Wavelength range: 240–300 nm
  Solution: 57 mg/mL of gadodiamide in water
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Solution A: Dissolve 14 mL of triethylamine and 5.7 mL of glacial acetic acid in 1 L of water.

Mobile phase: Transfer 50 mL of *Solution A* to a 1-L volumetric flask. Add 900 mL of water. Adjust with 1 N acetic acid or 1 N sodium hydroxide to a pH between 6.5 and 7.0. Dilute with water to volume.

Postcolumn reagent: Dissolve 325 mg of urea in a solution of 60 mg of arsenazo III acid in 550 mL of water previously acidified with 3.2 mL of nitric acid. Pass the solution through a filter of 0.45-µm pore size, wash the filter with 400 mL of water, and dilute with water to 1000 mL.

Standard solution: 0.18 mg/mL of USP Gadodiamide RS

Sample solution: Equivalent to a nominal concentration of 0.18 mg/mL of gadodiamide from Injection

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: Vis 658 nm
Column: 4.6-mm × 25-cm; 5-µm base-deactivated packing L1. [NOTE—A second pump mixes the *Mobile phase* with the *Postcolumn reagent* prior to detection via a T-junction.]
Column temperature: 20°–35° (system maintained at constant temperature)
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.5%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of gadodiamide ($C_{16}H_{26}GdN_5O_8$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of gadodiamide from the *Sample solution*
r_S = peak response of gadodiamide from the *Standard solution*
C_S = concentration of USP Gadodiamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of gadodiamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Mobile phase, and **Chromatographic system:** Prepare as directed in the *Assay.*

Postcolumn reagent: Dissolve 120 mg of arsenazo III acid in 400 mL of water previously acidified with 6.3 mL of nitric acid. Add 650 mg of urea, and mix to dissolve. Pass the solution through a filter of 0.45-µm pore size, washing the filter with 600 mL of water. Dilute with water to 1000 mL.

System suitability solution: 10 µg/mL of USP Gadodiamide Related Compound A RS, 10 µg/mL of USP Gadodiamide Related Compound B RS, and 2 mg/mL of USP Gadodiamide RS in an aqueous solution

Sample solution: Equivalent to a nominal concentration of 2 mg/mL of gadodiamide from Injection

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.0 between the gadodiamide and gadodiamide related compound A peaks; NLT 1.5 between gadodiamide related compound A and gadodiamide related compound B peaks
Relative standard deviation: NMT 10%
[NOTE—The tail of the gadodiamide peak may contain a small shoulder due to an isomer. The area of the shoulder should be included in the gadodiamide peak area.]

Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the volume of Injection taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity
r_T = sum of all peaks having a percentage greater than 0.10%

Acceptance criteria: See *Table 1*.

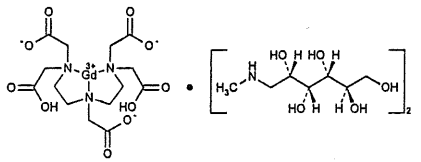
Table 1

Name	Acceptance Criteria, NMT (%)
Gadodiamide related compound A	2.0
Gadodiamide related compound B	2.0
Any other individual impurity	0.2
Total impurities (sum of all impurities other than gadodiamide related compounds A and B)	0.5

SPECIFIC TESTS
- **OSMOLALITY AND OSMOLARITY,** *Osmolality* ⟨785⟩: 650–1000 mOsmol/kg
- **pH** ⟨791⟩: 5.5–7.0
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.029 USP Endotoxin Unit/mg of gadodiamide
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose plastic or Type I glass containers. Store at controlled room temperature, protected from light. Do not freeze.
- **LABELING:** Label containers of Injection to direct the user to discard any unused portion. Label it to state its routes of administration. Label it to indicate "serious injury can occur if given by intrathecal route."
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Gadodiamide RS
  USP Gadodiamide Related Compound A RS
  Gadolinium sodium diethylenetriamine pentaacetic acid monomethylamide.
  $C_{15}H_{22}GdN_4NaO_9$ 582.60
  USP Gadodiamide Related Compound B RS
  Gadolinium disodium diethylenetriamine pentaacetic acid.
  $C_{14}H_{18}GdN_3Na_2O_{10}$ 591.54

Gadopentetate Dimeglumine Injection



DEFINITION
Gadopentetate Dimeglumine Injection is a sterile solution of gadopentetate dimeglumine in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of gadopentetate dimeglumine ($C_{14}H_{20}GdN_3O_{10} \cdot 2C_7H_{17}NO_5$). It may contain small amounts of Meglumine and Pentetic Acid as stabilizers, and it may contain suitable buffers. Gadopentetate Dimeglumine Injection intended for intravascular use contains no antimicrobial agents.

IDENTIFICATION

Change to read:

- **A.** **▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
  Standard solution: 74 mg/mL of USP Gadopentetate Monomeglumine RS
  Sample solution: 94 mg/mL of gadopentetate dimeglumine
- **B.** The absorption of the gadolinium emission line at 368.4 nm by the *Sample solution* in the test for *Content of Gadolinium* confirms the presence of gadolinium.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Mobile phase: Dissolve 1.37 g of tetrabutylammonium perchlorate in 1 L of a mixture of acetonitrile and water (12:88).
  Standard solution: 1.85 mg/mL of USP Gadopentetate Monomeglumine RS prepared as follows. Transfer a suitable quantity to a suitable volumetric flask containing 50% of the flask volume of 0.1% meglumine solution. Dilute to volume with water.
  Sample solution: Nominally 2.35 mg/mL of gadopentetate dimeglumine from Injection in water
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 195 nm
  Column: 4.6-mm × 12.5-cm; 5-µm packing L7
  Flow rate: 1.5 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Column efficiency: NLT 800 theoretical plates
  Tailing factor: NMT 3.5
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of gadopentetate dimeglumine ($C_{14}H_{20}GdN_3O_{10} \cdot 2C_7H_{17}NO_5$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Gadopentetate Monomeglumine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of gadopentetate dimeglumine, 938.02
M_{r2} = molecular weight of gadopentetate monomeglumine, 742.80

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS
- **CONTENT OF MEGLUMINE**
  Sample solution: Use the Injection.

USP Monographs

www.webofpharma.com

Instrumental conditions
Mode: Polarimetry (see *Optical Rotation* ⟨781⟩)
Light source: Sodium lamp
Analytical wavelength: 589 nm
Cell: 10 cm
Analysis: Determine the angular rotation (see *Optical Rotation* ⟨781⟩) of the *Sample solution*.
Calculate the amount of meglumine as a percentage of the labeled amount of gadopentetate dimeglumine ($C_{14}H_{20}GdN_3O_{10} \cdot 2C_7H_{17}NO_5$) in the portion of the Injection taken:

$$\text{Result} = [(100a/\alpha) \times (1/L)] \times F \times 100$$

a = observed angular rotation corrected for the *Blank* (degrees)
α = average specific rotation of meglumine, 24.9 deg.dL/g
L = label claim of Injection in mg/mL
F = unit conversion factor from g/dL to mg/mL, 10

Acceptance criteria: Meglumine content is 37.4%–45.8% of the labeled amount of gadopentetate dimeglumine.

• **CONTENT OF GADOLINIUM**
Cesium chloride solution: 100 mg/mL of cesium chloride in water
Blank solution: *Cesium chloride solution*, hydrochloric acid (spectrophotometric grade), and water (10:1:89)
Standard stock solution: Transfer 1.15 g of gadolinium (III) oxide to a 100-mL volumetric flask, add 2.0 mL of hydrochloric acid to dissolve, and dilute with water to volume.
Standard solution A: 600 µg/mL of gadolinium prepared as follows. Transfer 3.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, add 5.0 mL of *Cesium chloride solution* and 0.5 mL of hydrochloric acid (spectrophotometric grade), and dilute with water to volume.
Standard solution B: 800 µg/mL of gadolinium prepared as follows. Transfer 4.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, add 5.0 mL of *Cesium chloride solution* and 5.0 mL of hydrochloric acid (spectroscopic grade), and dilute with water to volume.
Standard solution C: 1000 µg/mL of gadolinium prepared as follows. Transfer 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, add 5.0 mL of *Cesium chloride solution* and 5.0 mL of hydrochloric acid (spectroscopic grade), and dilute with water to volume.
Sample solution: Treat a volume of Injection, equivalent to 469 mg of gadopentetate dimeglumine, with 0.2 mL of nitric acid in a porcelain crucible, concentrate on a hot plate, char with a burner, and ignite in a muffle furnace at 800° until all black particles disappear (approximately 1 h). Allow the residue to cool on a refractory surface for 5 min, then equilibrate to room temperature in a desiccator. Dissolve the white residue so obtained in a mixture of 1.0 mL of water and 1.0 mL of hydrochloric acid (spectrophotometric grade) with heating. Transfer this solution to a 100-mL volumetric flask, add 10.0 mL of *Cesium chloride solution*, and dilute with water to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectroscopy
Analytical wavelength: 368.4 nm at the gadolinium emission line
Lamp: Gadolinium hollow-cathode
Flame: Nitrous oxide–acetylene

Analysis
Samples: *Blank solution*, *Standard solutions*, and *Sample solution*
Blank the instrument with the *Blank solution*. Determine the absorbances of the *Standard solutions* and *Sample solution*. Plot the absorbances of the *Standard solutions* versus their concentrations, in µg/mL, of gadolinium, and draw the straight line best fitting the three plotted points. From the graph so obtained and the absorbance of the *Sample solution*, determine the concentration, in µg/mL, of gadolinium in the *Sample solution*.
Calculate the amount of gadolinium as a percentage of the labeled amount of gadopentetate dimeglumine ($C_{14}H_{20}GdN_3O_{10} \cdot 2C_7H_{17}NO_5$) in the portion of the Injection taken:

$$\text{Result} = [(C \times D)/(V \times L)] \times F \times 100$$

C = concentration of gadolinium in the *Sample solution* (µg/mL)
D = volume of the *Sample solution*, 100 mL
V = volume of Injection taken to prepare the *Sample solution* (mL)
L = label claim of the Injection (mg/mL)
F = conversion factor from µg to mg, 0.001

Acceptance criteria: Gadolinium content is 15.1%–18.4% of the labeled amount of gadopentetate dimeglumine.

SPECIFIC TESTS

• **LIMIT OF PENTETIC ACID**
Buffer: 50 g of sodium acetate and 10 mL of glacial acetic acid in a 1000-mL volumetric flask. Dilute with degassed water to volume. Adjust with 0.1 N sodium hydroxide or glacial acetic acid to a pH of 5.
Indicator solution: 0.508 mg/mL of xylenol orange in degassed water
Diluent: *Buffer*, *Indicator solution*, and water (30:3:167)
Titrant: 0.001 M (1 µmol/mL) gadolinium sulfate prepared by dissolving a suitable quantity of gadolinium sulfate (purity ≥99.9%) in water
Sample solution: Transfer volume of Injection equivalent to 938 mg of gadopentetate dimeglumine. Add 20 mL water and 10 mL *Diluent*. Adjust with 0.1 N sodium hydroxide or glacial acetic acid to a pH of 5.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Endpoint detection: Visual
Analysis: Titrate with *Titrant* until the color changes from yellow to reddish violet.
Calculate the amount of pentetic acid as a percentage in the portion of the Injection taken:

$$\text{Result} = [(V_T \times M \times F) \times (1/V_U)] \times 100$$

V_T = volume of *Titrant* consumed
M = molarity of *Titrant* (µmol/mL)
F = equivalent weight of pentetic acid, 0.7867 mg/µmol
V_U = injection volume used to prepare the *Sample solution*

Acceptance criteria: 0.027%–0.04%
• **pH** ⟨791⟩**:** 6.5–8.0
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 25 USP Endotoxin Units/mL of Injection.
• **STERILITY TESTS** ⟨71⟩**:** Meets requirements
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements

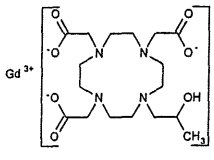
USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature. Do not freeze.
- **LABELING:** Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container. The labeling also states that it is not to be used if it contains solids.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Gadopentetate Monomeglumine RS

Gadoteridol



$C_{17}H_{29}GdN_4O_7$ 558.68

Gadolinium, [10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetato(3-)-N^1,N^4,N^7,N^{10},O^1,O^4,O^7,O^{10}]-.

(±)-[10-(2-Hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetato(3-)]gadolinium [120066-54-8].

» Gadoteridol contains not less than 97.0 percent and not more than 101.0 percent of $C_{17}H_{29}GdN_4O_7$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Gadoteridol RS
USP Gadoteridol Related Compound A RS
10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triaacetic acid.
$C_{17}H_{32}N_4O_7$ 404.46
USP Gadoteridol Related Compound B RS
1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid gadolinium salt.
USP Gadoteridol Related Compound C RS
1,4,7,10-tetraaza-11-oxo-bicyclo[8.2.2]tetradecane-4,7-diacetic acid.

Change to read:

Identification—

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)

Solution: 10 mg per mL.

Medium: water.

Water Determination, *Method I* ⟨921⟩: not more than 15%.

Limit of gadoteridol related compound A—

Buffer solution—Dissolve 0.60 g of tromethamine and 3.72 g of edetate disodium in 3 L of water, adjust with 5 N sodium hydroxide to a pH of 7.0, dilute with water to 4 L, and mix. Filter, and chill between 5° and 8°.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution,* acetonitrile, and tetrahydrofuran (98.8: 1.0: 0.2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Cupric acetate solution—Transfer 3.99 g of cupric acetate and 12.11 g of tromethamine to a 2-L volumetric flask, dissolve in 1500 mL of water, adjust with glacial acetic acid to a pH of 7.0, dilute with water to volume, and mix. Filter, and chill between 5° and 8°.

Standard stock solution—Dissolve an accurately weighed quantity of USP Gadoteridol Related Compound A RS in *Buffer solution,* and dilute quantitatively and stepwise with *Buffer solution* to obtain a solution having a known concentration of about 6 µg per mL.

Standard solution—Just prior to injection onto the column, dilute 1.0 mL of the *Standard stock solution* with 1.0 mL of *Cupric acetate solution* to obtain a solution having a known concentration of about 3 µg of USP Gadoteridol Related Compound A RS per mL. [NOTE—The copper–gadoteridol related compound A complex is stable for at least 2 hours, if maintained at about 5°.]

Test solution—Transfer about 30 mg of Gadoteridol, accurately weighed, to a test tube, add 1.0 mL of *Buffer solution,* and mix on a vortex mixer to dissolve. Add 1.0 mL of *Cupric acetate solution,* and mix. Immediately inject the solution into the chromatograph. [NOTE—The copper–gadoteridol related compound A complex is stable for at least 2 hours, if maintained at about 5°.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 15-cm column that contains packing L21. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for gadoteridol related compound A and 2.0 for copper-edetate; the resolution, *R,* between gadoteridol related compound A and copper-edetate is not less than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of gadoteridol related compound A in the portion of Gadoteridol taken by the formula:

$$100(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Gadoteridol Related Compound A RS in the *Standard stock solution; W* is the weight, in mg, of Gadoteridol taken to prepare the *Test solution;* and r_U and r_S are the gadoteridol related compound A peak responses obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.01% is found, calculated on the anhydrous basis.

Limit of free gadolinium (III)—

Buffer solution, Mobile phase, Diluent, Standard stock solution 1, Standard stock solution 2, Standard solution, and *Chromatographic system*—Proceed as directed for *Chromatographic purity, Test 1.*

Test solution—Transfer about 60 mg of Gadoteridol, accurately weighed, to a vial, add 2.0 mL of *Diluent,* and mix. Immediately place the vial in a bath maintained at about 5°.

Procedure—Proceed as directed for *Procedure* under *Chromatographic purity, Test 1.* Calculate the percentage of free gadolinium (III) in the portion of Gadoteridol taken by the formula:

$$200(157.25/334.38)(C/W)(r_U/r_S)$$

www.webofpharma.com

in which 157.25 is the molecular weight of gadolinium and 334.38 is the molecular weight of gadolinium acetate; *C* is the concentration, in mg per mL, of gadolinium acetate, calculated on the anhydrous basis, in the *Standard solution; W* is the weight, in mg, of Gadoteridol taken to prepare the *Test solution;* and r_U and r_S are the free gadolinium (III) peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.01% is found.

Limit of regioisomer—Using the chromatogram of the *Assay preparation* as obtained in the *Assay*, calculate the percentage of regioisomer peak at a relative retention time of about 1.2 in relation to that of the gadoteridol peak by the formula:

$$100r_R/(r_R + r_G)$$

in which r_R and r_G are the regioisomer and gadoteridol peak responses, respectively, in the *Assay preparation:* not more than 2.5% is found.

Chromatographic purity—

TEST 1: GADOLINIUM-CONTAINING IMPURITIES—

Buffer solution—Dissolve 10.2 g of monobasic potassium phosphate, 0.165 g of dibasic potassium phosphate, and 1.76 g of edetate disodium in 3000 mL of water, and filter.

Mobile phase—Prepare a degassed mixture of *Buffer solution* and acetonitrile (98:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Transfer 3.40 g of monobasic potassium phosphate, 4.21 g of dibasic potassium phosphate, and 0.584 g of edetate disodium to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Standard stock solution 1—Quantitatively dissolve an accurately weighed quantity of gadolinium (Gd III) acetate in water to obtain a solution having a concentration of about 0.4 mg per mL.

Standard stock solution 2—Prepare a solution of USP Gadoteridol Related Compound B RS in water to obtain a solution having a known concentration of about 0.6 mg per mL.

Standard solution—Transfer 1.0 mL of *Standard stock solution 1* and 2.0 mL of *Standard stock solution 2* to a 10-mL volumetric flask, and dilute with *Diluent* to volume. Transfer 1.0 mL of this solution to a vial, add 3.0 mL of *Diluent*, and mix.

Test solution—Transfer about 60 mg of Gadoteridol, accurately weighed, to a vial, add 2.0 mL of *Diluent*, and mix. Immediately place the vial in a bath maintained at about 5°.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a fluorometric detector operating at an excitation wavelength of 275 nm and an emission wavelength of 314 nm, and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for free gadolinium (III), 1.6 for 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid gadolinium sodium salt (gadoteridol related compound D), and 2.2 for gadoteridol related compound B; and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all of the peak responses. Allow the *Test solution* to elute for not less than 1.3 times the retention time of the gadoteridol peak. Calculate the percentage of gadoteridol related compound D in the portion of Gadoteridol taken by the formula:

$$200(C/W)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Gadoteridol Related Compound B RS, calculated on the anhydrous basis, in the *Standard solution; W* is the weight, in mg, of Gadoteridol taken to prepare the *Test solution;* and r_U and r_S are the gadoteridol related compound D peak responses obtained from the *Test solution* and the *Standard solution*, respectively. Calculate the percentage of gadoteridol related compound B in the portion of Gadoteridol taken by the formula:

$$200(C/W)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Gadoteridol Related Compound B RS, calculated on the anhydrous basis, in the *Standard solution; W* is the weight, in mg, of Gadoteridol taken to prepare the *Test solution;* and r_U and r_S are the gadoteridol related compound B peak responses obtained from the *Test solution* and *Standard solution*, respectively. Calculate the percentage of any other impurity in the portion of Gadoteridol taken by the formula:

$$200(C/W)(r_i/r_S)$$

in which r_i is the peak response of any other impurity obtained from the *Test solution;*and r_S is the peak response for gadoteridol related compound B obtained from the *Standard solution:* not more than 0.1% of any individual impurity is found, and not more than 0.3% of gadolinium-containing impurities is found.

TEST 2 (NONGADOLINIUM-CONTAINING IMPURITIES)—

50 mM Ammonium phosphate buffer—Dissolve 17.25 g of monobasic ammonium phosphate in 3000 mL of water, and filter.

pH 5.0 Buffer—Transfer 2000 mL of *50 mM Ammonium phosphate buffer* to a 2-L beaker, and adjust with ammonium hydroxide to a pH of 5.0.

pH 7.0 Buffer—Transfer 1000 mL of *50 mM Ammonium phosphate buffer* to a 1-L beaker, and adjust with ammonium hydroxide to a pH of 7.0.

Mobile phase—Prepare a filtered and degassed mixture of *pH 5.0 buffer* and acetonitrile (87:13). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Gadoteridol Related Compound C RS in *pH 7.0 Buffer* to obtain a solution having a known concentration of about 5 µg per mL.

System suitability solution—Prepare a solution in *pH 7.0 buffer* containing about 2 mg of USP Gadoteridol RS and 0.002 mg of USP Gadoteridol Related Compound C RS per mL.

Test solution—Transfer about 50 mg of Gadoteridol to a 25-mL volumetric flask, dissolve in and dilute with *pH 7.0 Buffer* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L18. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the responses as directed for *Procedure:* the relative retention times are about 1.0 for gadoteridol, 1.2 for 1,4,7,10-tetraaza-1,4,7-tris-(carboxymethyl)-11-oxo-bicyclo [8.2.2] tetradecanium chloride (gadoteridol related compound F), 1.5 for 1,4,7,10-tetraaza-13-oxo-bicyclo [8.2.1] tridecane-4,7-diacetic acid (gadoteridol related compound E), and 1.7 for gadoteridol related compound C; and the resolution, *R*, between gadoteridol and gadoteridol related compound C is not less than 5. Chromatograph the *Standard solution* as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 4.0%.

USP Monographs

www.webofpharma.com

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Allow the *Test solution* to elute for not less than twice the retention time of the gadoteridol peak. Calculate the percentage of gadoteridol related compound C in the portion of Gadoteridol taken by the formula:

$$2.5(C/W)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Gadoteridol Related Compound C RS in the *Standard solution*; W is the weight, in mg, of Gadoteridol taken to prepare the *Test solution*; and r_U and r_S are the peak responses for gadoteridol related compound C obtained from the *Test solution* and *Standard solution*, respectively. Calculate the percentages of gadoteridol related compound F and gadoteridol related compound E in the portion of Gadoteridol taken by the formula:

$$2.5(1/F)(C/W)(r_i/r_S)$$

in which F is the relative response factor for gadoteridol related compound F (0.68) or for gadoteridol related compound E (1.7); r_i is the peak response for gadoteridol related compound E or gadoteridol related compound F in the *Test solution*; and C, W, and r_S are as defined above. Calculate the percentage of any other impurity in the portion of Gadoteridol taken by the formula:

$$2.5(C/W)(r_i/r_S)$$

in which r_i is the response of any other impurity in the chromatogram of the *Test solution*; and C, W, and r_S are as defined above: not more than 0.1% of any other impurity is found, and the total of nongadolinium-containing impurities is not more than 0.3%, calculated on the anhydrous basis.

Other requirements—Where the label states that Gadoteridol is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ and *Bacterial endotoxins* under *Gadoteridol Injection*. Where the label states that Gadoteridol must be subjected to further processing during the preparation of injectable dosage form, it meets the requirements for *Bacterial endotoxins* under *Gadoteridol Injection*.

Assay—

Buffer solution—Dissolve 10.2 g of monobasic potassium phosphate and 0.165 g of dibasic potassium phosphate in 3 L of water, and filter.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (98:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Gadoteridol RS in *Buffer solution* to obtain a solution having a known concentration of about 0.6 mg per mL.

Assay preparation—Transfer about 60 mg of Gadoteridol, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Buffer solution* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a fluorometric detector operating at an excitation wavelength of 275 nm and an emission wavelength of 314 nm, and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, R, between the gadoteridol peak and the peak with a relative retention time of about 1.2 is not less than 1.2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{17}H_{29}GdN_4O_7$ in the portion of Gadoteridol taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration of USP Gadoteridol RS, in mg per mL, in the *Standard preparation;* and r_U and r_S are the gadoteridol peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Gadoteridol Injection

» Gadoteridol Injection is a sterile solution of Gadoteridol in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of gadoteridol ($C_{17}H_{29}GdN_4O_7$). It may contain buffers and stabilizers. Gadoteridol Injection intended for intravenous use contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose containers as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*, preferably of Type I glass. Store at controlled room temperature, and protect from light.

Labeling—Label containers of Injection intended for intravenous injection to direct the user to examine the product to ensure that all solids are dissolved, to discard the product if solids persist, and to discard any unused portion remaining in the container.

USP Reference standards ⟨11⟩—
USP Gadoteridol RS
USP Gadoteridol Related Compound A RS
10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triaacetic acid.
$C_{17}H_{32}N_4O_7$ 404.46

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 mg per mL.
Medium: water.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 8.3 USP Endotoxin Units per mL of Gadoteridol Injection.

pH ⟨791⟩: between 6.5 and 8.0.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Limit of free gadolinium (III)—

Buffer solution, *Mobile phase*, and *Diluent*—Proceed as directed for *Test 1* in the *Chromatographic purity* test under *Gadoteridol*.

www.webofpharma.com

Standard solution—Prepare a solution of gadolinium (Gd III) acetate in water to obtain a solution having a known concentration of about 0.4 mg of gadolinium acetate per mL. Transfer 1.0 mL of the solution to a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix. Transfer 1.0 mL of this solution to a small vial, add 3.0 mL of *Diluent*, and mix.

Test solution—Transfer an accurately measured volume of Injection, equivalent to about 150 mg of gadoteridol, to a small vial, dilute with *Diluent* to 5.0 mL, and mix. Immediately place in a bath maintained at about 5°.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a fluorometric detector operating at an excitation wavelength of 275 nm and an emission wavelength of 314 nm and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for about 1.5 times the retention time of the gadoteridol peak, and measure the peak responses. Calculate the percentage, by weight, of free gadolinium (III) in the volume of Injection taken by the formula:

$$500(157.25/334.38)(C/VP)(r_U/r_S)$$

in which 157.25 and 334.38 are the molecular weights of gadolinium and gadolinium acetate, respectively; C is the concentration, in mg per mL, of gadolinium (Gd III) acetate, calculated on the anhydrous basis, in the *Standard solution*; V is the volume, in mL, of Injection taken for the *Test solution*; P is the labeled potency of gadoteridol, in mg per mL, in the Injection; and r_U and r_S are the peak responses for free gadolinium (III) obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.02% is found.

Limit of gadoteridol related compound A—

Buffer solution, Mobile phase, Cupric acetate solution, Standard stock solution, Standard solution, and *Chromatographic system*—Proceed as directed for the *Limit of gadoteridol related compound A* test under *Gadoteridol.*

Test solution—Transfer an accurately measured volume of Injection, equivalent to about 30 mg of gadoteridol, to a test tube, dilute with chilled *Buffer solution* to 1.0 mL, and mix. Add 1.0 mL of chilled *Cupric acetate solution*, mix on a vortex mixer for about 10 seconds, and immediately inject as directed for *Procedure.*

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of gadoteridol related compound A in the Injection by the formula:

$$0.2(C/VP)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Gadoteridol Related Compound A RS in the *Standard solution*; V is the volume, in mL, of Injection taken for the *Test solution*; P is the labeled potency, in mg per mL, of gadoteridol in the Injection; and r_U and r_S are the peak responses of gadoteridol related compound A in the *Test solution* and the *Standard solution*, respectively: not more than 0.02% is found.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Buffer solution, Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Gadoteridol.*

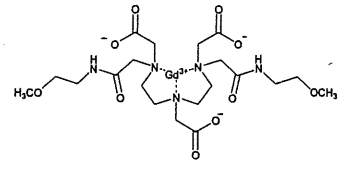
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 150 mg of gadoteridol, to a 250-mL volumetric flask, dilute with *Buffer solution* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg per mL, of gadoteridol ($C_{17}H_{29}GdN_4O_7$) in the volume of Injection taken by the formula:

$$250(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Gadoteridol RS in the *Standard preparation*; V is the volume of Injection taken, in mL; and r_U and r_S are the gadoteridol peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Gadoversetamide



$C_{20}H_{34}GdN_5O_{10}$ 661.76

(Gadoversetamide) [8,11-bis(carboxymethyl)-14-[2-[(2-methoxyethyl)amino]-2-oxoethyl]-6-oxo-2-oxa-5,8,11,14-tetraazahexadecan-16-oato(3-)], gadolinium.

[*N*,*N*-Bis[2-[(carboxymethyl)[[(2-methoxyethyl)car bamoyl] methyl]amino]ethyl]glycinato(3-)]gadolinium [131069-91-5].

» Gadoversetamide contains not less than 97.0 percent and not more than 102.0 percent of $C_{20}H_{34}GdN_5O_{10}$, calculated on an anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩**—**

USP Gadodiamide Related Compound B RS
Gadolinium disodium diethylenetriamine pentaacetic acid.
$C_{14}H_{18}GdN_3Na_2O_{10}$ 591.54

USP Gadoversetamide RS

USP Gadoversetamide Related Compound A RS
Hydrogen [8, 11,14-tris(carboxymethyl)-6-oxo-2-oxa-5,8,11,14-tetraazahexadecan-16-oato(4-)]gadolinium.

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

B: The lanthanide selectivity test detects gadolinium (III) in 0.1 N nitric acid with arsenazo (III). Prepare 1.5×10^{-4} M arsenazo (III) solution by dissolving 30 mg of arsenazo (III) and 160 mg of urea in 100 mL water, adding 1.6 mL of nitric acid,

www.webofpharma.com

and diluting with water to 250 mL. Add 10 mg of Gadoversetamide to 1.0 mL of the 1.5×10^{-4} M arsenazo (III) solution, and mix. The color changes from a wine red to green-blue, indicating the presence of gadolinium.

Bacterial Endotoxins Test ⟨85⟩ : not more than 15 USP Endotoxin Units per g of gadoversetamide.

Water Determination, *Method Ia* ⟨921⟩ : not more than 10.0% (w/w), a solvent mixture of methanol and formamide (9:1) being used.

Limits of free gadolinium (III) and total chelatable material—

MES buffer—Dissolve 97.6 g of 2-morpholinoethane sulfonic acid (MES) in about 950 mL of water, and mix. Adjust with 20% sodium hydroxide to a pH of 6, dilute with water to 1000 mL, and mix.

Edetate titrant: 0.002 M edetate disodium VS.

0.003 M Gadolinium (III) titrant—Transfer 0.790 g of gadolinium chloride to a 1000-mL volumetric flask, dilute with water to volume, and mix.

Test solution—Transfer about 5 g of Gadoversetamide, accurately weighed, into a 250-mL flask, and add 20 mL of water and 2 mL of hydrochloric acid. Stir, and heat to boiling. Rinse the sides of the flask with water. Add 50 mL of *MES buffer* and 100 to 150 µL of xylenol orange TS to impart a light yellow color. Heat to boiling, adjust with ammonium hydroxide to a pH of 6, and continue boiling for 2 minutes. If the solution is yellow, proceed as directed under *Uncomplexed chelatable material.* If the color is red-violet, proceed as directed under *Free gadolinium (III).*

Uncomplexed chelatable material—Continue boiling, and titrate with *0.003 M Gadolinium (III) titrant* to a red-violet endpoint that undergoes no further color change upon addition of more titrant. Record the volume of titrant used to reach the endpoint.

Free gadolinium (III)—Continue boiling and titrate with *Edetate titrant* to a yellow or yellow-orange endpoint that undergoes no further color change upon addition of more titrant. Record the volume of titrant used to reach the endpoint.

Calculations—If the *Test solution* was titrated with *Edetate titrant,* calculate the percentage of *Free gadolinium (III)* in the portion of Gadoversetamide taken by the formula:

$$(66.18/W)(V_{EU})(M_E)$$

in which W is the weight, in g, of Gadoversetamide taken; V_{EU} is the volume, in mL, of *Edetate titrant* used to titrate the *Test solution;* and M_E is the molarity of the *Edetate titrant.* If the sample was titrated with *0.003 M Gadolinium (III) titrant,* calculate the percentage of *Uncomplexed chelatable material* in the portion of Gadoversetamide taken by the formula:

$$(66.18/W)(V_{GU})(M_G)$$

in which W is as defined herein; V_{GU} is the volume, in mL, of *0.003 M Gadolinium (III) titrant* used to titrate the *Test solution;* and M_G is the molarity of the *0.003 M Gadolinium (III) titrant.* Not more than 0.05% of free gadolinium III and not more than 0.1% of uncomplexed chelatable material, both calculated on the anydrous basis, are found.

Limit of 2-methoxyethylamine—

Mobile phase—Add 2 mL of 5 M phosphoric acid to 550 mL of water, mix, and adjust with 10% (w/w) ammonium hydroxide to a pH of 5.0. Add 450 mL of acetonitrile, mix, filter, and degas.

0.4 M Borate buffer—Add 12.4 g of boric acid to 300 mL of water, and swirl to suspend. Add 100 mL of 1 N potassium hydroxide, and mix. Adjust with about 60 mL of 1 N potassium hydroxide to a pH of 10.0, dilute with water to 500 mL, and mix. Test the pH, and adjust if necessary.

o-Phthalaldehyde reagent—Dissolve 25 mg of *o*-phthalaldehyde in 0.75 mL of methanol, add 25 mL of *0.4 M Borate buffer* having a pH of 10.0 and 25 µL of 2-mercaptoethanol, and mix. [NOTE—Protect from light. Discard after 3 days.]

Standard solutions—Prepare aqueous solutions of 2-methoxyethylamine having known concentrations of about 1, 20, and 50 µg per mL, respectively. Derivatize by adding an equal volume of *o-Phthalaldehyde reagent* to each solution immediately before injection.

Test solution—Transfer about 250 mg of Gadoversetamide, accurately weighed, to a 5-mL volumetric flask, and dissolve in and dilute with water to volume. Derivatize the solution by combining equal volumes of *o-Phthalaldehyde reagent* and *Test solution* immediately before injection.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 335-nm detector and a 250-mm × 4.6-mm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solutions,* and record the chromatograms as directed for *Procedure:* the relative retention times of *o*-phthalaldehyde and 2-methoxyethylamine are about 0.6 and 1.0, respectively. Plot the concentration of 2-methoxyethylamine in each *Standard solution* versus its peak area, and perform a regression analysis to obtain a slope and an intercept. The correlation coefficient, r, is not less than 0.995, and the relative standard deviation for replicate injections of the 50 µg per mL *Standard solution* is not more than 5%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Test solution* and the *Standard solutions* into the chromatograph, record the chromatograms, and measure the peak responses. Determine the concentration, in µg per mL, of 2-methoxyethylamine in the *Test solution* from the standard response line. Calculate the percentage of 2-methoxyethylamine by the formula:

$$0.5C/W$$

in which C is the concentration, in µg per mL, of 2-methoxyethylamine obtained from the Standard response line; and W is the weight, in mg, of Gadoversetamide taken. Not more than 0.10% (w/w) of 2-methoxyethylamine is present, calculated on the anhydrous basis.

Limit of residual solvents—

Internal standard solution—Dilute butyl alcohol with water (3:5000).

Standard solutions—To four separate 5-mL volumetric flasks, transfer the following designated compositions:

Flask	Isopropyl alcohol	Acetonitrile	Internal standard
1	25 µg	25 µg	1.0 mL
2	100 µg	100 µg	1.0 mL
3	250 µg	250 µg	1.0 mL
4	500 µg	500 µg	1.0 mL

Dilute each flask with water to volume, and mix. The resulting *Standard solutions* contain about 5, 20, 50, and 100 µg of isopropyl alcohol and acetonitrile per mL.

Test solution—Transfer about 500 mg of Gadoversetamide, accurately weighed, to a 5-mL volumetric flask. Add 1.0 mL of *Internal standard solution,* dissolve in and dilute with water to volume, and mix.

www.webofpharma.com

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m capillary column with a 1.0-µm thickness of phase G35. Helium is used as the carrier gas, at a flow rate of about 5 mL per minute. The column temperature is maintained at 35° for 5 minutes, then increased at a rate of 15° per minute to 110°. The injection port temperature is maintained at 150°, and the detector temperature is maintained at 300°. Chromatograph the *Standard solutions,* and record the peak area ratios as directed for *Procedure:* the relative retention times are about 0.5 for isopropyl alcohol, 0.7 for acetonitrile, and 1.0 for butyl alcohol. Plot the concentration for each standard versus its peak area ratio, and perform a regression analysis. The correlation coefficient, *r,* is not less than 0.995 for each analyte; and the relative standard deviation for replicate injections of the 100 µg per mL *Standard solution* is not more than 5%.

Procedure—Separately inject equal volumes (about 2 µL) of the *Test solution* and the *Standard solutions* into the chromatograph, record the chromatograms, and measure the peak area ratios of the standard peak to the internal standard peak. Determine the concentration of isopropyl alcohol and acetonitrile from the respective standard response lines. Calculate the percentage (w/w) of each solvent in the portion of Gadoversetamide taken by the formula:

$$0.5C/W$$

in which *C* is the concentration, in µg per mL (obtained from the respective standard response line) of isopropyl alcohol and acetonitrile in the *Test solution;* and *W* is the weight (anhydrous), in mg, of the portion of Gadoversetamide taken: not more than 0.1% (w/w) of isopropyl alcohol is found; and not more than 0.025% (w/w) of acetonitrile is found. The total residual solvent content (sum of the % w/w isopropyl alcohol and the % w/w acetonitrile) does not exceed 0.1% w/w.

Related compounds—

Solution A—Dissolve 2.06 g of monobasic potassium phosphate and 18.6 mL of 20% w/w tetraethylammonium hydroxide in 950 mL of water. Adjust with phosphoric acid to a pH of 7, dilute with water to make 1000 mL, mix, filter, and degas.

Solution B—Prepare a filtered and degassed mixture of *Solution A* and acetonitrile (475:25).

Mobile phase—Use a mixture of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock solution—Transfer about 50 mg each of USP Gadoversetamide Related Compound A RS and USP Gadodiamide Related Compound B RS, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix. [NOTE—The solution may be stored for a week.]

Standard solutions—Prepare aqueous solutions of diluted *Standard stock solution* containing about 25, 150, and 250 µg of each Reference Standard per mL.

Test solution—Transfer about 250 mg of Gadoversetamide, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector and a metal-free 4.6- × 150-mm column that contains 5-µm packing L1. The flow rate is 1 mL per minute. The chromatograph is programmed to pump a mixture of *Solution A* to *Solution B* (97:3). The column temperature is maintained at about 25°. The relative retention times are about 0.6 for gadodiamide related compound B and 0.7 for gadoversetamide related compound A; the resolution, *R,* between gadodiamide related compound B and gadoversetamide related compound A is not less than 1.0; and the relative standard deviation for replicate injections is not more than 5% for the 250 µg per mL *Standard solution.*

Procedure—Separately inject equal volumes (about 50 µL) of the *Test solution* and the *Standard solutions* into the chromatograph. Allow 1 hour between injections to remove slow-eluting impurities. Determine the quantities, in µg per mL, of gadoversetamide related compound A and gadodiamide related compound B from the respective Standard response lines. Calculate the percentage of gadoversetamide related compound A in the portion of Gadoversetamide taken by the formula:

$$100C/V$$

in which *C* is the concentration of gadoversetamide related compound A, in µg per mL, obtained from the Standard response line; and *V* is the concentration of gadoversetamide, in µg per mL, in the *Test solution.* Not more than 1.0% (w/w) of gadoversetamide related compound A is found, calculated on the anhydrous basis. Calculate the percentage of gadodiamide related compound B in the portion of Gadoversetamide taken by the following formula:

$$92.2C/V$$

in which *C* is the concentration of gadodiamide related compound B, in µg per mL, obtained from the Standard response line; and *V* is as described herein. Not more than 0.5% (w/w) of gadodiamide related compound B is found, calculated on the anhydrous basis.

Assay—

Mobile phase—Dissolve 1.5 g of boric acid in about 950 mL of water, and mix. Adjust with ammonium hydroxide to a pH of 6.8, add 15 mL of acetonitrile, dilute with water to make 1000 mL, mix, filter, and degas.

Standard preparations—Prepare solutions of USP Gadoversetamide RS in *Mobile phase* having known concentrations of about 1.2, 1.0, and 0.8 mg per mL.

Assay preparation—Transfer about 100 mg of Gadoversetamide, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector and a metal-free 4.6- × 250-mm column that contains 5-µm packing L1. The column temperature is maintained at about 50°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparations,* and record the peak responses as directed for *Procedure.* Plot the concentration of each Standard versus its peak area and perform a regression analysis to obtain a slope and intercept for the Standard response line. The correlation coefficient, *r,* is not less than 0.995; and the relative standard deviation for replicate injections of the 1.0 mg per mL *Standard preparation* is not more than 2%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Assay preparation* and *Standard preparations* into the chromatograph, record the chromatograms, and measure the area of the gadoversetamide peak. Determine the quantity, in mg per mL, of gadoversetamide from the Standard response line. Calculate the quantity, in % (w/w), of $C_{20}H_{34}GdN_5O_{10}$ in the portion of Gadoversetamide taken by the formula:

$$10{,}000C/W$$

www.webofpharma.com

in which *C* is the concentration, in mg per mL, obtained from the Standard response line; and *W* is the weight (anhydrous), in mg, of the portion of Gadoversetamide taken to prepare the *Assay preparation.*

Gadoversetamide Injection

» Gadoversetamide Injection is a sterile solution of Gadoversetamide in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of gadoversetamide ($C_{20}H_{34}GdN_5O_{10}$). It may contain buffers and stabilizers. It contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose Type I glass containers or plastic syringes. Store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Gadoversetamide RS
USP Gadoversetamide Related Compound A RS
Hydrogen [8, 11,14-tris(carboxymethyl)-6-oxo-2-oxa-5,8,11,14-tetraazahexadecan-16-oato(4-)]gadolinium.

Bacterial Endotoxins Test ⟨85⟩ **:** not more than 5 USP Endotoxin Units per mL of gadoversetamide.

pH ⟨791⟩**:** between 5.5 and 7.5.

Particulate Matter in Injections ⟨788⟩ **:** meets the requirements for small-volume injections.

Nuclear Magnetic Resonance Spectroscopy ⟨761⟩—
Standard solutions—Dissolve an accurately weighed quantity of manganese (II) chloride tetrahydrate in water, and quantitatively dilute with water to obtain solutions having known concentrations of 0.9 mM, 2.7 mM, and 4.5 mM.

Test stock solution—Transfer 5.0 mL of the Injection to a 50-mL volumetric flask, dilute with water to volume, and mix.

Test solutions—Transfer 1.0, 2.0, 4.0, and 6.0 mL of *Test stock solution* to individual 100-mL volumetric flasks, dilute each with water to volume, and mix. These solutions have concentrations of 0.504 mM, 1.008 mM, 2.016 mM, and 3.024 mM, respectively, based on the label claim.

Apparatus—Use an NMR spectrometer with suitable sensitivity.

System suitability—Place a portion of each of the *Standard solutions* into a separate 10-mm specimen tube. Warm to 40° for not less than 10 minutes, and measure the resonance frequency (T_1), at 20 MHz. The average T_1 for replicate measurements must be within 5% of 156 ms for the 0.9 mM *Standard solution,* 52 ms for the 2.7 mM *Standard solution,* and 32 ms for the 4.5 mM *Standard solution.*

Procedure—Place an accurately measured portion of each *Test solution* into a 10-mm specimen tube. Warm to 40° for not less than 10 minutes, and measure the resonance frequency (T_1) of each *Test solution.* Plot $1/T_1$ versus the molarities of the *Test solutions,* and perform a regression analysis. The slope of the plotted line is the relaxivity. The relaxivity is between 4.0 and 5.0 sec^{-1} mM^{-1}.

Related compounds—
Solution A—Proceed as directed for *Mobile phase* in the test for *Related compounds* under *Gadoversetamide.*

Solution B—Prepare a filtered and degassed mixture of *Solution A* and acetonitrile (475:25).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solutions—Prepare aqueous solutions of USP Gadoversetamide Related Compound A RS having known concentrations of about 30 µg per mL, 150 µg per mL, and 360 µg per mL.

Test solution—Transfer 5.0 mL of the Injection to a 50-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the test for *Related compounds* under *Gadoversetamide.* The chromatograph is programmed as follows.

Time (minutes)	Solution A %	Solution B %	Elution
0–15	97	3	equilibration
15–16	97→0	3→100	linear gradient
16–26	0	100	isocratic
26–27	0→97	100→3	linear gradient
27–45	97	3	re-equilibration

Plot the concentration, in µg per mL, of each *Standard solution* versus its peak area, and perform a regression analysis to obtain a slope and intercept for the Standard response line. The relative standard deviation for replicate injections of the 360 µg per mL *Standard solution* is not more than 5%; and the correlation coefficient, *r,* of the regression analysis is not less than 0.995.

Procedure—Separately inject equal volumes (about 50 µL) of the *Test Solution,* the *Standard solutions,* and water (blank) into the chromatograph, record the chromatograms, and measure the peak responses. Allow about 1 hour between injections to remove slow-eluting impurities from the column. Calculate the percentage of gadoversetamide related compound A relative to the amount of gadoversetamide ($C_{20}H_{34}GdN_5O_{10}$) in the portion of Injection taken, based on the label claim, by the formula:

$$0.003C$$

in which *C* is the concentration of gadoversetamide related compound A in the *Test solution,* in µg per mL, obtained from the Standard response line: not more than 1.0% (w/w) of gadoversetamide related compound A is found.

Other requirements—It meets the requirements of the *Identification* tests under *Gadoversetamide.* It also meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase, Standard preparations, and *Chromatographic system*—Proceed as directed in the *Assay* under *Gadoversetamide.*

Assay preparation—Transfer about 3.0 mL of the Injection, accurately measured, to a 1000-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed in the *Assay* under *Gadoversetamide.* Calculate the quantity, in mg per mL, of gadoversetamide ($C_{20}H_{34}GdN_5O_{10}$) in the volume of Injection taken by the formula:

$$1000C/V$$

in which *C* is the concentration, in mg per mL, of gadoversetamide in the *Assay preparation,* obtained from the Standard response line; and *V* is the volume, in mL, of Injection taken.

www.webofpharma.com

Galantamine Extended-Release Capsules

DEFINITION

Galantamine Extended-Release Capsules contain galantamine hydrobromide ($C_{17}H_{21}NO_3 \cdot HBr$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of galantamine ($C_{17}H_{21}NO_3$).

[NOTE—Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them from light by using low-actinic glassware.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)

Sample: Prepare a potassium bromide dispersion as follows. Remove four beads from within one Capsule. Grind the beads into a fine powder, and combine with potassium bromide.

Standard: Prepare a potassium bromide dispersion using USP Galantamine Hydrobromide RS.

Acceptance criteria: The IR spectra of the *Sample* and the *Standard* exhibit similar absorption bands at 2800–2400 cm^{-1}, 1700–1500 cm^{-1}, and 850–750 cm^{-1}.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 4.0 g/L of monobasic potassium phosphate in water adjusted with 5 N sodium hydroxide TS to a pH of 6.5

Mobile phase: Acetonitrile and *Buffer* (10:90)

Standard stock solution: 0.62 mg/mL of USP Galantamine Hydrobromide RS (equivalent to 0.48 mg/mL of galantamine) prepared as follows. Transfer a suitable quantity of USP Galantamine Hydrobromide RS to a suitable flask, and dissolve in 20% of the flask volume of methanol. Dilute with *Buffer* to volume.

Standard solution: 0.048 mg/mL of galantamine from the *Standard stock solution* in *Buffer*

Sample stock solution: Prepare the solution using the appropriate nominal concentration of galantamine stated in *Table 1*. Transfer the contents of 10 Capsules to a suitable volumetric flask. Add 20% of the final flask volume of methanol, sonicate for 15 min, and stir for 20 min. Add a suitable volume of *Buffer* such that 80% of the final flask volume is filled, and stir for 90 min. Dilute with *Buffer* to volume.

Table 1

Capsule Strength (mg/Capsule)	Nominal Concentration of Galantamine (mg/mL)
8	0.32
16	0.32
24	0.48

Sample solution: Nominally 0.048 mg/mL of galantamine prepared as follows from the *Sample stock solution*. Transfer a suitable volume of the *Sample stock solution* to an appropriate volumetric flask, and dilute with *Buffer* to volume. Pass through a suitable filter of 0.45-µm pore size. Discard the first 5 mL, and use the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 288 nm
Column: 4.6-mm × 15.0-cm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.7
Relative standard deviation: NMT 1.0%

Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of galantamine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION ⟨711⟩**

Test 1
Medium: 0.05 M monobasic potassium phosphate, pH 6.5; 900 mL
Apparatus 2: 50 rpm with sinkers
[NOTE—A suitable sinker is catalog number CAPWHT-2S from www.qla-llc.com.]
Times: 1, 4, and 12 h
Buffer: 1 g/L of sodium 1-hexanesulfonate in water. Add 0.5 mL of phosphoric acid per L.
Mobile phase: Acetonitrile and *Buffer* (20:80)
Standard stock solution: 0.57 mg/mL of USP Galantamine Hydrobromide RS (equivalent to 0.44 mg/mL of galantamine) in *Medium*
Standard solution: (*L*/900) mg/mL of galantamine from the *Standard stock solution* in *Medium*, where *L* is the label claim of galantamine in mg/Capsule
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15.0-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: NLT 1.5 times the retention time of galantamine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of galantamine ($C_{17}H_{21}NO_3$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Calculate the percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of galantamine in the portion of sample withdrawn at time point i (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	20–40
2	4	40–65
3	12	NLT 75

The percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved at the times specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.05 M monobasic potassium phosphate, pH 6.5; 900 mL
Apparatus 2: 50 rpm
Times: 1, 4, and 12 h
Solution A: Transfer 0.5 mL of phosphoric acid to a 100-mL volumetric flask containing 50% of the flask volume of water. Dilute with water to volume.
Mobile phase: Acetonitrile and *Solution A* (7:93)
Standard stock solution: 0.23 mg/mL of USP Galantamine Hydrobromide RS (equivalent to 0.18 mg/mL of galantamine) in *Medium*
Standard solution: (L/900) mg/mL of galantamine from the *Standard stock solution* in *Medium*, where L is the label claim of galantamine, in mg/Capsule
Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 3.0-mm × 15.0-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 0.7 mL/min
Injection volume: 10 µL
Run time: NLT 1.6 times the retention time of galantamine
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of galantamine ($C_{17}H_{21}NO_3$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Calculate the percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of galantamine in the portion of sample withdrawn at time point i (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	22–38
2	4	50–70
3	12	NLT 80

The percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved at the times specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: pH 6.5 phosphate buffer (6.8 g/L of monobasic potassium phosphate and 0.56 g/L of sodium hydroxide

www.webofpharma.com

adjusted, if necessary, with phosphoric acid or 10 N sodium hydroxide TS to a pH of 6.5); 900 mL
Apparatus 2: 50 rpm, with stainless steel wire helix sinkers
Times: 1, 2, 4, and 12 h
Buffer: To each L of 6.8 g/L of monobasic potassium phosphate in water add 3.0 mL of triethylamine, and adjust the resulting solution with phosphoric acid to a pH of 2.5. Pass through a suitable membrane filter of 0.45-µm pore size, and use the filtrate.
Mobile phase: Acetonitrile and *Buffer* (8:92)
Standard stock solution: 0.23 mg/mL of USP Galantamine Hydrobromide RS (equivalent to 0.18 mg/mL of galantamine) in *Medium*
Standard solution: (*L*/900) mg/mL of galantamine from the *Standard stock solution* in *Medium*, where *L* is the label claim of galantamine, in mg/Capsule
Sample solution: Pass a portion of the solution under test through a suitable filter. Replace the portion of solution withdrawn with an equal volume of *Medium*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15.0-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 50 µL
Run time: NLT 2 times the retention time of galantamine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of galantamine ($C_{17}H_{21}NO_3$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Calculate the percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of galantamine in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	18–43
2	2	30–50
3	4	40–65
4	12	NLT 80

The percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved at the times specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: pH 6.5 phosphate buffer (6.8 g/L of monobasic potassium phosphate in water adjusted, if necessary, with 5 N sodium hydroxide TS to a pH of 6.5); 900 mL
Apparatus 2: 50 rpm, with sinkers
Times: 1, 4, and 12 h
Buffer: 6.8 g/L of monobasic potassium phosphate adjusted, if necessary, with 5 N sodium hydroxide TS to a pH of 7.5
Mobile phase: Acetonitrile and *Buffer* (15:85)
Standard stock solution: 0.11 mg/mL of USP Galantamine Hydrobromide RS (equivalent to 0.09 mg/mL of galantamine) in *Medium*
Standard solution: (*L*/900) mg/mL of galantamine from the *Standard stock solution* in *Medium*, where *L* is the label claim of galantamine, in mg/Capsule
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15.0-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.4 mL/min
Injection volume: 100 µL
Run time: NLT 1.5 times the retention time of galantamine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of galantamine ($C_{17}H_{21}NO_3$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Calculate the percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved at each time point (*i*):

www.webofpharma.com

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of galantamine in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 5*.

Table 5

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	NMT 40
2	4	45–70
3	12	NLT 75

The percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved at the times specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: pH 6.5 phosphate buffer (6.8 g/L of monobasic potassium phosphate adjusted, if necessary, with 0.2 N sodium hydroxide TS to a pH of 6.5); 900 mL
Apparatus 2: 50 rpm with sinkers
Times: 1, 4, and 12 h
Buffer: To each L of water add 7 mL of triethylamine.
Mobile phase: Methanol and *Buffer* (25:75), adjusted with phosphoric acid or a solution of triethylamine and water (5:95) to a pH of 6.5
Standard solution: 0.025 mg/mL of USP Galantamine Hydrobromide RS (equivalent to 0.02 mg/mL of galantamine) in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15.0-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: NLT 1.5 times the retention time of galantamine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of galantamine ($C_{17}H_{21}NO_3$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Calculate the percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of galantamine in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 6*.

Table 6

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	10–30
2	4	45–65
3	12	NLT 80

The percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved at the times specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.
Medium: pH 6.5 phosphate buffer [6.8 g/L of monobasic potassium phosphate and 0.46 g/L of sodium hydroxide in water adjusted, if necessary, with 0.2 N sodium hydroxide TS or hydrochloric acid and water (1.6: 98.4) to a pH of 6.5]; 900 mL
Apparatus 2: 50 rpm
Times: 1, 4, 10, and 12 h
Buffer: 2.7 g/L of monobasic potassium phosphate in water. To each liter, add 5 mL of triethylamine and adjust with phosphoric acid, if necessary, to a pH of 4.8.
Mobile phase: Methanol and *Buffer* (10:90)
Standard stock solution: 0.11 mg/mL of USP Galantamine Hydrobromide RS (equivalent to 0.09 mg/mL of galantamine) in *Medium*. Sonication may be used to promote dissolution.
Standard solution: (*L*/900) mg/mL of USP Galantamine Hydrobromide RS from the *Standard stock solution* in *Medium*, where *L* is the label claim of galantamine, in mg/Capsule
Sample solution: Pass a portion of the solution under test through a suitable filter. Replace the portion of solution withdrawn with an equal volume of *Medium*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°

www.webofpharma.com

Flow rate: 1.5 mL/min
Injection volume: 20 µL
Run time: NLT 2 times the retention time of galantamine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of galantamine ($C_{17}H_{21}NO_3$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Calculate the percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of galantamine in the portion of sample withdrawn at the specified time point (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 7*.

Table 7

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	15–35
2	4	45–65
3	10	NLT 70
4	12	NLT 80

The percentages of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A: 1.7 g/L of dibasic potassium phosphate and 3.0 g/L of monobasic potassium phosphate in water
Solution B: Acetonitrile

Mobile phase: See *Table 8*.

Table 8

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	97	3	0.7
22	69	31	0.7
24	25	75	1.2
27	25	75	1.2
29	97	3	0.7
35	97	3	0.7

Diluent: Methanol and *Solution A* (60:40)
System suitability solution: 0.31 mg/mL of USP Galantamine Hydrobromide Related Compounds Mixture RS in *Diluent*
Standard solution: 0.0015 mg/mL of USP Galantamine Hydrobromide RS (equivalent to 0.0012 mg/mL of galantamine) in *Diluent*
Sample solution: Nominally 0.24 mg/mL of galantamine from NLT 20 Capsules prepared as follows. Transfer a suitable portion of the contents from NLT 20 Capsules to a suitable volumetric flask, and dilute with *Diluent* to volume. Sonication and shaking may be used to promote dissolution. Allow the solution to sit for NLT 24 h, and then clarify the solution using a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 3.0-mm × 15.0-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: See *Table 8*.
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 9* for relative retention times.]
Suitability requirements
Resolution: NLT 3.4 between galantamine and 6*S*-galantamine (also known as 6α-hexahydrogalantamine), *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of galantamine from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of galantamine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Acceptance criteria: See *Table 9*. Disregard peaks less than 0.05%.

www.webofpharma.com

Table 9

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
N-Desmethyl galantamine[a, b]	0.57	0.5
Galantamine *N*-oxide[c]	0.75	0.5
Dihydrogalantamine[d, e]	0.87	—
Galantamine	1.0	—
6*S*-Galantamine[f]	1.1	0.2
Didehydrodeoxygalantamine[e, g]	1.9	—
Any unspecified degradation product	—	0.2
Total degradation products	—	1.2

[a] (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.
[b] This degradation product may be found if the drug substance is isolated from a natural source.
[c] (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol, *N*-oxide; also known as 6β-Hexahydrogalantamine.
[d] (4a*S*,6*R*,8a*S*)-4a,5,7,8,9,10,11,12-Octahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol; also known as 6β-Octahydrogalantamine.
[e] This is a process impurity and is listed for information only. It is controlled in the drug substance. It is not to be reported and is not to be included in the total degradation products.
[f] (4a*S*,6*S*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol; also known as 6α-Hexahydrogalantamine or *epi*-galantamine.
[g] (4a*S*,8a*S*)-9,10,11,12-Tetrahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepine; also known as Tetrahydrogalantamine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Galantamine Hydrobromide RS
  USP Galantamine Hydrobromide Related Compounds Mixture RS
  Galantamine hydrobromide;
  6β-Hexahydrogalantamine (galantamine *N*-oxide);
  (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol, *N*-oxide.
  $C_{17}H_{21}NO_4$ 303.35
  6β-Octahydrogalantamine (dihydrogalantamine);
  (4a*S*,6*R*,8a*S*)-4a,5,7,8,9,10,11,12-Octahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.
  $C_{17}H_{23}NO_3$ 289.37
  6α-Hexahydrogalantamine (6*S*-galantamine);
  (4a*S*,6*S*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.
  $C_{17}H_{21}NO_3$ 287.35
  Tetrahydrogalantamine (didehydrodeoxygalantamine);
  (4a*S*,8a*S*)-9,10,11,12-Tetrahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepine.
  $C_{17}H_{19}NO_2$ 269.34

Galantamine Oral Solution

DEFINITION

Galantamine Oral Solution contains an amount of galantamine hydrobromide ($C_{17}H_{21}NO_3 \cdot HBr$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of galantamine ($C_{17}H_{21}NO_3$). Galantamine Oral Solution also contains one or more suitable preservatives.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them from light by using low-actinic glassware.
Solution A: Acetonitrile and water (1:99)
Solution B: 5 mg/mL of ammonium acetate in *Solution A*
Solution C: Acetonitrile
Solution D: Water
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)	Solution D (%)
0	50	3	47
25	50	35	15
27	50	3	47
32	50	3	47

Diluent: 5 mg/mL of ammonium acetate in water
System suitability solution: 0.26 mg/mL of USP Galantamine Hydrobromide Related Compounds Mixture RS prepared as follows. Transfer a suitable quantity of USP Galantamine Hydrobromide Related Compounds Mixture RS to a suitable flask, and dissolve in 3% of the flask volume of methanol. Dilute with *Diluent* to volume.
Standard solution: 0.26 mg/mL of USP Galantamine Hydrobromide RS (equivalent to 0.2 mg/mL of galantamine) prepared as follows. Transfer a suitable quantity of USP Galantamine Hydrobromide RS to an appropriate flask, and dissolve in 3% of the flask volume of methanol. Dilute with *Diluent* to volume.
Sample solution: Nominally 0.2 mg/mL of galantamine from Oral Solution prepared as follows. Transfer a suitable volume of Oral Solution to an appropriate volumetric flask. Add 3% of the flask volume of methanol, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 3.5 times the retention time of galantamine
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 5 between galantamine and 6*S*-galantamine (also known as 6α-hexahydrogalantamine), *System suitability solution*
Tailing factor: NMT 1.3, *Standard solution*

www.webofpharma.com

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of galantamine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Deliverable Volume** ⟨698⟩**:** Meets the requirements

IMPURITIES

• **Organic Impurities**

Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them from light by using low-actinic glassware.

Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity solution: 0.5 µg/mL of USP Galantamine Hydrobromide RS prepared as follows. Transfer a suitable quantity of USP Galantamine Hydrobromide RS to an appropriate flask, and dissolve in 3% of the flask volume of methanol. Dilute with *Diluent* to volume.

System suitability

Samples: *System suitability solution, Standard solution,* and *Sensitivity solution*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 5 between galantamine and 6*S*-galantamine (also known as 6α-hexahydrogalantamine), *System suitability solution*

Tailing factor: NMT 1.3, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of galantamine from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of galantamine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.10%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
N-Desmethyl galantamine[a, b]	0.54	0.5
Galantamine *N*-oxide[c]	0.68	0.30
Dihydrogalantamine[d, e]	0.85	—
Galantamine	1.0	—
6*S*-Galantamine[f]	1.2	0.2
Narwedine[g, e]	1.6	—
Didehydrodeoxygalantamine[h, e]	2.0	—
Any unspecified degradation product	—	0.2
Total degradation products	—	1.0

[a] (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.

[b] This degradation product may be found if the drug substance is isolated from a natural source.

[c] (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol, *N*-oxide; also known as 6β-hexahydrogalantamine.

[d] (4a*S*,6*R*,8a*S*)-4a,5,7,8,9,10,11,12-Octahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol; also known as 6β-octahydrogalantamine.

[e] This is a process impurity and is listed for information only. It is controlled in the drug substance. It is not to be reported and is not to be included in the *Total degradation products*.

[f] (4a*S*,6*S*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol; also known as 6α-hexahydrogalantamine or *epi*-galantamine.

[g] (4a*S*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-one.

[h] (4a*S*,8a*S*)-9,10,11,12-Tetrahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepine; also known as tetrahydrogalantamine.

SPECIFIC TESTS

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/mL. The total yeasts and molds count does not exceed 5×10^1 cfu/mL. It meets the requirements for the test for absence of *Escherichia coli*.

• **pH** ⟨791⟩**:** 3.9–6.2

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature. Protect from freezing.

• **USP Reference Standards** ⟨11⟩

USP Galantamine Hydrobromide RS
USP Galantamine Hydrobromide Related Compounds Mixture RS
Galantamine hydrobromide.
6β-Hexahydrogalantamine.
(4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol, *N*-oxide.
$C_{17}H_{21}NO_4$ 303.35
6β-Octahydrogalantamine.

USP Monographs

www.webofpharma.com

(4a*S*,6*R*,8a*S*)-4a,5,7,8,9,10,11,12-Octahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.
$C_{17}H_{23}NO_3$ 289.37
6α-Hexahydrogalantamine.
(4a*S*,6*S*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.
$C_{17}H_{21}NO_3$ 287.35
Tetrahydrogalantamine.
(4a*S*,8a*S*)-9,10,11,12-Tetrahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepine.
$C_{17}H_{19}NO_2$ 269.34

Galantamine Tablets

DEFINITION

Galantamine Tablets contain an amount of Galantamine Hydrobromide equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of galantamine ($C_{17}H_{21}NO_3$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)**:** The spectrum of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Uniformity of Dosage Units*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer solution: 5.34 g/L of dibasic sodium phosphate dihydrate in water. Adjust with phosphoric acid to a pH of 6.5.
Solution A: Chromatographic methanol and *Buffer solution* (1:19)
Solution B: Chromatographic acetonitrile and chromatographic methanol (19:1)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40.0	75	25
45.0	60	40
46.0	40	60
55.0	40	60
56.0	100	0
61.0	100	0

Diluent: Dissolve 35.4 g of edetate disodium in 950 mL of water, and add 50 mL of chromatographic methanol. [NOTE—First dissolve in water, and then add chromatographic methanol.]
Standard solution: 0.62 mg/mL of USP Galantamine Hydrobromide RS in *Diluent*
Sample solution: 0.48 mg/mL of galantamine from powdered Tablets (NLT 10) in *Diluent*. Pass through a PTFE filter of 0.45-µm or finer pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of galantamine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION ⟨711⟩**

Test 1
Medium: Water; 500 mL
Apparatus 2: 50 rpm
Time: 20 min
Standard solution: (*L*/400) mg/mL of USP Galantamine Hydrobromide RS in *Medium*, where *L* is the label claim in mg
Sample solution: Pass portions of the solution through a suitable filter of 0.2-µm pore size.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 288 nm
Cell: 5-cm cell for 4-mg and 8-mg Tablets; 1-cm cell for 12-mg Tablets
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

www.webofpharma.com

V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium, Apparatus 2, Time, Standard solution, Sample solution, and **Analysis:** Proceed as directed for *Test 1*.
Tolerances: NLT 70% (Q) of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
Medium: Water; 500 mL
Apparatus 2: 50 rpm. Use peak vessels and adjust the position of the paddle so that the distance between the tip of the peak and the bottom of the paddle is 1 cm.
Time: 20 min
Buffer: Triethylamine and 3.45 g/L of monobasic sodium phosphate in water (1:1000). Adjust with phosphoric acid to a pH of 4.5.
Mobile phase: Chromatographic acetonitrile, chromatographic methanol, and *Buffer* (10:10:80), filtered and deaerated
Standard solution: (L/400) mg/mL of USP Galantamine Hydrobromide RS in water, where L is the label claim in mg
Sample solution: Pass a portion of the solution through a suitable filter of 0.45-µm pore size. Use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
Run time: Two times the retention time of galantamine
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

r_U = peak area of the *Sample solution*
r_S = peak area of the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27
V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩, *Content Uniformity*
Standard solution: 0.05 mg/mL of USP Galantamine Hydrobromide RS in 0.1 N hydrochloric acid
Sample solution: Add 1 Tablet to each appropriately sized volumetric flask to obtain a final galantamine concentration of 0.04 mg/mL. Add an appropriate amount of 0.1 N hydrochloric acid, equivalent to 75% of the total volume of the volumetric flask, and mechanically shake for 45 min. Dilute with 0.1 N hydrochloric acid to volume. Pass a portion of the solution through a suitable filter of 0.2-µm pore size, and use the filtrate.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: Maximum absorbance at about 289 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amount of galantamine ($C_{17}H_{21}NO_3$) dissolved in filtered portions of the *Sample solution* in comparison with the *Standard solution*.
Calculate the percentage of the labeled amount of galantamine ($C_{17}H_{21}NO_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27

Acceptance criteria: Meet the requirements for coated Tablets

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer solution, Solution A, Solution B, Mobile phase, Diluent, Standard solution, and **Sample solution:** Prepare as directed in the *Assay*.
System suitability solution: 0.6 mg/mL of USP Galantamine Hydrobromide Related Compounds Mixture RS in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between 6β-hexahydro galantamine and 6β-octahydrogalantamine, *System suitability solution*
Relative standard deviation: NMT 2.0% for galantamine, *Standard solution*
[NOTE—Identify the impurities using the approximate relative retention times given in *Table 2*.]
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Ignore the peak due to bromide near the void volume.]
Calculate the percentage of each of the impurities including the unspecified degradation impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (100/F)$$

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of galantamine from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of galantamine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of galantamine, 287.35
M_{r2} = molecular weight of galantamine hydrobromide, 368.27
F = relative response factor for each of the impurities relative to galantamine (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Desmethyl-galantamine[a]	0.41	1.1	0.5
6β-Hexahydro-galantamine (also known as galantamine *N*-oxide)[b]	0.73	1.1	0.75
6β-Octahydro-galantamine (also known as lycoramine)[c, d]	0.86	—	—
Galantamine hydrobromide	1.00	1.0	—
6α-Hexahydro-galantamine (also known as epigalantamine)[e]	1.15	1.1	0.5
Tetrahydro-galantamine[f, d]	2.09	—	—
Individual, unspecified degradation impurity	—	1.0	0.2
Total impurities	—	—	1.5

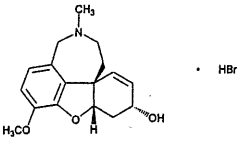
[a] (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.
[b] (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol, *N*-oxide.
[c] (4a*S*,6*R*,8a*S*)-4a,5,7,8,9,10,11,12-Octahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.
[d] This is a process impurity and is listed for information only. It is controlled in the drug substance.
[e] (4a*S*,6*S*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol.
[f] (4a*S*,8a*S*)-9,10,11,12-Tetrahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Galantamine Hydrobromide RS
  USP Galantamine Hydrobromide Related Compounds Mixture RS
  Contains galantamine hydrobromide, 6β-hexahydrogalantamine, 6β-octahydrogalantamine, 6α-hexahydrogalantamine, and tetrahydrogalantamine.

Galantamine Hydrobromide

Change to read:



$C_{17}H_{21}NO_3 \cdot HBr$ 368.27
6*H*-Benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol, 4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-, hydrobromide, (4a*S*,6*R*,8a*S*)-;
▲(4a*S*,6*R*,8a*S*)-3-Methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol hydrobromide;
(4a*S*,6*R*,8a*S*)-3-Methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-ol hydrobromide▲ (USP 1-Dec-2019) [1953-04-4].

DEFINITION

Galantamine Hydrobromide contains NLT 98.0% and NMT 102.0% of galantamine hydrobromide ($C_{17}H_{21}NO_3 \cdot HBr$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  [NOTE—Specimens are to be prepared using undried USP Galantamine Hydrobromide RS and the test article.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the *Assay*.

Change to read:

- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Bromide*
  Sample solution: ▲▲ (USP 1-Dec-2019) 7 mg/mL ▲of Galantamine Hydrobromide▲ (USP 1-Dec-2019) in water
  Acceptance criteria: Meets the requirements of test *B*

ASSAY

Change to read:

- **PROCEDURE**
  Diluent: Methanol and water▲(5:95)▲ (USP 1-Dec-2019)
  Buffer: 0.79 g/L of dibasic sodium phosphate dihydrate and 2.46 g/L of monobasic sodium phosphate anhydrous in water
  Solution A: Methanol and *Buffer* (5:95)
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
6.0	100	0
20.0	95	5
35.0	85	15

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
50.0	80	20
51.0	40	60
55.0	40	60
56.0	100	0
60.0	100	0

System suitability solution: 1 mg/mL of USP Galantamine Hydrobromide Related Compounds Mixture RS in *Diluent*
Standard solution: 1.0 mg/mL of USP Galantamine Hydrobromide RS in *Diluent*
Sample solution: 1.0 mg/mL of Galantamine Hydrobromide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 10-cm; 3.5-µm packing L1
Column temperature: 55°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—For relative retention times, see *Table 2*.]
Suitability requirements
Resolution: NLT 4.5 between galantamine and ▲6*S*-galantamine,▲ (USP 1-Dec-2019) *System suitability solution*
Tailing factor: NMT 2.0 for galantamine, *System suitability solution*
Relative standard deviation: ▲NMT 0.73%,▲ (USP 1-Dec-2019) *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of galantamine hydrobromide ($C_{17}H_{21}NO_3 \cdot HBr$) in the portion of Galantamine Hydrobromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Galantamine Hydrobromide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Change to read:

• **LIMIT OF PALLADIUM:** ▲Proceed as directed below or in *Elemental Impurities—Procedures* ⟨233⟩.▲ (USP 1-Dec-2019)
[NOTE—Perform this test only if palladium is a known inorganic impurity of the manufacturing process.]
Standard stock solution: 20 mg/L of palladium reference stock solution (NIST traceable) in water
Aqua regia: Under a hood, carefully mix hydrochloric acid and nitric acid (3:1).
[NOTE—To obtain each of the required Standard solutions, it is recommended that the required volume of *Standard stock solution* be mixed with a volume of *Aqua regia* equivalent to 5% of the final volume, followed by water.]
Standard solution A: 0.2 mg/L of palladium from the *Standard stock solution* in water
Standard solution B: 1.0 mg/L of palladium from the *Standard stock solution* in water
Standard solution C: 2.0 mg/L of palladium from the *Standard stock solution* in water
System suitability solution: Prepare a solution having a known concentration of 1.6 mg/L of palladium, as directed for the Standard solutions.
Sample solution: Weigh 1 g of Galantamine Hydrobromide. Transfer the sample to an appropriate digestion system, and digest using appropriate acids (e.g., nitric acid or mixtures of nitric acid and sulfuric acid and mixtures of nitric acid and hydrogen peroxide). After digestion, heat to dryness. Add 0.5 mL of *Aqua regia* and 2 mL of water. Warm gently to dissolve any residue. Allow to cool. Transfer quantitatively to a 10-mL volumetric flask, and dilute with water to volume.
Digestion blank solution: Prepare this solution following the procedure for the *Sample solution*, without the test article.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectroscopy (flame)
Analytical wavelength: 247.6 nm (0.2-nm slit width)
Lamp: Palladium hollow-cathode
Blank solution: Dilute 5 mL of *Aqua regia* with water to 100 mL.
System suitability
Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, *System suitability solution*, and *Blank solution*
Using the Standard solutions and *Blank solution*, construct a calibration curve.
Suitability requirements
Correlation coefficient: NLT 0.99
Recovery: 87.5%–112.5%, *System suitability solution*.
[NOTE—Recovery is calculated using the calibration curve.]
Analysis
Samples: *Sample solution* and *Digestion blank solution*
Calculate the concentration of palladium in the *Sample solution*, using the calibration curve, corrected for the *Digestion blank solution* and the sample weight. Calculate the amount of palladium in the Galantamine Hydrobromide taken to prepare the *Sample solution*.
Acceptance criteria: NMT 10 ppm

Change to read:

• **ORGANIC IMPURITIES**
Diluent, Buffer, Solution A, Solution B, Mobile phase, System suitability solution, ▲▲ (USP 1-Dec-2019) and **Chromatographic system:** Proceed as directed in the *Assay*.
▲**Sensitivity solution:** 0.5 µg/mL of USP Galantamine Hydrobromide RS in *Diluent*▲ (USP 1-Dec-2019)
Standard solution: 5.0 µg/mL of USP Galantamine Hydrobromide RS in *Diluent*
▲**Sample solution:** 1000 µg/mL of Galantamine Hydrobromide in *Diluent*
System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[NOTE—For relative retention times, see *Table 2*.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 4.5 between galantamine and *6S*-galantamine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution* ▲(USP 1-Dec-2019)

Analysis
Samples: *Standard solution* and *Sample solution* ▲▲(USP 1-Dec-2019)
Calculate the percentage of each impurity in the portion of Galantamine Hydrobromide taken, on the dried basis:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times [100/(100 - L)]$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of galantamine from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide RS in the *Standard solution* ▲(µg/mL)▲(USP 1-Dec-2019)
C_U = concentration of the *Sample solution* ▲(µg/mL)▲(USP 1-Dec-2019)
F = relative response factor (see *Table 2*)
L = loss on drying, ▲%▲(USP 1-Dec-2019)

Acceptance criteria: See *Table 2*. ▲Disregard the bromide peak near the void volume. The reporting threshold is 0.05%.▲(USP 1-Dec-2019)

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Desmethyl galantamine[a]	0.29	1.2	0.6
O-Desmethyl galantamine[b]	0.35	1.1	0.20
Galantamine *N*-oxide[c]	0.65	0.96	0.20
Dihydrogalantamine[d]	0.82	0.81	0.35
Galantamine	1.00	1.0	—
6S-Galantamine[e]	1.16	0.95	0.20
Narwedine[f]	1.64	1.9	0.15
▲Anhydrogalantamine▲(USP 1-Dec-2019)[g]	2.05	1.2	0.40
Any unspecified impurity	—	1.0	0.10
Total impurities[h]	—	—	1.0

▲[a] (4a*S*,6*R*,8a*S*)-3-Methoxy-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-ol.
[b] (4a*S*,6*R*,8a*S*)-11-Methyl-4a,5,9,10,11,12-Hexahydro-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-3,6-diol.
[c] (4a*S*,6*R*,8a*S*)-6-Hydroxy-3-methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepine 11-oxide.
[d] (4a*S*,6*R*,8a*S*)-3-Methoxy-11-methyl-4a,5,7,8,9,10,11,12-octahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-ol.
[e] (4a*S*,6*S*,8a*S*)-3-Methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-ol.
[f] (4a*S*,8a*S*)-3-Methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-one. This is a process impurity that may be found in galantamine hydrobromide isolated from a natural source.
[g] (4a*S*,8a*S*)-3-Methoxy-11-methyl-9,10,11,12-tetrahydro-4a*H*-benzo[2,3]benzofuro[4,3-*cd*]azepine.▲(USP 1-Dec-2019)
[h] Do not include the ▲*4R,6S,8R* isomer.▲(USP 1-Dec-2019)

Change to read:

• **▲LIMIT OF THE *4R,6S,8R* ISOMER▲** (USP 1-Dec-2019)
[NOTE—If Galantamine Hydrobromide is not isolated from a natural source, perform either *Procedure 1* or *Procedure 2*.]

Procedure 1
Background electrolyte solution: 8.9 g/L of dibasic sodium phosphate dihydrate in water. Adjust with phosphoric acid to a pH of 3.0.
Run buffer: 19.6 g/L of ▲alpha-cyclodextrin hydrate▲(USP 1-Dec-2019) in *Background electrolyte solution*. Pass the solution through a filter of 0.22-µm pore size.
Standard solution: 5 µg/mL of USP Galantamine Hydrobromide Racemic RS in water. Pass the solution through a filter of 0.22-µm pore size, discarding the first 8 mL.
Sample solution: ▲500 µg/mL▲(USP 1-Dec-2019) of Galantamine Hydrobromide in water. Pass the solution through a filter of 0.22-µm pore size, discarding the first 8 mL.
Capillary rinse procedure: Use separate *Run buffer* vials for the capillary rinse and sample analysis. Proceed as directed in *Table 3*.

Table 3

Step #	Solution/Gas	Time (min)
1	0.1 N sodium hydroxide ▲VS▲(USP 1-Dec-2019)	15
2	Water	10
3	Suitable gas	5

[NOTE—If a new or dry capillary is being used, rinse with 1 N sodium hydroxide ▲VS▲(USP 1-Dec-2019) for 30 min, followed by rinsing with water for 15 min. Dry it with air or nitrogen for 10 min.]

Electrophoretic system
Mode: CE
Detector: UV 214 nm
Column: 75-µm × 60-cm uncoated fused-silica
Column temperature: 20°
Applied voltage: 250 V/cm, positive polarity
Run time: 35 min

System suitability
Sample: *Standard solution*. [NOTE—For the purpose of identification, the ▲*4S,6R,8S* isomer▲(USP 1-Dec-2019) elutes at an approximate relative migration time (RMT) of 1.00, and the ▲*4R,6S,8R* isomer▲(USP 1-Dec-2019) elutes at an RMT of about 1.05.]
Suitability requirements
Resolution: NLT 2.5 between the two enantiomers
Relative standard deviation: NMT 10% for the ▲*4R, 6S,8R* isomer▲(USP 1-Dec-2019) peak
Measure the migration times and peak responses: the migration times for the ▲*4R,6S,8R* isomer▲(USP 1-Dec-2019) in the electropherograms ▲of▲(USP 1-Dec-2019) the *Sample solution* should not deviate by more than 5% of the migration time for the same component in the electropherogram of the *Standard solution*.

Analysis
Samples: *Standard solution* and *Sample solution*
Injection: [NOTE—Rinse the capillary between injections as follows: water for 5 min, followed by *Run buffer* for 5 min. Rinse times are based on a rinse pressure of 1.4 bar.]

www.webofpharma.com

Sample solution: 34.5 mbar for 4 s
Run buffer: 6.9 mbar for 5 s
Calculate the corrected peak responses using the formula:

$$\text{Result} = (r/m)$$

r = peak response
m = migration time of the peak (min)

Calculate the limit of the ▲*4R,6S,8R*▲ (USP 1-Dec-2019) isomer, in percent, in the portion of Galantamine Hydrobromide taken:

$$\text{Result} = (r_{CU}/r_{CS}) \times (C_S/C_U) \times P \times 100$$

r_{CU} = average corrected peak responses ▲of▲ (USP 1-Dec-2019) the ▲*4R,6S,8R*▲ (USP 1-Dec-2019) isomer from the *Sample solution*
r_{CS} = average corrected peak responses ▲of▲ (USP 1-Dec-2019) the ▲*4R,6S,8R*▲ (USP 1-Dec-2019) isomer from the *Standard solution*
C_S = concentration of USP Galantamine Hydrobromide Racemic RS in the *Standard solution* ▲(µg/mL)▲ (USP 1-Dec-2019)
C_U = concentration of Galantamine Hydrobromide in the *Sample solution* ▲(µg/mL)▲ (USP 1-Dec-2019)
P = chiral purity of USP Galantamine Hydrobromide Racemic RS, 0.5

Acceptance criteria: NMT 0.10% of the ▲*4R,6S,8R* isomer▲ (USP 1-Dec-2019)

Procedure 2
[NOTE—Use low-actinic glassware and vials. It is recommended that precautions be taken to protect all solutions from light.]
Buffer: 8.2 g/L of ▲anhydrous sodium acetate▲ (USP 1-Dec-2019) in water
Mobile phase: Acetonitrile and *Buffer* (2:98). Adjust with acetic acid to a pH of 6.5.
System suitability solution: 2.4 µg/mL of USP Galantamine Hydrobromide Racemic RS in water. [NOTE—This solution will contain about 1.2 µg/mL of the ▲*4R,6S,8R* isomer.▲ (USP 1-Dec-2019)]
Sample solution: 1.2 mg/mL of Galantamine Hydrobromide in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.0-mm × 15-cm; 5-µm packing L41. [NOTE—Alternatively, a 2.0-mm × 15.0-cm column containing 5-µm packing L41 can be used with a recommended flow rate of about 0.2 mL/min.]
Flow rate: 0.8 mL/min
Injection volume: 5 µL
▲Run time: NLT 3 times the retention time of galantamine▲ (USP 1-Dec-2019)
System suitability
Sample: *System suitability solution*. [NOTE—The ▲*4R,6S,8R* isomer▲ (USP 1-Dec-2019) elutes first as the minor peak followed by the major peak due to galantamine (which is the same as the ▲*4S,6S,8S* isomer▲ (USP 1-Dec-2019)).]
Suitability requirements
Resolution: NLT 3.0 between the ▲*4R,6S,8R* isomer▲ (USP 1-Dec-2019) and galantamine peaks
Relative standard deviation: NMT 5.0% for the ▲*4R,6S,8R* isomer▲ (USP 1-Dec-2019) peak

Analysis
Sample: *Sample solution*
Calculate the percentage of ▲*4R,6S,8R* isomer▲ (USP 1-Dec-2019) in the portion of Galantamine Hydrobromide taken:

$$\text{Result} = [{}^{\blacktriangle}r_{4R,6S,8R}/(r_{4R,6S,8R} + r_{4S,6R,8S})_{\blacktriangle\ (\text{USP 1-Dec-2019})}] \times 100$$

$^{\blacktriangle}r_{4R,6S,8R\blacktriangle\ (\text{USP 1-Dec-2019})}$ = peak area of the ▲*4R,6S,8R* isomer▲ (USP 1-Dec-2019) from the *Sample solution*
$^{\blacktriangle}r_{4S,6R,8S\blacktriangle\ (\text{USP 1-Dec-2019})}$ = peak area of galantamine from the *Sample solution*

Acceptance criteria: NMT 0.10% of the ▲*4R,6S,8R* isomer▲ (USP 1-Dec-2019)

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.5%

Change to read:

- **OPTICAL ROTATION** ▲⟨781S⟩▲ (USP 1-Dec-2019), *Procedures, Specific Rotation*
[NOTE—If Galantamine Hydrobromide is isolated from a natural source, perform the test for *Optical Rotation*.]
Sample solution: 20 mg/mL in water
Acceptance criteria: –90° to –100°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store at room temperature. Preserve in well-closed containers.

Change to read:

- **LABELING:** Label it to state if the source is naturally derived or is synthetic. If the source is not natural, perform either *Procedure 1* or *Procedure 2* of the test for ▲the *Limit of the 4R,6S,8R Isomer*.▲ (USP 1-Dec-2019) If the source is natural, perform the test for *Optical Rotation* ▲⟨781S⟩▲ (USP 1-Dec-2019) , *Procedures, Specific Rotation*.

Change to read:

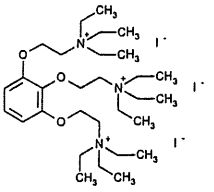
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Galantamine Hydrobromide RS
USP Galantamine Hydrobromide Racemic RS
▲(4a*S*,6*R*,8a*S*)-3-Methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-ol hydrobromide.
(4a*R*,6*S*,8a*R*)-3-Methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-ol hydrobromide.
$C_{17}H_{21}NO_3 \cdot HBr$ 368.27
[NOTE—This is 50:50 mixture of the *4S,6R,8S* and *4R,6S,8R* isomers.▲ (USP 1-Dec-2019)]
USP Galantamine Hydrobromide Related Compounds Mixture RS
▲Contains a mixture of the following 5 compounds:
Galantamine hydrobromide.
Galantamine *N*-oxide;
(4a*S*,6*R*,8a*S*)-6-Hydroxy-3-methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepine 11-oxide.
$C_{17}H_{21}NO_4$ 303.35
Dihydrogalantamine;

USP Monographs

www.webofpharma.com

(4a*S*,6*R*,8a*S*)-3-Methoxy-11-methyl-4a,5,7,8,9,10,11,12-octahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-ol.
$C_{17}H_{23}NO_3$ 289.37
6*S*-Galantamine;
(4a*S*,6*S*,8a*S*)-3-Methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-ol.
$C_{17}H_{21}NO_3$ 287.35
Anhydrogalantamine;
(4a*S*,8a*S*)-3-Methoxy-11-methyl-9,10,11,12-tetrahydro-4a*H*-benzo[2,3]benzofuro[4,3-*cd*]azepine.
$C_{17}H_{19}NO_2$ 269.34
[NOTE—The contents have previously been referred to as galantamine hydrobromide, 6β-hexahydrogalantamine, 6β-octahydrogalantamine, 6α-hexahydrogalantamine, and tetrahydrogalantamine, respectively.▲ (USP 1-Dec-2019)]

Gallamine Triethiodide



$C_{30}H_{60}I_3N_3O_3$ 891.53
Ethanaminium, 2,2′,2″-[1,2,3-benzenetriyltris(oxy)]tris[*N*,*N*,*N*-triethyl]-, triiodide.
[*v*-Phenenyltris(oxyethylene)]tris[triethylammonium] triiodide [65-29-2].

» Gallamine Triethiodide contains not less than 98.0 percent and not more than 101.0 percent of $C_{30}H_{60}I_3N_3O_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers, protected from light.
USP Reference standards ⟨11⟩—
USP Gallamine Triethiodide RS
Clarity and color of solution—A solution (1 in 50) is clear and colorless.

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: A solution (1 in 100) responds to the tests for *Iodide* ⟨191⟩.
pH ⟨791⟩: between 5.3 and 7.0, in a solution (1 in 50).
Loss on drying ⟨731⟩—Dry it at 100° for 4 hours: it loses not more than 1.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Assay—
Sodium perchlorate buffer—Prepare a 0.14 M solution of sodium perchlorate in water, and adjust with 10 N sodium hydroxide or 0.05 M phosphoric acid to a pH of 3.0.
Mobile phase—Prepare a filtered and degassed mixture of *Sodium perchlorate buffer* and acetonitrile (69:31). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Gallamine Triethiodide RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL.
Assay preparation—Transfer about 25 mg of Gallamine Triethiodide, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 200-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 1.4, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{30}H_{60}I_3N_3O_3$ in the portion of Gallamine Triethiodide taken by the formula:

$$25C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Gallamine Triethiodide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Gallamine Triethiodide Injection

» Gallamine Triethiodide Injection is a sterile solution of Gallamine Triethiodide in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of gallamine triethiodide ($C_{30}H_{60}I_3N_3O_3$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Gallamine Triethiodide RS
Identification—Evaporate a volume of Injection, equivalent to not less than 200 mg of gallamine triethiodide, to dryness. Take up the residue in warm alcohol, and pass through fine filter paper. Remove a portion of the filtrate, equivalent to about 100mg of gallamine triethiodide, and evaporate to dryness: the residue responds to the *Identification* tests under *Gallamine Triethiodide.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 5.0 USP Endotoxin Units per mg of gallamine triethiodide.
pH ⟨791⟩: between 6.5 and 7.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

www.webofpharma.com

Assay—

Sodium perchlorate buffer, Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay*— under *Gallamine Triethiodide.*

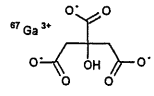
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of gallamine triethiodide, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{30}H_{60}I_3N_3O_3$ in the portion of Injection taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Gallamine Triethiodide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Gallium Citrate Ga 67 Injection



$C_6H_5{}^{67}GaO_7$

1,2,3-Propanetricarboxylic acid, 2-hydroxy-, gallium-^{67}Ga (1:1) salt.

Gallium-^{67}Ga citrate (1:1) [41183-64-6; 52260-70-5].

» Gallium Citrate Ga 67 Injection is a sterile aqueous solution of radioactive, essentially carrier-free gallium citrate Ga 67 suitable for intravenous administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{67}Ga as citrate expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 3.0 percent of the total radioactivity. It may contain a preservative or stabilizer.

Packaging and storage—Preserve in single-dose or multiple-dose containers.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{67}Ga as labeled gallium citrate expressed as total megabecquerels (MBq) (microcuries [µCi] or millicuries [mCi]) and concentration as megabecquerels (µCi or mCi) per mL at the time of calibration; the expiration date and time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calibrations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{67}Ga is 78.26 hours.

Bacterial Endotoxins Test ⟨85⟩—It meets the requirements of the *Bacterial Endotoxins Test,* the limit of endotoxin content being not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

pH ⟨791⟩: between 4.5 and 8.0.

Radiochemical purity—Place 10 to 20 µL of Injection about 3 cm from one end of a 3- × 55-cm strip of chromatographic paper (see *Chromatography* ⟨621⟩). While spots are wet, immediately develop the chromatogram at room temperature to the 14-cm mark by ascending chromatography, using a solvent system consisting of a mixture of 1.36 g of sodium acetate and 0.58 mL of glacial acetic acid in each 100 mL of water. Allow the strip to partially dry, cover with clear tape, and determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector: not less than 97.0% of the total radioactivity is found as gallium citrate when measured at the solvent front (R_F value equal to or greater than 0.9).

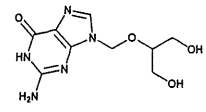
Radionuclide identification (see *Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of ^{67}Ga of known purity that exhibits major photopeaks having energies of 93.3, 184.6, and 300.2 KeV.

Radionuclidic purity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radionuclidic purity of the Injection: not less than 99% of the total radioactivity is present as ^{67}Ga at the time of calibration.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility,* the latter test being started on the day of manufacture, and except that it is not subject to the recommendation on *Container Content.*

Assay for radioactivity—Using a suitable counting assembly , determine the radioactivity in MBq (µCi or mCi) per mL of Gallium Ga 67 Injection by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

Ganciclovir



$C_9H_{13}N_5O_4$ 255.23

6*H*-Purin-6-one, 2-amino-1,9-dihydro-9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-;

9-[[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine [82410-32-0].

DEFINITION

Ganciclovir contains NLT 98.0% and NMT 102.0% of $C_9H_{13}N_5O_4$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 10 µg/mL in methanol

ASSAY

- **PROCEDURE**

Solution A: Trifluoroacetic acid and water (0.5 in 1000)

www.webofpharma.com

Mobile phase: Acetonitrile and *Solution A* (1:1)
System suitability solution: 0.1 mg/mL each of USP Ganciclovir RS and USP Ganciclovir Related Compound A RS in *Mobile phase*. [NOTE—Sonicate the solution if necessary.]
Standard solution: 0.22 mg/mL of USP Ganciclovir RS in *Mobile phase*
Sample solution: 0.22 mg/mL of Ganciclovir in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L9
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for ganciclovir related compound A and ganciclovir are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.4 between ganciclovir and ganciclovir related compound A
Column efficiency: NLT 5000 theoretical plates
Tailing factor: NMT 1.4
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_9H_{13}N_5O_4$ in the portion of Ganciclovir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ganciclovir RS in the *Standard solution* (mg/mL)
C_U = concentration of Ganciclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES
INORGANIC IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
ORGANIC IMPURITIES
• **Procedure**
Solution A, Mobile phase, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Sample solution: 0.22 mg/mL of Ganciclovir in *Mobile phase*
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Ganciclovir taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity in the *Sample solution*
r_T = sum of the responses of all the peaks

Acceptance criteria
Ganciclovir related compound A: NMT 0.5%
Total impurities: NMT 1.5%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 6.0%
[NOTE—Ganciclovir is extremely hygroscopic.]

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ganciclovir RS
USP Ganciclovir Related Compound A RS

Ganciclovir for Injection

DEFINITION
Ganciclovir for Injection is a freeze-dried powder prepared by the neutralization of Ganciclovir with the aid of Sodium Hydroxide. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ganciclovir ($C_9H_{13}N_5O_4$), calculated on the anhydrous basis.
[**CAUTION**—Handle Ganciclovir for Injection with great care because it is a potent cytotoxic agent and suspected carcinogen.]

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: 1.4 g/L of monobasic ammonium phosphate and 2.0 g/L of phosphoric acid in water
Internal standard solution: 0.15 mg/mL of hypoxanthine in water
Standard stock solution: 250 µg/mL of USP Ganciclovir RS in water
Standard solution: 0.05 mg/mL of USP Ganciclovir RS prepared as follows. Transfer 20.0 mL of the *Standard stock solution* and 10.0 mL of the *Internal standard solution* to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.
Sample stock solution: Nominally 1 mg/mL of ganciclovir, from Ganciclovir for Injection in water
Sample solution: Nominally 0.05 mg/mL of ganciclovir prepared as follows. Transfer 5.0 mL of *Sample stock solution* and 10.0 mL of *Internal standard solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for hypoxanthine and ganciclovir are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between hypoxanthine and ganciclovir
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the percentage of the labeled amount of ganciclovir ($C_9H_{13}N_5O_4$) in the portion of Ganciclovir for Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of ganciclovir to the internal standard from the *Sample solution*
R_S = peak response ratio of ganciclovir to the internal standard from the *Standard solution*
C_S = concentration of USP Ganciclovir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ganciclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% on the anhydrous basis

SPECIFIC TESTS

- **pH** ⟨791⟩: 10.8–11.4, in the solution constituted as directed in the labeling
- **Water Determination,** *Method I* ⟨921⟩
  Analysis: Proceed as directed in the chapter, except for the following modifications. Use a mixture of anhydrous formamide and methanol (1:1) instead of methanol as the titration vessel solvent. The reagent volume required to condition the titration vessel solvent is NMT 10% of the initial volume of solvent. The concentration of Ganciclovir for Injection in the titration vessel is NMT 7 mg/mL.
  Acceptance criteria: NMT 3.0%
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 0.84 Endotoxin unit/mg of Ganciclovir for Injection
- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements for small-volume injections
- **Sterility Tests** ⟨71⟩: Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **Injections and Implanted Drug Products** ⟨1⟩: At the time of use, it meets the requirements for *Constituted Solutions*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*. Store between 15° and 30°, unless otherwise specified by the manufacturer. Protect from moisture.
- **Labeling:** Label it to state that it is to be handled with great care because it is a potent cytotoxic agent and suspected carcinogen.
- **USP Reference Standards** ⟨11⟩
  USP Ganciclovir RS

Ganciclovir Compounded Oral Suspension

DEFINITION

Ganciclovir Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ganciclovir ($C_9H_{13}N_5O_4$).

Prepare Ganciclovir Compounded Oral Suspension 100 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Ganciclovir	10 g
Vehicle for Oral Solution (regular or sugar-free), *NF*, a sufficient quantity to make	100 mL

Add sufficient *Vehicle for Oral Solution* to wet the *Ganciclovir* powder, and triturate to form a smooth paste. Add additional *Vehicle for Oral Solution* to about half the final volume, and transfer the contents of the mortar to a calibrated bottle. Using additional *Vehicle for Oral Solution*, rinse the mortar, and transfer the contents, stepwise and quantitatively, to bring to final volume. Mix well.

[**Caution**—Avoid skin contact or inhalation of ganciclovir by using protective gloves and a fume hood or surgical mask.]

ASSAY

- **Procedure**

Solution A: 25-mM monobasic sodium phosphate solution. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: Acetonitrile and *Solution A* (2.5: 97.5). Filter and degas.
Internal standard solution: 0.4 mg/mL of hypoxanthine
Standard stock solution: 1.0 mg/mL of USP Ganciclovir RS
Standard solution: 6 µg/mL of ganciclovir and 4 µg/mL of hypoxanthine prepared from *Standard stock solution* and *Internal standard solution*
Sample solution: Transfer about 1 mL of Oral Suspension from each bottle to a plastic weighing cup, and weigh to determine density. [Note—The exact volume of Oral Suspension taken from each bottle is calculated by the suspension density.] Transfer the Oral Suspension to a 100-mL volumetric flask, and add 50 mL of water. Place the volumetric flask on a mechanical shaker for 30 min, and dilute with water to volume. Transfer 0.6 mL of this solution and 1 mL of the *Internal standard solution* to a 100-mL volumetric flask, and dilute with water to volume to obtain a solution with a nominal concentration of 6 µg/mL of ganciclovir and 4 µg/mL of hypoxanthine.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[Note—The relative retention times for hypoxanthine and ganciclovir are 0.75 and 1.0, respectively.]
Suitability requirements
Relative standard deviation: NMT 1.5% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ganciclovir ($C_9H_{13}N_5O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of ganciclovir to the internal standard from the *Sample solution*
R_S = peak response ratio of ganciclovir to the internal standard from the *Standard solution*
C_S = concentration of USP Ganciclovir RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ganciclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

USP Monographs

www.webofpharma.com

SPECIFIC TESTS
- **pH** ⟨791⟩: 4.0–5.0

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight, light-resistant containers. Store at controlled room temperature.
- **Beyond-Use Date:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature
- **Labeling:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date.*
- **USP Reference Standards** ⟨11⟩
  USP Ganciclovir RS

Absorbent Gauze

» Absorbent Gauze is cotton, or a mixture of cotton, and not more than 53.0 percent, by weight, of rayon, and is in the form of a plain woven cloth conforming to the standards set forth herein. Absorbent Gauze that has been rendered sterile is packaged to protect it from contamination.
[NOTE—Condition all Absorbent Gauze for not less than 4 hours in a standard atmosphere of 65 ± 2% relative humidity at 21 ± 1.1° before determining the weight, thread count, and absorbency. Remove the Absorbent Gauze from its wrappings before placing it in the conditioning atmosphere, and if it is in the form of bolts or rolls, cut the quantity necessary for the various tests from the piece, excluding the first two and the last two meters when the total quantity of Gauze available so permits.]

Packaging and storage—Preserve in well-closed containers. Absorbent Gauze that has been rendered sterile is so packaged that the sterility of the contents of the package is maintained until the package is opened for use.
Labeling—Its type or thread count, length, and width and the number of pieces contained are stated on the container, and the designation "non-sterilized" or "not sterilized" appears prominently thereon unless the Gauze has been rendered sterile, in which case it may be labeled to indicate that it is sterile. The package label of sterile Gauze indicates that the contents may not be sterile if the package bears evidence of damage or has been previously opened.
The name of the manufacturer, packer, or distributor is stated on the package.
General characteristics—Absorbent Gauze is white cloth of various thread counts and weights. It may be supplied in various lengths and widths, and in the form of rolls or folds.
The accompanying table designates for each commercial type the thread count and weight in g per square meter.

Type	Threads per 2.54 Cm		Average Count, Threads per 6.45 Sq. Cm	Weight,[1] g per Sq. Meter
	Warp	Filling		
I	41 to 47	33 to 39	76 to 84[2]	43.8 to 55.8
II	30 to 34	26 to 30	57 to 63	32.9 to 41.9
III	26 to 30	22 to 26	49 to 55	28.4 to 36.2
IV	22 to 26	18 to 22	41 to 47	24.5 to 31.1
V	20 to 24	16 to 20	37 to 43	22.5 to 28.8
VI	18 to 22	14 to 18	33 to 39	19.8 to 25.2
VII	18 to 22	8 to 14	27 to 35	18.1 to 23.1
VIII	12 to 16	8 to 12	21 to 27	12.1 to 15.5

[1] For Absorbent Gauze that contains purified rayon, increase these values by 2.5%.
[2] For Type I rolled gauze, the range is 75 to 85 threads per 6.45 sq. cm.

Thread count—If the dimensions of the piece permit, count the warp and filling threads of Absorbent Gauze in three separate 76.2-mm squares, not counting threads nearer any edge than one-tenth of the dimension of the fabric and not including the same threads in any two counts. For pieces not greater than 76.2 mm in either dimension, count all the threads in three different places in that dimension of the piece.
Average the three counts for the warp and filling, respectively: the average lies within the ranges tabulated under *General characteristics*.
For Absorbent Gauze packaged in rolls, count the number of warp and filling threads in areas of 1.27 cm square at five points evenly spread along the center line of the bandage, no point being within 30.5 cm of either end of the bandage.
Length—Unfold or unroll it, smooth it without stretching it, and measure its length along the center line: the length is not less than 98.0% of that stated on the label.
Width—Measure the width at each of the points selected for the *Thread:* the average of the three measurements is within 1.6 mm of the width stated on the label.
Weight—Weigh a piece of gauze of stated size: the weight, expressed in terms of g per m^2, meets the requirements for weight under *General characteristics*.
Absorbency—Fold about 0.1 m^2 into a 10-cm section. For Absorbent Gauze packaged in rolls, use the entire roll. Hold the folded or rolled Gauze horizontally almost in contact with the surface of water at approximately 25°, and allow it to drop lightly upon the water: complete submersion takes place in not more than 30 seconds.
Sterility Tests ⟨71⟩ —Absorbent Gauze that has been rendered sterile meets the requirements.
Dried and ignited residue, Acid or alkali, and Dextrin or starch, in water extract—Place 20 ± 0.1 g in 500 mL of water, and boil the mixture for 15 minutes, adding boiling water as necessary to maintain the original volume. Pour the water through a funnel into a 1000-mL volumetric flask, transfer the Absorbent Gauze to the funnel, press out the excess water with a glass rod, and wash it with two 250-mL portions of boiling water, pressing the gauze after each washing. Cool the combined washings, dilute to volume, and mix. Then apply the following tests.
Dried residue—Evaporate 400 mL of the extract, filtering if necessary, in a suitable dish on a steam bath, and dry the residue at 105° to constant weight: the weight of the residue so obtained does not exceed an amount, in mg, calculated by the formula:

$$80 - 0.6C$$

in which *C* is the corrected percentage of cotton (50 mg maximum, or 0.6%).
Ignited residue—Ignite the dried residue in a muffle furnace at a dull-red heat to constant weight: the weight of the ignited

USP Monographs

www.webofpharma.com

residue does not exceed an amount, in mg, calculated by the formula:

$$20 - 0.14C$$

in which *C* is the corrected percentage of cotton (13 mg maximum, or 0.16%).

Acid or alkali—To separate 200-mL portions of the extract, add 3 drops of phenolphthalein TS and 1 drop of methyl orange TS, respectively: no pink color develops in either portion.

Dextrin or starch—To a 200-mL portion of the extract add 1 drop of iodine TS: no red, violet, or blue color develops.

Residue on ignition—Place about 5 g, accurately weighed, in a suitable dish, and moisten with 2 N sulfuric acid. Gently heat the mixture until it is charred, then ignite more strongly until the carbon is completely consumed: the weight of the residue corresponds to not more than the percentage of the weight of the Gauze, calculated by the formula:

$$0.002C + 0.015(100 - C)$$

in which *C* is the corrected percentage of cotton (0.89% maximum).

Fatty matter—Pack 10 ± 0.01 g in a continuous-extraction thimble with a tared flask, and extract with ether for 5 hours, adjusting the rate so that the ether siphons not less than four times per hour. The ether extract in the flask shows no trace of blue, green, or brownish color. Evaporate the extract to dryness, and dry at 105° to constant weight: the weight of the residue does not exceed an amount, in mg, calculated by the formula:

$$0.4C + 30$$

in which *C* is the corrected percentage of cotton (70 mg maximum, or 0.7%).

Alcohol-soluble dyes—Pack 10 g in a narrow percolator, and extract slowly with alcohol until the percolate measures 50 mL: when observed downward in a column 20 cm in depth, the percolate may show a yellowish color, but neither a blue nor a green tint.

Cotton and rayon content—

Sulfuric acid solution (59.5% by weight)—Add sulfuric acid slowly to water until the specific gravity, determined at 20°, is between 1.4902 and 1.4956.

Procedure—Place about 500 mg of Absorbent Gauze, previously bleached and dried at 110° to constant weight and accurately weighed, in a glass-stoppered, 125-mL flask, add 50.0 mL of *Sulfuric acid solution*, and shake by mechanical means for 30 minutes. Pass the mixture through a tared sintered-glass crucible, using three 10-mL portions of *Sulfuric acid solution* to rinse the flask and applying suction each time to drain the acid. Wash the residue in the crucible with 50 mL of 2 N sulfuric acid, then wash it with water until the filtrate is neutral to litmus. Add 40 mL of 6 N ammonium hydroxide to the crucible, allow the residue to soak for 10 minutes, then apply suction to remove the liquid. Similarly wash the residue with three 50-mL portions of water, allowing the residue to soak for 15 minutes each time. Dry the residue at 105° to 110° to constant weight. Calculate *C*, the corrected percentage of cotton, taken by the formula:

$$[100(1.046I/G) - 1.6]$$

in which *I* is the weight, in mg, of the residue; *G* is the weight, in mg, of the portion of Absorbent Gauze taken; and 1.046 and 1.6 are empirical correction factors. Calculate *R*, the corrected percentage of rayon, taken by the formula:

$$100 - C.$$

Petrolatum Gauze

» Petrolatum Gauze is Absorbent Gauze saturated with White Petrolatum. The weight of the petrolatum in the gauze is not less than 70.0 percent and not more than 80.0 percent of the weight of petrolatum gauze. Petrolatum Gauze is sterile. It may be prepared by adding, under aseptic conditions, molten, sterile White Petrolatum to dry, sterile Absorbent Gauze, previously cut to size, in the ratio of 60 g of petrolatum to each 20 g of gauze.

Packaging and storage—Each Petrolatum Gauze unit is so packaged individually that the sterility of the unit is maintained until the package is opened for use.

Labeling—The package label bears a statement to the effect that the sterility of the Petrolatum Gauze cannot be guaranteed if the package bears evidence of damage or has been opened previously. The package label states the width, length, and type or thread count of the Gauze.

Sterility Tests ⟨71⟩: meets the requirements.

Other tests—The petrolatum recovered by draining in the *Assay* has the characteristics of and meets the requirements of the tests under *White Petrolatum*. The conditioned gauze obtained in the *Assay* meets the requirements of the tests for *Thread count—*, *Length—*, *Width—*, and *Weight—* under *Absorbent Gauze*.

Assay—Weigh not less than 20 units of Petrolatum Gauze, place them in a heated glass funnel, maintaining the temperature at approximately 75°, and allow the petrolatum to melt and drain from the funnel. Draining may be facilitated by pressing the gauze with a glass rod or porcelain spatula.

Wash the gauze on the funnel with successive portions of warm methyl chloroform until it is free from petrolatum, allow the residual methyl chloroform to evaporate spontaneously, condition the gauze in a standard atmosphere of 65 ± 2% relative humidity at 21 ± 1.1° for not less than 4 hours, and weigh. The difference between the weight of the gauze and that of the Petrolatum Gauze taken represents the weight of petrolatum.

Absorbable Gelatin Film

» Absorbable Gelatin Film is Gelatin in the form of a sterile, absorbable, water-insoluble film.

Packaging and storage—Preserve in a hermetically sealed or other suitable container in such manner that the sterility of the product is maintained until the container is opened for use.

Labeling—The package bears a statement to the effect that the sterility of Absorbable Gelatin Film cannot be guaranteed if the package bears evidence of damage, or if the package has been previously opened.

Sterility Tests ⟨71⟩ **:** meets the requirements.

Residue on ignition ⟨281⟩ **:** not more than 2.0%.

USP Monographs

www.webofpharma.com

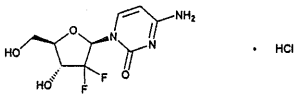
Proteolytic digest—Place 150 mg (±5 mg) in a glass-stoppered, 150-mL flask containing 100 mL of a 1 in 100 solution of pepsin in 0.1 N hydrochloric acid, previously warmed to 37°. Maintain at 37 ± 1°, and agitate gently every 30 minutes until digestion is complete: the average time of three proteolytic digest determinations is between 4 and 8 hours.

Absorbable Gelatin Sponge

» Absorbable Gelatin Sponge is Gelatin in the form of a sterile, absorbable, water-insoluble sponge.

Packaging and storage—Preserve in a hermetically sealed or other suitable container in such manner that the sterility of the product is maintained until the container is opened for use.
Labeling—The package bears a statement to the effect that the sterility of Absorbable Gelatin Sponge cannot be guaranteed if the package bears evidence of damage, or if the package has been previously opened.
Sterility Tests ⟨71⟩: meets the requirements.
Residue on ignition ⟨281⟩: not more than 2.0%.
Digestibility—Place a 50-mg piece in a beaker of water. Knead gently between the fingers until thoroughly wet, and until all the air has been removed, taking care not to break the tissue. Lift from the water, and remove the excess water with absorbent paper. Place the wetted sample in a 150-mL flask that contains 100 mL of a 1 in 100 solution of pepsin in 0.1 N hydrochloric acid previously warmed to 37°. Maintain at a temperature of 37°, and agitate gently and continuously until digestion is complete: the average digestion time of three determinations is not more than 75 minutes.
Water absorption—Cut a portion of about 10 mg from 1 Absorbable Gelatin Sponge, weigh accurately, and place in a beaker of water. Knead gently between the fingers until thoroughly wet, and until all air has been removed, taking care not to break the tissue. Lift the portion of sponge from the water, and blot twice by pressing firmly between two pieces of absorbent paper. Drop the expressed sponge into a tared weighing bottle containing about 20 mL of water, and allow to stand for 2 minutes. Lift the sponge from the water with a suitable hooked instrument, allow to drain over the weighing bottle for 5 seconds, and discard the sponge. Again weigh the weighing bottle and water: the loss in weight represents the weight of water absorbed by the sponge. Absorbable Gelatin Sponge absorbs not less than 35 times its weight of water.

Gemcitabine Hydrochloride



$C_9H_{11}F_2N_3O_4 \cdot HCl$ 299.66
Cytidine, 2′-deoxy-2′,2′-difluoro-, monohydrochloride; 2′-Deoxy-2′,2′-difluorocytidine monohydrochloride (β-isomer) [122111-03-9].

DEFINITION
Gemcitabine Hydrochloride contains NLT 97.5% and NMT 101.5% of gemcitabine hydrochloride ($C_9H_{11}F_2N_3O_4 \cdot HCl$), calculated on the as-is basis.

[**Caution**—Gemcitabine Hydrochloride is a potent cytotoxic agent. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020). Meets the requirements.
- **B. Identification Tests—General** ⟨191⟩, *Chemical Identification Tests, Chloride*: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**

Mobile phase: 13.8 g of monobasic sodium phosphate and 2.5 mL of *phosphoric acid* in 1 L of water. [Note—The pH of this solution is 2.4–2.6.]
System suitability solution: Transfer 10 mg of Gemcitabine Hydrochloride to a small vial, add 4 mL of 168 mg/mL of potassium hydroxide in methanol, cap tightly, and sonicate. Heat at 55° for 6–16 h, allow to cool, and transfer the contents to a 100-mL volumetric flask with successive washes of 1% *phosphoric acid*. Dilute with 1% (v/v) *phosphoric acid* to volume. [Note—This solution contains about 0.02 mg/mL of gemcitabine α-anomer.]
Standard solution: 0.1 mg/mL of USP Gemcitabine Hydrochloride RS in water
Sample solution: 0.1 mg/mL of Gemcitabine Hydrochloride in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 275 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for gemcitabine α-anomer and gemcitabine are about 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 8.0 between gemcitabine α-anomer and gemcitabine, *System suitability solution*
Tailing factor: NMT 1.5 for the gemcitabine peak, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of gemcitabine hydrochloride ($C_9H_{11}F_2N_3O_4 \cdot HCl$) in the portion of Gemcitabine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Gemcitabine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Gemcitabine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–101.5% on the as-is basis

USP Monographs

www.webofpharma.com

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

System suitability solution and **Chromatographic system:** Proceed as directed in the *Assay*.

Solution A: Use the *Mobile phase*, prepared as directed in the *Assay*.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	97	3
8	97	3
13	50	50
20	50	50
25	97	3

Standard solution: 0.002 mg/mL each of USP Gemcitabine Hydrochloride RS and USP Cytosine RS in water

Sample solution: 2 mg/mL of Gemcitabine Hydrochloride in water

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 8.0 between gemcitabine α-anomer and gemcitabine, *System suitability solution*

Tailing factor: NMT 1.5 for the gemcitabine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cytosine in the portion of Gemcitabine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of cytosine from the *Sample solution*

r_S = peak response of cytosine from the *Standard solution*

C_S = concentration of USP Cytosine RS in the *Standard solution* (mg/mL)

C_U = concentration of Gemcitabine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each impurity other than cytosine in the portion of Gemcitabine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of gemcitabine from the *Standard solution*

C_S = concentration of USP Gemcitabine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Gemcitabine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.02%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cytosine[a]	0.4	0.1
Gemcitabine α-anomer[b]	0.7	0.1
Gemcitabine	1.0	—
Any individual unspecified impurity	—	0.1
Total impurities	—	0.2

[a] 2(1*H*)-Pyrimidinone, 4-amino-.

[b] 4-Amino-1-(2-deoxy-2,2-difluoro-α-D-erythro-pentofuranosyl)-2(1*H*)-pyrimidinone.

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 10 mg/mL

Acceptance criteria: +43° to +50° at 20°

- **pH** ⟨791⟩

Sample solution: 10 mg/mL

Acceptance criteria: 2.0–3.0

- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Where the label states that Gemcitabine Hydrochloride is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Where the label states that Gemcitabine Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.05 USP Endotoxin Units/mg of gemcitabine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Cytosine RS

2(1*H*)-Pyrimidinone, 4-amino-.

$C_4H_5N_3O$ 111.10

USP Gemcitabine Hydrochloride RS

Gemcitabine for Injection

DEFINITION

Gemcitabine for Injection contains an amount of gemcitabine hydrochloride equivalent to NLT 95% and NMT 105% of the labeled amount of gemcitabine ($C_9H_{11}F_2N_3O_4$).

[**CAUTION**—Gemcitabine Hydrochloride is a potent cytotoxic agent. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

Medium: 0.14 M phosphate buffer with a pH of 2.5 prepared as follows. Add 13.8 g of monobasic sodium phosphate and 2.5 mL of phosphoric acid to 1 L of water.

Sample solution: 16 µg/mL of gemcitabine in *Medium*

www.webofpharma.com

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: 13.8 g of monobasic sodium phosphate and 2.5 mL of phosphoric acid in 1 L of water. [NOTE—The pH of this solution is 2.4–2.6.]

System suitability solution: Transfer 10 mg of gemcitabine hydrochloride to a small vial, add 4 mL of 168 mg/mL of potassium hydroxide in methanol, cap tightly, and sonicate. Heat at 55° for 6–16 h, allow to cool, and transfer the contents to a 100-mL volumetric flask with successive washes of 1% phosphoric acid. Dilute with 1% (v/v) phosphoric acid to volume. [NOTE—This solution contains about 0.02 mg/mL of gemcitabine α-anomer.]

Standard solution: 0.1 mg/mL of USP Gemcitabine Hydrochloride RS in water

Sample solution: Equivalent to 0.1 mg/mL of gemcitabine in water from Gemcitabine for Injection prepared as follows. Reconstitute a suitable number of vials with an appropriate amount of water, based on the labeled amount of gemcitabine.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for gemcitabine α-anomer and gemcitabine are about 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8.0 between gemcitabine α-anomer and gemcitabine, *System suitability solution*

Tailing factor: NMT 1.5 for the gemcitabine peak, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of gemcitabine ($C_9H_{11}F_2N_3O_4$) in the portion of Gemcitabine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Gemcitabine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of gemcitabine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of gemcitabine, 263.20

M_{r2} = molecular weight of gemcitabine hydrochloride, 299.66

Acceptance criteria: 95%–105%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS,** *Weight Variation* ⟨905⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

System suitability solution and **Chromatographic system:** Proceed as directed in the *Assay*.

Solution A: Use the *Mobile phase* as directed in the *Assay*.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	97	3
8	97	3
13	50	50
20	50	50
25	97	3

Standard solution: 2 µg/mL each of USP Gemcitabine Hydrochloride RS and USP Cytosine RS in water

Sample solution: Equivalent to 2 mg/mL of gemcitabine in water prepared by reconstituting the vial with an appropriate amount of water, based on the labeled amount of gemcitabine

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 8.0 between gemcitabine α-anomer and gemcitabine, *System suitability solution*

Tailing factor: NMT 1.5 for the gemcitabine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cytosine, expressed as a percentage of gemcitabine hydrochloride ($C_9H_{11}F_2N_3O_4 \cdot HCl$), in the portion of Gemcitabine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of cytosine from the *Sample solution*

r_S = peak response of cytosine from the *Standard solution*

C_S = concentration of USP Cytosine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of gemcitabine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of gemcitabine, 263.20

M_{r2} = molecular weight of gemcitabine hydrochloride, 299.66

Calculate the percentage of each impurity other than cytosine, expressed as a percentage of gemcitabine hydrochloride ($C_9H_{11}F_2N_3O_4 \cdot HCl$), in the portion of Gemcitabine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response of gemcitabine from the *Standard solution*

C_S = concentration of USP Gemcitabine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of gemcitabine in the *Sample solution* (mg/mL)

www.webofpharma.com

M_{r1} = molecular weight of gemcitabine, 263.20
M_{r2} = molecular weight of gemcitabine hydrochloride, 299.66

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.02%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cytosine[a]	0.4	0.1
Gemcitabine α-anomer[b]	0.7	0.1
Gemcitabine	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities	—	0.3

[a] 2(1*H*)-Pyrimidinone, 4-amino-.
[b] 2′-Deoxy-2′,2′-difluorocytidine (α-isomer).

SPECIFIC TESTS

- **PARTICULATE MATTER IN INJECTIONS ⟨788⟩:** It meets the requirements for small-volume injections.
- **pH ⟨791⟩**
  Sample solution: 40 mg/mL of gemcitabine in 0.9% sodium chloride solution
  Acceptance criteria: 2.7–3.3

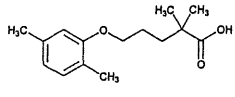
Change to read:

- **CLARITY OF SOLUTION**
  Sample solution: Dissolve it in the solvent and at the concentration recommended in the labeling.
  Analysis: Determine the turbidity by ratio turbidimetry within 15 min of reconstitution, corrected for a diluent blank (see ▲*Nephelometry and Turbidimetry* ⟨855⟩▲ (CN 1-May-2019)).
  Acceptance criteria: NMT 10 NTU
- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** It contains NMT 0.05 USP Endotoxin Unit/mg of gemcitabine.
- **STERILITY TESTS ⟨71⟩:** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements ⟨659⟩, Injection Packaging, Packaging for constitution.* Store at controlled room temperature. Do not refrigerate after reconstitution.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Cytosine RS
  2(1*H*)-Pyrimidinone, 4-amino-.
  $C_4H_5N_3O$ 111.10
  USP Gemcitabine Hydrochloride RS

Gemfibrozil



$C_{15}H_{22}O_3$ 250.33
Pentanoic acid, 5-(2,5-dimethylphenoxy)-2,2-dimethyl-; 2,2-Dimethyl-5-(2,5-xylyloxy)valeric acid [25812-30-0].

DEFINITION

Gemfibrozil contains NLT 98.0% and NMT 102.0% of gemfibrozil ($C_{15}H_{22}O_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: Add 10 mL of glacial acetic acid to 800 mL of methanol in a 1000-mL volumetric flask, dilute with water to volume, and pass through a membrane filter.
  System suitability solution: 0.2 mg/mL of gemfibrozil and 0.05 mg/mL of 2,5-dimethylphenol in *Mobile phase*
  Standard stock solution: 1 mg/mL of USP Gemfibrozil RS in methanol
  Standard solution: 0.2 mg/mL of USP Gemfibrozil RS in *Mobile phase* from the *Standard stock solution*
  Sample stock solution: 1 mg/mL of Gemfibrozil in methanol
  Sample solution: 0.2 mg/mL of Gemfibrozil in *Mobile phase* from the *Sample stock solution*
  Chromatographic system
  (See *Chromatography ⟨621⟩, System Suitability.*)
  Mode: LC
  Detector: UV 276 nm
  Column: 3.9-mm × 30-cm; packing L1
  Flow rate: 0.8 mL/min
  Injection volume: 10 µL
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Suitability requirements
  [NOTE—The elution order is 2,5-dimethylphenol, followed by gemfibrozil.]
  Resolution: NLT 8.0 between gemfibrozil and 2,5-dimethylphenol, *System suitability solution*
  Relative standard deviation: NMT 1.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of gemfibrozil ($C_{15}H_{22}O_3$) in the portion of Gemfibrozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Gemfibrozil RS in the *Standard solution* (mg/mL)
C_U = concentration of Gemfibrozil in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **ORGANIC IMPURITIES**
  Mobile phase: Add 10 mL of glacial acetic acid to 750 mL of methanol in a 1000-mL volumetric flask, dilute with water to volume, and pass through a membrane filter.
  Peak identification solution: 0.2 mg/mL of USP Gemfibrozil RS, 0.05 mg/mL of USP Gemfibrozil Related

www.webofpharma.com

Compound A RS, and 0.05 mg/mL of 2,5-dimethylphenol in *Mobile phase*
Standard stock solution: 0.1 mg/mL each of USP Gemfibrozil RS and USP Gemfibrozil Related Compound A RS in methanol
Standard solution: 0.01 mg/mL each of USP Gemfibrozil RS and USP Gemfibrozil Related Compound A RS in *Mobile phase* from the *Standard stock solution*
Sample solution: 10 mg/mL of Gemfibrozil in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 276 nm
Column: 4.0-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0% for each peak
Analysis
Chromatograph the *Peak identification solution*, and identify the components on the basis of their relative retention times. The relative retention times for 2,5-dimethylphenol, gemfibrozil, and gemfibrozil related compound A are 0.35, 1.0, and 2.1, respectively.
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of gemfibrozil related compound A in the portion of Gemfibrozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of gemfibrozil related compound A from the *Sample solution*
r_S = peak area of gemfibrozil related compound A from the *Standard solution*
C_S = concentration of USP Gemfibrozil Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Gemfibrozil in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Gemfibrozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*
r_S = peak area for gemfibrozil from the *Standard solution*
C_S = concentration of USP Gemfibrozil RS in the *Standard solution* (mg/mL)
C_U = concentration of Gemfibrozil in the *Sample solution* (mg/mL)

Acceptance criteria
Gemfibrozil related compound A: NMT 0.1%
Any other impurity: NMT 0.1%
Total impurities: NMT 0.5%

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.25%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Gemfibrozil RS
USP Gemfibrozil Related Compound A RS
(*E,Z*)-2,2-Dimethyl-5-[2,5-dimethyl-4-(propene-1-yl) phenoxy]valeric acid.
$C_{18}H_{26}O_3$ 290.40

Gemfibrozil Capsules

» Gemfibrozil Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of gemfibrozil ($C_{15}H_{22}O_3$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Gemfibrozil RS
Identification—Shake a portion of Capsule contents, equivalent to about 100 mg of gemfibrozil, with 10 mL of 0.1 N sodium hydroxide. Filter the mixture into a 50-mL centrifuge tube, and acidify the filtrate with 3 N sulfuric acid to obtain a copious precipitate. Centrifuge, and discard the clear solution. Wash the precipitate with small portions of water, and allow it to air-dry: the IR absorption spectrum of a potassium bromide dispersion of the precipitate, previously dried over silica gel for 4 hours, exhibits maxima only at the same wavelengths as those of a similar preparation of USP Gemfibrozil RS.

Dissolution ⟨711⟩—
Medium: 0.2 M pH 7.5 phosphate buffer prepared by dissolving 545 g of monobasic potassium phosphate in 5 L of water, adding 131 g of sodium hydroxide, diluting with water to about 19.5 L, and mixing well. Adjust with either 1 N phosphoric acid or 1 N sodium hydroxide to a pH of 7.5, and dilute with water to 20 L; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{15}H_{22}O_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm on filtered portions of the solution under test, suitably diluted with 1 N sodium hydroxide, in comparison with a Standard solution obtained as follows. Prepare a Standard stock solution of USP Gemfibrozil RS having a known concentration of about 0.33 mg per mL in *Medium*. [NOTE—Initially dissolve the USP Reference Standard in an amount of methanol not to exceed 1% of the volume of the Standard stock solution.] Quantitatively dilute the Standard stock solution with 1 N sodium hydroxide to obtain a Standard solution having a concentration estimated to correspond to that of the filtered and diluted solution under test.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{15}H_{22}O_3$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Assay—
Mobile phase, Standard preparation, and *System suitability preparation*—Proceed as directed in the *Assay* under *Gemfibrozil.*
Assay preparation—Remove, as completely as possible, the contents of not fewer than 20 Capsules, weigh, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of gemfibrozil, to a 100-mL volumetric flask, add about 80 mL of methanol, and shake to dissolve. Dilute with methanol to volume, mix, and filter. Transfer 5.0 mL of this clear solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

www.webofpharma.com

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Gemfibrozil*. Calculate the quantity, in mg, of $C_{15}H_{22}O_3$ in the portion of Capsules taken by the formula:

$$500C(r_U/r_S)$$

in which the terms are as defined therein.

Gemfibrozil Tablets

DEFINITION

Gemfibrozil Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of gemfibrozil ($C_{15}H_{22}O_3$).

IDENTIFICATION

- **A.**

Sample: 100 mg of gemfibrozil from a quantity of finely ground Tablets

Analysis: Shake the *Sample* with 10 mL of 0.1 N sodium hydroxide. Filter the mixture into a 50-mL centrifuge tube, and acidify the filtrate with 3 N sulfuric acid to obtain a copious precipitate. Centrifuge, and discard the clear solution. Wash the precipitate with small portions of water, and allow it to air-dry.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the precipitate, previously dried over silica gel for 4 h, exhibits maxima only at the same wavelengths as those of a similar preparation of USP Gemfibrozil RS.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Add 10 mL of glacial acetic acid to 800 mL of methanol in a 1000-mL volumetric flask, and dilute with water to volume.

System suitability solution: 0.2 mg/mL of USP Gemfibrozil RS and 0.05 mg/mL of 2,5-xylenol in *Mobile phase*

Standard stock solution: 1 mg/mL of USP Gemfibrozil RS in methanol

Standard solution: 0.2 mg/mL of USP Gemfibrozil RS from the *Standard stock solution* in *Mobile phase*

Sample stock solution: Nominally 1 mg/mL of gemfibrozil prepared as follows. Transfer the equivalent of 100 mg of gemfibrozil from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add about 80 mL of methanol and shake to dissolve. Dilute with methanol to volume and filter.

Sample solution: Nominally 0.2 mg/mL of gemfibrozil from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 0.8 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 8.0 between gemfibrozil and 2,5-xylenol, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of gemfibrozil ($C_{15}H_{22}O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Gemfibrozil RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of gemfibrozil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.2 M phosphate buffer prepared as follows. Dissolve 545 g of monobasic potassium phosphate in 5 L of water, add 131 g of sodium hydroxide, dilute with water to about 19.5 L, and mix well. Adjust with either 1 N phosphoric acid or 1 N sodium hydroxide to a pH of 7.5. Dilute with water to 20 L; 900 mL.

Apparatus 2: 50 rpm

Time: 30 min

Standard stock solution: A known concentration of USP Gemfibrozil RS in *Medium* prepared as follows. Dissolve USP Gemfibrozil RS in an amount of methanol not to exceed 1% of the total volume of the *Standard stock solution*. Dilute with *Medium* to volume.

Standard solution: A suitable concentration of USP Gemfibrozil RS from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with 1 N sodium hydroxide to a concentration similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 276 nm

Analysis: Calculate the percentage of the labeled amount of gemfibrozil ($C_{15}H_{22}O_3$) dissolved from UV absorbances of the *Sample solution* in comparison with those of the *Standard solution*.

Tolerances: NLT 80% (Q) of the labeled amount of gemfibrozil ($C_{15}H_{22}O_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, and **Standard stock solution:** Proceed as directed in the *Assay*.

Standard solution: 0.05 mg/mL of USP Gemfibrozil RS from the *Standard stock solution* in *Mobile phase*

Sensitivity solution: 0.005 mg/mL of USP Gemfibrozil RS from the *Standard solution* in *Mobile phase*

Sample solution: Nominally 10 mg/mL of gemfibrozil prepared as follows. Transfer 500 mg of gemfibrozil from NLT 20 finely powdered Tablets to a 50-mL volumetric flask, and add about 40 mL of *Mobile phase*. Sonicate and shake for 20 min. Dilute with *Mobile phase* to volume and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 276 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 3 times the retention time of gemfibrozil

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 8.0 between gemfibrozil and 2,5-xylenol, *System suitability solution*
Relative standard deviation: NMT 2.0% for the gemfibrozil peak, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of gemfibrozil from the *Standard solution*
C_S = concentration of USP Gemfibrozil RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of gemfibrozil in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: NMT 0.17%
Total impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Gemfibrozil RS

Gentamicin Uterine Infusion

» Gentamicin Uterine Infusion is a sterile solution of Gentamicin Sulfate in Water for Injection. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin. It may contain suitable buffers, preservatives, and sequestering agents.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
Labeling—Label Uterine Infusion to indicate that it is for veterinary use only. The label states that it must be diluted with 0.9% Sodium Chloride Irrigation before aseptic uterine infusion.
USP Reference standards ⟨11⟩—
USP Gentamicin Sulfate RS
Identification—It responds to the *Identification*— test under *Gentamicin Injection*, Uterine Infusion being used instead of Injection.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
pH ⟨791⟩: between 3.0 and 5.5.
Assay—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Uterine Infusion diluted quantitatively and stepwise with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (0.1 µg of gentamicin per mL).

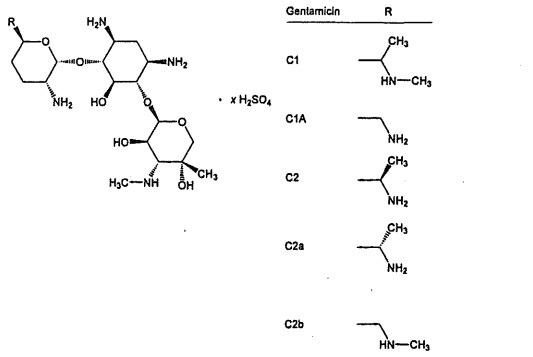
Gentamicin Injection

» Gentamicin Injection contains an amount of Gentamicin Sulfate equivalent to not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin. It may contain suitable buffers, preservatives, and sequestering agents, unless it is intended for intrathecal use, in which case it contains only suitable tonicity agents.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Gentamicin Sulfate RS
Identification—Apply separately a volume of Injection equivalent to 20 µg of gentamicin and the same volume of a similar preparation of USP Gentamicin Sulfate RS to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel having an average pore size of 6 nm. [NOTE—Dilute the Injection with water, if necessary, to obtain a test solution containing 1000 µg of gentamicin per mL. Where the Injection contains less than 1000 µg per mL, apply a volume of it, equivalent to 20 µg of gentamicin, to the chromatographic plate, in separate portions of not more than 20 µL each, each application being allowed to dry before the next is applied.] Place the plate in a suitable chromatographic chamber, and develop the chromatogram in a solvent system consisting of the lower phase of a mixture of chloroform, methanol, and ammonium hydroxide (20:13:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and expose the plate to vapors of iodine in a detection jar containing iodine crystals: the intensities and R_F values of the three principal spots obtained from the test solution correspond to those obtained from the Standard solution.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.71 USP Endotoxin Unit per mg of gentamicin.
pH ⟨791⟩: between 3.0 and 5.5.
Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Injection diluted quantitatively and stepwise with *Buffer B.3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (0.1 µg of gentamicin per mL).

www.webofpharma.com

Gentamicin Sulfate



Gentamicin sulfate (salt);
Gentamycin sulfate [1405-41-0].

DEFINITION

Gentamicin Sulfate is the sulfate salt, or a mixture of such salts, of the antibiotic substances produced by the growth of *Micromonospora purpurea*. It has a potency equivalent to NLT 590 µg/mg of gentamicin, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. Identification Tests—General,** *Sulfate* ⟨191⟩

ASSAY

- **Procedure**

Analysis: Proceed as directed for Gentamicin under *Antibiotics—Microbial Assays* ⟨81⟩.
Acceptance criteria: NLT 590 µg/mg of gentamicin, calculated on the dried basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 1.0%
- **Limit of Methanol** (if present)

Internal standard solution: 0.50% v/v of *n*-propyl alcohol
Standard solution: 0.25% v/v each of methanol and *n*-propyl alcohol
Control solution: 250 mg/mL of Gentamicin Sulfate
Sample solution: 250 mg/mL of Gentamicin Sulfate in a mixture of *Internal standard solution* and water (1:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 4-mm × 1.5-m; packed with support S3
Temperature
Column: Constant temperature between 120° and 140°
Injector: Constant temperature at least 50° higher than the column temperature
Detector: Constant temperature at least 50° higher than the column temperature
Carrier gas: Nitrogen
Flow rate: Constant flow rate between 30 and 40 mL/min
Injection type: Syringe with a polytef-tipped plunger
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between *n*-propyl alcohol and methanol

Analysis
Samples: *Standard solution*, *Sample solution*, and *Control solution*
Chromatograph the *Control solution*, and examine the chromatogram: if any peak is observed at a retention time corresponding to that of *n*-propyl alcohol, use the response of that peak to correct the *n*-propyl alcohol peak response of the *Sample solution*.
Calculate the percentage of methanol in the Gentamicin Sulfate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times D \times F$$

R_U = peak area response of methanol to *n*-propyl alcohol (corrected for any peak at the locus of the *n*-propyl alcohol peak in the *Control solution*) from the *Sample solution*
R_S = peak area response of methanol to *n*-propyl alcohol from the *Standard solution*
C_S = percentage of methanol in the *Standard solution*
C_U = concentration of Gentamicin Sulfate in the *Sample solution* (mg/mL)
D = density of methanol (g/mL)
F = conversion factor, 1000 mg/g

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

- **Content of Gentamicins**

Mobile phase: To 900 mL of carbonate-free water, add 7 mL of trifluoroacetic acid, 250 µL of pentafluoropropanoic acid, and 4 mL of 12.5 M sodium hydroxide (carbonate-free). Allow to equilibrate, and adjust with 0.5 M sodium hydroxide (carbonate-free) to a pH of 2.6. Add 15 mL of acetonitrile, and dilute with carbonate-free water to 1 L. If necessary, adjust the volume of acetonitrile in the *Mobile phase*. A total volume of up to 50 mL can be added per L of *Mobile phase*.
System suitability solution: 100 µg/mL of USP Gentamicin Sulfate RS and 20 µg/mL of USP Sisomicin Sulfate RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Gentamicin Sulfate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Pulsed amperometric electrochemical detector
Indicator electrode: Gold
Reference electrode: Silver–silver chloride
Auxiliary electrode: Stainless steel. [NOTE—If the cell body is made of stainless steel, it can be used as the auxiliary electrode.]
Waveform: See *Table 1*.

Table 1

Time (s)	Potential (V)	Integration
0.00	+0.05	—
0.10	+0.05	Begin
0.40	+0.05	End
0.41	+0.75	—
0.55	+0.75	—
0.56	−0.15	—
1.00	−0.15	—

Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°

www.webofpharma.com

Flow rate: 1 mL/min
Post-column reagent: 20 g/L sodium hydroxide (carbonate-free), degassed and introduced pulselessly using a 375-µL polymeric mixing coil. [NOTE—A suitable mixing coil is the knitted reaction coil, part number 043700, available from Dionex Corporation (www.dionex.com).]
Flow rate of post-column reagent: 0.3 mL/min
Injection volume: 20 µL
Run time: 1.2 times the retention time of gentamicin C_1

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between gentamicin C_2 and gentamicin C_{2b}

Analysis
Sample: *Sample solution*
Calculate the percentage of each gentamicin in the portion of Gentamicin Sulfate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area response corresponding to the particular gentamicin
r_T = sum of the area responses of the gentamicin C_{1a}, gentamicin C_2, gentamicin C_{2a}, gentamicin C_{2b}, and gentamicin C_1 peaks

Acceptance criteria: Identify peaks by the relative retention times in *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Garamine[a, b]	0.35	—
Sisomicin[a, c]	1.0	—
Gentamicin C_{1a}	1.1	10%–35%
Gentamicin C_2	1.8	25%–55%[d]
Gentamicin C_{2a}	2.3	
Gentamicin C_{2b}	2.0	25%–50%[e]
Gentamicin C_1	2.9	

[a] These compounds are listed for information only and are not to be reported in this test.
[b] 4-*O*-[3-Deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-2-deoxy-L-streptamine.
[c] *O*-3-Deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl-(1→4)-*O*-[2,6-diamino-2,3,4,6-tetradeoxy-α-D-glycero-hex-4-enopyranosyl-(1→6)]-2-deoxy-D-streptamine.
[d] The limit is for the sum of gentamicin C_2 and gentamicin C_{2a}.
[e] The limit is for the sum of gentamicin C_{2b} and gentamicin C_1.

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL
  Acceptance criteria: +107° to +121°
- **PH** ⟨791⟩
  Sample solution: 40 mg/mL
  Acceptance criteria: 3.5–5.5
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry it in vacuum at a pressure NMT 5 mm of mercury at 110° for 3 h.
  Acceptance criteria: NMT 18.0%
- **STERILITY TESTS** ⟨71⟩**:** Where the label states that Gentamicin Sulfate is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Where the label states that Gentamicin Sulfate is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.71 USP Endotoxin Unit/mg of gentamicin.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Gentamicin Sulfate RS
  USP Sisomicin Sulfate RS

Gentamicin Sulfate Cream

» Gentamicin Sulfate Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of gentamicin.

Packaging and storage—Preserve in collapsible tubes or in other tight containers, and avoid exposure to excessive heat.
USP Reference standards ⟨11⟩—
USP Gentamicin Sulfate RS
Identification—Shake a quantity of Cream, equivalent to about 5 mg of gentamicin, with a mixture of 200 mL of chloroform and 5 mL of water. Allow to separate, and filter the aqueous phase: the filtrate so obtained meets the requirements of the *Identification*— test under *Gentamicin Injection*.
Minimum fill ⟨755⟩: meets the requirements.
Assay—Proceed with Cream as directed in the *Assay*— under *Gentamicin Sulfate Ointment*.

Gentamicin Sulfate Ointment

» Gentamicin Sulfate Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of gentamicin.

Packaging and storage—Preserve in collapsible tubes or other tight containers, and avoid exposure to excessive heat.
USP Reference standards ⟨11⟩—
USP Gentamicin Sulfate RS
Identification—Shake a quantity of Ointment, equivalent to about 5 mg of gentamicin, with a mixture of 200 mL of chloroform and 5 mL of water. Allow to separate, and filter the aqueous layer: the filtrate so obtained meets the requirements of the *Identification*— test under *Gentamicin Injection*.
Minimum fill ⟨755⟩: meets the requirements.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.
Assay—Proceed with Ointment as directed under *Antibiotics —Microbial Assays* ⟨81⟩, using an accurately weighed quantity of Ointment, equivalent to about 1 mg of gentamicin, shaken with about 50 mL of ether in a separator, and extracted with

www.webofpharma.com

four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Gentamicin Sulfate Ophthalmic Ointment

DEFINITION

Gentamicin Sulfate Ophthalmic Ointment contains the equivalent of NLT 90.0% and NMT 135.0% of the labeled amount of gentamicin.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 1 mg/mL of USP Gentamicin Sulfate RS in water

Sample solution: Nominally 1 mg/mL of gentamicin from Ophthalmic Ointment prepared as follows. Shake a quantity of Ophthalmic Ointment, containing nominally 5 mg of gentamicin, with a mixture of 200 mL of chloroform and 5 mL of water. Allow to separate, and filter the aqueous layer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 20 µL

Developing solvent system: Mix chloroform, methanol, and ammonium hydroxide (20:13:10). Allow to separate, and use the lower layer.

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* to the plate. Place the plate in a chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate, air-dry, and expose it to vapors of iodine in a detection jar containing iodine crystals.

Acceptance criteria: The intensities and R_F values of the three principal spots of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY

• **PROCEDURE**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution: Shake a portion of Ophthalmic Ointment containing nominally 1 mg of gentamicin with about 50 mL of ether in a separator, and extract with four 20-mL portions of *Buffer B.3* (see the chapter). Combine the buffer extracts, and dilute with *Buffer B.3* to a suitable volume to obtain a *Test Dilution* with a gentamicin concentration that is nominally equivalent to the median level of the standard.

Analysis: Proceed as directed in the chapter.

Acceptance criteria: 90.0%–135.0%

SPECIFIC TESTS

• **STERILITY TESTS** ⟨71⟩**:** Meets the requirements

• **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Gentamicin Sulfate RS

Gentamicin Sulfate Ophthalmic Solution

» Gentamicin Sulfate Ophthalmic Solution is a sterile, buffered solution of Gentamicin Sulfate with preservatives. It contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of gentamicin.

Packaging and storage—Preserve in tight containers, and avoid exposure to excessive heat.

USP Reference standards ⟨11⟩—

USP Gentamicin Sulfate RS

pH ⟨791⟩: between 6.5 and 7.5.

Other requirements—It meets the requirements of the *Identification*— test under *Gentamicin Injection* and meets the requirements under *Sterility Tests* ⟨71⟩, when tested as directed in the section *Membrane Filtration* in *Test for Sterility of the Product To Be Examined*.

Assay—Proceed with Ophthalmic Solution as directed in the *Assay*— under *Gentamicin Injection*.

Gentamicin Sulfate and Betamethasone Acetate Ophthalmic Solution

» Gentamicin Sulfate and Betamethasone Acetate Ophthalmic Solution contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin and contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone acetate ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—

USP Betamethasone Acetate RS

USP Gentamicin Sulfate RS

Identification—

A: Apply 10 µL of Ophthalmic Solution and 10 µL of a Standard solution containing 5 mg per mL of USP Gentamicin Sulfate RS in water to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and in a paper-lined tank develop the chromatogram in a solvent system consisting of the lower phase mixture of dichloromethane, methanol, and ammonium hydroxide (1:1:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Locate the spots on the plate by placing it in a tank containing about 15 g of iodine crystals for 15 minutes: the R_F values of the three principal spots obtained from the test solution correspond to those obtained from the Standard solution.

www.webofpharma.com

B: The retention time of the major peak obtained in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay for betamethasone acetate*.
pH ⟨791⟩: between 5.5 and 7.0.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* in *Test for Sterility of the Product To Be Examined*.
Other requirements—It meets the requirements under *Antimicrobial Effectiveness Tests* ⟨51⟩.
Assay for gentamicin—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for betamethasone acetate—
Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (8:7). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Dissolve a quantity of *o*-phenylphenol in methanol to obtain a solution containing about 0.55 mg per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Betamethasone Acetate RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.45 mg per mL. Transfer 2.0 mL of this solution to a 10-mL volumetric flask, add 1.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix to obtain a solution having a known concentration of about 0.09 mg of USP Betamethasone Acetate RS per mL.
Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 2 mg of betamethasone acetate, to a 10-mL volumetric flask. Dilute with methanol to volume, and mix. Transfer a portion of this solution to a centrifuge tube, and centrifuge. Transfer 4.0 mL of the clear supernatant to a 10-mL volumetric flask. Add 1.0 mL of *Internal standard solution*, dilute with a mixture of methanol and water (1:1) to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-mm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*:the relative retention times are about 1.3 for *o*-phenylphenol and 1.0 for betamethasone acetate; the resolution, *R*, between the betamethasone acetate and *o*-phenylphenol peaks is not less than 3.9; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betamethasone acetate ($C_{24}H_{31}FO_6$) in each mL of the Ophthalmic Solution taken by the formula:

$$25(C/V)(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Betamethasone Acetate RS, calculated on the anhydrous basis, in the *Standard preparation*; *V* is the volume, in mL, of Ophthalmic Solution taken to prepare the *Assay preparation*; and R_U and R_S are the ratios of the betamethasone acetate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Gentamicin Sulfate and Betamethasone Valerate Ointment

» Gentamicin Sulfate and Betamethasone Valerate Ointment contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin and an amount of betamethasone valerate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$).

Packaging and storage—Preserve in collapsible tubes or other tight containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Betamethasone Valerate RS
USP Beclomethasone Dipropionate RS
USP Gentamicin Sulfate RS

Identification—
A: Transfer an amount of Ointment, equivalent to about 15 mg of gentamicin, to a centrifuge tube, and add 10 mL of a mixture of methanol and 0.1 N hydrochloric acid (4:1) and 25 mL of solvent hexane. Rotate for 30 minutes, and centrifuge. Discard the upper phase. Apply 25 µL of the lower phase and 25 µL of a Standard solution containing 3 mg per mL of USP Gentamicin Sulfate RS in a mixture of methanol and 0.1 N hydrochloric acid (4:1) to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of the lower phase of a mixture of chloroform, methanol, and ammonium hydroxide (1:1:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the spots to air-dry. Locate the spots on the plate by placing it in a tank containing about 15 g of iodine crystals for 15 minutes: the R_F values of the three principal spots obtained from the test solution correspond to those obtained from the Standard solution.
B: The retention time of the major peak obtained in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay for betamethasone*.
Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* species, and *Escherichia coli*.
Minimum fill ⟨755⟩: meets the requirements.
Assay for gentamicin—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed quantity of Ointment, equivalent to about 3 mg of gentamicin, shaken with about 50 mL of ether in a separator and extracted with three 25-mL portions of *Buffer B. 3*. Combine the aqueous extracts, and dilute quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for betamethasone—
Mobile phase—Prepare a filtered and degassed mixture of methanol and water (475:300). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluent—Transfer 25 mL of water to a 500-mL volumetric flask. Add 2.5 mL of glacial acetic acid, dilute with methanol to volume, and mix.

www.webofpharma.com

Internal standard solution—Dissolve a quantity of USP Beclomethasone Dipropionate RS in *Diluent* to obtain a solution containing about 0.4 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Betamethasone Valerate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.45 mg per mL. Transfer 5.0 mL of this solution to a stoppered vial, add 10.0 mL of *Internal standard solution*, and mix to obtain a solution having a known concentration of about 0.15 mg of USP Betamethasone Valerate RS per mL.

Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 2 mg of beta methasone, to a 50-mL centrifuge tube. Add 10.0 mL of *Internal standard solution* and 5.0 mL of *Diluent*, and shake vigorously for 10 minutes. Place the tube in an ice–methanol bath for 15 minutes, then centrifuge to separate the phases. Transfer the clear supernatant to a stoppered flask, and allow to warm to room temperature (*Assay preparation*).

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 2.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*:the relative retention times are about 1.5 for beclomethasone dipropionate and 1.0 for betamethasone valerate; the resolution, *R*, between the betamethasone valerate and beclomethasone dipropionate peaks is not less than 3.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Ointment taken by the formula:

$$(392.47/476.58)(15C)(R_U/R_S)$$

in which 392.47 and 476.58 are the molecular weights of betamethasone and betamethasone valerate, respectively; *C* is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation;* and R_U and R_S are the ratios of the betamethasone valerate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Gentamicin Sulfate and Betamethasone Valerate Otic Solution

» Gentamicin Sulfate and Betamethasone Valerate Otic Solution contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin and an amount of beta methasone valerate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$).

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—
USP Beclomethasone Dipropionate RS
USP Betamethasone Valerate RS
USP Gentamicin Sulfate RS

Identification—

A: Transfer an amount of Otic Solution, equivalent to about 3 mg of gentamicin, to a centrifuge tube. Dissolve an accurately weighed quantity of USP Gentamicin Sulfate RS quantitatively in water to obtain a solution having a concentration of about 5 mg per mL. Transfer 1.0 mL of this solution to a centrifuge tube. To each centrifuge tube add 3 mL of water and 4 g of potassium carbonate, and mix. Add 1 mL of isopropyl alcohol to each tube, mix, and centrifuge. Use the upper phases as the test solution and Standard solution, respectively. Separately apply 20 µL of each of these solutions to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of the lower phase of a mixture of methanol, dichloromethane, and ammonium hydroxide (1:1:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Locate the spots on the plate by placing it in a tank containing about 15 g of iodine crystals for 15 minutes: the R_F values of the three principal spots obtained from the test solution correspond to those obtained from the Standard solution.

B: The retention time of the major peak obtained in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation,* both relative to the internal standard, as obtained in the *Assay for betamethasone.*

pH ⟨791⟩: between 3.0 and 5.0.

Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* species, and *Escherichia coli.*

Assay for gentamicin—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Otic Solution diluted quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for betamethasone—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of methanol and glacial acetic acid (1000:1).

Internal standard solution—Dissolve a quantity of USP Beclomethasone Dipropionate RS in methanol to obtain a solution containing about 0.8 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Betamethasone Valerate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 1.2 mg per mL. Transfer 2.0 mL of this solution to a 10-mL volumetric flask, add 5.0 mL of *Internal standard solution,* dilute with *Diluent* to volume, and mix to obtain a solution having a known concentration of about 0.24 mg of USP Betamethasone Valerate RS per mL.

Assay preparation—Add 5.0 mL of *Internal standard solution* to a 10-mL volumetric flask. Transfer to the flask an accurately measured volume of Otic Solution, equivalent to about 2 mg of betamethasone, dilute with methanol to volume, and mix. Centrifuge a portion of this solution, and use the clear supernatant as the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed under *Procedure*:the relative retention times are about

www.webofpharma.com

1.6 for beclomethasone dipropionate and 1.0 for betamethasone valerate; the resolution, *R*, between the betamethasone valerate and beclomethasone dipropionate peaks is not less than 3.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betamethasone ($C_{22}H_{29}FO_5$) in each mL of the Otic Solution taken by the formula:

$$(392.47/476.58)(10C/V)(R_U/R_S)$$

in which 392.47 and 476.58 are the molecular weights of betamethasone and betamethasone valerate, respectively; *C* is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation*; *V* is the volume, in mL, of Otic Solution taken to prepare the *Assay preparation;* and R_U and R_S are the ratios of the betamethasone valerate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Gentamicin Sulfate and Betamethasone Valerate Topical Solution

» Gentamicin Sulfate and Betamethasone Valerate Topical Solution contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of gentamicin and an amount of betamethasone valerate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$).

Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Beclomethasone Dipropionate RS
USP Betamethasone Valerate RS
USP Gentamicin Sulfate RS

Identification—

A: Separately apply 50 µL of Topical Solution and 50 µL of a Standard solution containing 1.2 mg per mL of USP Gentamicin Sulfate RS in water to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of the lower phase of a mixture of methanol, chloroform, and ammonium hydroxide (1:1:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Locate the spots on the plate by placing it in a tank containing about 15 g of iodine crystals for 15 minutes: the R_F values of the three principal spots obtained from the test solution correspond to those obtained from the Standard solution.

B: The retention time of the major peak obtained in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay for betamethasone.*

pH ⟨791⟩: between 3.0 and 4.5.

Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* species, and *Escherichia coli.*

Assay for gentamicin—Proceed as directed for gentamicin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Topical Solution diluted quantitatively and stepwise with *Buffer B.3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for betamethasone—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of methanol and glacial acetic acid (1000:1).

Internal standard solution—Dissolve a quantity of USP Beclomethasone Dipropionate RS in methanol to obtain a solution containing about 0.6 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Betamethasone Valerate RS in *Diluent* and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.35 mg per mL. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with a mixture of methanol and water (4:1) to volume, and mix to obtain a solution having a known concentration of about 0.07 mg of USP Betamethasone Valerate RS per mL.

Assay preparation—Add 5.0 mL of *Internal standard solution* to a 25-mL volumetric flask. Transfer to the flask an accurately measured volume of Topical Solution, equivalent to about 1.4 mg of betamethasone, dilute with a mixture of methanol and water (4:1) to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:*the relative retention times are about 1.6 for beclomethasone dipropionate and 1.0 for betamethasone valerate; the resolution, *R*, between the betamethasone valerate and beclomethasone dipropionate peaks is not less than 4.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betamethasone ($C_{22}H_{29}FO_5$) in each mL of the Topical Solution taken by the formula:

$$(392.47/476.58)(25C/V)(R_U/R_S)$$

in which 392.47 and 476.58 are the molecular weights of betamethasone and betamethasone valerate, respectively; *C* is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation;* *V* is the volume, in mL, of Topical Solution taken to prepare the *Assay preparation;* and R_U and R_S are the ratios of the betamethasone valerate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

www.webofpharma.com

Gentamicin and Prednisolone Acetate Ophthalmic Ointment

DEFINITION

Gentamicin and Prednisolone Acetate Ophthalmic Ointment contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of gentamicin, and NLT 90.0% and NMT 110.0% of the labeled amount of prednisolone acetate ($C_{23}H_{30}O_6$).

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution: 1 mg/mL of USP Gentamicin Sulfate RS in water

Sample solution: Nominally 1 mg/mL of gentamicin from Ophthalmic Ointment prepared as follows. Shake a quantity of Ophthalmic Ointment, containing nominally 5 mg of gentamicin, with a mixture of 200 mL of chloroform and 5 mL of water. Allow to separate, and filter the aqueous layer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 20 µL

Developing solvent system: Mix chloroform, methanol, and ammonium hydroxide (20:13:10), allow to separate, and use the lower layer.

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Standard solution* and *Sample solution* to the plate. Place the plate in a chromatographic chamber, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and expose the plate to vapors of iodine in a detection jar containing iodine crystals.

Acceptance criteria: The intensities and R_F values of the three principal spots from the *Sample solution* correspond to those from the *Standard solution.*

• **B.** The retention time of the prednisolone acetate peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay* for *Prednisolone Acetate.*

ASSAY

• **Gentamicin**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Sample solution: Shake a portion of Ophthalmic Ointment containing nominally 1 mg of gentamicin with about 50 mL of ether in a separator, and extract with four 20-mL portions of *Buffer B.3* (see the chapter). Combine the buffer extracts, and dilute with *Buffer B.3* to a suitable volume to obtain a *Test Dilution* having a gentamicin concentration that is nominally equivalent to the median level of the standard.

Analysis: Proceed as directed in the chapter.

Acceptance criteria: 90.0%–120.0%

• **Prednisolone Acetate**

Mobile phase: Acetonitrile and water (40:60)

Internal standard solution: 2.7 mg/mL of fluorometholone acetate in methanol

Standard stock solution: 0.38 mg/mL of USP Prednisolone Acetate RS in methanol

Standard solution: 0.06 mg/mL of USP Prednisolone Acetate RS in methanol prepared as follows. Transfer 8.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add 25 mL of *n*-hexane, and shake. Add 2.0 mL of *Internal standard solution,* dilute with methanol to volume, and shake vigorously for 30 s. Allow the layers to separate, remove the upper *n*-hexane layer by aspiration, and discard the aspirate. Dilute the solution in the volumetric flask with methanol to volume. Centrifuge a portion of this solution, and use the clear supernatant.

Sample solution: Nominally 0.06 mg/mL of prednisolone acetate from Ophthalmic Ointment in methanol prepared as follows. Transfer a portion of Ophthalmic Ointment, containing nominally 3 mg of prednisolone acetate, to a 50-mL volumetric flask, add 25 mL of *n*-hexane, and shake. Add 2.0 mL of *Internal standard solution,* dilute with methanol to volume, and shake vigorously for 30 s. Allow the layers to separate, remove the upper *n*-hexane layer by aspiration, and discard the aspirate. Dilute the solution in the volumetric flask with methanol to volume. Centrifuge a portion of this solution, and use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 30 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the prednisolone acetate and fluorometholone acetate peaks

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of prednisolone acetate ($C_{23}H_{30}O_6$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area response ratio of prednisolone acetate to fluorometholone acetate from the *Sample solution*

R_S = peak area response ratio of prednisolone acetate to fluorometholone acetate from the *Standard solution*

C_S = concentration of USP Prednisolone Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of prednisolone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **Sterility Tests** ⟨71⟩**:** Meets the requirements

• **Other Requirements:** It meets the requirements for *Particulate and Foreign Matter* and *Container Contents* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter* and *Container Contents.*

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩

USP Gentamicin Sulfate RS

USP Prednisolone Acetate RS

www.webofpharma.com

Gentamicin and Prednisolone Acetate Ophthalmic Suspension

» Gentamicin and Prednisolone Acetate Ophthalmic Suspension is a sterile aqueous suspension containing Gentamicin Sulfate and Prednisolone Acetate. It contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amount of gentamicin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone acetate ($C_{23}H_{30}O_6$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Gentamicin Sulfate RS
USP Prednisolone Acetate RS

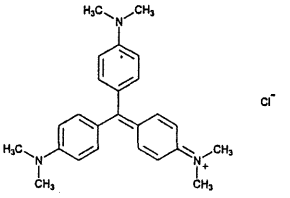
Identification—
A: It meets the requirements of the *Identification* test in *Gentamicin Injection.*
B: The retention time of the prednisolone acetate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for prednisolone acetate.*
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* in *Test for Sterility of the Product To Be Examined.*
pH ⟨791⟩: between 5.4 and 6.6.
Assay for gentamicin—Proceed with Ophthalmic Suspension as directed in the *Assay* in *Gentamicin Sulfate Injection.*

Assay for prednisolone acetate—
Diluting solvent—Mix 700 mL of methanol and 300 mL of water.
Mobile phase—Prepare a suitable mixture of water and acetonitrile (60:40), and pass through a suitable filter having a porosity of 1 µm or less. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer about 60 mg of USP Prednisolone Acetate RS, accurately weighed, to a 50-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 8.0 mL of this solution to a second 50-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix. This solution contains about 0.2 mg of USP Prednisolone Acetate RS per mL.
Assay preparation—Transfer an accurately measured volume of well-mixed Ophthalmic Suspension, equivalent to about 10 mg of prednisolone acetate, to a 50-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and measure the peak responses as directed under *Procedure:* the tailing factor for the analyte peak is not more than 1.25, the column efficiency is not less than 2000 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 30 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of prednisolone acetate ($C_{23}H_{30}O_6$), in each mL of the Ophthalmic Suspension taken by the formula:

$$50(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Prednisolone Acetate RS in the *Standard preparation;* V is the volume, in mL, of Ophthalmic Suspension taken; and r_U and r_S are the prednisolone acetate peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Gentian Violet



$C_{25}H_{30}ClN_3$ 407.98
Methanaminium, *N*-[4-[bis[4-(dimethylamino)phenyl] methylene]-2,5-cyclohexadien-1-ylidene]-*N*-methyl-, chloride;
C. I. Basic violet 3;
[4-[Bis[*p*-(dimethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]dimethylammonium chloride [548-62-9].

DEFINITION
Gentian Violet contains NLT 96.0% and NMT 100.5% of gentian violet ($C_{25}H_{30}ClN_3$), calculated on the anhydrous basis.

IDENTIFICATION
• **A.**
Sample: 1 mg
Analysis: Sprinkle the *Sample* on 1 mL of sulfuric acid.
Acceptance criteria: The *Sample* dissolves in the acid with an orange or brown-red color. When this solution is diluted cautiously with water, the color changes to brown, then to green, and finally to blue.
• **B.**
Sample: 20 mg
Analysis: Dissolve the *Sample* in 10 mL of water, and add 5 drops of hydrochloric acid. To 5 mL of this solution add tannic acid TS dropwise.
Acceptance criteria: A deep blue precipitate is formed.
• **C.**
Sample: The remainder of the solution prepared for *Identification* test *B*
Analysis: To the *Sample* add about 500 mg of zinc dust, and warm the mixture.
Acceptance criteria: Rapid decolorization occurs. Place a drop of the decolorized solution adjacent to a drop of 6 N ammonium hydroxide on a filter paper: a blue color is produced at the zone of contact.

ASSAY
• **PROCEDURE**
Sample solution: Transfer about 400 mg of Gentian Violet to a 300-mL conical flask, add 25 mL of water and 10 mL of hydrochloric acid, displace the air in the flask with carbon dioxide, and pass a stream of carbon dioxide through the flask. Add 50.0 mL of 0.1 N titanium trichloride VS, heat to boiling, and boil gently for 10 min, swirling the liquid occasionally. Cool the solution, and add 5 mL of ammonium thiocyanate solution (1 in 10).

www.webofpharma.com

Analysis
Sample: *Sample solution*
Titrate with 0.1 N ferric ammonium sulfate VS until a faint red color is produced. Perform a blank determination. Each mL of 0.1 N titanium trichloride is equivalent to 20.40 mg of gentian violet ($C_{25}H_{30}ClN_3$).
Acceptance criteria: 96.0%–100.5% on the anhydrous basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 1.5%
• **ARSENIC,** *Method I* ⟨211⟩
Test preparation: Mix 300 mg with 2.5 g each of powdered potassium nitrate and anhydrous sodium carbonate, and heat the mixture in a crucible until the organic matter is completely oxidized. Dissolve the cooled residue in 15 mL of 2 N sulfuric acid, and evaporate the solution by heating until copious white fumes begin to evolve. Dissolve the residue in 35 mL of water.
Analysis: Proceed as directed in the chapter.
Acceptance criteria: NMT 10 ppm
• **LEAD**
Sample solution: Place 1.0 g in a small Kjeldahl flask, add 5 mL of sulfuric acid, and insert a small funnel into the flask. Gently rotate the flask until the sulfuric acid has completely wetted the sample, then heat gently until complete carbonization has taken place. Allow to cool, and add, in small quantities, 5 mL of nitric acid. Again heat gently until copious white fumes are evolved. Allow to cool, add another 5 mL of nitric acid, and again heat until white fumes are evolved. Allow to cool, cautiously add about 25 mL of water, and boil for a few min. After cooling, neutralize to litmus paper with ammonium hydroxide, and add 5 mL of nitric acid. Transfer the solution to a 100-mL volumetric flask, and dilute with water to volume.
Analysis: Use 20 mL of this solution for the limit test for *Lead* ⟨251⟩. Perform a blank determination.
Acceptance criteria: NMT 30 ppm
• **ZINC**
Standard stock solution: 1 g/mL of zinc prepared as follows. Transfer 1 g of zinc to a 1000-mL volumetric flask, add 50 mL of nitric acid, and dilute with water to volume.
Standard solution: 0.50 µg/mL of zinc, from *Standard stock solution* in water
Sample solution: Weigh 0.50 g of Gentian Violet in a suitable tared crucible. Place in a low-temperature plasma ashing apparatus, and ash until a constant weight is attained. Pipet 10 mL of 6 N nitric acid into the crucible, and heat to dissolve the ash. Transfer the solution to a 500-mL volumetric flask, and dilute with water to volume. Prepare a reagent blank.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Apparatus: Atomic absorption spectrophotometer
Analytical wavelength: Zinc emission line, 213.9 nm
Lamp: Zinc
Flame: Air–acetylene
Blank: Water
Analysis
Samples: *Standard solution, Sample solution,* and reagent blank
Acceptance criteria: 0.05%; the absorbance of the *Sample solution,* corrected for that of the reagent blank, is NMT the absorbance of the *Standard solution,* similarly corrected.
• **ORGANIC IMPURITIES**
Sample solution A: 1 mg/mL of Gentian Violet in methanol
Sample solution B: 0.01 mg/mL of Gentian Violet in methanol from *Sample solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
Mode: TLC
Adsorbent: 0.25-mm layer of octadecylsilanized chromatographic silica gel
Application volume: 5 µL
Developing solvent system: Upper layer separated from a well-shaken mixture of butyl alcohol, glacial acetic acid, and water (80:20:100)
Analysis
Samples: *Sample solution A* and *Sample solution B*
Allow the spots to dry, and develop the chromatogram in a suitable chamber with a solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and visually locate the spots on the plate.
Acceptance criteria: 1.0%; *Sample solution A* exhibits a principal spot and NMT one secondary spot, which, if present in the chromatogram from *Sample solution A,* is not more intense than the principal spot from *Sample solution B.*

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 7.5%
• **ALCOHOL-INSOLUBLE SUBSTANCES**
Sample: 1.0 g
Analysis: Boil the *Sample* with 50 mL of alcohol under a reflux condenser for 15 min, pass through a tared filtering crucible, wash the residue on the filter with hot alcohol until the last washing is not colored violet, and dry the crucible at 105° for 1 h.
Acceptance criteria: NMT 1.0% of insoluble residue remains.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Gentian Violet Cream

DEFINITION
Gentian Violet Cream is Gentian Violet in a suitable cream base. It contains, in each 100 g, NLT 1.20 g and NMT 1.60 g of gentian violet ($C_{25}H_{30}ClN_3$).

IDENTIFICATION
• **A.**
Sample: 0.1 g
Analysis: Dissolve the *Sample* in 5 mL of sulfuric acid.
Acceptance criteria: An orange or brown-red color is produced.
• **B.** The visible absorption spectrum of the *Sample solution* used for measurement of absorbance in the *Assay* exhibits maxima at the same wavelengths as that of a similar solution of USP Gentian Violet RS, concomitantly measured.

ASSAY
• **PROCEDURE**
Standard solution: 13.5 µg/mL of USP Gentian Violet RS in hydrochloric acid
Sample stock solution: Transfer 500 mg of Cream to a 100-mL volumetric flask. Add 15 mL of hydrochloric acid, warm in a water bath to disperse the Cream thoroughly, cool, and add hydrochloric acid to volume. Pass the mixture through a sintered-glass filter of fine pore size.
Sample solution: Pipet 2 mL of the *Sample stock solution* into a 10-mL volumetric flask, and add hydrochloric acid to volume.
Blank: Hydrochloric acid

USP Monographs

www.webofpharma.com

Instrumental conditions
Analytical wavelength: 435 nm
Cell: 1 cm. [NOTE—Use quartz cells having tightly fitting covers to prevent the corrosion caused by hydrochloric acid.]
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in g/100 g, of gentian violet ($C_{25}H_{30}ClN_3$) in the portion of Cream taken:

$$\text{Result} = (A_U/A_S) \times C_S \times (1/W) \times V \times D \times (1/F) \times 100$$

A_U = absorbance from the *Sample solution*
A_S = absorbance from the *Standard solution*
C_S = concentration of USP Gentian Violet RS in the *Standard solution* (µg/mL)
W = weight of the sample in the *Sample stock solution*, 0.5 g
V = volume of the *Sample stock solution*, 100 mL
D = dilution factor, 5
F = conversion factor, 10^6 µg/g

Acceptance criteria: It contains, in each 100 g, 1.20–1.60 g of gentian violet ($C_{25}H_{30}ClN_3$).

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or other tight containers, and avoid exposure to excessive heat.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Gentian Violet RS

Gentian Violet Topical Solution

DEFINITION
Gentian Violet Topical Solution contains, in each 100 mL, NLT 0.95 g and NMT 1.05 g of gentian violet ($C_{25}H_{30}ClN_3$).

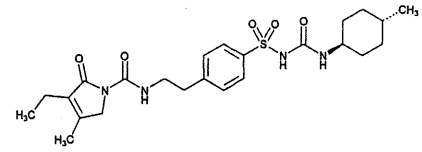
IDENTIFICATION
• **A.**
Sample solution: 1 in 5 dilution in water
Analysis: Add 5 drops of hydrochloric acid. To 5 mL of this solution add tannic acid TS dropwise.
Acceptance criteria: A deep blue precipitate is formed.
• **B.**
Sample solution: 1 in 5 dilution in water
Analysis: To the remaining solution from *Identification* test *A* add 500 mg of zinc dust, and warm the mixture.
Acceptance criteria: Rapid decolorization occurs. Place a drop of the decolorized solution adjacent to a drop of 6 N ammonium hydroxide on a filter paper. A blue color is produced at the zone of contact.

ASSAY
• **PROCEDURE**
Sample solution: Pipet 25 mL of Topical Solution into a 300-mL conical flask, add 25 mL of water and 10 mL of hydrochloric acid, displace the air in the flask with carbon dioxide, and pass a stream of carbon dioxide through the flask. Add 50.0 mL of 0.1 N titanium trichloride VS, heat to boiling, and boil gently for 10 min, swirling the liquid occasionally. Cool the solution, and add 5 mL of ammonium thiocyanate solution (1 in 10).
Analysis: Titrate with 0.1 N ferric ammonium sulfate VS until a faint red color is produced. Perform a blank determination. Each mL of 0.1 N titanium trichloride is equivalent to 20.40 mg of gentian violet ($C_{25}H_{30}ClN_3$).
Acceptance criteria: It contains, in each 100 mL, 0.95–1.05 g of gentian violet ($C_{25}H_{30}ClN_3$).

SPECIFIC TESTS
• **SOLUTION OF RESIDUE IN ALCOHOL**
Sample: 10 mL
Analysis: Evaporate the *Sample* on a steam bath to dryness.
Acceptance criteria: The residue dissolves completely in 10 mL of alcohol.
• **ALCOHOL DETERMINATION** ⟨611⟩: 8.0%–10.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.

Glimepiride



$C_{24}H_{34}N_4O_5S$ 490.62
1*H*-Pyrrole-1-carboxamide, 3-ethyl-2,5-dihydro-4-methyl-*N*-[2-[4-[[[[(4-methylcyclohexyl)amino]carbonyl]amino]sulfonyl]phenyl]ethyl]-2-oxo-, *trans*-;
1-[[4-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl]sulfonyl]-3-(*trans*-4-methylcyclohexyl)urea [93479-97-1].

DEFINITION
Glimepiride contains NLT 98.0% and NMT 102.0% of glimepiride ($C_{24}H_{34}N_4O_5S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020) [NOTE—Methods described in ⟨197K⟩ or ⟨197A⟩ may be used.]
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Dissolve 0.5 g of monobasic sodium phosphate in 500 mL of water. Adjust with phosphoric acid to a pH of 2.1–2.7, and add 500 mL of acetonitrile.
Diluent: Acetonitrile and water (4:1)
Standard solution: 0.2 mg/mL of USP Glimepiride RS in *Diluent*
System suitability stock solution: 0.1 mg/mL each of USP Glimepiride Related Compound B RS, USP Glimepiride Related Compound C RS, and USP Glimepiride Related Compound D RS in *Diluent*
System suitability solution: Dilute 1 mL of the *System suitability stock solution* with the *Standard solution* to 50 mL.
Sample solution: 0.2 mg/mL of Glimepiride in *Diluent*. [NOTE—Keep the *Sample solution* at a temperature not exceeding 12°, and store it no longer than 15 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

USP Monographs

www.webofpharma.com

Mode: LC
Detector: UV 228 nm
Column: 4-mm × 25-cm or 4.6-mm × 25-cm; 4- or 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 4.0 between glimepiride related compound B and glimepiride related compound C, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of glimepiride ($C_{24}H_{34}N_4O_5S$) in the portion of Glimepiride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Glimepiride RS in the *Standard solution* (mg/mL)
C_U = concentration of Glimepiride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%

• **LIMIT OF *cis*-ISOMER (GLIMEPIRIDE RELATED COMPOUND A)**

Mobile phase: Transfer 100 mL of isopropyl alcohol to a 1-L volumetric flask, add 1 mL of glacial acetic acid, and dilute with hexane to volume.
System suitability stock solution: Dissolve 1 mg of USP Glimepiride Related Compound A RS in 1 mL of methylene chloride. Add 3 mL of *Mobile phase*, and mix.
System suitability solution: Transfer 10 mg of USP Glimepiride RS into a 20-mL volumetric flask, dissolve in 5 mL of methylene chloride, and dilute with *Mobile phase* to volume. Transfer 5 mL of this solution to a separate flask, add 50 µL of the *System suitability stock solution*, and mix.
Sample solution: Transfer 10 mg of Glimepiride into a 20-mL volumetric flask, dissolve in 5 mL of methylene chloride, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 228 nm
Column: 3-mm × 15-cm; 5-µm packing L20
[NOTE—The analyses could also be performed with 4.6-mm × 15-cm, 4.6-mm × 25-cm, 4-mm × 12.5-cm, or 4-mm × 25-cm columns containing packing L20. It is recommended that the flow rate be adjusted to about 1.1 mL/min for a 4.6-mm column and to about 0.8 mL/min for a 4.0-mm column.]
Flow rate: 0.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for glimepiride *cis*-isomer and glimepiride are 0.9 and 1.0, respectively.]
Suitability requirements
Signal-to-noise ratio: NLT 15 for the glimepiride *cis*-isomer peak
Peak-to-valley ratio: NLT 2.0 for glimepiride *cis*-isomer and glimepiride
Analysis
Sample: *Sample solution*
Calculate the percentage of glimepiride *cis*-isomer in the portion of Glimepiride taken:

$$\text{Result} = r_{cis}/(r_{cis} + r_G) \times 100$$

r_{cis} = peak area of glimepiride *cis*-isomer from the *Sample solution*
r_G = peak area of glimepiride from the *Sample solution*

Acceptance criteria: NMT 0.8%

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, Standard solution, System suitability solution, Sample solution, and **System suitability:** Proceed as directed in the *Assay*.
Diluted sample solution 1: Dilute 5.0 mL of the *Sample solution* with *Diluent* to 100.0 mL. Dilute 5.0 mL of the solution obtained with *Diluent* to 50.0 mL. This solution contains about 0.001 mg/mL of glimepiride.
Diluted sample solution 2: Dilute 1.0 mL of *Diluted sample solution 1* with *Diluent* to 10.0 mL.
Chromatographic system: Proceed as directed in the *Assay*, except for the *Run time*.
Run time: 2.5 times the retention time of the glimepiride peak
Analysis
Samples: *Sample solution*, *Diluted sample solution 1*, and *Diluted sample solution 2*
Calculate the percentage of each impurity in the portion of Glimepiride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of glimepiride from *Diluted sample solution 1*
C_S = concentration of Glimepiride in *Diluted sample solution 1* (mg/mL)
C_U = concentration of Glimepiride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peak with an area less than that of the glimepiride peak from *Diluted sample solution 2*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Glimepiride sulfonamide (glimepiride related compound B)	0.2	0.4
Glimepiride urethane (glimepiride related compound C)	0.3	0.1
Glimepiride	1.0	—
Glimepiride 3-isomer (glimepiride related compound D)	1.1	0.2
Any individual unspecified impurity	—	0.1

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities, excluding glimepiride related compound B	—	0.5

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ic*

Sample solution: Dissolve 0.25 g of Glimepiride in dimethylformamide previously dried over a 2-mm molecular sieve with 0.4-nm pore size, and dilute with the same solvent to 5.0 mL.

Analysis: Use 1.0 mL of the *Sample solution*. Perform a blank determination, using 1.0 mL of the solvent.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store in well-closed containers, at a temperature not exceeding 25°.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Glimepiride RS

USP Glimepiride Related Compound A RS
Glimepiride *cis*-isomer;
1-[[*p*-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl]sulfonyl]-3-(*cis*-4-methylcyclohexyl)urea.
$C_{24}H_{34}N_4O_5S$ 490.62

USP Glimepiride Related Compound B RS
Glimepiride sulfonamide;
3-Ethyl-4-methyl-2-oxo-*N*-(4-sulfamoylphenethyl)-2,5-dihydro-1*H*-pyrrole-1-carboxamide.
$C_{16}H_{21}N_3O_4S$ 351.42

USP Glimepiride Related Compound C RS
Glimepiride urethane;
Methyl ({4-[2-(3-ethyl-4-methyl-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamido)ethyl]phenyl}sulfonyl) carbamate.
$C_{18}H_{23}N_3O_6S$ 409.46

USP Glimepiride Related Compound D RS
Glimepiride 3-isomer;
1-[[*m*-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl]sulfonyl]-3-(*trans*-4-methylcyclohexyl)urea.
$C_{24}H_{34}N_4O_5S$ 490.62

Glimepiride Tablets

DEFINITION

Glimepiride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

[NOTE—Store the solutions containing glimepiride for NMT 24 h.]

Mobile phase: Dissolve 0.5 g of monobasic sodium phosphate in 500 mL of water. Adjust with 10% phosphoric acid to a pH of 2.1–2.7, and add 500 mL of acetonitrile.

Diluent: Acetonitrile and water (9:1)

System suitability solution: 0.1 mg/mL of USP Glimepiride RS and 0.02 mg/mL each of USP Glimepiride Related Compound B RS and USP Glimepiride Related Compound C RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Glimepiride RS in *Diluent*

Sample solution: Transfer 5 whole Tablets into a suitable volumetric flask to prepare a solution of approximately 0.1 mg/mL of glimepiride, based on the label claim. Add water to 10% of the volume of the flask. Shake the flask to completely dissolve the Tablets. Add acetonitrile to about 70% of the volume of the flask, and swirl. Sonicate the samples in a water bath not to exceed 20° for NLT 5 min and NMT 10 min, with occasional shaking. Allow the solutions to come to room temperature, dilute with acetonitrile to volume, mix, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 228 nm. For *Identification B*, use a diode array detector in the range of 200–340 nm.

Column: 4-mm × 12.5-cm or 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for glimepiride related compound B, glimepiride related compound C, and glimepiride are 0.25, 0.35, and 1.0, respectively. Identify the glimepiride peak and the peaks due to the related compounds based on their relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between glimepiride related compound B and glimepiride related compound C, *System suitability solution*

Tailing factor: NMT 2.0 for the glimepiride peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Glimepiride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of glimepiride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: pH 7.8 phosphate buffer (0.58 g of monobasic potassium phosphate and 8.86 g of dibasic sodium phosphate, anhydrous, in 1000 mL of water, adjusted with 10% phosphoric acid or 1 N sodium hydroxide to a pH of 7.8); 900 mL

Apparatus 2: 75 rpm

www.webofpharma.com

Time: 15 min
Mobile phase: Prepare as directed in the *Assay*.
Diluting solution: Methanol and water (1:1)
Standard solution: Prepare a solution of USP Glimepiride RS in a mixture of acetonitrile and water (90:10) having a known concentration of about 0.125 mg/mL of glimepiride. Transfer 4.0 mL of this solution into a 200-mL volumetric flask, dilute with *Medium* to volume, and mix. Transfer 15.0 mL of this solution into a 50-mL volumetric flask, dilute with *Diluting solution* to volume, and mix. The final solution contains about 0.75 µg/mL of glimepiride.
Sample solution: Withdraw approximately 10 mL of the solution under test, and transfer to a centrifuge tube. Centrifuge for 5 min at 2500 rpm. Pipet 3.0 mL of the supernatant into a 10-mL volumetric flask, dilute with *Diluting solution* to volume, and mix.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 228 nm
Column: 4.0-mm × 12.5-cm or 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL
D = dilution factor of the *Sample solution*

Tolerances: NLT 80% (Q) of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: pH 7.8 phosphate buffer (add 250 mL of 0.2 M monobasic potassium phosphate to 223 mL of 0.2 M sodium hydroxide, dilute with water to 1 L, and adjust with 0.2 M sodium hydroxide or phosphoric acid to a pH of 7.8); 900 mL
Apparatus 2: 75 rpm
Time: 45 min
Buffer solution: 4.0 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 5.3.
Mobile phase: Acetonitrile and *Buffer solution* (1:1)
Diluent: Methanol and acetonitrile (1:1)
Standard stock solution: 0.22 mg/mL of USP Glimepiride RS in *Diluent*
Standard solution: (L/1000) mg/mL of USP Glimepiride RS in *Medium*, from the *Standard stock solution*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 10-cm; 5-µm packing L1
Flow rate: 1.3 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: pH 7.8 phosphate buffer (prepared as indicated for *Test 1*); 900 mL
Apparatus 2: 75 rpm
Time: 20 min
Buffer solution: 1.36 g/L of monobasic potassium phosphate in water. Adjust with 10% sodium hydroxide to a pH of 7.0 ± 0.05.
Mobile phase: *Buffer solution* and acetonitrile (675:325)
Standard stock solution: 0.22 mg/mL of USP Glimepiride RS in methanol
Standard solution: (L/1000) mg/mL of USP Glimepiride RS in *Medium*, from the *Standard stock solution*, where L is the label claim in mg/Tablet
Sample solution: Pass a portion of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 228 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) is dissolved.

www.webofpharma.com

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

[NOTE—Store the solutions containing glimepiride for NMT 24 h.]

Mobile phase and **Diluent:** Prepare as directed in the *Assay*.

System suitability solution: 4 µg/mL of USP Glimepiride RS and 2 µg/mL each of USP Glimepiride Related Compound B RS and USP Glimepiride Related Compound C RS in *Diluent*

Sensitivity solution: Transfer 5.0 mL of the *System suitability solution* into a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Sample solution: Finely powder NLT 10 Tablets, and transfer a portion of the powder to a 50-mL centrifuge tube. Add *Diluent* to prepare a solution containing 0.1 mg/mL of glimepiride, based on the label claim. Sonicate in a water bath at a temperature not to exceed 20° for NLT 5 min and NMT 10 min, with occasional mixing. Centrifuge the samples, and use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 228 nm

Column: 4-mm × 25-cm or 4.6-mm × 25-cm; 4- or 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 2 times the retention time of the glimepiride peak

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 4 between glimepiride related compound B and glimepiride related compound C, *System suitability solution*

Relative standard deviation: NMT 2.0% of the glimepiride peak, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Reporting threshold for impurities is 0.1%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Glimepiride related compound B	0.2	1.3	2.5
Glimepiride related compound C	0.3	1.0	0.5
Glimepiride	1.0	1.0	—
Any other individual impurity	—	1.0	0.5
Total impurities, excluding glimepiride related compound B	—	—	1.0
Total impurities, including glimepiride related compound B	—	—	3.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Glimepiride RS

USP Glimepiride Related Compound B RS

Glimepiride sulfonamide;

3-Ethyl-4-methyl-2-oxo-*N*-(4-sulfamoylphenethyl)-2,5-dihydro-1*H*-pyrrole-1-carboxamide.

$C_{16}H_{21}N_3O_4S$ 351.42

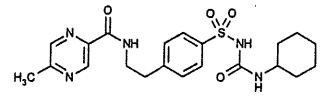
USP Glimepiride Related Compound C RS

Glimepiride urethane;

Methyl ({4-[2-(3-ethyl-4-methyl-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamido)ethyl]phenyl}sulfonyl) carbamate.

$C_{18}H_{23}N_3O_6S$ 409.46

Glipizide



$C_{21}H_{27}N_5O_4S$ 445.54

Pyrazinecarboxamide, *N*-[2-[4-[[[(cyclohexylamino)carbonyl]amino]sulfonyl]phenyl]ethyl]-5-methyl-.

1-Cyclohexyl-3-[[*p*-[2-(5-methylpyrazinecarboxamido)ethyl]phenyl]sulfonyl]urea [29094-61-9].

» Glipizide contains not less than 98.0 percent and not more than 102.0 percent of $C_{21}H_{27}N_5O_4S$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers, protected from light. Store at room temperature.

USP Reference standards ⟨11⟩—

USP Glipizide RS

USP Glipizide Related Compound A RS

N-{2-[(4-Aminosulfonyl)phenyl]ethyl}-5-methylpyrazinecarboxamide.

$C_{14}H_{16}N_4O_3S$ 320.37

USP Glipizide Related Compound B RS

6-Methyl-*N*-[2-(4-sulfamoylphenyl)ethyl]pyrazine-2-carboxamide.

$C_{14}H_{16}N_4O_3S$ 320.37

USP Monographs

www.webofpharma.com

USP Glipizide Related Compound C RS
1-Cyclohexyl-3-[[4-[2-[[(6-methylpyrazin-2-yl)carbonyl]amino]ethyl]phenyl]sulfonyl]urea.
$C_{21}H_{27}N_5O_4S$ 445.54

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 20 µg per mL.
Medium: methanol.
C: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Loss on drying ⟨731⟩—Dry it in vacuum at 100° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.4%.

Related compounds— [NOTE—Use low-actinic glassware for solutions containing glipizide and glipizide related compounds.]

TEST 1 (limit of methyl-*N*-4-[2-(5-methylpyrazine-2-carboxamido)ethyl] benzenesulfonyl carbamate)—
Buffer solution—Add 4.0 mL of *n*-butylamine to 1000 mL of water. Adjust with phosphoric acid to a pH of 3.00 ± 0.05.
Diluent—Prepare a mixture of water, acetonitrile, and methanol (3:1:1).
Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution,* acetonitrile, and methanol (3:1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard stock solution—Prepare a solution of USP Glipizide RS in methanol containing about 0.1 mg per mL.
Standard solution—Prepare a solution of USP Glipizide Related Compound A RS in methanol containing about 0.1 mg per mL. Pipet 2.0 mL of this solution into a 100-mL volumetric flask, add 2.0 mL of the *Standard stock solution,* dilute with *Diluent* to volume, and mix. This solution contains about 0.002 mg of USP Glipizide RS and about 0.002 mg of USP Glipizide Related Compound A RS per mL.
Test solution—Transfer about 25 mg of Glipizide, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Pipet 4.0 mL of this solution into a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The column temperature is maintained at 30°. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0% for each peak. [NOTE—The retention time of the glipizide peak is about 45 minutes; for the purpose of identification, the relative retention times for glipizide related compound A and glipizide are about 0.12 and 1.0, respectively.]
Procedure—Separately inject equal volumes (about 35 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. In the *Test solution,* identify the peaks that correspond to glipizide related compounds A, B, and C, based on their relative retention times: the relative retention time for glipizide related compound A is given above; glipizide related compound B, if present, elutes immediately after glipizide related compound A, and these two peaks are not resolved by this method; glipizide related compound C elutes at a relative retention time of approximately 1.1. Disregard these impurities when using *Test 1,* as they will be controlled using *Test 2.*
In the *Test solution,* identify the peak that corresponds to methyl-*N*-4-[2-(5-methylpyrazine-2-carboxamido)ethyl] benzenesulfonyl carbamate impurity, based on its relative retention time, which is about 0.18. Calculate the percentage of this impurity and any other individual impurity in the portion of Glipizide taken by the formula:

$$100(C_G/C_T)(r_i/r_{SG})$$

in which C_G is the concentration, in mg per mL, of glipizide in the *Standard solution;* C_T is the concentration, in mg per mL, of Glipizide in the *Test solution;* r_i is the peak response for each individual impurity obtained from the *Test solution;* r_{SG} is the glipizide peak response obtained from the *Standard solution.* Disregard any impurity peak that is less than 0.05%. Not more than 0.5% of any individual impurity is found.

TEST 2 (limit of related compounds A, B, and C)—
0.02 M Phosphate buffer, Mobile phase, Impurity standard stock solution, System suitability solution, Sensitivity solution, and *Chromatographic system*—Proceed as directed in the *Assay.*
Impurity standard solution—Accurately transfer 5.0 mL of the *Impurity standard stock solution* into a 100-mL volumetric flask, and dilute with methanol to volume. Accurately transfer 10.0 mL of the solution obtained into another 100-mL volumetric flask containing 20 mL of acetonitrile and 60 mL of *0.02 M Phosphate buffer.* Sonicate the solution for about 5 minutes, dilute with *0.02 M Phosphate buffer* to volume, and mix. This solution contains about 0.0005 mg of each of glipizide related compounds A, B, and C per mL.
Test solution—Use *Assay preparation.*
Procedure—Separately inject equal volumes (about 10 µL) of the *Impurity standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each of the glipizide related compounds A, B, or C in the portion of Glipizide taken by the formula:

$$100(C_S/C_T)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of the respective glipizide related compound in the *Impurity standard solution;* C_T is the concentration, in mg per mL, of Glipizide in the *Test solution;* r_U and r_S are the peak responses for the respective glipizide related compound obtained from the *Test solution* and the *Impurity standard solution,* respectively. Not more than 0.5% of any individual impurity is found; and not more than 1.5% of total impurities is found, the results for *Test 1* and *Test 2* being combined.

Assay— [NOTE—Use low-actinic glassware for solutions containing glipizide and glipizide related compounds.]
0.02 M Phosphate buffer—Dissolve 2.84 g of anhydrous dibasic sodium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 6.00 ± 0.05.
Mobile phase—Prepare a filtered and degassed mixture of *0.02 M Phosphate buffer,* acetonitrile, and methanol (70:20:10). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard stock preparation—Transfer accurately weighed amounts of USP Glipizide RS to a suitable volumetric flask, and dissolve in methanol with the aid of sonication for about 5 minutes to obtain a solution having a known concentration of about 1.0 mg of glipizide per mL.

USP Monographs

www.webofpharma.com

Standard preparation—Accurately transfer 10.0 mL of the *Standard stock preparation* into a 100-mL volumetric flask containing 20 mL of acetonitrile and 60 mL of *0.02 M Phosphate buffer.* Sonicate the solution for about 5 minutes, dilute with *0.02 M Phosphate buffer* to volume, and mix. This solution contains about 0.1 mg of glipizide per mL.

Impurity standard stock solution—Transfer accurately weighed amounts of USP Glipizide Related Compound A RS, USP Glipizide Related Compound B RS, and USP Glipizide Related Compound C RS to a suitable volumetric flask, and dissolve in methanol with the aid of sonication for about 5 minutes to obtain a solution having known concentrations of about 0.1 mg of each of glipizide related compounds A, B, and C per mL. [NOTE—Glipizide related compound A is *N*-{2-[(4-aminosulfonyl)phenyl]ethyl}-5-methyl-pyrazinecarboxamide; glipizide related compound B is 6-methyl-*N*-[2-(4-sulfamoylphenyl)ethyl]pyrazine-2-carboxamide; and glipizide related compound C is 1-cyclohexyl-3-[[4-[2- [[(6-methylpyrazin-2-yl)carbonyl] amino]ethyl]phenyl] sulfonyl]urea.]

System suitability stock solution—Transfer an accurately weighed amount of USP Glipizide RS to a suitable volumetric flask, dissolve in methanol with the aid of sonication for about 5 minutes, and add an accurately measured volume of *Impurity standard stock solution* to obtain a solution containing about 1.0 mg of glipizide and about 0.005 mg of each of glipizide related compounds A, B, and C per mL.

System suitability solution—Accurately transfer 10.0 mL of the *System suitability stock solution* into a 100-mL volumetric flask containing 20 mL of acetonitrile and 60 mL of *0.02 M Phosphate buffer.* Sonicate the solution for about 5 minutes, dilute with *0.02 M Phosphate buffer* to volume, and mix.

Sensitivity solution—Accurately transfer 5.0 mL of the *System suitability solution* into a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay stock preparation—Transfer about 100 mg of Glipizide, accurately weighed, to a 100-mL volumetric flask, dissolve in about 50 mL of methanol with the aid of sonication for about 5 minutes, dilute with methanol to volume, and mix.

Assay preparation—Accurately transfer 10.0 mL of the *Assay stock preparation* into a 100-mL volumetric flask containing 20 mL of acetonitrile and 60 mL of *0.02 M Phosphate buffer.* Sonicate the solution for about 5 minutes, dilute with *0.02 M Phosphate buffer* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L43. The column temperature is maintained at 40°. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the elution order is glipizide related compound A, glipizide related compound B, glipizide, and glipizide related compound C; the resolution, *R,* between glipizide related compound A and glipizide related compound B peaks is not less than 1.8, and the resolution, *R,* between glipizide and glipizide related compound C peaks is also not less than 1.8; the tailing factor for the glipizide peak is not more than 2.0; and the relative standard deviation for 5 replicate injections is not more than 1.5% for the glipizide peak and not more than 5.0% for each of the related compounds peaks. Chromatograph the *Sensitivity solution,* and record the peak responses as directed for *Procedure:* the signal-to-noise ratio for each of glipizide related compound A and glipizide related compound B peaks is not less than 15.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of $C_{21}H_{27}N_5O_4S$ in the portion of Glipizide taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of glipizide in the *Standard preparation* and the *Assay preparation,* respectively; and r_U and r_S are the glipizide peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Glipizide Tablets

» Glipizide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of glipizide ($C_{21}H_{27}N_5O_4S$).

Packaging and storage—Preserve in tight containers.

Labeling—When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

USP Reference standards ⟨11⟩—
USP Glipizide RS
USP Glipizide Related Compound A RS
N-{2-[(4-Aminosulfonyl)phenyl]ethyl}-5-methyl-pyrazinecarboxamide.
$C_{14}H_{16}N_4O_3S$ 320.37

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation,* as obtained in the *Assay.*

B: Transfer a quantity of finely powdered Tablets, equivalent to about 10 mg of glipizide, to a glass-stoppered centrifuge tube, add 10 mL of methanol, insert a stopper into the tube, and shake. Centrifuge the mixture, and use the clear supernatant as the test solution. Separately apply, as streaks about 7 cm in length, 100 µL of the test solution and 100 µL of a Standard solution of USP Glipizide RS in methanol containing 1 mg per mL, to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the streaks to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, ethyl acetate, and 98% formic acid (5:3:2) until the solvent front has moved to within 2.5 cm of the top of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 80° for 30 minutes. Cool the plate, spray it with 0.5% sodium hypochlorite solution, and allow the plate to air-dry. Spray the plate with alcohol, air-dry, and spray with a freshly prepared mixture of 1% soluble starch solution and 1% potassium iodide solution (1:1): the R_F value of the principal band obtained from the test solution corresponds to that obtained from the Standard solution.

Dissolution ⟨711⟩—
TEST 1—
Medium: simulated intestinal fluid TS (without pancreatin); 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.

Determine the amount of $C_{21}H_{27}N_5O_4S$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm of filtered portions of the solution under test, in comparison with a Standard solution having a known concentration of USP Glipizide RS in the same medium.
Tolerances—Not less than 80% *(Q)* of the label claim of $C_{21}H_{27}N_5O_4S$ is dissolved in 45 minutes.

www.webofpharma.com

TEST 2—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*. *Medium:* simulated intestinal fluid TS (without pancreatin), with pH adjusted to pH 7.5 ± 0.1 with 0.2 N sodium hydroxide; 900 mL.

Apparatus 2 and *Time*—Proceed as directed under *Test 1*.

Determine the amount of $C_{21}H_{27}N_5O_4S$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm of filtered portions of the solution under test, in comparison with a Standard solution having a known concentration of USP Glipizide RS in the same medium.

Tolerances—Not less than 80% *(Q)* of the label claim of $C_{21}H_{27}N_5O_4S$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements, the following procedure being used where the test for *Content Uniformity* is required.

Buffer, Mobile phase, and *Standard preparation*—Prepare as directed in the *Assay*.

Test preparation—Transfer 1 Tablet to an appropriate volumetric flask, add a volume of *Buffer* equal to one-half of the total flask volume, and shake by mechanical means for 10 minutes to allow the Tablet to disintegrate completely. Dilute with methanol to volume, and sonicate for 15 minutes to obtain a solution having a concentration of about 0.05 mg of glipizide per mL. Filter through a solvent-resistant filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay*. Chromatograph the *Standard preparation,* and record the peak responses as directed under *Procedure:* the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{27}N_5O_4S$ in the Tablet taken by the formula:

$$CV(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Glipizide RS in the *Standard preparation;* V is the volume, in mL, of the *Test preparation* taken; and r_U and r_S are the peak responses obtained from the *Test preparation* and the *Standard preparation,* respectively.

Related compounds—

Buffer and *Mobile phase*—Prepare as directed in the *Assay*.

Standard stock solution—Dissolve an accurately weighed quantity of USP Glipizide Related Compound A RS in methanol to obtain a solution having a known concentration of about 50 µg per mL.

Standard solution—Dissolve an accurately weighed quantity of USP Glipizide RS in methanol. To this solution add sufficient *Standard stock solution,* and dilute quantitatively with methanol to obtain a solution having known concentrations of about 100 µg per mL of USP Glipizide RS and about 0.5 µg per mL of USP Glipizide Related Compound A RS. Transfer 25 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer* to volume, and mix.

Test solution—Use the *Assay preparation*.

Chromatographic system—Prepare as directed in the *Assay*. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.2 for glipizide related compound A and 1.0 for glipizide; the resolution, *R,* between glipizide related compound A and glipizide is not less than 1.5; and the relative standard deviation for replicate injections is not more than 5% for glipizide related compound A.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of N-{2-[(4-aminosulfonyl)phenyl]ethyl}-5-methyl-pyrazinecarboxamide (glipizide related compound A) in the Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Glipizide Related Compound A RS in the *Standard solution;* and r_U and r_S are the peak responses of glipizide related compound A obtained from the *Test solution* and the *Standard solution,* respectively: not more than 2.0% of glipizide related compound A relative to the glipizide content, as determined in the *Assay,* is found.

Assay—

Buffer—Dissolve 13.8 g of monobasic sodium phosphate in water, and dilute with water to 1000 mL. Adjust with 2.0 N sodium hydroxide to a pH of 6.00 ± 0.05.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer* and methanol (55:45). Make adjustments if necessary (see *System suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Glipizide RS in methanol, and dilute quantitatively with methanol to obtain a solution having a known concentration of about 0.1 mg per mL. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer* to volume, and mix to obtain a solution having a known concentration of about 0.05 mg per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of glipizide, to a 100-mL volumetric flask. Add 50 mL of methanol, and place in an ultrasonic bath for 15 minutes. Dilute with *Buffer* to volume, and place in the ultrasonic bath for an additional 15 minutes. Filter through a solvent-resistant filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 225-nm detector and a 3.9-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{27}N_5O_4S$ in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Glipizide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Glipizide and Metformin Hydrochloride Tablets

DEFINITION

Glipizide and Metformin Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of glipizide ($C_{21}H_{27}N_5O_4S$) and metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$).

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197A▲** (CN 1-May-2020)

Sample: Transfer NLT 10 Tablets to a suitable container, add 10 mL of methanol, and shake to remove any Tablet coating. Drain the methanol, add 20 mL of water, and stir until the Tablets dissolve (1 h). Transfer the solution to a separatory funnel, and extract twice with 10-mL portions of chloroform, shaking for approximately 5 min. Transfer the lower organic layer into a beaker containing 3 to 4 g of anhydrous magnesium sulfate. Repeat the extraction of the solution in the separatory funnel two more times, each time using 20-mL portions of chloroform. Swirl the mixture in the beaker for 1 min. Filter, and collect the filtrate. Evaporate the solvent under vacuum, and dry the residue under vacuum for 4 h at 105°. Mound the residue onto a diamond cell.

Acceptance criteria: The IR spectrum exhibits maxima only at the same wavelengths as a similarly obtained spectrum of USP Glipizide RS.

• **B.** The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Glipizide*.

• **C.** The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Metformin Hydrochloride*.

ASSAY

• **GLIPIZIDE**

Solution A: 2.6 g/L of dibasic ammonium phosphate in water. Adjust with ammonium hydroxide to a pH of 8.0.

Solution B: Acetonitrile, water, and *Solution A* (1:14:5)

Solution C: Acetonitrile, water, and *Solution A* (2:1:1)

Diluent: Acetonitrile and water (60:40)

Standard stock solution: Transfer a weighed quantity of USP Glipizide RS to a suitable low-actinic volumetric flask. Dissolve first in acetonitrile, using 60% of the final volume, by sonicating for 20 min, then dilute with water to volume to obtain a solution having a concentration of 0.1 mg/mL of glipizide. [NOTE—The solution is stable for 2 weeks when stored at 5° and protected from light.]

Standard solution: Transfer 25.0 mL of *Standard stock solution* to a 200-mL low-actinic volumetric flask. Add 75 mL of *Diluent*, and dilute with water to volume to obtain a solution having a known glipizide concentration of 0.0125 mg/mL. [NOTE—The solution is stable for 2 weeks when stored at 5° and protected from light.]

System suitability solution: Transfer approximately 5 mg of USP Glipizide Related Compound A RS to a 500-mL volumetric flask, and fill halfway with acetonitrile. Sonicate for 30 min to dissolve, and dilute with acetonitrile to volume. Transfer 1 mL of this solution to a 50-mL low-actinic volumetric flask, and dilute with *Standard solution* to volume.

Sample solution: Transfer NLT 5 Tablets to a suitable volumetric flask, and fill halfway with *Diluent*. Sonicate for 30 min, and shake vigorously for another 30 min to dissolve. Dilute with water to volume, and mix to obtain a solution with a final glipizide concentration of 0.0125 mg/mL. Pass a portion of this solution through a nylon or PVDF filter of 0.2-µm pore size, and use the filtrate. [NOTE—The solution is stable for 2 weeks when stored at 5° and protected from light.]

Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
3	100	0
18	0	100
20	0	100
22	100	0
30	100	0

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 223 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection size: 50 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for glipizide related compound A and glipizide are 0.92 and 1.0, respectively.]
Suitability requirements
Resolution: Greater than 1.2 between glipizide related compound A and glipizide, *System suitability solution*
Relative standard deviation: Less than 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{21}H_{27}N_5O_4S$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Glipizide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of glipizide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

• **METFORMIN HYDROCHLORIDE**

Solution A: 9.41 g/L of sodium 1-hexanesulfonate in water, and adjust with trifluoroacetic acid to a pH of 2.0 (50 mM hexanesulfonic acid solution)

Solution B: Acetonitrile and water (40:60)

Mobile phase: *Solution A*, *Solution B*, and water (3:2:5)

Diluent pH 2.0: Acetonitrile, *Solution A*, and water (7:30:63)

Standard solution: 0.1 mg/mL of USP Metformin Hydrochloride RS in *Diluent pH 2.0*

System suitability solution: 5 µg/mL of USP Metformin Related Compound A RS in water. Pipet 0.5 mL of this solution into a 50-mL volumetric flask, and dilute with *Standard solution* to volume.

Sample solution: Dilute a portion of the *Sample solution*, obtained as directed in the *Assay* for *Glipizide*, with *Diluent pH 2.0*, to obtain a solution having a concentration of 0.1 mg/mL of metformin hydrochloride.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 218 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L11
Flow rate: 1 mL/min
Injection size: 25 µL

www.webofpharma.com

System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for metformin related compound A and metformin are 0.26 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between metformin related compound A and metformin, *System suitability solution*
Relative standard deviation: Less than 2.0%, determined from the metformin peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_4H_{11}N_5 \cdot HCl$ in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metformin hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Test 1
Medium: 0.05 M phosphate buffer, pH 6.8 ± 0.05. (Dissolve 12.96 g of monobasic potassium phosphate and 1.66 g of sodium hydroxide in approximately 400 mL of water, and dilute with water to 2000 mL. Adjust the pH, if necessary, with diluted sodium hydroxide); 1000 mL. [NOTE—Tight control of the pH is critical.]
Apparatus 2: 50 rpm
Time: 45 min for glipizide, and 30 min for metformin hydrochloride.
Determination of glipizide: Determine the amount of glipizide ($C_{21}H_{27}N_5O_4S$) dissolved as follows.
Solution A: Dissolve approximately 3.4 g of monobasic potassium phosphate in approximately 800 mL of water. Adjust with 10 N sodium hydroxide solution to a pH of 6.0 ± 0.1. Dilute with water to 1000 mL.
Mobile phase: Methanol and *Solution A* (13:12)
Standard stock solution: Transfer 50 mg of USP Glipizide RS to a 1000-mL low-actinic volumetric flask, and dissolve in 100 mL of methanol. Dilute with *Medium* to volume, and sonicate for 5 min. [NOTE—This solution is stable for 7 days at 5° when protected from light.]
Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a solution containing (L/1000) mg/mL, with L being the glipizide Tablet label claim, in mg.
Sample solution: After the specified time, withdraw 10 mL of the solution under test. Pass the solution through a suitable PVDF filter of 0.45-µm pore size or a glass fiber filter of 1.0-µm pore size, discarding the first mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms running for 8 min and measure the peak response for glipizide.
Calculate the percentage of $C_{21}H_{27}N_5O_4S$ dissolved:

$$\text{Result} = (r_U \times C_S \times V \times 100)/(r_S \times L)$$

r_U = peak response from the *Sample solution*
C_S = concentration of glipizide in the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
r_S = peak response from the *Standard solution*
L = label claim for glipizide (mg/Tablet)

Determination of metformin hydrochloride: Determine the amount of metformin hydrochloride dissolved by employing UV absorption at the wavelength of maximum absorbance at 233 nm on portions of the *Sample solution*, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Metformin Hydrochloride RS in the same *Medium*.
Calculate the amount of $C_4H_{11}N_5 \cdot HCl$ dissolved:

$$\text{Result} = (A_U \times C_S \times V \times 100)/(A_S \times L)$$

A_U = absorbance from the *Sample solution*
C_S = concentration of the *Standard solution* (mg/mL)
V = volume of *Medium*, 1000 mL
A_S = absorbance from the *Standard solution*
L = label claim for metformin hydrochloride (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{21}H_{27}N_5O_4S$ is dissolved in 45 min. NLT 80% (Q) of the labeled amount of $C_4H_{11}N_5 \cdot HCl$ is dissolved in 30 min.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: 0.05 M phosphate buffer, pH 6.8; 1000 mL
Apparatus 2: 50 rpm
Time: 60 min for both metformin hydrochloride and glipizide
pH 6.0 buffer solution: 4.3 g/L of octanesulfonic acid sodium salt and 6.9 g/L of monobasic monohydrate sodium phosphate in water, adjusted with diluted sodium hydroxide to a pH of 6.00 ± 0.05
Glipizide standard stock solution: 0.05 mg/mL of USP Glipizide RS in methanol
Standard solution: Transfer a quantity of USP Metformin Hydrochloride RS to a volumetric flask, add a suitable aliquot of *Glipizide standard stock solution*, and dilute with *Medium* to obtain a final concentration of (L/1000) mg/mL, where L is the Tablet label claim for both metformin hydrochloride and glipizide, in mg.
Sample solution: Pass a portion of the solution under test through a suitable polyethersulfone filter of 0.45-µm pore size, discarding the first few mL.
Mobile phase: Methanol and *pH 6.0 buffer solution* (1:1)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Sample compartment chiller: 4°
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.0 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*

www.webofpharma.com

Suitability requirements
Resolution: NLT 2 between the glipizide and metformin hydrochloride peaks
Relative standard deviation: NMT 2.0% for both glipizide and metformin hydrochloride
Analysis: Calculate the percentages of $C_{21}H_{27}N_5O_4S$ and $C_4H_{11}N_5 \cdot HCl$ dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response for glipizide or metformin hydrochloride from the *Sample solution*
r_S = peak response for glipizide or metformin hydrochloride from the *Standard solution*
C_S = concentration of glipizide or metformin hydrochloride in the *Standard solution* (mg/mL)
L = label claim for glipizide or metformin hydrochloride (mg/Tablet)
V = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (Q) of the labeled amounts of $C_{21}H_{27}N_5O_4S$ and $C_4H_{11}N_5 \cdot HCl$ is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES
ORGANIC IMPURITIES
• **Procedure 1: Glipizide**
Solution A, Solution B, Solution C, Sample solution, and **Chromatographic system:** Prepare as directed in the *Assay* for *Glipizide*.
Analysis
Sample: *Sample solution*
Calculate the percentage of glipizide related compound A (approximate relative retention time 0.92) and other individual impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity
r_T = sum of all the peak responses
F = relative response factor for each impurity equal to 1.4 for glipizide related compound A and 1.0 for all other peaks

Acceptance criteria
Glipizide related compound A: NMT 2.0%
Individual impurities: NMT 0.5% of any other individual glipizide related impurity (eluting after approximately 8 min)
Total impurities: NMT 1.0%, excluding glipizide related compound A. [NOTE—Disregard the broad peak due to metformin that elutes before 8 min. Disregard any peak observed in the blank, and disregard any peak less than 0.05%.]
• **Procedure 2: Metformin Hydrochloride**
Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Prepare as directed in the *Assay* for *Metformin Hydrochloride*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of all the peak responses

Acceptance criteria
Individual impurities: NMT 0.1%
Total impurities: NMT 0.5%
[NOTE—Disregard any peak less than 0.05%. Disregard any peak observed in the blank.]

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Glipizide RS
USP Glipizide Related Compound A RS
N-{2-[(4-Aminosulfonyl)phenyl]ethyl}-5-methylpyrazinecarboxamide.
$C_{14}H_{16}N_4O_3S$ 320.37
USP Metformin Hydrochloride RS
USP Metformin Related Compound A RS
1-Cyanoguanidine.

Immune Globulin

» Immune Globulin conforms to the regulations of the FDA concerning biologics (640.100 to 640.104) (see *Biologics* ⟨1041⟩). It is a sterile, nonpyrogenic solution of globulins that contains many antibodies normally present in adult human blood, prepared by pooling approximately equal amounts of material (source blood, plasma, serum, or placentas) from not less than 1000 donors. It contains not less than 15 g and not more than 18 g of protein per 100 mL, not less than 90.0 percent of which is gamma globulin. It contains 0.3 M glycine as a stabilizing agent and contains a suitable preservative. It has a potency of component antibodies of diphtheria antitoxin based on the U.S. Standard Diphtheria Antitoxin and a diphtheria test toxin, tested in guinea pigs (not less than 2 antitoxin units per mL), and antibodies for measles and poliovirus. It meets the requirements of the tests for heat stability in absence of gelation on heating, and for pH.

Packaging and storage—Preserve at a temperature between 2° and 8°.
Expiration date—The expiration date is not later than 3 years after date of issue from manufacturer's cold storage (5°, 3 years).
Labeling—Label it to state that passive immunization with Immune Globulin modifies hepatitis A, prevents or modifies measles, and provides replacement therapy in persons having hypo- or agammaglobulinemia, that it is not standardized with respect to antibody titers against hepatitis B surface antigen and that it should be used for prophylaxis of viral hepatitis type B only when the specific Immune Globulin is not available, that it may be of benefit in women who have been exposed to rubella in the first trimester of pregnancy but who would not consider a therapeutic abortion, and that it may be used in immunosuppressed patients for passive immunization against varicella if the specific Immune Globulin is not available. Label

www.webofpharma.com

it also to state that it is not indicated for routine prophylaxis or treatment of rubella, poliomyelitis, or mumps, or for allergy or asthma in patients who have normal levels of immunoglobulin, that the plasma units from which it has been derived have been tested and found non-reactive for hepatitis B surface antigen, and that it should not be administered intravenously but be given intramuscularly, preferably in the gluteal region.

Rh_o (D) Immune Globulin

» Rh_o (D) Immune Globulin conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile, nonpyrogenic solution of globulins derived from human blood plasma containing antibody to the erythrocyte factor Rh_o (D). It contains not less than 10 g and not more than 18 g of protein per 100 mL, not less than 90.0 percent of which is gamma globulin. It has a potency, determined by a suitable method, not less than that of the U.S. Reference Rh_o (D) Immune Globulin. It contains 0.3 M glycine as a stabilizing agent and contains a suitable preservative.

Packaging and storage—Preserve at a temperature between 2° and 8°.
Expiration date—The expiration date is not later than 6 months from the date of issue from manufacturer's cold storage, or not later than 1 year from the date of manufacture, as indicated on the label.

Glucagon

HSQGTFTSDY SKYLDSRRAQ DFVQWLMNT

$C_{153}H_{225}N_{43}O_{49}S$ 3482.75
Glucagon (human) [16941-32-5].

DEFINITION

Change to read:

Glucagon is a polypeptide hormone that has the property of increasing the concentration of glucose in the blood. It has the same structure (29 amino acids) as the hormone produced by the α-cells of the human pancreas. Glucagon is produced ▲from either synthetic or▲ (USP 1-Dec-2019) microbial processes using recombinant DNA (rDNA) technology. ▲When produced by microbial processes, the▲ (USP 1-Dec-2019) host cell–derived protein content and/or the host cell–derived or vector–derived DNA content are determined by validated methods. During the course of product development, it must be demonstrated that the manufacturing process produces Glucagon having a biological activity of NLT 0.80 USP Units/mg, using a validated bioassay approved by a competent authority. It contains NLT 90% and NMT 105% of glucagon ($C_{153}H_{225}N_{43}O_{49}S$), calculated on the anhydrous basis.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Change to read:

- **B.** Determine the peptide fragments, using the following peptide mapping procedure.

Ammonium bicarbonate buffer: Prepare a 1 M ammonium bicarbonate solution, and adjust with ammonia TS to a pH of 10.3. Prepare a mixture of 1 M ammonium bicarbonate and water (1:9).
Enzyme solution: 2 mg/mL of α-chymotrypsin (peptide mapping grade) in *Ammonium bicarbonate buffer*
Solution A: Prepare a degassed mixture of 0.5 mL of trifluoroacetic acid and 1000 mL of water.
Solution B: Prepare a degassed mixture of 0.5 mL of trifluoroacetic acid, 600 mL of ethanol, and 400 mL of water.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	53	47
45	0	100
46	100	0
75	100	0

Standard digest solution: Prepare a 5-mg/mL solution of USP rGlucagon RS in 0.01 M hydrochloric acid. Mix 200 µL of this solution with 800 µL of *Ammonium bicarbonate buffer*. To this solution add 25 µL of *Enzyme solution*, and place in a closed vial at about 37° for 2 h. Remove the vial, and stop the reaction immediately by adding 120 µL of glacial acetic acid.
Sample digest solution: Prepare a 5-mg/mL solution of Glucagon in 0.01 M hydrochloric acid. Proceed as directed for the *Standard digest solution*, beginning with "Mix 200 µL of this solution".
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.0-mm × 5-cm; 5-µm or less packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard digest solution*▲and *Sample digest solution*▲ (USP 1-Dec-2019)
Suitability requirements
Chromatogram similarity: ▲The chromatogram from the *Sample digest solution* shows a peak pattern similar to that of the chromatogram from the *Standard digest solution*. The peak pattern varies with respect to the purity of the chymotrypsin used for the digestion. If the chymotrypsin is very pure without trypsin, a chromatogram with four main peaks instead of five will be generated. Additional peptides due to minor cleavages may be seen, but where present, the pattern between the sample and reference is not different. [NOTE—The chromatogram from the USP rGlucagon RS certificate is provided as an example to

www.webofpharma.com

demonstrate the assignment of the predominant cleavage fragments.]

▲ (USP 1-Dec-2019)

Analysis

Samples: *Standard digest solution* and *Sample digest solution*

Acceptance criteria: The chromatographic profile of the *Sample digest solution* corresponds to that of the *Standard digest solution*.

ASSAY

Change to read:

• **PROCEDURE**

Solution A: Dissolve 16.3 g of potassium phosphate, monobasic in 750 mL of water, adjust with phosphoric acid to a pH of 2.7 (±0.05), add water to 800 mL, add 200 mL of acetonitrile, and degas.

Solution B: Prepare a degassed solution of acetonitrile and water (4:6).

Mobile phase: See *Table 2*. [NOTE—The ratio of *Solution A* to *Solution B* can be adjusted to obtain a retention time of about 21 min for the main peak.]

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	61	39
25[a]	61	39
29	12	88
30	12	88
31	61	39
▲45▲ (USP 1-Dec-2019)	61	39

[a] The end time of the isocratic elution can be adjusted so that the gradient begins after the fourth desamido peak elutes (relative retention time about 1.4). The rest of the program is then adjusted accordingly with this offset.

System suitability solution: Reconstitute a vial of USP rGlucagon RS in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.5 mg/mL. Let stand at 50° for 48 h. At least 7% total of all four desamido glucagons should be present in the solution.

Standard solution: Reconstitute a vial of USP rGlucagon RS in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.5 mg/mL.

Sample solution: 0.5 mg/mL of Glucagon in 0.01 N hydrochloric acid

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 3-mm × 15-cm; 3-µm packing L1

Column temperature: 45°

Flow rate: 0.5 mL/min

Injection volume: 15 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: Four peaks eluting after the glucagon peak that correspond to the desamido glucagons are clearly visible. The resolution between the main peak and the first eluting desamido peak is NLT 1.5, *System suitability solution*

Tailing factor: NMT 1.8 for the glucagon peak, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of glucagon ($C_{153}H_{225}N_{43}O_{49}S$) in the portion of Glucagon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90%–105% on the anhydrous basis

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Glucagon taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of the responses of all peaks

Acceptance criteria

Total of all four desamido glucagons: NMT 2.0%

Total impurities and related compounds: NMT 6.0%

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ic* **:** NMT 10%, determined on a 20- to 50-mg sample

Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** ▲The level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Glucagon is used can be met. Where the label states that Glucagon must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Glucagon is used can be met.▲ (USP 1-Dec-2019)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in airtight containers, protected from light, and store in a freezer.

Add the following:

▲• **LABELING:** The labeling states that the material is synthetic or of recombinant DNA origin.▲ (USP 1-Dec-2019)

• **USP REFERENCE STANDARDS** ⟨11⟩

USP rGlucagon RS

www.webofpharma.com

Glucagon for Injection

DEFINITION

Glucagon for Injection is a sterile lyophilized mixture of the hydrochloride of glucagon with one or more suitable buffering and stabilizing agents. It contains NLT 65% and NMT 110% of the labeled amount of glucagon.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. GLUCAGON BIOIDENTITY TESTS** ⟨123⟩**:** Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: Dissolve 16.3 g of monobasic potassium phosphate in 750 mL of water, adjust with phosphoric acid to a pH of 2.7 (± 0.05), add water to 800 mL, add 200 mL of acetonitrile, and degas.

Solution B: Prepare a degassed solution of acetonitrile and water (4:6).

Mobile phase: See *Table 1*. [NOTE—The ratio of *Solution A* to *Solution B* can be adjusted to obtain a retention time of about 21 min for the main peak.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	61	39
25[a]	61	39
29	12	88
30	12	88
31	61	39
70	61	39

[a] The end time of the isocratic elution can be adjusted so that the gradient begins after the 4th desamido peak elutes (relative retention time about 1.4). The rest of the program is then adjusted accordingly with this offset.

System suitability solution: Reconstitute a vial of USP rGlucagon RS in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.5 mg/mL. Let stand at 50° for 48 h. At least 7% total of all four desamido glucagons should be present in the solution.

Standard solution: Reconstitute a vial of USP rGlucagon RS in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.5 mg/mL.

Sample solution: Dissolve an adequate amount of Glucagon for Injection in order to obtain a 0.5-mg/mL concentration of glucagon in 0.01 N hydrochloric acid.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 3-mm × 15-cm; 3-µm or less packing L1

Column temperature: 45°

Flow rate: 0.5 mL/min

Injection volume: 15 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between the main peak and the first eluting desamido peak. Four peaks eluting after the glucagon peak that correspond to the desamido glucagons are clearly visible, *System suitability solution*

Tailing factor: NMT 1.8 for the glucagon peak, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of glucagon ($C_{153}H_{225}N_{43}O_{49}S$) in the portion of Glucagon for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 65%–110%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Sample solution: Dissolve the substance to be examined in water in order to obtain a concentration of 0.5 mg/mL of glucagon.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Glucagon for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
r_T = sum of the responses of all peaks

Acceptance criteria: NMT 14% total of all four desamido glucagons is found, and NMT 31% of total impurities and related compounds is found.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I, Method Ic* ⟨921⟩**:** NMT 4.0%
- **pH AND CLARITY OF SOLUTION:** Dissolve it in the solvent and in the concentration recommended in the labeling: the pH of the solution is between 1.7 and 3.5, and the solution is clear.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 10 USP Endotoxin Units/mg.
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions.*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for Constitution.*

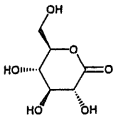
Change to read:

- **LABELING:** The labeling states that the material is of recombinant DNA ▲or of synthetic▲ (RB 1-Feb-2019) origin.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP rGlucagon RS

www.webofpharma.com

Gluconolactone



$C_6H_{10}O_6$ 178.14
D-Gluconic acid δ-lactone;
Glucono δ-lactone [90-80-2].

DEFINITION
Gluconolactone contains NLT 99.0% and NMT 101.0% of gluconolactone ($C_6H_{10}O_6$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

ASSAY
- **PROCEDURE**
  Sample: 600 mg
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Residual titration
  Titrant: 0.1 N sodium hydroxide VS
  Back-titrant: 0.1 N hydrochloric acid VS
  Endpoint detection: Visual
  Analysis: Dissolve the *Sample* in 100 mL of water in a 300-mL conical flask, add 50.0 mL of *Titrant*, and allow to stand for 15 min. Add phenolphthalein TS. Titrate excess alkali with *Back-titrant*. Perform a blank determination. Each milliliter of *Titrant* is equivalent to 17.81 mg of gluconolactone ($C_6H_{10}O_6$).
  Acceptance criteria: 99.0%–101.0%

IMPURITIES
- **LEAD** ⟨251⟩
  Test preparation: Prepare as directed in the chapter.
  Analysis: Use 10 mL of *Diluted standard lead solution* (10 µg of lead) for the procedure.
  Acceptance criteria: NMT 10 ppm
- **REDUCING SUBSTANCES**
  Sample solution: Transfer 10.0 g to a 400-mL beaker, add 40 mL of water, and swirl to dissolve. Add 2 drops of phenolphthalein TS, and neutralize with sodium hydroxide solution (1 in 2). Dilute with water to 50 mL, and add 50 mL of alkaline cupric tartrate TS. Heat so that the solution begins to boil in 4 min, and allow boiling to continue for 120 s. Pass the suspension through a filtering crucible of medium pore size, and wash the filter with three 5-mL portions of water. Place the crucible in an upright position in the original beaker, add 5 mL of water and 3 mL of nitric acid to the crucible, mix with a glass rod to ensure complete solution of the cuprous oxide, and wash the solution from the crucible into a beaker with the aid of 5 mL of water. Add bromine TS, usually 5–10 mL, until the solution becomes yellow in color, and dilute with water to 75 mL. Add a few glass beads, boil until the bromine has been driven off, and cool. Slowly add ammonium hydroxide until a deep blue color appears, then adjust with glacial acetic acid to a pH of 4, and add water to make 100 mL.
  Analysis: Add 4 g of potassium iodide to the *Sample solution*, and titrate with 0.1 N sodium thiosulfate VS, adding starch TS just before the endpoint is reached.
  Acceptance criteria: NMT 16.1 mL of 0.1 N sodium thiosulfate is consumed.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Gluconolactone RS

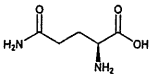
Glucose Enzymatic Test Strip

» Glucose Enzymatic Test Strip consists of the enzymes glucose oxidase and horseradish peroxidase, a suitable substrate for the reaction of hydrogen peroxide catalyzed by peroxidase, and other inactive ingredients impregnated and dried on filter paper. When tested in human urine containing known glucose concentrations, it reacts in the specified times to produce colors corresponding to the color chart provided.

Packaging and storage—Preserve in the original container, in a dry place, at controlled room temperature.
Identification—Remove the Strips from the container, and test them as directed in the instructions provided by the manufacturer, first in dextrose solution (1 in 50) and then in sucrose solution (1 in 50): color develops from the dextrose solution, but not from the sucrose solution.

Calibration—
Glucose standard solutions—Dissolve anhydrous dextrose in separate portions of freshly voided, normal, glucose-free human urine, previously adjusted to a pH of 6.0 with dilute formic acid (1 in 5), and with sodium hydroxide solution (1 in 2), respectively, to obtain separate solutions of the final concentrations corresponding to the color chart calibrations provided. Allow the standard solutions to stand for 1 hour prior to use.
Procedure—Remove the Strips from the container, and test each *Glucose standard solution* as directed in the instructions provided by the manufacturer: the colors formed on each of the Strips during the specified times match the colors on the color chart provided.

Glutamine



$C_5H_{10}N_2O_3$ 146.14
L-Glutamine;
2-Aminoglutaramic acid [56-85-9].

DEFINITION
Glutamine contains NLT 98.5% and NMT 101.5% of L-glutamine ($C_5H_{10}N_2O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Sample: 150 mg of Glutamine

Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with the *Titrant*. Perform a *Blank* determination.

Calculate the percentage of glutamine ($C_5H_{10}N_2O_3$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = volume of *Titrant* consumed by the *Sample* (mL)

V_B = volume of *Titrant* consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 146.1 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.3%

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Standard solution: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.7 g of Glutamine

Acceptance criteria: NMT 0.05%

• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Standard solution: 0.25 mL of 0.020 N sulfuric acid

Sample: 0.8 g of Glutamine

Acceptance criteria: NMT 0.03%

• **IRON** ⟨241⟩**:** NMT 30 ppm

• **RELATED COMPOUNDS**

Standard solution: 0.05 mg/mL of USP Glutamine RS in water

Sample solution: 10 mg/mL of Glutamine in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)

Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

Analysis: Dry the plate at 80° for 30 min. Spray the plate with *Spray reagent*, heat at 80° for 10 min, and examine under white light.

Acceptance criteria: No secondary spot of the *Sample solution* is larger or more intense than the principal spot of the *Standard solution* (NMT 0.5%).

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 40 mg/mL in water. Warm at 40° to dissolve.

Analysis: Proceed as directed in the chapter, except measure at 20°.

Acceptance criteria: +6.3° to +7.3°

• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 3 h: it loses NMT 0.3% of its weight.

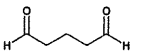
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Glutamine RS

Glutaral Concentrate



$C_5H_8O_2$ 100.12

Pentanedial;

Glutaraldehyde [111-30-8].

DEFINITION

Glutaral Concentrate is a solution of glutaraldehyde in Purified Water. It contains NLT 100.0% and NMT 104.0% of the labeled amount of glutaral ($C_5H_8O_2$). The labeled amount is 50.0 g of $C_5H_8O_2$ per 100 g of Concentrate.

IDENTIFICATION

• **A.**

Solution A: Add 4 mL of sulfuric acid to 0.8 g of 2,4-dinitrophenylhydrazine, then add 6 mL of water, dropwise, with swirling. When dissolution is essentially complete, add 20 mL of alcohol, and filter. The filtrate is the 2,4-dinitrophenylhydrazine reagent.

Analysis: Add 0.4 mL of Concentrate to 20 mL of *Solution A*, mix by swirling, and allow to stand for 5 min. Collect the precipitate on a filter, and rinse thoroughly with alcohol. Dissolve the precipitate in 20 mL of hot ethylene dichloride, filter, and cool the filtrate in an ice bath until crystallization occurs. Collect the precipitate on a filter. Redissolve the precipitate by refluxing with 30 mL of acetone, filter, and cool the filtrate in an ice bath until crystallization occurs. Collect the precipitate on a filter.

Acceptance criteria: The 2,4-dinitrophenylhydrazone so obtained melts at 185°–195°, within a 3° range (see *Melting Range or Temperature* ⟨741⟩).

ASSAY

Change to read:

• **PROCEDURE**

Solution A: 35 g/L of hydroxylamine hydrochloride prepared as follows. Dissolve 35 g of hydroxylamine hydrochloride in 150 mL of water in a 1000-mL volumetric flask. Add isopropyl alcohol to volume.

Solution B: Transfer 65 mL of triethanolamine to a glass-stoppered, 1000-mL volumetric flask, and add water to volume.

Analysis: To 500 mL of *Solution A* add 15 mL of a solution of bromophenol blue in alcohol (1 in 2500), and add *Solution B* from a buret to obtain a neutralized solution that appears greenish-blue by transmitted light. Transfer 65.0 mL of the neutralized solution to a glass-stoppered, 500-mL conical flask, add 50.0 mL of *Solution B*, purge with nitrogen, and insert the stopper. Add 1.2 g of Glutaral Concentrate. Insert the stopper, and allow to stand for 60 min, swirling the flask occasionally. Titrate with 0.5 N sulfuric acid VS to a greenish-blue endpoint, and perform a blank determination (see *Titrimetry* ⟨541⟩, *Residual Titrations*).

Calculate the percentage of the labeled amount of glutaral ($C_5H_8O_2$) in the Concentrate taken:

www.webofpharma.com

$$\text{Result} = \{[(V_B - V_S) \times N \times F]/W\} \times 100$$

V_B = volume of 0.5 N sulfuric acid VS consumed by the blank (mL)
V_S = volume of 0.5 N sulfuric acid VS consumed by the *Sample solution* (mL)
N = normality of the sulfuric acid
F = equivalency factor for glutaraldehyde, 0.05006 g/mEq
W = ▲nominal weight of glutaral▲ (ERR 1-Dec-2018) taken (g)

Acceptance criteria: 100.0%–104.0%

IMPURITIES

SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩: 1.126–1.135 at 20°
- **ACIDITY**

Analysis: Transfer 60.0 g to a conical flask, add phenolphthalein TS, and titrate with 0.10 N alcoholic potassium hydroxide to a pink endpoint that is permanent for NLT 15 s.

Acceptance criteria: NMT 40 mL of 0.10 N potassium hydroxide is consumed, corresponding to NMT 0.4% (w/w) of acid, calculated as acetic acid.

- **pH** ⟨791⟩: 3.7–4.5
- **CLARITY OF SOLUTION**

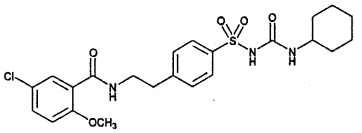
Analysis: Transfer 5.0 mL of Concentrate to a glass-stoppered, 100-mL graduated cylinder, add water to obtain 100 mL of mixture, insert the stopper, and mix by inverting the graduated cylinder several times. Allow the bubbles to rise, and view downward through the solution against a dark background.

Acceptance criteria: The solution is clear.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and avoid exposure to excessive heat.
- **LABELING:** The label states that this article is not intended for direct administration to humans or animals.

Glyburide



$C_{23}H_{28}ClN_3O_5S$ 494.00

Benzamide, 5-chloro-*N*-[2-[4-[[[(cyclohexylamino)carbon yl] amino]-sulfonyl]phenyl]ethyl]-2-methoxy-.
1-[[*p*-[2-(5-Chloro-*o*-anisamido)ethyl]phenyl]sulfonyl]-3-cyclohex-ylurea [10238-21-8].

» Glyburide contains not less than 98.0 percent and not more than 102.0 percent of $C_{23}H_{28}ClN_3O_5S$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Glyburide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for glyburide, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

Loss on drying—Dry it at 105° for 6 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.5%.

Chromatographic purity—

Mobile phase—Prepare as directed in the *Assay*.

Test solution—To about 10 mg of Glyburide, accurately weighed, add 10 mL of acetonitrile, and shake to dissolve. Add 4 mL of water, and mix.

Chromatographic system—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 3500 theoretical plates.

Procedure—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Glyburide taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all of the peaks: not more than 1.5% of any impurity, which elutes before glyburide, is found; not more than 0.5% of any other individual impurity is found; and not more than 2.0% of total impurities is found.

Assay—

Mobile phase—Dissolve 2.6 g of monobasic ammonium phosphate in 450 mL of water. Add 550 mL of acetonitrile, filter, and degas. Adjust, if necessary, with phosphoric acid or sodium hydroxide to a pH of 5.25 ± 0.30. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve progesterone in acetonitrile to obtain a solution containing about 0.2 mg per mL.

Standard preparation—To about 10 mg of USP Glyburide RS, accurately weighed, add 20.0 mL of *Internal standard solution*, and shake vigorously to dissolve. Add 4.0 mL of water, and mix.

Assay preparation—To about 10 mg of Glyburide, accurately weighed, add 20.0 mL of *Internal standard solution*, and shake vigorously to dissolve. Add 4.0 mL of water, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.4 for glyburide and 1.0 for progesterone; the resolution, *R*, between glyburide and progesterone is not less than 5.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the

USP Monographs

www.webofpharma.com

chromatograph, record the chromatograms, and measure the heights for the major peaks. Calculate the quantity, in mg, of $C_{23}H_{28}ClN_3O_5S$ in the portion of Glyburide taken by the formula:

$$W_S (R_U/R_S)$$

in which W_S is the weight, in mg, of USP Glyburide RS taken to prepare the *Standard preparation;* and R_U and R_S are the peak height ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Glyburide Tablets

DEFINITION

Glyburide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$).

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K▲** (CN 1-May-2020)

Sample: Grind a number of Tablets, equivalent to 15 mg of glyburide, to a fine powder. Add 30 mL of acetonitrile, and shake. Filter the mixture, evaporate the filtrate to dryness, and dry the residue under vacuum at 60° for 3 h.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **Procedure**

Mobile phase: Dissolve 2.6 g of monobasic ammonium phosphate in 450 mL of water. Add 550 mL of acetonitrile, filter, and degas. Adjust, if necessary, with phosphoric acid or sodium hydroxide to a pH of 5.25 ± 0.30.

System suitability stock solution: 0.1 mg/mL of USP Glyburide Related Compound A in acetonitrile

System suitability solution: To 10 mg of USP Glyburide RS add 20 mL of the *System suitability stock solution,* and shake vigorously to dissolve. Add 4.0 mL of water.

Standard solution: To 10 mg of USP Glyburide RS add 20.0 mL of acetonitrile, and shake vigorously to dissolve. Add 4.0 mL of water.

Sample solution: Transfer NLT 20 Tablets to a suitable container. Add water equivalent to 0.4 mL of water per mg of glyburide, and swirl to disperse and wet Tablet material. Then add acetonitrile equivalent to 2.0 mL of acetonitrile per mg of glyburide, and shake for 30 min. Centrifuge a portion of the suspension, and use the clear supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 2 mL/min
Injection volume: 10 µL

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for glyburide related compound A and glyburide are about 0.5 and 1.0, respectively.]

Suitability requirements
Resolution: NLT 4.0 between glyburide related compound A and glyburide
Relative standard deviation: NMT 2.0% for glyburide

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (W_S/V_S)/(W_U/V_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
W_S = weight of USP Glyburide RS, used to prepare the *Standard solution* (mg)
V_S = sum of volumes of acetonitrile and water used to prepare the *Standard solution* (mL)
W_U = nominal amount of glyburide used to prepare the *Sample solution* (mg)
V_U = sum of volumes of acetonitrile and water used to prepare the *Sample solution* (mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **Dissolution ⟨711⟩**

Test 1 (nonmicronized glyburide)
Medium: 0.05 M borate buffer, pH 9.5 (381.5 g of sodium borate and 19.1 g of sodium hydroxide in 20 L of water, and adjust with phosphoric acid to a pH of 9.5 ± 0.1); 500 mL
Apparatus 2: 75 rpm
Time: 45 min
Mobile phase: Acetonitrile and water (1:1) containing 4.0 mL of phosphoric acid per L of solution
Standard stock solution: 0.15 mg/mL of USP Glyburide RS in *Medium.* Sonicate for about 25 min to dissolve, and dilute with *Medium* to volume.
Standard solutions: Dilute the *Standard stock solution* with *Medium* to obtain 0.003 mg/mL (for Tablets labeled to contain 1.5 mg), 0.006 mg/mL (for Tablets labeled to contain 3.0 mg), 0.009 mg/mL (for Tablets labeled to contain 4.5 mg), and 0.012 mg/mL (for Tablets labeled to contain 6.0 mg).
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 215 nm
Column: 4.6-mm × 30-cm; 10-µm packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)

USP Monographs

www.webofpharma.com

V = volume of *Medium*, 500 mL

Tolerances: NLT 70% (Q) of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) is dissolved.

Test 2 (micronized glyburide): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.05 M phosphate buffer, pH 8.5 (6.8 g of monobasic potassium phosphate and 1.99 g of sodium hydroxide in 1 L of water, and adjust with diluted phosphoric acid or diluted sodium hydroxide to a pH of 8.5 ± 0.05); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Mobile phase: Acetonitrile and water containing 5 g/L of monobasic ammonium phosphate (480:520)
Standard stock solution: Transfer 67 mg of USP Glyburide RS to a 500-mL volumetric flask, dissolve in 40 mL of methanol with sonication for 5 min, and dilute with *Medium* to volume.
Standard solutions: Dilute the *Standard stock solution* with *Medium* to obtain solutions having known concentrations of 0.0017 mg/mL (for Tablets labeled to contain 1.5 mg), 0.0034 mg/mL (for Tablets labeled to contain 3 mg), 0.0047 mg/mL (for Tablets labeled to contain 4.5 mg), and 0.0067 mg/mL (for Tablets labeled to contain 6 mg).
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.5-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 4.0-mm × 25-cm; 10-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) is dissolved.

Test 3 (micronized glyburide): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 0.05 M phosphate buffer, pH 7.5 (40.8 g of monobasic potassium phosphate and 9.4 g of sodium hydroxide, in 6 L of water, and adjust with diluted sodium hydroxide to a pH of 7.5 ± 0.1); 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase: Proceed as directed in the *Assay*.
Diluent: Acetonitrile and water (5:1)
Standard stock solution: 0.67 mg/mL of USP Glyburide RS in *Diluent*
Standard solution: 6.7 µg/mL of USP Glyburide RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 2 mL/min
Injection volume: 75 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) is dissolved.

Test 4 (nonmicronized glyburide): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: 0.05 M borate buffer, pH 8.0, with 0.014 M hexadecyltrimethylammonium bromide (prepared by dissolving about 180.0 g of hexadecyltrimethylammonium bromide, 55.6 g of boric acid, 67.1 g of potassium chloride, and 2.8 g of sodium hydroxide in 1500 mL of water at 50° under vigorous stirring for several hours, cooling to room temperature, diluting with water to 2000 mL, adjusting with diluted hydrochloric acid or diluted sodium hydroxide to a pH of 8.00 ± 0.05, and diluting 50 mL of this solution with water to 900 mL); 900 mL
Apparatus 1: 50 rpm
Time: 45 min
Mobile phase: Acetonitrile and water (11:9) containing 5.2 g of monobasic ammonium phosphate for each 2 L
Standard stock solution: 0.27 mg/mL of USP Glyburide RS in alcohol. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume.
Standard solution: 2.8 µg/mL of USP Glyburide RS in *Medium* from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 5-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 25-cm; packing L7
Flow rate: 2 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the percentage of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) is dissolved.

Test 5 (micronized glyburide): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: 0.05 M phosphate buffer, pH 7.5 (40.8 g of monobasic potassium phosphate and 9.4 g of sodium hydroxide, in 6 L of water, and adjust with diluted sodium hydroxide to a pH of 7.5 ± 0.1); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Standard solution: USP Glyburide RS in *Medium* in a concentration similar to the one expected in the *Sample solution*
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Mobile phase, Diluent, Chromatographic system, System suitability, and **Analysis:** Proceed as directed for *Test 3*.
Tolerances: NLT 75% (*Q*) of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) is dissolved.

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase: Dissolve 0.087 g of dibasic potassium phosphate and 0.612 g of monobasic potassium phosphate in 550 mL of water to obtain a solution with a pH of 6.00 ± 0.05. Add 450 mL of methanol.
Diluent: Dissolve 0.871 g of dibasic potassium phosphate in 550 mL of water, and dilute with methanol to 1 L.
Standard stock solution: 0.1 mg/mL each of USP Glyburide RS and USP Glyburide Related Compound A RS in methanol
Standard solution: 0.0004 mg/mL each of USP Glyburide RS and USP Glyburide Related Compound A RS in *Mobile phase* from the *Standard stock solution*. This solution is stable for 48 h when stored at room temperature.
Sample solution: Weigh, and powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to about 20 mg of glyburide, to a 100-mL volumetric flask. Add 80 mL of *Diluent*, and sonicate for 15 min, then shake the flask for 10 min. Dilute with *Diluent* to volume, and mix. Centrifuge a portion of this solution, and use a clear supernatant. This solution is stable for 14 h when stored at room temperature.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 3.9-mm × 15-cm; 4-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for glyburide related compound A and glyburide are about 0.3 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 15 between glyburide related compound A and glyburide
Relative standard deviation: NMT 5% for glyburide related compound A
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of glyburide related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of glyburide related compound A from the *Sample solution*
r_S = peak response of glyburide related compound A from the *Standard solution*
C_S = concentration of USP Glyburide Related Compound A RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of glyburide in the *Sample solution* (mg/mL)

Acceptance criteria
Glyburide related compound A: NMT 5.3%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Glyburide RS
USP Glyburide Related Compound A RS
4-[2-(5-Chloro-2-methoxybenzamido)ethyl]benzenesulfonamide.
$C_{16}H_{17}ClN_2O_4S$ 368.83

Glyburide and Metformin Hydrochloride Tablets

DEFINITION

Glyburide and Metformin Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of glyburide ($C_{23}H_{28}ClN_3O_5S$) and metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$).

IDENTIFICATION

• **A. GLYBURIDE:** The retention time of the glyburide peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Glyburide*.
• **B. METFORMIN HYDROCHLORIDE:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Metformin Hydrochloride*.

ASSAY

• **GLYBURIDE**
Buffer: 28.8 g/L of monobasic ammonium phosphate
Mobile phase: Acetonitrile and *Buffer* (40:60). Adjust with 1 N sodium hydroxide to a pH of 5.3.
Diluent: Acetonitrile and water (50:50)
Standard stock solution: 0.25 mg/mL of USP Glyburide RS prepared as follows. Transfer a weighed amount of USP Glyburide RS to a suitable volumetric flask. Dissolve first in the acetonitrile, using 50% of the final volume, and then dilute with water to volume.

USP Monographs

www.webofpharma.com

Standard solution: 0.025 mg/mL of USP Glyburide RS in *Diluent*, from the *Standard stock solution*
System suitability solution 1: Prepare a solution containing 0.025 mg/mL of USP Glyburide Related Compound A RS in *Diluent*. Transfer 50 µL of this solution to a 50-mL volumetric flask, and dilute with *Standard solution* to volume.
System suitability solution 2: 5.0 mg/mL of USP Metformin Hydrochloride RS in *System suitability solution 1*
Sample solution: Dissolve NLT 5 Tablets in *Diluent* by stirring with a magnetic stirring bar for at least 1 h. Dilute to obtain a solution containing 0.025 mg/mL of glyburide, based on the label claim. Centrifuge a portion of this solution at 3000 rpm for 10 min and use the clear supernatant. [NOTE—Retain a portion of this solution for the *Assay* for *Metformin Hydrochloride*.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 100 µL
Run time: 1.25 times the retention time of glyburide
System suitability
Sample: *System suitability solution 2*
[NOTE—The relative retention time for glyburide related compound A is about 0.30 with respect to glyburide.]
Suitability requirements
Relative standard deviation: NMT 1.5% for the glyburide peak; NMT 10% for the glyburide related compound A peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of glyburide from the *Sample solution*
r_S = peak response of glyburide from the *Standard solution*
C_S = concentration of USP Glyburide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of glyburide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of glyburide

• **METFORMIN HYDROCHLORIDE**
Buffer: Transfer 1.0 g each of sodium heptanesulfonate and sodium chloride to a 2000-mL volumetric flask. Add 1800 mL of water, and adjust with 0.06 M phosphoric acid to a pH of 3.85. Dilute with water to volume.
Mobile phase: Acetonitrile and *Buffer* (10:90)
[NOTE—To improve the separation, the composition of acetonitrile and *Buffer* may be changed to 5:95, if necessary.]
Diluent: Acetonitrile and water (1:40)
Standard solution: 0.25 mg/mL of USP Metformin Hydrochloride RS in *Diluent*. [NOTE—Sonicate to achieve complete dissolution, if necessary.]
System suitability stock solution: 25 µg/mL each of USP Metformin Related Compound B RS and USP Metformin Related Compound C RS in *Diluent*
System suitability solution: Transfer 0.5 mL of the *System suitability stock solution* to a 50-mL volumetric flask, and dilute with *Standard solution* to volume.
Sample solution: Dilute with water a portion of the retained *Sample solution* from the *Assay* for *Glyburide* to obtain 0.25 mg/mL of metformin hydrochloride based on the label claim.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 218 nm
Column: 3.9-mm × 30-cm; 10-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for metformin related compound B, metformin, and metformin related compound C are about 0.86, 1.0, and 2.1–2.3, respectively. Metformin related compound C can have a variable retention time. The composition of acetonitrile and *Buffer* in *Mobile phase* may be changed to 5:95, if it elutes at a relative retention time of less than 2.1.]
Suitability requirements
Resolution: NLT 1.5 between metformin related compound B and metformin
Tailing factor: 0.8–2.0 for the metformin peak
Relative standard deviation: NMT 1.5% for the metformin peak; NMT 10% each for the metformin related compound B and metformin related compound C peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metformin from the *Sample solution*
r_S = peak response of metformin from the *Standard solution*
C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of metformin hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of metformin hydrochloride

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Glyburide
Medium: 0.05 M boric acid and 0.05 M potassium chloride solution. Prepare by dissolving 3.09 g of boric acid and 3.73 g of potassium chloride in 250 mL of water, adjust with 1 N sodium hydroxide to a pH of 9.5, and dilute with water to 1 L; 500 mL.
Apparatus 2: 75 rpm
Time: 30 min
Standard solution: 0.1 mg/mL of USP Glyburide RS prepared as follows. Transfer a weighed amount of USP Glyburide RS to a suitable volumetric flask, dissolve first in acetonitrile, using 20% of the final volume, then dilute with *Medium* to volume. Dilute further with *Medium* to obtain a solution having a glyburide

USP Monographs

www.webofpharma.com

concentration, in mg/mL, of (L/500), where L is the label claim of glyburide in mg/Tablet.

Sample solution: Sample per *Dissolution* ⟨711⟩. Pass a portion of the solution under test through a polypropylene filter of 0.45-µm pore size or a glass fiber filter of 1-µm pore size.

Buffer: 28.8 g/L of monobasic ammonium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (1:1). Adjust with 1 N sodium hydroxide to a pH of 5.3.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 200 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–2.0

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of glyburide from the *Sample solution*

r_S = peak response of glyburide from the *Standard solution*

C_S = concentration of USP Glyburide RS in the *Standard solution* (mg/mL)

L = label claim of glyburide (mg/Tablet)

V = volume of *Medium*, 500 mL

Tolerances: NLT 85% (Q) of the labeled amount of glyburide is dissolved.

Metformin hydrochloride

Medium: 0.05 M phosphate buffer, pH 6.8. Prepare by dissolving 6.8 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 0.2 N sodium hydroxide to a pH of 6.8 ± 0.1; 1000 mL.

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: Dissolve a quantity of USP Metformin Hydrochloride RS in *Medium*, to obtain a solution having a metformin hydrochloride concentration, in mg/mL, of (L/1000), where L is the label claim of metformin hydrochloride in mg/Tablet. Dilute further, if necessary, with *Medium*.

Sample solution: Sample per *Dissolution* ⟨711⟩. Pass a portion of the solution under test through a polypropylene filter of 0.45-µm pore size or a glass fiber filter of 1-µm pore size. Dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV-Vis

Analytical wavelength: 232 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 1000 mL

D = dilution factor for the *Sample solution*

Tolerances: NLT 85% (Q) of the labeled amount of metformin hydrochloride is dissolved.

Test 2

Glyburide

Medium: 0.05 M boric acid and 0.05 M potassium chloride solution. Prepare by dissolving 3.09 g of boric acid and 3.73 g of potassium chloride in 250 mL of water, adjust with 1 N sodium hydroxide to a pH of 9.5, and dilute with water to 1 L; 500 mL.

Apparatus 2: 75 rpm

Time: 30 min

Solution A: Dissolve 0.288 g of sodium lauryl sulfate in 700 mL water.

Mobile phase: Acetonitrile, *Solution A*, and triethylamine (300:700:2). Adjust with phosphoric acid to a pH of 7.2.

Standard stock solution: 0.1 mg/mL of USP Glyburide RS prepared in acetonitrile

Standard solution: Dilute *Standard stock solution* with *Medium* to obtain a solution having a glyburide concentration, in mg/mL, of (L/500), where L is the label claim of glyburide in mg/Tablet.

Sample solution: Sample per *Dissolution* ⟨711⟩. Pass a portion of the solution under test through a membrane filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 2 mL/min

Injection volume

For Tablets labeled to contain 5 mg/500 mg and 2.5 mg/500 mg: 20 µL

For Tablets labeled to contain 1.25 mg/250 mg: 50 µL

Run time: 8 min

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of glyburide ($C_{23}H_{28}ClN_3O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of glyburide from the *Sample solution*

r_S = peak response of glyburide from the *Standard solution*

C_S = concentration of USP Glyburide RS in the *Standard solution* (mg/mL)

L = label claim of glyburide (mg/Tablet)

V = volume of *Medium*, 500 mL

Supplement FCO

www.webofpharma.com

Tolerances: NLT 80% (*Q*) of the labeled amount of glyburide is dissolved.

Metformin hydrochloride

Medium: 0.05 M phosphate buffer, pH 6.8. Prepare by dissolving 6.8 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 0.2 N sodium hydroxide to a pH of 6.8 ± 0.1; 1000 mL.

Apparatus 2: 50 rpm

Time: 30 min

Solution A: Dissolve 0.288 g of sodium lauryl sulfate in 700 mL of water.

Mobile phase: Acetonitrile, *Solution A*, and triethylamine (300:700:2). Adjust with phosphoric acid to a pH of 7.2.

Standard solution

For Tablets labeled to contain 5 mg/500 mg and 2.5 mg/500 mg: 0.5 mg/mL of USP Metformin Hydrochloride RS in *Medium*. Sonicate before final dilution.

For Tablets labeled to contain 1.25 mg/250 mg: 0.25 mg/mL of USP Metformin Hydrochloride RS in *Medium*. Sonicate before final dilution.

Sample solution: Sample per *Dissolution* ⟨711⟩. Pass a portion of the solution under test through a membrane filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 2 mL/min

Injection volume

For Tablets labeled to contain 5 mg/500 mg and 2.5 mg/500 mg: 20 µL

For Tablets labeled to contain 1.25 mg/250 mg: 50 µL

Run time: 8 min

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 1000 mL

Tolerances: NLT 85% (*Q*) of the labeled amount of metformin hydrochloride is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements for *Weight Variation* for metformin hydrochloride and for *Content Uniformity* for glyburide

IMPURITIES

• **GLYBURIDE**

Buffer, Mobile phase, Diluent, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay* for *Glyburide*.

Standard solution: Dilute 1.0 mL of the *Standard solution* from the *Assay* for *Glyburide* with *Diluent* to 100 mL.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each glyburide impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each glyburide impurity from the *Sample solution*
r_S = peak response of glyburide from the *Standard solution*
C_S = concentration of USP Glyburide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of glyburide in the *Sample solution* (mg/mL)
F = relative response factor, 1.2 for glyburide related compound A, 1.0 for all other impurities

Acceptance criteria

[NOTE—Disregard any peak less than 0.05%, and disregard any peak observed in the blank.]

Glyburide related compound A: NMT 1.0%

Any other individual impurities: NMT 0.2%

Total impurities: NMT 0.50%, excluding glyburide related compound A

• **METFORMIN HYDROCHLORIDE**

Buffer, Mobile phase, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay* for *Metformin Hydrochloride*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each metformin impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each metformin impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria

[NOTE—Disregard any peak less than 0.05%, and disregard any peak observed in the blank.]

Individual metformin impurities: NMT 0.1%

Total impurities: NMT 0.5 %

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **LABELING:** When more than one dissolution test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Glyburide RS

USP Glyburide Related Compound A RS
4-[2-(5-Chloro-2-methoxybenzamido)ethyl] benzenesulfonamide.
$C_{16}H_{17}ClN_2O_4S$ 368.84

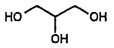
USP Metformin Hydrochloride RS

USP Metformin Related Compound B RS
1-Methylbiguanide hydrochloride.
$C_3H_9N_5 \cdot HCl$ 151.60

USP Metformin Related Compound C RS
Dimethylmelamine, or *N,N*-dimethyl-[1,3,5]triazine-2,4,6-triamine.
$C_5H_{10}N_6$ 154.17

www.webofpharma.com

Glycerin



$C_3H_8O_3$ 92.09
1,2,3-Propanetriol;
Glycerol [56-81-5].

DEFINITION
Glycerin contains NLT 99.0% and NMT 101.0% of $C_3H_8O_3$, calculated on the anhydrous basis.

IDENTIFICATION
[NOTE—Compliance is determined by meeting the requirements for *Identification* tests *A*, *B*, and *C*.]

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)
- **B. LIMIT OF DIETHYLENE GLYCOL AND ETHYLENE GLYCOL**

Standard solution: 2.0 mg/mL of USP Glycerin RS, 0.050 mg/mL of USP Ethylene Glycol RS, 0.050 mg/mL of USP Diethylene Glycol RS, and 0.10 mg/mL of 2,2,2-trichloroethanol (internal standard) in methanol
Sample solution: 50 mg/mL of Glycerin and 0.10 mg/mL of 2,2,2-trichloroethanol (internal standard) in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused-silica analytical column coated with 3.0-µm G43 stationary phase, and a deactivated split liner with glass wool
Temperature
Injector: 220°
Detector: 250°
Column: See the temperature program table.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	—	100	4
100	50	120	10
120	50	220	6

Carrier gas: Helium
Injection size: 1.0 µL
Flow rate: 4.5 mL/min
Injection type: Split ratio, about 10:1
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ethylene glycol, 2,2,2-trichloroethanol, diethylene glycol, and glycerin are about 0.3, 0.6, 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between diethylene glycol and glycerin
Analysis
Sample: *Sample solution*
Acceptance criteria: If a peak at the retention times for the diethylene glycol or ethylene glycol is present in the *Sample solution*, the peak response ratio relative to 2,2,2-trichloroethanol is NMT the peak response ratio for diethylene glycol or ethylene glycol relative to 2,2,2-trichloroethanol in the *Standard solution*; NMT 0.10% each for diethylene glycol and ethylene glycol is found.

- **C.** Examine the chromatograms obtained in *Identification* test *B*. The retention time of the glycerin peak of the *Sample solution* corresponds to that obtained in the *Standard solution*.

ASSAY
- **PROCEDURE**

Sodium periodate solution: Dissolve 60 g of sodium metaperiodate in sufficient water containing 120 mL of 0.1 N sulfuric acid to make 1000 mL. Do not heat to dissolve the periodate. If the solution is not clear, pass through a sintered-glass filter. Store the solution in a glass-stoppered, light-resistant container. Test the suitability of this solution as follows. Pipet 10 mL into a 250-mL volumetric flask, and dilute with water to volume. To 550 mg of Glycerin dissolved in 50 mL of water, add 50 mL of the diluted periodate solution with a pipet. For a blank, pipet 50 mL of the solution into a flask containing 50 mL of water. Allow the solutions to stand for 30 min, then to each add 5 mL of hydrochloric acid and 10 mL of potassium iodide TS, and rotate to mix. Allow to stand for 5 min, add 100 mL of water, and titrate with 0.1 N sodium thiosulfate, shaking continuously and adding 3 mL of starch TS as the endpoint is approached. The ratio of the volume of 0.1 N sodium thiosulfate required for the glycerin–periodate mixture to that required for the blank should be between 0.750 and 0.765.
Analysis: Transfer 400 mg of Glycerin to a 600-mL beaker, dilute with 50 mL of water, add bromothymol blue TS, and acidify with 0.2 N sulfuric acid to a definite green or greenish yellow color. Neutralize with 0.05 N sodium hydroxide to a definite blue endpoint, free from green color. Prepare a blank containing 50 mL of water, and neutralize in the same manner. Pipet 50 mL of the *Sodium periodate solution* into each beaker, mix by swirling gently, cover with a watch glass, and allow to stand for 30 min at room temperature (not exceeding 35°) in the dark or in subdued light. Add 10 mL of a mixture of equal volumes of ethylene glycol and water, and allow to stand for 20 min. Dilute each solution with water to 300 mL, and titrate with 0.1 N sodium hydroxide VS to a pH of 8.1 ± 0.1 for the specimen under assay and 6.5 ± 0.1 for the blank, using a pH meter. Each mL of 0.1 N sodium hydroxide, after correction for the blank, is equivalent to 9.210 mg of $C_3H_8O_3$.
Acceptance criteria: 99.0%–101.0% on the anhydrous basis

IMPURITIES
INORGANIC IMPURITIES
- **Chloride and Sulfate,** *Chloride* ⟨221⟩: A 7.0-g portion shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (NMT 10 ppm).
- **Chloride and Sulfate,** *Sulfate* ⟨221⟩: A 10-g portion shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (NMT 20 ppm).
- **RESIDUE ON IGNITION** ⟨281⟩: Heat 50 g in an open, shallow 100-mL porcelain dish until it ignites, and allow it to burn without further application of heat in a place free from drafts. Cool, moisten the residue with 0.5 mL of sulfuric acid, and ignite to constant weight: the weight of the residue does not exceed 5 mg (0.01%).

ORGANIC IMPURITIES
- **Procedure 1: Related Compounds**

System suitability solution: 0.5 mg/mL each of USP Diethylene Glycol RS and USP Glycerin RS
Sample solution: 50 mg/mL of Glycerin

USP Monographs

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m fused-silica analytical column coated with 3.0-µm G43 stationary phase, and an inlet liner having an inverted cup or spiral structure
Temperature
Injector: 220°
Detector: 250°
Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	—	100	—
100	7.5	220	4

Carrier gas: Helium
Injection size: 0.5 µL
Linear velocity: 38 cm/s
Injection type: Split ratio, about 10:1
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 7.0 between diethylene glycol and glycerin
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity, excluding any solvent peaks and diethylene glycol, in the portion of Glycerin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Acceptance criteria
Individual impurities: NMT 0.1%
Total impurities: NMT 1.0%
• **Procedure 2: Limit of Chlorinated Compounds**
Sample: 5 g of Glycerin
Analysis: Transfer the *Sample* into a dry, round-bottom, 100-mL flask. Add 15 mL of morpholine, and connect the flask by a ground joint to a reflux condenser. Reflux gently for 3 h. Rinse the condenser with 10 mL of water, receiving the washings in the flask, and cautiously acidify with nitric acid. Transfer the solution to a suitable comparison tube, add 0.50 mL of silver nitrate TS, and dilute with water to 50.0 mL.
Acceptance criteria: The turbidity is not greater than that of a blank to which 0.20 mL of 0.020 N hydrochloric acid has been added, the refluxing being omitted (NMT 30 ppm of Cl).
• **Procedure 3: Fatty Acids and Esters**
Sample solution: Mix 50 g of Glycerin with 50 mL of freshly boiled water and 5 mL of 0.5 N sodium hydroxide VS. Boil the mixture for 5 min, cool, and add phenolphthalein TS.
Analysis: Titrate the excess alkali with 0.5 N hydrochloric acid VS. Perform a blank determination (see *Titrimetry* ⟨541⟩, *Residual Titrations*).
Acceptance criteria: NMT 1 mL of 0.5 N sodium hydroxide is consumed.

SPECIFIC TESTS
• **COLOR:** When viewed downward against a white surface in a 50-mL color-comparison tube, the color is not darker than the color of a standard made by diluting 0.40 mL of ferric chloride CS with water to 50 mL and similarly viewed in a color-comparison tube of approximately the same diameter and color as that containing the Glycerin.
• **SPECIFIC GRAVITY** ⟨841⟩: NLT 1.249
• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 5.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Diethylene Glycol RS
USP Ethylene Glycol RS
USP Glycerin RS
1,2,3-Propanetriol.
$C_3H_8O_3$ 92.10

Glycerin Ophthalmic Solution

» Glycerin Ophthalmic Solution is a sterile, anhydrous solution of Glycerin, containing not less than 98.5 percent of glycerin ($C_3H_8O_3$). It may contain one or more suitable antimicrobial preservatives. [NOTE—In the preparation of this Ophthalmic Solution, use Glycerin that has a low water content, in order that the Ophthalmic Solution may comply with the *Water* limit. This may be ensured by using Glycerin having a specific gravity of not less than 1.2607, corresponding to a concentration of 99.5 percent.]
[NOTE—Do not use the Ophthalmic Solution if it contains crystals, or is cloudy or discolored, or contains a precipitate.]

Packaging and storage—Preserve in tight containers of glass or plastic, containing not more than 15 mL, protected from light. The container or individual carton is sealed and tamper-proof so that sterility is ensured at time of first use.
USP Reference standards ⟨11⟩—
USP Glycerin RS
Identification—It responds to the *IDENTIFICATION* test under *Glycerin*.
Sterility Tests ⟨71⟩: meets the requirements.
pH ⟨791⟩: between 4.5 and 7.5, determined potentiometrically in a solution prepared by the addition of 5 mL of Sodium Chloride Injection to 5 mL of Ophthalmic Solution.
Water Determination, *Method I* ⟨921⟩: not more than 1.0%.
Assay—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 3 g of glycerin, to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer a 3-mL portion to a conical flask, add 100.0 mL of a solution of potassium periodate (prepared by dissolving 3 g of potassium periodate in about 500 mL of warm water, cooling to room temperature, and then diluting with water to 1000 mL), swirl, and allow to stand at room temperature for 10 minutes. Add 4 g of sodium bicarbonate and 2 g of potassium iodide, and titrate immediately with 0.1 N potassium arsenite VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination, using water in place of the Ophthalmic Solution, and note the difference in volumes

www.webofpharma.com

required. Each mL of 0.1 N potassium arsenite is equivalent to 2.303 mg of glycerin ($C_3H_8O_3$).

Glycerin Oral Solution

» Glycerin Oral Solution contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of glycerin ($C_3H_8O_3$).

Packaging and storage—Preserve in tight containers.
Identification—Heat a few drops with about 500 mg of potassium bisulfate in a test tube: pungent vapors of acrolein are evolved.
pH ⟨791⟩: between 5.5 and 7.5.
Assay—Transfer an accurately measured volume of Oral Solution, equivalent to about 3 g of glycerin, to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer a 3-mL portion to a conical flask, add 100.0 mL of a solution of potassium periodate (prepared by dissolving 3 g of potassium periodate in about 500 mL of warm water, cooling to room temperature, and then diluting with water to 1000 mL), swirl, and allow to stand at room temperature for 10 minutes. Add 4 g of sodium bicarbonate and 2 g of potassium iodide, and titrate immediately with 0.1 N potassium arsenite VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination, using water in place of the Oral Solution, and note the difference in volumes required. Each mL of 0.1 N potassium arsenite is equivalent to 2.303 mg of glycerin ($C_3H_8O_3$).

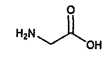
Glycerin Suppositories

» Glycerin Suppositories contain Glycerin solidified with Sodium Stearate. [NOTE—If preferred, the Sodium Stearate for Glycerin Suppositories may be prepared during the making of the Suppositories by the direct reaction between Stearic Acid and Sodium Bicarbonate, Sodium Carbonate, or Sodium Hydroxide, these being taken in the correct proportion.] Glycerin Suppositories contain not less than 75.0 percent and not more than 90.0 percent, by weight, of glycerin ($C_3H_8O_3$).

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Stearic Acid RS

Identification—
A: Dissolve 1 g of sodium borate in 100 mL of water, add 25 drops of phenolphthalein TS, and mix. To a test tube containing 0.5 mL of this solution add 2 drops of 1 Suppository that has been melted: the pink solution becomes colorless, and when it is heated the pink color reappears.
B: Disperse 12 Suppositories in about 125 mL of water in a 250-mL beaker on a hot plate. Cool, add 1.5 mL of hydrochloric acid, and pour the mixture into a 250-mL separator. Extract with 75 mL of hexanes, discarding the lower aqueous layer and collecting the organic layer in a beaker. Evaporate with the aid of a steam bath to near dryness: the IR absorption spectrum of a mineral oil dispersion of the residue so obtained exhibits maxima only at the same wavelengths as those of a mineral oil dispersion of USP Stearic Acid RS.
Water Determination, *Method I* ⟨921⟩: not more than 15.0%.
Assay—Transfer an accurately weighed quantity of Suppositories, equivalent to about 250 mg of glycerin, to a 250-mL volumetric flask. Dissolve in water, dilute with water to volume, and mix. Pipet 5 mL of this solution into a 250-mL conical flask, and add 50.0 mL of a reagent prepared by mixing 40 mL of dilute sulfuric acid (1 in 20) with 60 mL of potassium periodate solution (1 in 1000) acidified with 3 to 5 drops of sulfuric acid. Heat the solution on a steam bath for 15 minutes, cool to room temperature, and add 1 g of potassium iodide. Allow the flask to stand for 5 minutes, and titrate with 0.02 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination, using water in place of Suppositories, and note the difference in volumes required. Each mL of the difference in volume of 0.02 N sodium thiosulfate consumed is equivalent to 0.4604 mg of glycerin ($C_3H_8O_3$).

Glycine



$C_2H_5NO_2$ 75.07
Glycine [56-40-6].

DEFINITION
Glycine contains NLT 98.5% and NMT 101.5% of glycine ($C_2H_5NO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Sample: 150 mg of Glycine
Blank: 100 mL of glacial acetic acid
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Visual
Analysis: Dissolve the *Sample* in 100 mL of glacial acetic acid, and add 1 drop of crystal violet TS. Titrate with the *Titrant* to a green endpoint. Perform the *Blank* determination.
Calculate the percentage of glycine ($C_2H_5NO_2$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 75.07 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

USP Monographs

www.webofpharma.com

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*
  Standard solution: 0.10 mL of 0.020 N hydrochloric acid
  Sample: 1 g of Glycine
  Acceptance criteria: NMT 0.007%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*
  Standard solution: 0.20 mL of 0.020 N sulfuric acid
  Sample: 3 g of Glycine
  Acceptance criteria: NMT 0.0065%

Delete the following:

- **HEAVY METALS,** *Method I* ⟨231⟩: NMT 20 ppm (Official 1-Jan-2018)
- **HYDROLYZABLE SUBSTANCES**
  Sample solution: 100 mg/mL of Glycine
  Analysis: Boil 10 mL of the *Sample solution* for 1 min, and set aside for 2 h.
  Acceptance criteria: The solution appears as clear and as mobile as 10 mL of the same solution that has not been boiled.

Change to read:

- **RELATED COMPOUNDS**
  Solution A: Transfer 2.16 g of octanesulfonic acid sodium salt to a 1000-mL volumetric flask, add 900 mL of HPLC grade water and 2.0 mL of perchloric acid, and mix to dissolve. Adjust with 5 N sodium hydroxide solution to a pH of 2.2. Dilute with HPLC grade water to volume. Pass the solution through a membrane filter of 0.2-µm pore size.
  Solution B: Acetonitrile
  Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7	100	0
13	90	10
18	90	10
35	100	0
45	100	0

Standard solution: A mixture of 0.005 mg/mL each of USP Glycine RS, USP Diglycine RS, USP Triglycine RS, and glycine anhydride,[1] and 0.0025 mg/mL of monochloroacetic acid[2] in HPLC grade water. [NOTE—Monochloroacetic acid may be omitted from the *Standard solution* if the article being tested does not contain this substance.]
Sample solution: Transfer 125 mg of Glycine into a 25-mL volumetric flask, dissolve in and dilute with HPLC grade water to volume.
Blank: HPLC grade water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 25°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *Blank*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Interference peaks: Compare the chromatogram obtained from the *Standard solution* with that obtained from the *Blank*. Any peak area from the *Blank* that overlaps or co-elutes with the amino acid peak from the *Standard solution* is NMT 2.0% of that amino acid peak area.
Resolution: NLT 2.0 between the diglycine and triglycine peaks, *Standard solution*
Relative standard deviation: NMT 5.0% each for the specified peaks, *Standard solution*
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Separately inject the *Blank*, *Standard solution*, and *Sample solution* into the chromatograph. Compare the chromatogram from the *Sample solution* with that from the *Blank*. Disregard any peak observed in both the *Sample solution* and the *Blank*. Identify the amino acid impurities in the *Sample solution* by comparing with those specified in the *Standard solution*.
Separately calculate the percentage of each specified impurity in the portion of Glycine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of glycine anhydride, monochloroacetic acid, diglycine, or triglycine from the *Sample solution*
r_S = peak response of glycine anhydride, monochloroacetic acid, diglycine, or triglycine from the *Standard solution*
C_S = concentration of glycine anhydride, monochloroacetic acid, USP Diglycine RS, or USP Triglycine RS in the *Standard solution* (mg/mL)
C_U = concentration of Glycine in the *Sample solution* (mg/mL)

Separately calculate the percentage of iminodiacetic acid, hexamethylenetetramine, and any unspecified impurity in the portion of Glycine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of iminodiacetic acid, hexamethylenetetramine, or any unspecified impurity from the *Sample solution*
r_S = peak response of glycine from the *Standard solution*
C_S = concentration of USP Glycine RS in the *Standard solution* (mg/mL)
C_U = concentration of Glycine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. [NOTE—Disregard any impurity peak less than 0.05%.]

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Glycine anhydride	0.25	0.1
Monochloroacetic acid	0.44	▲a▲ (Postponed on 1-Aug-2018)

[1] Analytical grade with purity NLT 99.0%.
[2] Analytical grade with purity NLT 99.0%.

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Iminodiacetic acid	0.60	0.1
Glycine	1.00	—
Diglycine	1.70	0.1
Triglycine	1.80	0.1
Hexamethylenetetramine	2.47	0.1
Any unspecified impurity	—	▲[b]▲ (Postponed on 1-Aug-2018)
Total impurities	—	1.0

[a] The limit should be controlled as per International Council for Harmonisation (ICH) M7.
[b] The limit should be based on the maximum daily dose (MDD) of the drug products.

▲1S (USP41)

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in well-closed containers ▲at room temperature.▲ 1S (USP41)

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  ▲USP Diglycine RS▲ 1S (USP41)
  USP Glycine RS
  ▲USP Triglycine RS▲ 1S (USP41)

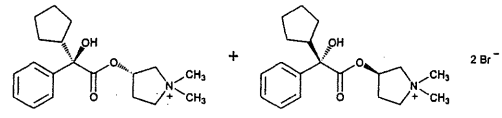
Glycine Irrigation

» Glycine Irrigation is a sterile solution of Glycine in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of glycine ($C_2H_5NO_2$).

Packaging and storage—Preserve in single-dose containers, preferably of Type I or Type II glass.
USP Reference standards ⟨11⟩—
USP Glycine RS
Identification—Evaporate a portion of Irrigation to dryness, and obtain the IR absorption spectrum of a mineral oil dispersion of the residue: it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Glycine RS.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.5 Endotoxin Unit per mL.
pH ⟨791⟩: between 4.5 and 6.5, determined potentiometrically on a portion to which 0.3 mL of saturated potassium chloride has been added for each 100 mL.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the container in which the solution is packaged may be designed to empty rapidly and may exceed 1000 mL in capacity.
Assay—Dilute an accurately measured volume of Irrigation, equivalent to about 150 mg of glycine, with water to 25 mL, and add 10 mL of formaldehyde TS, previously adjusted to a pH of 9.0, and 5 drops of mixed indicator solution (prepared by dissolving 75 mg of phenolphthalein and 25 mg of thymol blue in a mixture of equal volumes of alcohol and water to make 100 mL). Titrate with 0.1 N sodium hydroxide VS until the yellow color disappears and a faint violet color appears. Each mL of 0.1 N sodium hydroxide is equivalent to 7.507 mg of glycine ($C_2H_5NO_2$).

Glycopyrrolate

Change to read:



$C_{19}H_{28}BrNO_3$ ▲398.34▲ (USP 1-Dec-2019)
Pyrrolidinium, 3-[(*SR*)-(cyclopentylhydroxyphen ylacetyl) oxy]-1,1-dimethyl-, [*RS*-] bromide;
(*RS*)-[3-(*SR*)-Hydroxy-1,1-dimethylpyrrolidinium bromide] α-cyclopentylmandelate [▲51186-83-5▲ (USP 1-Dec-2019)].

DEFINITION
Glycopyrrolate contains NLT 98.0% and NMT 102.0% of glycopyrrolate ($C_{19}H_{28}BrNO_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)▲ or ⟨197A⟩: Meets the requirements▲ (USP 1-Dec-2019)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Bromide*
  Sample solution: 25 mg/mL
  Acceptance criteria: Meets the requirements

ASSAY

Change to read:

- **PROCEDURE**
  Buffer solution: Prepare a solution of 1.0 g of anhydrous sodium sulfate and 200 mg of sodium 1-hexanesulfonate monohydrate in 650 mL of water. To this solution add 3.0 mL of 1 N sulfuric acid, and mix.
  Mobile phase: Acetonitrile, methanol, and *Buffer solution* (20:15:65)
  Standard solution: 0.1 mg/mL of USP Glycopyrrolate RS in *Mobile phase*
  Sample solution: 0.1 mg/mL of Glycopyrrolate in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 222 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Column temperature: 40°

USP Monographs

www.webofpharma.com

Flow rate: 1.2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: ▲NMT 0.73%▲ (USP 1-Dec-2019)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of glycopyrrolate ($C_{19}H_{28}BrNO_3$) in the portion of Glycopyrrolate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of glycopyrrolate from the *Sample solution*
r_S = peak response of glycopyrrolate from the *Standard solution*
C_S = concentration of USP Glycopyrrolate RS in the *Standard solution* (mg/mL)
C_U = concentration of Glycopyrrolate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%

Change to read:

• **ORGANIC IMPURITIES, PROCEDURE 1**
[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 1* is recommended when ▲glycopyrrolate related compound A▲ (USP 1-Dec-2019) may be present (see *Table 2*); *Procedure 2* is recommended when didehydroglycopyrrolate, ▲glycopyrrolate related compound I,▲ (USP 1-Dec-2019) and ▲glycopyrrolate related compound L▲ (USP 1-Dec-2019) may be present (see *Table 4*).]
Buffer solution: Prepare a solution of 1.0 g of anhydrous sodium sulfate and 200 mg of sodium 1-hexanesulfonate monohydrate in 650 mL of water. To this solution add 3.0 mL of 1 N sulfuric acid, and mix.
Diluent: Prepare a solution of 1.0 g of anhydrous sodium sulfate, 6.8 g of monobasic potassium phosphate, and 200 mg of sodium 1-hexanesulfonate monohydrate in 650 mL of water. To this solution add 3.0 mL of 1 N sulfuric acid, 150 mL of methanol, and 200 mL of acetonitrile, and mix. Adjust with phosphoric acid to a pH of 2.8.
Solution A: Acetonitrile, methanol, and *Buffer solution* (20:15:65)
Solution B: Acetonitrile, methanol, and *Buffer solution* (50:15:35)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
25	10	90
35	10	90
37	100	0
45	100	0

Standard solution: 1.5 µg/mL each of USP Glycopyrrolate RS, USP Glycopyrrolate Related Compound A RS, USP Glycopyrrolate Related Compound B RS, and USP Glycopyrrolate Related Compound C RS in *Diluent*. Sonicate, if necessary, to facilitate dissolution.
Sample solution: 1.0 mg/mL of Glycopyrrolate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 222 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between glycopyrrolate and glycopyrrolate related compound B
Tailing factor: NMT 2.0 for the glycopyrrolate peak
Relative standard deviation: NMT 6.0% for each peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the glycopyrrolate related compounds A, B, and C in the portion of Glycopyrrolate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each related compound from the *Sample solution*
r_S = peak response of the corresponding related compound from the *Standard solution*
C_S = concentration of the corresponding Reference Standard in the *Standard solution* (mg/mL)
C_U = concentration of Glycopyrrolate in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Glycopyrrolate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of glycopyrrolate from the *Standard solution*
C_S = concentration of USP Glycopyrrolate RS in the *Standard solution* (mg/mL)
C_U = concentration of Glycopyrrolate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
▲Glycopyrrolate related compound A▲ (USP 1-Dec-2019)	0.45	0.15
Glycopyrrolate	1.00	—
▲Glycopyrrolate related compound B▲ (USP 1-Dec-2019)	1.14	0.15
▲Glycopyrrolate related compound C▲ (USP 1-Dec-2019)	2.68	0.15
Any individual unspecified impurity	—	0.10

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	0.50

▲▲ (USP 1-Dec-2019)

Change to read:

- **ORGANIC IMPURITIES, PROCEDURE 2**

Buffer solution: 3.4 g/L of monobasic potassium phosphate in water (25 mM monobasic potassium phosphate buffer). Adjust with phosphoric acid to a pH of 2.5 ± 0.2.
Diluent: Acetonitrile and *Buffer solution* (1:1)
Solution A: *Buffer solution*
Solution B: Methanol
Mobile phase: See *Table 3*. Return to original conditions, and equilibrate the system.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	95	5
7	39	61
8	39	61
11	15	85
11.5	15	85

System suitability solution: 0.5 mg/mL of USP Glycopyrrolate RS and 1.0 µg/mL each of benzoic acid, USP Glycopyrrolate Related Compound B RS, USP Glycopyrrolate Related Compound C RS, USP Glycopyrrolate Related Compound I RS, and USP Glycopyrrolate Related Compound L RS in *Diluent*
Standard solution: 1.0 µg/mL of USP Glycopyrrolate RS in *Diluent*
Sample solution: 0.5 mg/mL of Glycopyrrolate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 215 nm
Column: 2.1-mm × 10-cm; 1.7-µm packing L1
Column temperature: 45°
Flow rate: 0.42 mL/min
Injection volume: 2.5 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between glycopyrrolate and glycopyrrolate related compound B
Tailing factor: NMT 2.0 for the glycopyrrolate peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Glycopyrrolate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of glycopyrrolate from the *Standard solution*
C_S = concentration of USP Glycopyrrolate RS in the *Standard solution* (mg/mL)
C_U = concentration of Glycopyrrolate in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 4*)

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzoic acid	0.82	3.1	0.15
Didehydroglycopyrrolate[a]	0.89	1.0	0.15
Glycopyrrolate	1.00	—	—
▲Glycopyrrolate related compound B▲ (USP 1-Dec-2019)	1.06	1.4	0.15
▲Glycopyrrolate related compound I▲ (USP 1-Dec-2019)	1.22	1.4	0.15
▲Glycopyrrolate related compound C▲ (USP 1-Dec-2019)	1.32	1.9	0.15
▲Glycopyrrolate related compound L▲ (USP 1-Dec-2019)	1.52	1.8	0.15
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

▲▲ (USP 1-Dec-2019)
[a] 3-[2-(Cyclopent-1-en-1-yl)-2-hydroxy-2-phenylacetoxy]-1,1-dimethylpyrrolidin-1-ium bromide.

Change to read:

- **LIMIT OF ERYTHRO ISOMER**

Buffer solution: 2.8 g/L of monobasic sodium phosphate in water. Adjust with ▲2.5 M sodium hydroxide▲ (USP 1-Dec-2019) to a pH of 6.50 ± 0.05.
Mobile phase: Methanol, acetonitrile, and *Buffer solution* (50:10:40)
System suitability solution: 40 µg/mL each of USP Glycopyrrolate Erythro Isomer RS and USP Glycopyrrolate RS in *Mobile phase*
Standard solution: 0.01 mg/mL of USP Glycopyrrolate RS in *Mobile phase*
Sample solution: 0.5 mg/mL of Glycopyrrolate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 222 nm
Column: 4.0-mm × 25-cm; 5-µm packing L45
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 1.2 between the ▲glycopyrrolate▲ (USP 1-Dec-2019) erythro isomer and glycopyrrolate, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 6.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲glycopyrrolate▲ (USP 1-Dec-2019) erythro isomer in the portion of Glycopyrrolate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

USP Monographs

www.webofpharma.com

r_U = peak response of the ▲glycopyrrolate▲ (USP 1-Dec-2019) erythro isomer from the *Sample solution*
r_S = peak response of glycopyrrolate from the *Standard solution*
C_S = concentration of USP Glycopyrrolate RS in the *Standard solution* (mg/mL)
C_U = concentration of Glycopyrrolate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
▲Glycopyrrolate erythro isomer▲ (USP 1-Dec-2019)	0.89	0.4
Glycopyrrolate	1.00	—

▲▲ (USP 1-Dec-2019)

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Glycopyrrolate RS
  USP Glycopyrrolate Erythro Isomer RS
  (*RS*)-3-[(*RS*)-2-Cyclopentyl-2-hydroxy-2-phenylacetoxy]-1,1-dimethylpyrrolidinium bromide.
  $C_{19}H_{28}BrNO_3$ 398.33
  USP Glycopyrrolate Related Compound A RS
  5-Nitrobenzene-1,3-dicarboxylic acid.
  $C_8H_5NO_6$ 211.13
  USP Glycopyrrolate Related Compound B RS
  ▲[NOTE—May be available as a free base or a hydrochloride salt.]▲ (USP 1-Dec-2019)
  1-Methylpyrrolidin-3-yl-2-cyclopentyl-2-hydroxy-2-phenylacetate.
  $C_{18}H_{25}NO_3$ 303.40
  ▲1-Methylpyrrolidin-3-yl-2-cyclopentyl-2-hydroxy-2-phenylacetate hydrochloride.
  $C_{18}H_{25}NO_3 \cdot HCl$ 339.86▲ (USP 1-Dec-2019)
  USP Glycopyrrolate Related Compound C RS
  2-Cyclopentyl-2-hydroxy-2-phenylacetic acid.
  $C_{13}H_{16}O_3$ 220.26
  USP Glycopyrrolate Related Compound I RS
  (*RS*)-3-[(*SR*)-2-(4-Chlorophenyl)-2-cyclopentyl-2-hydroxyacetoxy]-1,1-dimethylpyrrolidin-1-ium bromide.
  $C_{19}H_{27}BrClNO_3$ 432.78
  USP Glycopyrrolate Related Compound L RS
  Methyl 2-cyclopentyl-2-hydroxy-2-phenylacetate.
  $C_{14}H_{18}O_3$ 234.29

Glycopyrrolate Injection

» Glycopyrrolate Injection is a sterile solution of Glycopyrrolate in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of glycopyrrolate ($C_{19}H_{28}BrNO_3$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Glycopyrrolate RS

Identification—
Spray reagent—Dissolve 2 g of bismuth subnitrate in a solution consisting of 100 mL of water and 25 mL of glacial acetic acid (*Solution A*). Dissolve 40 g of potassium iodide in 100 mL of water (*Solution B*). Add 10 mL of *Solution A* and 10 mL of *Solution B* to a solution consisting of 100 mL of water and 20 mL of glacial acetic acid, and mix.
Procedure—Pipet an amount of Injection equivalent to about 1 mg of glycopyrrolate into a 10-mL volumetric flask, dilute with water to volume, and mix to obtain the test solution. Prepare a Standard solution of USP Glycopyrrolate RS in water containing about 0.1 mg of glycopyrrolate per mL. Apply 30 µL of the test solution and 30 µL of the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of butyl alcohol, glacial acetic acid, and water (3:1:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and allow to air-dry. Spray the plate with *Spray reagent*, and allow to air-dry: the R_F value and color of the principal spot obtained from the test solution correspond to those obtained from the Standard solution.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 555.5 USP Endotoxin Units per mg of glycopyrrolate.
pH ⟨791⟩: between 2.0 and 3.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase—Dissolve 1.0 g of anhydrous sodium sulfate and 200 mg of sodium 1-pentanesulfonate in 615 mL of water in a 1000-mL volumetric flask. Add 3.0 mL of 1 N sulfuric acid, 235 mL of acetonitrile, and 150 mL of methanol, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Glycopyrrolate RS in *Mobile phase*, and dilute quantitatively with *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.
Resolution solution—Prepare a solution of benzaldehyde in *Mobile phase* containing about 0.5 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, dilute with *Standard preparation* to volume, and mix.
Assay preparation—Dilute a volume of Injection, quantitatively if necessary, with *Mobile phase* to obtain a solution having a concentration of about 0.2 mg of glycopyrrolate per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 222-nm detector and a 3.9-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure:* the resolution, *R*, between the

www.webofpharma.com

benzaldehyde and glycopyrrolate peaks is not less than 3.0. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 35 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of glycopyrrolate ($C_{19}H_{28}BrNO_3$) in each mL of the Injection taken by the formula:

$$C(L/D)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Glycopyrrolate RS in the *Standard preparation*; L is the labeled quantity, in mg per mL, of glycopyrrolate in the Injection; D is the concentration, in mg per mL, of glycopyrrolate in the *Assay preparation*, on the basis of the labeled quantity and the extent of dilution; and r_U and r_S are the glycopyrrolate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Glycopyrrolate Tablets

DEFINITION

Glycopyrrolate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of glycopyrrolate ($C_{19}H_{28}BrNO_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

- **PROCEDURE**

Buffer solution: Prepare a solution of 1.0 g of sodium sulfate anhydrous and 200 mg of sodium 1-hexanesulfonate monohydrate in 650 mL of water. To this solution add 3.0 mL of 1 N sulfuric acid, and mix.

Mobile phase: Acetonitrile, methanol, and *Buffer solution* (20:15:65)

Standard solution: 0.1 mg/mL of USP Glycopyrrolate RS in *Mobile phase*

Sample solution: ▲Nominally▲ (USP 1-Aug-2019) 0.1 mg/mL of glycopyrrolate in *Mobile phase*. Prepare by transferring 10 Tablets to a suitable volumetric flask. Add *Mobile phase* to 50% of the volume of the flask, and sonicate for 10 min or until the Tablets disintegrate completely. Add *Mobile phase* to 75% of the volume of the flask, shake mechanically for 30 min, and dilute with *Mobile phase* to volume. Centrifuge a portion of the solution, and pass the supernatant through a suitable filter, discarding the first few milliliters of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 222 nm. ▲For *Identification B*, use a diode array detector in the range of 210–400 nm.▲ (USP 1-Aug-2019)
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of glycopyrrolate ($C_{19}H_{28}BrNO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of glycopyrrolate from the *Sample solution*
r_S = peak response of glycopyrrolate from the *Standard solution*
C_S = concentration of USP Glycopyrrolate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of glycopyrrolate in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

Medium: Water; 500 mL
Apparatus 1: 100 rpm
Time: 45 min
Buffer solution: 1.0 g of sodium sulfate anhydrous and 200 mg of sodium 1-pentanesulfonate in 620 mL of water
Mobile phase: Acetonitrile, methanol, *Buffer solution*, and 1 N sulfuric acid (200:180:620:3)
Standard stock solution: 0.2 mg/mL of USP Glycopyrrolate RS in *Medium*. A small volume of methanol, not exceeding 20% of the final volume, can be used to solubilize glycopyrrolate.
Standard solution: (L/500) mg/mL of glycopyrrolate in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet. Prepare this solution fresh, and refrigerate immediately at 5°.
Sample solution: Pass a portion of the solution under test through a suitable filter, discarding the first few milliliters of the filtrate. Refrigerate the samples at 5°.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 5°
Column: 40°
Flow rate: 1.2 mL/min
Injection volume: 80 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the ▲▲ (USP 1-Aug-2019) percentage of the labeled amount of glycopyrrolate dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of ▲USP Glycopyrrolate RS▲ (USP 1-Aug-2019) in the *Standard solution* (mg/mL)
L = label claim (mg/Tablet)
V = volume of *Medium*, 500 mL

Tolerances: NLT 75% (Q) of the labeled amount of glycopyrrolate ($C_{19}H_{28}BrNO_3$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Buffer solution: Prepare a solution of 1.0 g of sodium sulfate anhydrous and 200 mg of sodium 1-hexanesulfonate monohydrate in 650 mL of water. To this solution add 3.0 mL of 1 N sulfuric acid, and mix.

Diluent: Prepare a solution of 1.0 g of sodium sulfate anhydrous, 6.8 g of monobasic potassium phosphate, and 200 mg of sodium 1-hexanesulfonate monohydrate in 650 mL of water. To this solution add 3.0 mL of 1 N sulfuric acid, 150 mL of methanol, and 200 mL of acetonitrile, and mix. Adjust with phosphoric acid to a pH of 2.8.

Solution A: Acetonitrile, methanol, and *Buffer solution* (20:15:65)

Solution B: Acetonitrile, methanol, and *Buffer solution* (50:15:35)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
25	10	90
35	10	90
37	100	0
45	100	0

Standard solution: 0.0015 mg/mL each of USP Glycopyrrolate RS, USP Glycopyrrolate Related Compound B RS, and USP Glycopyrrolate Related Compound C RS in *Diluent*. Sonicate, if necessary, to facilitate dissolution.

Sample solution: ▲Nominally▲ (USP 1-Aug-2019) 0.5 mg/mL of glycopyrrolate in *Diluent*. Prepare by transferring the equivalent of 25 mg of glycopyrrolate from a portion of NLT 20 powdered Tablets to a 50-mL volumetric flask. Add 30 mL of *Diluent*, sonicate for 10 min, shake mechanically for 30 min, and dilute with *Diluent* to volume. Centrifuge a portion of the solution, and pass the supernatant through a suitable filter, discarding the first few milliliters of the filtrate.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 222 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 50 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between glycopyrrolate and glycopyrrolate related compound B
Tailing factor: NMT 2.0 for the glycopyrrolate peak
Relative standard deviation: NMT 6.0% for the glycopyrrolate and glycopyrrolate related compound C peaks

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of glycopyrrolate related compound C in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of glycopyrrolate related compound C from the *Sample solution*
r_S = peak response of glycopyrrolate related compound C from the *Standard solution*
C_S = concentration of USP Glycopyrrolate Related Compound C RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of glycopyrrolate in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of glycopyrrolate from the *Standard solution*
C_S = concentration of USP Glycopyrrolate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of glycopyrrolate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
5-Nitroisophthalic acid[a]	0.45	—[b]
Glycopyrrolate	1.00	—
Glycopyrrolate base[c]	1.14	—[b]
Cyclopentylmandelic acid[d]	2.68	0.5
Any other individual impurity	—	0.2
Total impurities	—	1.2

[a] Glycopyrrolate related compound A.
[b] Disregard the peaks due to 5-nitroisophthalic acid and glycopyrrolate base, because these are process impurities and are controlled in the drug substance monograph.
[c] Glycopyrrolate related compound B.
[d] Glycopyrrolate related compound C.

ADDITIONAL REQUIREMENTS

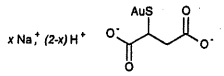
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

www.webofpharma.com

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Glycopyrrolate RS
USP Glycopyrrolate Related Compound B RS
▲[NOTE—May be available as a free base or a hydrochloride salt.]▲ (USP 1-Aug-2019)
1-Methylpyrrolidin-3-yl-2-cyclopentyl-2-hydroxy-2-phenylacetate.
$C_{18}H_{25}NO_3$ 303.40
▲1-Methylpyrrolidin-3-yl 2-cyclopentyl-2-hydroxy-2-phenylacetate hydrochloride.
$C_{18}H_{25}NO_3 \cdot HCl$ 339.86▲ (USP 1-Aug-2019)
USP Glycopyrrolate Related Compound C RS
2-Cyclopentyl-2-hydroxy-2-phenylacetic acid.
$C_{13}H_{16}O_3$ 220.26

Gold Sodium Thiomalate



$C_4H_3AuNa_2O_4S$ plus $C_4H_4AuNaO_4S$ 368.09
Butanedioic acid, mercapto-, monogold(1+) sodium salt.
Mercaptosuccinic acid, monogold(1+) sodium salt [12244-57-4].

» Gold Sodium Thiomalate is a mixture of the mono- and di-sodium salts of gold thiomalic acid. It contains not less than 44.8 percent and not more than 49.6 percent of Au. It contains not less than 49.0 percent and not more than 52.5 percent of Au on a dry, alcohol- and glycerin-free basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

Identification—
A: To 2 mL of a solution (1 in 10) add 1 mL of calcium nitrate solution (1 in 10): a white precipitate is formed, and it dissolves in 2 N nitric acid and reappears upon the addition of ammonium acetate TS.
B: To 2 mL of a solution (1 in 10) add 4 mL of silver nitrate TS: a yellowish precipitate is formed, and it dissolves completely in an excess of 6 N ammonium hydroxide.
C: To 2 mL of a solution (1 in 10) add 1 mL of 6 N ammonium hydroxide and 1 mL of 30 percent hydrogen peroxide, evaporate in a porcelain dish, and ignite. Add 20 mL of water to the ignited residue, and filter: particles of gold remain on the filter. Separate portions of the filtrate meet the requirements of the tests for *Sodium* ⟨191⟩ and for *Sulfate* ⟨191⟩.
pH ⟨791⟩**:** between 5.8 and 6.5, in a solution (1 in 10).
Loss on drying ⟨731⟩—Dry it at 60° and at a pressure not exceeding 5 mm of mercury for 2 hours: it loses not more than 8.0% of its weight.

Limit of alcohol—
Standard solution—Transfer 50 mg of dehydrated alcohol to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL to a 50-mL volumetric flask, and dilute with water to volume. This solution contains about 0.025 mg of alcohol per mL.
Test solution—Transfer about 50 mg of Gold Sodium Thiomalate, accurately weighed, to a 10-mL volumetric flask, and dilute with water to volume.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m fused-silica capillary column coated with a 3.0-µm film of phase G43. The column temperature is maintained at 40° for 8.5 minutes, then the temperature is increased at 30° per minute to 240°. The total chromatographic time is about 15 minutes. The injection port and detector block temperatures are maintained at 150°. The carrier gas is helium, flowing at a rate of about 2 mL per minute, and the split flow rate is about 20 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 4.6%.
Procedure—Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of alcohol (C_2H_5OH) in the portion of Gold Sodium Thiomalate taken by the formula:

$$100(C_a/C_b)(r_U/r_S)$$

in which C_a is the concentration, in mg per mL, of C_2H_5OH in the *Standard solution;* C_b is the concentration, in mg per mL, of Gold Sodium Thiomalate in the *Test solution;* and r_U and r_S are the alcohol peak responses obtained from the *Test solution* and the *Standard solution,* respectively. Not more than 4.0% is found.

Limit of glycerin—
[NOTE—This procedure is based on the absorption characteristics of a sodium–copper–glycerin complex. The stability of this complex prepared as directed herein is such that all measurements are to be taken within 1 hour. Thoroughly rinse all glassware used in this procedure with water to avoid large blank errors.]
Sodium hydroxide solution—Dissolve 23.6 g of sodium hydroxide in water to obtain 100 mL of solution.
Cupric chloride solution—Dissolve 3.8 g of cupric chloride in water to obtain 100 mL of solution.
Glycerin standard solutions—Dissolve an accurately weighed quantity of glycerin in water to obtain a solution having a known concentration of about 8 mg per mL. Pipet 1.0, 2.0, and 3.0 mL of this solution into a series of 10-mL volumetric flasks, followed by 4.0, 3.0, and 2.0 mL of water, respectively.
Reagent blank—Pipet 5.0 mL of water into a 10-mL volumetric flask.
Test solution—Dissolve about 400 mg Gold Sodium Thiomalate, accurately weighed, in 5.0 mL of water in a 10-mL volumetric flask.
Procedure—To each of the *Glycerin standard solutions,* the *Reagent blank,* and the *Test solution* add 1.0 mL of *Sodium hydroxide solution,* and mix. With vigorous shaking, and in increments of 0.1 mL, add *Cupric chloride solution,* checking for turbidity after each addition. After the solutions turn slightly turbid, add an excess of 0.1 mL of *Cupric chloride solution,* insert the stopper, and shake for 1 minute. Dilute with water to volume, and mix. Centrifuge the solutions in tapered, graduated 15-mL centrifuge tubes. The presence of 1 mm to 4 mm of copper hydroxide precipitate is observed. Using a suitable spectrophotometer equipped with 1-cm cells, and using water as a reference, measure the absorbance of the clear supernatant at a wavelength of 635 nm. Subtract the absorbance value of the *Reagent blank,* which is 0.040 or less, from the absorbance values of the *Glycerin standard solutions* and the *Test solution.* Plot the corrected absorbance readings

USP Monographs

www.webofpharma.com

of the *Glycerin standard solutions* against the corresponding weight of glycerin. From the standard curve so obtained, and the corrected absorbance of the *Test solution,* determine the weight of glycerin in the test specimen: not more than 5.5% is found.

Assay—Dissolve about 600 mg of Gold Sodium Thiomalate, accurately weighed, in water in a 25-mL volumetric flask, dilute with water to volume, and mix. Pass the entire solution through a clean, dry 0.5-µm filter into a clean, dry receiver. Pipet 20.0 mL of the filtrate into a 300-mL Kjeldahl flask, add 20 mL of nitric acid, and mix. To this solution add 15 mL of sulfuric acid slowly, with mixing. Heat over a low flame, gently at first, and then increase the heat until fumes of sulfur trioxide are evolved. Allow the flask and contents to cool to room temperature, add 30 mL of water slowly, with mixing, and 20 mL of hydrogen peroxide TS, again heat to fumes of sulfur trioxide, cool, and dilute with 30 mL of water. Pass the mixture through an ignited, tared filtering crucible, wash with water, heat the crucible and contents over a low flame to dry the precipitate, and ignite at 650 ± 50° to constant weight. The weight of the residue so obtained, multiplied by 1.25, is the weight of Au in the Gold Sodium Thiomalate taken.

Gold Sodium Thiomalate Injection

» Gold Sodium Thiomalate Injection is a sterile solution of Gold Sodium Thiomalate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of Gold Sodium Thiomalate.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and store protected from light.

Identification—It responds to *Identification* tests *A* and *B* under *Gold Sodium Thiomalate.*

pH ⟨791⟩**:** between 5.8 and 6.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—Transfer an accurately measured volume of Injection, equivalent to about 500 mg of gold sodium thiomalate, to a 300-mL Kjeldahl flask, and proceed as directed in the *Assay* under *Gold Sodium Thiomalate,* beginning with "add 20 mL of nitric acid." The weight of the gold so obtained, multiplied by 2.116, represents the weight of Gold Sodium Thiomalate in the portion of Injection taken.

Gonadorelin for Injection

DEFINITION

Gonadorelin for Injection is a sterile mixture of Gonadorelin Hydrochloride with suitable diluents. It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of gonadorelin ($C_{55}H_{75}N_{17}O_{13}$).

IDENTIFICATION

• **A.**

Buffer solution, Mobile phase, Standard solution 2, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*

Identity sample solution: Mix equal volumes of *Standard solution 2* and the *Sample solution.*

Analysis

Samples: *Standard solution 2, Sample solution,* and *Identity sample solution*

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of *Standard solution 2,* and the major peak of the *Identity sample solution* elutes as a single peak.

ASSAY

• **PROCEDURE**

[NOTE—Perform all manipulations involving the weighing of the gonadorelin hydrochloride sample and the Reference Standard in a low-humidity glove box.]

Buffer solution: 6.8 mg/mL of monobasic potassium phosphate in water. Adjust with 1 N potassium hydroxide to a pH of 6.5.

Mobile phase: Acetonitrile and *Buffer solution* (18:82)

Standard solution 1: 80 µg/mL of USP Gonadorelin Hydrochloride RS in *Mobile phase*

Standard solution 2: 100 µg/mL of USP Gonadorelin Hydrochloride RS in *Mobile phase*

Standard solution 3: 120 µg/mL of USP Gonadorelin Hydrochloride RS in *Mobile phase*

[NOTE—The *Standard solutions* may be stored in a refrigerator for 2 months. Remove suitable portions and warm to room temperature before use.]

Sample solution: Separately dissolve the contents of NLT 5 vials of Gonadorelin for Injection in *Mobile phase* to obtain a nominal concentration of 100 µg/mL of gonadorelin. Sonicate the vials for 5 min, and allow to cool to room temperature. Combine the solutions in the vials to obtain the *Sample solution.*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution 1*

[NOTE—The retention time for gonadorelin is 8–11 min.]

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 3.0% for gonadorelin

Analysis

Samples: *Standard solutions* and *Sample solution*

[NOTE—If more than five specimens are analyzed, reinject the *Standard solutions* before injecting further specimens of the *Sample solution.*]

Plot the responses of the gonadorelin peaks versus concentration, in mg/mL, of gonadorelin in each of the *Standard solutions,* and determine the regression line, using the least-squares method. The coefficient of variation from the regression line is NMT 3.0%. From the graph so obtained, determine the concentration, *C,* of gonadorelin in the *Sample solution* (mg/mL).

Calculate the percentage of the labeled amount of gonadorelin ($C_{55}H_{75}N_{17}O_{13}$) in the portion of Gonadorelin for Injection taken:

$$\text{Result} = C \times (M_{r1}/M_{r2}) \times (V + F) \times (100/L)$$

C = concentration of gonadorelin in the *Sample solution* as obtained from the regression line (µg/mL)

M_{r1} = molecular weight of gonadorelin, 1182.3

www.webofpharma.com

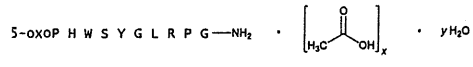
M_{r2} = molecular weight of gonadorelin hydrochloride, 1255.2
V = volume of *Mobile phase* used to prepare the *Sample solution* (mL)
F = correction factor for the volume created by the dissolved sample, 0.06 mL
L = label claim (µg)

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
- **pH** ⟨791⟩**:** 4.0–8.0, in a solution constituted as directed in the labeling
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 70 USP Endotoxin Units/mg.
- **OTHER REQUIREMENTS:** Meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, well-sealed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Gonadorelin Hydrochloride RS

Gonadorelin Acetate



$C_{55}H_{75}N_{17}O_{13} \cdot xC_2H_4O_2 \cdot yH_2O$ 1374.46
Luteinizing hormone-releasing factor acetate (salt) hydrate.
5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosylglycyl-L-leucyl-L-arginyl-L-prolylglycinamide acetate (salt) hydrate [52699-48-6; 33515-09-2].

» Gonadorelin Acetate is a synthetic polypeptide hormone having the property of stimulating the release of the luteinizing hormone from the hypothalamus. It contains not less than 80 percent by weight of $C_{55}H_{75}N_{17}O_{13}$, the remainder being acetic acid and water.
[NOTE—Gonadorelin Acetate is extremely hygroscopic. Protect from exposure to moisture, and store in a desiccator.]

Packaging and storage—Preserve in tight, well-sealed containers, protected from moisture. Store at a temperature of not more than 8°.
Labeling—Label it to indicate it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Gonadorelin Acetate RS
$C_{55}H_{75}N_{17}O_{13} \cdot xC_2H_4O_2 \cdot yH_2O$ 1182.3
(acetate free)
USP Gonadorelin Acetate Related Compound A RS
Gonadorelin free acid.
$C_{55}H_{74}N_{16}O_{14}$ 1183.3

Identification—
A: The monoisotopic mass by *Mass Spectrometry* ⟨736⟩ is 1181.6 ± 1 mass units.

B: The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution,* as obtained in the test for *Related compounds.*
Specific rotation ⟨781S⟩**:** between –54° and –66°, at 20°, calculated with reference to the peptide content determined in the *Assay.*
Test solution: 10 mg per mL, in 1% (v/v) acetic acid.
Water Determination, *Method Ic* ⟨921⟩ **:** not more than 7.0%, determined by directly introducing not less than 2 mg of the solid substance into the titrator.
Limit of fluoride— [NOTE—Use polypropylene vessels for preparation of solutions and standards.]
Standard solutions—Prepare a series of calibration standards containing 10, 1, 0.1, and 0.05 ppm fluoride dissolved in an ionic strength adjustment buffer suitable for the electrode in use (pH about 5).
Test solution—Dissolve between 3 and 5 mg of Gonadorelin Acetate in 1.375 mL of the same buffer as that used for the preparation of the *Standard solutions.*
Procedure—Using a fluoride ion-selective electrode connected to a pH/ion meter, measure the potential of each *Standard solution,* and plot the response versus the logarithm of the concentration. Determine the regression line using the least squares method. The test is considered valid if the slope of the curve is in the range of –54 to –60 mV per decade and the regression curve has a square of the correlation coefficient, r^2, not less than 0.995. From the calibration curve and the concentration of the *Test solution,* determine the amount of fluoride in the sample: not more than 0.1% (w/w) is found.

Acetic acid and trifluoroacetic acid—
Solution A—To 900 mL of water add 7.0 mL of phosphoric acid and 5.0 mL of concentrated ammonia. Mix, and dilute with water to 1000 mL, pass through a 0.45-µm filter, and degas. Add 20 mL of methanol, mix, and degas for an additional 2 minutes.
Solution B—Prepare a degassed mixture of acetonitrile and water (1:1).
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluent—Dilute 5.0 mL of phosphoric acid with water to 1000 mL, and mix thoroughly.
Trifluoroacetic acid stock solution—Add about 50 mL of water to a 100-mL volumetric flask with a stopper. Tare the stoppered flask on an analytical balance until there is no further significant drift in the reading. Carefully add 670 µL of trifluoroacetic acid to the flask, stopper immediately, and weigh. Dilute with water to volume.
Standard solutions—Accurately weigh out 150, 75, and 10 mg of sodium acetate trihydrate into three separate 100-mL volumetric flasks. Add 10 mL, 2 mL, and 100 µL, respectively, of the *Trifluoroacetic acid stock solution* to the flasks, and dilute each with *Diluent* to the 100-mL mark. Calculate the concentration, in mg per mL, of acetic acid in each *Standard solution* using the following equation:

$$0.00434W_A$$

in which W_A is the weight, in mg, of sodium acetate trihydrate taken. Calculate the concentration, in mg per mL, of trifluoroacetic acid in each *Standard solution* using the following equation:

$$0.0001(W_T V)$$

in which W_T is the weight, in mg, of trifluoroacetic acid used for preparation of the *Trifluoroacetic acid stock solution;* and V

www.webofpharma.com

is the volume, in mL, of *Trifluoroacetic acid stock solution* used to prepare the *Standard solution.*

Test solution—Prepare duplicate samples by accurately weighing out two separate aliquots of about 4.0 mg of Gonadorelin Acetate and dissolving each with 1 mL of the *Diluent.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column containing 5-µm packing L1. The flow rate is approximately 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–5	100	0	isocratic
5–6	100→0	0→100	linear
6–14	0	100	isocratic
14–15	0→100	100→0	return to initial
15–25	100	0	re-equilibration

Chromatograph the *Standard solutions,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 2000 theoretical plates for the trifluoroacetic acid peak and not less than 10,000 for the acetic acid peak; and the relative standard deviation for six replicate injections of the most concentrated *Standard solution* is not more than 2.0%.

Procedure—Inject in duplicate equal volumes (about 20 µL) of each of the *Standard solutions* followed by the duplicate *Test solutions.* Plot the peak areas of each of the components in the *Standard solutions* versus concentration, in mg per mL, and determine the regression line using the least squares method. The test is considered valid if the regression curves for both acetic acid and trifluoroacetic acid have a square of the correlation coefficient, r^2, not less than 0.995. From the resulting graph, determine the percentages of acetic acid and trifluoroacetic acid in the *Test solution:* between 8% and 12.5% of acetic acid is found, and not more than 0.25% of trifluoroacetic acid is found.

Related compounds—

Standard solution—Dissolve an accurately weighed quantity of USP Gonadorelin Acetate RS in water to obtain a solution having a known concentration of about 0.5 mg per mL.

System suitability solution—Dissolve an accurately weighed quantity of USP Gonadorelin Acetate Related Compound A RS in water to obtain a solution having a known concentration of about 0.5 mg per mL. Mix equal volumes of this solution and the *Standard solution.*

Test solution—Dissolve an accurately weighed quantity of Gonadorelin Acetate in water to obtain a solution having a known concentration of about 0.5 mg per mL.

SYSTEM 1—

Solvent 1—Mix 1 mL of trifluoroacetic acid with 1 L of water. Pass through a 0.45-µm filter, and degas.

Solvent 2—Mix 1 mL of trifluoroacetic acid with 1 L of acetonitrile.

Solution A—Prepare a mixture of *Solvent 1* and *Solvent 2* (95:5).

Solution B—Prepare a mixture of *Solvent 2* and *Solvent 1* (60:40).

Chromatographic system (see *Chromatography* ⟨621⟩)—The HPLC is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is approximately 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	91	9	initial
0–25	91→45	9→55	linear
25	45→91	55→9	return to initial
25–30	91	9	re-equilibrium

SYSTEM 2—

Mobile phase—Add 47 mL of phosphoric acid and 55 mL of triethylamine to 4 L of water, and adjust with phosphoric acid or triethylamine to a pH of 2.5, as appropriate. Pass through a 0.45-µm filter, and degas. Add acetonitrile to obtain a 13% (v/v) concentration of acetonitrile.

Chromatographic system (see *Chromatography* ⟨621⟩)—The HPLC is equipped with a 215-nm detector and a 4.6-mm × 10-cm column that contains 5-µm packing L1. The flow rate is approximately 1.5 mL per minute using isocratic elution and having a run time of 50 minutes.

Using both *System 1* and *System 2* chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure.* The *Standard solution* is used only to identify the gonadorelin acetate peak. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between gonadorelin acetate and gonadorelin acetate related compound A is not less than 2.0; the column efficiency is not less than 75,000 theoretical plates for *System 1* and not less than 3000 theoretical plates for *System 2;* the tailing factor is not more than 2.0 for both *System 1* and *System 2;* and the relative standard deviation for five replicate injections is not more than 2.0%.

Procedure—Inject equal volumes (about 20 µL) of each of the *Standard solution,* the *System suitability solution,* and the *Test solution,* followed by a co-injection of the *Test solution* with the *Standard solution,* into both *System 1* and *System 2.* Include blank injections between the different solutions. Integrate all peaks in order to obtain a baseline similar to that in the blank chromatograms, disregarding any peaks due to the solvent, counter-ion, and baseline artifacts. Using peak areas, and including all peaks greater than 0.05%, calculate the percentage of each impurity in the portion of Gona dorelin Acetate taken: not more than 1% of any single impurity is found, and not more than 2% of total impurities is found.

Amino acid analysis—Proceed as directed in the *Assay.* Express the content of each amino acid in µmoles, and calculate the total number of µmoles of Gonadorelin Acetate in the test sample as directed in the *Assay.* By dividing the number of µmoles of each amino acid by the total number of µmoles of Gonadorelin Acetate in the test sample, the relative proportions of amino acids are found: serine, 0.7 to 1.05; glutamic acid, 0.95 to 1.05; proline, 0.95 to 1.05; glycine, 1.9 to 2.1; leucine, 0.9 to 1.1; tyrosine, 0.7 to 1.05; histidine, 0.95 to 1.05; and arginine, 0.95 to 1.05. Isoleucine and lysine are absent; not more than traces of other amino acids except tryptophan are detected.

Assay—(see *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩). [NOTE—The following method is given for informational purposes; any validated amino acid analysis method can be used.]

Standardize the instrument with a mixture containing equal molar per volume amounts (except for L-cystine which is half the molar amount) of glycine and the L-form of the following amino acids: lysine, threonine, alanine, leucine, histidine, serine, valine, tyrosine, arginine, glutamic acid, methionine, phenylalanine, aspartic acid, proline, isoleucine, tryptophan, and cystine.

Assay preparation (see *Protein Hydrolysis, Method 1* ⟨1052⟩)—Accurately weigh out between 0.4 and 1.0 mg of

www.webofpharma.com

Gonadorelin Acetate in glass ampuls. Add a minimum of 1.0 mL of *Hydrolysis Solution* containing 4% phenol, freeze the sample ampul, and flame seal under vacuum. Hydrolyze at 110° for about 22 hours. After hydrolysis, dry the test sample under vacuum to remove any residual acid. To the ampul add 2 mL of a buffer solution that is suitable for the amino acid analyzer, and pass through a filter having a 0.45-µm porosity.

Procedure—Prepare a co-injection of the Standard solution and the test sample. Inject a suitable volume into the amino acid analyzer, and record and measure the responses for each amino acid peak. Express the content of each amino acid in µmoles. The total number of µmoles of gonadorelin acetate in the test sample is calculated by summing the number of µmoles for glutamic acid, proline, glycine, leucine, tyrosine, histidine, and arginine, and dividing by eight. Calculate the percentage of $C_{55}H_{75}N_{17}O_{13}$ in the portion of Gonadorelin Acetate taken by the formula:

$$118.23(N/W)$$

in which N is the total number of µmoles of gonadorelin acetate; and W is the weight of the sample in mg.

Gonadorelin Hydrochloride



$C_{55}H_{75}N_{17}O_{13}$ ×HCl 1182.30 (free base)
Gonadorelin hydrochloride;
5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosylglycyl-L-leucyl-L-arginyl-L-prolylglycinamide hydrochloride salt [51952-41-1].

DEFINITION

Gonadorelin Hydrochloride is a synthetic polypeptide hormone having the property of stimulating the release of the luteinizing hormone from the hypothalamus. It contains NLT 95.0% and NMT 102.0% of gonadorelin ($C_{55}H_{75}N_{17}O_{13}$), calculated on the anhydrous and hydrochloride-free basis.

[**CAUTION**—Gonadorelin Hydrochloride is extremely hygroscopic. Protect from exposure to moisture, and store in a desiccator.]

IDENTIFICATION

• **A.** The monoisotopic mass by *Mass Spectrometry* ⟨736⟩ is 1181.6 ± 1 mass units.

[NOTE—This quantity corresponds to $m/z = 1182.6 \pm 1$ for $(M + H)^+$ or $m/z = 591.8 \pm 0.5$ for $(M + 2H)^{2+}$.]

• **B. HPLC**

Buffer, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Identity sample solution: Mix equal volumes of the *Standard solution* and the *Sample solution*.

Analysis

Samples: *Standard solution, Sample solution,* and *Identity sample solution*

Examine the chromatograms of the *Standard solution*, the *Sample solution*, and the *Identity sample solution*.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, and the major peak of the *Identity sample solution* elutes as a single peak.

ASSAY

• **PROCEDURE**

[NOTE—Perform all manipulations involving the weighing of the gonadorelin hydrochloride and the Reference Standard in a low-humidity glove box.]

Buffer: Dissolve 6.8 g of monobasic potassium phosphate in water and dilute to 1 L. Adjust with 1 N potassium hydroxide to a pH of 6.5.

Mobile phase: Acetonitrile and *Buffer* (18:82)

Standard solution: 0.10 mg/mL of USP Gonadorelin Hydrochloride RS in *Mobile phase*

[NOTE—*Standard solution* may be stored in a refrigerator for 2 months. Remove suitable portions and warm to room temperature before use.]

Sample solution: 0.10 mg/mL of Gonadorelin Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

[NOTE—Condition the column with *Mobile phase* until a stable baseline is obtained.]

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Retention time: 8–11 min for the gonadorelin peak

Tailing factor: NMT 2.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of gonadorelin ($C_{55}H_{75}N_{17}O_{13}$) in the portion of Gonadorelin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times [100/(100 - W - \text{HCl})] \times 100$$

r_U = peak response of gonadorelin from the *Sample solution*

r_S = peak response of gonadorelin from the *Standard solution*

C_S = concentration of USP Gonadorelin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Gonadorelin Hydrochloride in the *Sample solution* (mg/mL)

F = conversion factor, (1182.3/1255.2)

W = water content in Gonadorelin Hydrochloride sample (%)

HCl = hydrochloride content in Gonadorelin Hydrochloride sample (%) [NOTE—%HC = %Cl × (36.46/35.45).]

Acceptance criteria: 95.0–102.0% on the anhydrous and hydrochloride-free basis

OTHER COMPONENTS

• **COUNTER ION: CHLORIDE**

Sample: 25 mg of Gonadorelin Hydrochloride

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.01 N silver nitrate VS

Blank: To 1 mL of methanol, add 10 mL of water and 1 drop of glacial acetic acid.

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 1 mL of methanol, and add 10 mL of water and 1 drop of glacial acetic acid. Titrate with *Titrant* and perform a blank determination.

www.webofpharma.com

Calculate the percentage of chloride (Cl^-) in the portion of Gonadorelin Hydrochloride taken:

$$\text{Result} = [(V_S - V_B) \times N \times F \times 100]/W$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (Eq/L = mEq/mL)
F = equivalency factor for chloride, 35.45 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 4.0%–6.0%

IMPURITIES

• **GONADORELIN RELATED IMPURITIES**

[NOTE—The *Sample solution* may be stored at room temperature for up to 20 min or at 4° for up to 8 h.]

Solution A: Dissolve 6.8 g of monobasic potassium phosphate in water and dilute to 1 L. Adjust with 1 N potassium hydroxide to a pH of 6.5.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	82	18
20	82	18
30	30	70
35	30	70
38	100	0

[NOTE—Pump *Solution A* through the column at a flow rate of 1 mL/min for 30 min or until a stable baseline is obtained, then inject 100 µL of *Solution A*, and run the gradient elution program to completion to condition the column. Again inject 100 µL of *Solution A*, and run the gradient elution program to completion.]

Standard stock solution: 1 mg/mL of USP Gonadorelin Hydrochloride RS in *Solution A*

Standard solution: 5 µg/mL of USP Gonadorelin Hydrochloride RS in *Solution A* from *Standard stock solution*

System suitability solution: 0.01 mg/mL of USP Gonadorelin Acetate Related Compound A RS and 1 mg/mL of USP Gonadorelin Hydrochloride RS in *Solution A*

Sample solution: 1 mg/mL of Gonadorelin Hydrochloride in *Solution A*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 100 µL

Autosampler temperature: 4°

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for gonadorelin related compound A and gonadorelin are 0.98 and 1.0, respectively.]

Suitability requirements

Retention time: 24–30 min for the gonadorelin peak, *Standard solution*

Resolution: The valley point between gonadorelin related compound A and gonadorelin is visible, *System suitability solution*.

Tailing factor: NMT 2.0, *Standard solution*

[NOTE—If necessary, adjust the flow rate to between 0.8 and 2 mL/min, or, alternatively, change by NMT 3% the percentages of *Solution A* and *Solution B* at 3 min and at 20 min.]

Analysis

Samples: *Solution A*, *Standard solution*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Gonadorelin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of gonadorelin from the *Standard solution*
C_S = concentration of USP Gonadorelin Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Gonadorelin Hydrochloride in the *Sample solution* (mg/mL)
F = conversion factor, 0.001 mg/µg

Acceptance criteria

Any individual impurity: NMT 2.0%

Total impurities: NMT 3.0%

• **ACETIC ACIDS IN PEPTIDES** ⟨503⟩**:** NMT 1.0%

• **LIMIT OF TRIFLUOROACETIC ACID**

Perform if trifluoroacetic acid is used in the manufacturing process.

Buffer: 7.0 mL/L of phosphoric acid and 5.0 mL/L of ammonium hydroxide in water

Solution A: Methanol and *Buffer* (2:100)

Solution B: Acetonitrile and water (1:1)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
6	0	100
14	0	100
15	100	0
25	100	0

Diluent: 5 mL/L of phosphoric acid in water

Trifluoroacetic acid stock solution: 10 mg/mL of trifluoroacetic acid in water prepared as follows. Add about 50 mL of water to a 100-mL volumetric flask with a stopper. Tare the stoppered flask on an analytical balance until there is no further significant drift in the reading. Transfer 670 µL of trifluoroacetic acid to the flask, stopper immediately, and weigh. Dilute with water to volume.

Standard solutions: Transfer 10 mL, 2 mL, and 100 µL of the *Trifluoroacetic acid stock solution* into three separate 100-mL volumetric flasks, and dilute each with *Diluent* to obtain *Standard solutions* having known concentrations, respectively, of about 1 mg/mL, 0.2 mg/mL, and 0.01 mg/mL of trifluoroacetic acid in *Diluent*.

Sample solution: 4.0 mg/mL of Gonadorelin Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

USP Monographs

www.webofpharma.com

Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solutions*
Suitability requirements
Correlation coefficient (r^2): NLT 0.995, determined by plotting the peak areas of the *Standard solutions* versus concentration, in mg/mL, and determining the regression line using the least-squares method
Relative standard deviation: NMT 2.0%, the most concentrated *Standard solution*
Analysis
Samples: *Standard solutions* and *Sample solution*
From the graph of the *Standard solutions* and the concentration of the *Sample solution*, determine the percentages of trifluoroacetic acid in the sample.
Acceptance criteria: NMT 0.25%

• **LIMIT OF FLUORIDE**
Perform if fluoride is used in the manufacturing process. Use polypropylene vessels for preparation of sample and Standards.
Standard solutions: Prepare a series of calibration standards containing 0.05, 0.1, 1, and 10 ppm of fluoride dissolved in an ionic strength adjustment buffer suitable for the electrode in use (pH of 5).
Sample solution: 2.2–3.6 mg/mL of Gonadorelin Hydrochloride in the same buffer as that used to prepare the *Standard solutions*
Analysis: Using a fluoride ion-selective electrode connected to a pH/ion meter, measure the potential of each *Standard solution*, and plot the response versus the logarithm of the concentration. Determine the regression line using the least-squares method. The slope of the calibration curve is –54 to –60 mV/decade, and the square of the correlation coefficient curve (r^2) is NLT 0.995.
From the calibration curve and the concentration of the *Sample solution*, determine the amount of fluoride in the sample.
Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 7.0%
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 70 USP Endotoxin Units/mg
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 100 cfu/g. The total yeast and mold count is NMT 100 cfu/g.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, well-sealed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Gonadorelin Hydrochloride RS
USP Gonadorelin Acetate Related Compound A RS
Gonadorelin free acid.
$C_{55}H_{74}N_{16}O_{14}$ 1183.3

Chorionic Gonadotropin

[9002-61-3].

DEFINITION

Chorionic Gonadotropin is a gonad-stimulating polypeptide hormone obtained from the urine of pregnant women. Its potency is NLT 1500 USP Chorionic Gonadotropin Units in each mg, and NLT 80.0% and NMT 125.0% of the potency stated on the label.

ASSAY

• **PROCEDURE**
Diluent: Saline TS, freshly prepared to contain 1 mg/mL of bovine serum albumin and adjusted with sodium hydroxide TS to a pH of 6.9–8.0
Standard stock solution: 10 Units/mL of USP Human Chorionic Gonadotropin RS in *Diluent*
Standard solutions: From the *Standard stock solution*, prepare three *Standard solutions* in *Diluent* such that the respective concentrations of chorionic gonadotropin constitute a geometric series such as 1:1.2:1.44 or 1:2:4 and such that the activity in each mL lies within the range of 0.1–1.0 Unit.
Sample solutions: Following the analysis outlined for the *Standard stock solution* and *Standard solutions*, prepare solutions of Chorionic Gonadotropin to obtain three *Sample solutions* corresponding to those of the *Standard solutions*.
Animals: Select 20- to 23-day-old female rats, but restrict the selection so that no rat is more than 30% heavier than the lightest. House the animals under uniform conditions of temperature, lighting, feeding, and watering. Mark the animals for identification, and divide them at random into groups of the same number but NLT 10 animals. Assign one group to each of the three *Standard solutions* and three *Sample solutions*, respectively.
Analysis: Inject each rat subcutaneously in the dorsal area with 0.20 mL of the solution to which it was assigned, at approximately the same time on each of 3 consecutive days. On the afternoon of the fifth day, sacrifice the animals, and excise the uterus from each animal by cutting through the cervix, stripping off the surrounding tissue, and severing at the utero-tubal junction. Gently press out the uterine fluid on moistened absorbent paper, and weigh the uterus to the nearest 0.2 mg, using a suitable balance.
Calculation: Tabulate the observed uterine weight for each rat, designated by the symbol y, for each dosage group of f rats. If the data from one or more rats are missing, adjust to groups of equal size by suitable means. Total the values of y in each group, and designate each total as T, subscripts 1–3 for the three successive dosage levels and subscripts S and U for the *Standard solutions* and the *Sample solutions*, respectively. Test for parallelism and linearity between the results for the *Standard solutions* compared to the *Sample solutions* by suitable statistical methods.
Determine the logarithm of potency of the sample taken:

$$\text{Result} = (4iT_a/3T_b) + \log R$$

i = interval between successive log doses of both the *Standard solution* and the *Sample solution*

$$T_a = \Sigma(T_U - T_S)$$

$$T_b = \Sigma(T_3 - T_1)$$

$$R = v_S/v_U$$

v_S = high dose of the Standard (USP Units)
v_U = high dose of the sample (mL)

Compute the log confidence interval L (see *Design and Analysis of Biological Assays* ⟨111⟩). If the confidence interval is more than 0.1938, which corresponds at P = 0.95 to confidence limits of 80% and 125% of the computed potency, repeat the *Assay* until the combined

USP Monographs

www.webofpharma.com

data of two or more assays, redetermined as described under *Design and Analysis of Biological Assays* ⟨111⟩, *Combination of Independent Assays*, meet this limit.

Acceptance criteria: Potency is NLT 1500 USP Chorionic Gonadotropin Units/mg, and 80.0%–125.0% of the potency stated on the label.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 5.0%
- **ESTROGENIC ACTIVITY**

Sample solution: Dissolve a suitable quantity in saline TS to obtain an equivalent of 1000 USP Chorionic Gonadotropin Units/mL.

Analysis: Into each of five rats that have been ovariectomized NLT 2 weeks previously, inject subcutaneously 0.25 mL of the *Sample solution* in the morning and in the afternoon of 2 successive days. On each of the 3 following days, take a vaginal smear from each animal.

Acceptance criteria: The requirements of the test are met if the cellular elements in the smears consist mainly of leukocytes and a few nucleated epithelial cells, but no cornified epithelial cells.

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Contains NMT 0.03 USP Endotoxin Unit/USP Chorionic Gonadotropin Unit
- **STERILITY TESTS** ⟨71⟩: Where the label states that it is sterile, it meets the requirements.
- **ACUTE TOXICITY**

Sample solution: Prepare by dissolving a suitable quantity in sterile, pyrogen-free saline TS to obtain a solution containing 2000 USP Chorionic Gonadotropin Units/mL.

Analysis: Select five healthy mice, weighing 18–22 g. Inject intravenously a dose of 0.5 mL of the *Sample solution* into each of the mice. Observe the animals over the 48 h following the injection.

Acceptance criteria: The requirements of the test are met if, at the end of 48 h, all of the animals survive and NMT 1 of the animals shows outward symptoms of a toxic reaction. If more than 1 of the animals show outward signs of a toxic reaction or if NMT 2 of the animals die, repeat the test on 10 additional, similar animals. If all of the animals of the repeat test survive for 48 h and show no symptoms of a toxic reaction, the requirements of the test are met.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, preferably of Type I glass, in a refrigerator.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Human Chorionic Gonadotropin RS

Chorionic Gonadotropin for Injection

DEFINITION

Chorionic Gonadotropin for Injection is a sterile, dry mixture of Chorionic Gonadotropin with suitable diluents and buffers. Its potency is NLT 80.0% and NMT 125.0% of the potency stated on the label in USP Chorionic Gonadotropin Units. It may contain an antimicrobial agent.

ASSAY

- **PROCEDURE**

Diluent: Saline TS, freshly prepared to contain 1 mg/mL of bovine serum albumin and adjusted with sodium hydroxide TS to a pH of 6.9–8.0

Standard stock solution: 10 Units/mL of USP Human Chorionic Gonadotropin RS in *Diluent*

Standard solutions: From the *Standard stock solution*, prepare three *Standard solutions* in *Diluent* such that the respective concentrations of chorionic gonadotropin constitute a geometric series such as 1: 1.2: 1.44 or 1:2:4 and such that the activity in each mL lies within the range of 0.1–1.0 Unit.

Sample solutions: Dilute a portion of the solution prepared for the test for *Estrogenic Activity* quantitatively and stepwise with *Diluent* to obtain three *Sample solutions* corresponding to those of the *Standard solutions*.

Animals: Select 20- to 23-day-old female rats, but restrict the selection so that no rat is more than 30% heavier than the lightest. House the animals under uniform conditions of temperature, lighting, feeding, and watering. Mark the animals for identification, and divide them at random into groups of the same number but NLT 10 animals. Assign one group to each of the three *Standard solutions* and three *Sample solutions*, respectively.

Analysis: Inject each rat subcutaneously in the dorsal area with 0.20 mL of the solution to which it was assigned, at approximately the same time on each of 3 consecutive days. On the afternoon of the fifth day, sacrifice the animals, and excise the uterus from each animal by cutting through the cervix, stripping off the surrounding tissue, and severing at the utero-tubal junction. Gently press out the uterine fluid on moistened absorbent paper, and weigh the uterus to the nearest 0.2 mg, using a suitable balance.

Calculation: Tabulate the observed uterine weight for each rat, designated by the symbol y, for each dosage group of f rats. If the data from one or more rats are missing, adjust to groups of equal size by suitable means. Total the values of y in each group, and designate each total as T, subscripts 1 to 3 for the three successive dosage levels, and subscripts S and U for the *Standard solutions* and *Sample solutions*, respectively. Test for parallelism and linearity between the results for the *Standard solutions* compared to the *Sample solutions* by suitable statistical methods.

Determine the logarithm of potency of the Injection:

$$\text{Result} = (4iT_a/3T_b) + \log R$$

i = interval between successive log doses of the *Standard solution* and *Sample solution*

$$T_a = \Sigma(T_U - T_S)$$

$$T_b = \Sigma(T_3 - T_1)$$

$$R = v_S/v_U$$

v_S = high dose of the Standard (USP Units)

v_U = high dose of the sample (mL)

Compute the log confidence interval, L (see *Design and Analysis of Biological Assays* ⟨111⟩). If the confidence interval is more than 0.1938, which corresponds at P = 0.95 to confidence limits of 80% and 125% of the computed potency, repeat the *Assay* until the combined data of two or more assays, redetermined as described under *Design and Analysis of Biological Assays* ⟨111⟩, *Combination of Independent Assays*, meet this limit.

Acceptance criteria: 80.0%–125.0% of the potency stated on the label

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS**

Analysis: Open 10 containers, and accurately weigh each individual container and its contents, taking care to preserve the identity of each container and removing any labeling that might be altered in weight during the removal of the container contents. Remove the contents of each

www.webofpharma.com

container by rinsing thoroughly with water, dry at 105° to constant weight, and reweigh. Calculate for each container the net weight of its contents by subtracting the weight of the dry, empty container from its initial gross weight. Determine the average weight of the contents and the relative standard deviation.

Acceptance criteria: The requirements are met if the weight of the contents of each container does not deviate from the average weight by more than 5.0%, and the relative standard deviation of the 10 containers is NMT 3.0%. If the requirements of the test are not met, test 20 additional containers. The requirements are met if the net weight of NMT 1 container of the 30 deviates by more than 7.5% from the average weight of the contents of the 30 containers and the relative standard deviation of the 30 containers is NMT 3.3%.

SPECIFIC TESTS

- **pH** ⟨791⟩: The pH of the solution prepared for the test for *Estrogenic Activity* is between 6.0 and 8.0.
- **Estrogenic Activity**

Sample solution: Reconstitute as directed in the labeling.

Analysis: Into each of five rats that have been ovariectomized NLT 2 weeks previously, inject subcutaneously 0.25 mL of the *Sample solution* in the morning and in the afternoon of 2 successive days. On each of the 3 following days, take a vaginal smear from each animal.

Acceptance criteria: The requirements of the test are met if the cellular elements in the smears consist mainly of leucocytes and a few nucleated epithelial cells, but no cornified epithelial cells.

- **Other Requirements:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.
- **Bacterial Endotoxins Test** ⟨85⟩: It contains NMT 0.03 USP Endotoxin Unit/USP Chorionic Gonadotropin Unit.
- **Sterility Tests** ⟨71⟩: Meets the requirements
- **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution* .
- **Labeling:** Label it to indicate the expiration date.

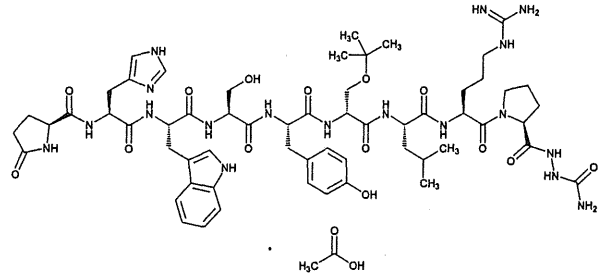
Change to read:

- **USP Reference Standards** ⟨11⟩

▲▲ (ERR 1-Jul-2018)

USP Human Chorionic Gonadotropin RS

Goserelin Acetate



$C_{59}H_{84}N_{18}O_{14} \cdot xC_2H_4O_2$

Luteinizing hormone-releasing factor (pig), 6-[*O*-(1,1-dimethylethyl)-D-serine]-10-deglycinamide-, 2-(aminocarbonyl)hydrazide, acetate (salt) [145781-92-6].

1-(5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-*O*-*tert*-butyl-D-seryl-L-leucyl-L-arginyl-L-prolyl)semicarbazide (Goserelin) 1269.41

[65807-02-5].

DEFINITION

Goserelin Acetate is a synthetic nonapeptide analog of the hypothalmic decapeptide, gonadorelin. It is obtained by chemical synthesis and is available as an acetate salt. It contains NLT 94.5% and NMT 103.0% of goserelin ($C_{59}H_{84}N_{18}O_{14}$), calculated on the anhydrous and acetic acid-free basis.

IDENTIFICATION

- **A.** The retention time of the goserelin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Mobile phase: Prepare a filtered and degassed mixture of water, acetonitrile, and trifluoroacetic acid (1600:400:1).

Standard solution: 1 mg/mL of USP Goserelin Acetate RS in water

Diluted standard solution: Transfer 1 mL of the *Standard solution* to a 10-mL volumetric flask, and dilute with water to volume.

Resolution solution: Dissolve the contents of a vial of USP Goserelin Related Compound A RS in water to obtain a concentration of 0.1 mg/mL, and mix with an equal volume of *Diluted standard solution*.

System suitability solution: Dissolve the contents of a vial of USP Goserelin System Suitability Mixture RS with 1 mL of water.

Sample solution: 1 mg/mL of Goserelin Acetate in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Column temperature: 50°–55°

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Samples: *Standard solution*, *Resolution solution*, and *System suitability solution*.

Suitability requirements

[NOTE—Two minor peaks are visible prior to the elution of the principal peak, *System suitability solution*.]

USP Monographs

www.webofpharma.com

Resolution: NLT 7.0 between the goserelin and goserelin related compound A (4-D-ser-goserelin) peaks, *Resolution solution*
Retention time: 40–50 min for the goserelin peak, *Resolution solution*
Relative standard deviation: NMT 2.0% for the goserelin peak, from replicate injections, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of goserelin ($C_{59}H_{84}N_{18}O_{14}$) in the portion of Goserelin Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 94.5%–103.0% of goserelin ($C_{59}H_{84}N_{18}O_{14}$) on the anhydrous, acetic acid-free basis

OTHER COMPONENTS

• **LIMIT OF ACETIC ACID**

Mobile phase: Transfer 49.04 g of sulfuric acid to a 1000-mL volumetric flask, dilute with water to volume, and mix. Accurately transfer 20 mL of this solution to a 2000-mL volumetric flask, dilute with water to volume, mix, filter, and degas.
Standard stock solution: Transfer 2.0 mL of glacial acetic acid to a 500-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Sample solution: Dissolve about 20 mg of Goserelin Acetate, accurately weighed, in 2–3 mL of *Mobile phase*. Connect a 1-mL cartridge containing packing L44 to a 1-mL cartridge containing packing L2, which is then attached to a suitable vacuum apparatus. With the vacuum applied, wash the cartridge combination with 2 mL of methanol followed by 15 mL of *Mobile phase*, and discard the washings. Quantitatively apply the solution containing the sample to the cartridge combination, and wash through the cartridge system with several small volumes of *Mobile phase*. Collect the solution and washings in a 10-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*).
[NOTE—Condition the column for about 24 h until a stable baseline is obtained.]
Mode: LC
Detector: UV 206 nm
Column: 7.8-mm × 30-cm; packing L17
Column temperature: 65°
Flow rate: 0.8 mL/min
Injection size: 100 µL
System suitability:
Sample: *Standard solution*
[NOTE—The retention time of the acetic acid peak is about 11 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 3.1%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetic acid in the portion of Goserelin Acetate taken by the formula:

$$\text{Result} = (r_U/r_S) \times (1/W) \times (1.049/5)$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
W = sample weight of Goserelin Acetate taken to prepare the *Sample solution* (g) and corrected (for the purposes of the calculation) to eliminate the water content, which is determined immediately prior to the test
1.049 = weight of glacial acetic acid (g/mL)

Acceptance criteria: 4.5%–15.0%

IMPURITIES

• **ORGANIC IMPURITIES: RELATED COMPOUNDS**

Mobile phase, Standard solution, Diluted standard solution, Resolution solution, System suitability solution, Sample solution, and **Chromatographic system:** Prepare as directed in the *Assay*.
Diluted sample solution: Transfer 1 mL of the *Sample solution* into a 100-mL volumetric flask, and dilute with water to volume.
System suitability
Samples: *Resolution solution* and *System suitability solution*
[NOTE—The retention time for the goserelin peak is between 40 and 50 min, *Resolution solution*. See *Table 1* for the relative retention times, *Resolution solution*. Two peaks, corresponding to decarbamoylgoserelin and 2-D-his-goserelin, eluting prior to the principal peak, are visible, *System suitability solution*.]

Table 1

Name	Relative Retention Time
4-D-ser-Goserelin	0.67
Decarbamoylgoserelin	0.89
5-D-tyr-Goserelin	0.92
2-D-his-Goserelin	0.94
Goserelinare	1.0

Resolution: NLT 7.0, *Resolution solution*
Column efficiency: NLT 2000 theoretical plates, *System suitability solution*
Tailing factor: NMT 2.0, *System suitability solution*
Relative standard deviation: NMT 2.0%, *System suitability solution*
Analysis
Samples: *Sample solution* and *Diluted sample solution*
Calculate the percentage of goserelin-related impurities in the portion of Goserelin Acetate taken:

$$\text{Result} = r_I/r_U$$

r_I = peak response for any individual impurity in the *Sample solution*
r_U = peak response of the main goserelin peak in the *Diluted sample solution*

Acceptance criteria: NMT 1.0% of decarbamoylgoserelin is found, NMT 0.5% of any other impurity is found, and NMT 2.5% of total impurities is found.

SPECIFIC TESTS

• **NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY** ⟨761⟩, *Amino Acid Content*
[NOTE—Concentrations of goserelin in both the *Standard solution* and the *Sample solution* must be the same (within 5% of each other) but can be adjusted

USP Monographs

www.webofpharma.com

based on the quality of the ^{13}C spectrum obtained. The spectra must be acquired under the same conditions for both the *Standard solution* and the *Sample solution*. The spectra obtained are of sufficient quality to allow quantification of the integrals of the resonances specified below to be obtained. Integrals and spectra of both the *Standard solution* and the *Sample solution* can be repeated and averaged.]

Standard solution: Dissolve USP Goserelin Acetate RS in deuterium oxide to obtain a solution having a known concentration of about 10% w/v, and adjust with deuterated acetic acid-d4 to a pH of 4.

Sample solution: Prepare a 10% w/v solution of Goserelin Acetate in deuterium oxide, and adjust with deuterated acetic acid-d4 to a pH of 4.

Analysis: Obtain a ^{13}C, proton-decoupled NMR spectrum of both the *Standard solution* and the *Sample solution*. The spectra from both solutions are qualitatively similar, and all the resonances from the spectrum of the *Standard solution* are present in the spectrum of the *Sample solution* and have the same chemical shift values (±0.1 ppm for goserelin, ±0.5 ppm for acetate). Identify any other resonances in the spectrum of the *Sample solution*. The relative amino acid ratio between the *Standard solution* and the *Sample solution* can be calculated as follows. Integrate the resonances at the approximate ppm corresponding to each amino acid in *Table 2*.

Table 2

Amino Acids	Resonances (ppm)
Azo-glycine	162.2
Histidine	118.4
Tyrosine	116.7
tert-Butyl serine	62.5
Serine	62.2
Tryptophan	55.7
Arginine	41.8
Pyroglutamic acid	26.3
Proline	26.0
Leucine	23.5

Calculate the ratio of each of the amino acids from the integrals of the *Standard solution* and the *Sample solution* by the formula:

$$\text{Result} = r_U/r_S$$

r_U = integral of the resonance of a designated amino acid from the *Sample solution*

r_S = integral of the resonance of a designated amino acid from the *Standard solution*

Acceptance criteria: The resulting ratios fall within the following limits: histidine, tyrosine, *tert*-butyl serine, serine, tryptophan, arginine, pyroglutamic acid, proline, and leucine 0.9–1.1; azo-glycine 0.8–1.2.

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 2 mg/mL, in water, calculated on the anhydrous and acetic acid-free basis
  Acceptance criteria: Between −52° and −56°
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: It contains NMT 16 USP Endotoxin Units/mg of goserelin, if intended for use in the manufacture of parenteral dosage forms without a further, appropriate procedure for the removal of bacterial endotoxins.
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 10.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a refrigerator.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Goserelin Acetate RS
  USP Goserelin Related Compound A RS
  USP Goserelin System Suitability Mixture RS

Goserelin Implants

DEFINITION

Goserelin Implants are extended-release formulations of a sterile dispersion of Goserelin Acetate in a matrix of D,L-lactic and glycolic acids copolymer (PLGA) that is subcutaneously injected. The content of the PLGA and other ingredients is process-specific, and their identity and properties are determined by validated methods. The quality of Goserelin Acetate meets the compendial standard. Goserelin Implants contain NLT 90.0% and NMT 110.0% of the labeled amount of goserelin ($C_{59}H_{84}N_{18}O_{14}$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Product-Related Substances and Impurities, Procedure 1*.

ASSAY

- **PROCEDURE**
  Buffer: 0.1 M sodium perchlorate solution, pH 2.1. [NOTE—This solution can be prepared as follows. Weigh 167.5 g of 60% (w/v) perchloric acid into a 1-L volumetric flask, then cool the flask on an ice bath. Add 1 M sodium hydroxide to volume. Transfer 100 mL of this 1 M sodium perchlorate solution to a 1-L volumetric flask and add water to volume. Adjust with 20% (w/v) sodium hydroxide to a pH of 2.1.]
  Mobile phase: Acetonitrile and *Buffer* (92:8)
  Diluent: Acetonitrile and water (85:15)
  System suitability solution: 2.0 mg/mL each of USP Goserelin Acetate RS and USP Goserelin Related Compound A RS in *Diluent*
  Standard solution: 2.0 mg/mL of USP Goserelin Acetate RS in *Diluent*
  Sample solution: Nominally 2.0 mg/mL of goserelin in *Diluent* prepared by adding 10 Implants to a suitable volumetric flask. Add *Diluent* to about 70% of the total volume, and sonicate to dissolve the sample. Cool to room temperature, and dilute with *Diluent* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 280 nm
  Column: 7.8-mm × 30-cm; 5-µm packing L33
  Flow rate: 1.0 mL/min
  Injection volume: 5 µL
  Run time: 90 min
  System suitability
  Samples: *System suitability solution* and *Standard solution*

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 4.0 between the main goserelin and goserelin related compound A peaks, *System suitability solution*
Tailing factor: NMT 1.5 for the goserelin peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of goserelin ($C_{59}H_{84}N_{18}O_{14}$) in the portion of Implants taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of goserelin from the *Sample solution*
r_S = peak response of goserelin from the *Standard solution*
C_S = concentration of USP Goserelin Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of goserelin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
For 3.6-mg Implants
Medium: pH 7.4 phosphate/citrate buffer prepared as follows. Dissolve 25.8 g/L of anhydrous disodium hydrogen phosphate, 1.92 g/L of citric acid, and 0.2 g/L of sodium azide in water. Adjust with anhydrous disodium hydrogen phosphate or citric acid to a pH of 7.4. Pass through a sterile filter of NMT 0.2-µm pore size into a sterile container; 50 mL
Apparatus: 120-mL flat-bottomed, borosilicate glass jar with a tight plastic cap. Incubate at 39 ± 0.5°.
[NOTE—Suitable procedures should be carried out to remove/minimize microbiological contamination on the glassware immediately before the test, e.g., rinse with methanol and dry at 90° for 2 h.]
Times: 168, 336, 408, 504, and 672 h
Sample solutions: Place 5 Implants in each jar, and add 50 mL of *Medium* at ambient temperature to each jar. Place the jars in the incubator. At the times specified, remove the jars from the incubator and allow to cool for 1 h. Swirl gently to mix, and withdraw 5 mL of aliquot. Replace the aliquot withdrawn with the same volume of *Medium* at ambient temperature into the jar, and return to the incubator 2 h after removal. Dilute each sample with *Medium* (1:1).
For 10.8-mg Implants
Medium: pH 7.4 phosphate buffered saline prepared as follows. Dissolve 8 g/L of sodium chloride, 0.19 g/L of potassium dihydrogen phosphate, 1.38 g/L of anhydrous disodium hydrogen phosphate, and 0.2 g/L of sodium azide in water. Adjust with 2 M hydrochloric acid to a pH of 7.4. Pass through a sterile filter of NMT 0.2-µm pore size into a sterile container; 50 mL.
Apparatus: 120-mL flat-bottomed, borosilicate glass jar with a tight plastic cap. Incubate at 39 ± 0.5°.
[NOTE—Suitable procedures should be carried out to remove/minimize microbiological contamination on the glassware immediately before the test, e.g., rinse with methanol and dry at 90° for 2 h.]
Times: 72, 336, 840, 1344, and 2016 h
Sample solutions: Transfer 50 mL of *Medium* to the jars, seal, and warm to 39 ± 0.5° overnight in the incubator. Place a single Implant in each jar, and return to the incubator. At the times specified, remove the jars from the incubator, swirl gently to mix, and withdraw 5 mL of aliquot at 24, 72, 168, and 264 h, and 20 mL of aliquot at 336, 504, 672, 840, 1008, 1176, 1344, 1512, 1680, 1848, and 2016 h. Replace the aliquot withdrawn with the same volume of prewarmed *Medium* into the jar, and return to the incubator. [NOTE—At each sampling time point, the UV absorbance must be measured so that the goserelin content of the test aliquot can be used in the calculation of cumulative release.]
Standard solution: 0.1 mg/mL of USP Goserelin Acetate RS in *Medium*
Analytical wavelength: 280 nm
[NOTE—Bandwidth is 10 nm.]
Path length cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Sample solutions* and *Standard solution*
Calculate each *Sample solution* concentration of goserelin taken at each sampling time point, C_x:

$$\text{Result} = (A_U/A_S) \times C_S$$

A_U = absorbance of each *Sample solution* taken at each sampling time point
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Goserelin Acetate RS in the *Standard solution* (mg/mL)

Calculate the cumulative amount of goserelin ($C_{59}H_{84}N_{18}O_{14}$) dissolved, as a percentage of the labeled amount of the dose, at the specified times:

$$\text{Result} = \frac{[(C_x \times V_0 \times D) + \sum_{n=1}^{x-1} (C_n \times V_n \times D)]}{W} \times 100$$

C_x = concentration of goserelin in each *Sample solution* taken at each sampling time point (mg/mL)
V_0 = volume of the dissolution *Medium*, 50 mL
D = dilution factor
C_n = concentration of goserelin in each *Sample solution* taken at n sampling time point (mg/mL), n must be ≤ x–1
V_n = volume of each *Sample solution* taken at n sampling time point (mL)
W = amount of goserelin in the sample (mg)

Tolerances: See *Table 1*.

Table 1

Time (h)	For 3.6-mg Implants Only Amount Dissolved (%)	For 10.8-mg Implants Only Amount Dissolved (%)	
	Each of the Three Replicates (Individually)	Mean of the 6 Implants	Individual Implant
72	—	10–25	5–30
168	NMT 20	—	—
336	25–55	15–40	10–45
408	35–75	—	—
504	65–90	—	—
672	85–105	—	—

USP Monographs

www.webofpharma.com

Table 1 *(continued)*

Time (h)	For 3.6-mg Implants Only Amount Dissolved (%)	For 10.8-mg Implants Only Amount Dissolved (%)	
	Each of the Three Replicates (Individually)	Mean of the 6 Implants	Individual Implant
840	—	20–55	15–60
1344	—	60–85	55–90
2016	—	NLT 75	NLT 70

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Mobile phase: 2.72 mg/mL of potassium dihydrogen phosphate in a mixture of water and methanol (45:55). Adjust with phosphoric acid to a pH of 3.0.

Diluent: Acetonitrile and water (85:15)

Standard solution: 0.2 mg/mL of USP Goserelin Acetate RS in *Diluent*

Sample solution: Nominally 0.2 mg/mL of goserelin in *Diluent* prepared by dissolving 1 Implant in a suitable volume of *Diluent* with the aid of sonication

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1.8 mL/min

Injection volume: 10 µL

Run time: 12 min

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for the goserelin peak

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of goserelin ($C_{59}H_{84}N_{18}O_{14}$) in the portion of Implant taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of goserelin from the *Sample solution*

r_S = peak response of goserelin from the *Standard solution*

C_S = concentration of USP Goserelin Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of goserelin in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **PROCEDURE 1**

Mobile phase: 2.72 mg/mL of potassium dihydrogen phosphate in a mixture of acetonitrile and water (27:73). Adjust with phosphoric acid to a pH of 3.0.

Diluent: Acetonitrile and water (85:15)

System suitability solution: 20 µg/mL each of USP Goserelin Acetate RS and USP Goserelin Related Compound A RS in *Diluent*

Sensitivity solution: 3 µg/mL of USP Goserelin Acetate RS in *Diluent*

Standard solution: 20 µg/mL of USP Goserelin Acetate RS in *Diluent*

Sample solution: Nominally 2.0 mg/mL of goserelin prepared by dissolving 10 Implants in *Diluent* with the aid of sonication

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.8 mL/min

Injection volume: 5 µL

Run time: 60 min

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

[NOTE—The retention times for goserelin related compound A and goserelin in the *System suitability solution* are approximately 17 and 22 min, respectively.]

Suitability requirements

Resolution: NLT 4.5 between the main goserelin and goserelin related compound A peaks, *System suitability solution*

Tailing factor: NMT 2.0 for the goserelin peak, *Standard solution*

Relative standard deviation: NMT 4.5%, *Standard solution*

Signal-to-noise ratio: NLT 5, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Implants taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F$$

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of goserelin from the *Standard solution*

C_S = concentration of USP Goserelin Acetate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of goserelin in the *Sample solution* (mg/mL)

F = conversion factor, 0.1

Acceptance criteria

Any individual impurity: NMT 1.0%

Total impurities: NMT 4.0% from *Procedure 1*

• **PROCEDURE 2**

Buffer, Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity solution: 6.5 µg/mL of USP Goserelin Acetate RS in *Diluent*

Peak identification solution: Degrade Implants at 90° for 4 h. Dissolve the degraded Implants in *Diluent* with the aid of sonication to obtain a solution that contains approximately 2 mg/mL of goserelin.

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 4.0 between the main goserelin and goserelin related compound A peaks, *System suitability solution*

Tailing factor: NMT 1.5 for the goserelin peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

USP Monographs

www.webofpharma.com

Signal-to-noise ratio: NLT 5, *Sensitivity solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Peak identification solution*

Identify the *O*-glycolyl-ser[4]-goserelin and polymer envelope peaks using the chromatogram of the *Peak identification solution* and the relative retention times listed in *Table 2*.

Calculate the percentage of any individual impurity in the portion of Implants taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of goserelin from the *Standard solution*

C_S = concentration of USP Goserelin Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of goserelin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Polymer envelope[a]	0.10–0.60	5.5
O-Glycolyl-ser[4]-goserelin	0.93	2.0
Any unspecified impurity	—	1.0
Total impurities[b]	—	10.0

[a] *O*-Lactide/glycolide copolymer chain to ser[4] of goserelin. It is calculated as the sum of all peaks eluted in the specified relative retention time range.
[b] Total impurities is the sum of impurities from *Procedure 2* and impurity C from *Procedure 3*.

• **PROCEDURE 3**

[NOTE—Perform this test if impurity C is present.]

Buffer: Prepare as directed in the *Assay*.

Mobile phase: Acetonitrile and *Buffer* (87.5:12.5)

Diluent: Acetonitrile and water (85:15)

System suitability solution: Weigh 4 mg of USP Goserelin Acetate RS, add 250 µL of trifluoroacetic acid, and leave to stand for 24 h. Dissolve in 20 mL of *Diluent* to obtain a solution containing goserelin related compound B. [NOTE—Goserelin related compound B is des-*t*-butyl-goserelin.]

Sensitivity solution: 3 µg/mL of USP Goserelin Acetate RS in *Diluent*

Standard solution: 20 µg/mL of USP Goserelin Acetate RS in *Diluent*

Sample solution: Nominally 2.0 mg/mL of goserelin in *Diluent* prepared by adding 10 Implants to a suitable volumetric flask. Add *Diluent* to about 70% of the total volume, and sonicate to dissolve the sample. Cool to room temperature, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 7.8-mm × 30-cm; 5-µm packing L33

Flow rate: 2.0 mL/min

Injection volume: 50 µL

Run time: 30 min

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Retention: The retention time for goserelin related compound B is between 19 and 24 min, *System suitability solution*

Signal-to-noise ratio: NLT 5, *Sensitivity solution*

Analysis

Samples: *System suitability solution*, *Standard solution*, and *Sample solution*

Identify the impurity C peak with a retention time relative to goserelin related compound B of 1.1 using the chromatogram of the *System suitability solution*. [NOTE—Impurity C is a specified, but unidentified, impurity.]

Calculate the percentage of impurity C in the portion of Implants taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of impurity C from the *Sample solution*

r_S = peak response of goserelin from the *Standard solution*

C_S = concentration of USP Goserelin Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of goserelin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

OTHER COMPONENTS

• **ACETIC ACID CONTENT**

Standard stock solution: 6.25 mg/mL of USP Glacial Acetic Acid RS in dimethylformamide

Internal standard stock solution: Transfer 1.0 mL of *n*-hexadecane to a 50-mL volumetric flask containing approximately 30 mL of dimethylformamide. Dilute with dimethylformamide to volume.

Standard solution: Transfer 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and add approximately 50 mL of dimethylformamide. Add 5.0 mL of the *Internal standard stock solution*, and dilute with dimethylformamide to volume.

Sample solution: Weigh 150 mg of Implants into a 5-mL volumetric flask. Add approximately 1 mL of dimethylformamide and sonicate to dissolve. Add 250 µL of the *Internal standard stock solution*, and dilute with dimethylformamide to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 10-m; fused-silica capillary that contains a 0.3-µm film of phase G35

Temperatures

Injection port: 200°

Column: See *Table 3*.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	0.1
50	30	200	3

Detector: 250°

Carrier gas: Helium

Flow rate: 1.3 mL/min

Injection volume: 1 µL

www.webofpharma.com

Split ratio: 100:30
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 15 between acetic acid and *n*-hexadecane
Tailing factor: NMT 2.0 for acetic acid
Relative standard deviation: NMT 6% for the response ratio of acetic acid to *n*-hexadecane
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetic acid in the portion of Implants taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of acetic acid to *n*-hexadecane from the *Sample solution*
R_S = peak response ratio of acetic acid to *n*-hexadecane from the *Standard solution*
C_S = concentration of USP Glacial Acetic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the goserelin implant sample in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.5%

SPECIFIC TESTS
- **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ic*
Sample solution: 27-mg/mL solution of Implant in dry dimethylformamide
Analysis: Determine the water content of 1.0-mL of the *Sample solution*. Perform a blank determination and make any necessary correction.
Acceptance criteria: NMT 2.5%
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 350 USP Endotoxin Units/Implant
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to be Examined, Membrane Filtration*: Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, tight, light-resistant single-unit containers at controlled room temperature.
- **LABELING:** The label states the amount of the peptide (goserelin) in mg in each implant.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Glacial Acetic Acid RS
USP Endotoxin RS
USP Goserelin Acetate RS
USP Goserelin Related Compound A RS
4-D-Serine goserelin.

Gramicidin

Gramicidin.
Gramicidin [1405-97-6].

» Gramicidin is an antibacterial substance produced by the growth of *Bacillus brevis* Dubos (Fam. Bacillaceae). It may be obtained from tyrothricin. It has a potency of not less than 900 µg of gramicidin per mg, calculated on the dried basis.

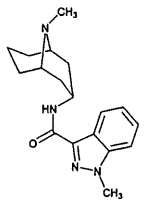
Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Gramicidin RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests,*** ⟨197⟩*Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Solution: 50 µg per mL.
Medium: alcohol.
Melting temperature, *Class Ia* ⟨741⟩: not lower than 229°, determined after drying.
Crystallinity ⟨695⟩: meets the requirements.
Loss on drying ⟨731⟩—Dry about 100 mg in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 3.0% of its weight.
Residue on ignition ⟨281⟩: not more than 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.
Assay—Proceed with Gramicidin as directed under *Antibiotics—Microbial Assays* ⟨81⟩.

Granisetron



$C_{18}H_{24}N_4O$ 312.41
1*H*-Indazole-3-carboxamide, 1-methyl-*N*-(9-methyl-9-azabicyclo[3.3.1]-non-3-yl)-, *endo*-;
1-Methyl-*N*-(9-methyl-*endo*-9-azabicyclo[3.3.1]non-3-yl)-1*H*-indazole-3-carboxamide [109889-09-0].

DEFINITION
Granisetron contains NLT 98.0% and NMT 102.0% of granisetron ($C_{18}H_{24}N_4O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Mobile phase: Dilute 1.6 mL of phosphoric acid with water to 800 mL, and then add 200 mL of acetonitrile. Add 1.0 mL of hexylamine. Adjust with triethylamine to a pH of 7.5 ± 0.05 (about 4 mL is needed).
System suitability solution: Transfer 2 mL of a solution containing about 1.0 mg/mL of granisetron in *Mobile phase* to a colorless glass vial, stopper it, and either expose the solution to sunlight for 4 h or place it under a UV lamp for 16 h (granisetron undergoes partial degradation to granisetron related compound C). A degradation of at least about 0.3% of granisetron to granisetron related compound C must be obtained, as shown by the appearance of a corresponding peak in the chromatogram.

www.webofpharma.com

If it is not obtained, again expose the solution to sunlight or place it under a UV lamp.
Standard solution: 1.0 mg/mL of USP Granisetron RS in *Mobile phase*
Sample solution: 1.0 mg/mL of Granisetron in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 305 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.5 between granisetron and granisetron related compound C, *System suitability solution*
Tailing factor: NMT 2.0 for the granisetron peak, *System suitability solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of granisetron ($C_{18}H_{24}N_4O$) in the portion of Granisetron taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Granisetron RS in the *Standard solution* (mg/mL)
C_U = concentration of Granisetron in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

• **LIMIT OF GRANISETRON RELATED COMPOUND E**
Diluent: Acetonitrile and water (80:20)
Standard solution: 0.075 mg/mL of USP Granisetron Related Compound E RS in *Diluent*
[NOTE—USP Granisetron Related Compound E RS is the acetate salt of (1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]nonan-3-amine. Use the correction factor stated on the label of the USP Reference Standard to calculate the concentration, as appropriate.]
Sample solution: 50 mg/mL of Granisetron in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Ethyl acetate, isopropyl alcohol, and ammonium hydroxide (50: 30: 6.5)
Analysis
Samples: *Standard solution* and *Sample solution*
Proceed as directed in the chapter and develop the chromatogram until the solvent front has moved about half of the length of the plate. Dry the plate in air, and expose it to iodine vapor for 30 min.
Acceptance criteria: Any spot corresponding to granisetron related compound E from the *Sample solution* is not more intense than the corresponding spot from the *Standard solution* (NMT 0.15%).

• **ORGANIC IMPURITIES**
[NOTE—Protect all solutions containing granisetron from light.]
Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.
Standard solution: 0.001 mg/mL of USP Granisetron RS in *Mobile phase*
Sample solution: 1.0 mg/mL of Granisetron in *Mobile phase*. Inject this solution within 4 h of preparation.
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.5 between granisetron and granisetron related compound C, *System suitability solution*
Tailing factor: NMT 2.0 for the granisetron peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Granisetron taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of granisetron from the *Standard solution*
C_S = concentration of USP Granisetron RS in the *Standard solution* (mg/mL)
C_U = concentration of Granisetron in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. The reporting threshold for impurities is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
1-Methylindazole acid (granisetron related compound D)[a]	0.4	0.10
1-Desmethyl granisetron (granisetron related compound B)[b]	0.5	0.15
Granisetron 2-methyl isomer (granisetron related compound A)[c]	0.7	0.15
9-Desmethyl granisetron (granisetron related compound C)[d]	0.8	0.2
Granisetron	1.0	—
Any other individual impurity	—	0.10
Total impurities	—	1.0

[a] 1-Methyl-1*H*-indazole-3-carboxylic acid.
[b] *N*-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide.
[c] 2-Methyl-*N*-[1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide.
[d] *N*-[(1*R*,3*r*,5*S*)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide.

SPECIFIC TESTS

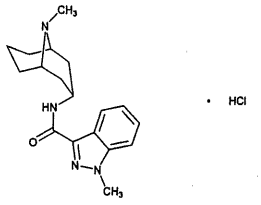
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.5%

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Granisetron RS
  USP Granisetron Related Compound E RS
  Granisetron amine acetate;
  (1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]nonan-3-amine acetate.
  $C_9H_{18}N_2 \cdot C_2H_4O_2$ 214.31

Granisetron Hydrochloride



$C_{18}H_{24}N_4O \cdot HCl$ 348.87

1*H*-Indazole-3-carboxamide, 1-methyl-*N*-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-, monohydrochloride, *endo*-.

1-Methyl-*N*-(9-methyl-*endo*-9-azabicyclo[3.3.1]non-3-yl)-1*H*-indazole-3-carboxamide monohydrochloride [107007-99-8].

» Granisetron Hydrochloride contains not less than 97.0 percent and not more than 102.0 percent of $C_{18}H_{24}N_4O \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, protected from light. Store at room temperature.

USP Reference standards ⟨11⟩—
USP Granisetron Hydrochloride RS
USP Granisetron Related Compound A RS
(2-Methyl-*N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide).
USP Granisetron Related Compound B RS
(*N*-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide).
USP Granisetron Related Compound E RS
((1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]nonan-3-amine, acetate salt).

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: It meets the requirements of the test for *Chloride* ⟨191⟩.

pH ⟨791⟩**:** between 4.0 and 6.5, in a solution in carbon dioxide-free water, containing 10 mg per mL.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Limit of granisetron related compound E—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Diluent: a mixture of acetonitrile and water (80:20).

Test solution—Transfer 250 mg of Granisetron Hydrochloride to a 5-mL volumetric flask, and dissolve in and dilute with *Diluent* to volume.

Standard solution—Dissolve an accurately weighed quantity of USP Granisetron Related Compound E RS in *Diluent* to obtain a solution containing 0.25 mg per mL. [NOTE—USP Granisetron Related Compound E RS is the acetate salt of (1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]nonan-3-amine. Use the correction factor stated on the label of the USP Reference Standard to calculate the concentration, as appropriate.]

Application volume: 2 µL.

Developing solvent system—A mixture of ethyl acetate, isopropyl alcohol, and ammonium hydroxide (50: 30: 6.5).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩, and develop the chromatogram until the solvent front has moved about half of the length of the plate. Dry the plate in air, and expose it to iodine vapor for 30 minutes. Any spot corresponding to granisetron related compound E obtained from the *Test solution* is not more intense than the corresponding spot obtained from the *Standard solution* (0.5%).

Related compounds—

[NOTE—Protect all solutions containing granisetron hydrochloride from light.]

Mobile phase and *System suitability solution*—Proceed as directed in the *Assay*.

Standard solution—Dissolve an accurately weighed quantity of USP Granisetron Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.005 mg per mL.

Identification solution—Prepare a solution in *Mobile phase* containing 0.01 mg per mL of USP Granisetron Related Compound A RS and 0.005 mg per mL of USP Granisetron Related Compound B RS.

Test solution—Use the *Assay preparation*, prepared as directed in the *Assay*.

Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay*. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between granisetron related compound C and granisetron is not less than 3.5; and the tailing factor for the granisetron peak is not more than 2.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 10%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution*, the *Identification solution*, and the *Test solution* into the chromatograph, record the chromatograms for about two times the retention time of granisetron, identify the peaks on the basis of their relative retention times as given in *Table 1* and measure the peak responses. Calculate the percentage of each impurity in the portion of Granisetron Hydrochloride taken by the formula:

$$100(1/F)(C_S/C_T)(r_U/r_S)$$

in which F is the relative response factor given in *Table 1* C_S and C_T are the concentrations, in mg per mL, of granisetron hydrochloride in the *Standard solution* and the *Test solution*, respectively; r_U is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for granisetron obtained from the *Standard solution:* in addition to not exceeding the limits in *Table 1* not more than 1.0% of total impurities is found. Disregard any peak observed in the blank. The reporting level for impurities is 0.05%.

Assay—

Mobile phase—Dilute 1.6 mL of phosphoric acid with water to 800 mL, add 200 mL of acetonitrile, and mix. Add 1.0 mL

USP Monographs

www.webofpharma.com

Table 1 (Granisetron Hydrochloride)

Name	Relative Retention Time	Relative Response Factor (*F*)	Limit (%)
Granisetron related compound D[1]	0.4	1.0	0.1
Granisetron related compound B[2]	0.5	0.59	0.5
Granisetron related compound A[3]	0.7	1.0	1.0
Granisetron related compound C[4]	0.8	1.0	0.2
Granisetron	1.0	n/a	n/a
Any other individual impurity	n/a	1.0	0.1

[1] 1-Methyl-1*H*-indazole-3-carboxylic acid.
[2] *N*-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide.
[3] 2-Methyl-*N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide.
[4] *N*-[(1*R*,3*r*,5*S*)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide.

of hexylamine, and mix. Adjust with triethylamine to a pH of 7.5 ± 0.05 (about 4 mL is needed). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Granisetron Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL.

System suitability solution—Transfer 2 mL of a solution in *Mobile phase* containing about 1.0 mg of Granisetron Hydrochloride per mL to a colorless glass vial, stopper it, and either expose the solution to sunlight for 4 hours or place it under a UV lamp for 16 hours (granisetron undergoes partial degradation to granisetron related compound C). A degradation of at least about 0.3% of granisetron to granisetron related compound C must be obtained, as shown by the appearance of a corresponding peak in the chromatogram. If it is not obtained, again expose the solution to sunlight or place it under a UV lamp.

Assay preparation—Transfer about 50 mg of Granisetron Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 305-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The column temperature is maintained at 40°. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between granisetron related compound C and granisetron is not less than 3.5; and the tailing factor for the granisetron peak is not more than 2.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{18}H_{24}N_4O \cdot HCl$ in the portion of Granisetron Hydrochloride taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of granisetron hydrochloride in the *Standard preparation* and the *Assay preparation,* respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Granisetron Hydrochloride Injection

» Granisetron Hydrochloride Injection is a sterile solution of Granisetron Hydrochloride in Water for Injection. It contains the equivalent of not less than 93.0 percent and not more than 107.0 percent of the labeled amount of granisetron ($C_{18}H_{24}N_4O$). It may contain suitable preservatives.

Packaging and storage—Preserve in single-dose or multiple-dose containers, protected from light, and store at controlled room temperature.

Labeling—It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.* Label it to indicate the name and the quantity of any added preservative.

USP Reference standards ⟨11⟩—
USP Granisetron Hydrochloride RS
USP Granisetron Related Compound B RS
N-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide.
USP Granisetron Related Compound C RS
N-[(1*R*,3*r*,5*S*)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide hydrochloride.
USP Granisetron Related Compound D RS
1-Methyl-1*H*-indazole-3-carboxylic acid.

Identification—
A: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Developing solvent*—Prepare a mixture of methylene chloride, alcohol, water, and ammonium hydroxide (60:40:5:2).
Standard solution—Dissolve an accurately weighed quantity of USP Granisetron Hydrochloride RS in water or alcohol to obtain a solution having a concentration of granisetron that matches the concentration of granisetron in the *Test solution.* To calculate the concentration of granisetron in the *Standard solution,* use the molecular weights of granisetron (312.41) and granisetron hydrochloride (348.87).
Test solution—Use the undiluted Injection.
Procedure—Separately apply equal volumes of the *Standard solution* and the *Test solution,* equivalent to about 4 to 5 µg of granisetron, to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture, dry the spots under a current of warm air for about 5 minutes, and develop the plate in a paper-lined chromatographic chamber equilibrated with *Developing solvent* prior to use. Allow the chromatogram to develop until the solvent front has moved about 15 cm. Remove the plate, dry the plate under a current of warm air, and examine the

www.webofpharma.com

plate under short-wavelength UV light: the principal spot from the *Test solution* corresponds in appearance and R_F value to that of the *Standard solution.*

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 25 USP Endotoxin Units per mg of granisetron.

Sterility Tests ⟨71⟩ **:** meets the requirements.

pH ⟨791⟩**:** between 4.0 and 6.0.

Particulate Matter in Injections ⟨788⟩ **:** meets the requirements for small-volume injections.

Related compounds— [NOTE—Perform the determination under subdued light, and use amber autosampler vials and low-actinic glassware.]

Buffer solution pH 2.0, Mobile phase, System suitability preparation, and *Chromatographic system*—Prepare as directed in the *Assay.*

Standard solution—Use the *Standard preparation* prepared as directed in the *Assay.*

Test solution—Use the undiluted Injection.

Procedure—Separately inject equal volumes (about 15/*L* µL), where *L* is the labeled amount, in mg, of granisetron per mL of Injection of the *Standard solution* and the *Test solution,* into the chromatograph. Record the chromatograms for at least three times the retention time of the granisetron peak, identify the impurities listed in *Table 1*and measure the peak responses. Calculate the percentage of each impurity relative to the labeled content of granisetron in the portion of Injection taken by the formula:

$$100(1/F)(312.41/348.87)(C_S/C_T)(r_i/r_S)$$

in which *F* is the relative response factor as listed in *Table 1;* 312.41 and 348.87 are the molecular weights of granisetron and granisetron hydrochloride, respectively; C_S is the concentration, in mg per mL, of granisetron hydrochloride in the *Standard solution;* C_T is the concentration, in mg per mL, of granisetron in the *Test solution,* based on the label claim; r_i is the peak response of each impurity obtained from the *Test solution;* and r_S is the peak response of the granisetron peak, obtained from the *Standard solution.* Disregard the peak due to granisetron related compound A that elutes at the relative retention time of about 0.5–0.6 as this impurity is controlled in the drug substance monograph. Not more than 0.7% of granisetron related compound C is found, not more than 1.3% of total specified impurities is found, and not more than 0.5% of any unspecified impurity is found. The reporting level for impurities is 0.1%.

Table 1

Name	Relative Retention Time	Relative Response Factor (*F*)
Granisetron related compound A[1]	0.5–0.6	—
Granisetron related compound B[2]	0.7	0.8
Granisetron	1.0	—
Granisetron related compound C[3]	1.2	1.0
Granisetron related compound D[4]	2.1–2.3	1.5

[1] 2-Methyl-*N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide.

[2] *N*-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide.

[3] *N*-[(1*R*,3*r*,5*S*)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide.

[4] 1-Methyl-1*H*-indazole-3-carboxylic acid.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay— [NOTE—Perform the determination under subdued light, and use amber autosampler vials and low-actinic glassware.]

Buffer solution pH 2.0—Dissolve 15.6 g of monobasic sodium phosphate dihydrate in 900 mL of water, adjust with phosphoric acid to a pH of 2.0, and dilute with water to 1000 mL.

Mobile phase—Prepare a mixture of *Buffer solution pH 2.0,* methanol, and tetrahydrofuran (75: 24: 1.1), mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability preparation—Dissolve suitable quantities of USP Granisetron Hydrochloride RS, USP Granisetron Related Compound B RS, USP Granisetron Related Compound C RS, and USP Granisetron Related Compound D RS in a mixture of water and methanol (75:25), to obtain a solution containing about 0.1 mg of each component per mL. Dilute with water to obtain a solution containing about *L* µg of each component per mL, where *L* is the labeled amount, in mg, of granisetron per mL of Injection.

Standard preparation—Dissolve an accurately weighed quantity of USP Granisetron Hydrochloride RS in water to obtain a solution having a known concentration of about (0.11 × *L*) mg of granisetron hydrochloride per mL, where *L* is the labeled amount, in mg, of granisetron per mL of Injection.

Assay preparation—Use the Injection diluted 1:10 with water.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 300-nm detector and a 4.6-mm × 15-cm column that contains 4-µm polar endcapped packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *System suitability preparation,* and identify the components based on the information listed in *Table 1.* Record the peak responses as directed for *Procedure:* the resolution, *R,* between the granisetron and granisetron related compound C peaks is not less than 2. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing for the granisetron peak is not more than 3; and the relative standard deviation for a minimum of six replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 15/*L* µL), where *L* is the labeled amount, in mg, of granisetron per mL of Injection, of the *Standard preparation* and the *Assay preparation* into the chromatograph. Record the chromatograms for at least three times the retention time of the granisetron peak, and measure the responses for the major peaks. Calculate the percentage of the labeled amount of granisetron ($C_{18}H_{24}N_4O$) in each mL of the Injection by the formula:

$$100(312.41/348.87)(C/C_U)(r_U/r_S)$$

in which 312.41 and 348.87 are the molecular weights of granisetron and granisetron hydrochloride, respectively; *C* is the concentration, in mg per mL, of granisetron hydrochloride in the *Standard preparation;* C_U is the nominal concentration, in mg per mL, of granisetron in the *Assay* preparation; and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

USP Monographs

www.webofpharma.com

Granisetron Hydrochloride Compounded Oral Suspension

DEFINITION

Granisetron Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of granisetron ($C_{18}H_{24}N_4O$). Prepare Granisetron Hydrochloride Compounded Oral Suspension containing 0.05 mg/mL of granisetron as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Granisetron (as Granisetron Hydrochloride)	5 mg (5.6 mg)
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, *NF*, and Vehicle for Oral Suspension, *NF*, a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number of tablets in a suitable mortar, and comminute the tablets to a fine powder with a pestle, or add *Granisetron Hydrochloride* powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a *Granisetron Hydrochloride* suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and 50 mM monobasic sodium dihydrogen phosphate (3:17). Adjust with phosphoric acid to a pH of 7.0. Make adjustments if necessary (see *Chromatography* ⟨621⟩, *System Suitability*).

Standard stock solution: 1.0 mg/mL of USP Granisetron Hydrochloride RS

Standard solution: Transfer 2.5 mL of *Standard stock solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution containing 25 µg/mL of granisetron hydrochloride.

Sample solution: Shake the Oral Suspension thoroughly by hand. Pipet 5.0 mL into a 10-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 300 nm

Column: 4.6-mm × 15-cm; 5-µm packing L10

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time of the granisetron peak is 7.0 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for the replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of granisetron ($C_{18}H_{24}N_4O$) in the volume of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Granisetron Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of granisetron in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of granisetron, 312.41

M_{r2} = molecular weight of granisetron hydrochloride, 348.87

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 4.0–4.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature or when stored in a refrigerator
- **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Granisetron Hydrochloride RS

Granisetron Hydrochloride Tablets

» Granisetron Hydrochloride Tablets contain an amount of Granisetron Hydrochloride equivalent to not less than 92.0 percent and not more than 108.0 percent of the labeled amount of granisetron ($C_{18}H_{24}N_4O$).

Packaging and storage—Preserve in well-closed containers, protected from light. Store at controlled room temperature.

USP Reference standards ⟨11⟩—

USP Granisetron Hydrochloride RS

USP Granisetron Related Compound B RS
N-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide.

USP Granisetron Related Compound C RS
N-[(1*R*,3*r*,5*S*)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide hydrochloride.

USP Granisetron Related Compound D RS
1-Methyl-1*H*-indazole-3-carboxylic acid.

Identification—

A: Thin-Layer Chromatographic Identification Test ⟨201⟩—

Developing solvent—Prepare a mixture of methylene chloride, alcohol, water, and ammonium hydroxide (60:40:5:2).

Standard solution—Dissolve an accurately weighed quantity of USP Granisetron Hydrochloride RS in 0.1 N hydrochloric acid to obtain a solution containing 0.44 mg of granisetron hydrochloride per mL.

Test solution—Transfer a number of Tablets, equivalent to about 2 mg of granisetron, to a suitable container, add 5.0 mL of 0.1 N hydrochloric acid, and sonicate for about 3 minutes. Pass through a 0.45-µm filter.

Procedure—Separately apply 20 µL each of the *Standard solution* and the *Test solution* to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture, dry the spots under a current of warm air for about 5 minutes, and develop the plate in a paper-lined chromatographic chamber equilibrated with *Developing solvent*. Allow the chromatogram to develop until the solvent front has moved about 15 cm. Remove the plate,

www.webofpharma.com

dry the plate under a cold air stream for about 10 minutes, and examine the plate under short-wavelength UV light: the principal spot from the *Test solution* corresponds in appearance and R_F value to that of the *Standard solution.*

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—

Medium: pH 6.5 phosphate buffer, prepared by dissolving 6.8 g of monobasic potassium phosphate in 800 mL of water, adjusting to pH 6.5 with 1 N sodium hydroxide, and diluting to 1 L with water; 500 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Buffer solution pH 2.0, Mobile phase, Diluent, System suitability preparation, and *Standard preparation*—Prepare as directed in the *Assay.*

Standard solution—Transfer 5.0 mL of the *Standard preparation* to a 250-mL volumetric flask, and dilute with *Diluent* to volume.

Test solution—Pass a portion of the solution under test through a 0.45-µm filter. If necessary, further dilute 5 mL of this solution with *Diluent* to (5 × *L*) mL, where *L* is the Tablet label claim, in mg.

Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay.* Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor is not less than 0.8 and not more than 1.5; and the relative standard deviation for a minimum of six replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the amount of $C_{18}H_{24}N_4O$ dissolved by the formula:

$$(312.41/348.87)(r_U \times C_S \times 500 \times D \times 100)/(r_S \times L)$$

in which 312.41 and 348.87 are the molecular weights of granisetron and granisetron hydrochloride, respectively; r_U and r_S are the peak responses for the *Test solution* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of the *Standard solution;* 500 is the volume, in mL, of the *Medium; D* is the dilution factor of the *Test solution;* 100 is the conversion factor to percentage; and *L* is the Tablet label claim, in mg.

Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{18}H_{24}N_4O$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Related compounds— [NOTE—Perform the determination under subdued light and use amber autosampler vials and low-actinic glassware.]

Buffer solution pH 2.0, Mobile phase, Diluent, System suitability preparation, and *Chromatographic system*—Prepare as directed in the *Assay.*

Standard solution—Use the *Standard preparation,* prepared as directed in the *Assay.*

Test solution—Use the *Assay preparation,* prepared as directed in the *Assay.*

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for at least three times the retention time of the granisetron peak, identify the impurities listed in *Table 1* and measure the peak responses. Calculate the percentage of each impurity relative to the labeled content of granisetron in the portion of Tablets taken by the formula:

$$100(1/F)(312.41/348.87)(C_S/C_U)(r_i/r_S)$$

in which *F* is the relative response factor as listed in *Table 1* 312.41 and 348.87 are the molecular weights of granisetron and granisetron hydrochloride, respectively; C_S is the concentration, in mg per mL, of granisetron hydrochloride in the *Standard solution;* C_U is the concentration, in mg per mL, of granisetron in the *Test solution,* based on the label claim; r_i is the peak response of each impurity obtained from the *Test solution;* and r_S is the peak response of the granisetron peak, obtained from the *Standard solution.* Disregard the peak due to granisetron related compound A that elutes at the relative retention time of about 0.5–0.6, as this impurity is controlled in the drug substance monograph. Not more than 0.7% of granisetron related compound C is found, not more than 1.3% of total specified impurities is found, and not more than 0.5% of any unspecified impurity is found. The reporting level for impurities is 0.1%.

Table 1

Name	Relative Retention Time	Relative Response Factor (*F*)
Granisetron related compound A[1]	0.5–0.6	—
Granisetron related compound B[2]	0.7	0.8
Granisetron	1.0	—
Granisetron related compound C[3]	1.2	1.0
Granisetron related compound D[4]	2.1–2.3	1.5

[1] 2-Methyl-*N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide.
[2] *N*-[(1*R*,3*r*,5*S*)-9-Methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide.
[3] *N*-[(1*R*,3*r*,5*S*)-9-Azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide.
[4] 1-Methyl-1*H*-indazole-3-carboxylic acid.

Assay— [NOTE—Perform the determination under subdued light and use amber autosampler vials and low-actinic glassware.]

Buffer solution pH 2.0—Dissolve 15.6 g of monobasic sodium phosphate dihydrate in 900 mL of water, adjust with phosphoric acid to a pH of 2.0, and dilute with water to 1000 mL.

Mobile phase—Prepare a mixture of *Buffer solution pH 2.0,* methanol, and tetrahydrofuran (75: 24: 1.1), mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Use *Buffer solution pH 2.0.*

System suitability preparation—Dissolve suitable amounts of USP Granisetron Hydrochloride RS, USP Granisetron Related Compound B RS, USP Granisetron Related Compound C RS, and USP Granisetron Related Compound D RS in *Diluent,* to obtain a solution having about 0.1 mg of granisetron hydrochloride per mL and about 0.01 mg of each of granisetron related compounds B, C, and D.

Standard preparation—Dissolve an accurately weighed quantity of USP Granisetron Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 0.11 mg of granisetron hydrochloride per mL.

Assay preparation—Prepare a solution containing about 0.1 mg of granisetron (base) per mL, based on the label claim, using the following procedure: fill a suitable volumetric flask with *Diluent,* add 5 Tablets and sonicate for approximately 20 minutes until the Tablets disintegrate completely. Pass a portion of this solution through a 0.45-µm membrane filter, discarding the first few mL.

USP Monographs

www.webofpharma.com

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 300-nm detector, and 4.6-mm × 15-cm column that contains 4-µm polar endcapped packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *System suitability preparation,* and identify the components based on the information listed in *Table 1*. Record the peak responses as directed for *Procedure:* the column efficiency, determined from the granisetron peak, is not less than 1200 theoretical plates; the tailing factor for the granisetron peak is not less than 0.8 and not more than 1.5; the resolution, *R,* between the granisetron and granisetron related compound C peaks is not less than 2. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for a minimum of six replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and measure the responses for the major peaks. Calculate the percentage of the labeled amount of granisetron ($C_{18}H_{24}N_4O$) in each Tablet taken by the formula:

$$100(312.41/348.87)(CV/NL)(r_U/r_S)$$

in which 312.41 and 348.87 are the molecular weights of granisetron and granisetron hydrochloride, respectively; *C* is the concentration, in mg per mL, of granisetron hydrochloride in the *Standard preparation; V* is the volume, in mL, of the *Assay preparation, N* is the number of Tablets taken to prepare the *Assay preparation; L* is the Tablet label claim, in mg; and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Green Soap

DEFINITION

Green Soap is a potassium soap made by the saponification of suitable vegetable oils, excluding coconut oil and palm kernel oil, without the removal of glycerin.

Prepare Green Soap as follows.

Vegetable Oil	380 g
Oleic Acid	20 g
Potassium Hydroxide	91.7 g
Glycerin	50 mL
Purified Water, a sufficient quantity to make	1000 g

Mix the *Vegetable Oil* and the *Oleic Acid,* and heat the mixture to about 80°. Dissolve the *Potassium Hydroxide* in a mixture of the *Glycerin* and 100 mL of *Purified Water,* and add the solution while hot to the hot oil mixture. Stir the mixture vigorously until emulsified, then heat with continuous stirring until the mixture is homogeneous and a test portion will dissolve to give a clear solution in hot water. Add hot *Purified Water* to bring the preparation to final weight. Continue stirring until the Soap is homogeneous.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method II* ⟨921⟩
  Sample: 5 g, quickly weighed, in the flask of the toluene moisture apparatus
  Analysis: Place 250 mL of toluene and 10 g of anhydrous barium chloride in the flask. Proceed as directed in the chapter.
  Acceptance criteria: The volume of water found corresponds to NMT 52.0% by weight of the Soap taken.
- **ALCOHOL-INSOLUBLE SUBSTANCES**
  Sample: 5 g, rapidly and accurately weighed
  Analysis: Dissolve the *Sample* in 100 mL of hot neutralized alcohol, collect the residue, if any, on a tared filter, thoroughly wash it with hot neutralized alcohol, and dry at 105° for 1 h. Retain the solution for the test for *Free Alkali Hydroxides,* and retain the residue for the test for *Alkali Carbonates.*
  Acceptance criteria: The weight of the residue so obtained is NMT 3.0% of the weight of the Soap taken.
- **FREE ALKALI HYDROXIDES**
  Sample: Combined filtrate and washings obtained in the test for *Alcohol-Insoluble Substances*
  Analysis: To the *Sample* add 0.5 mL of phenolphthalein TS. If a pink color is produced, titrate the solution with 0.1 N sulfuric acid VS until the pink color is just discharged. Each mL of 0.1 N sulfuric acid is equivalent to 5.611 mg of potassium hydroxide.
  Acceptance criteria: The volume of 0.1 N sulfuric acid VS consumed corresponds to NMT 0.25% of potassium hydroxide.
- **ALKALI CARBONATES**
  Sample: Residue obtained in the test for *Alcohol-Insoluble Substances*
  Analysis: Wash the filter containing the *Sample* with 50 mL of boiling water, cool, add methyl orange TS, and titrate the filtrate with 0.1 N sulfuric acid VS.
  Acceptance criteria: NMT 0.5 mL of 0.10 N sulfuric acid per g of Soap taken is required (0.35% as potassium carbonate).
- **UNSAPONIFIED MATTER**
  Sample: A solution of Soap in hot water (1 in 20)
  Acceptance criteria: Solution is nearly clear.
- **FATS AND FIXED OILS,** *Acid Value* ⟨401⟩
  Sample: 30 g
  Analysis: Dissolve the *Sample* in 300 mL of hot water in a beaker, add gradually 60 mL of 2 N sulfuric acid, and heat on a steam bath until the liberated acids form a transparent layer. Decant the fatty acids into a separator, and wash them with 50-mL portions of hot water until the last washing, when cool, is neutral to methyl orange TS. Transfer the fatty acids to a dry beaker, and allow them to stand in a warm oven until any water that may be present has separated. Then pass the acids through a dry filter in a warm oven. Determine the acid value of about 1 g, accurately weighed, of the fatty acids.
  Acceptance criteria: NMT 205
- **FATS AND FIXED OILS,** *Iodine Value* ⟨401⟩
  Sample: 30 g
  Analysis: Dissolve the *Sample* in 300 mL of hot water in a beaker, add gradually 60 mL of 2 N sulfuric acid, and heat on a steam bath until the liberated acids form a transparent layer. Decant the fatty acids into a separator, and wash them with 50-mL portions of hot water until the last washing, when cool, is neutral to methyl orange TS. Transfer the fatty acids to a dry beaker, and allow them to stand in a warm oven until any water that may be present has separated. Then pass the acids through a dry filter in a warm oven. Determine the iodine value of 150–200 mg, accurately weighed, of the fatty acids.
  Acceptance criteria: NLT 85

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in well-closed containers.

www.webofpharma.com

Green Soap Tincture

DEFINITION
Prepare Green Soap Tincture as follows.

Green Soap	650 g
Suitable Essential Oil(s)	—
Alcohol	316 mL
Purified Water, a sufficient quantity to make	1000 mL

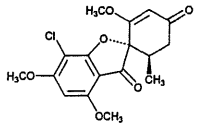
Mix the *Suitable Essential Oil(s)* and *Alcohol*. Dissolve the *Green Soap* in the mixture by stirring or agitation. Allow the solution to stand for 24 h, filter through paper, and add sufficient *Purified Water* to bring to final volume.

IDENTIFICATION
• **A.**
Sample: 10 mL
Analysis: To 10 mL of water in a flask add the *Sample*, and carefully add 1 mL of sulfuric acid.
Acceptance criteria: The fatty material separates.

OTHER COMPONENTS
• **ALCOHOL DETERMINATION,** *Method II* ⟨611⟩**:** 28.0%–32.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight containers.

Griseofulvin



$C_{17}H_{17}ClO_6$ 352.77
Spiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione, 7-chloro-2′,4,6-trimethoxy-6′-methyl-, (1′*S-trans*)-;
7-Chloro-2′,4,6-trimethoxy-6′β-methylspiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione [126-07-8].

DEFINITION
Griseofulvin has a potency of NLT 900 µg/mg of griseofulvin ($C_{17}H_{17}ClO_6$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, tetrahydrofuran, and water (35:5:60). Degas for 5 min before use, and stir continuously during use.
Standard stock solution: 1.25 mg/mL of USP Griseofulvin RS in methanol
Standard solution: 0.125 mg/mL of USP Griseofulvin RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: 1.24 mg/mL of Griseofulvin in methanol
Sample solution: 0.124 mg/mL of Griseofulvin in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L10
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of griseofulvin ($C_{17}H_{17}ClO_6$) in the portion of Griseofulvin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Griseofulvin RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of griseofulvin in USP Griseofulvin RS (µg/mg)

Acceptance criteria: NLT 900 µg/mg

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 217°–224°
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in dimethylformamide
Acceptance criteria: +348° to +364°
• **CRYSTALLINITY** ⟨695⟩**:** Meets the requirements
• **LOSS ON DRYING** ⟨731⟩
Sample: 100 mg
Analysis: Dry at 60° for 3 h in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury.
Acceptance criteria: NMT 1.0%
• **PERMEABILITY DIAMETER**
Sample solution: 1.819 ± 0.001 g of Griseofulvin
Analysis: Determine the apparent particle size in µm by the air-permeation method, using a suitable subsieve sizer. Transfer the sample to the compression tube of the apparatus. Compact with moderate pressure so that a uniform porosity is achieved. Pass dry compressed air through the tube, and measure the air pressure with a water manometer. Read the porosity, and calculate the apparent particle size from the instrument equation. Repeat the porosity readings at successively higher degrees of compaction until the apparent particle size reaches a minimum value.
Calculate the observed permeability diameter taken, in m^2/g:

$$\text{Result} = 6/(1.455 \times M \times F)$$

M = minimum apparent particle size
F = factor, interpolation used if necessary, to correct the apparent particle size to the true particle size at a given porosity reading (see *Table 1*)

www.webofpharma.com

Table 1

Porosity Reading	*F*	Porosity Reading	*F*
0.80	1.3771	0.56	1.7353
0.76	1.4142	0.52	1.8528
0.72	1.4573	0.48	2.0076
0.68	1.5082	0.44	2.2203
0.64	1.5690	0.40	2.5298
0.60	1.6432	—	—

Concomitantly determine the observed permeability diameter of a similar preparation of USP Griseofulvin Permeability Diameter RS.
Calculate the permeability diameter of the Griseofulvin taken:

$$\text{Result} = O_U \times (A_S/O_S)$$

O_U = observed permeability diameter of the specimen
A_S = assigned permeability diameter of USP Griseofulvin Permeability Diameter RS
O_S = observed permeability diameter of USP Griseofulvin Permeability Diameter RS

Acceptance criteria: 1.3–1.7 m^2/g

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Griseofulvin RS
  USP Griseofulvin Permeability Diameter RS

Griseofulvin Capsules

DEFINITION
Griseofulvin Capsules contain NLT 90.0% and NMT 115.0% of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile, tetrahydrofuran, and water (35:5:60). Degas for 5 min before use, and stir continuously during use.
Standard stock solution: 1.25 mg/mL of USP Griseofulvin RS in methanol
Standard solution: 0.125 mg/mL of USP Griseofulvin RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: 1.25 mg/mL of griseofulvin prepared as follows. Remove, as completely as possible, the contents of NLT 20 Capsules. Transfer a portion of the powder, equivalent to 125 mg of griseofulvin, to a 100-mL volumetric flask. Add 70 mL of methanol, shake by mechanical means for 30 min, and dilute with methanol to volume. Filter a portion of this solution, discarding the first 5 mL of the filtrate.
Sample solution: Nominally 0.125 mg/mL of griseofulvin in *Mobile phase* from the *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L10
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Griseofulvin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of griseofulvin in the *Sample solution* (mg/mL)
P = potency of griseofulvin in USP Griseofulvin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: 5.4 mg/mL of sodium lauryl sulfate in water; 1000 mL
Apparatus 2: 50 rpm
Time: 30 min
Diluent: Methanol and water (40:10)
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Diluent*, if necessary.
Standard solution: USP Griseofulvin RS at a known concentration similar to that in the *Sample solution*, prepared in the same medium
Instrumental conditions
Mode: UV
Analytical wavelength: 291 nm
Analysis
Samples: *Sample solution* and *Standard solution*
Determine the amount of griseofulvin ($C_{17}H_{17}ClO_6$) dissolved.
Tolerances: NLT 80% (Q) of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity
Standard solution: 10 µg/mL of USP Griseofulvin RS in methanol
Sample solution: Nominally 10 µg/mL of griseofulvin in methanol prepared as follows. Transfer the contents of 1 Capsule to a suitable container; add a volume of methanol sufficient to yield a concentration of griseofulvin NMT 1 mg/mL; shake by mechanical means for 1 h, or longer if necessary, to disperse the specimen completely; and sonicate for 1 min. Centrifuge a portion of this solution, and quantitatively dilute a measured volume of the clear supernatant to obtain a solution containing 10 µg/mL of griseofulvin.
Blank: Methanol
Instrumental conditions
Mode: UV
Analytical wavelength: 292 nm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*

www.webofpharma.com

Calculate the percentage of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times P \times F \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Griseofulvin RS in the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)
P = potency of griseofulvin in USP Griseofulvin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: Meet the requirements

SPECIFIC TESTS
- **Loss on Drying** ⟨731⟩
  Sample: 100 mg
  Analysis: Dry at 60° for 3 h in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** The label indicates that the griseofulvin contained is known as griseofulvin (microsize).
- **USP Reference Standards** ⟨11⟩
  USP Griseofulvin RS

Griseofulvin Oral Suspension

DEFINITION
Griseofulvin Oral Suspension contains NLT 90.0% and NMT 115.0% of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$). It contains one or more suitable colors, diluents, flavors, preservatives, and wetting agents.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **Procedure**
  Solution A: 100 mg/mL of sodium chloride in water
  Mobile phase: Acetonitrile, tetrahydrofuran, and water (35:5:60). Degas for 5 min before use, and stir continuously during use.
  Standard stock solution: 1.25 mg/mL of USP Griseofulvin RS in methanol
  Standard solution: 0.125 mg/mL of USP Griseofulvin RS in *Mobile phase* from the *Standard stock solution*
  Sample stock solution: Nominally 1.25 mg/mL of griseofulvin prepared as follows. Transfer a measured volume of Oral Suspension, freshly mixed and free from air bubbles and equivalent to 125 mg of griseofulvin, to a glass-stoppered, 50-mL centrifuge tube. Add 20 mL of methylene chloride and 20 mL of *Solution A*. Insert the stopper into the tube, and mix by rotating the tube for 10 min. Separate the phases by centrifugation, carefully remove the lower methylene chloride layer with a needle and syringe, and filter through methylene chloride-prerinsed anhydrous sodium sulfate into a 100-mL volumetric flask. Repeat the extraction with two additional 20-mL portions of methylene chloride, combining the extracts in the volumetric flask. Dilute with methylene chloride to volume.
  Sample solution: Nominally 0.125 mg/mL of griseofulvin prepared as follows. Transfer 5.0 mL of *Sample stock solution* to a 50-mL volumetric flask, and evaporate on a steam bath under a stream of nitrogen to dryness. Transfer 4.0 mL of *Mobile phase* to the flask, swirl to dissolve the residue, and dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.6-mm × 25-cm; packing L10
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Griseofulvin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of griseofulvin in the *Sample solution* (mg/mL)
P = potency of griseofulvin in USP Griseofulvin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
- **Uniformity of Dosage Units** ⟨905⟩**:** Meets the requirements for oral suspension packaged in single-unit containers
- **Deliverable Volume** ⟨698⟩**:** Meets the requirements

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 5.5–7.5

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** The label indicates that the griseofulvin contained is known as griseofulvin (microsize).
- **USP Reference Standards** ⟨11⟩
  USP Griseofulvin RS

Griseofulvin Tablets

DEFINITION
Griseofulvin Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, tetrahydrofuran, and water (35:5:60). Degas for 5 min before use, and stir continuously during use.

Standard stock solution: 1.25 mg/mL of USP Griseofulvin RS in methanol

Standard solution: 0.125 mg/mL of USP Griseofulvin RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: Nominally 1.25 mg/mL of griseofulvin in methanol prepared as follows. Transfer the required number of finely powdered Tablets, based on the labeled amount, to a suitable volumetric flask and shake for at least 30 min in methanol. Dilute with methanol to volume, mix, and pass through a suitable filter.

Sample solution: 0.125 mg/mL of griseofulvin in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L10

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Griseofulvin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)
P = potency of griseofulvin in USP Griseofulvin RS (µg/mg)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: Water containing 40.0 mg/mL of sodium lauryl sulfate; 1000 mL

Apparatus 2: 75 rpm

Time: 90 min

Diluent: Methanol and water (40:10)

Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Diluent*, if necessary.

Standard solution: USP Griseofulvin RS at a known concentration similar to that of the *Sample solution*, prepared in the same *Medium*

Analysis: Determine the percentage of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) dissolved using UV absorption at the wavelength of maximum absorbance at about 291 nm.

Tolerances: NLT 75% (*Q*) of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 4% sodium lauryl sulfate in water; 1000 mL

Apparatus 2: 50 rpm

Time: 45 min

Diluent: Methanol and water (40:10)

Standard solution: 10 µg/mL of USP Griseofulvin RS in *Diluent*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Diluent*, if necessary, to obtain a concentration similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 291 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Griseofulvin RS in the *Standard solution* (µg/mL)
V = volume of the *Medium*, 1000 mL
D = dilution factor of the *Sample solution*
L = label claim (mg/Tablet)

Tolerances: NLT 80% (*Q*) of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Standard solution: 10 µg/mL of USP Griseofulvin RS in methanol

Sample solution: Transfer 1 Tablet to a suitable container; add a measured volume of methanol sufficient to yield a concentration of griseofulvin NMT 1 mg/mL; shake by mechanical means for 1 h, or longer if necessary, to disperse the specimen completely; and sonicate for 1 min. Centrifuge a portion of this solution, and quantitatively dilute a volume of the clear supernatant to obtain a *Sample solution* containing about 10 µg/mL of griseofulvin.

Blank: Methanol

Instrumental conditions

Mode: UV

Analytical wavelength: 292 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times P \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Griseofulvin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of the *Sample solution* (µg/mL)
P = potency of griseofulvin in USP Griseofulvin RS (µg/mL)

Acceptance criteria: Meet the requirements

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry the sample at 60° for 3 h in a capillary-stoppered bottle under vacuum.

Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

www.webofpharma.com

• **LABELING:** The label indicates that the griseofulvin contained is known as griseofulvin (microsize). When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Griseofulvin RS

Ultramicrosize Griseofulvin Tablets

DEFINITION
Ultramicrosize Griseofulvin Tablets are composed of ultramicrosize crystals of Griseofulvin dispersed in Polyethylene Glycol 6000 or dispersed by other suitable means. They contain NLT 90.0% and NMT 115.0% of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, tetrahydrofuran, and water (35:5:60). Degas for 5 min before use, and stir continuously during use.
Standard stock solution: 1.25 mg/mL of USP Griseofulvin RS in methanol
Standard solution: 0.125 mg/mL of USP Griseofulvin RS in *Mobile phase* from the *Standard stock solution*
Sample stock solution: Nominally 1.25 mg/mL of griseofulvin prepared as follows. Weigh and powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 125 mg of griseofulvin, to a 100-mL volumetric flask. Add 70 mL of methanol, shake by mechanical means for 30 min, and dilute with methanol to volume. Filter a portion of this solution, discarding the first 5 mL of the filtrate.
Sample solution: Nominally 0.125 mg/mL of griseofulvin in *Mobile phase* from the *Sample stock solution* prepared as follows. Dilute 5.0 mL of the clear filtrate from the *Sample stock solution* with *Mobile phase* to 50 mL.
Chromatographic system
(see *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L10
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Griseofulvin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of griseofulvin in the *Sample solution* (mg/mL)
P = potency of griseofulvin in USP Griseofulvin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 5.4 g/L of sodium lauryl sulfate in water; 1000 mL
Apparatus 2: 75 rpm
Time: 45 min
Diluent: Methanol and water (40:10)
Sample solution: Sample per *Dissolution* ⟨711⟩. Dilute with *Diluent,* if necessary.
Standard solution: USP Griseofulvin RS at a known concentration similar to that in the *Sample solution,* prepared in the same medium
Instrumental conditions
Mode: UV
Analytical wavelength: 291 nm
Analysis
Samples: *Sample solution* and *Standard solution*
Determine the amount of griseofulvin ($C_{17}H_{17}ClO_6$) dissolved.
Tolerances: NLT 80% (*Q*) of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Standard solution: 10 µg/mL of USP Griseofulvin RS in methanol
Sample solution: Nominally 10 µg/mL of griseofulvin in methanol prepared as follows. Transfer 1 Tablet to a suitable container; add a volume of methanol sufficient to yield a concentration of griseofulvin NMT 1 mg/mL; shake by mechanical means for 1 h, or longer if necessary, to disperse the specimen completely; and sonicate for 1 min. Centrifuge a portion of this solution, and quantitatively dilute a measured volume of the clear supernatant to obtain a solution containing 10 µg/mL of griseofulvin.
Blank: Methanol
Instrumental conditions
Mode: UV
Analytical wavelength: 292 nm
Analysis
Samples: *Standard solution, Sample solution,* and *Blank*
Calculate the percentage of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times P \times F \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Griseofulvin RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of griseofulvin in USP Griseofulvin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: Meet the requirements

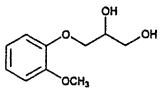
SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Sample: 100 mg
Analysis: Dry at 60° for 3 h in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury.
Acceptance criteria: NMT 5.0%

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Griseofulvin RS

Guaifenesin



$C_{10}H_{14}O_4$ 198.22

1,2-Propanediol, 3-(2-methoxyphenoxy)-(±)-;
(±)-3-(*o*-Methoxyphenoxy)-1,2-propanediol [93-14-1].

DEFINITION
Guaifenesin contains NLT 98.0% and NMT 102.0% of guaifenesin ($C_{10}H_{14}O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: 197U▲ (CN 1-May-2020)

Sample solution: 40 µg/mL in methanol
Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**

Solution A: Glacial acetic acid and water (1:99)
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
32	50	50
35	80	20

System suitability solution: 0.5 mg/mL of USP Guaifenesin RS and 0.02 mg/mL of USP Guaiacol RS in *Solution B*
Standard solution: 0.5 mg/mL of USP Guaifenesin RS in *Solution B*
Sample solution: 0.5 mg/mL of Guaifenesin in *Solution B*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 276 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 3 between guaifenesin and guaiacol, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of guaifenesin ($C_{10}H_{14}O_4$) in the portion of Guaifenesin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of guaifenesin from the *Sample solution*
r_S = peak response of guaifenesin from the *Standard solution*
C_S = concentration of USP Guaifenesin RS in the *Standard solution* (mg/mL)
C_U = concentration of Guaifenesin in the *Sample solution* (mg/mL)

To this value, add the percentage of guaifenesin β-isomer found in the test for *Organic Impurities*.
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Change to read:

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Sample solution: 2 mg/mL of Guaifenesin in *Solution B*
Diluted sample solution: 0.02 mg/mL of Guaifenesin from the *Sample solution* in *Solution B*
Analysis
Samples: *Sample solution* and *Diluted sample solution*
[NOTE—See *Table 2* for relative retention times.]
[NOTE—All of the peaks are baseline resolved.]
Calculate the percentage of each individual impurity in the portion of Guaifenesin taken:

$$\text{Result} = (r_U/r_S) \text{ ▲}\times (C_S/C_U)\text{▲ (ERR 1-Nov-2018)} \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of guaifenesin from the *Diluted sample solution*
▲C_S = concentration of guaifenesin in the *Diluted sample solution*
C_U = concentration of guaifenesin in the *Sample solution*▲ (ERR 1-Nov-2018)
F = relative response factor

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Guaifenesin β-isomer[a]	0.9	1.0	1.5
Guaifenesin	1.0	—	—
Guaiacol	1.4	1.6	0.03
Any individual unspecified impurity	—	1.0	0.5
Total impurities[b]	—	—	1.0

[a] 2-(2-Methoxyphenoxy)-1,3-propanediol.
[b] Excluding guaifenesin β-isomer and guaiacol.

USP Monographs

www.webofpharma.com

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum (NLT 10 mm of mercury) at 60° to a constant weight.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Guaiacol RS
USP Guaifenesin RS

Guaifenesin Capsules

» Guaifenesin Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guaifenesin ($C_{10}H_{14}O_4$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Guaifenesin RS

Identification—
A: Triturate a portion of the contents of Capsules, equivalent to about 100 mg of guaifenesin, with 10 mL of chloroform, filter, and evaporate 1 mL of the filtrate on a watch glass. Mix the residue with 1 drop of formaldehyde and a few drops of sulfuric acid: a deep cherry-red to purple color is produced.
B: The retention time of the guaifenesin peak in the chromatogram of the *Assay preparation* corresponds to that of the guaifenesin peak in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: water; 900 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{10}H_{14}O_4$ dissolved, employing the procedure set forth in the *Assay*, making any necessary modifications.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{10}H_{14}O_4$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—
Mobile phase, Benzoic acid solution, Resolution solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay*— under *Guaifenesin Tablets*.
Assay preparation—Transfer, as completely as possible, the contents of not less than 20 Capsules to a suitable tared container, and determine the average content weight per Capsule. Mix the combined contents, and transfer an accurately weighed portion, equivalent to about 200 mg of guaifenesin, to a 100-mL volumetric flask. Proceed as directed for *Assay preparation* in the *Assay*— under *Guaifenesin Tablets*, beginning with "add about 60 mL of water."
Procedure—Proceed as directed for *Procedure* in the *Assay* — under *Guaifenesin Tablets*. Calculate the quantity, in mg, of guaifenesin ($C_{10}H_{14}O_4$) in the portion of Capsule contents taken by the formula:

$$5C(r_U/r_S)$$

in which the terms are as defined therein.

Guaifenesin Compounded Injection, Veterinary

DEFINITION
Guaifenesin Compounded Injection, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of guaifenesin ($C_{10}H_{14}O_4$).
Prepare Guaifenesin Compounded Injection, Veterinary 50 mg/mL as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩.)

Guaifenesin	50 g
Propylene Glycol	20 mL
Dextrose Injection (5%), a sufficient quantity to make	1000 mL

Accurately weigh the *Guaifenesin* and transfer it into a sterile depyrogenated beaker. Add the *Propylene Glycol* to the *Guaifenesin* and mix well to wet. Add about 200 mL of the *Dextrose Injection (5%)* to the guaifenesin mixture and heat the mixture briefly, not to exceed 50°, for solubilization. Bring to final volume with the *Dextrose Injection (5%)*. Pass through an inline sterile membrane filter of 0.22-µm pore size into a sterile polyvinyl chloride intravenous bag. [NOTE—Use a sterilizing filter that is compatible with glycol-containing solutions.]

ASSAY
• **PROCEDURE**
Solution A: Dissolve 2.5 mL of glacial acetic acid in 1000 mL of water.
Mobile phase: Methanol and *Solution A* (35:65)
Diluent: Methanol and water (35:65)
Standard solution: 1 mg/mL of guaifenesin prepared from USP Guaifenesin RS in *Diluent*
Sample solution: Transfer 1 mL of Injection, Veterinary into a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix well to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 276 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 25°
Flow rate: 1.0 mL/min
Injection volume: 15 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time for guaifenesin is about 16.4 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of guaifenesin ($C_{10}H_{14}O_4$) in the portion of Injection, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of guaifenesin from the *Sample solution*
r_S = peak response of guaifenesin from the *Standard solution*

USP Monographs

www.webofpharma.com

C_S = concentration of USP Guaifenesin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of guaifenesin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.6–4.6
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: It meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.05 USP Endotoxin Units/mg of guaifenesin
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: It meets the requirements.

Change to read:

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Package in a sterile polyvinyl chloride intravenous bag. Store at controlled room temperature or at 37°. [NOTE—Do not store at cold temperatures as precipitation may occur.]
- **BEYOND-USE DATE:** ▲In the absence of passing a sterility and endotoxins test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test and endotoxins test is passed, NMT 90 days after the date on which it was compounded when stored at controlled room temperature or NMT 7 days after the date on which it was compounded when stored at 37°.▲ (CN 1-May-2020)
- **LABELING:** Label it to indicate that it is for veterinary use only. The label states that it is intended for injection only by the intravenous route. Label it to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Guaifenesin RS

Guaifenesin for Injection

» Guaifenesin for Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guaifenesin ($C_{10}H_{14}O_4$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, and store at controlled room temperature.
Labeling—It meets the requirements for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*. Label it to indicate that it is for veterinary use only. The label states that it is intended for injection only by the intravenous route in horses.
USP Reference standards ⟨11⟩—
USP Guaifenesin RS

Change to read:

Identification, ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.05 Endotoxin Unit per mg of guaifenesin.

Assay—
Standard preparation—Dissolve an accurately weighed quantity of USP Guaifenesin RS in chloroform to obtain a solution having a known concentration of about 40 µg per mL.
Assay preparation—Constitute a container of Guaifenesin for Injection with a volume of warm (30° to 35°) water, accurately measured, corresponding to the volume of solvent specified in the labeling. Transfer an accurately measured volume of the constituted solution, equivalent to about 100 mg of guaifenesin, to a 250-mL separator containing 10 mL of a saturated solution of sodium bicarbonate. Extract with four 25-mL portions of chloroform, combining the chloroform extracts in a second 250-mL separator. Wash the combined chloroform extracts with 5 mL of 1 N hydrochloric acid. Filter the washed chloroform extracts through chloroform-moistened filter paper, collecting the filtrate in a 100-mL volumetric flask. Dilute with chloroform to volume, and mix. Transfer 4.0 mL of this solution to a second 100-mL volumetric flask, dilute with chloroform to volume, and mix.
Procedure—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the wavelength of maximum absorbance at about 276 nm, using chloroform to zero the instrument. Calculate the quantity, in mg, of guaifenesin ($C_{10}H_{14}O_4$) in each mL of the constituted solution of Guaifenesin for Injection taken by the formula:

$$2.5(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Guaifenesin RS in the *Standard preparation*, V is the volume, in mL, of constituted solution taken to prepare the *Assay preparation*, and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Guaifenesin Oral Solution

» Guaifenesin Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guaifenesin ($C_{10}H_{14}O_4$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Guaifenesin RS
Identification—The retention time of the guaifenesin peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.
pH ⟨791⟩: between 2.3 and 3.0.
Alcohol Determination, *Method I* ⟨611⟩ ***(if present):*** between 90.0% and 115.0% of the labeled amount of C_2H_5OH.

Assay—
Mobile phase, Benzoic acid solution, Resolution solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Guaifenesin Tablets*.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 200 mg of

USP Monographs

www.webofpharma.com

guaifenesin, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 45 mL of methanol, dilute with water to volume, and mix.

Procedure—Proceed as directed in the *Assay* under *Guaifenesin Tablets*. Calculate the quantity, in mg, of guaifenesin ($C_{10}H_{14}O_4$) in each mL of the Oral Solution taken by the formula:

$$5C/V(r_U/r_S)$$

in which V is the volume, in mL, of Oral Solution taken; and the other terms are as defined therein.

Guaifenesin Tablets

» Guaifenesin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guaifenesin ($C_{10}H_{14}O_4$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Guaifenesin RS

Identification—

A: Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of guaifenesin, with 10 mL of chloroform, filter, and evaporate 1 mL of the filtrate on a watch glass. Mix the residue with 1 drop of formaldehyde and a few drops of sulfuric acid: a deep cherry-red to purple color is produced.

B: The retention time of the guaifenesin peak in the chromatogram of the *Assay preparation* corresponds to that of the guaifenesin peak in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.

Procedure—Determine the amount of $C_{10}H_{14}O_4$ dissolved in filtered portions of the solution under test from UV absorbances at the wavelength of maximum absorbance at about 274 nm in comparison with a Standard solution having a known concentration of USP Guaifenesin RS in the same medium.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{10}H_{14}O_4$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase—Prepare a suitable filtered and degassed mixture of water, methanol, and glacial acetic acid (60: 40: 1.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Benzoic acid solution—Dissolve a suitable quantity of benzoic acid in methanol to obtain a solution containing about 2 mg per mL.

Resolution solution—Dissolve a suitable quantity of guaifenesin in water, with shaking, to obtain a solution containing about 2 mg per mL. Transfer 2.0 mL of this solution and 5.0 mL of *Benzoic acid solution* to a 100-mL volumetric flask, add 40 mL of methanol, dilute with water to volume, and mix to obtain a solution containing about 40 µg of guaifenesin and 100 µg of benzoic acid per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Guaifenesin RS quantitatively in water, with shaking, to obtain a solution having a known concentration of about 2 mg per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 45 mL of methanol, dilute with water to volume, and mix to obtain a *Standard preparation* having a known concentration of about 40 µg per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of guaifenesin, to a 100-mL volumetric flask, add about 60 mL of water, and shake for about 15 minutes. Dilute with water to volume, filter if necessary to obtain a clear solution, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 45 mL of methanol, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 276-nm detector and a 4.6-mm × 25-cm column that contains 10-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed under *Procedure:* the resolution, *R,* between the guaifenesin and benzoic acid peaks is not less than 3.0 (the relative retention times are about 0.7 for guaifenesin and 1.0 for benzoic acid). Chromatograph the *Standard preparation,* and record the peak responses as directed under *Procedure:* the relative standard deviation for replicate injections is not more than 2.5%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of guaifenesin ($C_{10}H_{14}O_4$) in the portion of Tablets taken by the formula:

$$5C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Guaifenesin RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Guaifenesin and Codeine Phosphate Oral Solution

» Guaifenesin and Codeine Phosphate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of guaifenesin ($C_{10}H_{14}O_4$) and codeine phosphate ($C_{18}H_{21}NO_4 \cdot H_3PO_4 \cdot ½H_2O$).

Packaging and storage—Preserve in tight, light-resistant containers, at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Codeine Phosphate RS
USP Guaifenesin RS

Identification—

A: The retention time of the codeine peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for codeine phosphate.*

B: The retention time of the guaifenesin peak in the chromatogram of the *Assay preparation* corresponds to that in

USP Monographs

www.webofpharma.com

the chromatogram of the *Standard preparation,* as obtained in the *Assay for guaifenesin.*

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

pH ⟨791⟩**:** between 2.3 and 3.0 if it contains alcohol, 5.0 to 5.5 if it does not contain alcohol.

Alcohol Determination ⟨611⟩ ***(if present)***—
Standard stock solution—Dilute 10.0 mL of dehydrated alcohol with water to 100.0 mL.
Internal standard solution—Dilute 10 mL of acetone with water to 100 mL.
Standard preparation—Transfer 10.0 mL of *Standard stock solution* and 8.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with water to volume, and mix. This solution contains 0.02% (v/v) of alcohol.
Test preparation—Transfer 25/*k* mL of Oral Solution (*k* being the labeled percentage [v/v] of C_2H_5OH in the Oral Solution), accurately measured, to a 25-mL volumetric flask, add 2.0 mL of *Internal standard solution,* dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with water to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 1.8-m column that contains 5% liquid phase G16 on 100- to 120-mesh support S1A, and is programmed to maintain the column temperature at 50° for 5 minutes after injection, to increase to 100° at the rate of 20° per minute, to maintain the temperature at 100° for 2 minutes, and then to return to 50° before the next injection. The injection port is maintained at about 200°, and the detector block is maintained at about 250°. The carrier gas is dry helium flowing at a rate of about 30 mL per minute.
Chromatograph the *Standard preparation,* and record the chromatogram as directed for *Procedure:* the relative retention times are about 0.3 for acetone and 1.0 for alcohol (C_2H_5OH); the resolution, *R,* between the acetone and alcohol peaks is not less than 2; the tailing factors for the acetone and alcohol peaks are not more than 1.5; and the relative standard deviation for replicate injections is not more than 2%.
Procedure—Separately inject equal volumes (about 2 µL) of the *Test preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage (v/v) of alcohol (C_2H_5OH) in the portion of Oral Solution taken by the formula:

$$(1250P/V)(R_U/R_S)$$

in which *P* is the percentage (v/v) of alcohol (C_2H_5OH) in the *Standard preparation; V* is the volume, in mL, of Oral Solution taken to prepare the *Test preparation;* and R_U and R_S are the peak response ratios of alcohol to that of acetone obtained from the *Test preparation* and the *Standard preparation,* respectively: between 90.0% and 115.0% of the labeled amount of alcohol (C_2H_5OH) is found.

Assay for codeine phosphate—
Internal standard solution—Place about 45 mg of hydrocodone bitartrate in a flask containing about 5 mL of water, add 1 mL of 0.5 N sodium hydroxide and 50 mL of chloroform, insert the stopper into the flask, and shake by mechanical means for about 20 minutes. Allow the layers to separate, and filter the chloroform layer.
Standard preparation—Prepare a Standard stock solution of USP Codeine Phosphate RS in 0.1 N hydrochloric acid having a known concentration of about 2 mg per mL. Transfer 5.0 mL of this Standard stock solution to a suitable flask. Add 2 mL of 2.5 N sodium hydroxide, 8.0 mL of *Internal standard solution,* and 40 mL of chloroform, insert the stopper, and shake by mechanical means for 1 hour. Allow the layers to separate, and collect the chloroform layer.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 10 mg of codeine phosphate, to a 200-mL flask containing about 5 mL of water, and swirl. Add 2 mL of 2.5 N sodium hydroxide, 8.0 mL of *Internal standard solution,* and 40 mL of chloroform, insert the stopper, and shake by mechanical means for 1 hour. Allow the layers to separate, and collect the chloroform layer.
Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 0.6-m column that contains 3% liquid phase G3 on 100- to 120-mesh support S1A, and is stabilized for isothermal operation. The column temperature is maintained at about 210°, the injection port temperature at about 250°, and the detector block temperature at about 300°. The carrier gas is dry helium flowing at a rate of about 25 mL per minute. Chromatograph the *Standard preparation,* and record the chromatogram as directed for *Procedure:* the relative retention times are about 0.75 for codeine and 1.0 for hydrocodone; the resolution, *R,* between the codeine and hydrocodone peaks is not less than 1; and the relative standard deviation for replicate injections is not more than 2%.
Procedure—Separately inject equal volumes (about 2 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of codeine phosphate hemihydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$) in each mL of the Oral Solution taken by the formula:

$$(406.37/397.37)(5C/V)(R_U/R_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively; *C* is the concentration, in mg per mL, of USP Codeine Phosphate RS in the Standard stock solution used to prepare the *Standard preparation; V* is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation;* and R_U and R_S are the peak response ratios of the codeine peak to the hydrocodone peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for guaifenesin—
Internal standard solution—Prepare a solution of dipropyl phthalate in chloroform containing about 12.5 mg per mL.
Standard preparation—Prepare a Standard stock solution of USP Guaifenesin RS in 0.1 N hydrochloric acid having a known concentration of about 4 mg per mL. Transfer 5.0 mL of this Standard stock solution to a suitable glass-stoppered flask, add 2 mL of 2.5 N sodium hydroxide, 8.0 mL of *Internal standard solution,* and 40 mL of chloroform, insert the stopper, and shake by mechanical means for 1 hour. Allow the layers to separate, and collect the chloroform layer. Extract the aqueous layer with two 20-mL portions of chloroform, and combine the three chloroform extracts. Transfer 5.0 mL of the combined chloroform extracts to a suitable glass-stoppered flask, add 1 mL of trifluoroacetic anhydride, and allow to stand for not less than 1 hour.
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 20 mg of guaifenesin, to a 200-mL flask containing about 5 mL of water, and swirl. Add 2 mL of 2.5 N sodium hydroxide, 8.0 mL of

www.webofpharma.com

Internal standard solution, and 40 mL of chloroform, insert the stopper, and shake by mechanical means for 1 hour. Allow the layers to separate, and collect the chloroform layer. Extract the aqueous layer with two 20-mL portions of chloroform, and combine the three chloroform extracts. Transfer 5.0 mL of the combined chloroform extracts to a suitable glass-stoppered flask, add 1 mL of trifluoroacetic anhydride, and allow to stand for not less than 1 hour.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm × 1.2-m column that contains 3% liquid phase G6 on 100- to 120-mesh support S1A. The column temperature is maintained at about 170°, the injection port temperature is maintained at about 250°, and the detector block temperature is maintained at about 300°. The carrier gas is dry helium flowing at a rate of about 45 mL per minute. Chromatograph the *Standard preparation,* and record the chromatogram as directed for *Procedure:* the relative retention times are about 0.6 for the trifluoroacetyl derivative of guaifenesin and 1.0 for the trifluoroacetyl derivative of dipropyl phthalate; the resolution, *R,* between the guaifenesin and dipropyl phthalate peaks is not less than 1; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 1 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of guaifenesin ($C_{10}H_{14}O_4$) in the portion of Oral Solution taken by the formula:

$$(W/V)(R_U/R_S)$$

in which W is the quantity, in mg, of USP Guaifenesin RS taken to prepare the *Standard preparation; V* is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation;* and R_U and R_S are the peak response ratios of the guaifenesin peak to the dipropyl phthalate peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Guaifenesin and Pseudoephedrine Hydrochloride Capsules

» Guaifenesin and Pseudoephedrine Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of guaifenesin ($C_{10}H_{14}O_4$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Guaifenesin RS
USP Pseudoephedrine Hydrochloride RS

Identification—

A: The retention time of the guaifenesin peak relative to that of the benzoic acid peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay for guaifenesin.*

B: The retention time of the pseudoephedrine peak in the chromatogram of the *Assay preparation* relative to that of the dextromethorphan peak corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay for pseudoephedrine hydrochloride.*

Uniformity of dosage units ⟨905⟩—

Procedure for content uniformity of pseudoephedrine hydrochloride—Proceed as directed in the *Assay for pseudoephedrine hydrochloride,* preparing the *Assay preparation* as follows. Transfer 1 Capsule to a 100-mL volumetric flask, add about 50 mL of water, heat on a steam bath for about 5 minutes, and allow to cool. Add 5.0 mL of *Internal standard solution,* dilute with water to volume, and mix.

Assay for guaifenesin—

Mobile phase—Prepare a mixture of water, methanol, and glacial acetic acid (60: 40: 1.5). Make any necessary adjustments (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Prepare a solution of benzoic acid in methanol containing about 2 mg per mL.

Standard stock solution—Prepare a solution in water having known concentrations of about 20 mg of USP Guaifenesin RS and 20*J* mg of USP Pseudoephedrine Hydrochloride RS per mL, *J* being the ratio of the labeled amount, in mg, of pseudoephedrine hydrochloride to the labeled amount, in mg, of guaifenesin per Capsule.

Standard preparation—Transfer 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution and 5.0 mL of *Internal standard solution* to a second 100-mL volumetric flask, add about 40 mL of methanol, dilute with water to volume, and mix. Each mL of this solution contains about 0.1 mg of USP Guaifenesin RS, 0.1*J* mg of USP Pseudoephedrine Hydrochloride RS, and 0.1 mg of benzoic acid.

Assay preparation—Transfer an accurately counted number of Capsules, equivalent to about 2000 mg of guaifenesin, to a 100-mL volumetric flask, add about 50 mL of water, and heat on a steam bath for about 15 minutes. Allow to cool, dilute with water to volume, and mix. Transfer 10.0 mL of this stock solution to a second 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution and 5.0 mL of *Internal standard solution* to a third 100-mL volumetric flask, add about 40 mL of methanol, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 276-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed under *Procedure:* the relative retention times are about 0.65 for guaifenesin and 1.0 for benzoic acid; the resolution, *R,* between the guaifenesin peak and the benzoic acid peak is not less than 3.0; the tailing factors for the guaifenesin peak and the benzoic acid peak are not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.5%.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of guaifenesin ($C_{10}H_{14}O_4$) in each Capsule taken by the formula:

$$20{,}000(C/N)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Guaifenesin RS in the *Standard preparation; N* is the number of Capsules taken; and R_U and R_S are the ratios of the guaifenesin peak response to the benzoic acid peak response obtained from the *Assay preparation* and the *Standard preparation,* respectively.

www.webofpharma.com

Assay for pseudoephedrine hydrochloride—

Mobile phase—To 3.5 g of docusate sodium add 500 mL of methanol, 350 mL of water, 145 mL of tetrahydrofuran, and 5 mL of glacial acetic acid, mix, and pass through a filter having a porosity of 0.5 µm or less. Make any necessary adjustments (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Prepare a solution of dextromethorphan hydrobromide in methanol containing about 1.2 mg per mL.

Standard stock solution—Prepare as directed in the *Assay for guaifenesin*.

Standard preparation—Transfer 10.0 mL of *Standard stock solution* and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains about 2 mg of USP Guaifenesin RS, 2*J* mg of USP Pseudoephedrine Hydrochloride RS, and 0.06 mg of dextromethorphan hydrobromide.

Assay preparation—Transfer 10.0 mL of the stock solution used to prepare the *Assay preparation* in the *Assay for guaifenesin* and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 263-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*:the relative retention times are about 0.55 for pseudoephedrine and 1.0 for dextromethorphan; the resolution, *R*, between the pseudoephedrine peak and the dextromethorphan peak is not less than 1.5; the tailing factors for the pseudoephedrine peak and the dextromethorphan peak are not more than 1.5 and 2.5, respectively; and the relative standard deviation for replicate injections determined for the pseudoephedrine peak is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in each Capsule taken by the formula:

$$1000(C/N)(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*; *N* is the number of Capsules taken; and R_U and R_S are the ratios of the pseudoephedrine peak area response to the dextromethorphan peak area response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Guaifenesin, Pseudoephedrine Hydrochloride, and Dextromethorphan Hydrobromide Capsules

» Guaifenesin, Pseudoephedrine Hydrochloride, and Dextromethorphan Hydrobromide Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of guaifenesin ($C_{10}H_{14}O_4$), pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$), and dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Dextromethorphan Hydrobromide RS
USP Guaifenesin RS
USP Pseudoephedrine Hydrochloride RS

Identification—

A: The retention time of the guaifenesin peak relative to that of the benzoic acid peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay for guaifenesin*.

B: The retention times of the pseudoephedrine and dextromethorphan peaks in the chromatogram of the *Assay preparation* relative to that of the brompheniramine peak correspond to those in the chromatogram of the *Standard preparation* as obtained in the *Assay for pseudoephedrine hydrochloride and dextromethorphan hydrobromide*.

Uniformity of dosage units ⟨905⟩—

Procedure for content uniformity of pseudoephedrine hydrochloride and *dextromethorphan hydrobromide*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride and dextromethorphan hydrobromide,* preparing the *Assay preparation* as follows. Transfer 1 Capsule to a 100-mL volumetric flask, add about 50 mL of water, heat on a steam bath for about 15 minutes, and allow to cool. Add 5.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

Assay for guaifenesin—

Mobile phase, Internal standard solution, Standard stock solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay for guaifenesin*— under *Guaifenesin and Pseudoephedrine Hydrochloride Capsules*.

Assay preparation—Transfer an accurately counted number of Capsules, equivalent to about 2000 mg of guaifenesin, to a 100-mL volumetric flask, add about 50 mL of water, and heat on a steam bath for about 15 minutes. Allow to cool, dilute with water to volume, and mix (stock solution). Transfer 10.0 mL of this stock solution to a second 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution and 5.0 mL of *Internal standard solution* to a third 100-mL volumetric flask, add about 40 mL of methanol, dilute with water to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of guaifenesin ($C_{10}H_{14}O_4$) in each Capsule taken by the formula:

$$20{,}000(C/N)(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Guaifenesin RS in the *Standard preparation*, *N* is the number of Capsules taken, and R_U and R_S are the ratios of the guaifenesin peak area response to the benzoic acid peak area response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for pseudoephedrine hydrochloride and dextromethorphan hydrobromide—

Mobile phase—To 3.5 g of docusate sodium add 500 mL of methanol, 350 mL of water, 145 mL of tetrahydrofuran, and 5 mL of glacial acetic acid, mix, and pass through a filter having a porosity of 0.5 µm or less. Make any necessary

www.webofpharma.com

adjustments (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Prepare a solution of brompheniramine maleate in methanol containing about 0.3 mg per mL.

Standard stock solution—Prepare a solution in water having known concentrations of about 20 mg of USP Guaifenesin RS, 20*J* mg of USP Pseudoephedrine Hydrochloride RS per mL, and 20*J′* mg of USP Dextromethorphan Hydrobromide RS per mL, *J* being the ratio of the labeled amount, in mg, of pseudoephedrine hydrochloride to the labeled amount, in mg, of guaifenesin per Capsule, and *J′* being the ratio of the labeled amount, in mg, of dextromethorphan hydrobromide to the labeled amount, in mg, of guaifenesin per Capsule.

Standard preparation—Transfer 10.0 mL of *Standard stock solution* and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Assay preparation—Transfer 10.0 mL of the stock solution used to prepare the *Assay preparation* in the *Assay for guaifenesin* and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 263-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.4 for pseudoephedrine, 0.75 for dextromethorphan, and 1.0 for brompheniramine; the resolution, *R*, between the peaks is not less than 1.5; the tailing factors for the pseudoephedrine peak, the dextromethorphan peak, and the brompheniramine peak are not more than 1.5, 2.5, and 3.0, respectively; and the relative standard deviation for replicate injections determined for the pseudoephedrine peak is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in each Capsule taken by the formula:

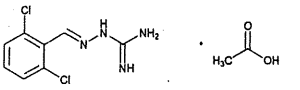
$$1000(C/N)(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*; *N* is the number of Capsules taken; and R_U and R_S are the ratios of the pseudoephedrine peak area response to the brompheniramine peak area response obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr \cdot H_2O$) in each Capsule taken by the formula:

$$1000(370.33/352.31)(C/N)(R_U/R_S)$$

in which 370.33 and 352.31 are the molecular weights of dextromethorphan hydrobromide and anhydrous dextromethorphan hydrobromide, respectively; *C* is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*; *N* is the number of Capsules taken; and R_U and R_S are the ratios of the dextromethorphan peak area response to the brompheniramine peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Guanabenz Acetate



$C_8H_8Cl_2N_4 \cdot C_2H_4O_2$ 291.13

Hydrazinecarboximidamide, 2-[(2,6-dichlorophenyl) methylene]-, monoacetate;
[(2,6-Dichlorobenzylidene)amino]guanidine monoacetate [23256-50-0].

DEFINITION

Guanabenz Acetate contains NLT 98.0% and NMT 101.5% of the labeled amount of guanabenz acetate ($C_{10}H_{12}N_4O_2Cl_2$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

- **PROCEDURE**

Sample solution: 4 mg/mL of Guanabenz Acetate in glacial acetic acid

Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N perchloric acid VS
Endpoint detection: Potentiometric

Analysis: Titrate 50 mL of the *Sample solution* with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 29.12 mg of guanabenz acetate ($C_{10}H_{12}N_4O_2Cl_2$).

Acceptance criteria: 98.0%–101.5%

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Diluent: A mixture of formic acid and methanol (1 in 2000)

Aminoguanidine bicarbonate solution: Transfer 100 mg of aminoguanidine bicarbonate to a test tube, add 0.05 mL of formic acid, and warm gently to dissolve. Quantitatively transfer the contents of the test tube to a 10-mL volumetric flask, and dilute with methanol to volume.

Standard solution A: 0.1 mg/mL of USP Guanabenz Acetate RS in *Diluent* prepared as follows. Transfer a suitable amount of USP Guanabenz Acetate RS to a suitable volumetric flask, and dissolve in about 50% of total volume of *Diluent*. Add 0.1 mL of *Aminoguanidine bicarbonate solution* per mg of USP Guanabenz Acetate RS, and dilute with *Diluent* to volume.

Standard solution B: 0.05 mg/mL of USP Guanabenz Acetate RS in *Diluent* from *Standard solution A*

Standard solution C: 0.02 mg/mL of USP Guanabenz Acetate RS in *Diluent* from *Standard solution A*

Sample solution: 10 mg/mL of Guanabenz Acetate in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform, methanol, and ammonium hydroxide (60:40:1)

www.webofpharma.com

Analysis
Samples: *Diluent, Standard solution A, Standard solution B, Standard solution C,* and *Sample solution*
Allow the chromatographic chamber to equilibrate in *Developing solvent system* for at least 30 min before use. Prewash a plate coated with *Adsorbent* by placing it in the chromatographic chamber, allowing the solvent front to rise to the top of the plate, dry it in air, and activate it by heating at 105° for 20 min. Within 30 min after preparation, separately apply portions of each of the *Standard solutions*, the *Sample solution*, and the *Diluent*. Allow the spots to dry, and place the plate in the chromatographic chamber. When the solvent has moved about three-fourths of the length of the plate, remove the plate, and allow it to air-dry for about 30 min. Examine the plate under short-wavelength UV light. Estimate the amount of any secondary spots, disregarding spots that have the same R_F as those from the *Diluent*, observed in the chromatogram of the *Sample solution* by comparison with each *Standard solution*. Place the plate in a chamber saturated with iodine vapors for about 10 min. Remove and examine the plate. Estimate the amount of any spot in the chromatogram of the *Sample solution* that has an R_F corresponding to the R_F of the spot produced by the aminoguanidine bicarbonate by comparison with each *Standard solution*.
Acceptance criteria: No individual secondary spot is greater in size or intensity than the spot produced by *Standard solution B* (0.5%), and the total of any such spots observed is NMT 1%.

Change to read:

• **LIMIT OF 2,6-DICHLOROBENZALDEHYDE**
Internal standard solution 1: 1 mg/mL of *p*-chlorobenzaldehyde in chloroform
Internal standard solution 2: 0.1 mg/mL of *p*-chlorobenzaldehyde in chloroform from *Internal standard solution 1*
Standard stock solution: 1 mg/mL of 2,6-dichlorobenzaldehyde in chloroform
Standard solution: 0.4 mg/mL of 2,6-dichlorobenzaldehyde from the *Standard stock solution* and 0.1 mg/mL of *p*-chlorobenzaldehyde from *Internal standard solution 1* in chloroform
Sample solution: Transfer 200 mg of Guanabenz Acetate to a 30-mL glass-stoppered centrifuge tube. Add 10 mL of 0.1 N hydrochloric acid, and shake to dissolve. Add 1.0 mL of *Internal standard solution 2*, and shake. Centrifuge, and transfer a portion of the lower layer to a stoppered container. The lower layer must be removed within 10 min of adding the acid to the centrifuge tube.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: ▲1.8-m▲ (ERR 1-Jun-2019) × 3-mm; packed with 20% phase G1 on 80–100 mesh S1A support
Temperatures
Injection port: 225°
Detector: 250°
Column: 190°
Carrier gas: Nitrogen
Flow rate: 30 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for *p*-chlorobenzaldehyde and 2,6-dichlorobenzaldehyde are 0.5 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between 2,6-dichlorobenzaldehyde and *p*-chlorobenzaldehyde
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The relative peak response ratio from the *Sample solution* does not exceed that from the *Standard solution* (0.2%).

SPECIFIC TESTS
• **PH** ⟨791⟩
Sample solution: 7 mg/mL
Acceptance criteria: 5.5–7.0
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum at 60° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Guanabenz Acetate RS

Guanabenz Acetate Tablets

DEFINITION

Change to read:

Guanabenz Acetate Tablets contain ▲an amount of guanabenz acetate equivalent to▲ (USP 1-May-2019) NLT 90.0% and NMT 110.0% of the labeled amount of guanabenz ($C_8H_8N_4Cl_2$).

IDENTIFICATION
• **A.**
Sample: Transfer an amount of powdered Tablets, equivalent to 8 mg of guanabenz, to a 60-mL separator. Add 10 mL of 0.1 N hydrochloric acid, and shake to disperse the powder. Shake the mixture with three 10-mL portions of chloroform, discarding the chloroform phase each time. Add 5 mL of 1 N sodium hydroxide, and extract with two 25-mL portions of ether, filtering the ether extracts. Evaporate the combined extracts to dryness with the aid of a current of air.
Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the *Sample* exhibits maxima at the same wavelengths as those of a similar preparation of USP Guanabenz Acetate RS.

Add the following:

• **▲B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**
Mobile phase: Methanol, phosphoric acid, and water (430:3:570)
Diluent: Dissolve 8.2 g of sodium acetate in 20 mL of water, add 5.7 mL of glacial acetic acid, and dilute with methanol to 1 L.

www.webofpharma.com

System suitability solution: Transfer 30 mg of guanabenz acetate to a 100-mL stoppered flask. Add about 50 mL of 0.1 N hydrochloric acid, heat on a steam bath for 60 min, and allow the solution to cool.

Standard solution: 0.1 mg/mL of USP Guanabenz Acetate RS in *Diluent* prepared as follows. Transfer a suitable amount of USP Guanabenz Acetate RS to a suitable volumetric flask. Add 10% of the total volume of water, shake to dissolve the solids, and dilute with *Diluent* to volume.

Sample solution: Nominally 0.08 mg/mL of guanabenz prepared as follows. Transfer 10 Tablets to a 500-mL volumetric flask. Add 50 mL of water, stir by mechanical means until the solids are well dispersed, add 400 mL of *Diluent*, and stir for 45 min. Dilute with *Diluent* to volume, and centrifuge a portion of the mixture until a clear supernatant is obtained. If necessary, dilute a portion of the supernatant quantitatively with a mixture of *Diluent* and water (90:10).

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: ▲3.9-mm▲ (USP 1-May-2019) × 30-cm; ▲10-µm▲ (USP 1-May-2019) packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

▲Run time: NLT 6 times the retention time of the guanabenz peak▲ (USP 1-May-2019)

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.6 between guanabenz and the peak eluting before it, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of guanabenz ($C_8H_8N_4Cl_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Guanabenz Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of guanabenz in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of guanabenz, 231.08

M_{r2} = molecular weight of guanabenz acetate, 291.13

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 1000 mL

Apparatus 2: 50 rpm

Time: 60 min

Standard solution: A known concentration of USP Guanabenz Acetate RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium* to a concentration similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: Maximum absorbance at about 272 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of guanabenz ($C_8H_8N_4Cl_2$) dissolved.

Tolerances: NLT 75% (*Q*) of the labeled amount of guanabenz ($C_8H_8N_4Cl_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Standard solution: Dilute 2 mL of the *Standard solution* from the *Assay* with *Diluent* to 100 mL.

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of any impurity observed having a relative retention time corresponding to the component eluting before guanabenz from the *System suitability solution*.

Acceptance criteria: NMT 2%

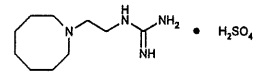
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Guanabenz Acetate RS

Guanethidine Monosulfate



$C_{10}H_{22}N_4 \cdot H_2SO_4$ 296.39

Guanidine, [2-(hexahydro-1(2*H*)-azocinyl)ethyl]-, sulfate (1:1);

[2-(Hexahydro-1(2*H*)-azocinyl)ethyl]guanidine sulfate (1:1) [645-43-2].

DEFINITION

Guanethidine Monosulfate contains NLT 97.0% and NMT 103.0% of guanethidine monosulfate ($C_{10}H_{22}N_4 \cdot H_2SO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

• **B.**

Sample solution: 2.5 mg dissolved in 10 mL of water

Analysis: To the *Sample solution* add 2 mL of a solution prepared by dissolving 500 mg of 1-naphthol, 3 g of sodium hydroxide, and 8 g of sodium carbonate in water to make 50 mL, and 1 mL of a solution of 2,3-butanedione (1 in 2000). Allow to stand at room temperature.

Acceptance criteria: An intense, pinkish-red color develops.

ASSAY

• **PROCEDURE**

Solution A: 10 mg/mL of sodium nitroferricyanide and 10 mg/mL of potassium ferricyanide in water

Standard solution: 1 mg/mL of USP Guanethidine Monosulfate RS in 1 N sulfuric acid

www.webofpharma.com

Sample solution: 1 mg/mL of Guanethidine Monosulfate in 1 N sulfuric acid
Blank: 1 N sulfuric acid
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: Maximum absorbance at about 500 nm
Cell: 1 cm
Analysis
Samples: *Standard solution, Sample solution,* and *Blank*
Pipet 2 mL each of the *Standard solution, Sample solution,* and *Blank* into separate glass-stoppered, 40-mL centrifuge tubes. Add 10.0 mL of water to each tube. Add 10.0 mL of *Solution A,* and mix. Add 4.0 mL of 1 N sodium hydroxide to each tube, mix, and allow to stand for 20 min, accurately timed.
Calculate the percentage of guanethidine monosulfate ($C_{10}H_{22}N_4 \cdot H_2SO_4$) in the portion of Guanethidine Monosulfate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Guanethidine Monosulfate RS in the *Standard solution* (mg/mL)
C_U = concentration of Guanethidine Monosulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° to constant weight.
Acceptance criteria: NMT 0.5%
• **PH** ⟨791⟩
Sample solution: 20 mg/mL
Acceptance criteria: 4.7–5.7

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Guanethidine Monosulfate RS

Guanethidine Monosulfate Tablets

DEFINITION
Guanethidine Monosulfate Tablets contain an amount of guanethidine monosulfate ($C_{10}H_{22}N_4 \cdot H_2SO_4$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of guanethidine sulfate [$(C_{10}H_{22}N_4)_2 \cdot H_2SO_4$].

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
Sample: Transfer a quantity of powdered Tablets, equivalent to about 120 mg of guanethidine monosulfate, to a glass-stoppered flask. Add 20 mL of water, shake by mechanical means for 30 min, and filter, discarding the first few mL of the filtrate. Transfer 10 mL of the filtrate to a separator. Add 2 mL of 0.1 N sodium hydroxide and 2 mL of a saturated solution of picric acid in 0.1 N sodium hydroxide, and mix. Extract with 20 mL of chloroform, filter the chloroform extract through cotton, and collect in a beaker. Evaporate the chloroform extract with the aid of a stream of nitrogen to dryness.
Acceptance criteria: Meet the requirements

ASSAY
• **PROCEDURE**
Solution A: Transfer 12.4 g of boric acid to a 1-L volumetric flask, dissolve in 100 mL of 1.0 N sodium hydroxide, and dilute with water to volume.
Buffer: *Solution A* and 0.1 N sodium hydroxide (400:600). Adjust with 1.0 N sodium hydroxide or 1.0 N hydrochloric acid to a pH of 12.3 ± 0.1.
Picrate reagent: 15 g/L of picric acid prepared as follows. Transfer 15 g of picric acid to a 1-L volumetric flask, dissolve in 750 mL of 0.1 N sodium hydroxide, and dilute with water to volume.
Standard solution: 0.3 mg/mL of USP Guanethidine Monosulfate RS in 0.1 N sulfuric acid
Sample solution: 0.25 mg/mL of guanethidine sulfate in 0.1 N sulfuric acid prepared as follows. Transfer a quantity from NLT 20 finely powdered Tablets, equivalent to 50 mg of guanethidine sulfate, to a 200-mL volumetric flask. Add 150 mL of 0.1 N sulfuric acid, shake by mechanical means for 30 min, and dilute with 0.1 N sulfuric acid to volume. Filter, and discard the first 25 mL of the filtrate.
Blank: Chloroform
Instrumental conditions
Mode: UV-Vis
Analytical wavelength: Maximum absorbance at about 412 nm
Analysis
Samples: *Standard solution, Sample solution,* and *Blank*
Pipet 5 mL each of the *Standard solution* and the *Sample solution* into separate 125-mL separators. To each separator, add 20 mL of *Buffer* and 20 mL of *Picrate reagent.* Extract with three 20-mL portions of chloroform, filtering the extracts through chloroform-prerinsed cotton, and collect the extracts in low-actinic 100-mL volumetric flasks. Rinse the cotton with 20 mL of chloroform, adding the rinsings to the volumetric flasks, and dilute with chloroform to volume. Determine the absorbances of the solutions.
Calculate the percentage of the labeled amount of guanethidine sulfate [$(C_{10}H_{22}N_4)_2 \cdot H_2SO_4$] in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Guanethidine Monosulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of guanethidine monosulfate in the *Sample solution* (mg/mL)
M_{r1} = one-half the molecular weight of guanethidine sulfate, 247.35
M_{r2} = molecular weight of guanethidine monosulfate, 296.39

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 500 mL
Apparatus 1: 100 rpm
Time: 45 min

USP Monographs

www.webofpharma.com

Analysis: Determine the percentage of the labeled amount of guanethidine sulfate $[(C_{10}H_{22}N_4)_2 \cdot H_2SO_4]$ dissolved using the *Assay* procedure and making any necessary modifications.

Tolerances: NLT 75% (*Q*) of the labeled amount of guanethidine sulfate $[(C_{10}H_{22}N_4)_2 \cdot H_2SO_4]$ is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Guanethidine Monosulfate RS

Guanfacine Tablets

» Guanfacine Tablets contain an amount of Guanfacine Hydrochloride ($C_9H_9Cl_2N_3O \cdot HCl$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of guanfacine ($C_9H_9Cl_2N_3O$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Guanfacine Hydrochloride RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay.*

B: It responds to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, the test solution and the Standard solution being prepared at a concentration of 2 mg per mL in methanol, and a solvent system consisting of a mixture of ethyl acetate, glacial acetic acid, and water (5:2:2) being used.

Dissolution ⟨711⟩—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_9H_9Cl_2N_3O$ dissolved, employing the procedure set forth in the *Assay* and making any necessary modifications.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_9H_9Cl_2N_3O$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

pH 2.5 Diethylamine phosphate solution—Add 10.3 mL of diethylamine to about 70 mL of water. Adjust with phosphoric acid to a pH of 2.5, dilute with water to 100 mL, and mix.

Reagent solution—Dissolve an accurately weighed quantity of 2,6-dichlorophenylacetic acid in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 18 µg per mL.

Mobile phase—Dissolve 600 mg of monobasic potassium phosphate and 3 mL of *pH 2.5 Diethylamine phosphate solution* in 480 mL of water, and mix. Adjust with 0.2 N sodium hydroxide to a pH of 4.0. While swirling, add 520 mL of acetonitrile. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Prepare a solution of butylparaben in *Mobile phase* containing 0.5 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Guanfacine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.23 mg per mL. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, and add 5.0 mL each of the *Reagent solution* and the *Internal standard solution.* Dilute with *Mobile phase* to volume, and mix.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of guanfacine, to a 100-mL volumetric flask. Add 50 mL of *Mobile phase,* and heat on a steam bath for 5 minutes. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of *Internal standard solution,* dilute with *Mobile phase* to volume, and mix.

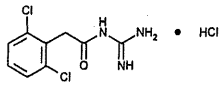
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.4 for guanfacine, 0.6 for 2,6-dichlorophenylacetic acid, and 1.0 for butylparaben; the resolution, *R,* between guanfacine and 2,6-dichlorophenylacetic acid is not less than 1.5, and the resolution, *R,* between 2,6-dichlorophenylacetic acid and butylparaben is not less than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of guanfacine ($C_9H_9Cl_2N_3O$) in the portion of Tablets taken by the formula:

$$(246.09/282.55)(0.25C)(R_U/R_S)$$

in which 246.09 and 282.55 are the molecular weights of guanfacine and guanfacine hydrochloride, respectively; *C* is the concentration, in µg per mL, of USP Guanfacine Hydrochloride RS in the *Standard preparation;* and R_U and R_S are the peak response ratios of guanfacine to butylparaben obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Guanfacine Hydrochloride



$C_9H_9Cl_2N_3O \cdot HCl$ 282.55

Benzeneacetamide, *N*-(aminoiminomethyl)-2,6-dichloro-, monohydrochloride.

N-Amidino-2-(2,6-dichlorophenyl)acetamide monohydrochloride [29110-48-3].

» Guanfacine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_9H_9Cl_2N_3O \cdot HCl$, calculated on the dried basis. [**CAUTION**—Guanfacine Hydrochloride is a potent antihypertensive drug. Minimize flying dust, and avoid all bodily and respiratory contact with this substance.]

www.webofpharma.com

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Guanfacine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—

Spray reagent—[Caution—Avoid contact with o-tolidine. Prepare and use this Spray reagent in a well-ventilated hood.] Dissolve 50 mg of o-tolidine in 100 mL of alcohol, and mix.

Chlorine chamber—Transfer 1.5 g of potassium permanganate to a 100-mL beaker, dissolve in and dilute with water to volume, and mix. Transfer 25 mL of this solution to a beaker, and place the beaker inside a chromatographic chamber. Pipet 10 mL of hydrochloric acid into the beaker, and cover the chamber.

Developing solvent system—Prepare a fresh mixture of ethyl acetate, glacial acetic acid, and acetonitrile (70:25:3).

Standard solutions—Dissolve accurately weighed quantities of USP Guanfacine Hydrochloride RS and guanidine hydrochloride in methanol to obtain a solution having a known concentration of 0.4 mg each of USP Guanfacine Hydrochloride RS and guanidine hydrochloride per mL. Quantitatively dilute this solution with methanol to obtain *Standard solutions* having the following compositions:

Standard Solution	Dilution	Concentration (µg RS and Guanidine Hydrochloride per mL)	Percentage (% for Comparison with Test Specimen)
1	(undiluted)	400	2.0
2	(1 in 2)	200	1.0
3	(1 in 4)	100	0.5
4	(1 in 8)	50	0.25

Test solution—Dissolve an accurately weighed quantity of Guanfacine Hydrochloride in methanol to obtain a solution having a concentration of about 20 mg per mL.

Procedure—Use a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩ coated with a 0.25-mm layer of chromatographic silica gel. Prewash the plates by placing in a chromatographic chamber saturated with *Developing solvent system*. Remove the plates from the chamber, and allow to dry. Separately apply 10 µL each of the *Standard solutions* and the *Test solution* to the chromatographic plate. Allow the spots to dry, and develop the chromatogram in *Developing solvent* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for about 1 hour. Examine the plate under short-wavelength UV light. Place the dried plate in the *Chlorine chamber* for 15 minutes, remove, and allow the excess chlorine to evaporate by air drying for 5 minutes. Spray the plate with *Spray reagent*, and examine: any spot due to guanidine hydrochloride observed in the chromatogram of the *Test solution* is not greater in size or intensity than the guanidine hydrochloride spot obtained from *Standard solution 3* (0.5%); no other individual impurity spot observed in the chromatogram of the *Test solution* is greater in size or intensity than the guanfacine hydrochloride spot obtained from *Standard solution 4* (0.25%); and the sum of all impurities found, including guanidine hydrochloride, is not more than 1.0%.

Chromatographic purity—

Spray reagent 1—Prepare a mixture of tertiary butyl alcohol and water (9:1).

Spray reagent 2—Dissolve 5 g of 4,4′-tetramethyldiaminodiphenylmethane in 20 mL of glacial acetic acid, add 10 mL of water, and mix (*Solution 1*). Dissolve 6 g of potassium iodide in 120 mL of water, and mix (*Solution 2*). Dissolve 0.3 g of ninhydrin in 10 mL of glacial acetic acid, dilute with water to 100 mL, and mix (*Solution 3*). Mix *Solution 1* and *Solution 2*, and add 9 mL of *Solution 3*.

Developing solvent system—Prepare a fresh mixture of hexanes, diisopropyl ether, toluene, and glacial acetic acid (60:30:5:3).

Reference solutions—Dissolve an accurately weighed quantity of 2,6-dichlorophenylacetic acid in a mixture of methanol and water (9:1) to obtain a solution having a concentration of 1 mg per mL (*Reference solution 1*). Quantitatively dilute this solution with a mixture of methanol and water (9:1) to obtain *Reference solution 2* and *Reference solution 3* having known concentrations of 0.5 and 0.25 mg per mL of 2,6-dichlorophenylacetic acid, respectively.

Test solution—Prepare a solution of Guanfacine Hydrochloride in a mixture of methanol and water (9:1), containing 100 mg per mL.

Procedure—Use a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Prewash the plates by placing in a chromatographic chamber saturated with *Developing solvent system*. Remove the plates from the chamber, and allow to dry. Separately apply 25 µL of each of the *Reference solutions* and the *Test solution* to the chromatographic plate. Allow the spots to dry, and develop the chromatograms in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the plate to air-dry for 30 minutes. Examine the plate under short-wavelength UV light. Spray the plate with *Spray reagent 1*, wait for 1 minute, and then spray with *Spray reagent 2*. Place the wet plate under short-wavelength UV light for 10 minutes, remove, and observe under white light: no spot observed in the chromatogram of the *Test solution*, other than that due to guanfacine hydrochloride, is greater in size or intensity than the principal spot obtained from *Reference solution 2* (0.5%); and the sum of all impurities found is not more than 1.0%.

Assay—

Dilute phosphoric acid—Prepare a mixture of water and phosphoric acid (4:1).

Buffer solution—Dissolve 68 g of monobasic potassium phosphate in water, dilute with water to 1000 mL, and mix. Dilute 100 mL of this solution with water to 1000 mL, add 5 mL of triethylamine, mix, and adjust with *Dilute phosphoric acid* to a pH of 3.0.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (79:21). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Guanfacine Hydrochloride RS in a mixture of acetonitrile and water (3:1) to obtain a solution having a known concentration of about 1 mg of USP Guanfacine Hydrochloride RS per mL. Transfer 2.0 mL of this solution to a

www.webofpharma.com

50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer an accurately weighed quantity of about 50 mg of Guanfacine Hydrochloride to a 50-mL volumetric flask, dissolve in and dilute with a mixture of acetonitrile and water (3:1) to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure:* the capacity factor, *k'*, is between 2 and 5; the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_9H_9Cl_2N_3O \cdot HCl$ in the portion taken by the formula:

$$1.25C(r_U/r_S)$$

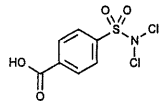
in which C is the concentration, in µg per mL, of USP Guanfacine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the guanfacine hydrochloride peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Gutta Percha

» Gutta Percha is the coagulated, dried, purified latex of the trees of the genera *Palaquium* and *Payena* and most commonly *Palaquium gutta* (Hooker) Baillon (Fam. Sapotaceae).

Packaging and storage—Preserve under water in well-closed containers, protected from light.
Residue on ignition ⟨281⟩: not more than 1.7%.

Halazone



$C_7H_5Cl_2NO_4S$ 270.09
Benzoic acid, 4-[(dichloroamino)sulfonyl]-;
p-(Dichlorosulfamoyl)benzoic acid [80-13-7].

DEFINITION
Halazone contains NLT 91.5% and NMT 100.5% of $C_7H_5Cl_2NO_4S$, calculated on the dried basis.

IDENTIFICATION
• **PROCEDURE**
Sample: 100 mg
Analysis: Add the *Sample* to 5 mL of a sodium bromide solution (1 in 10).
Acceptance criteria: Bromine is liberated from the mixture.

ASSAY
• **PROCEDURE**
Sample: 150 mg
Analysis: Add the *Sample* to 10 mL of 2.5 N sodium hydroxide in a 250-mL iodine flask, and stir well to dissolve. Add 75 mL of water, then promptly add 15 mL of potassium iodide solution (1 in 10), and mix. Acidify with 10 mL of 6 N acetic acid, and titrate immediately with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 6.752 mg of $C_7H_5Cl_2NO_4S$.
Acceptance criteria: 91.5%–100.5% on the dried basis

IMPURITIES
ORGANIC IMPURITIES
• **Procedure: Readily Carbonizable Substances Test** ⟨271⟩
Sample: 100 mg
Analysis: Dissolve the *Sample* in 0.5 mL of sulfuric acid.
Acceptance criteria: No blackening occurs, although some effervescence may take place.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample over phosphorus pentoxide for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Halazone Tablets for Solution

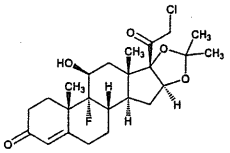
» Halazone Tablets for Solution contain not less than 90.0 percent and not more than 135.0 percent of the labeled amount of $C_7H_5Cl_2NO_4S$.

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—Label the Halazone Tablets for Solution to indicate that they are not intended to be swallowed.
Identification—Finely powder a number of Halazone Tablets for Solution, equivalent to about 150 mg of halazone: a portion of the powder, equivalent to about 100 mg of halazone, responds to the *IDENTIFICATION* test under *Halazone*.
Disintegration ⟨701⟩: 10 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements, except that if the average value of the dosage units tested is between 100.0 percent and 135.0 percent, *Criterion* (*B*) (*3*) applies.
pH ⟨791⟩: not less than 7.0, in a solution of 1 Halazone Tablet for Solution, containing 4 mg of halazone, in 200 mL of water.
Assay—Transfer a counted number of Halazone Tablets for Solution, equivalent to about 160 mg of halazone, to a suitable container, and proceed as directed in the *ASSAY* under *Halazone*. Each mL of 0.1 N sodium thiosulfate is equivalent to 6.752 mg of $C_7H_5Cl_2NO_4S$.

USP Monographs

www.webofpharma.com

Halcinonide



$C_{24}H_{32}ClFO_5$ 454.96

Pregn-4-ene-3,20-dione, 21-chloro-9-fluoro-11-hydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (11β,16α)-;
21-Chloro-9-fluoro-11β,16α,17-trihydroxypregn-4-ene-3,20-dione cyclic 16,17-acetal with acetone [3093-35-4].

DEFINITION

Halcinonide contains NLT 97.0% and NMT 102.0% of halcinonide ($C_{24}H_{32}ClFO_5$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and water (50:50)
Diluent: Acetonitrile and water (2:1)
Standard solution: 0.3 mg/mL of USP Halcinonide RS in *Diluent*
Sample solution: 0.3 mg/mL of Halcinonide in *Diluent*. [NOTE—Sonication may be needed to aid in the dissolution of the *Standard solution* and *Sample solution*.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5
Relative standard deviation: NMT 0.85% for six replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of halcinonide ($C_{24}H_{32}ClFO_5$) in the portion of Halcinonide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Halcinonide RS in the *Standard solution* (mg/mL)
C_U = concentration of Halcinonide in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0%

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Solution A: 10 mM of ammonium acetate in water
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20.0	10	90
22.0	10	90
22.1	90	10
25.0	90	10

Diluent 1: Acetonitrile and water (60:30)
Diluent 2: Acetonitrile and water (10:90)
Standard stock solution: 0.2 mg/mL of USP Halcinonide RS in *Diluent 1*
Standard solution: 0.1 µg/mL of USP Halcinonide RS from *Standard stock solution* in *Diluent 2*
Sample stock solution: 0.2 mg/mL of Halcinonide in *Diluent 1*
Sample solution: 0.1 mg/mL of Halcinonide from *Sample stock solution* in *Diluent 2*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 2.1-mm × 15-cm; 1.7-µm packing L1
Flow rate: 0.3 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Relative standard deviation: NMT 2.8%
Signal-to-noise ratio: NLT 50
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual unspecified impurity in the portion of Halcinonide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual unspecified impurity from the *Sample solution*
r_S = peak response of halcinonide from the *Standard solution*
C_S = concentration of USP Halcinonide RS in the *Standard solution* (µg/mL)
C_U = concentration of Halcinonide in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dihydrotriamcinolone[a]	0.5	—
Halcinonide	1.0	—
Any individual unspecified impurity	—	0.10

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total impurities	—	3.0

[a] 9-Fluoro-11β,16α,17,21-tetrahydroxypregna-4-ene-3,20-dione.

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 20 mg/mL in chloroform
  Acceptance criteria: +150° to +160°
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry under vacuum at 100° for 3 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Halcinonide RS

Halcinonide Cream

DEFINITION
Halcinonide Cream is Halcinonide in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of halcinonide ($C_{24}H_{32}ClFO_5$).

IDENTIFICATION
- **A.** The retention time of the halcinonide peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV-Vis spectrum of the halcinonide peak of the *Sample solution* corresponds to that of the *Diluted standard stock solution*, as obtained in the test for *Organic Impurities*.

ASSAY
- **PROCEDURE**
  Mobile phase: Acetonitrile and water (50:50)
  Diluent: Acetonitrile and water (2:1), saturated with hexanes. [NOTE—Prepare on the day of use.]
  Internal standard solution: 150 µg/mL of progesterone in hexanes-saturated *Diluent*
  Standard stock solution: 0.2 mg/mL of USP Halcinonide RS in *Diluent*
  Standard solution: 0.02 mg/mL of USP Halcinonide RS in hexanes-saturated *Diluent* prepared as follows. Transfer 5.0 mL of *Standard stock solution* to a 50-mL volumetric flask. Add 4.0 mL of *Internal standard solution*, and dilute with hexanes-saturated *Diluent* to volume.
  Sample solution: Nominally 0.02 mg/mL of halcinonide in hexanes-saturated *Diluent* prepared as follows. Transfer a quantity of Cream equivalent to 0.5 mg of halcinonide to a glass-stoppered, 50-mL centrifuge tube. Add 12 mL of hexanes-saturated *Diluent* and 20 mL of hexanes, and shake for 1 min. Place in a heated ultrasonic bath at 58 ± 2° for 20 min, initially shaking for 1–2 min to ensure dispersion, and at 5-min intervals thereafter, on a vibratory mixer. Cool, centrifuge, and transfer the lower layer to a 25-mL volumetric flask. Add 5 mL of hexanes-saturated *Diluent* to the tube, mix for 1 min, then centrifuge. Transfer the lower layer to the volumetric flask, and repeat the extraction with an additional 5 mL of hexanes-saturated *Diluent*, combining the extracts in the flask. Add 2.0 mL of *Internal standard solution* to the flask, dilute with hexanes-saturated *Diluent* to volume, and mix. If necessary, clarify a portion of the solution by centrifugation.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for halcinonide and progesterone are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 1.5 between halcinonide and progesterone
Relative standard deviation: NMT 3.0%, peak response ratio of halcinonide to progesterone
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of halcinonide ($C_{24}H_{32}ClFO_5$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of halcinonide to progesterone from the *Sample solution*
R_S = peak response ratio of halcinonide to progesterone from the *Standard solution*
C_S = concentration of USP Halcinonide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of halcinonide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

IMPURITIES
- **ORGANIC IMPURITIES**
  Solution A: 10 mM of ammonium acetate in water
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20.0	10	90
22.0	10	90
22.1	90	10
25.0	90	10

Diluent 1: Acetonitrile and water (60:30), saturated with hexanes
Diluent 2: Acetonitrile and water (10:90)
Standard stock solution: 0.2 mg/mL of USP Halcinonide RS in *Diluent 1*
Diluted standard stock solution: 0.1 mg/mL of USP Halcinonide RS in *Diluent 2* from the *Standard stock solution*
Standard solution: 0.2 µg/mL of USP Halcinonide RS in *Diluent 2* from the *Standard stock solution*
Sample stock solution: Nominally 0.2 mg/mL of halcinonide in *Diluent 1* prepared as follows. Transfer a portion of Cream equivalent to 4 mg of halcinonide to a 50-mL centrifuge tube. Add 10 mL of *Diluent 1* and 20 mL of hexanes heated at 58 ± 2° for 20 min. Shake for NLT 1 min initially to ensure dispersion and at 5-min intervals

USP Monographs

www.webofpharma.com

thereafter. Cool, centrifuge at 3000 rpm for 10 min, and transfer the lower layer to the 20-mL volumetric flask. Repeat the extraction with an additional 5 mL of *Diluent 1* and 20 mL of hexanes each time. Dilute with *Diluent 1* to volume.

Sample solution: 0.1 mg/mL of halcinonide from *Sample stock solution* in *Diluent 2*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. For *Identification* test *B*, use a diode array detector in the range of 200–450 nm.
Column: 2.1-mm × 15-cm; 1.7-µm packing L1
Flow rate: 0.3 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Relative standard deviation: NMT 2.4%
Signal-to-noise ratio: NLT 50

Analysis
Samples: *Diluted standard stock solution*, *Standard solution*, and *Sample solution*
[NOTE—The *Diluted standard stock solution* is used for *Identification* test *B*.]
Calculate the percentage of any individual unspecified impurity in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual unspecified impurity from the *Sample solution*
r_S = peak response of halcinonide from the *Standard solution*
C_S = concentration of USP Halcinonide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of halcinonide in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dihydrotriamcinolone[a]	0.5	—
Halcinonide	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities	—	3.0

[a] Drug substance process impurity included in the table for identification only.

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: Meets the requirements for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature. Avoid excessive heat.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Halcinonide RS

Halcinonide Ointment

DEFINITION
Halcinonide Ointment is Halcinonide in a suitable ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of halcinonide ($C_{24}H_{32}ClFO_5$).

IDENTIFICATION
- **A.** The retention time of the halcinonide peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the halcinonide peak of the *Sample solution* corresponds to that of the *Diluted standard stock solution*, as obtained in the test for *Organic Impurities*.

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile and water (50:50)
Internal standard solution: 6 µg/mL of butylparaben in acetonitrile
Standard stock solution: 0.04 mg/mL of USP Halcinonide RS in *Internal standard solution*
Standard solution: 0.02 mg/mL of USP Halcinonide RS from *Standard stock solution* prepared as follows. Mix equal volumes of *Mobile phase* and *Standard stock solution*.
Sample solution: Nominally 0.02 mg/mL of halcinonide in *Mobile phase* prepared as follows. Transfer an equivalent to 1 mg of halcinonide from a quantity of Ointment to a glass-stoppered, 50-mL centrifuge tube, and add 25.0 mL of *Internal standard solution* and 5.0 mL of hexane. Place in a water bath at 58 ± 2° for 3 min, then mix in a vortex mixer for 1 min until the sample is well dispersed. Repeat the above-specified heating and mixing step once. Cool in an ice–methanol bath for 15 min, or until the two phases separate, centrifuging if necessary. Transfer 5.0 mL of the lower layer to a 15-mL centrifuge tube, and add 5.0 mL of *Mobile phase*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for butylparaben and halcinonide are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between halcinonide and butylparaben
Relative standard deviation: NMT 3.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of halcinonide ($C_{24}H_{32}ClFO_5$) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of halcinonide to butylparaben from the *Sample solution*
R_S = peak response ratio of halcinonide to butylparaben from the *Standard solution*
C_S = concentration of USP Halcinonide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of halcinonide in the *Sample solution* (mg/mL)

www.webofpharma.com

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: 10 mM ammonium acetate in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20.0	10	90
22.0	10	90
22.1	90	10
25.0	90	10

Diluent 1: Acetonitrile and water (60:30), saturated with hexane

Diluent 2: Acetonitrile and water (10:90)

Standard stock solution: 0.2 mg/mL of USP Halcinonide RS in *Diluent 1*

Diluted standard stock solution: 0.1 mg/mL of USP Halcinonide RS in *Diluent 2* from the *Standard stock solution*

Standard solution: 0.2 µg/mL each of USP Halcinonide RS in *Diluent 2* from the *Standard stock solution*

Sample stock solution: Nominally 0.2 mg/mL of halcinonide in *Diluent 1* prepared as follows. Transfer a portion of Ointment equivalent to 4 mg of halcinonide to a 50-mL centrifuge tube. Add 10 mL of *Diluent 1* and 20 mL of hexane heated at 58 ± 2° for 20 min. Shake for NLT 1 min initially to ensure dispersion and at 5-min intervals thereafter. Cool, centrifuge at 3000 rpm for 10 min, and transfer the lower layer to a 20-mL volumetric flask. Repeat the extraction with an additional 5 mL of *Diluent 1* and 20 mL of hexane each time. Dilute with *Diluent 1* to volume.

Sample solution: 0.1 mg/mL of halcinonide from the *Sample stock solution* in *Diluent 2*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm. For *Identification* test *B*, use a diode array detector in the range of 200–450 nm.

Column: 2.1-mm × 15-cm; 1.8-µm packing L1

Flow rate: 0.3 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Relative standard deviation: NMT 2.4%

Signal-to-noise ratio: NLT 50

Analysis

Samples: *Diluted standard stock solution*, *Standard solution*, and *Sample solution*

[NOTE—The *Diluted standard stock solution* is used for *Identification* test *B*.]

Calculate the percentage of any individual unspecified impurity in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response of halcinonide from the *Standard solution*

C_S = concentration of USP Halcinonide RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of halcinonide in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dihydrotriamcinolone[a]	0.5	—
Halcinonide	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities	—	3.0

[a] Drug substance process impurity included in the table for identification only.

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: Meets the requirements for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature. Avoid excessive heat.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Halcinonide RS

Halcinonide Topical Solution

» Halcinonide Topical Solution is Halcinonide in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{24}H_{32}ClFO_5$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—

USP Halcinonide RS

Identification—It responds to the *IDENTIFICATION* test under *Halcinonide Cream*.

Microbial enumeration tests ⟨61⟩ **and *Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Assay—

Mobile phase—Mix approximately equal volumes of acetonitrile and water, adjusting the ratio of solvents as necessary to achieve acceptable chromatography.

Internal standard solution—Transfer 15 mg of Progesterone to a 50-mL volumetric flask. Dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and mix.

Standard preparation—Transfer about 20 mg of USP Halcinonide RS, accurately weighed, to a 100-mL volumetric flask, dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL

USP Monographs

www.webofpharma.com

volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

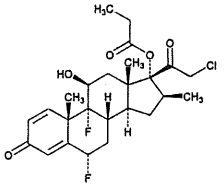
Assay preparation—Transfer an accurately measured quantity of Topical Solution, equivalent to about 1 mg of halcinonide, to a 50-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

Chromatographic system and *Procedure*—Proceed as directed in the *ASSAY* under *Halcinonide Cream*. Calculate the quantity, in mg, of $C_{24}H_{32}ClFO_5$ in the portion of Topical Solution taken by the formula:

$$50C(R_U/R_S)$$

in which the terms are as defined therein.

Halobetasol Propionate



$C_{25}H_{31}ClF_2O_5$ 484.96

Pregna-1,4-diene-3,20-dione, 21-chloro-6,9-difluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)-, (6α,11β,16β)-; 21-Chloro-6α,9-difluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate [66852-54-8].

DEFINITION

Halobetasol Propionate contains NLT 98.0% and NMT 102.0% of halobetasol propionate ($C_{25}H_{31}ClF_2O_5$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Acetonitrile and water (90:110)
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	64.5	35.5
22	64.5	35.5
23	100	0
30	100	0

Standard solution: 0.2 mg/mL of USP Halobetasol Propionate RS in acetonitrile

Sample solution: 0.2 mg/mL of Halobetasol Propionate in acetonitrile

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 0.8 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Tailing factor: 0.9–1.1
Suitability requirements
Relative standard deviation: NMT 0.73%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of halobetasol propionate ($C_{25}H_{31}ClF_2O_5$) in the portion of Halobetasol Propionate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of halobetasol propionate from the *Sample solution*
r_S = peak response of halobetasol propionate from the *Standard solution*
C_S = concentration of USP Halobetasol Propionate RS in the *Standard solution* (mg/mL)
C_U = concentration of Halobetasol Propionate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase, Standard solution, and **Sample solution:** Prepare as directed in the *Assay*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 0.8 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 15,000 theoretical plates
Tailing factor: 0.9–1.1
Relative standard deviation: NMT 0.73%

Analysis
Sample: *Sample solution*
Calculate the percentage of any impurity in the portion of Halobetasol Propionate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

USP Monographs

www.webofpharma.com

Table 2

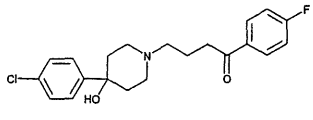
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
21-Chloro diflorasone[a]	0.75	0.15
21-Acetate 17-propionate diflorasone[b]	0.88	0.15
11-Propionate 21-chloro diflorasone[c]	0.95	0.15
Halobetasol propionate	1.0	—
9-Chloro halobetasol propionate[d]	1.12	0.15
6-Chloro halobetasol propionate[e]	1.24	0.15
Any other individual impurity	—	0.10
Total impurities	—	1.0

[a] 21-Chloro-6α,9-difluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione.
[b] 6α,9-Difluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 21-acetate 17-propionate.
[c] 21-Chloro-6α,9-difluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 11-propionate.
[d] 9,21-Dichloro-6α,-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate.
[e] 6α,21-Dichloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate.

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry a sample under vacuum at 105° for 3 h.
  Acceptance criteria: NMT 1.0%
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 10 mg/mL in dioxane
  Acceptance criteria: +87° to +99°

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store between 2° and 8°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Halobetasol Propionate RS

Haloperidol



$C_{21}H_{23}ClFNO_2$ 375.86
1-Butanone, 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-;
4-[4-(*p*-Chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone [52-86-8].

DEFINITION
Haloperidol contains NLT 98.0% and NMT 102.0% of haloperidol ($C_{21}H_{23}ClFNO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY
- **PROCEDURE**
  Sample solution: 5 mg/mL of Haloperidol in glacial acetic acid
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.05 N perchloric acid VS
  Endpoint detection: Visual
  Analysis: To 25 mL of the *Sample solution* add 3 drops of *p*-naphtholbenzein TS, and titrate with *Titrant*. Perform a blank determination, and make any necessary correction. Calculate the percentage of haloperidol ($C_{21}H_{23}ClFNO_2$) in the portion of sample taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the sample (mL)
V_B = *Titrant* volume consumed by the blank (mL)
N = actual normality of the *Titrant* (meq/mL)
F = equivalency factor, 375.86 mg/meq
W = sample weight (mg)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**
  Prepare the solutions immediately before use, and protect from light.
  Solution A: 17 g/L of tetrabutylammonium hydrogen sulfate
  Solution B: Acetonitrile
  Mobile phase: See *Table 1*. [NOTE—The dwell volume is 1.2 mL.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
2	90	10
17	50	50
22	50	50

System suitability solution: 10 mg/mL of USP Haloperidol RS and 20 µg/mL each of USP Haloperidol Related Compound A RS and USP Haloperidol Related Compound B RS in methanol. [NOTE—Haloperidol related compound A is used for identification purposes only.]
Standard solution: 50 µg/mL of USP Haloperidol RS in methanol
Sample solution: 10 mg/mL of Haloperidol in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 3.0 between the haloperidol related compound B and haloperidol peaks

USP Monographs

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Haloperidol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of an individual impurity from the *Sample solution*
r_S = peak response of haloperidol from the *Standard solution*
C_S = concentration of USP Haloperidol RS in the *Standard solution* (mg/mL)
C_U = concentration of Haloperidol in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. [NOTE—Disregard any peak with an area less than 0.05% of the main peak.]

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Haloperidol related compound B	0.9	1.4	0.3
Haloperidol	1.0	—	—
Haloperidol related compound A	1.6	1.0	0.2
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.5

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample under vacuum at 60° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Haloperidol RS
USP Haloperidol Related Compound A RS
4,4′-Bis[4-*p*-chlorophenyl)-4-hydroxypiperidino] butyrophenone.
$C_{32}H_{36}Cl_2N_2O_3$ 567.56
USP Haloperidol Related Compound B RS
4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(2-fluorophenyl)butan-1-one.
$C_{21}H_{23}ClFNO_2$ 375.86

Haloperidol Injection

DEFINITION
Haloperidol Injection is a sterile solution of Haloperidol in Water for Injection, prepared with the aid of Lactic Acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$). It may contain a suitable preservative.

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer solution: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0.
Mobile phase: Methanol and *Buffer solution* (55:45)
Standard stock solution: 1 mg/mL of USP Haloperidol RS in methanol. Sonicate to aid in dissolution.
Standard solution: 0.2 mg/mL of USP Haloperidol RS in *Mobile phase* from the *Standard stock solution*
Sample solution: Nominally, 0.2 mg/mL of haloperidol in *Mobile phase* from a volume of Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 247 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 0.8 mL/min
Injection size: 10 µL
Run time: 2.5 times the retention time of haloperidol
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Haloperidol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of haloperidol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 71.4 USP Endotoxin Units/mg of haloperidol.
• **PH** ⟨791⟩**:** 3.0–3.8
• **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Haloperidol RS

Haloperidol Oral Solution

DEFINITION
Haloperidol Oral Solution is a solution of Haloperidol in Water, prepared with the aid of Lactic Acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer solution: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0.

Mobile phase: Methanol and *Buffer solution* (55:45)

Standard stock solution: 1 mg/mL of USP Haloperidol RS in methanol. Sonicate to aid in dissolution.

Standard solution: 0.2 mg/mL of USP Haloperidol RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Nominally, 0.2 mg/mL of haloperidol in *Mobile phase* from a volume of Haloperidol Oral Solution. Filter a portion to use in the analysis.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 247 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 0.8 mL/min

Injection size: 10 µL

Run time: 2.5 times the retention time of haloperidol

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Haloperidol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of haloperidol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DELIVERABLE VOLUME** ⟨698⟩**:** Meets the requirements for oral solution packaged in multiple-unit containers

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements for oral solution packaged in single-unit containers

SPECIFIC TESTS

• **PH** ⟨791⟩**:** 2.75–3.75

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Haloperidol RS

Haloperidol Tablets

DEFINITION

Haloperidol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol and 0.05 M monobasic potassium phosphate buffer (60:40). Adjust with 1 N sodium hydroxide or phosphoric acid to a pH of 4.0.

Standard solution: 0.1 mg/mL of USP Haloperidol RS in *Mobile phase*

Sample solution: Nominally 0.1 mg/mL of Haloperidol prepared as follows. Transfer an equivalent of about 10 mg of haloperidol from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add 60 mL of *Mobile phase*, sonicate for 10 min, and shake by mechanical means for about 1 h. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 20 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection size: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*

r_S = peak response of the *Standard solution*

C_S = concentration of USP Haloperidol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of haloperidol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Simulated gastric fluid TS without enzyme; 900 mL

Apparatus 1: 100 rpm

Time: 60 min

Mobile phase: Prepare as directed in the *Assay*.

Standard solution: A known concentration of USP Haloperidol RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

www.webofpharma.com

Tolerances: NLT 80% (*Q*) of the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Standard solution: 20 µg/mL of USP Haloperidol RS in warm methanol

Sample solution: 20 µg/mL of haloperidol from 1 finely powdered Tablet in warm methanol. Shake for 15 min and filter, discarding the first 20 mL of the filtrate.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 245 nm

Cell: 1 cm

Blank: Methanol

Analysis: Concomitantly determine the absorbances of the *Standard solution* and *Sample solution* at the wavelength of maximum absorbance.

Calculate the percentage of the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Haloperidol RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of haloperidol in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

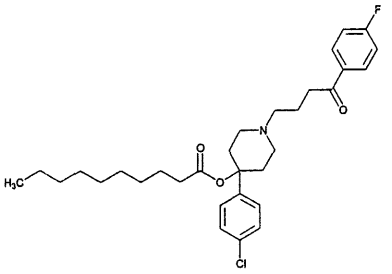
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Haloperidol RS

Haloperidol Decanoate



$C_{31}H_{41}ClFNO_3$ 530.11

Decanoic acid, 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinyl ester;

Decanoic acid, ester with 4-[4-(p-chlorophenyl)-4-hydroxy piperidino]-4′-fluorobutyrophenone [74050-97-8].

DEFINITION

Haloperidol Decanoate contains NLT 97.0% and NMT 103.0% of $C_{31}H_{41}ClFNO_3$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak in the chromatogram of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chloride*

Sample solution: Mix 0.1 g of the sample with 0.5 g of anhydrous sodium carbonate in a porcelain crucible. Heat over open flame for 10 min. Allow to cool. Dissolve the residue in 5 mL of dilute nitric acid, and filter. Dilute 1 mL of the filtrate with 1 mL of water.

Acceptance criteria: Meets the requirements of test *A*

ASSAY

• **PROCEDURE**

Solution A: 27 g/L of tetrabutylammonium hydrogen sulfate in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
30	40	60
35	40	60
40	80	20
45	80	20

Standard solution: 0.2 mg/mL of USP Haloperidol Decanoate RS in methanol

Sample solution: 0.2 mg/mL of Haloperidol Decanoate in methanol

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4-mm × 10-cm; 3-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of Haloperidol Decanoate ($C_{31}H_{41}ClFNO_3$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Haloperidol Decanoate RS in the *Standard solution* (mg/mL)

C_U = concentration of Haloperidol Decanoate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

www.webofpharma.com

• **ORGANIC IMPURITIES**

Solution A, Solution B, and **Mobile phase:** Proceed as directed in the *Assay*.

System suitability solution: 0.05 mg/mL each of USP Haloperidol Decanoate RS and USP Bromperidol Decanoate RS in methanol

Standard stock solution: 0.2 mg/mL of USP Haloperidol Decanoate RS in methanol

Standard solution: 0.05 mg/mL of USP Haloperidol Decanoate RS in methanol, from *Standard stock solution*

Sample solution: 10 mg/mL of Haloperidol Decanoate in methanol

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between haloperidol decanoate and bromperidol decanoate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Haloperidol Decanoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of haloperidol decanoate from the *Standard solution*

C_S = concentration of USP Haloperidol Decanoate RS in the *Standard solution* (mg/mL)

C_U = concentration of Haloperidol Decanoate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Haloperidol[a]	0.09	0.50
Haloperidol octanoate[b]	0.6	0.50
Haloperidol nonanoate[c]	0.79	0.50
Haloperidol decanoate-dechloro analog[d]	0.89	0.50
2-Fluorohaloperidol decanoate[e]	0.97	0.50
Haloperidol decanoate	1.0	—
Bromperidol decanoate[f]	1.05	—
Haloperidol undecanoate[g]	1.10	0.50
Haloperidol decanoate-3-ethyl analog[h]	1.13	0.50
Haloperidol decanoate-4-piperidinol analog[i]	1.17	0.50
Haloperidol dodecanoate[j]	1.21	0.50
Haloperidol decanoate-3-chlorobiphenyl analog[k]	1.22	0.50
Haloperidol decanoate-4-chlorobiphenyl analog[l]	1.24	0.50
Any other individual, unspecified impurity	—	0.10
Total impurities	—	1.0

[a] 4-[4-(4-Chlorophenyl)-4-hydroxypiperidino]-4-fluorobutyrophenone.
[b] 4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)piperidin-4-yl octanoate.
[c] 4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)piperidin-4-yl nonanoate.
[d] 1-[4-(4-Fluorophenyl)-4-oxobutyl]-4-phenylpiperidin-4-yl decanoate.
[e] 4-(4-Chlorophenyl)-1-(4-(2-fluorophenyl)-4-oxobutyl)piperidin-4-yl decanoate.
[f] Used only for system suitability.
[g] 4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)piperidin-4-yl undecanoate.
[h] 4-(4-Chlorophenyl)-1-[4-(3-ethyl-4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate.
[i] 4-(4-Chlorophenyl)-1-(4-{4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]phenyl}-4-oxobutyl)piperidin-4-yl decanoate.
[j] 4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)piperidin-4-yl dodecanoate.
[k] 4-(3′-Chlorobiphenyl-4-yl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate.
[l] 4-(4′-Chlorobiphenyl-4-yl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩: Dry a sample in a vacuum at 30° under phosphorous pentoxide desiccant: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in light-resistant, tight containers. Store at room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Bromperidol Decanoate RS

Decanoic acid, 4-(4-bromophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinyl ester.

$C_{31}H_{41}BrFNO_3$ 574.56

USP Haloperidol Decanoate RS

Halothane



$C_2HBrClF_3$ 197.38

Ethane, 2-bromo-2-chloro-1,1,1-trifluoro-, (±)-;
(±)-2-Bromo-2-chloro-1,1,1-trifluoroethane [151-67-7].

DEFINITION

Halothane contains NLT 0.008% and NMT 0.012% of thymol, by weight, as a stabilizer.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197S**▲ (CN 1-May-2020)

Sample solution: 1 in 25

Medium: Carbon disulfide

Acceptance criteria: Meets the requirements

OTHER COMPONENTS

• **THYMOL CONTENT**

Buffer: Use *pH 8.0 Alkaline Borate Buffer* (see *Reagents, Indicators, and Solutions—Solutions*).

www.webofpharma.com

Chlorimide solution: 4 mg/mL of 2,6-dibromoquinonechlorimide in dehydrated alcohol. [NOTE—Prepare a fresh solution for each assay.]
Standard thymol solution: 0.1 mg/mL of thymol in 0.25 N sodium hydroxide
Standard solution A: Pipet 1 mL of *Standard thymol solution* into a 100-mL volumetric flask, and add 0.25 N sodium hydroxide to make the final volume 5 mL. Add 10 mL of *Buffer*, mix by gentle swirling, and add 1 mL of *Chlorimide solution*. Allow to stand for 15 min, accurately timed. Add 3 mL of 0.25 N sodium hydroxide, and dilute with water to volume.
Standard solution B: Pipet 3 mL of *Standard thymol solution* into a 100-mL volumetric flask. Proceed as directed for *Standard solution A*, beginning with "add 0.25 N sodium hydroxide...".
Standard solution C: Pipet 5 mL of *Standard thymol solution* into a 100-mL volumetric flask. Proceed as directed for *Standard solution A*, beginning with "Add 10 mL of *Buffer*...".
Sample solution: Place 2 mL of Halothane in a 100-mL volumetric flask containing 5 mL of 0.25 N sodium hydroxide, and mix by gentle swirling. Evaporate the halothane under a stream of nitrogen, and add 10 mL of *Buffer* and 1 mL of *Chlorimide solution*. Swirl gently, and allow to stand for 15 min, accurately timed. Add 3 mL of 0.25 N sodium hydroxide, and add water to volume.
Blank solution: Pipet 5.0 mL of 0.25 N sodium hydroxide into a 100-mL volumetric flask. Add 10 mL of *Buffer*, mix by gentle swirling, and add 1 mL of *Chlorimide solution*. Allow to stand for 15 min, accurately timed. Add 3 mL of 0.25 N sodium hydroxide, and dilute with water to volume.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Vis
Analytical wavelength: 590 nm
Analysis
Samples: *Standard solution A, Standard solution B, Standard solution C, Sample solution,* and *Blank solution*
Measure the absorbances of *Standard solution A, Standard solution B,* and *Standard solution C* relative to the *Blank solution*. Plot the readings, and draw the curve of best fit. Read the absorbance of the *Sample solution*, and by reference to the Standard thymol curve, calculate the percentage of thymol in the weight of Halothane taken.
Acceptance criteria: 0.008%–0.012%, by weight

IMPURITIES

- **LIMIT OF NONVOLATILE RESIDUE**

Analysis: Evaporate 50 mL in a tared dish on a steam bath to dryness, dry the residue at 105° for 2 h, and weigh.
Acceptance criteria: NMT 1 mg of the residue remains.

- **CHLORIDE AND BROMIDE**

Analysis: Shake 25 mL with 25 mL of water for 5 min, and allow the liquids to separate completely. Draw off the water layer, and to 10 mL add 1 drop of nitric acid and 5 drops of silver nitrate TS.
Acceptance criteria: No opalescence is produced.

- **ORGANIC IMPURITIES**

Standard solution: Add 1.0 µL of 1,1,2-trichloro-1,2,2-trifluoroethane to 20.0 mL of Halothane.
Sample solution: Halothane
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 3-m × 2-mm stainless steel; 20% G24 on support S1AB
Temperatures
Column: 60°
Injection port: 200°
Detector: 200°
Carrier gas: Nitrogen
Flow rate: 15 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention times for 1,1,2-trichloro-1,2,2-trifluoroethane and halothane are 5 and 13 min, respectively.]
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: 0.005%; the total area of all peaks (except that of halothane) recorded from the *Sample solution* does not exceed that due to the added 1,1,2-trichloro-1,2,2-trifluoroethane in the *Standard solution*.

SPECIFIC TESTS

- **ACIDITY OR ALKALINITY**

Analysis: Shake 20 mL with 20 mL of carbon dioxide-free water for 3 min, and allow the layers to separate.
Acceptance criteria: The aqueous layer requires NMT 0.1 mL of 0.010 N sodium hydroxide or NMT 0.6 mL of 0.010 N hydrochloric acid for neutralization, using bromocresol purple TS as the indicator.

- **DISTILLING RANGE,** *Method II* ⟨721⟩**:** NLT 95% distills within a 1° range between 49° and 51°, and NLT 100% distills between 49° and 51°, a correction factor of 0.040°/mm being applied as necessary.
- **REFRACTIVE INDEX** ⟨831⟩**:** 1.369–1.371 at 20°
- **SPECIFIC GRAVITY** ⟨841⟩**:** 1.872–1.877 at 20°
- **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.03%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, preferably of Type NP glass, and avoid exposure to excessive heat. Dispense it only in the original container.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Halothane RS

Helium

He 4.00
Helium.
Helium [7440-59-7].

» Helium contains not less than 99.0 percent, by volume, of He.

Packaging and storage—Preserve in cylinders. [NOTE—Reduce the container pressure by means of a regulator. Measure the gases with a gas volume meter downstream from the detector tube in order to minimize contamination or change of the specimens.]
Identification—The flame of a burning splinter of wood is extinguished when inserted into an inverted test tube filled with Helium. [NOTE—Use caution.] A small balloon filled with Helium shows decided buoyancy.
Odor—Carefully open the container valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose: no appreciable odor is discernible.
Carbon monoxide—Pass 1000 ± 50 mL through a carbon monoxide detector tube (see under *Reagents* in the section *Reagents, Indicators, and Solutions*) at the rate specified for the

USP Monographs

www.webofpharma.com

tube: the indicator change corresponds to not more than 0.001%.

Air—Not more than 1.0% of air is present, determined as directed in the *Assay*.

Assay—Introduce a specimen of Helium into a gas chromatograph by means of a gas sampling valve. Select the operating conditions of the gas chromatograph such that the standard peak signal resulting from the following procedure corresponds to not less than 70% of the full-scale reading. Preferably, use an apparatus corresponding to the general type in which the column is 6 m in length and 4 mm in inside diameter and is packed with porous polymer beads, which permits complete separation of nitrogen and oxygen from Helium, although the nitrogen and oxygen may not be separated from each other. Use industrial grade helium (99.99%) as the carrier gas, with a thermal-conductivity detector, and control the column temperature: the peak response produced by the assay specimen exhibits a retention time corresponding to that produced by an air-helium certified standard (see under *Reagents* in the section *Reagents, Indicators, and Solutions*), and indicates not more than 1.0% of air when compared to the peak response of the air-helium certified standard, and not less than 99.0%, by volume, of He.

Heparin Lock Flush Solution

DEFINITION

Heparin Lock Flush Solution is a sterile preparation of Heparin Sodium Injection with sufficient Sodium Chloride to make it isotonic with blood. Heparin sodium used in the manufacture of Heparin Lock Flush Solution complies with the compendial requirements stated in the Heparin Sodium monograph. The potency of Heparin Lock Flush Solution is NLT 90.0% and NMT 120.0% of the potency stated on the label in terms of USP Heparin Units. It contains NMT 1.00% of sodium chloride (NaCl). It may contain a suitable preservative.

ASSAY

- **ANTI-FACTOR IIa POTENCY**

(See *Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated and Low Molecular Weight Heparins* ⟨208⟩, *Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated Heparin, Anti-Factor IIa Activity for Unfractionated Heparin*.)

Acceptance criteria: 90.0%–120.0%

- **SODIUM CHLORIDE**

Sample solution: Pipet 10 mL of Solution into a suitable container, dilute with water to about 150 mL, and add 1.5 mL of potassium chromate TS.

Analysis: Titrate with 0.1 N silver nitrate. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride (NaCl).

Acceptance criteria: NMT 1.00%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Units/mL
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **pH** ⟨791⟩: 5.0–7.5
- **OTHER REQUIREMENTS:** It meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose, prefilled syringes or containers; in multiple-dose containers, preferably of Type I glass; or in suitable plastic containers.

Change to read:

- **LABELING** ⟨7⟩, *Labels and Labeling for Injectable Products, ▲Quantity and Total Volume for Injectable Drug Products Packaged in Single- and Multiple-Dose Containers*▲ (CN 1-May-2019): ▲For injectable drug products greater than 1 mL, whether packaged in single- or multiple-dose containers, the quantity per total volume should be the primary and prominent expression on the principal display panel of the label, followed in close proximity by quantity per milliliter enclosed by parentheses. For containers that hold a volume of less than 1 mL, the quantity per fraction of a milliliter should be the only expression of strength. For containers that hold a volume equal to 1 mL, the strength should be expressed as quantity/mL, not quantity/1 mL.▲ (USP 1-May-2019) For further information, see the entirety of *Injections and Implanted Drug Products* ⟨1⟩. Label it to indicate the organ and species from which the heparin sodium is derived. The label also states that the Solution is intended for maintenance of patency of intravenous injection devices only, and that it is not to be used for anticoagulant therapy. The label also states that in the case of the Solution having a concentration of 10 USP Heparin Units/mL it may alter, and that in the case of higher concentrations it will alter, the results of the blood coagulation tests.

Anticoagulant Heparin Solution

DEFINITION

Anticoagulant Heparin Solution is a sterile solution of Heparin Sodium in Sodium Chloride Injection. Its potency is NLT 90.0% and NMT 110.0% of the potency stated on the label in terms of USP Heparin Units. It contains NLT 0.85% and NMT 0.95% of sodium chloride (NaCl). It may be buffered. It contains no antimicrobial agents.

Prepare Anticoagulant Heparin Solution as follows.

Heparin Sodium	75,000 Units
Sodium Chloride Injection, sufficient quantity to make	1000 mL

Add the Heparin Sodium, in solid form or in solution, to the Sodium Chloride Injection, mix, filter if necessary, and sterilize.

ASSAY

- **ANTI-FACTOR IIa POTENCY**

pH 8.4 buffer: Dissolve 6.10 g of tris(hydroxymeth yl) aminomethane, 10.20 g of sodium chloride, 2.80 g of edetate sodium, and, if suitable, between 0 and 10.00 g of polyethylene glycol 6000 and/or 2.00 g of bovine serum albumin in 800 mL of water. [NOTE—2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin.] Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 mL.

Antithrombin solution: Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution of 5 Antithrombin IU/mL. Dilute this solution with *pH 8.4 buffer* to obtain a solution having a concentration of 0.125 Antithrombin IU/mL.

Thrombin human solution: Reconstitute thrombin human (factor IIa) (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to give 20 Thrombin IU/mL, and

www.webofpharma.com

dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 5 Thrombin IU/mL. [NOTE—The thrombin should have a specific activity of NLT 750 IU/mg.]

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic thrombin substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a concentration of 1.25 mM.

Stopping solution: 20% (v/v) solution of acetic acid

Standard solutions: Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water, and dilute with *pH 8.4 buffer* to obtain at least four dilutions in the concentration range between 0.005 and 0.03 USP Heparin Unit/mL.

Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of Anticoagulant Heparin Solution similar to those obtained for the *Standard solutions*.

Analysis

[NOTE—The procedure can also be performed using alternative platforms.]

For each dilution of the *Standard solutions* and the *Sample solutions*, at least duplicate samples should be tested. Label a suitable number of tubes, depending on the number of replicates to be tested. For example, if five blanks are to be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, T3, and T4 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, B2, T1, T2, T3, T4, B3, T1, T2, T3, T4, B4, S1, S2, S3, S4, B5.] Note that after each addition of a reagent, the incubation mixture should be mixed without allowing bubbles to form. Add twice the volume (100–200 µL) of *Antithrombin solution* to each tube containing one volume (50–100 µL) of either the *pH 8.4 buffer* or an appropriate dilution of the *Standard solutions* or the *Sample solutions*. Mix, but do not allow bubbles to form. Incubate at 37° for at least 1 min. Add to each tube 25–50 µL of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100 µL of *Chromogenic substrate solution*. Please note that all reagents, *Standard solutions*, and *Sample solutions* should be prewarmed to 37° just before use. Two different types of measurements can be recorded:

1. Endpoint measurement: Stop the reaction after at least 1 min with 50–100 µL of *Stopping solution*. Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩). The RSD over the blank readings is less than 10%.
2. Kinetic measurement: Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩). Calculate the change in absorbance/min (ΔOD/min). The blanks for kinetic measurement are also expressed as ΔOD/min and should give the highest values because they are carried out in the absence of heparin. The RSD over the blank readings is less than 10%.

Calculations: The statistical models for *Slope ratio assay* or *Parallel-line assay* can be used, depending on which model best describes the correlation between concentration and response.

Parallel-line assay: For each series, calculate the regression of the absorbance or change in absorbance/min against log concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of Anticoagulant Heparin Solution in USP Units/mL using statistical methods for parallel-line assays.

Slope ratio assay: For each series, calculate the regression of the log absorbance or the log change in absorbance/min against concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of Anticoagulant Heparin Solution in USP Units/mL using statistical methods for slope ratio assays.

Acceptance criteria: 90.0%–110.0% of the potency stated on the label in terms of USP Heparin Units.

- **SODIUM CHLORIDE**

Sample solution: Solution and potassium chromate TS (5:1)

Analysis: Titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl.

SPECIFIC TESTS

- **pH** ⟨791⟩**:** Between 5.0 and 7.5
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 2.5 USP Endotoxin Units/mL.
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, of colorless, transparent Type I or Type II glass, or of a suitable plastic material (see ▲*Medical Devices—Bacterial Endotoxin and Pyrogen Tests* ⟨161⟩).▲ (CN 1-May-2019)
- **LABELING:** Label it in terms of USP Heparin Units, and to indicate the number of mL of Solution required per 100 mL of whole blood.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Heparin Sodium for Assays RS

Heparin Sodium

DEFINITION

Heparin Sodium is the sodium salt of sulfated glycosaminoglycans present as a mixture of heterogeneous molecules varying in molecular weights that retains a combination of activities against different factors of the blood clotting cascade. It is present in mammalian tissues and is usually obtained from the intestinal mucosa or other suitable tissues of domestic mammals used for food by humans. The sourcing of heparin material must be specified in compliance with applicable regulatory requirements. The manufacturing process should be validated to demonstrate clearance and inactivation of relevant infectious and adventitious agents (e.g., viruses, TSE agents). See *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* ⟨1050⟩ for general guidance on viral safety evaluation. The heparin manufacturing process should also be validated to demonstrate clearance of lipids. It is composed of polymers of alternating derivatives of α-D-glucosamido (*N*-sulfated, *O*-sulfated, or *N*-acetylated) and *O*-sulfated uronic acid (α-L-iduronic acid or β-D-glucuronic acid). The component activities of the mixture are in ratios corresponding to those shown by USP Heparin Sodium for Assays RS. Some of these components have the property of prolonging the clotting time of blood. This occurs mainly through the formation of a complex of each component with the plasma proteins antithrombin and heparin cofactor II to potentiate the inactivation of thrombin

www.webofpharma.com

(factor IIa). Other coagulation proteases in the clotting sequence, such as activated factor X (factor Xa), are also inhibited. The ratio of anti-factor Xa activity to anti-factor IIa potency is between 0.9 and 1.1. The potency of Heparin Sodium, calculated on the dried basis, is NLT 180 USP Heparin Units in each mg.

IDENTIFICATION

• **A. ^{1}H NMR SPECTRUM**

(See *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩.)

Standard solution: NLT 20 mg/mL of USP Heparin Sodium Identification RS in deuterium oxide with 0.002% (w/v) deuterated trimethylsilylpropionic (TSP) acid sodium salt

System suitability solution: Prepare 0.3% (w/w) USP Oversulfated Chondroitin Sulfate RS in the *Standard solution*.

Sample solution: NLT 20 mg/mL of Heparin Sodium in deuterium oxide with 0.002% (w/v) deuterated TSP. [NOTE—EDTA may be added to the *Sample solution* to NMT 12 µg/mL. In the event that EDTA is added to the *Sample solution*, spectra should be recorded and compared both with and without addition of EDTA.]

Instrumental conditions

(See *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩.)

Mode: NMR, pulsed (Fourier transform)

Frequency: NLT 500 MHz (for ^{1}H)

Temperature: 20°–30°

System suitability

Samples: *Standard solution* and *System suitability solution*

Using a pulsed (Fourier transform) NMR spectrometer operating at NLT 500 MHz for ^{1}H, acquire a free induction decay (FID) using NLT 16 scans using a 90° pulse, an acquisition time of NLT 2 s, and at least a 10-s delay. Record the ^{1}H NMR spectra of the *Standard solution* and the *System suitability solution* at a stable temperature between 20°–30°. Collect the ^{1}H NMR spectrum with a spectral window of at least 10 to −2 ppm and without spinning. The number of transients should be adjusted until the signal-to-noise ratio of the *N*-acetyl heparin signal in the *Standard solution* is at least 1000/1 in the region near 2 ppm. The *Standard solution* shall be run at least daily when *Sample solutions* are being run. For all samples, the TSP methyl signal should be set to 0.00 ppm. The chemical shift for the *N*-acetyl resonance of heparin and oversulfated chondroitin sulfate in the *System suitability solution* should be observed at 2.05 ± 0.02 and 2.16 ± 0.03 ppm, respectively. Record the ^{1}H NMR spectrum of the *Sample solution* at a stable temperature between 20°–30°. Draw a baseline from 8.00 ppm to 0.10 ppm. The ppm values for H1 of GlcNAc/GlcNS, 6S (signal 1), H1 of IdoA2S (signal 2), the H2 of GlcNS (signal 3), and the methyl of GlcNAc (signal 4) of heparin are present at 5.42, 5.21, 3.28 (doublet centered at 3.28 ppm), and 2.05 ppm, respectively.[1] The chemical shifts of these signals do not differ by more than ±0.03 ppm. Measure the signal heights above the baseline of signal 1 and signal 2, and calculate the mean of these signal heights. Other signals of variable heights and ppm values, attributable to heparin and HOD, may be seen between signal 2 and 4.55 ppm. Residual solvent signals may be observed in the 0.10–3.75 range. Heparin Sodium must meet the requirements stated in *Residual Solvents* ⟨467⟩.

Suitability requirements

Number of transients: Adjust until the signal-to-noise ratio of the *N*-acetyl heparin signal in the *Standard solution* is at least 1000/1 in the region near 2 ppm.

Chemical shift: The TSP methyl signal should be set to 0.00 ppm for all samples.

Chemical shifts (for the *N*-acetyl resonance of heparin and oversulfated chondroitin sulfate in the ***System suitability solution***): Should be observed at 2.05 ± 0.02 and 2.16 ± 0.03 ppm, respectively

Analysis

Sample: *Sample solution*

Acceptance criteria: No unidentified signals greater than 4% of the mean of the height of signals 1 and 2 are present in the following ranges: 0.10–2.00, 2.10–3.20, and 5.70–8.00 ppm. No signals greater than 200% of the mean of the height of signals 1 and 2 are present in the 3.75–4.55 ppm for porcine heparin.

• **B. CHROMATOGRAPHIC IDENTITY**

Solution A: Dissolve 0.8 g of monobasic sodium phosphate dihydrate in 2 L of water, and adjust with phosphoric acid to a pH of 3.0. Pass the solution through a membrane filter with a 0.45-µm pore size, and degas before use.

Solution B: Dissolve 0.8 g of monobasic sodium phosphate dihydrate and 280 g of sodium perchlorate monohydrate in 2 L of water, and adjust with phosphoric acid to a pH of 3.0. Pass the solution through a membrane filter with a 0.45-µm pore size, and degas before use.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
30	10	90
31	80	20
45	80	20

Standard solution: NLT 20 mg/mL of USP Heparin Sodium Identification RS in water

System suitability solution: Prepare 0.1% (w/w) USP Oversulfated Chondroitin Sulfate RS and 0.5% (w/w) USP Dermatan Sulfate RS in the *Standard solution*.

Sample solution: NLT 20 mg/mL of Heparin Sodium in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 202 nm

Column: 2-mm × 25-cm; packing L81[2]

Guard column: 2-mm × 5-cm; packing L61

Column temperature: Maintain columns at 40°

Flow rate: 0.22 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The retention times for dermatan sulfate, heparin, and oversulfated chondroitin sulfate are about 17, 22, and 30 min, respectively.]

Suitability requirements

Resolution: NLT 1.0 between the dermatan sulfate and heparin peaks, and NLT 1.5 between the heparin and oversulfated chondroitin sulfate peaks

[1] GlcNAc, *N*-acetylated glucosamine; GlcNS, *N*-sulfated glucosamine; S, sulfate; IdoA, iduronic acid; GlcN, glucosamine; GalN, galactosamine.

[2] L81—A hydroxide-selective, strong anion-exchange resin consisting of a highly cross-linked core of 9 µm porous particles having a pore size of 2000 Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene with a latex coating composed of 70 nm diameter microbeads (6% crosslinked) bonded with alkanol quaternary ammonium ions (A suitable column is Dionex IonPac AS11-HC available from www.thermofisher.com).

USP Monographs

www.webofpharma.com

Relative standard deviation: NMT 2% for the heparin peak area determined from three replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms, and measure the retention times for the major peaks.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.

• **C. Anti-Factor Xa to Anti-Factor IIa Ratio**

ANTI-FACTOR XA AND ANTI-FACTOR IIA ASSAYS FOR UNFRACTIONATED AND LOW MOLECULAR WEIGHT HEPARINS ⟨208⟩, *Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated Heparin*

Acceptance criteria: 0.9–1.1

• **D. Molecular Weight Determinations**

1 M ammonium acetate solution: Accurately weigh 77.1 g of ammonium acetate, and dissolve in 1 L of water.

1% sodium azide solution: Dissolve 1 g of sodium azide in 100 mL of water.

Mobile phase: Transfer 100 mL of *1 M ammonium acetate solution* to a 1-L volumetric flask, add 20 mL of *1% sodium azide solution*, and dilute with water to volume. Filter using a nylon membrane with a 0.2-µm pore size prior to use.

Calibration solution: Prepare by dissolving 10 mg of the USP Heparin Sodium Molecular Weight Calibrant RS in 2 mL of *Mobile phase*, and filter using a nylon membrane with a 0.2-µm pore size.

System suitability solution: 5 mg/mL of USP Heparin Sodium Identification RS in *Mobile phase*. Filter using a nylon membrane with a 0.2-µm pore size.

Sample solution: Dissolve about 10 mg of Heparin Sodium sample in 2 mL of *Mobile phase*, and filter using a nylon membrane with a 0.2-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

[NOTE—The temperature of the refractive index detector must be set at the same temperature as the *Column temperature*.]

Mode: LC

Detector: Refractive index

Columns: One 7.8-mm × 30-cm, 8-µm packing L59 in series with a 7.8-mm × 30-cm, 5-µm packing L59[3]

Guard column: 6-mm × 4-cm; 7-µm packing L59

Column temperature: 30°

Flow rate: 0.6 mL/min ± 0.1%

Column equilibration: 0.6 mL/min for 2 h

Injection volume: 20 µL

System suitability

Samples: *Calibration solution* and *System suitability solution* (duplicate injections)

Suitability requirements

Weight-average molecular weight (M_w): Take the mean of the calculated M_w from the duplicate injections of the *System suitability solution*, and round to the nearest 100 Da. The chromatographic system is suitable if the M_w of the *System suitability* sample is within 500 Da of the labeled value as stated in the USP Certificate for USP Heparin Sodium Identification RS.

Peak molecular weights (M_p): The peak molecular weights (M_p) of the duplicate injections of the *System suitability solution* do not differ by more than 5% of the upper value.

Resolution: There is baseline resolution between the heparin and salt peaks.

Calibration curve: The linear regression coefficient of the calibration curve fitted to the Broad Standard Table values must be NLT 0.990, using a third-order polynomial equation.

Analysis

Samples: Inject 20 µL of the *System suitability solution* (duplicate injections), *Sample solution* (duplicate injection), and *Calibration solution* (single injection), and record the chromatograms for a length of time to ensure complete elution, including salt and solvent peaks (about 50 min). [NOTE—The calibrant, standard, or sample of heparin will give a broad heparin peak between about 20 and 40 min, followed by a later eluting narrow salt peak, as illustrated in the USP Certificate for USP Heparin Sodium Molecular Weight Calibrant RS.]

Calculations: Calculate the total area under the heparin peak in the *Calibration solution* chromatogram, and the cumulative area at each point under the peak as a percent of the total. Do not include the salt peak. Using the Broad Standard Table provided in the USP Certificate for USP Heparin Sodium Molecular Weight Calibrant RS, identify those points in the chromatogram for which the percent cumulative area is closest to the percent fractions listed in the Table, and assign the molecular weight (*MW*) in the Table to the corresponding retention time (*RT*) in the chromatogram. For the set of retention times and molecular weights identified, fit log(*MW*) vs. *RT* to a third-order polynomial function using suitable gel permeation chromatography (GPC) software [or: find values of *a*, *b*, *c*, and *d* such that $\log(MW) = a + b(RT) + c(RT)^2 + d(RT)^3$].

Using the same GPC software, for each of the duplicate chromatograms of the *System suitability solution* and the *Sample solution*, with the calibration function derived as described above, calculate M_w according to the following formula:

$$M_w = \Sigma(RI_i M_i)/\Sigma RI_i$$

where the detector response at each point is defined as RI_i and the *MW* at each point as M_i. Round the mean value of M_w to the nearest 100 Da.

Using the same GPC software, determine for each of the duplicate *Sample solution* chromatograms: the percentage of heparin with molecular weight in the range 8,000–16,000, $M_{8000\text{–}16000}$, the percentage of heparin with molecular weight in the range 16,000–24,000, $M_{16000\text{–}24000}$, and the percentage of heparin with molecular weight greater than 24,000, M_{24000}. Round the mean percentage values to the nearest 1%.

Acceptance criteria: M_{24000} is NMT 20%, M_w is between 15,000 Da and 19,000 Da, and the ratio of $M_{8000\text{–}16000}$ to $M_{16000\text{–}24000}$ is NLT 1.0.

• **E.** A solution of Heparin Sodium imparts an intense yellow color to a nonluminous flame.

ASSAY

• **Anti-Factor IIa Potency**

ANTI-FACTOR XA AND ANTI-FACTOR IIA ASSAYS FOR UNFRACTIONATED AND LOW MOLECULAR WEIGHT HEPARINS ⟨208⟩, *Anti-Factor IIa Activity for Unfractionated Heparin*

Acceptance criteria: The potency of Heparin Sodium, calculated on the dried basis, is NLT 180 USP Heparin Units in each mg.

[3] The method was validated using a guard column TSK SWXL 6-mm × 4-cm, 7-µm in series with two analytical columns: TSK G4000 SWXL 7.8- × 30-cm, 8-µm in series with a TSK G3000 SWXL 7.8- × 30-cm, 5-µm diameter.

USP Monographs

www.webofpharma.com

OTHER COMPONENTS

- **NITROGEN DETERMINATION** ⟨461⟩, *Method I*: 1.3%–2.5%, calculated on the dried basis, using the procedure for *Nitrates and Nitrites Absent*

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: 28.0%–41.0%
- **LIMIT OF GALACTOSAMINE IN TOTAL HEXOSAMINE** (a measure of dermatan sulfate and other galactosamine containing impurities)

Mobile phase: 14 mM potassium hydroxide

Glucosamine standard solution: 1.6 mg/mL of USP Glucosamine Hydrochloride RS in 5 N hydrochloric acid

Galactosamine standard solution: 16 µg/mL of USP Galactosamine Hydrochloride RS in 5 N hydrochloric acid

Standard solution: Mix equal volumes of the *Glucosamine standard solution* and the *Galactosamine standard solution*.

Hydrolyzed standard solution: Transfer 5 mL of the *Standard solution* to a 7-mL screw-cap test tube, cap, and heat for 6 h at 100°. Cool to room temperature, and dilute with water (1 in 100).

Sample solution: Transfer 12 mg of Heparin Sodium to a 7-mL screw-cap test tube, dissolve in 5 mL of 5 N hydrochloric acid, and cap.

Hydrolyzed sample solution: Heat the *Sample solution* for 6 h at 100°. Cool to room temperature, and dilute with water (1 in 100).

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: HPIC

Detector: Pulsed amperometric detector, set to the following waveform. See *Table 2*.

Table 2

Step	Time (s)	Potential (V)	Integration
1	0.00	+0.1	—
2	0.20	+0.1	Begins
3	0.40	+0.1	Ends
4	0.41	−2.0	—
5	0.42	−2.0	—
6	0.43	+0.6	—
7	0.44	−0.1	—
8	0.50	−0.1	—

Column: 3-mm × 3-cm amino acid trap column in series with a 3-mm × 3-cm guard column and a 3-mm × 15-cm column that contains packing L69

Column temperature: 30°

Flow rate: 0.5 mL/min

Pre-equilibration: At least 60 min with *Mobile phase*

Injection volume: 10 µL

Elution: 10 min with *Mobile phase*

Column cleaning: At least 10 min with 100 mM potassium hydroxide

Equilibration: At least 10 min with *Mobile phase* before each injection

System suitability

Sample: *Hydrolyzed standard solution*

Suitability requirements

Resolution: NLT 2 between the galactosamine and glucosamine peaks

Column efficiency: NLT 2000 theoretical plates for glucosamine

Tailing factor: Between 0.8 and 2.0 for the galactosamine and glucosamine peaks

Analysis

Samples: *Hydrolyzed standard solution* and *Hydrolyzed sample solution*

Record the chromatograms, and measure the responses for the peaks at the retention time of galactosamine and glucosamine.

Calculate the response ratio of galactosamine to glucosamine ($GalN_R$) in the *Hydrolyzed standard solution*:

$$\text{Result} = (GalN_B/GalN_W) \times (GlcN_W/GlcN_B)$$

$GalN_B$ = peak area of galactosamine from the *Hydrolyzed standard solution*

$GalN_W$ = weight of galactosamine for the *Standard solution*

$GlcN_W$ = weight of glucosamine for the *Standard solution*

$GlcN_B$ = peak area of glucosamine from the *Hydrolyzed standard solution*

Calculate the percentage of galactosamine in the portion of total hexosamine taken:

$$\text{Result} = \{[(GalN_U/GalN_R)]/[(GalN_U/GalN_R) + GlcN_U]\} \times 100$$

$GalN_U$ = peak area of galactosamine from the *Hydrolyzed sample solution*

$GalN_R$ = response ratio of galactosamine

$GlcN_U$ = peak area of glucosamine from the *Hydrolyzed sample solution*

Acceptance criteria: The percent galactosamine peak area of the total hexosamine of the *Hydrolyzed sample solution* must be NMT 1%.

- **NUCLEOTIDIC IMPURITIES**

Solution A: Dissolve 3.08 g of ammonium acetate in 2 L of water, and adjust with glacial acetic acid to a pH of 4.4 ± 0.2. Degas for 2 min under vacuum with sonication before use.

Solution B: 100% acetonitrile. Degas for 1 min under vacuum with sonication before use.

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	98	2
5.00	98	2
15.00	80	20
20.00	80	20
20.10	98	2
25.00	98	2

Nucleoside identification solution: Accurately weigh and transfer about 25 mg each of uridine, guanosine, cytidine, thymidine, 2′-deoxyadenosine, 2′-deoxyguanosine, 2′-deoxycytidine, and 5-methyl-2′-deoxycytidine into a 200-mL volumetric flask, add approximately 185 mL of water, and dissolve with sonication and vortexing, if necessary. Dilute with water to volume, and mix. Transfer 2.0 mL of this solution into a 100-mL volumetric flask, dilute with water to volume, and mix.

Adenosine stock solution: Accurately weigh and transfer 25 mg of USP Adenosine RS into a 100-mL volumetric flask, add approximately 85 mL of water, and dissolve with

www.webofpharma.com

sonication and vortexing, if necessary. Dilute with water to volume, and mix.

Standard solution: Transfer 2.0 mL of the *Adenosine stock solution* into a 200-mL volumetric flask, dilute with water, and mix.

System suitability solution: Transfer 2.0 mL of the *Standard solution* into a 100-mL volumetric flask, dilute with water to volume, and mix.

Reaction buffer: Accurately weigh and transfer 0.41 g of magnesium chloride hexahydrate, 0.24 g of tris (hydroxymethyl)amino methane, and 0.58 g of sodium chloride into a 100-mL volumetric flask, dissolve in 75 mL of water, and mix. Adjust with 1 N hydrochloric acid to a pH of 7.9 ± 0.1. Dilute with water to volume, and mix.

PDE I diluent: Transfer 5.0 mL of glycerol and 5.0 mL of the *Reaction buffer* into a 20-mL flask, and vortex to mix.

PDE I solution: 0.1 unit/µL of phosphodiesterase I (PDE I) in *PDE I diluent*. Store at –20°.

Enzyme digest solution: Add 10 µL of Benzonase,[4] 222 Units of alkaline phosphase (AP), and 125 µL of *PDE I solution* to 5.0 mL of *Reaction buffer*. Store at –20°.

Blank: Transfer 100 µL of water and 100 µL of *Enzyme digest solution* into a 250-µL HPLC vial, and mix with a micropipette. Incubate NLT 60 min in the autosampler at 37° before injection.

Sample solution: Accurately weigh and transfer 400 mg of Heparin Sodium into a 20-mL volumetric flask, dilute with water to volume, and mix. Transfer 100 µL of this solution and 100 µL of *Enzyme digest solution* into a 250-µL HPLC vial, and mix. Incubate NLT 60 min in the autosampler at 37° before injection.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Column: 4.6-mm × 15-cm; 4-µm packing L1

Detector: UV 260 nm

Autosampler temperature: 37 ± 1°

Column temperature: 20 ± 3°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 25 min

System suitability

Samples: *Nucleoside identification solution*, *Standard solution*, and *System suitability solution*

Suitability requirements

Resolution: The resolution between the 2′-deoxycytidine peak and the uridine peak is NLT 1.3 for the injection of the *Nucleoside identification solution*.

Relative standard deviation: Inject six replicates of the *Standard solution*, and record the chromatograms. The percent relative standard deviation (%RSD) of the areas of the adenosine peak is NMT 10%.

Signal-to-noise ratio: The *S/N* of the adenosine peak in the *System suitability solution* is NLT 10.

Analysis

Samples: Water, *Blank*, *Nucleoside identification solution*, *Standard solution*, *System suitability solution*, and *Sample solution*

Record the chromatograms.

Calculate the area reject value, *Q*:

$$Q = (10 \times A_{sss})/(S/N)$$

A_{sss} = peak area of adenosine in the *System suitability solution*

S/N = signal-to-noise ratio of the adenosine peak in the *System suitability solution*

For the *Standard solution*, calculate the concentration of adenosine, in mg/mL:

$$C_S = W_S/DF$$

C_S = concentration of adenosine in the *Standard solution* (mg/mL)

W_S = weight of USP Adenosine RS (mg)

DF = 10,000 (dilution factor)

Calculate the percentage of nucleotidic impurities:

$$\text{Result} = \Sigma[(C_S/A_S) \times A_I \times (MW_{ratio}/RRF_i)] \times (DF/W_{sample}) \times 100$$

C_S = concentration of adenosine in the *Standard solution* (mg/mL)

A_S = average peak area ($n = 6$) of adenosine in the *Standard solution*

A_I = peak area of each impurity above *Q* in the *Sample solution*

MW_{ratio} = see *Table 4*

RRF_i = relative response factor for the corresponding peak (see *Table 4*)

DF = dilution factor, 40

W_{sample} = sample weight of Heparin Sodium (mg)

Table 4

Name	Relative Retention Time	Relative Response Factor	MW_{ratio}
Cytidine	0.28	0.53	1.2548
2′-Deoxycytidine	0.38	0.56	1.2727
Uridine	0.40	0.75	1.2537
5-Methyl-2′-deoxycytidine	0.66	0.25	1.2569
Guanosine	0.81	0.74	1.2188
2′-Deoxyguanosine	0.89	0.83	1.2319
Thymidine	0.92	0.68	1.2558
Adenosine	1.00	1.00	1.2319
2′-Deoxyadenosine	1.04	1.09	1.2466
Others	—	1.00	1.0000

Acceptance criteria: NMT 0.1% (w/w) is found.

• **ABSENCE OF OVERSULFATED CHONDROITIN SULFATE**

A. Proceed as directed in *Identification A*. No features associated with oversulfated chondroitin sulfate are found between 2.12 and 3.00 ppm.

B. Proceed as directed in *Identification B*. No peaks corresponding to oversulfated chondroitin sulfate should be detected eluting after the heparin peak.

• **PROTEIN IMPURITIES**

[NOTE—Treatment for interfering substances is only required for samples previously tested with a protein content greater than 0.1%. *Spiked sample* should be prepared and assayed only if the treatment for interfering substances is performed.]

Standard stock solution: 2.0 mg/mL of bovine serum albumin in water

Standard solutions: Dilute portions of the *Standard stock solution* with water to obtain NLT 5 standard solutions having concentrations between 0.010 and 0.050 mg/mL of bovine serum albumin, the concentrations being evenly spaced.

[4] A suitable ultrapure *Serratia marcescens* nuclease (EC 3.1.30.2) must be ≥99% containing ≥25 units/µL.

www.webofpharma.com

System suitability standard: Dilute a portion of the *Standard stock solution* with water to obtain a solution containing 0.030 mg/mL of bovine serum albumin.
Sample solution: 30 mg/mL of Heparin Sodium in water. Prepare in triplicate.
Spiked sample: Using an appropriate dilution scheme and the *Standard stock solution*, prepare a *Spiked sample* containing 30 mg/mL Heparin Sodium and 0.030 mg/mL bovine serum albumin in water.
Blank: Water
Lowry reagent A: Prepare a solution of 10 g/L of sodium hydroxide in water and a solution of 50 g/L of sodium carbonate in water. Mix equal volumes (2V:2V) of each solution, and dilute with water to 5V.
Lowry reagent B: Prepare a solution of 29.8 g/L of disodium tartrate dihydrate in water. Prepare a solution of 12.5 g/L of cupric sulfate in water. Mix equal volumes of both solutions (2V:2V), and dilute with water to 5V.
Lowry reagent C: Mix 50 volumes of *Lowry reagent A* with 1 volume of *Lowry reagent B*. Prepare fresh daily.
Diluted Folin–Ciocalteu's phenol reagent: Dilute Folin–Ciocalteu's phenol reagent 1–2 times with water. The dilution should be chosen such that the pH of the samples (i.e., *Standard solution* and *Sample solution* after addition of *Lowry reagent C* and the *Diluted Folin–Ciocalteu's phenol reagent*) is 10.3 ± 0.3.
Sodium deoxycholate reagent: Prepare a solution of sodium deoxycholate in water having a concentration of 150 mg in 100 mL.
Trichloroacetic acid reagent: Prepare a solution of trichloroacetic acid in water having a concentration of 72 g in 100 mL.
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
To 1 mL each of *Standard solution*, *Sample solution*, and *Blank*, add 5 mL of *Lowry reagent C*. Mix. Allow to stand at room temperature for 10 min. Add 0.5 mL of *Diluted Folin–Ciocalteu's phenol reagent* to each solution, mix immediately, and allow to stand at room temperature for NLT 30 min. Determine the absorbance at the wavelength of maximum absorbance at 750 nm with a suitable spectrophotometer, using the solution from the *Blank* to set the instrument to zero and ensuring that all samples and standards absorbances are measured after the same final incubation time. To remove interfering substances, add 0.1 mL of *Sodium deoxycholate reagent* to 1 mL of a solution of the protein under test. Mix on a vortex mixer, and allow to stand at room temperature for 10 min. Add 0.1 mL of *Trichloroacetic acid reagent*, and mix on a vortex mixer. Centrifuge at a speed that ensures removal of visible particulate matter. [NOTE—NLT 14,100 RCF should be used. Appropriate centrifuge speed should be determined by each laboratory.] The supernatant should be essentially free of visible particulates. A pellet may not be visible. If the interfering substances method is used, dissolve the protein residue in 1 mL of *Lowry reagent C*.
Calculations: Using the linear regression method, plot the absorbances of the solutions from the *Standard solutions* versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the absorbance of the *Sample solution*, determine the concentration of protein in the *Sample solution*.
System suitability: The correlation coefficient (*r*) for a linear fit of all standards is NLT 0.99. The percent RSD between triplicate sample results is NMT 10%. If the sample absorbances are lower than the standard curve, the percent RSD specification is not required. The percent recovery of the *System suitability standard* is 90%–110%. If the interfering substances treatment is performed, the percent recovery of the *Spiked sample* is 85%–115%.
Acceptance criteria: NMT 0.1% (w/w) is found.

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 0.03 USP Endotoxin Units/USP Heparin Unit.
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry a sample in a vacuum at 60° for 3 h.
Acceptance criteria: It loses NMT 5.0% of its weight.
- **pH** ⟨791⟩**:** 5.0–7.5 in a solution (1 in 100)
- **STERILITY TESTS** ⟨71⟩**:** Where it is labeled as sterile, it meets the requirements.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store below 40°, preferably at room temperature.
- **LABELING:** Label it to indicate the tissue and the animal species from which it is derived.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Adenosine RS
USP Oversulfated Chondroitin Sulfate RS
USP Dermatan Sulfate RS
USP Galactosamine Hydrochloride RS
USP Glucosamine Hydrochloride RS
USP Heparin Sodium Identification RS
USP Heparin Sodium Molecular Weight Calibrant RS

Heparin Sodium Injection

DEFINITION
Heparin Sodium Injection is a sterile solution of Heparin Sodium in Water for Injection. Heparin sodium used in the manufacture of Heparin Sodium Injection complies with the compendial requirements stated in the Heparin Sodium monograph. Heparin Sodium Injection exhibits NLT 90.0% and NMT 110.0% of the potency stated on the label in terms of USP Heparin Units per milliliter.

ASSAY
- **ANTI-FACTOR IIa POTENCY**
(See *Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated and Low Molecular Weight Heparins* ⟨208⟩, *Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated Heparin*, *Anti-Factor IIa Activity for Unfractionated Heparin*.)
Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.03 USP Endotoxin Units/USP Heparin Unit
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **pH** ⟨791⟩**:** 5.0–7.5
- **OTHER REQUIREMENTS:** Meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and store between 20° and 25° at controlled room temperature.

Change to read:

- **LABELING** ⟨7⟩, *Labels and Labeling for Injectable Products*, ▲*Quantity and Total Volume for Injectable Drug Products Packaged in Single- and Multiple-Dose Containers*▲ (CN 1-May-2019)**:** ▲For injectable drug products

www.webofpharma.com

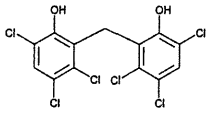
greater than 1 mL, whether packaged in single- or multiple-dose containers, the quantity per total volume should be the primary and prominent expression on the principal display panel of the label, followed in close proximity by quantity per milliliter enclosed by parentheses. For containers that hold a volume of less than 1 mL, the quantity per fraction of a milliliter should be the only expression of strength. For containers that hold a volume equal to 1 mL, the strength should be expressed as quantity/mL, not quantity/1 mL.▲ (USP 1-May-2019) For further information, see the entirety of *Injections and Implanted Drug Products* ⟨1⟩. Label it to indicate the tissue and the animal species from which it is derived.

Hepatitis B Immune Globulin

» Hepatitis B Immune Globulin conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile, nonpyrogenic solution free from turbidity, consisting of globulins derived from the blood plasma of human donors who have high titers of antibodies against hepatitis B surface antigen. It contains not less than 10.0 g and not more than 18.0 g of protein per 100 mL, of which not less than 80 percent is monomeric immunoglobulin G, having no ultracentrifugally detectable fragments, nor aggregates having a sedimentation coefficient greater than 12S. It contains 0.3 M glycine as a stabilizing agent, and it contains a suitable preservative. It has a potency per mL not less than that of the U.S. Reference Hepatitis B Immune Globulin tested by an approved radioimmunoassay for the detection and measurement of antibody to hepatitis B surface antigen. It has a pH between 6.4 and 7.2, measured in a solution diluted to contain 1 percent of protein with 0.15 M sodium chloride. It meets the requirements of the test for heat stability.

Packaging and storage—Preserve at a temperature between 2° and 8°.
Expiration date—Its minimum expiration date is not later than 1 year after the date of manufacture, such date being that of the first valid potency test of the product.
Labeling—Label it to state that it is not for intravenous injection.

Hexachlorophene



$C_{13}H_6Cl_6O_2$ 406.90
Phenol, 2,2′-methylenebis[3,4,6-trichloro]-;
2,2′-Methylenebis[3,4,6-trichlorophenol] [70-30-4].

DEFINITION
Hexachlorophene contains NLT 98.0% and NMT 100.5% of hexachlorophene ($C_{13}H_6Cl_6O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.**

Sample solution: 5 mg of Hexachlorophene in 5 mL of alcohol
Analysis: To the *Sample solution* add 1 drop of ferric chloride TS.
Acceptance criteria: A transient purple color is produced immediately.

ASSAY
- **PROCEDURE**

Sample: 1.5 g of Hexachlorophene
Analysis: Dissolve the *Sample* in 25 mL of alcohol, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 40.69 mg of hexachlorophene ($C_{13}H_6Cl_6O_2$).
Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN**

[**CAUTION**—Because 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is an extremely toxic substance, exercise all necessary precautions in the conduct of this procedure.]
Standard solution: 0.01 µg/mL of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin[1]
Sample solution: Dissolve 10.0 g of Hexachlorophene in 50 mL of methanol, transfer to a 1-L separator with the aid of 25 mL of methanol, add 25 mL of 2.5 N lithium hydroxide and 225 mL of water, and extract with two 200-mL portions of freshly distilled *n*-hexane. Dry the combined *n*-hexane extracts over anhydrous sodium sulfate, filter, and evaporate on a rotary evaporator at a bath temperature not exceeding 40° to a volume of 15 mL. Transfer this solution in portions to a 12-mL centrifuge tube, concentrating each time in a gentle stream of nitrogen in a warm water bath to a volume of 1 mL. Rinse the flask with 15 mL of *n*-hexane, and evaporate similarly. Wash down the walls of the tube with 10 mL of *n*-hexane, and again evaporate to a volume of 1.0 mL.

Cool, and transfer to a microcolumn that has been prepared in the following manner. Place a small plug of glass wool in a 5-mm × 15-mm pipet, add a small quantity of sand and 1.0 g of basic alumina, tap several times to pack down the alumina, and heat in a vacuum oven at 110° for 3 h. Store under vacuum.

Elute the column with 10 mL of a mixture of *n*-hexane and methylene chloride (9:1), using a portion to rinse the tube. Collect the eluate in a 12-mL graduated centrifuge tube, and concentrate in a gentle stream of nitrogen in a warm water bath to a volume of 1.0 mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC-MS (see *Mass Spectrometry* ⟨736⟩)

[1] A solution in anisole is available commercially from KOR Isotopes, Div. of ECO, Inc., 56 Rogers St., Cambridge, MA 02142. This solution may be diluted with a mixture of *n*-hexane and methylene chloride (9:1) to the required concentration.

www.webofpharma.com

Detector: Multiple-ion
Column: 2-mm × 1-m glass; liquid phase G1 on support S1
Temperatures
Column: 250°
Injection port: 300°
Carrier gas: Helium
Flow rate: 40 mL/min
Injection volume: 2.0 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: NMT 1 ppb; the sum of the peak heights at mass values of 320, 322, and 324 of the *Sample solution* is not greater than the sum of the peak heights at the same mass values of the *Standard solution.*

SPECIFIC TESTS
- **MELTING RANGE OR TEMPERATURE** ⟨741⟩**:** 161°–167°
- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry a sample at 105° for 4 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hexachlorophene RS

Hexachlorophene Cleansing Emulsion

DEFINITION
Hexachlorophene Cleansing Emulsion is Hexachlorophene in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hexachlorophene ($C_{13}H_6Cl_6O_2$). It contains no coloring agents.

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Chloroform and methanol (1:1)
Standard solution: 6 mg/mL of USP Hexachlorophene RS in *Diluent*
Sample solution: Place a volume of Cleansing Emulsion, equivalent to 150 mg of hexachlorophene, in a glass-stoppered, 25-mL graduated cylinder. Dilute with *Diluent* to volume, and allow to stand for 5 min.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography.*)
Adsorbent: 0.25-mm layer of silica gel
Application volume: 10 µL
Developing solvent system: Toluene and glacial acetic acid (9:1)
Spray reagent: Dilute nitric acid (1 in 5)
Analysis: Develop in the *Developing solvent system* until the solvent front has moved 10 cm above the point of application. Remove the plate, mark the solvent front, and evaporate the solvent in a current of warm air. Spray the plate with *Spray reagent,* and warm on a hot plate until yellow spots appear.
Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution.*

ASSAY
- **PROCEDURE**

Standard stock solution: 1 mg/mL of USP Hexachlorophene RS in methanol
Standard solution: 0.03 mg/mL of USP Hexachlorophene RS prepared as follows. Pipet 3 mL of *Standard stock solution* into a 100-mL volumetric flask. Add 1 mL of dilute hydrochloric acid (1 in 10), and add methanol to volume.
Sample stock solution: Nominally 0.3 mg/mL of hexachlorophene in methanol. Filter the solution through paper, taking adequate precautions to prevent evaporation.
Sample solution: 0.03 mg/mL of hexachlorophene prepared as follows. Pipet a 10-mL aliquot of the *Sample stock solution* into a 100-mL volumetric flask. Add 1 mL of dilute hydrochloric acid (1 in 10), and add methanol to volume.
Instrumental conditions
Mode: UV
Analytical wavelength: UV 299 nm
Cell: 1 cm
Blank: Methanol and hydrochloric acid (99:1)
Analysis: Concomitantly determine the absorbances of the *Sample solution* and the *Standard solution.*
Calculate the percentage of the labeled amount of hexachlorophene ($C_{13}H_6Cl_6O_2$) in the portion of Cleansing Emulsion taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
- **pH** ⟨791⟩

Analysis: Place 20 mL of well-shaken Cleansing Emulsion and 10 mL of water in a glass-stoppered, 50-mL graduated cylinder, and determine the pH in a suitable pH meter, using a glass electrode and preferably a sleeve-type calomel electrode.
Acceptance criteria: 5.0–6.0

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant, nonmetallic containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hexachlorophene RS

Hexachlorophene Liquid Soap

DEFINITION
Hexachlorophene Liquid Soap is a solution of Hexachlorophene in a 10.0%–13.0% solution of a potassium soap. It contains, in each 100 g, NLT 225 mg and NMT 260 mg of hexachlorophene ($C_{13}H_6Cl_6O_2$). It may contain suitable water hardness controls.
[NOTE—The inclusion of nonionic detergents in Hexachlorophene Liquid Soap in amounts greater than 8% on a total weight basis may decrease the bacteriostatic activity of the Liquid Soap.]

IDENTIFICATION
- **A.**

Sample: 2 g

www.webofpharma.com

Analysis: Pour the *Sample* into a beaker, and add, with stirring, dilute hydrochloric acid (1 in 100) until the mixture is just acid to litmus. To 10 mL of the mixture, in a beaker, add 10 mL of chloroform. Add 3 or 4 drops of ferric chloride TS, and allow to stand.
Acceptance criteria: The chloroform layer becomes purple.

• **B.**
Sample: 2 mL of the mixture prepared in *Identification* test *A*, in a test tube
Analysis: Add 2 mL of acetone to the *Sample*. Add 1 mL of titanium trichloride solution (1 in 5), and shake vigorously.
Acceptance criteria: A yellow oil separates.

ASSAY

• **PROCEDURE**
Alkaline buffer: Dissolve 6.07 g of tris(hydroxymethyl) aminomethane in 900 mL of methanol. Add 25.0 mL of dilute hydrochloric acid (1 in 10), and dilute with water to 1 L.
Solution A: Nominally 0.25 mg/mL of hexachlorophene prepared as follows. Transfer a portion of Liquid Soap containing the equivalent of about 100 mg of hexachlorophene to a 100-mL volumetric flask, and add alcohol to volume. Transfer 25.0 mL of this solution to a 100-mL volumetric flask, add 90% methanol to volume, and filter if necessary.
Control: Add 10.0 mL of *Solution A* to a 50-mL volumetric flask, and fill to volume with 0.3 M acetic acid in 90% methanol containing 0.1% of hydrochloric acid.
Standard solution: Transfer 50 mg of USP Hexachlorophene RS to a 100-mL volumetric flask, dissolve in 10 mL of alcohol, and dilute with *Alkaline buffer* to volume. Preserve in a tight container.
Standard hexachlorophene solutions: To 50-mL volumetric flasks add, by pipet and in duplicate, 2-, 3-, 4-, 5-, 6-, and 7-mL portions of the *Standard solution*. To one flask of each pair of duplicates, add to volume acidified 90% methanol containing, in each 100 mL, 5 mL of acetic acid and 0.3 mL of hydrochloric acid. To the second flask of each pair, add *Alkaline buffer* to volume. Arrange the two series of standard hexachlorophene solutions in pairs according to their hexachlorophene content.
Sample solution: Add 10.0 mL of *Solution A* to a 50-mL volumetric flask, and fill with *Alkaline buffer* to volume.
Blank: Corresponding acid solution from *Standard hexachlorophene solutions*
Instrumental conditions
Mode: UV
Analytical wavelength: 312 nm
Analysis
Samples: *Control*, *Standard hexachlorophene solutions*, *Sample solution*, and *Blank*
Determine the absorbance of the *Standard hexachlorophene solutions*, using the corresponding concentration of acid solution as the *Blank*. Plot the observed absorbance scale against the corresponding concentration of hexachlorophene, in mg/100 mL, on the abscissa scale.
Determine the absorbance of the *Sample solution*, using the *Control* as the blank. From the observed absorbance, calculate the weight of hexachlorophene in the Liquid Soap taken.
Acceptance criteria: 225–260 mg of hexachlorophene in each 100 g

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

• **WATER DETERMINATION,** *Method II* ⟨921⟩
Sample: 5 g
Analysis: Place the *Sample*, quickly weighed to the nearest centigram, in the distilling flask of the apparatus described in the chapter. The Liquid Soap is most conveniently weighed in a boat of metal foil, of a size that will just pass through the neck of the flask. Place 250 mL of toluene and 10 g of anhydrous barium chloride in the flask, connect the flask through a ground-glass joint to the distilling apparatus, fill the receiving tube with toluene, and determine the water as directed, beginning with "Heat the flask gently".
Acceptance criteria: The volume of water found corresponds to between 86.5% and 90.0% by weight of the portion of Liquid Soap taken.

• **ALCOHOL-INSOLUBLE SUBSTANCES**
Sample: 5 g
Analysis: Place the *Sample*, quickly weighed, in 100 mL of hot neutralized alcohol. Dissolve and collect the residue, if any, on a tared filter, thoroughly wash it with hot neutralized alcohol, and dry at 105° for 1 h. Retain this solution and the residue for use in the tests for *Free Alkali Hydroxides* and *Alkali Carbonates*.
Acceptance criteria: The weight of the residue obtained does not exceed 3.0% of the weight of Liquid Soap taken.

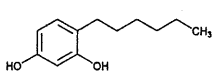
• **FREE ALKALI HYDROXIDES**
Sample solution: To the combined filtrate and washings obtained in the test for *Alcohol-Insoluble Substances* add 0.5 mL of phenolphthalein TS.
Analysis: If a pink color is produced, titrate the solution with 0.1 N sulfuric acid VS until the pink color is just discharged. Each mL of 0.1 N sulfuric acid is equivalent to 5.61 mg of potassium hydroxide (KOH).
Acceptance criteria: The volume of 0.1 N sulfuric acid consumed corresponds to NMT 0.05% of potassium hydroxide (KOH).

• **ALKALI CARBONATES**
Sample solution: Wash the filter containing the alcohol-insoluble substances from the *Alcohol-Insoluble Substances* test with 50 mL of boiling water, cool, and add methyl orange TS.
Analysis: Titrate the filtrate with 0.1 N sulfuric acid VS.
Acceptance criteria: NMT 0.5 mL of 0.1 N sulfuric acid per g of Liquid Soap originally taken is required [0.35% calculated as potassium carbonate (K_2CO_3)].

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **LABELING:** Solutions of higher concentrations of hexachlorophene and potassium soap, in which the ratios of these components are consistent with the official limits, may be labeled "For the preparation of Hexachlorophene Liquid Soap, USP", provided that the label indicates that the Liquid Soap is a concentrate and that directions are given for dilution to the official strength.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hexachlorophene RS

Hexylresorcinol



$C_{12}H_{18}O_2$ 194.27
1,3-Benzenediol, 4-hexyl-;
4-Hexylresorcinol [136-77-6].

www.webofpharma.com

DEFINITION

Hexylresorcinol, dried over silica gel for 4 h, contains NLT 98.0% and NMT 100.5% of hexylresorcinol ($C_{12}H_{18}O_2$).

[**CAUTION**—Hexylresorcinol is irritating to the oral mucosa and respiratory tract and to the skin, and its solution in alcohol has vesicant properties.]

IDENTIFICATION

• **A.**

Sample solution: 1 mL of a saturated solution

Analysis: To the *Sample solution*, add 1 mL of nitric acid.

Acceptance criteria: A light red color appears.

• **B.**

Sample solution: 1 mL of a saturated solution

Analysis: To the *Sample solution*, add 1 mL of bromine TS.

Acceptance criteria: A yellow, flocculent precipitate is formed. Add 2 mL of 6 N ammonium hydroxide. The precipitate dissolves, producing a yellow solution.

ASSAY

• **PROCEDURE**

Sample solution: Dissolve 70–100 mg of Hexylresorcinol, previously dried over silica gel for 4 h and weighed, in 10 mL of methanol in a 250-mL iodine flask. Add 30.0 mL of *Titrant*, then add quickly 5 mL of hydrochloric acid, and insert the stopper in the flask immediately. Cool the flask under running water to room temperature, shake vigorously for 5 min, then set aside for 5 min. Add 6 mL of potassium iodide TS around the stopper, cautiously loosen the stopper, again insert the stopper tightly, and swirl gently. Add 1 mL of chloroform.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Residual titration

Titrant: 0.1 N bromine VS

Back-titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Titrate the liberated iodine with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination.

Calculate the percentage of hexylresorcinol ($C_{12}H_{18}O_2$) in the sample taken. Each mL of *Titrant* is equivalent to 4.857 mg of hexylresorcinol ($C_{12}H_{18}O_2$).

Acceptance criteria: 98.0%–100.5%, dried over silica gel for 4 h

IMPURITIES

• **MERCURY**

Select all reagents for this test to have as low a content of mercury as practicable, and store all reagent solutions in containers of borosilicate glass. Glassware used in this test shall be specially cleaned by being soaked in warm 8 N nitric acid for 30 min and rinsed with water. Keep flasks for this determination separate from other flasks, and use only for mercury determinations.

Standard solution: Transfer 34.0 mg of mercuric chloride to a 250-mL volumetric flask. Add 1 drop of hydrochloric acid, add water to dissolve, and dilute with water to volume. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, add 1 drop of hydrochloric acid, and dilute with water to volume. Transfer 1.0 mL of this solution to a 500-mL volumetric flask, add 1 drop of hydrochloric acid, and dilute with water to volume.

Sample solution: Transfer 134 mg to a 250-mL beaker, and cautiously add 10 mL of 11 N nitric acid and 10 mL of 18 N sulfuric acid. Digest, with the aid of heat, in a well-ventilated hood until the evolution of brown fumes ceases. Cautiously add an additional 10 mL of 11 N nitric acid, and continue heating until no more fumes are evolved. Cool, transfer to a 200-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 253.65 nm

Lamp: Mercury hollow-cathode (and an absorption cell that permits the flameless detection of mercury)

Analysis: Transfer 100 mL of *Standard solution* to a 300-mL mercury analysis reaction vessel, and add 2 drops of potassium permanganate solution (1 in 20) (the solution should be purple; add additional permanganate solution dropwise, if necessary). Add 5 mL of 11 N nitric acid, stir, and allow to stand for NLT 15 s. Add 5 mL of 18 N sulfuric acid, stir, and allow to stand for NLT 45 s. Add 5 mL of hydroxylamine hydrochloride solution (3 in 200), stir, and allow to stand until the solution turns light yellow or colorless. Add 5 mL of stannous chloride solution (1 in 10). Disregard the presence of insoluble matter in this solution. Mix before use. Immediately insert the aerator connected to the air pump. Connect in a closed system, with a circulating air pump, a calcium chloride drying tube and an aerator inserted in a 300-mL reaction vessel so that air passed through the treated solution contained in the reaction vessel evaporates any metallic mercury present. In a similar manner, treat 100 mL of the *Sample solution* and 100 mL of water (reagent blank), and determine the maximum absorbances at the same wavelength. Check the zero setting of the instrument frequently.

Acceptance criteria: 3 ppm; the absorbance of the solution from the *Sample solution* does not exceed that of the solution from the *Standard solution*.

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

• **RESORCINOL AND OTHER PHENOLS**

Sample: 1 g

Analysis: Shake the *Sample* with 50 mL of water for a few min, filter, and to the filtrate add 3 drops of ferric chloride TS.

Acceptance criteria: No red or blue color is produced.

SPECIFIC TESTS

• **ACIDITY**

Sample: 250 mg

Analysis: Dissolve the *Sample* in 500 mL of water, add methyl red TS, and titrate with 0.020 N sodium hydroxide.

Acceptance criteria: NMT 1.0 mL is required for neutralization.

• **MELTING RANGE OR TEMPERATURE,** *Class I* ⟨741⟩: 62°–67°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Hexylresorcinol Lozenges

DEFINITION

Hexylresorcinol Lozenges contain NLT 90.0% and NMT 110.0% of the labeled amount of hexylresorcinol ($C_{12}H_{18}O_2$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 3.4 g of monobasic potassium phosphate in 850 mL of water. Adjust with phosphoric acid to a pH of

www.webofpharma.com

3.0 ± 0.05, dilute with water to 1000 mL, mix, and pass through a suitable filter of 0.5-µm or finer pore size.
Mobile phase: Methanol and *Buffer* (65:35)
Internal standard solution: 0.25 mg/mL of hexanophenone in *Mobile phase*
Standard stock solution: 0.4 mg/mL of USP Hexylresorcinol RS in *Mobile phase*
Standard solution: Transfer 10.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. This solution contains 0.08 mg/mL of USP Hexylresorcinol RS.
Sample solution: Nominally 0.08 mg/mL of hexylresorcinol prepared as follows. Transfer the equivalent to 4 mg of hexylresorcinol from Lozenges (NLT 20 Lozenges, weighed and pulverized) to a 50-mL volumetric flask. Add 10.0 mL of *Internal standard solution* and 20 mL of *Mobile phase*, and shake until dissolved. Dilute with *Mobile phase* to 50 mL, and mix. Pass a portion of this solution through a suitable filter of 0.5-µm or finer pore size, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 15-cm; packing L7
Column temperature: 37 ± 2°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution* and *Sample solution*
[NOTE—The relative retention times for hexylresorcinol and hexanophenone are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.2 between the hexylresorcinol peak and the nearest adjacent peak, *Sample solution*
Column efficiency: NLT 1500 theoretical plates, *Standard solution*
Tailing factor: 0.9–1.4, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hexylresorcinol ($C_{12}H_{18}O_2$) in the portion of Lozenges taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

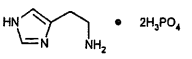
R_U = peak response ratio of hexylresorcinol to hexanophenone in the *Sample solution*
R_S = peak response ratio of hexylresorcinol to hexanophenone in the *Standard solution*
C_S = concentration of USP Hexylresorcinol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hexylresorcinol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hexylresorcinol RS

Histamine Phosphate



$C_5H_9N_3 \cdot 2H_3PO_4$ 307.14
1*H*-Imidazole-4-ethanamine, phosphate (1:2).
Histamine phosphate (1:2) [51-74-1].

» Histamine Phosphate contains not less than 98.0 percent and not more than 101.0 percent of $C_5H_9N_3 \cdot 2H_3PO_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

Identification—
A: Dissolve 0.10 g in a mixture of 7 mL of water and 3 mL of 1 N sodium hydroxide, and add the solution to a mixture of 50 mg of sulfanilic acid, 10 mL of water, 2 drops of hydrochloric acid, and 2 drops of sodium nitrite solution (1 in 10): a deep red color is produced.
B: Dissolve 50 mg in 5 mL of hot water, add a hot solution of 50 mg of picrolonic acid in 10 mL of alcohol, and allow to crystallize. Filter the crystals with suction, wash with a small amount of ice-cold water, and dry at 105° for 1 hour: the crystals so obtained melt between 250° and 254°, with decomposition.
C: A solution (1 in 10) responds to the tests for *Phosphate* ⟨191⟩.
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 3.0% of its weight.
Assay—Dissolve about 150 mg, accurately weighed, of Histamine Phosphate in 10 mL of water. Add 5 mL of chloroform and 25 mL of alcohol, then add 10 drops of thymolphthalein TS, and titrate with 0.2 N sodium hydroxide VS. Each mL of 0.2 N sodium hydroxide is equivalent to 15.36 mg of $C_5H_9N_3 \cdot 2H_3PO_4$.

Histamine Phosphate Injection

» Histamine Phosphate Injection is a sterile solution of Histamine Phosphate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_5H_9N_3 \cdot 2H_3PO_4$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.
USP Reference standards ⟨11⟩—
USP Histamine Dihydrochloride RS

Identification—
A: Evaporate a volume of Injection, equivalent to about 2 mg of histamine phosphate, on a steam bath to dryness, dissolve the residue in 0.5 mL of water, and add 0.5 mL of 1 N sodium hydroxide. Add 2 drops of sodium nitrite solution (1 in 10), and add 1 mL of a solution prepared by mixing 50 mg of sulfanilic acid with 10 mL of water containing 2 drops of hydrochloric acid: an orange-red color is produced.
B: To 1 mL of Injection, equivalent to not less than 1 mg of histamine phosphate (concentrate a larger volume by

USP Monographs

www.webofpharma.com

evaporation, if necessary), add ammonium molybdate TS dropwise: a yellow precipitate, which is soluble in ammonia TS, is formed.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 125.0 USP Endotoxin Units per mg of histamine phosphate.

pH ⟨791⟩: between 3.0 and 6.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Histamine Dihydrochloride RS in water, and quantitatively dilute with water to obtain a solution having a known concentration of 20 µg per mL, equivalent to 33.4 µg of histamine phosphate.

Assay preparation—Dilute an accurately measured volume of Injection, equivalent to about 1.65 mg of histamine phosphate, with water in a 50-mL volumetric flask to volume, and mix.

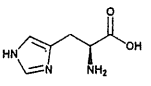
If phenol is present, prepare the *Assay preparation* as follows. Dilute an accurately measured volume of Injection, equivalent to about 1.65 mg of histamine phosphate, with water to about 25 mL. Heat the solution on a steam bath until the odor of phenol is no longer perceptible, adding water as required to maintain a volume of about 15 mL. Transfer to a 50-mL volumetric flask, cool, dilute with water to volume, and mix.

Procedure—Pipet 5 mL each of the *Standard preparation* and the *Assay preparation* into separate, 10-mL volumetric flasks, to each add 1 mL of sodium borate solution (1 in 100), followed by 1 mL of a freshly prepared solution of 50 mg of β-naphthoquinone-4-sodium sulfonate in 10 mL of water. Place the flasks in boiling water for 10 minutes, then immerse them for 5 minutes in water maintained between 5° and 10°. To each flask, add 1 mL of acid-formaldehyde (made by adding 0.5 mL of formaldehyde TS to a mixture of 45 mL of 1 N hydrochloric acid and 10 mL of glacial acetic acid and diluting with water to 80 mL), mix, add 1 mL of 0.1 N sodium thiosulfate, then dilute with water to volume, and mix. Concomitantly and immediately determine the absorbances of both solutions at the wavelength of maximum absorbance at about 460 nm, with a suitable spectrophotometer, against a reagent blank. Calculate the quantity, in mg, of $C_5H_9N_3 \cdot 2H_3PO_4$ in each mL of the Injection taken by the formula:

$$C(0.0835/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Histamine Dihydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Histidine



$C_6H_9N_3O_2$ 155.15

L-Histidine [71-00-1].

DEFINITION

Histidine contains NLT 98.5% and NMT 101.5% of L-histidine ($C_6H_9N_3O_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K.▲** (CN 1-May-2020) The *Sample* and USP L-Histidine RS are previously recrystallized from 80% alcohol.

ASSAY

- **PROCEDURE**

Sample: 150 mg of Histidine

Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate very slowly with the *Titrant*. Perform the *Blank* determination.

Calculate the percentage of histidine ($C_6H_9N_3O_2$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 155.2 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.4%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Standard solution: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.73 g of Histidine

Acceptance criteria: NMT 0.05%

- **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.33 g of Histidine

Acceptance criteria: NMT 0.03%

- **IRON** ⟨241⟩: NMT 30 ppm
- **RELATED COMPOUNDS**

System suitability solution: 0.4 mg/mL each of USP L-Histidine RS and USP L-Proline RS

Standard solution: 0.05 mg/mL of USP L-Histidine RS in water. [NOTE—This solution has a concentration equivalent to 0.5% of the *Sample solution*.]

Sample solution: 10 mg/mL of Histidine in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)

Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

System suitability

Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

Analysis

Samples: *System suitability solution*, *Standard solution*, and *Sample solution*

USP Monographs

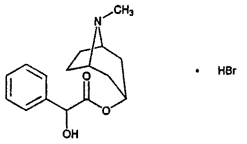
www.webofpharma.com

After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.
Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.
Individual impurities: NMT 0.5%
Total impurities: NMT 2.0%

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
  Sample solution: 110 mg/mL in 6 N hydrochloric acid
  Acceptance criteria: +12.6° to +14.0°
- **pH** ⟨791⟩
  Sample solution: 20 mg/mL solution
  Acceptance criteria: 7.0–8.5
- **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° for 3 h: it loses NMT 0.2% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP L-Histidine RS
  USP L-Proline RS

Homatropine Hydrobromide



$C_{16}H_{21}NO_3 \cdot HBr$ 356.25
Benzeneacetic acid, α-hydroxy-, 8-methyl-8-azabicyclo-[3.2.1]oct-3-yl ester, hydrobromide, *endo*-(±)-.
1αH,5αH-Tropan-3α-ol mandelate (ester) hydrobromide [51-56-9].

» Homatropine Hydrobromide contains not less than 98.0 percent and not more than 102.0 percent of $C_{16}H_{21}NO_3 \cdot HBr$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Homatropine Hydrobromide RS
USP Scopolamine Hydrobromide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩*, Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).
B: It responds to the tests for *Bromide* ⟨191⟩.
pH ⟨791⟩**:** between 5.7 and 7.0, in a solution (1 in 50).
Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 1.5% of its weight.
Residue on ignition ⟨281⟩**:** not more than 0.25%.

Change to read:

Limit of tropine—
Adsorbent: 0.2-mm layer of chromatographic silica gel mixture.
Diluent—Prepare a mixture of methanol and water (9:1).
Test solution—Transfer about 0.2 g of Homatropine Hydrobromide to a 5-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.
Standard solution—Dilute 0.5 mL of the *Test solution* with *Diluent* to 100.0 mL.
Tropine reference solution—Prepare a solution of tropine having a concentration of about 0.4 mg per mL ▲in *Diluent*.▲ (ERR 1-Jan-2019)
Application volume: 1 µL.
Developing solvent system: a mixture of ethyl acetate, anhydrous formic acid, and water (67: 16.5: 16.5).
Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩, applying the *Test solution*, the *Standard solution*, and the *Tropine reference solution*. Spray the plate with Dragendorff's reagent, followed by hydrogen peroxide TS, and immediately cover with a glass plate of the same size. Examine the plate no later than 5 to 10 minutes after spraying. In the chromatogram obtained from the *Test solution*, identify the spot corresponding to the principal spot in the chromatogram of the *Tropine reference solution:* this spot is not more intense than the spot obtained from the *Standard solution:* not more than 0.5% of tropine is found.

Chromatographic purity—
Buffer solution, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay*.
Standard solution—Use the *Standard preparation*, prepared as directed in the *Assay*.
Test solution—Use the *Assay preparation*, prepared as directed in the *Assay*.
Procedure—Separately inject a volume (about 7 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Continue the elution for 2.2 times the retention time of the homatropine peak. Disregard the peak for the bromide ion, which appears close to the solvent peak. Calculate the percentage of each impurity in the portion of Homatropine Hydrobromide taken by the formula:

$$100(r_i/r_s)$$

in which r_i and r_s are the peak response for each impurity and the sum of all peak responses, respectively, obtained from the *Test solution*. In addition to not exceeding the limits for each impurity in *Table 1*, not more than 0.1% of any other individual impurity is found; and not more than 1.0% of total impurities is found.

Table 1

Impurity	Relative Retention Time	Limit (%)
Mandelic acid	0.3	0.1
Dehydrohomatropine	0.9	0.5
Scopolamine	1.1	0.1
Atropine	1.9	0.1

Assay—
Buffer solution—Dissolve 6.8 g of monobasic potassium phosphate and 7.0 g of sodium 1-heptanesulfonate

USP Monographs

www.webofpharma.com

monohydrate in 1000 mL of water, adjust with 3 M phosphoric acid to a pH of 2.7, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (67:33).

Standard preparation—Dissolve an accurately weighed quantity of USP Homatropine Hydrobromide RS in *Mobile phase* to obtain a solution having a concentration of about 2 mg per mL.

System suitability solution—Prepare a solution of USP Scopolamine Hydrobromide RS having a concentration of about 0.1 mg per mL. Transfer 10 mL of this solution to a 100-mL volumetric flask, add 0.5 mL of the *Standard preparation,* and dilute with *Mobile phase* to volume.

Assay preparation—Transfer about 100 mg of Homatropine Hydrobromide, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 10-cm column that contains 3-µm packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 40°. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.0%. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution between homatropine and scopolamine peaks is not less than 1.5.

Procedure—Separately inject equal volumes (about 7 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{16}H_{21}NO_3 \cdot HBr$ in the portion of Homatropine Hydrobromide taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Homatropine Hydrobromide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Homatropine Hydrobromide Ophthalmic Solution

» Homatropine Hydrobromide Ophthalmic Solution is a sterile, buffered, aqueous solution of Homatropine Hydrobromide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{16}H_{21}NO_3 \cdot HBr$. It may contain suitable antimicrobial agents.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Homatropine Hydrobromide RS

Identification—

A: Proceed with Ophthalmic Solution as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩. The specified results are obtained.

B: It responds to the tests for *Bromide* ⟨191⟩.

Sterility Tests ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 2.5 and 5.0.

Assay—

Standard preparation—Accurately weigh about 50 mg of USP Homatropine Hydrobromide RS, dissolve in water, and dilute with water in a volumetric flask to 100 mL. Dilute 10.0 mL of this solution with water to 50.0 mL to obtain a solution having a known concentration of about 100 µg per mL. Prepare this solution fresh.

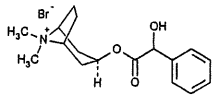
Assay preparation—Transfer a portion of Ophthalmic Solution, equivalent to 50 mg of homatropine hydrobromide, to a 100-mL volumetric flask, and dilute with water to volume. Dilute 10.0 mL of this solution with water to 50.0 mL.

Procedure—Transfer duplicate 2-mL portions of the *Standard preparation* and of the *Assay preparation* to separate glass-stoppered, 40-mL centrifuge tubes. To one set of two tubes add 3 mL of water and 1 mL of sodium hydroxide solution (1 in 100). Heat these tubes in a boiling water bath for 20 minutes, and allow to cool to room temperature. To the remaining set of tubes, which serve as blanks for the *Standard preparation* and the *Assay preparation,* respectively, add 4 mL of water. To each tube, add 2 mL of approximately 0.2 M ceric sulfate in diluted sulfuric acid (prepared by dissolving 12.6 g of ceric ammonium sulfate in 50 mL of water and 3 mL of sulfuric acid, and diluting with water to 100 mL) and 20.0 mL of isooctane. Shake by mechanical means for 15 minutes, allow the layers to separate, and remove the isooctane from each tube. Concomitantly determine the absorbances of the isooctane solutions from the hydrolyzed aliquots in 1-cm cells at the wavelength of maximum absorbance at about 242 nm, with a suitable spectrophotometer, against the respective blanks. Calculate the quantity, in mg, of $C_{16}H_{21}NO_3 \cdot HBr$ in the portion of Ophthalmic Solution taken by the formula:

$$0.5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Homatropine Hydrobromide RS in the *Standard preparation;* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Homatropine Methylbromide



$C_{17}H_{24}BrNO_3$ 370.28

8-Azoniabicyclo[3.2.1]octane, 3-(hydroxyphenylacetyl)oxy-8,8-dimethyl-, bromide, *endo*-(±)-;
3α-Hydroxy-8-methyl-1α*H*,5α*H*-tropanium bromide mandelate;
(1*R*,3*s*,5*S*)-3-[[(2*RS*)-2-Hydroxy-2-phenylacetyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide [80-49-9].

DEFINITION

Homatropine Methylbromide contains NLT 98.0% and NMT 102.0% of $C_{17}H_{24}BrNO_3$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

[NOTE—If differences are observed, dissolve the specimen and the Reference Standard separately in

www.webofpharma.com

methanol, and recrystallize by adding dioxane to each solution.]

• **B. IDENTIFICATION TESTS—GENERAL,** *Bromide* ⟨191⟩
Sample solution: 50 mg/mL in water
Acceptance criteria: Meets the requirements

ASSAY

PROCEDURE

Solution A: 3.4 g/L of monobasic potassium phosphate and 5 g/L of 1-pentanesulfonic acid sodium salt in water. Adjust with a 330-g/L solution of phosphoric acid to a pH of 3.0.
Solution B: Acetonitrile and *Solution A* (3:2)
Diluent: Acetonitrile and *Solution A* (9:41)
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	70	30
2	70	30
15	30	70
15.1	70	30
20	70	30

System suitability solution: 0.01 mg/mL each of USP Homatropine Methylbromide RS and USP Homatropine Hydrobromide RS in *Diluent*
Standard solution: 2.0 mg/mL of USP Homatropine Methylbromide RS in *Diluent*
Sample solution: 2.0 mg/mL of Homatropine Methylbromide in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1.4 mL/min
Injection size: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for homatropine methylbromide and homatropine hydrobromide are 1.0 and 1.14, respectively.]
Suitability requirements
Resolution: NLT 2.5 between homatropine methylbromide and homatropine hydrobromide, *System suitability solution*
Tailing factor: NMT 1.5 for homatropine methylbromide peak, *System suitability solution*
Relative standard deviation: NMT 1%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{17}H_{24}BrNO_3$ in the portion of Homatropine Methylbromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of Homatropine Methylbromide from the *Sample solution*
r_S = peak response of homatropine methylbromide from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

• **Procedure**
Solution A, Solution B, Diluent, Mobile phase, System suitability solution, and **Sample solution:** Proceed as directed in the *Assay*.
Standard solution: 0.01 mg/mL of USP Homatropine Methylbromide RS in *Diluent*
Chromatographic system: Proceed as directed in the *Assay*, except for injection size.
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.5 between homatropine methylbromide and homatropine hydrobromide
Tailing factor: NMT 1.5 for the homatropine methylbromide peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Homatropine Methylbromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of homatropine methylbromide from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

[NOTE—Reporting level for impurities is 0.05%.]
Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 1.0%
[NOTE—Disregard the peak due to the bromide ion that elutes close to the solvent peak at about 1 min.]

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methyldehydrohomatropine bromide[a]	0.94	0.5
Homatropine methylbromide	1.0	—
Homatropine hydrobromide	1.1	0.5
Any other individual impurity	—	0.1

[a] (1*R*,3*s*,5*S*)-3-[[(2*RS*)-2-Hydroxy-2-phenylacetyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]oct-6-ene.

SPECIFIC TESTS

• **PH** ⟨791⟩
Sample solution: 50 mg/mL in carbon dioxide-free water
Acceptance criteria: 4.5–6.5
• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample at 105° to constant weight: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Homatropine Methylbromide RS
USP Homatropine Hydrobromide RS

www.webofpharma.com

Homatropine Methylbromide Tablets

» Homatropine Methylbromide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{24}BrNO_3$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Homatropine Methylbromide RS

Identification—Shake a quantity of finely powdered Tablets, equivalent to about 10 mg of homatropine methylbromide, with 15 mL of a mixture of equal volumes of methanol and water for 10 minutes, and filter. Evaporate the filtrate on a steam bath to dryness, and dry at 105° for 1 hour. The residue of homatropine methylbromide so obtained melts between 190° and 198° (see *Class I* under *Melting Range or Temperature* ⟨741⟩), the temperature at which distinct liquefaction of the specimen is first observed being taken as the beginning of melting.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{17}H_{24}BrNO_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 258 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Homatropine Methylbromide RS in the same medium.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{17}H_{24}BrNO_3$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—
Standard preparation—Transfer about 25 mg of USP Homatropine Methylbromide RS, accurately weighed, to a 50-mL volumetric flask, add water to volume, and mix. Transfer 10.0 mL of this solution to a second 50-mL volumetric flask, dilute with water to volume, and mix. The concentration of USP Homatropine Methylbromide RS in the *Standard preparation* is about 100 µg per mL.
Test preparation—Transfer 1 finely powdered Tablet to a volumetric flask, suitably sized such that when the specimen is diluted to volume, the concentration is equivalent to about 100 µg of homatropine methylbromide per mL. Add water to about one-half of the volume of the flask, shake for 10 minutes, dilute with water to volume, mix, and filter, discarding the first 10 mL of filtrate. Use the subsequent filtrate as directed in the *Procedure*.
Procedure—Transfer 2.0 mL each of the *Standard preparation* and the *Test preparation* to separate glass-stoppered, 50-mL flasks. To each flask, add 0.1 mL of sodium hydroxide solution (1 in 10), and heat in a water bath at 80° for 15 minutes. Cool to room temperature, add 2.0 mL of 0.2 M ceric ammonium sulfate in 1 N sulfuric acid, and mix. To each flask, add 20.0 mL of *n*-hexane, and shake for 15 minutes. Decant the hexane layers into separate 1-cm cells, and concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 242 nm, with a suitable spectrophotometer, using *n*-hexane as the blank. Calculate the quantity, in mg, of $C_{17}H_{24}BrNO_3$ in the Tablet by the formula:

$$(TC/D)(A_U/A_S)$$

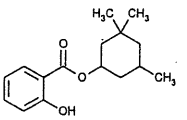
in which T is the labeled quantity, in mg, of homatropine methylbromide in the Tablet; C is the concentration, in µg per mL, of USP Homatropine Methylbromide RS in the *Standard preparation*; D is the concentration, in µg per mL, of the *Test preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

Assay—
Standard preparation—Transfer about 25 mg of USP Homatropine Methylbromide RS, accurately weighed, to a 50-mL volumetric flask, dissolve in water, dilute with water to volume, and mix.
Assay preparation—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 12.5 mg of homatropine methylbromide, and shake with 10 mL of water at frequent intervals during 30 minutes. Filter under reduced pressure through a sintered-glass crucible into a test tube placed in the suction flask under the filtering funnel, and wash under suction with several small portions of water. Transfer the contents of the test tube to a 25-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Transfer 10.0 mL each of the *Standard preparation* and the *Assay preparation* to separate test tubes, to each add 1 mL of 5 N sulfuric acid and 2 mL of ammonium reineckate TS, shake gently but well, and allow to stand for 1 hour. Filter through a sintered-glass crucible with suction, using portions of the filtrate to transfer the precipitate completely to the filter, and wash it with three 2-mL portions of ice-cold water. Completely dissolve the precipitate by pouring over it 1-mL portions of acetone with the application of suction, receiving the solution in a 10-mL volumetric flask, add acetone to volume, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 525 nm, with a suitable spectrophotometer, using acetone as the blank. Calculate the quantity, in mg, of $C_{17}H_{24}BrNO_3$ in the portion of Tablets taken by the formula:

$$0.025C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Homatropine Methylbromide RS in the *Standard preparation*; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Homosalate



$C_{16}H_{22}O_3$ 262.34
Benzoic acid, 2-hydroxy, 3,3,5-trimethylcyclohexyl ester; 3,3,5-Trimethylcyclohexyl salicylate [118-56-9].

DEFINITION
Homosalate contains NLT 90.0% and NMT 110% of $C_{16}H_{22}O_3$, calculated on the as-is basis.

USP Monographs

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

ASSAY

• **PROCEDURE**

Standard solution: 5.0 mg/mL of USP Homosalate RS in chloroform

Sample solution: 5.0 mg/mL of Homosalate in chloroform

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m column coated with a 1-µm film of G27

Temperature

Injector: 270°

Detector: 270°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	6	220	5

Carrier gas: Hydrogen

Flow rate: 6 mL/min

Injection size: 1 µL

Split ratio: 1:10

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for the *cis*-isomer and the *trans*-isomer are about 0.98 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.0 between the *cis*-isomer and the *trans*-isomer

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of homosalate ($C_{16}H_{22}O_3$) in the portion of Homosalate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses for the *cis*- and *trans*-isomers from the *Sample solution*

r_S = sum of the peak responses for the *cis*- and *trans*-isomers from the *Standard solution*

C_S = concentration of USP Homosalate RS in the *Standard solution* (mg/mL)

C_U = concentration of Homosalate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110% on the as-is basis

SPECIFIC TESTS

• **SPECIFIC GRAVITY** ⟨841⟩: 1.049–1.053

• **REFRACTIVE INDEX** ⟨831⟩: 1.516–1.519 at 20°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Homosalate RS

Hyaluronidase Injection

» Hyaluronidase Injection is a sterile solution of dry, soluble enzyme product, prepared from mammalian testes and capable of hydrolyzing mucopolysaccharides of the type of hyaluronic acid, in Water for Injection. It contains not less than 90.0 percent of the labeled amount of USP Hyaluronidase Units. Hyaluronidase Injection contains not more than 0.25 µg of tyrosine for each USP Hyaluronidase Unit. It may contain suitable stabilizers.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and store in a refrigerator.

USP Reference standards ⟨11⟩—

USP Hyaluronidase RS

USP L-Tyrosine RS

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 2.30 USP Endotoxin Units per USP Hyaluronidase Unit.

pH ⟨791⟩**:** between 6.4 and 7.4.

Limit of tyrosine—Transfer an accurately measured volume, equivalent to about 120 USP Hyaluronidase Units, to a 15-mL centrifuge tube calibrated at 6 mL, and evaporate at 105° to dryness. Proceed as directed in *Limit of tyrosine* under *Hyaluronidase for Injection* beginning with "Add 200 µL of 6 N sodium hydroxide," except to read Hyaluronidase Injection for Hyaluronidase for Injection in the second paragraph. Calculate the quantity, in µg, of tyrosine in the volume of Injection taken by the formula:

$$45(A_U/A_S)$$

in which A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively: not more than 0.25 µg of tyrosine is found for each USP Hyaluronidase Unit.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Acetate buffer solution, Phosphate buffer solution, Hydrolyzed gelatin, Diluent for hyaluronidase solutions, Serum stock solution, Serum solution, Potassium hyaluronate stock solution, Hyaluronate solution, and *Standard solution*—Prepare as directed in the *Assay* under *Hyaluronidase for Injection.*

Assay preparation—Quantitatively dilute an accurately measured volume of Injection with cold *Diluent for hyaluronidase solutions,* on the basis of trial or experience, so that the observed absorbances with the three dilutions of the Injection fall on the upper, linear part of the standard curve prepared as directed in the *Procedure.*

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Hyaluronidase for Injection* The potency, in USP Hyaluronidase Units, of the portion of Injection taken is the average of the six activity values read from the standard curve.

Hyaluronidase for Injection

» Hyaluronidase for Injection is a sterile, dry, soluble enzyme product prepared from mammalian testes

www.webofpharma.com

and capable of hydrolyzing mucopolysaccharides of the type of hyaluronic acid. Its potency, in USP Hyaluronidase Units, is not less than the labeled potency. Hyaluronidase for Injection contains not more than 0.25 µg of tyrosine for each USP Hyaluronidase Unit. It may contain a suitable stabilizer.

Packaging and storage—Preserve in *Containers for Sterile Solids* as described under *Injections and Implanted Drug Products* ⟨1⟩, preferably of Type I or Type III glass, and store at controlled room temperature.

USP Reference standards ⟨11⟩—
USP Hyaluronidase RS
USP L-Tyrosine RS

Sterility Tests ⟨71⟩ **:** meets the requirements.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 2.30 USP Endotoxin Units per USP Hyaluronidase Unit.

Limit of tyrosine—Dissolve the entire contents of 1 or more containers of Hyaluronidase for Injection in sufficient water, accurately measured, to give a concentration of about 60 USP Hyaluronidase Units per mL. Transfer 2.0 mL of the solution to a 15-mL centrifuge tube calibrated at 6 mL, and evaporate at 105° to dryness. Add 200 µL of 6 N sodium hydroxide, and heat with steam under pressure at 121° for 3 hours. Add 300 µL of 7 N sulfuric acid, then add 1.5 mL of water and 1.5 mL of a 15 in 100 solution of mercuric sulfate in 5 N sulfuric acid. Heat on a steam bath for 10 minutes, and cool to room temperature. Add 1 mL of 7 N sulfuric acid and 1 mL of sodium nitrite solution (1 in 500), with shaking. Add water to make 6 mL, mix, centrifuge, and decant the supernatant. Twenty minutes after diluting to 6 mL, determine the absorbance of the supernatant at 540 nm, with a suitable spectrophotometer.

Repeat the preceding test, using the same quantities of the same reagents and in the same manner but omitting the Hyaluronidase for Injection and replacing the 1.5 mL of water with 1.5 mL of a solution of USP L-Tyrosine RS in 0.4 N sulfuric acid containing 30 µg in each mL.

Calculate the quantity, in µg, of tyrosine in the 2-mL aliquot of the solution of Hyaluronidase for Injection taken by the formula:

$$45(A_U/A_S)$$

in which A_U and A_S are the absorbances of the solution from Hyaluronidase for Injection and the Standard solution, respectively: not more than 0.25 µg of tyrosine is found for each USP Hyaluronidase Unit.

Assay—

Acetate buffer solution—Dissolve 14 g of potassium acetate and 20.5 mL of glacial acetic acid in water to make 1000 mL.

Phosphate buffer solution—Dissolve 2.5 g of monobasic sodium phosphate, 1.0 g of anhydrous dibasic sodium phosphate, and 8.2 g of sodium chloride in water to make 1000 mL.

Hydrolyzed gelatin—Dissolve 50 g of bacteriological gelatin in 1000 mL of water, heat in an autoclave at 121° for 90 minutes, and freeze-dry the solution.

Diluent for hyaluronidase solutions—Mix 250 mL of *Phosphate buffer solution* with 250 mL of water and, within 2 hours before use, dissolve 330 mg of *Hydrolyzed gelatin* in the mixture.

Serum stock solution—Constitute dried horse serum with water to its original volume, and dilute it with 9 volumes of *Acetate buffer solution.* Adjust with 4 N hydrochloric acid to a pH of 3.1, and allow the solution to stand at room temperature for 18 to 24 hours. Store the solution at 0° to 4°, and use within 30 days.

Serum solution—On the day of the assay, dilute 1 volume of *Serum stock solution* with 3 volumes of *Acetate buffer solution,* and adjust to room temperature.

Potassium hyaluronate stock solution—Prepare a stock solution to contain, in each mL, 500 µg of potassium hyaluronate, previously dried in vacuum over phosphorus pentoxide for 48 hours. Do not keep the hyaluronate over phosphorus pentoxide indefinitely. The use of a weighing bottle is desirable. Store the solution at a temperature not exceeding 5°, and use within 30 days.

Hyaluronate solution—On the day of the assay, dilute 1 volume of *Potassium hyaluronate stock solution* with 1 volume of *Phosphate buffer solution.*

Standard solution—Dissolve a suitable quantity of USP Hyaluronidase RS, accurately weighed, in cold *Diluent for hyaluronidase solutions* to obtain a solution having a known concentration of about 1.5 USP Hyaluronidase Units in each mL. Prepare this solution immediately before use in the assay.

Assay solution—Dissolve the contents of 1 container of Hyaluronidase for Injection by adding cold *Diluent for hyaluronidase* directly to the container. On the basis of trial or experience, dilute the solution so prepared with cold *Diluent for hyaluronidase* so that the observed absorbances with the three dilutions of Hyaluronidase for Injection fall on the upper, linear part of the standard curve prepared as directed below.

Procedure—Prepare a standard concentration-response curve by adding to each of twelve 16- × 100-mm test tubes 500 µL of *Hyaluronate solution.* To each of two of the tubes add, respectively, 500, 400, 300, 200, 100, and 0 µL of *Diluent for hyaluronidase solutions.* If quantities of *Standard solution* other than those indicated below are used, change the above-specified quantities of *Diluent for hyaluronidase solutions* so that the final volume of solution in each tube, after the addition of the *Standard solution,* is 1.00 mL.

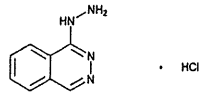
At 30-second intervals, accurately timed, add to each of two tubes 0, 100, 200, 300, 400, and 500 µL, respectively, of *Standard solution.* Mix the contents by gentle shaking, and place each tube in a water bath maintained at 37 ± 0.2°. After 30 ± 0.25 minutes, remove each tube, in order, from the water bath at 30-second intervals, and immediately add 4.0 mL of *Serum solution.* Shake the tube, and allow to stand at room temperature for 30 ± 2 minutes. Again shake the tube, and determine the absorbance at 640 nm, with a suitable spectrophotometer. Perform a blank determination but omit the hyaluronate, and make any necessary correction. Plot the average absorbance value for each level against the hyaluronidase activity expressed in Units, and draw the smooth curve that best fits the plotted points.

Concurrently, to six test tubes add 500 µL of *Hyaluronate solution* and sufficient *Diluent for hyaluronidase solutions* so that the final volume, after the addition of the *Assay solution,* is 1.00 mL. Add, at 30-second intervals, sufficient *Assay solution* so that duplicate tubes contain about 0.30, 0.50, and 0.70 Unit, respectively. Shake each tube gently, and treat as directed in the preceding paragraph for the *Standard solution,* beginning with "place each tube in a water bath." Measure the absorbances in the spectrophotometer, and make any necessary correction for the blank. The potency of Hyaluronidase for Injection is the average of the six activity values read from the standard curve.

USP Monographs

www.webofpharma.com

Hydralazine Hydrochloride



$C_8H_8N_4 \cdot HCl$ 196.64

Phthalazine, 1-hydrazino-, monohydrochloride;
1-Hydrazinophthalazine monohydrochloride [304-20-1].

DEFINITION

Hydralazine Hydrochloride contains NLT 98.0% and NMT 102.0% of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*

Sample solution: 0.25 mg/mL of Hydralazine Hydrochloride in water

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 1.44 g of sodium dodecyl sulfate and 0.75 g of tetrabutylammonium bromide in 770 mL of water, and add 230 mL of acetonitrile. Adjust with 0.1 N sulfuric acid to a pH of 3.0.

Diluent: 0.1 N acetic acid

System suitability stock solution: 0.25 mg/mL of USP Hydralazine Hydrochloride RS and 0.05 mg/mL of USP Phthalazine RS in *Diluent*

System suitability solution: 25 µg/mL of USP Hydralazine Hydrochloride RS and 5 µg/mL of USP Phthalazine RS in *Diluent* from the *System suitability stock solution*

Standard stock solution: 0.4 mg/mL of USP Hydralazine Hydrochloride RS in *Diluent*

Standard solution: 40 µg/mL of USP Hydralazine Hydrochloride RS in *Diluent* from the *Standard stock solution*

Sample stock solution: 0.4 mg/mL of Hydralazine Hydrochloride in *Diluent*

Sample solution: 40 µg/mL of Hydralazine Hydrochloride in *Diluent* prepared as follows. Transfer a suitable amount of the *Sample stock solution* to a suitable volumetric flask. Dilute with *Diluent* to volume and filter, discarding the first 10 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.0-mm × 25-cm; 10-µm packing L10

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for phthalazine and hydralazine hydrochloride are 0.65 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between the phthalazine and hydralazine peaks, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$) in the portion of Hydralazine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydralazine from the *Sample solution*

r_S = peak response of hydralazine from the *Standard solution*

C_S = concentration of USP Hydralazine Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = concentration of Hydralazine Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Mobile phase, Diluent, and **System suitability solution:** Prepare as directed in the *Assay*.

Standard solution: 0.001 mg/mL of USP Hydralazine Hydrochloride RS in *Diluent*. Sonication may be necessary for complete dissolution.

Sample solution: 0.5 mg/mL of Hydralazine Hydrochloride in *Diluent* prepared as follows. Transfer a suitable amount of Hydralazine Hydrochloride to a suitable volumetric flask. Add *Diluent* to fill about 60% of the total volume, and sonicate to dissolve. Cool, and dilute with *Diluent* to volume.

Chromatographic system: Proceed as directed in the *Assay*, except for *Injection volume*.

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between the phthalazine and hydralazine peaks, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Hydralazine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of hydralazine from the *Standard solution*

C_S = concentration of USP Hydralazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Hydralazine Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
1-Phthalazinone	0.55	2.3	0.15

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Formyl benzoic acid	0.60	1.1	0.15
Phthalazine	0.75	3.6	0.15
1-Chlorophthalazine	0.83	1.5	0.15
Hydralazine	1.00	—	—
Any unspecified impurity	—	1.0	0.10
Total impurities[a]	—	—	1.0

[a] Total impurities include specified and unspecified impurities.

• **Limit of Hydrazine**

Buffer: Dissolve 5.82 g of dibasic sodium phosphate and 3.81 g of monobasic potassium phosphate in 1 L of water, and adjust with either 1 N sodium hydroxide or 1 N phosphoric acid to a pH of 7.0 ± 0.1.

Mobile phase: Dissolve 300 mg of edetate disodium in 300 mL of water in a 1-L volumetric flask. Dilute with acetonitrile to volume.

Benzaldehyde solution: Transfer 1.0 mL of benzaldehyde to a 100-mL volumetric flask, and dilute with a mixture of methanol and water (9:1) to volume.

Acetonitrile solution: Transfer 300 mL of water to a 1000-mL volumetric flask, and dilute with acetonitrile to volume.

Standard stock solution: 0.65 mg/mL of hydrazine dihydrochloride in water

Standard solution 1: 0.325 µg/mL of hydrazine dihydrochloride in water from *Standard stock solution*

Standard solution 2: Transfer 1.0 mL of *Standard solution 1* to a 10-mL flask. Add 4.0 mL of *Benzaldehyde solution,* and shake by mechanical means for 20 min.

Standard solution 3: Transfer 2.0 mL of *Standard solution 2* to a 5-mL volumetric flask, and dilute with *Acetonitrile solution* to volume.

Extraction column: Use a freshly conditioned solid phase extraction column containing benzenesulfonic acid strong cation-exchange packing with a sorbent-mass to column volume ratio of 500 mg/3 mL, or equivalent. The column is conditioned as follows. Wash the column with two 2.0-mL portions of hexanes, and dry under vacuum for 2 min. Wash the column with two 2.0-mL portions of methanol, two 2.0-mL portions of water, and two 2.0-mL portions of *Buffer.* At no time after the hexanes wash should the column be allowed to dry out.

Sample solution: Transfer about 20 mg of Hydralazine Hydrochloride to a 10-mL reaction vessel, and dissolve in 1.0 mL of water. Add 4.0 mL of *Benzaldehyde solution,* and shake by mechanical means for 20 min. Pipet 2.0 mL of this solution into the *Extraction column* and elute into a 5-mL volumetric flask. Wash the *Extraction column* with two 1.5-mL portions of the *Acetonitrile solution,* collecting the washings with the eluate, and dilute with *Acetonitrile solution* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 310 nm

Column: 4.0-mm × 25-cm; 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution 3*

[NOTE—The relative retention times for hydralazine and hydrazine are about 1.0 and 1.5, respectively.]

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution 3* and *Sample solution*

Calculate the percentage of hydrazine in the portion of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$) taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of hydrazine from the *Sample solution*

r_S = peak response of hydrazine from *Standard solution 3*

C_S = concentration of hydrazine dihydrochloride in *Standard solution 3* (µg/mL)

C_U = concentration of Hydralazine Hydrochloride in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of hydrazine, 32.05

M_{r2} = molecular weight of hydrazine dihydrochloride, 104.97

Acceptance criteria: NMT 0.001%

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: 20 mg/mL of Hydralazine Hydrochloride in water

Acceptance criteria: 3.5–4.2

• **Loss on Drying** ⟨731⟩

Analysis: Dry at 110° for 15 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩

USP Hydralazine Hydrochloride RS

USP Phthalazine RS

$C_8H_6N_2$ 130.15

Hydralazine Hydrochloride Injection

DEFINITION

Hydralazine Hydrochloride Injection is a sterile solution of Hydralazine Hydrochloride in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$).

IDENTIFICATION

• **A. Infrared Absorption**

Sample: A volume of Injection, equivalent to 60 mg of hydralazine hydrochloride. Mix the *Sample* with a quantity of 1 N hydrochloric acid sufficient to prepare 25 mL of solution. Place 20 mL of this solution in a separator, wash with 10 mL of methylene chloride, and discard the methylene chloride washing. Mix the aqueous solution in the separator with 2 mL of sodium nitrite solution (14 mg/mL). Add 10 mL of methylene chloride, shake by mechanical means for 5 min, and allow the layers to separate. Pass the methylene chloride layer through a filter of anhydrous sodium sulfate, previously washed with methylene chloride, and collect the solution in a 50-mL beaker. Evaporate to dryness with the aid of gentle heat and a stream of dry nitrogen.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue so obtained

USP Monographs

www.webofpharma.com

exhibits maxima only at the same wavelengths as those of USP Hydralazine Hydrochloride RS similarly treated and prepared.

ASSAY

• **PROCEDURE**

Sample solution: Transfer an amount equivalent to 100 mg of hydralazine hydrochloride from a suitable volume of Injection in a 250-mL iodine flask. Add 20 mL of hydrochloric acid, cool to room temperature, and add 5 mL of chloroform.

Analysis: Titrate with 0.02 M potassium iodate VS until the purple color of iodine disappears from the chloroform, adding the last portion of the potassium iodate solution dropwise and agitating the mixture vigorously and continuously. Each mL of 0.02 M potassium iodate is equivalent to 3.933 mg of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$).

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.4–4.4

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 1.45 USP Endotoxin Units/mg

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections

• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Hydralazine Hydrochloride RS

Hydralazine Hydrochloride Compounded Oral Solution

DEFINITION

Hydralazine Hydrochloride Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$).

Prepare Hydralazine Hydrochloride Compounded Oral Solution of the designated percentage strength as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Hydralazine Hydrochloride	
For 0.1% Oral Solution	100 mg
For 1.0% Oral Solution	1.0 g
Sorbitol Solution (70%)	40 g
Methylparaben	65 mg
Propylparaben	35 mg
Propylene Glycol	10 g
Aspartame	50 mg
Purified Water, a sufficient quantity to make	100 mL

Dissolve the *Hydralazine Hydrochloride* in 30 mL of *Purified Water*, add the *Aspartame*, and shake or stir until the solids have dissolved. Add the *Sorbitol Solution*. In a separate container, dissolve an aliquot portion of an intimate homogeneous mixture of accurately weighed quantities of *Methylparaben* and *Propylparaben* in the *Propylene Glycol*, and, with stirring, add this mixture to the solution containing the *Hydralazine Hydrochloride*. Add sufficient *Purified Water* to make the preparation measure 100 mL, and mix.

[NOTE—Hydralazine reacts with many flavors; do not add flavors when compounding.]

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.0–5.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in a suitable light-resistant glass or plastic bottle, with a child-resistant closure. Store in a refrigerator.

• **BEYOND-USE DATE:** NMT 30 days after the date on which it was compounded when stored in a refrigerator

• **LABELING:** Label it to state the *Beyond-Use Date* and that it is to be stored in a refrigerator.

Hydralazine Hydrochloride Tablets

DEFINITION

Hydralazine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$).

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Sample: Transfer an amount equivalent to about 100 mg of hydralazine hydrochloride from finely powdered Tablets to a glass-stoppered flask. Add 40 mL of 1 N hydrochloric acid, shake by mechanical means for 5 min, and filter. Discard the first few milliliters of the filtrate. Place 20 mL of the filtrate in a separator, wash with 10 mL of methylene chloride, and discard the washing. Mix the aqueous solution in the separator with 2 mL of 14 mg/mL sodium nitrite solution, add 10 mL of methylene chloride, shake by mechanical means for 5 min, and allow the layers to separate. Pass the methylene chloride layer through a filter of anhydrous sodium sulfate previously washed with methylene chloride and collect the solution in a 50-mL beaker. Evaporate to dryness with the aid of gentle heat and a stream of dry nitrogen.

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Dissolve 1.44 g of sodium dodecyl sulfate and 0.75 g of tetrabutylammonium bromide in 770 mL of water and add 230 mL of acetonitrile. Adjust with 0.1 N sulfuric acid to a pH of 3.0.

Diluent: 0.1 N acetic acid

System suitability solution: 25 µg/mL of USP Hydralazine Hydrochloride RS and 5 µg/mL of USP Phthalazine RS in *Diluent*

Standard solution: 40 µg/mL of USP Hydralazine Hydrochloride RS in *Diluent*

Sample stock solution: Nominally 0.4 mg/mL of hydralazine hydrochloride from NLT 20 finely powdered Tablets in *Diluent*. Centrifuge the solution and use the clear supernatant.

www.webofpharma.com

Sample solution: Nominally 40 µg/mL of hydralazine hydrochloride in *Diluent* from the *Sample stock solution*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.0-mm × 25-cm; 10-µm packing L10
Flow rate: 1 mL/min
Injection volume: 25 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for phthalazine and hydralazine are about 0.65 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between the phthalazine and hydralazine peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydralazine from the *Sample solution*
r_S = peak response of hydralazine from the *Standard solution*
C_S = concentration of USP Hydralazine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of hydralazine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: A known concentration of USP Hydralazine Hydrochloride RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter and dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 260 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$) dissolved.
Tolerances: NLT 75% (*Q*) of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Diluent and **System suitability solution:** Prepare as directed in the *Assay*.
Mobile phase: Dissolve 1.44 g of sodium dodecyl sulfate and 0.75 g of tetrabutylammonium bromide in 770 mL of water, adjust with 0.1 N sulfuric acid to a pH of 3.0, and add 230 mL of acetonitrile.
Standard stock solution: 0.1 mg/mL of USP Hydralazine Hydrochloride RS in *Diluent*, prepared as follows. To a suitable amount of USP Hydralazine Hydrochloride RS, add 60% of the total volume of *Diluent*, sonicate to dissolve, cool to room temperature, and then dilute with *Diluent* to volume.
Standard solution: 0.001 mg/mL of USP Hydralazine Hydrochloride RS in *Diluent* from the *Standard stock solution*
Sample solution: Nominally 0.5 mg/mL of hydralazine hydrochloride from NLT 20 powdered Tablets in *Diluent*, prepared as follows. To a suitable amount of powdered Tablets, add 60% of the total volume of *Diluent* and sonicate for 15 min with occasional swirling. Cool to room temperature, dilute with *Diluent* to volume, and pass through a suitable filter of 0.45-µm pore size.
Chromatographic system: Proceed as directed in the *Assay* except for the following.
Injection volume: 20 µL
Run time: NLT 5.8 times the retention time of hydralazine

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between the phthalazine and hydralazine peaks, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any unspecified degradation product from the *Sample solution*
r_S = peak response of hydralazine from the *Standard solution*
C_S = concentration of USP Hydralazine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydralazine hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
1-Phthalazinone[a]	0.55	2.3	—
2-Formylbenzoic acid[a]	0.60	1.1	—
Phthalazine[a]	0.75	3.6	—
1-Chlorophthalazine[a]	0.83	1.5	—
Hydralazine	1.00	—	—
Any unspecified degradation product	—	1.0	0.20
Total impurities[b]	—	—	1.5

[a] Process-related impurity.
[b] Total impurities include process-related impurities and degradation products.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hydralazine Hydrochloride RS

www.webofpharma.com

USP Phthalazine RS
$C_8H_6N_2$ 130.15

Hydrochloric Acid Compounded Injection

Change to read:

DEFINITION
Hydrochloric Acid Compounded Injection contains NLT 90% and NMT 110% of the labeled amount of hydrochloric acid (HCl), equivalent to NLT 328 mg and NMT 401 mg of hydrochloric acid (HCl) in 100 mL.
Prepare Hydrochloric Acid Compounded Injection, 0.1 N, as follows (see *Pharmaceutical Compounding—Sterile Preparations* ⟨797⟩).

Hydrochloric Acid, 1.0 N	10 mL
Sodium Chloride Injection, 0.45% or 0.9%, a sufficient quantity to make	100 mL

Prepare *1.0 N Hydrochloric Acid* by adding Hydrochloric Acid to *Purified Water* in an appropriate volumetric flask with continuous stirring. Allow the solution to cool to room temperature, add sufficient *Purified Water* to bring to final volume, and continue mixing for 1 min. Pass through a compatible filter of 0.45-µm pore size to reduce particulate matter load. Accurately measure the nonsterile *1.0 N Hydrochloric Acid* with an appropriate glass or plastic device, and accurately dilute to final volume with *0.45% or 0.9% Sodium Chloride Injection*. Sterilize the solution by passing through a sterile acid-compatible membrane filter of 0.2-µm pore size into a sterile intravenous polypropylene container in an ISO Class 5 environment. ▲▲ (CN 1-May-2020)

ASSAY
• **PROCEDURE**
Sample: 25 mL
Blank: 25 mL of water
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.5 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Place the *Sample* in a conical flask, add methyl red TS, and titrate with *Titrant*.
Calculate the weight of hydrochloric acid in 100 mL:

$$\text{Result} = [(V_S - V_B) \times N \times F] \times D$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 36.46 mg/mEq
D = dilution factor, 4

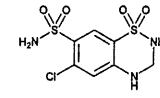
Acceptance criteria: 328–401 mg in 100 mL

SPECIFIC TESTS
• **pH** ⟨791⟩: 1.0–1.2
• **STERILITY TESTS** ⟨71⟩: It meets the requirements when tested as directed under *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 1.5 USP Endotoxin Units/mL

Change to read:

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in a single-dose polypropylene container. Store at controlled room temperature or in a refrigerator.
• **BEYOND-USE DATE:** ▲In the absence of passing a sterility test and endotoxins test, the BUDs in ⟨797⟩ apply. If prepared as a Category 2 CSP and a sterility test and endotoxins test is passed, NMT 120 days when stored at controlled room temperature or in a refrigerator.▲ (CN 1-May-2020)
• **LABELING:** Label it Hydrochloric Acid Compounded Injection, 0.1 N. Label it to state that this is a single-dose container. Label it to state the *Beyond-Use Date*.

Hydrochlorothiazide



$C_7H_8ClN_3O_4S_2$ 297.74
2H-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-3,4-dihydro-, 1,1-dioxide;
6-Chloro-3,4-dihydro-*2H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [58-93-5].

DEFINITION
Hydrochlorothiazide contains NLT 98.0% and NMT 102.0% of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Potassium bromide–hydrochlorothiazide mixture, previously heated at 105° for 2 h
Acceptance criteria: Meets the requirements

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 10 µg/mL in methanol
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Buffer: 2.76 g of monobasic sodium phosphate in a 1000-mL volumetric flask. Add 990 mL of water. Adjust with phosphoric acid to a pH of 2.7 ± 0.1, and dilute with water to volume.
Diluent: Acetonitrile and *Buffer* (3:7)
Solution A: Acetonitrile and methanol (3:1)
Solution B: Anhydrous formic acid in water (5 in 1000)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	3	97
5	3	97

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
14	36	64
18	3	97
20	3	97

System suitability solution: 0.32 mg/mL of USP Hydrochlorothiazide RS, 0.0032 mg/mL of USP Chlorothiazide RS, and 0.0032 mg/mL of USP Benzothiadiazine Related Compound A RS in *Diluent*; sonicate if necessary to dissolve. Pass a portion through a filter of 0.45-µm or finer pore size.

Standard solution: 0.32 mg/mL of USP Hydrochlorothiazide RS in *Diluent*. Sonicate if necessary to dissolve. Pass a portion through a filter of 0.45-µm or finer pore size.

Sample solution: 0.32 mg/mL of Hydrochlorothiazide in *Diluent*, prepared as follows. Transfer 32 mg of Hydrochlorothiazide into a 100-mL volumetric flask. Add 70 mL of *Diluent*, sonicate for 10 min if necessary to dissolve, and allow to cool to ambient temperature. Dilute with *Diluent* to volume. Pass a portion through a filter of 0.45-µm or finer pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 5-cm; 3.5-µm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2*for the relative retention times.]

Chromatograph the *Diluent* to check for interference by system-related peaks.

Suitability requirements

Resolution: NLT 2.0 between benzothiadiazine related compound A and chlorothiazide and NLT 1.5 between chlorothiazide and hydrochlorothiazide, *System suitability solution*

Tailing factor: NMT 1.5 for the peaks for benzothiadiazine related compound A, chlorothiazide, and hydrochlorothiazide, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Hydrochlorothiazide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydrochlorothiazide from the *Sample solution*

r_S = peak response of hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

C_U = concentration of Hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩

Sample solution: Shake 0.50 g with 40 mL of water for 5 min, and filter.

Acceptance criteria: 0.035%; the filtrate shows no more chloride than corresponds to 0.25 mL of 0.020 N hydrochloric acid.

• **SELENIUM** ⟨291⟩

Sample: 200 mg

Acceptance criteria: NMT 30 ppm

• **ORGANIC IMPURITIES**

Diluent, Solution A, Solution B, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Quantitative limit solution: 0.16 µg/mL of USP Hydrochlorothiazide RS in *Diluent*. Sonicate if necessary to dissolve.

Sample solution: 0.32 mg/mL of Hydrochlorothiazide in *Diluent*, prepared as follows. Transfer 32 mg of Hydrochlorothiazide into a 100-mL volumetric flask. Add 70 mL of *Diluent*, sonicate for 10 min if necessary to dissolve, and allow to cool to ambient temperature. Dilute with *Diluent* to volume, mix, and pass a portion through a filter of 0.45-µm or finer pore size.

System suitability

Samples: *System suitability solution* and *Quantitative limit solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between benzothiadiazine related compound A and chlorothiazide and NLT 1.5 between chlorothiazide and hydrochlorothiazide, *System suitability solution*

Tailing factor: NMT 1.5 for the peaks for benzothiadiazine related compound A, chlorothiazide, and hydrochlorothiazide, *System suitability solution*

Relative standard deviation: NMT 5.0% for benzothiadiazine related compound A and chlorothiazide, *System suitability solution*; NMT 25% for hydrochlorothiazide, based on 3 replicate injections, *Quantitative limit solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Hydrochlorothiazide taken:

$$\text{Result} = (R_U/R_T) \times 100$$

R_U = ratio of the peak area for each impurity to its response factor

R_T = sum of the ratios of all the peak areas to their respective response factors

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A	0.5	0.54	1.0
Chlorothiazide	0.8	0.63	0.5
Hydrochlorothiazide	1.0	—	—
5-Chlorohydrochlorothiazide	2.1	—	0.5
Hydrochlorothiazide dimer[a]	2.6	—	0.5
Any other individual impurity	—	1.0	0.5

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities	—	—	0.9[b]

[a] 6-Chloro-*N*-[(6-chloro-7-sulfamoyl-2,3-dihydro-4*H*-1,2,4-benzothiadiazine-4-yl 1,1-dioxide)methyl]3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide.
[b] Excluding benzothiadiazine related compound A.

SPECIFIC TESTS
- **Loss on Drying** ⟨731⟩
  Analysis: Dry a sample at 105° for 1 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨11⟩
  USP Benzothiadiazine Related Compound A RS
  4-Amino-6-chloro-1,3-benzenedisulfonamide.
  $C_6H_8ClN_3O_4S_2$ 285.73
  USP Chlorothiazide RS
  USP Hydrochlorothiazide RS

Hydrochlorothiazide Capsules

DEFINITION
Hydrochlorothiazide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

- **Procedure**
  Buffer: 13.8 g/L of monobasic sodium phosphate
  Mobile phase: Acetonitrile and *Buffer* (10:90). Adjust with 10% (v/v) phosphoric acid to a pH of 3.0. ▲▲ (USP 1-May-2019)
  System suitability solution: 0.15 mg/mL each of USP Hydrochlorothiazide RS and USP Chlorothiazide RS in *Mobile phase*. Sonicate to completely dissolve.
  Standard stock solution: 0.50 mg/mL ▲of USP Hydrochlorothiazide RS▲ (USP 1-May-2019) prepared as follows. ▲To a suitable amount of USP Hydrochlorothiazide RS in a suitable volumetric flask add acetonitrile to 10% of the final volume and sonicate to dissolve. Dilute with *Buffer* to volume.▲ (USP 1-May-2019)
  Standard solution: 50 µg/mL ▲of USP Hydrochlorothiazide RS▲ (USP 1-May-2019) in *Mobile phase* from the *Standard stock solution*▲▲ (USP 1-May-2019)
  Sample stock solution: ▲Nominally▲ (USP 1-May-2019) 0.25 mg/mL of hydrochlorothiazide prepared as follows. Transfer ▲NLT 10▲ (USP 1-May-2019) Capsules into a suitable volumetric flask. Add water to 10% of the final volume, and sonicate for 10 min with vigorous shaking. Add *Buffer* to 20% of the final volume, and again sonicate for 10 min. Add acetonitrile up to 40% of the final volume, and sonicate for 30 min. Dilute with *Buffer* to volume, and pass through a suitable filter of 0.45-µm pore size.
  Sample solution: 50 µg/mL of hydrochlorothiazide in *Mobile phase* from the *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 272 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2019)
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: ▲2 mL/min▲ (USP 1-May-2019)
  Injection volume: 20 µL
  ▲Run time: NLT 1.5 times the retention time of hydrochlorothiazide▲ (USP 1-May-2019)
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  ▲[NOTE—The relative retention times for chlorothiazide and hydrochlorothiazide are 0.80 and 1.0, respectively.]▲ (USP 1-May-2019)
  Suitability requirements
  Resolution: NLT 2.0 between chlorothiazide and hydrochlorothiazide, *System suitability solution*
  Tailing factor: NMT 2.0, *Standard solution*
  ▲▲ (USP 1-May-2019)
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydrochlorothiazide from the *Sample solution*
r_S = peak response of hydrochlorothiazide from the *Standard solution*
C_S = concentration of ▲USP Hydrochlorothiazide RS▲ (USP 1-May-2019) in the *Standard solution* (µg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **Dissolution** ⟨711⟩
  Test 1
  Medium: 0.1 N hydrochloric acid; 900 mL
  Apparatus 1: 100 rpm
  Time: 30 min
  Standard solution: 6.75 µg/mL ▲of USP Hydrochlorothiazide RS▲ (USP 1-May-2019) prepared as follows. Dissolve a quantity of USP Hydrochlorothiazide RS in 10% of the flask volume of acetonitrile, and dilute with *Medium*. Sonicate to completely dissolve.
  Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.

www.webofpharma.com

Instrumental conditions
Mode: UV
Analytical wavelength: 272 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of ▲USP Hydrochlorothiazide RS▲ (USP 1-May-2019) in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 30 min
Standard stock solution: 0.35 mg/mL of USP Hydrochlorothiazide RS in *Medium*. An amount of acetonitrile, not exceeding 25% of the final volume, may be used to help solubilize hydrochlorothiazide.
Standard solution: (L/900) mg/mL of ▲USP Hydrochlorothiazide RS▲ (USP 1-May-2019) in *Medium*, from the *Standard stock solution*, where L is the label claim in mg/Capsule
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.
Empty capsules solution: Place 10 Capsules into a 900-mL volumetric flask. Slowly add 800 mL of *Medium* pre-heated to 37°, and stir until dissolved. Cool to room temperature, and dilute with *Medium* to volume.
Instrumental conditions
Mode: UV
Analytical wavelength: 272 nm
Cell: 1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = [(A_U - A_{EC})/A_S] \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*
A_{EC} = absorbance of the *Empty capsules solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of ▲USP Hydrochlorothiazide RS▲ (USP 1-May-2019) in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
Buffer, Mobile phase, ▲▲ (USP 1-May-2019) and **System suitability solution:** Prepare as directed in the *Assay*.
Standard stock solution: 0.25 mg/mL ▲of USP Hydrochlorothiazide RS▲ (USP 1-May-2019) prepared as follows. ▲To a suitable amount of USP Hydrochlorothiazide RS in a suitable volumetric flask add acetonitrile to 10% of the final volume, and sonicate to dissolve. Dilute with *Buffer* to volume.▲ (USP 1-May-2019)
Standard solution: 0.25 µg/mL of USP Hydrochlorothiazide RS in *Mobile phase* from the *Standard stock solution*
Sample solution: Use the *Sample stock solution* as prepared in the *Assay*.
▲Chromatographic system: Proceed as directed in the *Assay* except for the *Run time*.
Run time: NLT 4 times the retention time of hydrochlorothiazide▲ (USP 1-May-2019)
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for the relative retention times for chlorothiazide and hydrochlorothiazide.]
Suitability requirements
Resolution: NLT 2.0 between chlorothiazide and hydrochlorothiazide, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
▲▲ (USP 1-May-2019)
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ▲benzothiadiazine related compound A and any unspecified impurity▲ (USP 1-May-2019) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of ▲benzothiadiazine related compound A or any unspecified impurity▲ (USP 1-May-2019) from the *Sample solution*
r_S = peak response of hydrochlorothiazide from the *Standard solution*
C_S = concentration of ▲USP Hydrochlorothiazide RS▲ (USP 1-May-2019) in the *Standard solution* (µg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: ▲▲ (USP 1-May-2019) See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A[aa]▲ (USP 1-May-2019)	0.65	0.61	1.0
Chlorothiazide▲[b]▲ (USP 1-May-2019)	0.80	—	—▲▲ (USP 1-May-2019)
Hydrochlorothiazide	1.0	1.0	—
5-Chlorohydrochlorothiazide[b,c]	2.88	—	—▲▲ (USP 1-May-2019)

www.webofpharma.com

Table 1 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any ▲unspecified impurity▲ (USP 1-May-2019)	—	1.0	0.2
Total impurities▲[d]▲ (USP 1-May-2019)	—	—	1.5

▲[a] 4-Amino-6-chloro-1,3-benzenedisulfonamide.▲ (USP 1-May-2019)
▲[b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities for the drug product.▲ (USP 1-May-2019)
▲[c] 5,6-Dichloro-3,4-dihydro-2*H*-benzothiadiazine-7-sulfonamide 1,1-dioxide▲ (USP 1-May-2019)
[d] Total impurities include benzothiadiazine related compound A and all ▲unspecified impurities.▲ (USP 1-May-2019)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

Change to read:

- **LABELING:** ▲▲ (USP 1-May-2019) The labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Chlorothiazide RS
  ▲▲ (USP 1-May-2019)
  USP Hydrochlorothiazide RS

Add the following:

▲Hydrochlorothiazide Compounded Oral Suspension

DEFINITION

Hydrochlorothiazide Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

Prepare Hydrochlorothiazide Compounded Oral Suspension 2.5 mg/mL and 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

For Hydrochlorothiazide Compounded Oral Suspension Containing 2.5 mg/mL

Hydrochlorothiazide powder	0.25 g
Ora-Blend,[a] a sufficient quantity to make	100 mL

[a] Perrigo, Allergan, MI.

For Hydrochlorothiazide Compounded Oral Suspension Containing 10 mg/mL

Hydrochlorothiazide powder	1 g
Ora-Blend,[a] a sufficient quantity to make	100 mL

[a] Perrigo, Allergan, MI.

Place the *Hydrochlorothiazide powder* in a suitable container and triturate to a fine powder. Add a small amount of *Ora-Blend* and mix well to form a smooth paste. Add a sufficient amount of *Ora-Blend* to make the contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the remainder of the *Ora-Blend*. Add sufficient *Ora-Blend* to bring to final volume. Shake to mix well.

ASSAY

- **PROCEDURE**

Solution A: 80 mM of monobasic sodium phosphate monohydrate adjusted with phosphoric acid to a pH of 2.9
Solution B: Methanol and water (20:80)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
6	80	20
6.5	50	50
11.5	50	50
12	80	20
17	80	20

Standard solution: Transfer 20 mg of USP Hydrochlorothiazide RS to a 200-mL volumetric flask. Add 40 mL of methanol and sonicate for 20 min. Dilute with water to volume and mix.

Sample solution
For Oral Suspension 2.5 mg/mL: Transfer 2 mL of Oral Suspension to a 50-mL volumetric flask, add 10 mL of methanol, and sonicate for 20 min. Dilute with water to volume and mix. Centrifuge an aliquot for 10 min and transfer the supernatant to an HPLC vial.
For Oral Suspension 10 mg/mL: Transfer 1 mL of Oral Suspension to a 100-mL volumetric flask, add 20 mL of methanol, and sonicate for 20 min. Dilute with water to volume and mix. Centrifuge an aliquot for 10 min and transfer the supernatant to an HPLC vial.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 227 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 4°
Column: 25°
Flow rate: 2.0 mL/min
Injection volume: 15 µL

System suitability
Sample: *Standard solution*
[NOTE—The retention time for hydrochlorothiazide is about 4.8 min.]
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0% for replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak response of hydrochlorothiazide from the *Sample solution*
r_S = peak response of hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
• **pH** ⟨791⟩**:** 3.7–4.7

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
• **Beyond-Use Date:** NMT 90 days after the date on which it was compounded, when stored in a refrigerator or at controlled room temperature
• **Labeling:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date.*
• **USP Reference Standards** ⟨11⟩
USP Hydrochlorothiazide RS▲ (USP 1-May-2020)

Hydrochlorothiazide Tablets

DEFINITION
Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION
• **A. Infrared Absorption**
Sample: Transfer an amount, equivalent to 50 mg of hydrochlorothiazide from finely powdered Tablets, to a 50-mL volumetric flask. Add 20 mL of sodium hydroxide solution (1 in 125), and shake vigorously for 15 min. Dilute with the same solvent to volume, mix, and filter, discarding the first few mL of the filtrate. Transfer 5 mL of the filtrate to a 125-mL separator, and add 5 mL of hydrochloric acid (1 in 10). Extract with 50 mL of ether, pass the ether extract through a small, dry, folded filter paper, and evaporate to dryness. Add 5 mL of alcohol, and again evaporate to dryness.
Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue obtained from the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydrochlorothiazide RS previously dissolved in alcohol and recovered by evaporating the solution to dryness.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
• **Procedure**
Mobile phase: Acetonitrile and 0.1 M monobasic sodium phosphate (1:9). Adjust with phosphoric acid to a pH of 3.0 ± 0.1, and filter.
System suitability solution: 0.15 mg/mL of chlorothiazide and 0.15 mg/mL of USP Hydrochlorothiazide RS in *Mobile phase.* [Note—A volume of acetonitrile not exceeding 10% of the total volume of solution may be used to dissolve the USP Reference Standard.]
Standard solution: 0.15 mg/mL of USP Hydrochlorothiazide RS in *Mobile phase*
Sample solution: Nominally 0.15 mg/mL of hydrochlorothiazide prepared as follows. Equivalent to 30 mg of hydrochlorothiazide from finely powdered Tablets (NLT 20) in a 200-mL volumetric flask. Add 20 mL of *Mobile phase,* sonicate for 5 min, and add 20 mL of acetonitrile. Sonicate for 5 min, add 50 mL of *Mobile phase,* and shake by mechanical means for 10 min. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 10 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for chlorothiazide and hydrochlorothiazide are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between chlorothiazide and hydrochlorothiazide, *System suitability solution*
Relative standard deviation: NMT 1.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Dissolution** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 60 min
Standard solution: USP Hydrochlorothiazide RS at a known concentration in *Medium*
Sample solution: Pass a portion of solution under test through a suitable filter. Dilute with *Medium* as necessary in comparison with the *Standard solution.*
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: About 272 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 60% (*Q*) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES
• **Organic Impurities**
Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*

www.webofpharma.com

Standard solution: 1.5 µg/mL of USP Benzothiadiazine Related Compound A RS in *Mobile phase*. [NOTE—A volume of acetonitrile not exceeding 10% of the total volume of the solution may be used to dissolve the USP Reference Standard.]

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 1.0%

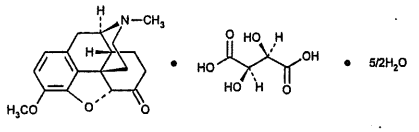
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73

USP Hydrochlorothiazide RS

Hydrocodone Bitartrate



$C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$ 494.49

Morphinan-6-one, 4,5-epoxy-3-methoxy-17-methyl-, (5α)-, [*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1), hydrate (2:5).

4,5α-Epoxy-3-methoxy-17-methylmorphinan-6-one tartrate (1:1) hydrate (2:5) [34195-34-1; 6190-38-1].

Anhydrous 449.46

[143-71-5].

» Hydrocodone Bitartrate, dried in vacuum at 105° for 2 hours, contains not less than 98.0 percent and not more than 102.0 percent of $C_{18}H_{21}NO_3 \cdot C_4H_6O_6$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—

USP Dihydrocodeine Bitartrate RS

USP Hydrocodone Bitartrate RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)

Solution: 100 µg per mL.

Medium: 0.1 N sulfuric acid.

Specific rotation ⟨781S⟩**:** between –79° and –84°.

Test solution: 20 mg, undried, per mL, in water. Calculate the result on the basis of the undried aliquot.

pH ⟨791⟩**:** between 3.2 and 3.8, in a solution (1 in 50).

Loss on drying—Dry it in vacuum at 105° for 2 hours [NOTE—See the *Note* in the *Assay* for precautions regarding handling of the dried material.] : it loses not less than 7.5% and not more than 12.0% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Chloride—To 10 mL of a solution (1 in 100), acidified with nitric acid, add a few drops of silver nitrate TS: no opalescence is produced immediately.

Assay— [NOTE—Dry both the USP Hydrocodone Bitartrate RS and the Hydrocodone Bitartrate materials in vacuum at 105° for 2 hours. Immediately transfer the dried materials to a desiccator containing phosphorus pentoxide. Weigh each dried material individually within 1 minute, and proceed with the *Assay*.]

Mobile phase—Prepare a mixture of acetonitrile, water, and diethylamine (800:4:1). Prepare a filtered and degassed mixture of this solution and methanol (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 10 mg of previously dried USP Hydrocodone Bitartrate RS, accurately weighed, to a 10-mL volumetric flask, add 5 mL of water, and mix to dissolve. Dilute with methanol to volume, and mix to obtain a solution having a known concentration of about 1 mg per mL.

Assay preparation—Transfer an accurately weighed quantity of previously dried Hydrocodone Bitartrate, equivalent to about 100 mg of hydrocodone bitartrate, $C_{18}H_{21}NO_3 \cdot C_4H_6O_6$, to a 100-mL volumetric flask, add 50 mL of water, and mix to dissolve. Dilute with methanol to volume, and mix.

Resolution solution—Prepare a solution in methanol containing about 0.4 mg of USP Dihydrocodeine Bitartrate RS and 0.6 mg of USP Hydrocodone Bitartrate RS per mL. Prepare a mixture of this solution and water (1:1).

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains packing L3. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure:* the relative retention times are about 0.7 for hydrocodone and 1.0 for dihydrocodeine; and the resolution, *R*, between hydrocodone and dihydrocodeine is not less than 3.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{21}NO_3 \cdot C_4H_6O_6$ in the portion of Hydrocodone Bitartrate taken by the formula:

$$(100C)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Hydrocodone Bitartrate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

www.webofpharma.com

Hydrocodone Bitartrate Tablets

» Hydrocodone Bitartrate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocodone bitartrate disesquihydrate ($C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Hydrocodone Bitartrate RS
Identification—Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Phosphate buffer–bromothymol blue solution—Dissolve 3.40 g of monobasic sodium phosphate in about 50 mL of water, add 18 mL of 0.1 N sodium hydroxide, dilute with water to 500 mL, and mix. Adjust the solution, if necessary, with 1 N sodium hydroxide or 1 N phosphoric acid (*Buffer solution*) to a pH of 5.8 ± 0.1. Mix 31.2 mg of bromothymol blue with 1.0 mL of 0.1 N sodium hydroxide, add *Buffer solution* to obtain 500 mL of solution, and mix.
Standard solution—Dissolve an accurately weighed quantity of USP Hydrocodone Bitartrate RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 10 µg per mL.
Test solution—Pipet into a 125-mL separator a volume of a filtered portion of the solution under test that is estimated to contain about 0.4 mg of hydrocodone bitartrate. Add 2 drops of 6 N ammonium hydroxide, and extract with three 25-mL portions of chloroform. Filter the extracts through about 3 g of anhydrous sodium sulfate supported on filter paper, and combine the filtered extracts in a 100-mL volumetric flask. Add chloroform through the filter to volume, and mix.
Procedure—Pipet 10 mL of the *Standard solution* into a 125-mL separator, and add 25.0 mL of chloroform. Pipet 25 mL of the *Test solution* into a second 125-mL separator, and add 10.0 mL of water. Pipet 25 mL of chloroform and 10 mL of water into a third 125-mL separator to provide a blank. Treat each mixture as follows. Add 30 mL of *Phosphate buffer–bromothymol blue solution*, and shake vigorously for not less than 15 minutes. Allow the layers to separate, and pass the chloroform layer through filter paper, discarding the first 10 mL of the filtrate. Determine the absorbances of the clear filtrates in 3-cm cells at the wavelength of maximum absorbance at about 415 nm, using the solution from the blank to set the spectrometer. Calculate the amount of $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$ dissolved by the formula:

$$(494.49/449.46)100(C_S/V_U)(A_U/A_S)$$

in which 494.49 and 449.46 are the molecular weights of the hydrated and anhydrous forms of hydrocodone bitartrate, respectively; C_S is the concentration, in µg per mL, of USP Hydrocodone Bitartrate RS in the *Standard solution*; V_U is the volume of the filtered solution under test that is estimated to contain 0.4 mg of hydrocodone bitartrate; and A_U and A_S are the absorbances of the solutions from the *Test solution* and the *Standard solution*, respectively.
Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Transfer 1 finely powdered Tablet to a 50-mL volumetric flask, add 0.1 N sulfuric acid to volume, and mix. Filter if necessary, discarding the first 20 mL of the filtrate. Concomitantly determine the absorbances of this solution and a solution of USP Hydrocodone Bitartrate RS in the same medium, having a known concentration of about 100 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 280 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid as the blank. Calculate the quantity, in mg, of $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2\ ½H_2O$ in the Tablet taken by the formula:

$$(494.49/449.46)(TC/D)(A_U/A_S)$$

in which 494.49 and 449.46 are the molecular weights of the hydrated and anhydrous forms of hydrocodone bitartrate, respectively; T is the labeled quantity, in mg, of hydrocodone bitartrate in the Tablet; C is the concentration, in µg per mL, of USP Hydrocodone Bitartrate RS in the Standard solution; D is the concentration, in µg per mL, of the solution from the Tablet, based upon the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.
Assay—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of hydrocodone bitartrate, to a 250-mL separator with the aid of 10 mL of water. Add a small piece of red litmus paper, then add, dropwise, 6 N ammonium hydroxide until the litmus paper turns blue (about 3 drops). Extract with 25-, 25-, 20-, 20-, 15-, and 15-mL portions of chloroform, and filter the chloroform extracts through a small pledget of cotton into a 250-mL conical flask. Evaporate the combined chloroform extracts almost to dryness, remove the flask from the steam bath, and evaporate the remainder of the chloroform with the aid of a current of air. Dissolve the residue in 80 mL of glacial acetic acid, warming, if necessary. Cool, and titrate with 0.02 N perchloric acid VS, determining the endpoint potentiometrically (see *Titrimetry* ⟨541⟩). Perform a blank determination, and make any necessary correction. Each mL of 0.02 N perchloric acid is equivalent to 9.890 mg of $C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$.

Hydrocodone Bitartrate and Acetaminophen Tablets

» Hydrocodone Bitartrate and Acetaminophen Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of hydrocodone bitartrate disesquihydrate ($C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$) and acetaminophen ($C_8H_9NO_2$).

Packaging and storage—Preserve in tight, light-resistant containers.
Labeling—The labeling indicates the *Dissolution* test with which the product complies.
USP Reference standards ⟨11⟩—
USP Acetaminophen RS
USP Hydrocodone Bitartrate RS

www.webofpharma.com

Identification—

A: Finely powder 1 Tablet, and transfer about half of the powder to a test tube. Add 1 mL of 1 N sodium hydroxide and 10 mL of water, and centrifuge. Add 5 or 6 drops of ferric chloride TS: a deep blue color develops, and almost immediately a gray-black precipitate forms *(presence of acetaminophen).*

B: The retention times of the hydrocodone bitartrate peak and the acetaminophen peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—

TEST 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1.*

Medium: pH 5.8 ± 0.05 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Proceed as directed in the *Assay,* making any necessary modifications.

Tolerances—Not less than 80% *(Q)* each of the labeled amounts of acetaminophen ($C_8H_9NO_2$) and hydrocodone bitartrate ($C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$) are dissolved in 30 minutes.

TEST 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2.*

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus, Time, and *Procedure*—Proceed as directed under *Test 1.*

Tolerances—Not less than 80% *(Q)* each of the labeled amounts of acetaminophen ($C_8H_9NO_2$) and hydrocodone bitartrate ($C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$) is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Assay—

Buffer solution—Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (85:15), and add 0.2 mL of triethylamine per L of *Mobile phase.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Hydrocodone bitartrate standard stock preparation—Dissolve an accurately weighed quantity of USP Hydrocodone Bitartrate RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 35 µg per mL.

Standard preparation—Transfer about 38 mg of USP Acetaminophen RS, accurately weighed, to a 50-mL volumetric flask. Add an accurately measured volume of *Hydrocodone bitartrate standard stock preparation* containing about 38000*J* µg of USP Hydrocodone Bitartrate RS, *J* being the ratio of the labeled amount, in mg, of hydrocodone bitartrate to that of acetaminophen per Tablet, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.76 mg of USP Acetaminophen RS per mL and about 760*J* µg of USP Hydrocodone Bitartrate RS per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 76 mg of acetaminophen, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Pass a portion of this mixture through a membrane having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a detector set at 210 nm for hydrocodone bitartrate and 295 nm for acetaminophen and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for acetaminophen and 2.0 for hydrocodone; the resolution, *R,* between hydrocodone and acetaminophen is not less than 5.0; the tailing factor for the hydrocodone peak is not more than 1.6; and the relative standard deviation for replicate injections determined from both acetaminophen and hydrocodone is not more than 2.0% each.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. From the responses obtained at 210 nm, calculate the quantity, in mg, of hydrocodone bitartrate ($C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$) in the portion of Tablets taken by the formula:

$$(494.49/449.46)(0.1C)(r_U/r_S)$$

in which 494.49 is the molecular weight of hydrocodone bitartrate disesquihydrate; 449.46 is the molecular weight of anhydrous hydrocodone bitartrate; *C* is the concentration, in µg per mL, of USP Hydrocodone Bitartrate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively. From the responses obtained at 295 nm, calculate the quantity, in mg, of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Hydrocodone Bitartrate and Homatropine Methylbromide Tablets

DEFINITION

Hydrocodone Bitartrate and Homatropine Methylbromide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of hydrocodone bitartrate disesquihydrate ($C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$) and homatropine methylbromide ($C_{17}H_{24}BrNO_3$).

[NOTE—Use of silanized autosampler vials such as dimethyldichlorosilane vials[1] is required for *Dissolution Test 1* and *Test 2,* the *Limit* tests, and the *Assay* to prevent drug degradation.]

IDENTIFICATION

- **A.** The UV absorption spectra of the hydrocodone bitartrate and homatropine methylbromide peaks of the *Sample solution* and those of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay.*
- **B.** The retention times of the hydrocodone bitartrate and homatropine methylbromide peaks of the *Sample solution* correspond to those of the *Standard solution,* as obtained in the *Assay.*

[1] A suitable grade is available from Analytical Research and Testing, Somerville, NJ; Fax: 908-725-8848.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: 0.005 M dibasic potassium phosphate. Adjust with phosphoric acid to a pH of 6.4 ± 0.01.

Mobile phase: Acetonitrile and *Buffer* (30:170)

Standard solution: 0.2 mg/mL of USP Hydrocodone Bitartrate RS and 0.06 mg/mL of USP Homatropine Methylbromide RS in *Mobile phase*

Sample solution: Nominally 0.2 mg/mL of hydrocodone bitartrate and 0.06 mg/mL of homatropine methylbromide prepared as follows. Transfer a portion of fine powder from NLT 20 Tablets to a suitable volumetric flask. Add 60% of the final volume of *Mobile phase*, sonicate for 15 min, and then shake with a wrist-action shaker for an additional 15 min. Dilute with *Mobile phase* to volume. Pass the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

Mode: LC

Detectors

Assay: UV 230 nm

Identification test A: Diode array, UV 200–400 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for homatropine methylbromide and hydrocodone bitartrate are about 0.44 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between hydrocodone bitartrate and homatropine methylbromide

Relative standard deviation: NMT 3.0% for each analyte

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of homatropine methylbromide ($C_{17}H_{24}BrNO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of homatropine methylbromide from the *Sample solution*

r_S = peak response of homatropine methylbromide from the *Standard solution*

C_S = concentration of USP Homatropine Methylbromide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of homatropine methylbromide in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of hydrocodone bitartrate disesquihydrate ($C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of hydrocodone bitartrate from the *Sample solution*

r_S = peak response of hydrocodone bitartrate from the *Standard solution*

C_S = concentration of USP Hydrocodone Bitartrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrocodone bitartrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of hydrocodone bitartrate disesquihydrate, 494.49

M_{r2} = molecular weight of anhydrous hydrocodone bitartrate, 449.46

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: Water; 900 mL, deaerated

Apparatus 2: 50 rpm

Time: 30 min

Buffer and **Mobile phase:** Prepare as directed in the *Assay*.

Standard solution: 0.0055 mg/mL of USP Hydrocodone Bitartrate RS and 0.00165 mg/mL of USP Homatropine Methylbromide RS in *Medium*

Sample solution: Pass the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system: Proceed as directed in the *Assay*, with the following exception.

Injection volume: 250 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 13 between homatropine methylbromide and hydrocodone bitartrate

Relative standard deviation: NMT 3.0% for each analyte

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of hydrocodone bitartrate disesquihydrate ($C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$) and homatropine methylbromide ($C_{17}H_{24}BrNO_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of hydrocodone bitartrate or homatropine methylbromide from the *Sample solution*

r_S = peak area of hydrocodone bitartrate or homatropine methylbromide from the *Standard solution*

C_S = concentration of USP Hydrocodone Bitartrate RS or USP Homatropine Methylbromide RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim for each drug substance (mg/Tablet)

M_{r1} = molecular weight of hydrocodone bitartrate disesquihydrate, 494.49

M_{r2} = molecular weight of anhydrous hydrocodone bitartrate, 449.46

Tolerances: NLT 80% (Q) of the labeled amounts of hydrocodone bitartrate disesquihydrate ($C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$) and homatropine methylbromide ($C_{17}H_{24}BrNO_3$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 45 min

Mobile phase: 1.4 g/L of octanesulfonic acid sodium salt and 0.1% phosphoric acid in a filtered and degassed mixture of acetonitrile and water (1:3)

Standard solution A: 0.50 mg/mL of USP Hydrocodone Bitartrate RS in *Mobile phase*

Standard solution B: 0.15 mg/mL of USP Homatropine Methylbromide RS in *Mobile phase*

www.webofpharma.com

System suitability solution: 0.01 mg/mL of USP Hydrocodone Bitartrate RS and 0.003 mg/mL of USP Homatropine Methylbromide RS prepared as follows. Transfer adequate amounts of *Standard solution A* and *Standard solution B* to a suitable volumetric flask. Add 21% of the total volume of *Mobile phase*, and dilute with *Medium* to volume.

Sample solution: Pass a 20-mL portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL. Mix thoroughly 15.0 mL of the filtrate with 5.0 mL of *Mobile phase*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 212 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2.0 mL/min
Injection volume: 200 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.2 between homatropine methylbromide and hydrocodone bitartrate
Tailing factor: NMT 1.5 for each drug substance
Relative standard deviation: NMT 3.0% for homatropine methylbromide; NMT 2.0% for hydrocodone bitartrate

Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Calculate the percentage of the labeled amounts of hydrocodone bitartrate disesquihydrate ($C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$) and homatropine methylbromide ($C_{17}H_{24}BrNO_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of hydrocodone bitartrate or homatropine methylbromide from the *Sample solution*
r_S = peak area of hydrocodone bitartrate or homatropine methylbromide from the *Standard solution*
C_S = concentration of USP Hydrocodone Bitartrate RS or USP Homatropine Methylbromide RS in the *Standard solution* (mg/mL)
D = dilution factor of the *Sample solution*
V = volume of *Medium*, 500 mL
L = label claim for each drug substance (mg/Tablet)
M_{r1} = molecular weight of hydrocodone bitartrate disesquihydrate, 494.49
M_{r2} = molecular weight of anhydrous hydrocodone bitartrate, 449.46

Tolerances: NLT 75% (Q) of the labeled amounts of hydrocodone bitartrate disesquihydrate ($C_{18}H_{21}NO_3 \cdot C_4H_6O_6 \cdot 2½H_2O$) and homatropine methylbromide ($C_{17}H_{24}BrNO_3$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meets the requirements

IMPURITIES

• **Limit of Dihydrocodeine Bitartrate, Hydrocodone Diol, and Related Substances**

Buffer: 0.005 M sodium 1-octanesulfonate. Adjust with glacial acetic acid to a pH of 2.5 ± 0.1.

Mobile phase: Methanol and *Buffer* (40:60). Add 0.5 mL/L of triethylamine.

System suitability stock solution: 0.02 mg/mL each of hydrocodone diol and USP Dihydrocodeine Bitartrate RS in *Mobile phase*

System suitability solution: 0.1 µg/mL each of hydrocodone diol and USP Dihydrocodeine Bitartrate RS in *Mobile phase* from the *System suitability stock solution*

Standard solution and **Sample solution:** Proceed as directed in the *Assay*.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 200 µL
Run time: NLT 1.7 times the retention time of hydrocodone bitartrate

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between hydrocodone diol and dihydrocodeine bitartrate
Relative standard deviation: NMT 5.0% for both hydrocodone diol and dihydrocodeine bitartrate

Analysis
Sample: *Sample solution*
Calculate the percentages of hydrocodone diol and dihydrocodeine bitartrate in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of either hydrocodone diol or dihydrocodeine bitartrate from the *Sample solution*
r_T = peak response of hydrocodone bitartrate from the *Sample solution*

Calculate the percentage of each individual related substance in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any individual related substance from the *Sample solution* with a relative retention time of NLT 0.42 in relation to the retention time of hydrocodone bitartrate
r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Hydrocodone diol[a]	0.67	0.5
Dihydrocodeine bitartrate	0.75	1.0
Hydrocodone bitartrate	1.0	—
Any individual related substance	—	0.5
Total impurities	—	1.5

[a] 4,5-Dihydroxy-3-methoxy-17-methylmorphinan-6-one.

• **Limit of Homatropine Hydrobromide and Related Substances**

Buffer: 0.005 M dibasic potassium phosphate. Adjust with phosphoric acid to a pH of 6.4 ± 0.01.

www.webofpharma.com

Mobile phase: Acetonitrile and *Buffer* (30:170). Filter and degas.
Standard solution: 0.6 µg/mL of homatropine hydrobromide in *Mobile phase*
Sample solution: Proceed as directed in the *Assay*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 1.6 times the retention time of hydrocodone bitartrate
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Sample: *Sample solution*
Calculate the percentage of homatropine hydrobromide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of homatropine hydrobromide from the *Sample solution*
r_T = peak response of homatropine methylbromide from the *Sample solution*

Calculate the percentage of each individual related substance in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any individual related substance from the *Sample solution* with a relative retention time less than 0.44 in relation to the retention time of hydrocodone bitartrate
r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

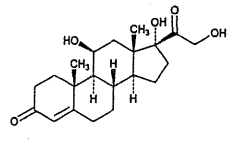
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Hydrocodone diol[a]	0.39	—
Dihydrocodeine bitartrate[b]	0.40	—
Homatropine methylbromide	0.44	—
Homatropine hydrobromide	0.53	0.5
Hydrocodone bitartrate	1.0	—
Any individual related substance	—	0.5
Total impurities[b]	—	1.5

[a] 4,5-Dihydroxy-3-methoxy-17-methylmorphinan-6-one.
[b] Impurities are quantified in the test for *Limit of Dihydrocodeine Bitartrate, Hydrocodone Diol, and Related Substances* are not included in Total impurities.

• **LIMIT OF TROPINE**
Standard stock solution: 150 µg/mL of tropine in diethyl ether
Standard solution 1: 75 µg/mL of tropine from the *Standard stock solution* in diethyl ether
Standard solution 2: 37.5 µg/mL of tropine from the *Standard solution 1* in diethyl ether
Standard solution 3: 18.75 µg/mL of tropine from the *Standard solution 2* in diethyl ether
Standard solution 4: 9.38 µg/mL of tropine from the *Standard solution 3* in diethyl ether
Sample solution: Finely powder 25 Tablets, and add to a centrifuge tube. Pipet 5.0 mL of diethyl ether into the centrifuge tube, mix on a vortex mixer for 5 min, centrifuge, and use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Application volume: 500 µL
Developing solvent system: Alcohol and ammonium hydroxide (400:100)
Spray reagent: Dissolve 300 mg of platinic acid in 3 mL of diluted hydrochloric acid. Add 97 mL of water and 100 mL of 6% potassium iodide in water, and mix.
Analysis
Samples: *Standard stock solution*, *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, *Standard solution 4*, and *Sample solution*
Apply the *Standard stock solution*, *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, *Standard solution 4*, and *Sample solution* to a TLC plate and proceed as directed in the chapter. After the plate has dried, position it in a chamber saturated with iodine vapor for about 30 min, then place it in a hood to allow the iodine to sublime from the plate, and spray the plate with *Spray reagent* until spots appear.
Acceptance criteria: Any spot from the *Sample solution* occurring at an R_F value corresponding to tropine is not greater in size or intensity than the corresponding spot from *Standard solution 2* (0.5%); NMT 0.5% of tropine.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Dihydrocodeine Bitartrate RS
USP Homatropine Methylbromide RS
USP Hydrocodone Bitartrate RS

Hydrocortisone



$C_{21}H_{30}O_5$ 362.46
Pregn-4-ene-3,20-dione, 11,17,21-trihydroxy-, (11β)-;
Cortisol [50-23-7].

DEFINITION
Hydrocortisone contains NLT 97.0% and NMT 102.0% of $C_{21}H_{30}O_5$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)

USP Monographs

www.webofpharma.com

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Analytical wavelength: 242 nm
Sample solution: 10 µg/mL in methanol
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 2.5%.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, methanol, and water (25:25:50)
Diluent: Methanol and water (1:1)
Internal standard solution: 1 mg/mL of propylparaben in methanol
Standard stock solution: 1 mg/mL of USP Hydrocortisone RS in methanol
Standard solution: 2.0 mL of *Standard stock solution* and 2.0 mL of *Internal standard solution*. Dilute with *Diluent* to 50 mL.
Sample stock solution: 1 mg/mL of Hydrocortisone in methanol
Sample solution: 2.0 mL of *Sample stock solution* and 2.0 mL of *Internal standard solution*. Dilute with *Diluent* to 50 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for hydrocortisone and propylparaben are about 1.0 and 1.8, respectively.]
Suitability requirements
Resolution: NLT 9.0 between the hydrocortisone and propylparaben peaks
Column efficiency: NLT 3000 theoretical plates for hydrocortisone
Tailing factor: NMT 1.2
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Hydrocortisone taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of hydrocortisone to the internal standard from the *Sample solution*
R_S = peak response ratio of hydrocortisone to the internal standard from the *Standard solution*
C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)
C_U = concentration of Hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION ⟨281⟩**
Sample: 100 mg
Acceptance criteria: NMT 0.5%
• **ORGANIC IMPURITIES**
Mobile phase: Butyl chloride, tetrahydrofuran, methanol, glacial acetic acid, and water (890: 56: 28: 24: 0.4)
Diluent: Butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (81.5: 10: 8: 0.5)
Standard solution: 40 µg/mL of USP Hydrocortisone RS in *Diluent*. [NOTE—Sonicate for 5 min.]
Sample solution: 2 mg/mL of Hydrocortisone in *Diluent*. [NOTE—Sonicate for 5 min.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3-µm packing L3
Flow rate: 1.5 mL/min
Injection size: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 5%
Analysis
Samples: *Standard solution*, *Sample solution*, and *Diluent*. [NOTE—Ignore artifact peaks.]
Calculate the percentage of impurities in the portion of Hydrocortisone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = individual impurity peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)
C_U = concentration of Hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: NMT 0.5%
Total impurities: NMT 2.0%

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL, in dioxane
Acceptance criteria: +150° to +156°
• **LOSS ON DRYING ⟨731⟩:** Dry a sample at 105° for 3 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS ⟨11⟩**
USP Hydrocortisone RS

Hydrocortisone Cream

DEFINITION
Hydrocortisone Cream is Hydrocortisone in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**
Sample solution: Transfer a portion of Cream, equivalent to 5 mg of hydrocortisone, to a flask. Add 5 mL of alcohol, and heat on a steam bath for 5 min, with frequent shaking. Cool, and filter. Use the filtrate.
Analysis: Proceed as directed in the chapter.
Acceptance criteria: Meets the requirements

www.webofpharma.com

ASSAY

• **PROCEDURE**

Diluent: Dilute methanol (1 in 2)

Mobile phase: Acetonitrile and water (25:75)

Standard stock solution: 500 µg/mL of USP Hydrocortisone RS in methanol

Standard solution: 50 µg/mL of USP Hydrocortisone RS prepared by mixing *Standard stock solution* and *Diluent* (1:9). [NOTE—If methanol is used in the final dilution of the *Sample solution*, similarly use methanol instead of aqueous methanol in the final dilution of the *Standard solution*.]

Sample solution: Transfer a quantity of Cream, nominally equivalent to 10 mg of hydrocortisone, to a 150-mL beaker. Add 40 mL of methanol, and heat on a steam bath while stirring to melt and disperse the Cream. Cool to room temperature, and filter through glass wool into a 100-mL volumetric flask. Repeat the extraction with two 20-mL portions of methanol, combining the filtrates in the 100-mL volumetric flask. Add methanol to volume, and mix. Quantitatively dilute one volume of this solution with an equal volume of water, and pass through a membrane filter of 5-µm pore size. If precipitation occurs on dilution with water and the solution is still cloudy after filtration, dilute the initial *Sample solution* with methanol instead of water. Pass this solution through a membrane filter of 5-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Injection volume: 10–25 µL

System suitability

[NOTE—Adjust the composition of the *Mobile phase* so that the retention time of hydrocortisone is about 10 min.]

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0% for five replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of hydrocortisone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Hydrocortisone RS

Hydrocortisone Gel

DEFINITION

Hydrocortisone Gel is Hydrocortisone in a suitable hydroalcoholic gel base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

IDENTIFICATION

Delete the following:

▲• **THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Sample solution: Transfer a quantity of Gel, equivalent to 5 mg of hydrocortisone, to a flask. Add 5 mL of alcohol, and heat on a steam bath for 5 min, with frequent shaking. Cool, and filter. Use the filtrate.

Acceptance criteria: Meets the requirements▲ (USP 1-May-2020)

Add the following:

▲• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

ASSAY

Change to read:

• ▲**PROCEDURE**

Solution A: 0.1% glacial acetic acid in water

Solution B: 0.1% glacial acetic acid in acetonitrile

Solution C: Acetonitrile, glacial acetic acid, and water (40:1:60)

Solution D: Tetrahydrofuran and water (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	74	26
11.0	74	26
20.0	55	45
30.0	30	70
30.1	74	26
35.0	74	26

Diluent: *Solution C* and *Solution D* (80:20)

System suitability solution: 0.3 mg/mL of USP Hydrocortisone RS and 6 µg/mL of USP Prednisolone RS in *Diluent*. Sonicate to dissolve, if necessary.

Standard solution: 0.3 mg/mL of USP Hydrocortisone RS in *Diluent*. Sonicate to dissolve, if necessary.

Sample solution: Nominally 0.3 mg/mL of hydrocortisone prepared as follows. Transfer 3 mg of hydrocortisone from a portion of Gel to a 10-mL volumetric flask. Add 2 mL of *Solution D* and vortex for about 3 min or until the sample is thoroughly dispersed. Add about 6 mL of *Solution C* and

www.webofpharma.com

sonicate for about 10 min with intermittent vortexing and shaking. Dilute with *Solution C* to volume. Centrifuge a portion of the solution to obtain a clear supernatant. [NOTE—The use of a centrifuge speed of 3500 rpm for 30 min may be suitable.] Use the supernatant.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 246 nm. For *Identification B*, use a diode array detector in the range of 210–400 nm.
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 30°
Flow rate: 0.8 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between hydrocortisone and prednisolone, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL) ▲(USP 1-May-2020)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**
Solution A, Solution B, Solution C, Solution D, Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Sensitivity solution: 0.3 µg/mL of USP Hydrocortisone RS in *Diluent*. Sonicate to dissolve, if necessary.
Standard solution: 0.0015 mg/mL of USP Hydrocortisone RS in *Diluent*. Sonicate to dissolve, if necessary.

System suitability
Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between hydrocortisone and prednisolone, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of cortisone or hydrocortisone glyoxal analog in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of cortisone or hydrocortisone glyoxal analog from the *Sample solution*
r_S = peak response of hydrocortisone from the *Standard solution*
C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Calculate the percentage of each unspecified impurity in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*
r_S = peak response of hydrocortisone from the *Standard solution*
C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
6β-Hydroxyhydrocortisone[a, b]	0.27	—	—
7α-Hydroxyhydrocortisone[c, b]	0.33	—	—
Hydrocortisone glyoxal analog[d]	0.81	0.89	0.5
Hydrocortisone-6-ene[e, b]	0.87	—	—
Prednisolone[b]	0.95	—	—
Hydrocortisone	1.00	—	—
Cortisone[f]	1.16	0.96	0.5
Cortodoxone[g, b]	1.79	—	—
Hydrocortisone acetate[b]	1.92	—	—
Any individual unspecified impurity	—	—	0.5
Total impurities	—	—	2.0

[a] 6β,11β,17,21-Tetrahydroxypregn-4-ene-3,20 dione.
[b] Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities of the drug product.
[c] 7α,11β,17,21-Tetrahydroxypregn-4-ene-3,20-dione.
[d] 11β,17-Dihydroxy-3,20-dioxopregn-4-en-21-al.
[e] 11β,17,21-Trihydroxypregna-4,6-diene-3,20-dione.
[f] 17,21-Dihydroxypregna-4-ene-3,11,20-trione.
[g] 17,21-Dihydroxypregn-4-ene-3,20-dione. ▲(USP 1-May-2020)

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

USP Monographs

www.webofpharma.com

SPECIFIC TESTS

Add the following:

▲• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count is NMT 10^2 cfu/g. The total yeasts and molds count is NMT 10^1 cfu/g. It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.▲ (USP 1-May-2020)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hydrocortisone RS
  ▲USP Prednisolone RS▲ (USP 1-May-2020)

Hydrocortisone Lotion

DEFINITION

Hydrocortisone Lotion is Hydrocortisone in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Standard solution: 500 µg/mL of USP Hydrocortisone RS in methanol

Sample solution: Transfer a quantity of Lotion, nominally equivalent to 5 mg of hydrocortisone, to a separator containing 10 mL of methylene chloride. Shake for 1 min, and allow the layers to separate. Filter the methylene chloride extract onto a suitable chromatographic column that has been packed with 2 g of activated magnesium silicate. Wash the column with 25 mL of methylene chloride with the aid of slight air pressure, discarding the washings, and elute the hydrocortisone with 10 mL of methanol.

Chromatographic system

Developing solvent system: Chloroform, methanol, and water (180:15:1)

Analysis: Proceed as directed in the chapter.

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (21:21:58)

Standard stock solution: 0.1 mg/mL of USP Hydrocortisone RS in alcohol

Standard solution: 0.05 mg/mL of USP Hydrocortisone RS from *Standard stock solution* in water

System suitability stock solution: 0.05 mg/mL of propylparaben in alcohol

System suitability solution: 5 µg/mL of propylparaben and 45 µg/mL of USP Hydrocortisone RS, prepared by diluting 1 mL of the *System suitability stock solution* with *Standard solution* to 10 mL

Sample stock solution: Nominally 0.1 mg/mL of hydrocortisone, prepared as follows. Shake a 100-mL portion of Lotion to ensure homogeneity, and allow to stand until the entrapped air rises. Invert carefully, and transfer a quantity of this Lotion, freshly mixed but free from air bubbles, nominally equivalent to 10 mg of hydrocortisone, to a 40-mL beaker, and add 30 mL of alcohol. Warm gently until the Lotion is dispersed, and cool to room temperature. Filter the mixture into a 100-mL volumetric flask through a pledget of cotton previously moistened with alcohol. Rinse the beaker with two 20-mL portions of alcohol, and collect the washings in the same volumetric flask. Dilute with alcohol to volume.

Sample solution: Nominally 0.05 mg/mL of hydrocortisone from *Sample stock solution* in water

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between propylparaben and hydrocortisone, *System suitability solution*
Column efficiency: NLT 1000 theoretical plates for hydrocortisone, *System suitability solution*
Tailing factor: NMT 1.2 for hydrocortisone, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Lotion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: It meets the requirements for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hydrocortisone RS

Hydrocortisone Ointment

DEFINITION

Hydrocortisone Ointment is Hydrocortisone in a suitable ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Sample solution: Transfer a portion of Ointment, equivalent to 5 mg of hydrocortisone, to a flask. Add 10 mL

www.webofpharma.com

of methanol, and heat on a steam bath for 5 min with frequent shaking. Cool to solidify the ointment base, and filter. Use the filtrate.

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Diluent: Dilute alcohol (1 in 2)

Mobile phase: Acetonitrile and water (25:75)

Standard stock solution: 500 µg/mL of USP Hydrocortisone RS in alcohol

Standard solution: 50 µg/mL of USP Hydrocortisone RS prepared by mixing *Standard stock solution* and *Diluent* (1:9). [NOTE—If alcohol is used in the final dilution of the *Sample solution*, similarly use alcohol instead of aqueous alcohol in the final dilution of the *Standard solution*.]

Sample solution: Transfer a quantity of Ointment, nominally equivalent to 10 mg of hydrocortisone, to a 150-mL beaker. Add 40 mL of alcohol, and heat on a steam bath while stirring to melt and disperse the Ointment. Cool to room temperature, and filter through glass wool into a 100-mL volumetric flask. Repeat the extraction with two 20-mL portions of alcohol, combining the filtrates in the 100-mL volumetric flask. Add alcohol to volume, and mix. Quantitatively dilute one volume of this solution with an equal volume of water, and pass through a membrane filter of 5-µm pore size. If precipitation occurs on dilution with water, and the solution is still cloudy after filtration, dilute the initial *Sample solution* with alcohol instead of water. Pass this solution through a membrane filter of 5-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Injection volume: 10–25 µL

System suitability

[NOTE—Adjust the composition of the *Mobile phase* so that the retention time of hydrocortisone is about 10 min.]

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0% for 5 replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of hydrocortisone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED ORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Hydrocortisone RS

Hydrocortisone Rectal Suspension

» Hydrocortisone Rectal Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**

USP Hydrocortisone RS

Identification, *Thin-Layer Chromatographic Identification Test* ⟨201⟩**—**

Test solution—Use the *Assay preparation*, except to omit addition of the *Internal standard solution*.

pH ⟨791⟩: between 5.5 and 7.0.

Assay—

Mobile phase—Mix 55 mL of a solution of water in methanol (5 in 100) with 1.0 mL of glacial acetic acid, dilute with water-washed 1,2-dichloroethane to 1000 mL, and mix. Degas before using. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve 200 mg of acetaminophen in 4 mL of methanol, dilute with water-washed 1,2-dichloroethane to 200 mL, and mix. Keep the solution tightly stoppered and protected from light.

Standard preparation—Accurately weigh about 8 mg of USP Hydrocortisone RS, add 4 mL of methanol and 4.0 mL of *Internal standard solution*, dilute with chloroform to 100.0 mL, and mix to obtain a solution having a known concentration of about 0.08 mg of USP Hydrocortisone RS per mL.

Assay preparation—Transfer an accurately weighed quantity of Rectal Suspension, equivalent to about 8 mg of hydrocortisone, to a separator. Extract with four 20-mL portions of chloroform, filtering each portion through chloroform-washed cotton into a 100-mL volumetric flask. Add 4 mL of methanol and 4.0 mL of *Internal standard solution*, dilute with chloroform to volume, and mix. Pass the extract through a 0.5-µm porosity polytef membrane filter, discarding the first 20 mL of the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L3. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 1.3 for acetaminophen and 1.0 for hydrocortisone; the resolution, *R*, between the analyte and internal standard is not less than 2.5; the column efficiency determined from the analyte peak is not less than 5000 theoretical plates; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Rectal Suspension taken by the formula:

www.webofpharma.com

$$100C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Hydrocortisone Tablets

DEFINITION

Hydrocortisone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197M▲** (CN 1-May-2020)

Sample: Powder a number of Tablets, equivalent to 50 mg of hydrocortisone, and digest with 15 mL of solvent hexane for 15 min. Decant the solvent hexane as completely as possible, and extract the residue first with 10 mL of solvent hexane, then with 10 mL of peroxide-free ether in the same manner as before, and discard the extracts. Digest the final residue with 25 mL of dehydrated alcohol for 15 min with frequent agitation. Filter, and evaporate the alcohol extract on a steam bath to dryness. Use the residue.

Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)

Internal standard solution: 0.06 mg/mL of USP Prednisone RS in water-saturated chloroform

Standard solution: 0.1 mg/mL of USP Hydrocortisone RS in *Internal standard solution*

Sample solution: Nominally 0.1 mg/mL of hydrocortisone from NLT 10 finely powdered Tablets, prepared as follows. Transfer a portion of the powder, equivalent to 5 mg of hydrocortisone, to a suitable container, and add 50.0 mL of the *Internal standard solution.* Shake vigorously for 30 min, and centrifuge a portion. Use the clear supernatant.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L3

Flow rate: 0.9 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between hydrocortisone and prednisone

Relative standard deviation: NMT 2.0% for four replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of hydrocortisone to the internal standard from the *Sample solution*

R_S = peak response ratio of hydrocortisone to the internal standard from the *Standard solution*

C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Hydrocortisone RS at a known concentration in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* if necessary.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: Maximum absorbance at about 248 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the amount of hydrocortisone ($C_{21}H_{30}O_5$) dissolved.

Tolerances: NLT 70% (Q) of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Mobile phase, Internal standard solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*

Sample solution: Nominally 0.1 mg/mL of hydrocortisone, prepared as follows. Transfer 1 Tablet to a suitable container, and add 0.3 mL of water directly onto the Tablet. Allow the Tablet to stand for about 5 min. Shake the container to break up the Tablet, and sonicate briefly to ensure complete disintegration. Add a few small glass beads and 50.0 mL of the *Internal standard solution* to the container. Shake the container for about 30 min. Dilute an accurately measured volume of the clear supernatant with a known, accurately measured volume of the *Internal standard solution* to obtain the desired concentration. Shake the contents of the container to mix.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) in the Tablet taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of hydrocortisone to the internal standard from the *Sample solution*

R_S = peak response ratio of hydrocortisone to the internal standard from the *Standard solution*

C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

USP Monographs

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hydrocortisone RS
USP Prednisone RS

Hydrocortisone and Acetic Acid Otic Solution

DEFINITION
Hydrocortisone and Acetic Acid Otic Solution is a solution of Hydrocortisone and Glacial Acetic Acid in a suitable nonaqueous solvent. It contains NLT 90.0% and NMT 120.0% of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$), and NLT 85.0% and NMT 130.0% of the labeled amount of acetic acid ($C_2H_4O_2$).

IDENTIFICATION
• **A.**
Analysis: Dilute 5 mL of Otic Solution with 10 mL of water, and adjust with 1 N sodium hydroxide to a pH of about 7. Add ferric chloride TS.
Acceptance criteria: A deep red color is produced, and it is destroyed by the addition of hydrochloric acid.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay* for *Acetic Acid*.
• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Hydrocortisone*.

ASSAY
• **ACETIC ACID**
Internal standard solution: Dilute 2.0 mL of anisole with methanol to 100 mL.
Standard stock solution: 20 mg/mL of glacial acetic acid in methanol
Standard solution: 10 mg/mL of glacial acetic acid in methanol, prepared as follows. Transfer a sufficient volume of the *Standard stock solution* to a volumetric flask of suitable size, add 20% of the flask volume of the *Internal standard solution*, and dilute with methanol to volume.
Sample solution: Nominally 10 mg/mL of glacial acetic acid, prepared as follows. Transfer a sufficient volume of Otic Solution to a volumetric flask of suitable size, add 20% of the flask volume of the *Internal standard solution*, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 2-mm × 1.8-m glass; packed with 20% liquid phase G35 on support S1A
Carrier gas: Nitrogen
Flow rate: 25 mL/min
Temperatures
Injection port: 180°
Detector: 220°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
115	0	115	12
115	35	190	3

Injection volume: 4 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for anisole and acetic acid are 1.0 and 1.5, respectively.]
Suitability requirements
Resolution: NLT 1.5 between anisole and acetic acid
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of acetic acid ($C_2H_4O_2$) in the portion of Otic Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of acetic acid to the internal standard from the *Sample solution*
R_S = peak response ratio of acetic acid to the internal standard from the *Standard solution*
C_S = concentration of glacial acetic acid in the *Standard solution* (mg/mL)
C_U = nominal concentration of acetic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 85.0%–130.0%
• **HYDROCORTISONE**
Diluent: Dilute alcohol (1 in 2)
Mobile phase: Acetonitrile and water (30:70)
Standard solution: 0.5 mg/mL of USP Hydrocortisone RS in *Diluent*
Sample solution: Nominally equivalent to 0.5 mg/mL of hydrocortisone from Otic Solution in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0% for four replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Otic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–120.0%

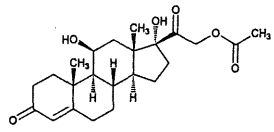
SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: Otic Solution and water (1:1)
Acceptance criteria: 2.0–4.0

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hydrocortisone RS

Hydrocortisone Acetate



$C_{23}H_{32}O_6$ 404.50
Pregn-4-ene-3,20-dione, 21-(acetyloxy)-11,17-dihydroxy-, (11β)-;
Cortisol 21-acetate [50-03-3].

DEFINITION
Hydrocortisone Acetate contains NLT 97.0% and NMT 102.0% of $C_{23}H_{32}O_6$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M▲** (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)
Analytical wavelength: 242 nm
Sample solution: 10 µg/mL in methanol
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 2.5%.

ASSAY
• **PROCEDURE**
Mobile phase: Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30)
Standard solution: 0.10 mg/mL of USP Hydrocortisone Acetate RS in *Mobile phase*
Sample solution: 0.10 mg/mL of Hydrocortisone Acetate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L3
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of hydrocortisone acetate ($C_{23}H_{32}O_6$) in the portion of Hydrocortisone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydrocortisone acetate from the *Sample solution*
r_S = peak response of hydrocortisone acetate from the *Standard solution*
C_S = concentration of USP Hydrocortisone Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Hydrocortisone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩
Sample: 100 mg
Acceptance criteria: NMT 0.5%
• **ORGANIC IMPURITIES**
Solution A: Acetonitrile and water (20:80)
Solution B: Acetonitrile and water (70:30)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
25	10	90
30	10	90
35	90	10
40	90	10

Diluent: Acetonitrile, glacial acetic acid, and water (700:1:300)
Standard solution: 5 µg/mL of USP Hydrocortisone Acetate RS in *Diluent*
Sample solution: 1 mg/mL of Hydrocortisone Acetate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Hydrocortisone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydrocortisone Acetate RS in the *Standard solution* (mg/mL)
C_U = concentration of Hydrocortisone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: NMT 1.0%
Total impurities: NMT 2.0%

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL in dioxane
Acceptance criteria: Between +158° and +167° at 20°

USP Monographs

www.webofpharma.com

• **LOSS ON DRYING** ⟨731⟩**:** Dry a sample in vacuum at 60° for 3 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hydrocortisone Acetate RS
Pregn-4-ene-3,20-dione, 21-(acetyloxy)-11,17-dihydroxy-, (11β)-.
$C_{23}H_{32}O_6$ 404.50

Hydrocortisone Acetate Cream

DEFINITION
Hydrocortisone Acetate Cream is Hydrocortisone Acetate in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Standard solution: 250 µg/mL of USP Hydrocortisone Acetate RS in methanol
Sample solution: To 1 g of Cream add 40.0 mL of a 35% solution of acetonitrile in methanol, and shake until dissolved. To 20.0 mL of this solution add 10.0 mL of isooctane, and mix. Allow the layers to separate, and use the bottom layer.
Developing solvent system: Ethyl acetate, toluene, and acetone (140:40:13)
Analysis: Heat the prepared chromatographic plate at 105° for 10 min, and cool. Develop in the *Developing solvent system* in a paper-lined chromatographic chamber equilibrated in an atmosphere of ammonia vapor.
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Mobile phase: Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30)
Standard solution: 0.10 mg/mL of USP Hydrocortisone Acetate RS in *Mobile phase*
Sample solution: Transfer a quantity of Cream, nominally equivalent to 25 mg of hydrocortisone acetate, to a suitable container, add 100.0 mL of tetrahydrofuran, and shake until the Cream dissolves. Transfer 10.0 mL of the resulting solution to another container, and add 15.0 mL of *Mobile phase*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; 10-µm packing L3
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydrocortisone Acetate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hydrocortisone Acetate RS

Hydrocortisone Acetate Lotion

» Hydrocortisone Acetate Lotion is Hydrocortisone Acetate in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩**—**
USP Hydrocortisone Acetate RS
Identification—It responds to the *Identification*— test under *Hydrocortisone Acetate Ointment*.
Minimum fill ⟨755⟩: meets the requirements.

Assay—
Mobile phase—Prepare a filtered and degassed solution containing butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Hydrocortisone Acetate RS in water-saturated chloroform to obtain a solution having a known concentration of about 0.10 mg per mL.
Assay preparation—Transfer an accurately weighed quantity of Lotion, equivalent to about 2.5 mg of hydrocortisone acetate, to a closable container. Add 25.0 mL of water-saturated chloroform and about 10 glass beads. Securely close the container, and shake vigorously for approximately 15 minutes. Centrifuge, and use the clear, lower chloroform layer.
Procedure—Introduce equal volumes of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph fitted with a 254-nm detector. Typically the apparatus is fitted with a 4-mm × 30-cm column

www.webofpharma.com

containing packing L3 and operated at room temperature. Six replicate injections of the *Standard preparation* show a relative standard deviation of not more than 2.0%. Calculate the quantity, in mg, of hydrocortisone acetate ($C_{23}H_{32}O_6$) in the portion of Lotion taken by the formula:

$$25C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone Acetate RS in the *Standard preparation*; and r_U and r_S are the hydrocortisone acetate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Hydrocortisone Acetate Ointment

» Hydrocortisone Acetate Ointment is Hydrocortisone Acetate in a suitable ointment base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{23}H_{32}O_6$.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Hydrocortisone Acetate RS
Identification—Transfer a quantity of Ointment, equivalent to about 5 mg of hydrocortisone acetate, to a flask, add 5 mL of methanol, and heat on a steam bath for 5 minutes with frequent mixing. Cool to solidify the ointment base, and filter. Using the filtrate as the test solution, proceed as directed under *Thin-layer Chromatographic Identification Test* ⟨201⟩. Locate the spots by spraying the dried plate with a 70% methanolic sulfuric acid solution. Heat the plate for 20 to 30 minutes at 90°, allow to cool, and view under long-wavelength UV light: the R_F value and fluorescence of the principal spot obtained from the test solution correspond to those obtained from the Standard solution.
Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
Minimum fill ⟨755⟩: meets the requirements.
Assay—Proceed with Ointment as directed in the *Assay* under *Hydrocortisone Acetate Lotion*.

Hydrocortisone Butyrate

$C_{25}H_{36}O_6$ 432.55
Pregn-4-ene-3,20-dione, 11,21-dihydroxy-17- (1-oxobutoxy)-, (11β)-;
Cortisol 17-butyrate;
11β,17,21-Trihydroxypregn-4-ene-3,20-dione 17-butyrate [13609-67-1].

DEFINITION
Hydrocortisone Butyrate contains NLT 97.0% and NMT 102.0% of hydrocortisone butyrate ($C_{25}H_{36}O_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 10 µg/mL in methanol
Analytical wavelength: 242 nm
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile, glacial acetic acid, and water (76:1:124)
Diluent A: Tetrahydrofuran and glacial acetic acid (1000:1)
Diluent B: Methanol, water, and glacial acetic acid (500:500:1)
System suitability stock solution: 0.1 mg/mL each of USP Hydrocortisone Butyrate RS and propyl 4-hydroxybenzoate in *Diluent A*
System suitability solution: 0.02 mg/mL each of USP Hydrocortisone Butyrate RS and propyl 4-hydroxybenzoate in *Diluent B*, from *System suitability stock solution*
Standard stock solution: 0.1 mg/mL of USP Hydrocortisone Butyrate RS in *Diluent A*
Standard solution: 0.02 mg/mL of USP Hydrocortisone Butyrate RS in *Diluent B*, from *Standard stock solution*
Sample stock solution: 0.1 mg/mL of Hydrocortisone Butyrate in *Diluent A*
Sample solution: 0.02 mg/mL of Hydrocortisone Butyrate in *Diluent B*, from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.0-mm × 10-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 5 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for propyl 4-hydroxybenzoate and hydrocortisone butyrate are 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4.0 between propyl 4-hydroxybenzoate and hydrocortisone butyrate, *System suitability solution*
Column efficiency: NLT 4000 theoretical plates, *Standard solution*
Tailing factor: NMT 1.6, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of hydrocortisone butyrate ($C_{25}H_{36}O_6$) in the portion of Hydrocortisone Butyrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Hydrocortisone Butyrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Hydrocortisone Butyrate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: 1 g of monobasic potassium phosphate in 1000 mL of water, adjusted with 45% potassium hydroxide to a pH of 5.5

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
12.5	35	65
15.5	35	65
20.5	80	20
22.5	80	20

Diluent: Acetonitrile and *Solution A* (80:20)

Standard solution: 0.5 mg/mL of USP Hydrocortisone Butyrate RS in *Diluent*

Sample solution: 1 mg/mL of Hydrocortisone Butyrate in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: 2 mL/min

Injection volume: 5 µL

System suitability

Samples: *Standard solution* and *Sample solution*

Suitability requirements

Resolution: NLT 1.0 between hydrocortisone butyrate and any impurity

Column efficiency: NLT 10,000 theoretical plates

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Hydrocortisone Butyrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of hydrocortisone butyrate from the *Standard solution*
C_S = concentration of USP Hydrocortisone Butyrate RS in the *Standard solution* (mg/mL)
C_U = concentration of Hydrocortisone Butyrate in the *Sample solution* (mg/mL)

Acceptance criteria

Disregard any peak having a percentage of 0.05% or less.

Any individual impurity: NMT 1.0%

Total impurities: NMT 2.0%

SPECIFIC TESTS

• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample solution: 10 mg/mL, in chloroform

Acceptance criteria: +47° to +54° at 20°

• **LOSS ON DRYING** ⟨731⟩

Sample: Dry Hydrocortisone Butyrate under vacuum at 78° for 3 h.

Acceptance criteria: NMT 1.0%

• **COMPLETENESS OF SOLUTION** ⟨641⟩

Sample solution: 1 g in 10 mL of dichloromethane

Acceptance criteria: The *Sample solution* is clear.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Hydrocortisone Butyrate RS

Hydrocortisone Butyrate Cream

DEFINITION

Hydrocortisone Butyrate Cream is Hydrocortisone Butyrate in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone butyrate ($C_{25}H_{36}O_6$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, glacial acetic acid, and water (76:1:124)

Diluent A: Tetrahydrofuran and glacial acetic acid (1000:1)

Diluent B: Methanol, water, and glacial acetic acid (500:500:1)

System suitability stock solution: 0.1 mg/mL each of USP Hydrocortisone Butyrate RS and propyl 4-hydroxybenzoate in *Diluent A*

System suitability solution: 0.02 mg/mL each of USP Hydrocortisone Butyrate RS and propyl 4-hydroxybenzoate in *Diluent B*, from *System suitability stock solution*

Standard stock solution: 0.1 mg/mL of USP Hydrocortisone Butyrate RS in *Diluent A*

Standard solution: 0.02 mg/mL of USP Hydrocortisone Butyrate RS in *Diluent B*, from *Standard stock solution*

Sample solution: Nominally 0.02 mg/mL of hydrocortisone butyrate, prepared as follows. Transfer a sufficient quantity of Cream to a volumetric flask of suitable size, add 20% of the flask volume of *Diluent A*, shake by mechanical means for 30 min, and dilute with *Diluent B* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.0-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for propyl 4-hydroxybenzoate and hydrocortisone butyrate are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between propyl 4-hydroxybenzoate and hydrocortisone butyrate, *System suitability solution*

Column efficiency: NLT 4000 theoretical plates, *Standard solution*

www.webofpharma.com

Tailing factor: NMT 1.6, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrocortisone butyrate ($C_{25}H_{36}O_6$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydrocortisone Butyrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone butyrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **Minimum Fill** ⟨755⟩: Meets the requirements

SPECIFIC TESTS
- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **pH** ⟨791⟩: 3.5–4.5

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨11⟩
  USP Hydrocortisone Butyrate RS

Hydrocortisone Hemisuccinate

$C_{25}H_{34}O_8 \cdot H_2O$ 480.55
Pregn-4-ene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11, 17-dihydroxy-, (11β)-, monohydrate.
Cortisol 21-(hydrogen succinate) monohydrate [83784-20-7].
Anhydrous 462.54
[2203-97-6].

» Hydrocortisone Hemisuccinate contains not less than 97.0 percent and not more than 103.0 percent of $C_{25}H_{34}O_8$, calculated on the dried basis. It contains one molecule of water of hydration or is anhydrous.

Packaging and storage—Preserve in tight containers.
Labeling—Label it to indicate whether it is hydrous or anhydrous.
USP Reference standards ⟨11⟩—
USP Hydrocortisone Hemisuccinate RS
USP Fluorometholone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 20 µg per mL.
Medium: alcohol.
Absorptivities at 242 nm, calculated on the dried basis, do not differ by more than 3.0%.
Specific rotation ⟨781S⟩: between +124° and +134°.
Test solution: 10 mg per mL, in acetone.
Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: the anhydrous form loses not more than 1.0% of its weight, and the hydrous form loses not more than 4.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Chromatographic purity—
Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and methanol (700:285:15). Add 3.0 mL of glacial acetic acid per Liter of this solution. Mix thoroughly. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Diluting solution—Prepare a mixture of water, acetonitrile, tetrahydrofuran, and glacial acetic acid (500:250:250:1). Mix thoroughly.
Standard solution—Dissolve an accurately weighed quantity of USP Hydrocortisone Hemisuccinate RS in *Diluting solution*, and dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 6.6 µg per mL.
Test solution—Transfer about 6.6 mg of Hydrocortisone Hemisuccinate, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix. [Note—Samples should be maintained at 5° or colder during analysis.]
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 5000 theoretical plates; and the relative standard deviation for replicate injections is not more than 5.0%.
Procedure—Inject a volume (about 15 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Hydrocortisone Hemisuccinate taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found. Disregard any peak representing less than 0.05%.

Assay—
Internal standard solution—Prepare a solution of USP Fluorometholone RS in tetrahydrofuran containing about 3 mg per mL.
Mobile phase—Prepare a filtered mixture of butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer an accurately weighed amount of USP Hydrocortisone Hemisuccinate RS to a suitable container to obtain a solution containing 0.6 mg per mL. Add an accurately measured volume of *Internal standard solution* so that the *Standard preparation* contains 10% *Internal standard solution*. Dilute with chloroform containing 3% glacial acetic acid to volume.

USP Monographs

www.webofpharma.com

Assay preparation—Transfer about 30 mg of Hydrocortisone Hemisuccinate, accurately weighed, to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, and dilute with chloroform containing 3% glacial acetic acid to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L3. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between hydrocortisone hemisuccinate and the internal standard is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 6 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{25}H_{34}O_8$ in the portion of Hydrocortisone Hemisuccinate taken by the formula:

$$50C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Hydrocortisone Hemisuccinate RS in the *Standard preparation;* and R_U and R_S are the peak area ratios of hydrocortisone hemisuccinate to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Hydrocortisone Sodium Phosphate

$C_{21}H_{29}Na_2O_8P$ 486.40
Pregn-4-ene-3,20-dione, 11,17-dihydroxy-21-(phosphono oxy)-, disodium salt, (11β)-.
Cortisol 21-(disodium phosphate) [6000-74-4].

» Hydrocortisone Sodium Phosphate contains not less than 96.0 percent and not more than 102.0 percent of $C_{21}H_{29}Na_2O_8P$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Hydrocortisone RS
USP Hydrocortisone Phosphate Triethylamine RS
$C_{21}H_{31}O_8P \cdot C_6H_{15}N$ 543.64

Identification—
A: Evaporate 15 mL of a methylene chloride solution of it, prepared as directed under *Procedure* in the *Assay*, on a steam bath to dryness, and dissolve the residue in 1 mL of methylene chloride. Proceed as directed in *Identification* test *B* under *Hydrocortisone Sodium Phosphate Injection*, beginning with "Apply 5 µL of this solution."
B: The residue from the ignition of about 20 mg of it responds to the tests for *Phosphate* ⟨191⟩ and for *Sodium* ⟨191⟩.

Phosphate ions—
Standard phosphate solution—Dissolve 143.3 mg of dried monobasic potassium phosphate, KH_2PO_4, in water to make 1000.0 mL. This solution contains the equivalent of 0.10 mg of phosphate (PO_4) in each mL.
Phosphate reagent A—Dissolve 5 g of ammonium molybdate in 1 N sulfuric acid to make 100 mL.
Phosphate reagent B—Dissolve 350 mg of *p*-methylaminophenol sulfate in 50 mL of water, add 20 g of sodium bisulfite, mix to dissolve, and dilute with water to 100 mL.
Procedure—Dissolve about 50 mg of Hydrocortisone Sodium Phosphate, accurately weighed, in a mixture of 10 mL of water and 5 mL of 2 N sulfuric acid contained in a 25-mL volumetric flask, by warming if necessary. Add 1 mL each of *Phosphate reagent A* and *Phosphate reagent B*, dilute with water to 25 mL, mix, and allow to stand at room temperature for 30 minutes. Similarly and concomitantly, prepare a standard solution, using 5.0 mL of *Standard phosphate solution* instead of the 50 mg of the substance under test. Concomitantly determine the absorbances of both solutions in 1-cm cells at 730 nm, with a suitable spectrophotometer, using water as the blank. The absorbance of the test solution is not more than that of the standard solution. The limit is 1.0% of phosphate (PO_4).
Chloride (as NaCl)—Dissolve about 3 g, accurately weighed, in 75 mL of water, add 1 mL of nitric acid, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using a glass silver-silver chloride electrode system. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl. Not more than 1.00% of NaCl is found.
Specific rotation, pH, and free hydrocortisone—Place about 2.5 g in a tared 50-mL flask, and weigh accurately (W_U). Add 25 mL of carbon dioxide-free water, and again weigh (W_S).Calculate the quantity, in mg, of anhydrous hydrocortisone sodium phosphate in each g of solution taken by the formula:

$$1000W_U(1 - L/100)/W_S$$

in which W_U is the weight of Hydrocortisone Sodium Phosphate taken, *L* is the average percentage of *Loss on drying*, and W_S is the weight of the solution in carbon dioxide-free water. Use this as the *Test preparation* for the following tests.

Specific rotation ⟨781S⟩: between +121° and +129°, determined in a solution prepared by weighing accurately 5.0 mL of *Test preparation* and diluting with pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) to 50.0 mL.

pH ⟨791⟩: between 7.5 and 10.5, in a solution prepared by diluting a portion of *Test preparation* with 9 volumes of carbon dioxide-free water.

Free hydrocortisone—Dilute 1 mL of *Test preparation* with carbon dioxide-free water to 100 mL. Pipet 5 mL of this solution into a glass-stoppered, 50-mL tube, add 25.0 mL of methylene chloride, insert the stopper, and mix by gentle shaking. Prepare a 1 in 500,000 solution of USP Hydrocortisone RS in methylene chloride. Similarly, shake 25 mL of this solution with 5 mL of water. Allow to stand until the methylene chloride layers are clear (about 5 minutes). Determine the absorbances of the methylene chloride solutions in 1-cm cells at 239 nm, with a suitable spectrophotometer, using methylene chloride as the blank. The absorbance of the *Test preparation* does not exceed that of the Standard solution (1.0%).
Loss on drying ⟨731⟩—Dry it in vacuum at 80° for 5 hours: the average percentage weight loss for two determinations (*L*) does not exceed 5.0%.

Assay—
pH 9 buffer with magnesium—Mix 3.1 g of boric acid and 500 mL of water in a 1-liter volumetric flask, add 21 mL of 1 N sodium hydroxide and 10 mL of 0.1 M magnesium chloride, dilute with water to volume, and mix.
Alkaline phosphatase solution—Transfer 250 mg of alkaline phosphatase enzyme to a 25-mL volumetric flask, and dissolve

www.webofpharma.com

by diluting with *pH 9 buffer with magnesium* to volume. Prepare this solution fresh daily.

Standard preparation—Dissolve about 50 mg of USP Hydrocortisone Phosphate Triethylamine RS, accurately weighed, in carbon dioxide-free water to make 25.0 mL.

Assay preparation—Weigh accurately, in g, 2.0 mL of the *Test preparation*, prepared as directed under *Specific rotation, pH, and Free hydrocortisone*, into a tared 100-mL volumetric flask, and dilute with carbon dioxide-free water that has been saturated with methylene chloride to volume. Pipet 10 mL of this solution into a 125-mL separator, and extract with two 25-mL portions of water-washed methylene chloride, discarding the washings.

Procedure—Weigh accurately 1.0 mL each of the *Standard preparation* (W_S) and the *Assay preparation* (W_A) into separate tared 100-mL volumetric flasks. To each flask, and to a similar flask containing 1.0 mL of water to provide a blank, add 1.0 mL of *Alkaline phosphatase solution* and then 50 mL of methylene chloride, and insert the stopper. Allow the flasks to stand at room temperature (not below 25°) for 2 hours with gentle mixing about every 15 minutes. Add 1 mL of dilute hydrochloric acid (1 in 10) to each flask, and mix gently. Add methylene chloride to each flask until the interfaces are at the 100-mL marks, and mix gently. Remove the aqueous layers by aspiration. Determine the absorbances of the methylene chloride solutions obtained from the *Standard preparation* and the *Assay preparation* at 239 nm, with a suitable spectrophotometer, using the methylene chloride solution blank to set the instrument. Calculate the percentage of $C_{21}H_{29}Na_2O_8P$, on the dried basis, taken by the formula:

$$100(A_U/A_S)(C_S/C_A)0.895(W_S/W_A)$$

in which A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively; C_A and C_S are the corresponding concentrations, in mg per mL, of those preparations; and 0.895 is the ratio of the molecular weight of hydrocortisone sodium phosphate to that of hydrocortisone phosphate triethylamine.

Hydrocortisone Sodium Phosphate Injection

» Hydrocortisone Sodium Phosphate Injection is a sterile, buffered solution of Hydrocortisone Sodium Phosphate in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

USP Reference standards ⟨11⟩—
USP Hydrocortisone RS
USP Hydrocortisone Phosphate Triethylamine RS
$C_{21}H_{31}O_8P \cdot C_6H_{15}N$ 543.64

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—

Solution: 20 µg per mL, USP Hydrocortisone Phosphate Triethylamine RS being used to prepare the Standard solution.

Medium: water.

B: Place 5 mL of the *Assay preparation*, obtained as directed in the *Assay*, in a glass-stoppered, 50-mL tube, and add 5 mL of a solution prepared by dissolving 50 mg of alkaline phosphatase enzyme in 50 mL of *pH 9 buffer with magnesium* prepared as directed in the *Assay* under *Hydrocortisone Sodium Phosphate*. Allow to stand at room temperature for 2 hours, with occasional mixing, and extract with 25 mL of methylene chloride. Evaporate 15 mL of the methylene chloride extract on a steam bath to dryness, and dissolve the residue in 0.5 mL of methylene chloride. Apply 5 µL of this solution and 5 µL of a solution of USP Hydrocortisone RS in methylene chloride containing 300 µg per mL to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a tank completely lined with filter paper, using a solvent system consisting of a mixture of 50 parts of chloroform, 50 parts of acetone, and 1 part of water, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, mark the solvent front, and dry. Spray the plate with dilute sulfuric acid (1 in 2), and heat at 105° until brown or black spots appear: the R_F value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.25 USP Endotoxin Units per mg of hydrocortisone.

pH ⟨791⟩: between 7.5 and 8.5.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Phenylhydrazine hydrochloride solution—Dissolve 65 mg of phenylhydrazine hydrochloride in 100 mL of dilute sulfuric acid (3 in 5), add 50 mL of isopropyl alcohol, and mix. Prepare this solution fresh daily.

Standard preparation—Dissolve a suitable quantity of USP Hydrocortisone Phosphate Triethylamine RS, accurately weighed, in water, and dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 110 µg per mL.

Assay preparation—Pipet a volume of Injection, equivalent to about 100 mg of hydrocortisone sodium phosphate, into a 100-mL volumetric flask, and dilute with water to volume. Pipet 10 mL of this solution into a separator, wash the solution with two 25-mL portions of methylene chloride, and discard the washings. Transfer the aqueous layer to a 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Pipet 2 mL each of the *Standard preparation* and the *Assay preparation* into separate glass-stoppered, 50-mL conical flasks. To each flask, and to a similar flask containing 2.0 mL of water to provide a blank, add 10.0 mL of *Phenylhydrazine hydrochloride solution*, and mix. Place the flasks in a water bath maintained at a temperature of 60° for 2 hours, then cool the solutions to room temperature. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 410 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of $C_{21}H_{30}O_5$ in each mL of the Injection taken by the formula:

$$0.667(C/V)(A_U/A_S)$$

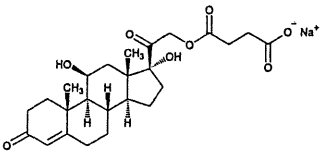
in which 0.667 is the ratio of the molecular weight of hydrocortisone to that of hydrocortisone phosphate triethylamine; C is the concentration, in µg per mL, of USP

USP Monographs

www.webofpharma.com

Hydrocortisone Phosphate Triethylamine RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Hydrocortisone Sodium Succinate



$C_{25}H_{33}NaO_8$ 484.51

Pregn-4-ene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11,17-dihydroxy-, monosodium salt, (11β)-;
Cortisol 21-(sodium succinate) [125-04-2].

DEFINITION

Hydrocortisone Sodium Succinate contains NLT 97.0% and NMT 102.0% of total steroids, calculated as hydrocortisone sodium succinate ($C_{25}H_{33}NaO_8$), on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption**

Sample: Transfer 100 mg of Hydrocortisone Sodium Succinate to a suitable container, and dissolve in 10 mL of water. In rapid succession, add 1 mL of 3 N hydrochloric acid, shake briefly, immediately decant the aqueous layer, and wash the precipitate with two additional 10-mL portions of water, each time removing the water by decanting. Remove as much of the water as possible, spread the precipitate in a suitable container, and dry under vacuum at 60° for 3 h.

Acceptance criteria: The IR spectrum of a mineral oil dispersion of the precipitate so obtained exhibits maxima only at the same wavelengths as those of a similar preparation of USP Hydrocortisone Hemisuccinate RS.

Change to read:

- **B. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: 20 µg/mL in methanol

Analytical wavelength: 242 nm

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

- **C.** Sodium compounds impart an intense yellow color to a nonluminous flame.

ASSAY

- **Assay for Steroids** ⟨351⟩

Blue tetrazolium solution: 5 mg/mL of blue tetrazolium in alcohol

Tetramethylammonium hydroxide solution: Tetramethylammonium hydroxide TS in alcohol (1 in 10)

Standard solution: 12.5 µg/mL of USP Hydrocortisone Hemisuccinate RS in alcohol

Sample solution: 12.5 µg/mL of Hydrocortisone Sodium Succinate in alcohol

Blank solution: Alcohol

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank solution*

Transfer 20.0-mL aliquots of the *Samples* to separate glass-stoppered, 50-mL conical flasks. To each flask add 2.0 mL of *Blue tetrazolium solution*, mix, and add 4.0 mL of *Tetramethylammonium hydroxide solution*. Allow to stand in the dark for 90 min, and add 1.0 mL of glacial acetic acid. Concomitantly determine the absorbances of the solutions from the *Sample solution* and the *Standard solution* at about 525 nm, with a suitable spectrophotometer, against the blank.

Calculate the percentage of total steroids, as hydrocortisone sodium succinate ($C_{25}H_{33}NaO_8$), in the portion of Hydrocortisone Sodium Succinate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Hydrocortisone Hemisuccinate RS in the *Standard solution* (µg/mL)
C_U = concentration of Hydrocortisone Sodium Succinate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of hydrocortisone sodium succinate, 484.51
M_{r2} = molecular weight of hydrocortisone hemisuccinate, 462.53

Acceptance criteria: 97.0%–102.0% on the dried basis

SPECIFIC TESTS

- **Sodium Content**

Sample: 1 g

Analysis: Dissolve the *Sample*, with gentle heating, in 75 mL of glacial acetic acid. Add 20 mL of dioxane, then add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Each mL of 0.1 N perchloric acid is equivalent to 2.299 mg of sodium (Na).

Acceptance criteria: 4.60%–4.84% on the dried basis

- **Optical Rotation** ⟨781S⟩, *Procedures*, *Specific Rotation*

Sample solution: 10 mg/mL in alcohol

Acceptance criteria: +140° to +150°

- **Loss on Drying** ⟨731⟩

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards** ⟨11⟩

USP Hydrocortisone Hemisuccinate RS

Hydrocortisone Sodium Succinate for Injection

DEFINITION

Hydrocortisone Sodium Succinate for Injection is a sterile mixture of Hydrocortisone Sodium Succinate and suitable buffers. It may be prepared from Hydrocortisone Sodium Succinate, or from Hydrocortisone Hemisuccinate with the aid of Sodium Hydroxide or Sodium Carbonate. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) in single-compartment containers, or in the volume of solution designated on the label of containers that are constructed to hold, in separate compartments, the Hydrocortisone Sodium Succinate for Injection and a solvent.

www.webofpharma.com

IDENTIFICATION

• **A. Infrared Absorption**

Sample: Transfer a quantity of Hydrocortisone Sodium Succinate for Injection, equivalent to 100 mg of hydrocortisone sodium succinate, to a suitable container, and dissolve in 10 mL of water. In rapid succession, add 1 mL of 3 N hydrochloric acid, shake briefly, immediately decant the aqueous layer, and wash the precipitate with two additional 10-mL portions of water, each time removing the water by decanting. Remove as much of the water as possible, spread the precipitate in a suitable container, and dry under vacuum at 60° for 3 h.

Acceptance criteria: The IR spectrum of a mineral oil dispersion of the precipitate so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydrocortisone Hemisuccinate RS.

ASSAY

• **Procedure**

Mobile phase: Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)

Internal standard solution: 3 mg/mL of USP Fluorometholone RS in tetrahydrofuran

Diluent: Glacial acetic acid in chloroform (3 in 100)

Solution A: 0.30 mg/mL of USP Hydrocortisone RS in *Diluent*

Standard solution: 0.65 mg/mL of USP Hydrocortisone Hemisuccinate RS, prepared as follows. Transfer 32.5 mg of USP Hydrocortisone Hemisuccinate RS to a 50-mL volumetric flask. Add by pipet 5.0 mL of *Internal standard solution* and 5.0 mL of *Solution A*. Dilute with *Diluent* to volume.

Sample solution: Mix the constituted solutions prepared from the contents of 10 vials of Hydrocortisone Sodium Succinate for Injection. Transfer a volume, equivalent to 50 mg of hydrocortisone from the resulting constituted solution, to a suitable flask containing 10.0 mL of *Internal standard solution*, and dilute with *Diluent* to 100.0 mL. Shake thoroughly for 5 min, then allow the phases to separate, discarding the upper phase.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L3

Flow rate: 1.0 mL/min

Injection volume: 6 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between hydrocortisone hemisuccinate and the internal standard

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—The order of elution of peaks is that from the internal standard, hydrocortisone hemisuccinate, and successive smaller peaks representing free hydrocortisone and hydrocortisone 17-hemisuccinate, the relative retention times of which are about 1.0, 1.5, 2.0, and 2.5, respectively.]

Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Hydrocortisone Sodium Succinate for Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = ratio of the summation of the peak areas of hydrocortisone hemisuccinate and hydrocortisone 17-hemisuccinate to the peak area of the internal standard from the *Sample solution*

R_S = ratio of the summation of the peak areas of hydrocortisone hemisuccinate and hydrocortisone 17-hemisuccinate to the peak area of the internal standard from the *Standard solution*

C_S = concentration of USP Hydrocortisone Hemisuccinate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

M_{r1} = molecular weight of hydrocortisone, 362.46

M_{r2} = molecular weight of hydrocortisone hemisuccinate, 462.53

To this percentage add the percentage of free hydrocortisone found in the test for *Free Hydrocortisone*.

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **Free Hydrocortisone**

Analysis: Using the chromatograms obtained in the *Assay*, measure the areas of the peaks from the internal standard and free hydrocortisone.

Calculate the percentage of free hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Hydrocortisone Sodium Succinate for Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of the free hydrocortisone to the internal standard from the *Sample solution*

R_S = peak area ratio of the free hydrocortisone to the internal standard from the *Standard solution*

C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NMT 6.7% of the labeled amount of hydrocortisone

PERFORMANCE TESTS

• **Uniformity of Dosage Units** ⟨905⟩**:** Meets the requirements

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: A solution containing the equivalent of 50 mg/mL of hydrocortisone

Acceptance criteria: 7.0–8.0

• **Loss on Drying** ⟨731⟩

Sample: Dry at 105° for 3 h.

Acceptance criteria: NMT 2.0%

• **Bacterial Endotoxins Test** ⟨85⟩**:** Contains NMT 1.25 USP Endotoxin Units/mg of hydrocortisone

• **Particulate Matter in Injections** ⟨788⟩**:** Meets the requirements for small-volume injections

• **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

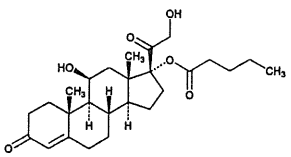
• **Sterility Tests** ⟨71⟩**:** Meets the requirements

• **Other Requirements:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
- **LABELING:** Label it to indicate that the constituted solution prepared from Hydrocortisone Sodium Succinate for Injection is suitable for use only if it is clear, and that the solution is to be discarded after 3 days. Label it to indicate that it was prepared by freeze-drying, having been filled into its container in the form of a true solution.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Fluorometholone RS
  USP Hydrocortisone RS
  USP Hydrocortisone Hemisuccinate RS

Hydrocortisone Valerate



$C_{26}H_{38}O_6$ 446.58

Pregn-4-ene-3,20-dione, 11,21-dihydroxy-17-[(1-oxopentyl) oxy]-, (11β)-;
Cortisol 17-valerate;
11β,17,21-Trihydroxypregn-4-ene-3,20-dione 17-valerate [57524-89-7].

DEFINITION
Hydrocortisone Valerate contains NLT 97.0% and NMT 102.0% of hydrocortisone valerate ($C_{26}H_{38}O_6$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile and water (45:55)
Internal standard solution: 2.0 mg/mL of ethyl benzoate in methanol
Standard stock solution: 0.5 mg/mL of USP Hydrocortisone Valerate RS in methanol. Prepare immediately before use.
Standard solution: 0.1 mg/mL of USP Hydrocortisone Valerate RS in methanol, prepared as follows. Pipet 2 mL of the *Standard stock solution* and 2 mL of the *Internal standard solution* into a 10-mL volumetric flask, and dilute with methanol to volume.
Sample stock solution: 1 mg/mL of Hydrocortisone Valerate in methanol
Sample solution: 0.1 mg/mL of Hydrocortisone Valerate in methanol, prepared as follows. Pipet 1 mL of the *Sample stock solution* and 2 mL of the *Internal standard solution* into a 10-mL volumetric flask, and dilute with methanol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ethyl benzoate and hydrocortisone valerate are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between ethyl benzoate and hydrocortisone valerate
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of hydrocortisone valerate ($C_{26}H_{38}O_6$) in the portion of Hydrocortisone Valerate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of hydrocortisone valerate to the internal standard from the *Sample solution*
R_S = peak response ratio of hydrocortisone valerate to the internal standard from the *Standard solution*
C_S = concentration of USP Hydrocortisone Valerate RS in the *Standard solution* (mg/mL)
C_U = concentration of Hydrocortisone Valerate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩

Sample: Dry at 105° for 3 h.
Acceptance criteria: NMT 1.0%
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩

Sample: 10 mg/mL in dioxane
Acceptance criteria: +37° to +43°

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hydrocortisone Valerate RS

Hydrocortisone Valerate Cream

DEFINITION
Hydrocortisone Valerate Cream is Hydrocortisone Valerate in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone valerate ($C_{26}H_{38}O_6$).

IDENTIFICATION
- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Samples: *Standard solution* and *Sample solution* from the *Assay*
Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**

Mobile phase: Acetonitrile and water (45:55)
Internal standard solution: 2.0 mg/mL of ethyl benzoate in methanol
Standard stock solution: 0.5 mg/mL of USP Hydrocortisone Valerate RS in methanol. Prepare immediately before use.

USP Monographs

www.webofpharma.com

Standard solution: 0.1 mg/mL of USP Hydrocortisone Valerate RS in methanol, prepared as follows. Pipet 2 mL of the *Standard stock solution* and 2 mL of the *Internal standard solution* into a 10-mL volumetric flask, and dilute with methanol to volume.
Sample solution: Nominally 0.1 mg/mL of hydrocortisone valerate, prepared as follows. Transfer a quantity of Cream, equivalent to 1 mg of hydrocortisone valerate, to a screw-capped tube. Add 8.0 mL of a mixture of methanol and water (3:1), and swirl to disperse. Heat at 80° for 1 min, swirl again, and allow to cool to room temperature. Add 2.0 mL of the *Internal standard solution*. Centrifuge for 5 min, and filter, if necessary, to obtain a clear supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ethyl benzoate and hydrocortisone valerate are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between ethyl benzoate and hydrocortisone valerate
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrocortisone valerate ($C_{26}H_{38}O_6$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of hydrocortisone valerate to the internal standard from the *Sample solution*
R_S = peak response ratio of hydrocortisone valerate to the internal standard from the *Standard solution*
C_S = concentration of USP Hydrocortisone Valerate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone valerate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hydrocortisone Valerate RS

Hydrocortisone Valerate Ointment

DEFINITION
Hydrocortisone Valerate Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone valerate ($C_{26}H_{38}O_6$) in a suitable ointment base.

IDENTIFICATION
• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩
Samples: *Standard solution* and *Sample solution* from the *Assay*
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Mobile phase: Acetonitrile and water (45:55)
Internal standard solution: 2.0 mg/mL of ethyl benzoate in methanol
Standard stock solution: 0.5 mg/mL of USP Hydrocortisone Valerate RS in methanol. Prepare immediately before use.
Standard solution: 0.1 mg/mL of USP Hydrocortisone Valerate RS in methanol, prepared as follows. Pipet 2 mL of the *Standard stock solution* and 2 mL of the *Internal standard solution* into a 10-mL volumetric flask, and dilute with methanol to volume.
Sample solution: Nominally 0.1 mg/mL of hydrocortisone valerate, prepared as follows. Transfer a quantity of Ointment, equivalent to 1 mg of hydrocortisone valerate, to a screw-capped tube. Add 8.0 mL of a mixture of methanol and water (3:1), and swirl to disperse. Heat in a steam bath until melted (about 30 s), swirl again, and allow to cool to room temperature. Add 2.0 mL of the *Internal standard solution*. Centrifuge for 5 min, and filter the supernatant, if necessary, to obtain a clear solution.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for ethyl benzoate and hydrocortisone valerate are about 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between ethyl benzoate and hydrocortisone valerate
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrocortisone valerate ($C_{26}H_{38}O_6$) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of hydrocortisone valerate to the internal standard from the *Sample solution*
R_S = peak response ratio of hydrocortisone valerate to the internal standard from the *Standard solution*
C_S = concentration of USP Hydrocortisone Valerate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone valerate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **MINIMUM FILL** ⟨755⟩**:** Meets the requirements

SPECIFIC TESTS
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total microbial count does not exceed 10^2 cfu/g. It meets the requirements

USP Monographs

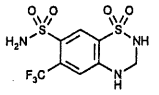
www.webofpharma.com

of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hydrocortisone Valerate RS

Hydroflumethiazide



$C_8H_8F_3N_3O_4S_2$ 331.29

2*H*-1,2,4-Benzothiadiazine-7-sulfonamide, 3,4-dihydro-6-(trifluoromethyl)-, 1,1-dioxide.

3,4-Dihydro-6-(trifluoromethyl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [135-09-1].

» Hydroflumethiazide contains not less than 98.0 percent and not more than 102.0 percent of $C_8H_8F_3N_3O_4S_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP 2,4-Disulfamyl-5-trifluoromethylaniline RS
$C_7H_8F_3N_3O_4S_2$ 319.29
USP Hydroflumethiazide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020), previously dried over silica gel for 4 hours.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 10 µg per mL.
Medium: methanol.

Melting range, *Class I* ⟨741⟩: between 270° and 275°.

pH ⟨791⟩: between 4.5 and 7.5, in a 1-in-100 dispersion in water.

Water Determination, *Method I* ⟨921⟩: not more than 1.0%.

Residue on ignition ⟨281⟩: not more than 1.0%.

Selenium ⟨291⟩: 0.003%.

Diazotizable substances—

Standard preparation—Transfer 10.0 mg of USP 2,4-Disulfamyl-5-trifluoromethylaniline RS to a 50-mL volumetric flask, dissolve in and dilute with acetone to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

Test preparation—Transfer 100 mg to a 50-mL volumetric flask, dissolve in 5 mL of acetone, dilute with water to volume, and mix.

Procedure—Transfer 5.0 mL each of the *Standard preparation*, the *Test preparation*, and a solution of acetone in water (1 in 10) to provide the blank, to separate 25-mL volumetric flasks. To each flask add 2.0 mL of dilute hydrochloric acid (1 in 5), and immediately add 1 mL of freshly prepared sodium nitrite solution (1 in 100). Mix, and allow to stand for 5 minutes. Add 1 mL of freshly prepared ammonium sulfamate solution (1 in 10) to each flask, mix, and allow to stand for 1 minute, with frequent swirling. Add 1 mL of a freshly prepared solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride (1 in 1000), and mix. After 1 minute, dilute with water to volume, and mix. Concomitantly, and within 5 minutes after mixing, taking care to establish the same elapsed time for each solution, determine the absorbances of the solutions in 1-cm cells at 518 nm, with a suitable spectrophotometer, using the blank to set the instrument: the absorbance of the solution from the *Test preparation* does not exceed that of the solution from the *Standard preparation*, corresponding to not more than 1.0% of diazotizable substances.

Assay—Transfer about 50 mg of Hydroflumethiazide, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 2.0 mL of this solution to a second 100-mL volumetric flask, dilute with methanol to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Hydroflumethiazide RS in the same medium having a known concentration of about 10 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_8H_8F_3N_3O_4S_2$ in the Hydroflumethiazide taken by the formula:

$$5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Hydroflumethiazide RS in the Standard solution; and A_U and A_S are the absorbances of the solution of Hydroflumethiazide and the Standard solution, respectively.

Hydroflumethiazide Tablets

» Hydroflumethiazide Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of hydroflumethiazide ($C_8H_8F_3N_3O_4S_2$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Hydroflumethiazide RS

Identification—Finely powder a number of Tablets, equivalent to about 100 mg of hydroflumethiazide, and place the powder in a 35-mL, screw-capped centrifuge tube. Add 30 mL of acetone, cap the tube, and allow it to stand for 30 minutes, with occasional shaking. Centrifuge, and decant the supernatant into a 100-mL beaker. Evaporate on a steam bath to dryness, add 10 mL of sodium hydroxide solution (1 in 250) to the residue, and mix. Transfer the liquid to a 125-mL separator. Rinse the beaker with 5 mL of water, and add the rinsing to the main portion. Add 50 mL of anhydrous ethyl ether to the separator, insert the stopper, shake vigorously for 2 minutes, releasing pressure as necessary, and allow the phases to separate. Draw off the lower phase, retaining any emulsion in the separator, and pass it through a membrane filter having a 0.2- to 2-µm porality. Add dilute hydrochloric acid (1 in 10) dropwise to the filtrate in a 50-mL beaker, stirring well and checking the pH with wide-range test paper after each drop. [NOTE—Crystallization begins at about pH 5. Rubbing the bottom of the beaker with a glass stirring rod helps to initiate crystallization.] When precipitation is

www.webofpharma.com

complete, decant and discard the supernatant, and wash the precipitate with 5 mL of water. Decant and discard the wash water, and dry the precipitate at 105° for 30 minutes: the IR spectrum of a potassium bromide dispersion of the dried material exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydroflumethiazide RS.

Dissolution ⟨711⟩—
Medium: dilute hydrochloric acid (1 in 100); 900 mL.
Apparatus 2: 50 rpm.
Time: 60 minutes.
Procedure—Determine the amount of $C_8H_8F_3N_3O_4S_2$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 273 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Hydroflumethiazide RS in the same medium.
Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_8H_8F_3N_3O_4S_2$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Crush 1 Tablet and quantitatively transfer to a 100-mL volumetric flask, add about 50-mL of methanol, and shake until disintegration is complete. Dilute with methanol to volume, mix, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate with methanol to obtain a solution containing approximately 10 µg of hydroflumethiazide per mL. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Hydroflumethiazide RS, in the same medium having a known concentration of about 10 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_8H_8F_3N_3O_4S_2$ in the Tablet taken by the formula:

$$(TC/D)(A_U/A_S)$$

in which T is the labeled quantity, in mg, of hydroflumethiazide in the Tablet; C is the concentration, in µg per mL, of USP Hydroflumethiazide RS in the Standard solution; D is the concentration, in µg per mL, of hydroflumethiazide in the test solution, based upon the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—
Standard preparation—Transfer about 30 mg of USP Hydroflumethiazide RS, accurately weighed, to a 100-mL volumetric flask, add sodium hydroxide solution (1 in 100) to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with sodium hydroxide solution (1 in 100) to volume, and mix. The concentration of USP Hydroflumethiazide RS in the *Standard preparation* is about 15 µg per mL.
Chromatographic column—Proceed as directed in *Chromatography* ⟨621⟩, *General Procedures, Column Chromatography*, packing a chromatographic tube with two segments of packing material. The lower segment is a mixture of 1 g of *Solid Support* and 1 mL of sodium hydroxide solution (1 in 100), and the upper segment is a mixture prepared as directed under *Assay preparation*.
Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of hydroflumethiazide, to a 50-mL volumetric flask, add about 35 mL of sodium hydroxide solution (1 in 100), shake vigorously, dilute with sodium hydroxide solution (1 in 100) to volume, and mix. Mix 2.0 mL of this solution with 3 g of *Solid Support* as directed under *Chromatographic column*, and transfer to the column. Wash the column with 50 mL of water-saturated chloroform, then with 50 mL of water-saturated ether, and discard the eluates. Elute the hydroflumethiazide from the column with 100 mL of glacial acetic acid in ether (1 in 1000), collecting the eluate in a 250-mL separator. Add 100 mL of a 1 in 1000 solution of glacial acetic acid in ether to a second 250-mL separator to provide a blank, and treat each as follows: Add 60 mL of isooctane to each separator, mix, and extract the resulting solution with three 50-mL portions of sodium hydroxide solution (1 in 100), collecting the extracts in a 200-mL volumetric flask. Dilute with sodium hydroxide solution (1 in 100) to volume, and mix.
Procedure—Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, using the blank. Calculate the quantity, in mg, of hydroflumethiazide ($C_8H_8F_3N_3O_4S_2$) in the portion of Tablets taken by the formula:

$$5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Hydroflumethiazide RS in the *Standard preparation*; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Hydrogen Peroxide Concentrate

H_2O_2 34.01
Hydrogen peroxide [7722-84-1].

DEFINITION
Hydrogen Peroxide Concentrate contains NLT 29.0% and NMT 32.0%, by weight, of hydrogen peroxide (H_2O_2). It contains NMT 0.05% of a suitable preservative or preservatives.
[**CAUTION**—Hydrogen Peroxide Concentrate is a strong oxidant.]

IDENTIFICATION
• **A.**
Sample: 1 mL of Concentrate
Analysis: Shake the *Sample* with 10 mL of water containing 1 drop of 2 N sulfuric acid, and add 2 mL of ether. Subsequently add a drop of potassium dichromate TS.
Acceptance criteria: The addition of potassium dichromate TS produces an evanescent blue color in the water layer, which upon agitation and standing passes into the ether layer.

ASSAY
• **PROCEDURE**
Sample solution: Weigh about 1 mL of Concentrate in a 100-mL volumetric flask, and dilute with water to volume.
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N potassium permanganate VS
Endpoint detection: Visual
Analysis: To 20.0 mL of the *Sample solution* add 20 mL of 2 N sulfuric acid, and titrate with *Titrant*. Each mL of 0.1 N potassium permanganate is equivalent to 1.701 mg of hydrogen peroxide (H_2O_2).
Acceptance criteria: 29.0%–32.0%, by weight

USP Monographs

www.webofpharma.com

IMPURITIES

• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample solution: 1.5 g of Concentrate, diluted to 25 mL
Acceptance criteria: 50 ppm; the *Sample solution* shows no more chloride than 0.10 mL of 0.020 N hydrochloric acid.

• **LIMIT OF NONVOLATILE RESIDUE**
Sample: 20 mL of Concentrate, previously shaken
Analysis: Evaporate the *Sample* on a steam bath to dryness, and dry the residue at 105° for 1 h.
Acceptance criteria: NMT 30 mg of residue

SPECIFIC TESTS

• **ACIDITY**
Sample solution: 25 g of Concentrate, diluted to 250 mL
Analysis: To 25 mL of the *Sample solution* add phenolphthalein TS, and titrate with 0.10 N sodium hydroxide.
Acceptance criteria: NMT 2.5 mL is required for neutralization.

• **LIMIT OF PRESERVATIVE**
Sample: 90 mL of Concentrate, well mixed
Analysis: Extract the *Sample* in a separator with a mixture of chloroform and ether (3:2), using 50, 25, and 25 mL, respectively. Evaporate the combined extracts at room temperature in a tared glass dish to dryness, and dry over silica gel for 2 h.
Acceptance criteria: 0.05%; the residue, if any, weighs NMT 50 mg.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in partially filled containers having a small vent in the closure, and store in a cool place.

• **LABELING:** Label it to indicate the name and amount of any added preservative. Label it to state that this article is not intended for direct administration to humans or animals.

Hydrogen Peroxide Topical Solution

H_2O_2 34.01
Hydrogen peroxide [7722-84-1].

DEFINITION

Hydrogen Peroxide Topical Solution contains, in each 100 mL, NLT 2.5 g and NMT 3.5 g of hydrogen peroxide (H_2O_2). It contains NMT 0.05% of a suitable preservative or preservatives.

IDENTIFICATION

• **A.**
Sample: 1 mL
Analysis: Shake the *Sample* with 10 mL of water containing 1 drop of 2 N sulfuric acid, and add 2 mL of ether. Subsequently add a drop of potassium dichromate TS.
Acceptance criteria: The addition of potassium dichromate TS produces an evanescent blue color in the water layer, which upon agitation and standing passes into the ether layer.

ASSAY

• **PROCEDURE**
Sample: 2 mL
Titrimetric system
Mode: Direct titration
Titrant: 0.1 N potassium permanganate VS
Endpoint detection: Visual
Analysis: Pipet the *Sample* into a suitable flask containing 20 mL of water. Add 20 mL of 2 N sulfuric acid, and titrate with *Titrant*. Each mL of 0.1 N potassium permanganate is equivalent to 1.701 mg of hydrogen peroxide (H_2O_2).
Acceptance criteria: 2.5–3.5 g in 100 mL

IMPURITIES

• **BARIUM**
Sample: 10 mL
Analysis: To the *Sample* add two drops of 2 N sulfuric acid.
Acceptance criteria: No turbidity or precipitate is produced within 10 min.

• **LIMIT OF NONVOLATILE RESIDUE**
Sample: 20 mL, previously shaken
Analysis: Evaporate the *Sample* on a steam bath to dryness, and dry the residue at 105° for 1 h.
Acceptance criteria: NMT 30 mg of the residue

SPECIFIC TESTS

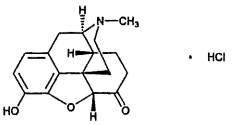
• **ACIDITY**
Sample: 25 mL
Analysis: To the *Sample* add phenolphthalein TS, and titrate with 0.10 N sodium hydroxide.
Acceptance criteria: NMT 2.5 mL is required for neutralization.

• **LIMIT OF PRESERVATIVE**
Sample: 100 mL, well mixed
Diluent: Chloroform and ether (3:2)
Analysis: Extract the *Sample* in a separator with *Diluent*, using 50, 25, and 25 mL, respectively. Evaporate the combined extracts at room temperature in a tared glass dish to dryness, and dry over silica gel for 2 h.
Acceptance criteria: 0.05%; the residue, if any, weighs NMT 50 mg.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, at controlled room temperature.

Hydromorphone Hydrochloride



$C_{17}H_{19}NO_3 \cdot HCl$ 321.80
Morphinan-6-one, 4,5-epoxy-3-hydroxy-17-methyl-, hydrochloride, (5α)-;
4,5α-Epoxy-3-hydroxy-17-methylmorphinan-6-one hydrochloride [71-68-1].

DEFINITION

Hydromorphone Hydrochloride contains NLT 98.0% and NMT 102.0% of hydromorphone hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Chloride*
Sample solution: 1 in 20
Acceptance criteria: Meets the requirements

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in 1000 mL of methanol and water (1:9). Add 1.0 mL of triethylamine and adjust with phosphoric acid to a pH of 2.5 ± 0.1.

Solution B: 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in 1000 mL of methanol and water (1:1). Add 1.0 mL of triethylamine and adjust with phosphoric acid to a pH of 2.5 ± 0.1.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	94	6
25	94	6
30	0	100
32	0	100
32.1	94	6
35	94	6

Diluent: Phosphoric acid and water (1:1000)

Standard solution: 0.4 mg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*

Sample solution: 0.4 mg/mL of Hydromorphone Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 45°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of hydromorphone hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl$) in the portion of Hydromorphone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydromorphone from the *Sample solution*

r_S = peak response of hydromorphone from the *Standard solution*

C_S = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Hydromorphone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%

[NOTE—On the basis of the synthetic route, perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*. *Organic Impurities, Procedure 2* is recommended when hydromorphone aldol dimer and 7,8′-bishydromorphone may be present.]

• **ORGANIC IMPURITIES, PROCEDURE 1**

Solution A, Solution B, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	94	6
25	94	6
40	20	80
70	20	80
75	94	6
90	94	6

System suitability solution: 0.8 mg/mL each of USP Hydromorphone Hydrochloride RS and USP Hydromorphone Related Compound A RS in *Diluent*. The solution should be kept in a cool place protected from light.

Standard solution: 4 µg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*

Sample solution: 0.8 mg/mL of Hydromorphone Hydrochloride in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between the hydromorphone related compound A and hydromorphone peaks, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Diluent*, *Standard solution*, and *Sample solution*

Calculate the percentage of any specified or unspecified impurity in the portion of Hydromorphone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual specified or unspecified impurity from the *Sample solution*

r_S = peak response of hydromorphone from the *Standard solution*

C_S = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Hydromorphone Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard peaks corresponding to those obtained from the *Diluent*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
8-Hydroxyhydromorphone[a]	0.50	1.0	0.15
Dihydromorphine (DHM)[b]	0.61	1.0	0.5
Morphine[c]	0.65	1.8	0.15
Hydromorphone *N*-oxide[d]	0.79	1.0	0.15

www.webofpharma.com

Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Hydromorphone related compound A[e]	0.93	1.4	0.1
Hydromorphone	1.0	—	—
8,14-Dihydrooripavine[f]	1.66	1.0	0.15
6β-Tetrahydrooripavine[f]	1.71	1.0	0.15
2,2′-Bishydromorphone[g]	2.02	1.7	0.15
Individual unspecified impurities	—	1.0	0.1
Total impurities	—	—	1.0

[a] 4,5α-Epoxy-17-methylmorphinan-3,8-diol-6-one.
[b] 4,5α-Epoxy-17-methylmorphinan-3,6α-diol.
[c] 7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol.
[d] 4,5α-Epoxy-3-hydroxy-17-methylmorphinan-6-one *N*-oxide.
[e] 7,8-Didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan-6-one.
[f] 8,14-Dihydrooripavine and 6β-tetrahydrooripavine are process impurities from another process and are controlled only if present.
[g] (5β)-3-Hydroxy-2-[(5α)-3-hydroxy-17-methyl-6-oxo-4,5-epoxymorphinan-2-yl]-17-methyl-4,5-epoxymorphinan-6-one.

• **ORGANIC IMPURITIES, PROCEDURE 2**
Diluent: Phosphoric acid and water (1:100)
Solution A: Mix 3520 mL of water, 18.40 g of monobasic ammonium phosphate, and 4.32 g of sodium 1-octanesulfonate. Add 4.0 mL of triethylamine, adjust with phosphoric acid to a pH of 2.90, and add 480 mL of acetonitrile.
Solution B: Acetonitrile and water (160:40)
Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	80	20
21	100	0
30	100	0

System suitability solution: 0.15 mg/mL of USP Hydromorphone Hydrochloride RS and 0.1 mg/mL of USP Morphine Sulfate RS in *Diluent*. The solution should be kept in a cool place protected from light.
Standard solution: 15 µg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*
Sample solution: 3 mg/mL of Hydromorphone Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 15-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: 30 min
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 4.0 between the morphine and hydromorphone peaks, *System suitability solution*
Tailing factor: NMT 1.5 for the hydromorphone peak, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Diluent*, *Standard solution*, and *Sample solution*
Calculate the percentage of any specified or unspecified impurity in the portion of Hydromorphone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual specified or unspecified impurity from the *Sample solution*
r_S = peak response of hydromorphone from the *Standard solution*
C_S = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Hydromorphone Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 5*)

Acceptance criteria: See *Table 5*. Disregard peaks corresponding to those obtained from the *Diluent*.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dihydromorphine (DHM)[a]	0.63	1.0	0.5
Morphine[b]	0.70	1.0	0.5
Hydromorphone	1.0	—	—
2,2′-Bishydromorphone[c]	1.82	2.0	0.5
Hydromorphone hydrochloride aldol dimer[d]	2.12	1.0	0.5
7,8′-Bishydromorphone[e]	2.32	1.0	0.5
Individual unspecified impurities	—	1.0	0.10
Total impurities	—	—	2.0

[a] 4,5α-Epoxy-17-methylmorphinan-3,6α-diol.
[b] 7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol.
[c] (5β)-3-Hydroxy-2-[(5α)-3-hydroxy-17-methyl-6-oxo-4,5-epoxymorphinan-2-yl]-17-methyl-4,5-epoxymorphinan-6-one.
[d] (5α)-7-[(5α)-3,6-Dihydroxy-17-methyl-4,5-epoxymorphinan-6-yl]-3-hydroxy-17-methyl-4,5-epoxymorphinan-6-one.
[e] (5α)-3-Hydroxy-8-[(5α)-3-hydroxy-17-methyl-6-oxo-4,5-epoxymorphinan-7-yl]-17-methyl-4,5-epoxymorphinan-6-one.

SPECIFIC TESTS
• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 50 mg/mL
Acceptance criteria: Between −136° and −139°
• **ACIDITY**
Sample: 300 mg
Analysis: Dissolve the *Sample* in 10 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide VS.
Acceptance criteria: NMT 0.30 mL is required to produce a yellow color.
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 1.5%

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **LABELING:** The labeling states with which *Organic Impurities* procedure the article complies, if other than *Procedure 1*.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hydromorphone Hydrochloride RS
  USP Hydromorphone Related Compound A RS
  7,8-Didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan-6-one perchlorate.
  $C_{17}H_{17}NO_3 \cdot HClO_4$ 383.78
  USP Morphine Sulfate RS

Hydromorphone Hydrochloride Injection

» Hydromorphone Hydrochloride Injection is a sterile solution of Hydromorphone Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of hydromorphone hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Hydromorphone Hydrochloride RS

Identification—Place a volume of Injection, equivalent to about 10 mg of hydromorphone hydrochloride, in a separator. Extract with four 10-mL portions of chloroform, and discard the extracts. Add 1 mL of sodium carbonate TS, and extract with three 10-mL portions of chloroform. Filter the chloroform extracts into a glass-stoppered, 50-mL flask, and evaporate on a steam bath with the aid of a current of air to dryness. Dissolve the residue in 1 mL of chloroform: the IR absorption spectrum of the solution so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydromorphone Hydrochloride RS.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 88.0 USP Endotoxin Units per mg of hydromorphone hydrochloride.

pH ⟨791⟩: between 3.5 and 5.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Standard preparation—Using an accurately weighed quantity of USP Hydromorphone Hydrochloride RS, prepare a solution in water having a known concentration of about 0.2 mg per mL.

Assay preparation—Quantitatively dilute an accurately measured volume of Injection, if necessary, with water to obtain a solution containing about 0.2 mg per mL.

Procedure—Transfer 20.0 mL each of the *Standard preparation* and the *Assay preparation* to separate 50-mL volumetric flasks. To each flask add, with mixing, 1.0 mL of hydrochloric acid and 1.0 mL of sodium nitrite solution (1 in 20). Insert the stoppers, allow to stand for 40 to 45 minutes, with occasional swirling, then add 2 mL of ammonium hydroxide, and mix. Allow to stand for 2 minutes, then dilute with water to volume, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 440 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of hydromorphone hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl$) in each mL of the Injection taken by the formula:

$$(V_A/V_I)(C)(A_U/A_S)$$

in which V_A is the volume, in mL, of the *Assay preparation*; V_I is the volume, in mL, of Injection taken to prepare the *Assay preparation*; C is the concentration, in mg per mL, of USP Hydromorphone Hydrochloride RS in the *Standard preparation*; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Hydromorphone Hydrochloride Oral Solution

DEFINITION

Hydromorphone Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of hydromorphone hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl$). It may contain suitable preservatives.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: Phosphoric acid and water (1:1000)

Solution A: 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in methanol and water (1:9). To each liter of this solution add 1.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.5 ± 0.1.

Solution B: 1.0 mg/mL of sodium 1-heptanesulfonate monohydrate in methanol and water (1:1). To each liter of this solution add 1.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.5 ± 0.1.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
24	5	95
25	85	15
30	85	15

The *Standard solution* and *Sample solution* should be kept in a cool place, protected from light.

Standard solution: 0.08 mg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.08 mg/mL of hydromorphone hydrochloride obtained by diluting a suitable volume of Oral Solution in *Diluent*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 5-cm; 3.5-µm packing L1
Column temperature: 45°

USP Monographs

www.webofpharma.com

Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for the hydromorphone peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydromorphone hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydromorphone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• ORGANIC IMPURITIES

Diluent, Solution A, and **Solution B:** Prepare as directed in the *Assay*.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	94	6
25	94	6
40	20	80
70	20	80
75	94	6
90	94	6

The *System suitability solution*, *Quantitation limit solution*, *Standard solution*, and *Sample solution* should be kept in a cool place, protected from light.
System suitability solution: 0.8 mg/mL of USP Hydromorphone Hydrochloride RS and 0.8 µg/mL of USP Hydromorphone Related Compound A RS in *Diluent*
Quantitation limit solution: 0.4 µg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*
Standard solution: 4 µg/mL of USP Hydromorphone Hydrochloride RS in *Diluent*
Sample solution: Nominally 0.4 mg/mL of hydromorphone hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution*, *Quantitation limit solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 1.0 between the hydromorphone related compound A and hydromorphone peaks, *System suitability solution*
Signal-to-noise ratio: NLT 10:1, *Quantitation limit solution*
Tailing factor: NMT 1.5 for the hydromorphone peak, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Diluent*, *Standard solution*, and *Sample solution*
Calculate the percentage of any specified or unspecified degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each degradation product found, including those in *Table 3*, from the *Sample solution*
r_S = peak response of hydromorphone from the *Standard solution*
C_S = concentration of USP Hydromorphone Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydromorphone hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for the corresponding individual specified or unspecified impurity (see *Table 3*)

Calculate the total degradation products by summing the percentage of all individual specified and unspecified degradation products determined to be at a level of 0.1% or greater, excluding the known process impurities, as indicated in *Table 3*.
Acceptance criteria: See *Table 3*. Disregard peaks corresponding to those from the *Diluent*, peaks that elute before a relative retention time of about 0.50, except for any peak with a relative retention time of about 0.34, and peaks that elute at the relative retention times of the process-related substances designated in *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Specified and unidentified degradation product	0.34	1.0	0.2
8-Hydroxy-hydromorphone[a, b]	0.50	—	—
Dihydromorphine (DHM)[a, c]	0.61	—	—
Morphine[a, d]	0.65	—	—
Hydromorphone *N*-oxide[e, f]	0.79	0.87	0.2
Hydromorphone	1.0	—	—
2,2′-Bis-hydromorphone dihydrochloride[e, g]	2.02	1.7	0.2
Individual unspecified degradation products	—	1.0	0.2

USP Monographs

www.webofpharma.com

Table 3 *(continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total degradation products	—	—	1.0

[a] Process impurity.
[b] 4,5α-Epoxy-17-methylmorphinan-3,8-diol-6-one.
[c] 4,5α-Epoxy-17-methylmorphinan-3,6α-diol.
[d] 7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol.
[e] Degradation product.
[f] 4,5α-Epoxy-3-hydroxy-17-methylmorphinan-6-one *N*-oxide.
[g] 2,2′-Bihydromorphone.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/mL, and the total yeasts and molds count does not exceed 10 cfu/mL. It meets the requirements of the test for the absence of *Escherichia coli*.
- **PH** ⟨791⟩**:** 3.5–6.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.
- **LABELING:** Identify in the product labeling any preservative used in the Oral Solution.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hydromorphone Hydrochloride RS
  USP Hydromorphone Related Compound A RS
  7,8-Didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan-6-one hydrochloride.
  $C_{17}H_{17}NO_3$ 319.78

Hydromorphone Hydrochloride Tablets

» Hydromorphone Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydromorphone hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Hydromorphone Hydrochloride RS
USP Morphine Sulfate RS

Identification—Place a quantity of finely powdered Tablets, equivalent to about 10 mg of hydromorphone hydrochloride, in a separator, and proceed as directed in the *Identification* test under *Hydromorphone Hydrochloride Injection*, beginning with "Extract with four 10-mL portions of chloroform".

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.

Determine the amount of $C_{17}H_{19}NO_3 \cdot HCl$ dissolved by employing the following method.

Buffer solution, *Mobile phase*, and *System suitability solution*—Proceed as directed in the *Assay*.

Test solution—Withdraw a 15-mL portion of the solution under test, filter, and discard the first few mL of the filtrate.

Standard solution—Dissolve an accurately weighed quantity of USP Hydromorphone Hydrochloride RS in water at a concentration similar to that of the *Test solution*.

Chromatographic system—Proceed as directed in the *Assay*, except to inject the *Standard solution* instead of the *Standard preparation* to obtain the relative standard deviation for replicate injections of not more than 5.0%.

Procedure—Proceed as directed in the *Assay*, except to use an injection volume of about 200 µL.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{17}H_{19}NO_3 \cdot HCl$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Buffer solution—Dissolve 10 g of sodium dodecyl sulfate and 20 mL of glacial acetic acid in 1.2 liters of water, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (33:17). Make adjustments if necessary.

System suitability solution—Dissolve suitable quantities of USP Morphine Sulfate RS and USP Hydromorphone Hydrochloride RS in water to obtain a solution containing about 30 and 40 µg of each per mL, respectively.

Standard preparation—Dissolve an accurately weighed quantity of USP Hydromorphone Hydrochloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 40 µg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer a portion of the powder, equivalent to about 4 mg of hydromorphone hydrochloride, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, sonicate if necessary, and mix. Filter a portion of the solution using a glass fiber filter, and discard the first 5 mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between morphine and hydromorphone is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor for the hydromorphone hydrochloride peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of hydromorphone hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl$) in the portion of Tablets taken by the formula:

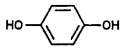
$$0.1C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Hydromorphone Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

USP Monographs

www.webofpharma.com

Hydroquinone



$C_6H_6O_2$ 110.11
1,4-Benzenediol.
Hydroquinone [123-31-9].

» Hydroquinone contains not less than 99.0 percent and not more than 100.5 percent of $C_6H_6O_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Hydroquinone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Prepare a solution of it in methanol containing approximately 1 mg per mL, and prepare a similar solution of USP Hydroquinone RS. Apply 5 µL of each solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of equal volumes of methanol and chloroform until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Heat on a hot plate or under a lamp until spots appear: the R_F value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

C: A 1 in 40,000 solution in methanol exhibits an absorbance maximum at 293 ± 2 nm.
Melting range ⟨741⟩: between 172° and 174°.
Water Determination, *Method I* ⟨921⟩: not more than 0.5%.
Residue on ignition ⟨281⟩: not more than 0.5%.
Assay—Dissolve about 250 mg of Hydroquinone, accurately weighed, in a mixture of 100 mL of water and 10 mL of 0.1 N sulfuric acid, add 3 drops of diphenylamine TS, and titrate with 0.1 N ceric sulfate VS until a red-violet endpoint is reached. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N ceric sulfate is equivalent to 5.506 mg of $C_6H_6O_2$.

Hydroquinone Cream

» Hydroquinone Cream contains not less than 94.0 percent and not more than 106.0 percent of the labeled amount of hydroquinone ($C_6H_6O_2$).

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Hydroquinone RS
Identification—Dissolve a portion of Cream equivalent to about 50 mg of hydroquinone, in a mixture of equal volumes of methanol and chloroform to make 50 mL: a 5-µL portion of this solution responds to *Identification*— test *B:* under *Hydroquinone.*
Minimum fill ⟨755⟩: meets the requirements.

Assay—
Standard preparation—Dissolve a suitable quantity of USP Hydroquinone RS in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution having a known concentration of about 10 µg per mL.
Assay preparation—Transfer an accurately weighed portion of Cream, equivalent to about 20 mg of hydroquinone, to a 100-mL beaker. Triturate the Cream with 50 mL of methanol, and pass the liquid through folded filter paper, previously washed with methanol, into a 500-mL volumetric flask. Repeat the trituration and filtration. Dilute, by washing the contents of the filter paper with methanol through the paper into the volumetric flask, to volume, and mix. Pipet 25 mL of this solution into a 100-mL volumetric flask, add methanol to volume, and mix.
Procedure—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 293 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of hydroquinone ($C_6H_6O_2$) in each g of the Cream taken by the formula:

$$2000(C/W)(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Hydroquinone RS in the *Standard preparation*; W is the weight, in g, of Cream taken; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Hydroquinone Topical Solution

» Hydroquinone Topical Solution contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of hydroquinone ($C_6H_6O_2$).

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Hydroquinone RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay.*
pH ⟨791⟩: between 3.0 and 4.2.

Assay—
Mobile phase—Mix 55 volumes of methanol and 45 volumes of water.
Standard preparation—Transfer about 250 mg of USP Hydroquinone RS, accurately weighed, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Assay preparation—Transfer an accurately measured volume of Topical Solution, equivalent to about 30 mg of hydroquinone, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing

www.webofpharma.com

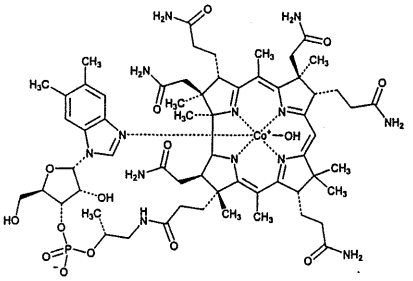
L1. The flow rate is about 0.8 mL per minute. Chromatograph three replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure:* the relative standard deviation is not more than 3.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. The retention time is about 4 minutes for hydroquinone. Calculate the quantity, in mg, of hydroquinone ($C_6H_6O_2$) in each mL of the Topical Solution taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Hydroquinone RS in the *Standard preparation*; V is the volume, in mL, of Topical Solution taken; and r_U and r_S are the peak responses of hydroquinone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Hydroxocobalamin



$C_{62}H_{89}CoN_{13}O_{15}P$ 1346.36

Cobinamide, dihydroxide, dihydrogen phosphate (ester), mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosyl-1*H*-benzimidazole;

Cobinamide dihydroxide dihydrogen phosphate (ester), mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosylbenzimidazole [13422-51-0].

DEFINITION

Hydroxocobalamin contains NLT 95% and NMT 102.0% of hydroxocobalamin ($C_{62}H_{89}CoN_{13}O_{15}P$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B. COBALT**

Sample: 1 mg of Hydroxocobalamin

Analysis: Fuse the *Sample* with 50 mg of potassium pyrosulfate in a porcelain crucible. Cool, break up the mass with a glass rod, add 3 mL of water, and boil until dissolved. Add 1 drop of phenolphthalein TS, and add 2 N sodium hydroxide dropwise until a pink color appears. Add 0.5 g of sodium acetate, 0.5 mL of 1 N acetic acid, and 0.5 mL of a 10-mg/mL solution of nitroso R salt. Add 0.5 mL of hydrochloric acid, and boil for 1 min.

Acceptance criteria: A red or orange-red color appears immediately after the addition of nitroso R salt. The red or orange-red color persists after boiling with the addition of hydrochloric acid.

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer solution: 15.6 g/L of sodium dihydrogen phosphate in water. Adjust with phosphoric acid (1 in 100) to a pH of 3.0.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Methanol (%)	Buffer solution (%)	Water (%)
0	8	10	82
20	8	10	82
40	40	10	50
45	8	10	82
50	8	10	82

Diluent: Methanol, *Buffer solution*, and water (8:10:82)

Standard solution: 0.1 mg/mL of USP Hydroxocobalamin Chloride RS in *Diluent*. Mix to homogenize.

Sample solution: 0.1 mg/mL of Hydroxocobalamin in *Diluent*. Mix to homogenize.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 351 nm

Column: 4.6-mm × 10-cm and 4.6-mm × 10-cm placed in series; packing L1[1]

Column temperature: 30°

Flow rate: 2.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time of hydroxocobalamin is about 16 min.]

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 1.0% from replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of hydroxocobalamin ($C_{62}H_{89}CoN_{13}O_{15}P$) in the portion of Hydroxocobalamin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Hydroxocobalamin Chloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Hydroxocobalamin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of hydroxocobalamin, 1346.4

M_{r2} = molecular weight of hydroxocobalamin chloride, 1382.8

[1] Chromolith columns (Merck) are based on a reversed phase employing monolithic C_{18}-bonded silica with bimodal pore structure (macropore 2 µm; mesopore 13 nm). Example: Merck Chromolith Performance RP-18 endcapped, 4.6-mm × 10-cm, product # 1021290001.

USP Monographs

www.webofpharma.com

Acceptance criteria: 95%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **RELATED COMPOUNDS**

Buffer solution, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in *Assay*.

System suitability solution: 0.75 mg/mL of USP Hydroxocobalamin Chloride RS in *Diluent*

Solution for quantitation: 7.5 µg/mL of USP Hydroxocobalamin Chloride RS in *Diluent*, diluted from the *System suitability solution*

Solution for the disregard limit: 0.75 µg/mL of USP Hydroxocobalamin Chloride RS in *Diluent*, diluted from the *Solution for quantitation*

Sample solution: 0.75 mg/mL of Hydroxocobalamin in *Diluent*

System suitability

Sample: *System suitability solution*

Suitability requirement

Peak-to-valley ratio:[2] NLT 2.0 between hydroxocobalamin and its closest impurity

Analysis

Sample: *Sample solution*

Measure the peak responses of the *Sample solution*. Disregard the peaks with a response smaller than 0.7 times that of the peak of the *Solution for the disregard limit*.

Calculate the percentage of each impurity in the portion of Hydroxocobalamin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response from the *Solution for quantitation*

C_S = concentration of USP Hydroxocobalamin Chloride RS in the *Solution for quantitation*

C_U = concentration of Hydroxocobalamin in the *Sample solution*

F = response factor, 0.8 for cyanocobalamin and 1.0 for any other impurity; *Sample solution*

M_{r1} = molecular weight of hydroxocobalamin, 1346.4

M_{r2} = molecular weight of hydroxocobalamin chloride, 1382.8

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
B6-Hydroxymethyl derivative	0.45	0.50
B5-Hydroxymethyl derivative	0.53	0.40
Impurity	0.70	0.30
Impurity	0.75	0.40

[2] Peak-to-valley ratio:

$$p/v = H_p/H_v$$

p/v = peak-to-valley ratio

H_p = height above the extrapolated baseline of the minor peak

H_v = height above the extrapolated baseline at the lowest point of the curve separating the minor and major peaks

(See *Chromatography* ⟨621⟩, *Peak-to-Valley Ratio*.) NLT 2.0 between hydroxocobalamin and its closest impurity

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Impurity	0.82	0.40
Impurity	0.93	1.1
C8 Epimer	1.25	1.0
Impurity	1.37–1.51	0.50
Cyanocobalamin	1.90	1.0
Any other impurity	—	0.20
Sum of all impurities	—	4.0

• **RESIDUAL SOLVENTS** ⟨467⟩

Acceptance criteria

Acetone: NMT 0.5%. [NOTE—For *Acceptance criteria* for any other residual solvents, see the chapter.]

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: 20 mg/mL of solution

Acceptance criteria: 8.0–10.0

• **WATER DETERMINATION,** *Method Ia* ⟨921⟩**:** 14.0%–18.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a cool, dry place. Do not freeze.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Hydroxocobalamin Chloride RS

Hydroxocobalamin Injection

» Hydroxocobalamin Injection is a sterile solution of Hydroxocobalamin in Water for Injection. It contains not less than 95.0 percent and not more than 115.0 percent of the labeled amount of hydroxocobalamin ($C_{62}H_{89}CoN_{13}O_{15}P$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—

USP Cyanocobalamin (Crystalline) RS

Identification—Dilute 3.0 mL of Injection with pH 4.0 buffer (prepared by dissolving 2.61 g of sodium acetate and 20.5 g of sodium chloride in 5.25 mL of glacial acetic acid and sufficient water to make 1500 mL of solution) to 100 mL: the UV-visible absorption spectrum of this solution exhibits maxima at 352 ± 2 nm and 525 ± 2 nm. The ratio A_{352}/A_{525} is between 2.7 and 3.3.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.4 USP Endotoxin Unit per µg of hydroxocobalamin.

pH ⟨791⟩: between 3.5 and 5.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

pH 9.3 Buffer—Dissolve 23.8 g of sodium borate and 402 mg of boric acid in sufficient water to make 1500 mL of solution, and mix.

Standard preparation—Dissolve a suitable quantity of USP Cyanocobalamin (Crystalline) RS, accurately weighed, in *pH 9.3 Buffer* and dilute quantitatively, and stepwise if necessary,

www.webofpharma.com

to obtain a solution having a known concentration of about 30 µg per mL.

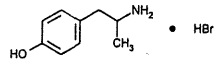
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of hydroxocobalamin, to a 50-mL volumetric flask containing about 25 mL of *pH 9.3 Buffer*. Add 5.0 mL of potassium cyanide solution (1 in 10,000), allow to stand at room temperature for 30 minutes, dilute with *pH 9.3 Buffer* to volume, and mix. Transfer 15.0 mL of this solution to a second 50-mL volumetric flask, dilute with *pH 9.3 Buffer* to volume, and mix.

Procedure—Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 361 nm, with a suitable spectrophotometer, using *pH 9.3 Buffer* as the blank. Calculate the quantity, in mg, of hydroxocobalamin ($C_{62}H_{89}CoN_{13}O_{15}P$) in each mL of the Injection taken by the formula:

$$(1346.36/1355.37)(0.1667C/V)(A_U/A_S)$$

in which 1346.36 and 1355.37 are the molecular weights of hydroxocobalamin and cyanocobalamin, respectively; C is the concentration, in µg per mL, of USP Cyanocobalamin (Crystalline) RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Hydroxyamphetamine Hydrobromide



$C_9H_{13}NO \cdot HBr$ 232.12

Phenol, 4-(2-aminopropyl)-, hydrobromide.

(±)-*p*-(2-Aminopropyl)phenol hydrobromide [306-21-8].

» Hydroxyamphetamine Hydrobromide contains not less than 98.0 percent and not more than 101.5 percent of $C_9H_{13}NO \cdot HBr$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Hydroxyamphetamine Hydrobromide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Dissolve about 500 mg of ammonium molybdate in 10 mL of sulfuric acid, and add to this solution about 2 mg of Hydroxyamphetamine Hydrobromide: an intense blue color is produced *(distinction from similar amino compounds such as amphetamine and methamphetamine, which, lacking a phenolic hydroxyl, do not undergo this reaction)*.

C: Dissolve about 200 mg in 2 mL of water, and add a solution of 500 mg of potassium carbonate in 2 mL of water. Extract with two 10-mL portions of ether, allow the clear ether solution to evaporate to dryness, and dry at about 80°: the hydroxyamphetamine so obtained melts between 124° and 127° (see *Class I* under *Melting Range or Temperature* ⟨741⟩).

D: To a solution of about 10 mg of it in 10 mL of water add 1 mL of 2 N nitric acid, then add silver nitrate TS: a pale yellow precipitate is formed, and it is slightly soluble in 6 N ammonium hydroxide.

Melting range ⟨741⟩: between 189° and 192°.

Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Bromide content—Accurately weigh about 400 mg, and dissolve in 50 mL of water. Add 50 mL of methanol and 10 mL of glacial acetic acid, then add eosin Y TS, and titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 7.990 mg of Br: the content of Br, calculated on the dried basis, is between 33.6% and 35.2%.

Ordinary impurities ⟨466⟩—
Test solution: methanol.
Standard solution: methanol.
Eluant: a mixture of toluene, methanol, and ammonium hydroxide (10: 4: 0.25).
Visualization: 1.

Assay—Dissolve about 400 mg of Hydroxyamphetamine Hydrobromide, accurately weighed, in a mixture of 10 mL of glacial acetic acid and 10 mL of mercuric acetate TS, warming slightly, if necessary, to effect solution. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 23.21 mg of $C_9H_{13}NO \cdot HBr$.

Hydroxyamphetamine Hydrobromide Ophthalmic Solution

» Hydroxyamphetamine Hydrobromide Ophthalmic Solution is a sterile, buffered, aqueous solution of Hydroxyamphetamine Hydrobromide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_9H_{13}NO \cdot HBr$. It contains a suitable antimicrobial agent.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Hydroxyamphetamine Hydrobromide RS

Identification—

A: Dissolve about 500 mg of ammonium molybdate in 10 mL of sulfuric acid, and add 0.2 mL of Ophthalmic Solution: an intense blue color is produced *(distinction from similar amino compounds such as amphetamine and methamphetamine, which, lacking a phenolic hydroxyl, do not undergo this reaction)*.

B: The dried diacetylhydroxyamphetamine obtained in the *Assay* melts between 96° and 100° (see *Class I* under *Melting Range or Temperature* ⟨741⟩), but the range between beginning and end of melting does not exceed 2.0°.

C: It responds to *Identification*— test *D:* under *Hydroxyamphetamine Hydrobromide*.

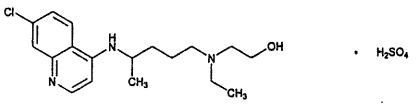
D: Dilute a volume of Ophthalmic Solution, equivalent to about 50 mg of hydroxyamphetamine hydrobromide, with 0.01 N hydrochloric acid to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩, using sodium carbonate TS in place of 1 N sodium hydroxide,

USP Monographs

www.webofpharma.com

beginning with "Transfer the liquid to a separator": the Ophthalmic Solution meets the requirements of the test.
Sterility Tests ⟨71⟩: meets the requirements.
pH ⟨791⟩: between 4.2 and 6.0.
Assay—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 100 mg of hydroxyamphetamine hydrobromide, to a 125-mL separator. Wash the solution with 15 mL of chloroform, and discard the washing. Rinse the stopper and the mouth of the separator with a few drops of water. Add 1.05 g of sodium bicarbonate, preventing it from coming in contact with the mouth of the separator, and swirl until most of the bicarbonate has dissolved. By means of a 1-mL syringe, rapidly inject 0.5 mL of acetic anhydride directly into the contents of the separator. Immediately insert the stopper in the separator, and shake vigorously until the evolution of carbon dioxide has ceased (7 to 10 minutes), releasing the pressure as necessary through the stopcock. Allow to stand for 5 minutes, and extract the solution with five 10-mL portions of chloroform, filtering each extract through a pledget of cotton, previously washed with chloroform, into a tared 100-mL beaker. Evaporate the combined chloroform extracts on a steam bath in a current of air or stream of nitrogen to dryness. Dry the residue at 80° for 90 minutes, cool in a desiccator, and weigh. The weight of the diacetylhydroxyamphetamine so obtained, multiplied by 0.9866, represents the weight of $C_9H_{13}NO \cdot HBr$ in the volume of Ophthalmic Solution taken.

Hydroxychloroquine Sulfate



$C_{18}H_{26}ClN_3O \cdot H_2SO_4$ 433.95
Ethanol, 2-[[4-[(7-chloro-4-quinolinyl)amino]pentyl]ethyl] amino-, (±)-, sulfate (1:1) (salt);
(±)-2-[[4-[(7-Chloro-4-quinolyl)amino]pentyl]ethylamino] ethanol sulfate (1:1) (salt) [747-36-4].

DEFINITION
Hydroxychloroquine Sulfate contains NLT 98.0% and NMT 102.0% of hydroxychloroquine sulfate ($C_{18}H_{26}ClN_3O \cdot H_2SO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

Sample solution: 10 µg/mL of Hydroxychloroquine Sulfate in dilute hydrochloric acid (1 in 100)
Acceptance criteria: Meets the requirements

Change to read:

- **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩,** *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
- **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩

Sample solution: 10 mg/mL of Hydroxychloroquine Sulfate in water
Acceptance criteria: Meets the requirements

ASSAY
- **PROCEDURE**

Standard solution: 10 µg/mL of USP Hydroxychloroquine Sulfate RS prepared as follows. Dissolve a quantity of USP Hydroxychloroquine Sulfate RS in water to obtain a solution of 20 mg/mL, and dilute with hydrochloric acid (1 in 100) to final concentration.
Sample solution: 10 µg/mL of Hydroxychloroquine Sulfate prepared as follows. Dissolve a quantity of Hydroxychloroquine Sulfate in water to obtain a solution of 20 mg/mL, and dilute with hydrochloric acid (1 in 100) to final concentration.
Instrumental conditions
Mode: UV
Analytical wavelength: 343 nm
Cell: 1 cm
Blank: Dilute hydrochloric acid (1 in 100)
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Determine the absorbances of the *Sample solution* and the *Standard solution*.
Calculate the percentage of hydroxychloroquine sulfate ($C_{18}H_{26}ClN_3O \cdot H_2SO_4$) in the portion of Hydroxychloroquine Sulfate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (µg/mL)
C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **ORDINARY IMPURITIES ⟨466⟩**

Standard solution: Prepare in 10% water in methanol.
Sample solution: Prepare in 10% water in methanol.
Eluant: A mixture of alcohol, water, and ammonium hydroxide (80:16:4)
Visualization: 1
Acceptance criteria: Meets the requirements

SPECIFIC TESTS
- **LOSS ON DRYING ⟨731⟩**

Analysis: Dry a sample at 105° for 2 h.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Hydroxychloroquine Sulfate RS

Hydroxychloroquine Sulfate Tablets

DEFINITION
Hydroxychloroquine Sulfate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of hydroxychloroquine sulfate ($C_{18}H_{26}ClN_3O \cdot H_2SO_4$).

IDENTIFICATION
- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES ⟨181⟩**

Sample solution: Nominally 20 mg/mL of hydroxychloroquine sulfate in water prepared as follows. Triturate a quantity of finely powdered Tablets, equivalent to about 1 g of hydroxychloroquine sulfate, with 50 mL of

www.webofpharma.com

water, and filter (retain the remainder of the filtrate for *Identification* test *B*).
Acceptance criteria: The clear filtrate meets the requirements.
• **B. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩**:** The clear filtrate obtained from *Identification* test *A* meets the requirements.
• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol, acetonitrile, water, and phosphoric acid (100:100:800:2). Add 96 mg of sodium 1-pentanesulfonate in the resulting solution, and filter.
Diluent: Methanol and water (1:1)
Standard stock solution: 1 mg/mL of USP Hydroxychloroquine Sulfate RS in *Diluent*
Standard solution: 0.05 mg/mL of USP Hydroxychloroquine Sulfate RS from the *Standard stock solution* in *Mobile phase*
System suitability stock solution: 1 mg/mL of chloroquine phosphate in methanol
System suitability solution: Transfer 5.0 mL of the *System suitability stock solution* to a 100-mL volumetric flask, add 5.0 mL of *Standard stock solution*, and dilute with *Mobile phase* to volume.
Sample stock solution: Nominally 1 mg/mL of hydroxychloroquine sulfate prepared as follows. Transfer nominally 200 mg of hydroxychloroquine sulfate, from finely powdered Tablets (NLT 20), to a 200-mL volumetric flask. Add 150 mL of *Diluent*, and mix. Sonicate, with intermittent shaking, for 15 min, and cool to room temperature. Dilute with *Diluent* to volume, and filter.
Sample solution: 0.05 mg/mL from the *Sample stock solution* in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5- to 10-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.8 between chloroquine and hydroxychloroquine, *System suitability solution*
Relative standard deviation: NMT 1.5% for replicate injections of the *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydroxychloroquine sulfate ($C_{18}H_{26}ClN_3O \cdot H_2SO_4$) in the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

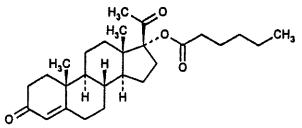
r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydroxychloroquine Sulfate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydroxychloroquine sulfate in the *Samplesolution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Standard solution: USP Hydroxychloroquine Sulfate RS in *Medium*
Sample solution: Pass a portion of the solution through a suitable filter. Dilute with *Medium*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: 343 nm
Analysis: Determine the percentage of the labeled amount of hydroxychloroquine sulfate ($C_{18}H_{26}ClN_3O \cdot H_2SO_4$).
Tolerances: NLT 70% (*Q*) of the labeled amount of hydroxychloroquine sulfate ($C_{18}H_{26}ClN_3O \cdot H_2SO_4$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hydroxychloroquine Sulfate RS

Hydroxyprogesterone Caproate



$C_{27}H_{40}O_4$ 428.60
Pregn-4-ene-3,20-dione, 17-[(1-oxohexyl)oxy]-.
17-Hydroxypregn-4-ene-3,20-dione hexanoate [630-56-8].

» Hydroxyprogesterone Caproate contains not less than 97.0 percent and not more than 103.0 percent of $C_{27}H_{40}O_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Hydroxyprogesterone Caproate RS

Change to read:

Identification, ▲ ***Spectroscopic Identification Tests*** ⟨ 197 ⟩ , *Infrared Spectroscopy* **: *197K*** ▲(CN 1-May-2020)•
Melting range ⟨741⟩**:** between 120° and 124°.
Specific rotation ⟨781S⟩**:** between +58° and +64°.
Test solution: 10 mg per mL, in chloroform.
Water Determination, *Method I* ⟨921⟩ **:** not more than 0.1%.
Free *n*-caproic acid—Dissolve 0.20 g in 25 mL of alcohol that previously has been neutralized to a faint pink color following the addition of 2 or 3 drops of phenolphthalein TS. Promptly titrate with 0.020 N sodium hydroxide: not more than 0.50 mL of 0.020 N sodium hydroxide is required (0.58%).

USP Monographs

www.webofpharma.com

Ordinary impurities ⟨466⟩—
Test solution: chloroform.
Standard solution: chloroform.
Eluant: a mixture of chloroform and ethyl acetate (3:1).
Visualization: 5; then view under long-wavelength UV light.

Assay—Transfer about 50 mg of Hydroxyprogesterone Caproate, accurately weighed, to a 100-mL volumetric flask, add alcohol to volume, and mix. Dilute 2.0 mL of this solution with alcohol to volume in a second 100-mL volumetric flask, and mix. Dissolve in alcohol a suitable quantity of USP Hydroxyprogesterone Caproate RS, and dilute quantitatively and stepwise with alcohol to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 240 nm, using alcohol as the blank. Calculate the quantity, in mg, of $C_{27}H_{40}O_4$ in the Hydroxyprogesterone Caproate taken by the formula:

$$5C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Hydroxyprogesterone Caproate RS in the Standard solution; and A_U and A_S are the absorbances of the solution of Hydroxyprogesterone Caproate and the Standard solution, respectively.

Hydroxyprogesterone Caproate Injection

DEFINITION

Hydroxyprogesterone Caproate Injection is a sterile solution of Hydroxyprogesterone Caproate in a suitable vegetable oil. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydroxyprogesterone caproate ($C_{27}H_{40}O_4$).

IDENTIFICATION

• **A.**
Sample solution: Transfer a volume of Injection, equivalent to 125 mg of hydroxyprogesterone caproate, to a 60-mL separator containing 10 mL of solvent hexane, 8 mL of methanol, and 2 mL of water. Insert the stopper, shake for 2 min, and allow the phases to separate. The lower layer is the *Sample solution*.
Analysis: To 3 mL of the *Sample solution* add sulfuric acid dropwise until a color develops, then add 3 mL of methanol.
Acceptance criteria: A purple color develops and the solution, when viewed under long-wavelength UV light, exhibits a pale yellow fluorescence.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Mobile phase: Methanol and water (80:20)
Standard solution: 0.05 mg/mL of USP Hydroxyprogesterone Caproate RS in methanol
Sample solution: Nominally 0.05 mg/mL of hydroxyprogesterone caproate from an appropriate volume of Injection in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 242 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Run time: Twice the retention time of hydroxyprogesterone caproate
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 4000 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydroxyprogesterone caproate ($C_{27}H_{40}O_4$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydroxyprogesterone Caproate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydroxyprogesterone caproate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.5 µg/mL of USP Hydroxyprogesterone Caproate RS in methanol
Sample stock solution: Transfer 2.0 mL of Injection, equivalent to 500 mg of hydroxyprogesterone caproate, to a 100-mL volumetric flask and dilute with methanol to volume.
Sample solution: Nominally 0.25 mg/mL of hydroxyprogesterone caproate prepared as follows. Transfer an appropriate amount of *Sample stock solution* to a suitable volumetric flask and dilute with methanol to volume.
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0%
Signal-to-noise ratio: NLT 10
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of hydroxyprogesterone or any individual unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each corresponding degradation product from the *Sample solution*
r_S = peak response of hydroxyprogesterone caproate from the *Standard solution*
C_S = concentration of USP Hydroxyprogesterone Caproate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of hydroxyprogesterone caproate in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

www.webofpharma.com

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Hydroxyprogesterone[a]	0.4	1.3	1.0
Hydroxyprogesterone caproate	1.0	—	—
Individual unspecified degradation product	—	1.0	0.2
Total impurities	—	—	2.0

[a] 17-Hydroxypregn-4-ene-3,20-dione.

SPECIFIC TESTS
- **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 0.2%
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type III glass. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hydroxyprogesterone Caproate RS

Hydroxypropyl Cellulose Ocular System

DEFINITION
Hydroxypropyl Cellulose Ocular System contains NLT 85.0% and NMT 115.0% of the labeled amount of Hydroxypropyl Cellulose. It contains no other substance. It is sterile.

IDENTIFICATION
- **INFRARED ABSORPTION**
  Sample solution: A 10-mg/mL solution in methanol, based on the labeled amount of Hydroxypropyl Cellulose. Evaporate 2 drops of the solution on a silver chloride plate so that it forms a thin film.
  Acceptance criteria: The infrared absorption spectrum of the film exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydroxypropyl Cellulose RS.

ASSAY
- **PROCEDURE**
  Standard stock solution: 0.25 mg/mL of USP Hydroxypropyl Cellulose RS prepared as follows: Weigh 25 mg of USP Hydroxypropyl Cellulose RS into a 100-mL volumetric flask. Dissolve in 80 mL of water. Mix well by agitating on a mechanical shaker until completely dissolved. Add one drop of methanol to dispel the foam and dilute with water to volume. [NOTE—Stirring overnight before diluting to volume is recommended.]
  Standard solution: 0.05 mg/mL of hydroxypropyl cellulose in water, from *Standard stock solution*
  Sample stock solution: 0.25 mg/mL of hydroxypropyl cellulose prepared using the same procedure as the *Standard stock solution*
  Sample solution: 0.05 mg/mL of hydroxypropyl cellulose in water, from *Sample stock solution*
  Spectrometric conditions
  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
  Mode: UV-Vis
  Analytical wavelength: 620 nm
  Cell length: 1.0 cm, quartz
  Analysis
  Samples: *Standard solution* and *Sample solution*

Separately pipet 2 mL of the *Standard solution*, the *Sample solution*, and water, to provide a blank, into individual 50-mL centrifuge tubes. Add to each tube, 6.0 mL of a 0.5 mg/mL solution of anthrone in sulfuric acid, and mix on a vortex mixer. Let the centrifuge tubes cool for approximately 40 min and remix. Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*. [NOTE—Prepare anthrone in sulfuric acid solution just before use in low-actinic glassware, and mix well before adding to the tube. Use it within 12 h of preparation. Avoid contact between the glassware and the paper products during analysis; the cellulose in the paper will react with the sulfuric acid and alter the results.]

Calculate the percentage of hydroxypropyl cellulose in the Ocular System:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of hydroxypropyl cellulose in the *Sample solution* (mg/mL)

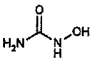
Acceptance criteria: 85.0%–115.0%

PERFORMANCE TESTS
- **WEIGHT VARIATION**
  Analysis: Determine the weight of each of a sufficient number of Systems.
  Acceptance criteria: NMT 1 out of 20 Systems varies more than 25% from the average or, failing that, NMT 6 out of 60 (including the original 20) vary more than 25% (but none more than 35%) from the average weight.

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose containers, at a temperature not exceeding 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hydroxypropyl Cellulose RS

Hydroxyurea



$CH_4N_2O_2$ 76.05
Urea, hydroxy-.
Hydroxyurea [127-07-1].

» Hydroxyurea contains not less than 97.0 percent and not more than 103.0 percent of $CH_4N_2O_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers, in a dry atmosphere.
USP Reference standards ⟨11⟩—
USP Hydroxyurea RS

www.webofpharma.com

Change to read:

Identification, ▲ ***Spectroscopic Identification Tests*** ⟨ 197 ⟩ **,** *Infrared Spectroscopy* **: *197K*** ▲(CN 1-May-2020)•

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.50%.

Urea and related compounds—

Developing solvent—Shake equal volumes of isobutyl alcohol and water in a separator, and allow the layers to separate. Use the upper layer as the *Mobile phase* and the lower layer as the *Stationary phase.*

p-Dimethylaminobenzaldehyde solution, 1%—Dissolve 1.0 g of *p*-dimethylaminobenzaldehyde in 50 mL of alcohol, add 2 mL of hydrochloric acid, and dilute with alcohol to 100.0 mL.

pH 6.5 Buffer solution—Mix 700 mL of 0.2 M dibasic sodium phosphate and 300 mL of 0.1 M citric acid.

Standard preparation—Prepare a solution of urea in water, containing 0.1 mg per mL.

Test preparation—Dissolve 10.0 mg of Hydroxyurea in 1.0 mL of water.

Procedure—Treat a suitable chromatographic paper strip (Whatman No. 1 or equivalent) by dipping it in *pH 6.5 Buffer solution.* Dry the paper strip, and apply 100 µL of the *Test preparation* and 50 µL of the *Standard preparation.* Place the strip in a chromatographic chamber for descending chromatography containing the *Stationary phase* in the bottom of the chamber and the *Mobile phase* in the trough. Develop for 24 hours, remove the strip from the chamber, air-dry, and develop again for 24 hours. Remove the strip, air-dry, spray with *p-Dimethylaminobenzaldehyde solution, 1%,* and heat at 90° for 1 to 2 minutes. Not more than two spots, other than the major component, are present in the *Test preparation,* and their intensities are not greater than the intensity of the spot from the *Standard preparation* (0.5% of each impurity). The R_f values relative to hydroxyurea, the principal spot, are 0.65 and 1.26 (urea).

Assay—

Solution A—Dissolve 1.7 g of tetrabutylammonium hydrogen sulfate and 1.74 g of dibasic potassium phosphate, anhydrous, in 1000 mL of water, and adjust with 1 N sodium hydroxide or 85% phosphoric acid to a pH of 5.0.

Solution B: methanol.

Mobile phase—Prepare a solution of filtered, degassed *Solution A* and *Solution B* (8.5: 1.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Dissolve accurately weighed quantities of USP Hydroxyurea RS and hydroxylamine hydrochloride in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.4 mg per mL of each.

Standard preparation—Dissolve an accurately weighed quantity of USP Hydroxyurea RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.4 mg per mL.

Assay preparation—Transfer about 200 mg of Hydroxyurea, accurately weighed, to a 500-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 0.5 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the hydroxylamine and hydroxyurea peaks is not less than 1.5; for the hydroxyurea peak, the column efficiency is not less than 5000; and the tailing factor is not more than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $CH_4N_2O_2$ in the portion of Hydroxyurea taken by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Hydroxyurea RS in the *Standard preparation;* and r_U and r_S are the peak responses of the hydroxyurea peaks obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Hydroxyurea Capsules

» Hydroxyurea Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydroxyurea ($CH_4N_2O_2$).

Packaging and storage—Preserve in tight containers, in a dry atmosphere.

USP Reference standards ⟨11⟩—
USP Hydroxyurea RS

Identification—Transfer a portion of the Capsule contents, equivalent to about 30 mg of hydroxyurea, to a suitable centrifuge tube, and add 10 mL of anhydrous methanol. Mix, and centrifuge for 3 minutes. Transfer 1.0 mL of the clear supernatant to a mortar containing 500 mg of potassium bromide, triturate to a homogeneous blend, dry in a vacuum desiccator at 60° for 3 hours, and prepare a suitable disk: the IR absorption spectrum exhibits maxima only at the same wavelengths as that of a similar preparation of USP Hydroxyurea RS.

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Time: 30 minutes.

Procedure—Determine the amount of $CH_4N_2O_2$ dissolved, employing the procedure set forth in the *Assay,* making any necessary modifications.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $CH_4N_2O_2$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Solution A, Solution B, Mobile phase, Resolution solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Hydroxyurea.*

Assay preparation—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Grind to a fine powder, and transfer an accurately weighed portion of the powder, equivalent to about 200 mg of hydroxyurea, to a 500-mL volumetric flask. Add about 300 mL of *Mobile phase,* sonicate for 10 minutes, stir with the aid of a magnetic stirrer for 30 minutes, sonicate for an additional 10 minutes, and dilute as necessary with *Mobile phase* to volume. Filter a portion of the resulting solution, discarding the first 2 mL of the filtrate.

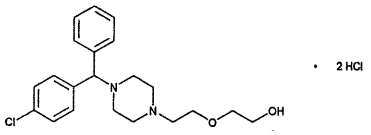
www.webofpharma.com

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Hydroxyurea*. Calculate the quantity, in mg, of hydroxyurea ($CH_4N_2O_2$) in the portion of Capsules taken by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Hydroxyurea RS in the *Standard preparation;* and r_U and r_S are the peak responses for the *Assay preparation* and the *Standard preparation,* respectively.

Hydroxyzine Hydrochloride



$C_{21}H_{27}ClN_2O_2 \cdot 2HCl$ 447.83

Ethanol, 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-, dihydrochloride, (±)-;
(±)-2-[2-[4-(*p*-Chloro-α-phenylbenzyl)-1-piperazinyl]ethoxy] ethanol dihydrochloride [2192-20-3].

DEFINITION

Hydroxyzine Hydrochloride contains NLT 98.0% and NMT 102.0% of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.**

Sample solution: 2.5 mg/mL of Hydroxyzine Hydrochloride in water

Analysis: To 10 mL of the *Sample solution*, add 2 drops of nitric acid and 1 mL of silver nitrate TS.

Acceptance criteria: A curdy, white precipitate, insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, separates (presence of chloride).

ASSAY

- **Procedure**

Solution A: Trifluoroacetic acid and water (0.1: 99.9)

Solution B: Trifluoroacetic acid and acetonitrile (0.05: 99.95)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
12	55	45
16	55	45
21	20	80
25	20	80
26	90	10
30	90	10

Diluent: Acetonitrile and water (30:70)

Standard solution: 0.05 mg/mL of USP Hydroxyzine Hydrochloride RS in *Diluent*

Sample solution: 0.05 mg/mL of Hydroxyzine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 2.1-mm × 15-cm; 1.8-µm packing L1

Flow rate: 0.3 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) in the portion of Hydroxyzine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydroxyzine from the *Sample solution*

r_S = peak response of hydroxyzine from the *Standard solution*

C_S = concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Hydroxyzine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** ⟨281⟩: NMT 0.5%
- **Organic Impurities**

Solution A, Solution B, Mobile phase, and **Diluent:** Prepare as directed in the *Assay*.

Standard solution: 1.5 µg/mL each of USP Hydroxyzine Hydrochloride RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS in *Diluent*

Sample solution: 500 µg/mL of Hydroxyzine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detectors

Decloxizine, hydroxyzine related compound A, and hydroxyzine: UV 230 nm

4-Chlorobenzophenone: UV 254 nm

Column: 2.1-mm × 15-cm; 1.8-µm packing L1

Flow rate: 0.3 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 5.0 between hydroxyzine related compound A and hydroxyzine
Relative standard deviation: NMT 3.0% for hydroxyzine related compound A, hydroxyzine, and 4-chlorobenzophenone
Analysis
Samples: *Standard solution* and *Sample solution*
For impurities detected at UV 230 nm
Calculate the percentage of hydroxyzine related compound A in the portion of Hydroxyzine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydroxyzine related compound A from the *Sample solution*
r_S = peak response of hydroxyzine related compound A from the *Standard solution*
C_S = concentration of USP Hydroxyzine Related Compound A RS in the *Standard solution* (µg/mL)
C_U = concentration of Hydroxyzine Hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of decloxizine or any individual unspecified impurity in the portion of Hydroxyzine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of decloxizine or any other individual impurity from the *Sample solution*
r_S = peak response of hydroxyzine from the *Standard solution*
C_S = concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = concentration of Hydroxyzine Hydrochloride in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

For 4-chlorobenzophenone detected at UV 254 nm
Calculate the percentage of 4-chlorobenzophenone in the portion of Hydroxyzine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 4-chlorobenzophenone from the *Sample solution*
r_S = peak response of 4-chlorobenzophenone from the *Standard solution*
C_S = concentration of USP 4-Chlorobenzophenone RS in the *Standard solution* (µg/mL)
C_U = concentration of Hydroxyzine Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.03%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Decloxizine[a]	0.87	0.68	0.3
Hydroxyzine related compound A	0.96	—	0.3
Hydroxyzine	1.0	—	—
4-Chlorobenzophenone	1.4	—	0.2
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	0.75

[a] 2-[2-(4-Benzhydrylpiperazin-1-yl)ethoxy]ethanol; also known as 2-{2-[4-(diphenylmethyl)piperazin-1-yl]ethoxy}ethanol.

SPECIFIC TESTS
• **WATER DETERMINATION** ⟨921⟩: NMT 5.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature. Protect from light.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP 4-Chlorobenzophenone RS
4-Chlorobenzophenone.
$C_{13}H_9ClO$ 216.66
USP Hydroxyzine Hydrochloride RS
USP Hydroxyzine Related Compound A RS
1-[(4-Chlorophenyl)phenylmethyl]piperazine; Also known as *p*-Chlorobenzhydrylpiperazine.
$C_{17}H_{19}ClN_2$ 286.80

Hydroxyzine Hydrochloride Injection

DEFINITION
Hydroxyzine Hydrochloride Injection is a sterile solution of Hydroxyzine Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$).

IDENTIFICATION
• **A.**
Standard solution: 20 µg/mL of USP Hydroxyzine Hydrochloride RS in 0.1 N hydrochloric acid
Sample solution: Nominally 20 µg/mL of hydroxyzine hydrochloride from Injection in 0.1 N hydrochloric acid
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*, concomitantly measured.
• **B.** The retention time of the main peak in the *Sample solution* corresponds to that of the hydroxyzine peak from the *Standard solution* as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Protect the *Standard solution* and *Sample solution* from light.
Buffer: 2 g/L of dibasic potassium phosphate and 8 g/L of monobasic potassium phosphate adjusted with 10 N potassium hydroxide to a pH of 6.6
Mobile phase: Methanol and *Buffer* (65:35)
Standard solution: 0.25 mg/mL of USP Hydroxyzine Hydrochloride RS and 0.5 µg/mL of USP 4-Chlorobenzophenone RS in *Mobile phase*
Sample solution: Nominally 0.25 mg/mL of hydroxyzine hydrochloride from Injection in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4-mm × 30-cm; packing L1
Flow rate: 2 mL/min

www.webofpharma.com

Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for 4-chlorobenzophenone and hydroxyzine are 0.75 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the 4-chlorobenzophenone and hydroxyzine peaks
Tailing factor: NMT 2.5 for the 4-chlorobenzophenone and hydroxyzine peaks
Relative standard deviation: NMT 2.0% for the hydroxyzine peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydroxyzine from the *Sample solution*
r_S = peak response of hydroxyzine from the *Standard solution*
C_S = concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydroxyzine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **LIMIT OF 4-CHLOROBENZOPHENONE**
Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of 4-chlorobenzophenone in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 4-chlorobenzophenone in the *Sample solution*
r_S = peak response of 4-chlorobenzophenone in the *Standard solution*
C_S = concentration of USP 4-Chlorobenzophenone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydroxyzine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2%

SPECIFIC TESTS
• **pH** ⟨791⟩: 3.5–6.0
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 3.6 USP Endotoxin Units/mg of hydroxyzine hydrochloride
• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, protected from light. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP 4-Chlorobenzophenone RS
$C_{13}H_9ClO$ 216.66
USP Hydroxyzine Hydrochloride RS

Hydroxyzine Hydrochloride Oral Solution

DEFINITION
Hydroxyzine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the hydroxyzine peak of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: Trifluoroacetic acid and water (0.1: 99.9)
Solution B: Trifluoroacetic acid and acetonitrile (0.05: 99.95)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
12	60	40
16	60	40
21	20	80
25	20	80
26	90	10
30	90	10

Diluent: Acetonitrile and water (30:70)
Standard solution: 0.05 mg/mL of USP Hydroxyzine Hydrochloride RS in *Diluent*
Sample stock solution: Nominally 0.5 mg/mL of hydroxyzine hydrochloride from Oral Solution in *Diluent* prepared as follows. Transfer a portion of Oral Solution, equivalent to 25 mg of hydroxyzine hydrochloride, to a 50-mL volumetric flask. Dissolve and dilute with *Diluent* to volume.
Sample solution: Nominally 0.05 mg/mL of hydroxyzine hydrochloride from the *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 2.1-mm × 15-cm; 1.8-µm packing L1
Column temperature: 30°
Flow rate: 0.3 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) in the portion of Oral Solution taken:

USP Monographs

www.webofpharma.com

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydroxyzine from the *Sample solution*
r_S = peak response of hydroxyzine from the *Standard solution*
C_S = concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydroxyzine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
For single-unit containers
Acceptance criteria: Meets the requirements
• **DELIVERABLE VOLUME** ⟨698⟩
For multiple-unit containers
Acceptance criteria: Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, and **Diluent:** Prepare as directed in the *Assay*.
Standard stock solution: 25.0 µg/mL each of USP Hydroxyzine Hydrochloride RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS in *Diluent*
Standard solution: 1.0 µg/mL each of USP Hydroxyzine Hydrochloride RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS from the *Standard stock solution* in *Diluent*
Sample solution: Nominally 500 µg/mL of hydroxyzine hydrochloride from Oral Solution in *Diluent* prepared as follows. Transfer a portion of Oral Solution, equivalent to 25 mg of hydroxyzine hydrochloride, to a 50-mL volumetric flask. Dissolve and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Hydroxyzine related compound A and hydroxyzine hydrochloride: UV 230 nm
4-Chlorobenzophenone: UV 254 nm
Column: 2.1-mm × 15-cm; 1.8-µm packing L1
Column temperature: 30°
Flow rate: 0.3 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between hydroxyzine related compound A and hydroxyzine
Relative standard deviation: NMT 3.0% for hydroxyzine related compound A, hydroxyzine, and 4-chlorobenzophenone
Analysis
Samples: *Standard solution* and *Sample solution*
For impurities detected at UV 230 nm
Calculate the percentage of any individual unspecified degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified degradation product from the *Sample solution*
r_S = peak response of hydroxyzine from the *Standard solution*
C_S = concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of hydroxyzine hydrochloride in the *Sample solution* (µg/mL)

For 4-chlorobenzophenone detected at UV 254 nm
Calculate the percentage of 4-chlorobenzophenone in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 4-chlorobenzophenone from the *Sample solution*
r_S = peak response of 4-chlorobenzophenone from the *Standard solution*
C_S = concentration of USP 4-Chlorobenzophenone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of hydroxyzine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Hydroxyzine related compound A[a]	0.94	—
Hydroxyzine	1.0	—
4-Chlorobenzophenone	1.4	0.2
Any individual unspecified degradation product	—	0.2
Total degradation products	—	0.75

[a] This is a process impurity that is controlled in the drug substance. It is not to be reported or included in the total degradation products.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP 4-Chlorobenzophenone RS
4-Chlorobenzophenone.
$C_{13}H_9ClO$ 216.66
USP Hydroxyzine Hydrochloride RS
USP Hydroxyzine Related Compound A RS
p-Chlorobenzhydrylpiperazine;
Also known as 1-[(4-Chlorophenyl)phenylmethyl] piperazine.
$C_{17}H_{19}ClN_2$ 286.80

Hydroxyzine Hydrochloride Tablets

DEFINITION

Hydroxyzine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$).

IDENTIFICATION

• **A.** The retention time of the main peak of the *Sample solution* corresponds to that of the hydroxyzine peak of the *Standard solution*, as obtained in the *Assay*.

www.webofpharma.com

• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Trifluoroacetic acid and water (0.1: 99.9)
Solution B: Trifluoroacetic acid and acetonitrile (0.05: 99.95)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
12	55	45
16	55	45
21	20	80
25	20	80
26	90	10
30	90	10

Diluent: Acetonitrile and water (30:70)
Standard solution: 0.05 mg/mL of USP Hydroxyzine Hydrochloride RS in *Diluent*
Sample stock solution: Nominally 0.5 mg/mL of hydroxyzine hydrochloride from Tablets in *Diluent* prepared as follows. Transfer a portion of finely powdered Tablets (NLT 10), equivalent to 50 mg of hydroxyzine hydrochloride, to a 100-mL volumetric flask and add 80 mL of *Diluent*. Sonicate for 30 min to dissolve and dilute with *Diluent* to volume. Centrifuge the solution and use the supernatant. [NOTE—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]
Sample solution: Nominally 0.05 mg/mL of hydroxyzine hydrochloride from the *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 2.1-mm × 15-cm; 1.8-µm packing L1
Flow rate: 0.3 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydroxyzine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: USP Hydroxyzine Hydrochloride RS in *Medium*
Sample solution: Use a filtered portion of the solution under test. Dilute with *Medium*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 230 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) dissolved from the UV absorbances.
Tolerances: NLT 75% (*Q*) of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) is dissolved.
Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: Water; 250 mL
Apparatus 3: 30 dips/min
Time: 45 min
Standard solution: USP Hydroxyzine Hydrochloride RS in *Medium*
Sample solution: Use a filtered portion of the solution under test. Dilute with *Medium*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 230 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) dissolved from the UV absorbances.
Tolerances: NLT 75% (*Q*) of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, and **Diluent:** Proceed as directed in the *Assay*.
Standard stock solution: 25.0 µg/mL each of USP Hydroxyzine Hydrochloride RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS in *Diluent*
Standard solution: 1.0 µg/mL each of USP Hydroxyzine Hydrochloride RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS from the *Standard stock solution* in *Diluent*
Sample solution: Nominally 500 µg/mL of hydroxyzine hydrochloride from Tablets prepared as follows. Transfer a portion of finely powdered Tablets (NLT 10), equivalent to 50 mg of hydroxyzine hydrochloride, to a 100-mL volumetric flask. Add 80 mL of *Diluent*. Sonicate 30 min to dissolve and dilute with *Diluent* to volume. Centrifuge the solution and use the supernatant. [NOTE—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detectors
Hydroxyzine and hydroxyzine related compound A: UV 230 nm
4-Chlorobenzophenone: UV 254 nm
Column: 2.1-mm × 15-cm; 1.8-µm packing L1
Flow rate: 0.3 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for the relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between hydroxyzine related compound A and hydroxyzine
Relative standard deviation: NMT 3.0% each for hydroxyzine related compound A, hydroxyzine, and 4-chlorobenzophenone
Analysis
Samples: *Standard solution* and *Sample solution*
For degradation products detected at UV 230 nm
Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of hydroxyzine from the *Standard solution*
C_S = concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of hydroxyzine hydrochloride in the *Sample solution* (µg/mL)

For 4-chlorobenzophenone detected at UV 254 nm
Calculate the percentage of 4-chlorobenzophenone in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of 4-chlorobenzophenone from the *Sample solution*
r_S = peak response of 4-chlorobenzophenone from the *Standard solution*
C_S = concentration of USP 4-Chlorobenzophenone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of hydroxyzine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.03%.

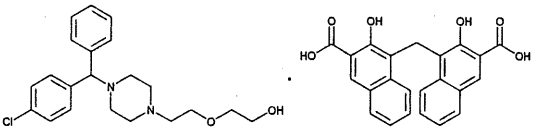
Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Decloxizine[a]	0.87	—
Hydroxyzine related compound A[a]	0.96	—
Hydroxyzine	1.0	—
4-Chlorobenzophenone	1.4	0.2
Any individual unspecified degradation product	—	0.3
Total degradation products	—	0.5

[a] These are process impurities that are controlled in the drug substance. They are not to be reported or included in the total degradation products.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP 4-Chlorobenzophenone RS
4-Chlorobenzophenone.
$C_{13}H_9ClO$ 216.66
USP Hydroxyzine Hydrochloride RS
USP Hydroxyzine Related Compound A RS
1-[(4-Chlorophenyl)phenylmethyl]piperazine; Also known as *p*-Chlorobenzhydrylpiperazine.
$C_{17}H_{19}ClN_2$ 286.80

Hydroxyzine Pamoate



$C_{21}H_{27}ClN_2O_2 \cdot C_{23}H_{16}O_6$ 763.27
Ethanol, 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-, (±)-, compd. with 4,4′-methylenebis[3-hydroxy-2-naphthalenecarboxylic acid] (1:1);
(±)-2-[2-[4-(*p*-Chloro-α-phenylbenzyl)-1-piperazinyl]ethoxy] ethanol 4,4′-methylenebis[3-hydroxy-2-naphthoate] (1:1) [10246-75-0].

DEFINITION
Hydroxyzine Pamoate contains NLT 97.0% and NMT 102.0% of hydroxyzine pamoate ($C_{21}H_{27}ClN_2O_2 \cdot C_{23}H_{16}O_6$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*▲ (CN 1-May-2020)**:** [NOTE—Methods described in ⟨197K⟩ or ⟨197A⟩ may be used.]
- **B.** The retention time of the hydroxyzine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Solution A: To each L of water, add 1.0 mL of stronger ammonia water.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
6	80	20
20	20	80
25	20	80
26	80	20

www.webofpharma.com

Table 1 *(continued)*

Time (min)	Solution A (%)	Solution B (%)
30	80	20

Diluent: Acetonitrile and *Solution A* (80:20)
System suitability solution: 1 µg/mL of USP Hydroxyzine Pamoate RS, 3 µg/mL of USP Hydroxyzine Related Compound A RS, and 1 µg/mL of USP 4-Chlorobenzophenone RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Hydroxyzine Pamoate RS in *Diluent*
Sample solution: 0.1 mg/mL of Hydroxyzine Pamoate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
[NOTE—Rinse the column with a solution of acetonitrile and water (20:80) and then with a solution of acetonitrile and water (80:20) after each analysis.]
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention time for 4-chlorobenzophenone is 1.1. See *Table 2* for the relative retention times for other compounds.]
Suitability requirements
Resolution: NLT 1.5 between hydroxyzine and hydroxyzine related compound A; NLT 1.5 between hydroxyzine related compound A and 4-chlorobenzophenone, *System suitability solution*
Tailing factor: NMT 1.5 for hydroxyzine, *Standard solution*
Relative standard deviation: NMT 0.73% for hydroxyzine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of hydroxyzine pamoate ($C_{21}H_{27}ClN_2O_2 \cdot C_{23}H_{16}O_6$) in the portion of Hydroxyzine Pamoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydroxyzine from the *Sample solution*
r_S = peak response of hydroxyzine from the *Standard solution*
C_S = concentration of USP Hydroxyzine Pamoate RS in the *Standard solution* (mg/mL)
C_U = concentration of Hydroxyzine Pamoate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%
• **ORGANIC IMPURITIES**
Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 1 µg/mL of USP Hydroxyzine Pamoate RS, 3 µg/mL of USP Hydroxyzine Related Compound A RS, and 1 µg/mL of USP 4-Chlorobenzophenone RS in *Diluent*
Sample solution: 1000 µg/mL of Hydroxyzine Pamoate in *Diluent*
System suitability
Sample: *Standard solution*
[NOTE—The relative retention time for 4-chlorobenzophenone is 1.1. See *Table 2* for the relative retention times for other compounds.]
Suitability requirements
Resolution: NLT 1.5 between hydroxyzine and hydroxyzine related compound A; NLT 1.5 between hydroxyzine related compound A and 4-chlorobenophenone
Relative standard deviation: NMT 5.0% each for hydroxyzine and hydroxyzine related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of hydroxyzine related compound A in the portion of Hydroxyzine Pamoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydroxyzine related compound A from the *Sample solution*
r_S = peak response of hydroxyzine related compound A from the *Standard solution*
C_S = concentration of USP Hydroxyzine Related Compound A RS in the *Standard solution* (µg/mL)
C_U = concentration of Hydroxyzine Pamoate in the *Sample solution* (µg/mL)

Calculate the percentage of decloxizine and each unspecified impurity in the portion of Hydroxyzine Pamoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of decloxizine or each unspecified impurity from the *Sample solution*
r_S = peak response of hydroxyzine from the *Standard solution*
C_S = concentration of USP Hydroxyzine Pamoate RS in the *Standard solution* (µg/mL)
C_U = concentration of Hydroxyzine Pamoate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pamoic acid[a]	0.05	—
Decloxizine[b]	0.90	0.15
Hydroxyzine	1.0	—
Hydroxyzine related compound A	1.05	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	0.50

[a] This peak is due to the pamoate counterion; hence it is not an impurity and should not be included in the total impurities.
[b] 2-[2-(4-Benzhydrylpiperazin-1-yl)ethoxy]ethanol.

SPECIFIC TESTS

• **WATER DETERMINATION** ⟨921⟩, *Method I*: NMT 5.0%

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store below 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP 4-Chlorobenzophenone RS
4-Chlorobenzophenone.
$C_{13}H_9ClO$ 216.66

USP Hydroxyzine Related Compound A RS
1-[(4-Chlorophenyl)phenylmethyl]piperazine.
$C_{17}H_{19}ClN_2$ 286.80

USP Hydroxyzine Pamoate RS

Hydroxyzine Pamoate Capsules

DEFINITION

Hydroxyzine Pamoate Capsules contain hydroxyzine pamoate ($C_{21}H_{27}ClN_2O_2 \cdot C_{23}H_{16}O_6$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$).

IDENTIFICATION

- **A.** The retention time of the hydroxyzine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the hydroxyzine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: To each L of water, add 1.0 mL of stronger ammonia water.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
6	80	20
20	20	80
25	20	80
26	80	20
30	80	20

Diluent: Acetonitrile and *Solution A* (80:20)
System suitability solution: 1 µg/mL of USP Hydroxyzine Pamoate RS (equivalent to 0.6 µg/mL of hydroxyzine hydrochloride), 3 µg/mL of USP Hydroxyzine Related Compound A RS, and 1 µg/mL of USP 4-Chlorobenzophenone RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Hydroxyzine Pamoate RS (equivalent to 0.06 mg/mL of hydroxyzine hydrochloride) in *Diluent*
Sample stock solution: Nominally 1.0 mg/mL of hydroxyzine pamoate from Capsules (equivalent to 0.6 mg/mL of hydroxyzine hydrochloride) prepared as follows. Transfer a portion of the powder from NLT 10 Capsules to a suitable volumetric flask. Add 75% of the flask volume of *Diluent*, sonicate for NLT 15 min, and shake by mechanical means for NLT 30 min. Dilute with *Diluent* to volume. Centrifuge a portion of the solution and use the supernatant. [NOTE—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]
Sample solution: Nominally 0.1 mg/mL of hydroxyzine pamoate (equivalent to 0.06 mg/mL of hydroxyzine hydrochloride) from *Sample stock solution* in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 3.5-µm packing L1.
[NOTE—Rinse the column with a solution of acetonitrile and water (20:80) and then with a solution of acetonitrile and water (80:20) after each analysis.]
Column temperature: 45°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for decloxizine and 4-chlorobenzophenone are 0.90 and 1.1, respectively. See *Table 2* for the relative retention times for other compounds.]
Suitability requirements
Resolution: NLT 1.5 between hydroxyzine and hydroxyzine related compound A; NLT 1.5 between hydroxyzine related compound A and 4-chlorobenophenone, *System suitability solution*
Tailing factor: NMT 1.5 for hydroxyzine, *Standard solution*
Relative standard deviation: NMT 0.73% for hydroxyzine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) in the portion of Hydroxyzine Pamoate Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of hydroxyzine from the *Sample solution*
r_S = peak response of hydroxyzine from the *Standard solution*
C_S = concentration of USP Hydroxyzine Pamoate RS in the *Standard solution* (mg/mL)
C_U = equivalent concentration of hydroxyzine hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of hydroxyzine hydrochloride, 447.83
M_{r2} = molecular weight of hydroxyzine pamoate, 763.27

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Mobile phase: Methanol and 0.05 M monobasic sodium phosphate (60:40)
Standard solution: USP Hydroxyzine Hydrochloride RS in *Medium*
Sample solution: Use a filtered portion of the solution under test. Dilute with *Medium*, if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 232 nm
Column: 4.6-mm × 25-cm; 10-µm packing L9
Flow rate: 1.9 mL/min

USP Monographs

www.webofpharma.com

Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times (1/L) \times 100$$

r_U = peak response of hydroxyzine from the *Sample solution*
r_S = peak response of hydroxyzine from the *Standard solution*
C_S = concentration of USP Hydroxyzine Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*, if needed
V = volume of the *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 75% (Q) of the labeled amount of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2 \cdot 2HCl$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Organic Impurities**

Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 1 µg/mL of USP Hydroxyzine Pamoate RS (equivalent to 0.6 µg/mL of hydroxyzine hydrochloride), 3 µg/mL of USP Hydroxyzine Related Compound A RS, and 1 µg/mL of USP 4-Chlorobenzophenone RS in *Diluent*

Sample solution: Nominally 1000 µg/mL of hydroxyzine pamoate from Capsules (equivalent to 600 µg/mL of hydroxyzine hydrochloride) prepared as follows. Transfer a portion of the powder from NLT 10 Capsules to a suitable volumetric flask. Add 75% of the flask volume of *Diluent*, sonicate for NLT 15 min, and shake by mechanical means for NLT 30 min. Dilute with *Diluent* to volume. Centrifuge a portion of the solution and use the supernatant. [Note—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]

System suitability
Sample: *Standard solution*
[Note—The relative retention times for decloxizine and 4-chlorobenzophenone are 0.90 and 1.1, respectively. See *Table 2* for the relative retention times for other compounds.]
Suitability requirements
Resolution: NLT 1.5 between hydroxyzine and hydroxyzine related compound A; NLT 1.5 between hydroxyzine related compound A and 4-chlorobenzophenone
Relative standard deviation: NMT 5.0% each for hydroxyzine and hydroxyzine related compound A
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of hydroxyzine related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydroxyzine related compound A from the *Sample solution*
r_S = peak response of hydroxyzine related compound A from the *Standard solution*
C_S = concentration of USP Hydroxyzine Related Compound A RS in the *Standard solution* (µg/mL)
C_U = equivalent concentration of hydroxyzine hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of hydroxyzine from the *Standard solution*
C_S = concentration of USP Hydroxyzine Pamoate RS in the *Standard solution* (µg/mL)
C_U = equivalent concentration of hydroxyzine hydrochloride in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of hydroxyzine hydrochloride, 447.83
M_{r2} = molecular weight of hydroxyzine pamoate, 763.27

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pamoic acid[a]	0.05	—
Hydroxyzine	1.0	—
Hydroxyzine related compound A	1.05	0.2
Any individual unspecified degradation product	—	0.20
Total degradation products	—	1.0

[a] This peak is due to the pamoate counterion; hence it is not an impurity and should not be included in the total degradation products.

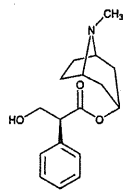
ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers. Store at controlled room temperature.

• **USP Reference Standards** ⟨11⟩
USP 4-Chlorobenzophenone RS
4-Chlorobenzophenone.
$C_{13}H_9ClO$ 216.66
USP Hydroxyzine Hydrochloride RS
USP Hydroxyzine Related Compound A RS
1-[(4-Chlorophenyl)phenylmethyl]piperazine.
$C_{17}H_{19}ClN_2$ 286.80
USP Hydroxyzine Pamoate RS

www.webofpharma.com

Hyoscyamine



$C_{17}H_{23}NO_3$ 289.37

Benzeneacetic acid, α-(hydroxymethyl)-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, [3(*S*)-*endo*-];
1α*H*,5α*H*-Tropan-3α-ol (–)-tropate (ester) [101-31-5].

DEFINITION

Hyoscyamine contains NLT 98.0% and NMT 101.0% of hyoscyamine ($C_{17}H_{23}NO_3$), calculated on the dried basis.
[**CAUTION**—Handle Hyoscyamine with exceptional care since it is highly potent.]

IDENTIFICATION

• **A.**
Standard solution: Transfer 36 mg of USP Hyoscyamine Sulfate RS to a 60-mL separator with the aid of 5 mL of water. Add 1.5 mL of 1 N sodium hydroxide and 10 mL of chloroform to the separator. Shake for 1 min, allow the layers to separate, and pass the chloroform extracts through a filter of about 2 g of anhydrous granular sodium sulfate supported on a pledget of glass wool. Extract the aqueous layer with two additional 10-mL portions of chloroform, filtering and combining with the main extract. Evaporate the chloroform solution under reduced pressure to dryness, and dissolve the residue in 10 mL of carbon disulfide.
Sample solution: Prepare as directed in the *Standard solution* using 30 mg of Hyoscyamine.
Acceptance criteria: The IR absorption spectrum (determined in a 1-mm cell) of the *Sample solution* exhibits maxima only at the same wavelengths as those of the *Standard solution*.

• **B.**
Sample: 60 mg
Analysis: Dissolve the *Sample* in 1 mL of 0.2 N hydrochloric acid, and add gold chloride TS, dropwise with shaking, until a definite precipitate separates. Add a small amount of 3 N hydrochloric acid, dissolve the precipitate with the aid of heat, and then allow to cool.
Acceptance criteria: Lustrous golden yellow scales are formed (distinction from atropine and scopolamine).

ASSAY

• **PROCEDURE**
Sample: 500 mg
Analysis: Dissolve the *Sample* in 50 mL of glacial acetic acid, and add 1 drop of crystal violet TS. Titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 28.94 mg of hyoscyamine ($C_{17}H_{23}NO_3$).
Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **LIMIT OF FOREIGN ALKALOIDS AND OTHER IMPURITIES**
Standard solution: 24 mg/mL of USP Hyoscyamine Sulfate RS in methanol
Sample solution A: 20 mg/mL of Hyoscyamine in methanol
Sample solution B: 1 mg/mL of Hyoscyamine from *Sample solution A* in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.5-mm layer of chromatographic silica gel
Developing solvent system: Chloroform, acetone, and diethylamine (5:4:1)
Spray reagent: Potassium iodoplatinate TS
Analysis: Apply 25 µL of *Sample solution A*, 1 µL of *Sample solution B*, and 5 µL of the *Standard solution* to a suitable thin-layer chromatographic plate. Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by spraying with the *Spray reagent*.
Acceptance criteria: The R_F value of the principal spot of each *Sample solution* corresponds to that of the *Standard solution*. No secondary spot of *Sample solution A* exhibits intensity equal to or greater than the principal spot of *Sample solution B* (0.2%).

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** ⟨741⟩: 106°–109°
• **OPTICAL ROTATION**, *Specific Rotation* ⟨781S⟩
Sample solution: 10 mg/mL, in dilute alcohol (1 in 2)
Acceptance criteria: –20° to –23°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum over silica gel to constant weight.
Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hyoscyamine Sulfate RS

Hyoscyamine Tablets

DEFINITION

Hyoscyamine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of hyoscyamine ($C_{17}H_{23}NO_3$).

IDENTIFICATION

• **A.**
Sample: Macerate a quantity of powdered Tablets, equivalent to 5 mg of hyoscyamine, with 20 mL of water. Filter, and transfer the filtrate to a separator. Render the solution alkaline with 6 N ammonium hydroxide, and extract the alkaloid with 50 mL of methylene chloride. Divide the methylene chloride layer into two equal portions, and evaporate each to dryness. Save one portion for use in *Identification* test *B*.
Analysis: To one portion of the *Sample* add 2 drops of nitric acid, and evaporate on a steam bath to dryness. Cool, and add 10 mL of acetone to dissolve the residue. Add a few drops of alcoholic potassium hydroxide TS.
Acceptance criteria: A violet color is produced.

• **B.**
Sample: The second portion of the *Sample* prepared in *Identification* test *A*
Analysis: Dissolve the *Sample* in 1 mL of 0.1 N hydrochloric acid, and add gold chloride TS, dropwise with shaking, until a definite precipitate separates. Slowly heat until the precipitate dissolves, and allow the solution to cool.
Acceptance criteria: Lustrous, golden yellow scales are formed.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: Dissolve 34.8 g of dibasic potassium phosphate in 900 mL of water, and adjust to a pH of 9.0 by the addition of 3 N hydrochloric acid or 1 N sodium hydroxide.

Internal standard solution: 0.5 mg/mL of homatropine hydrobromide in water. Prepare fresh daily.

Standard stock solution: 0.1 mg/mL of USP Hyoscyamine Sulfate RS. Prepare fresh daily.

Standard solution: Pipet 10.0 mL of *Standard stock solution* into a separator, add 2.0 mL of *Internal standard solution* and 5.0 mL of *Buffer*, and adjust with 1 N sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under nitrogen to dryness. Dissolve the residue in 2.0 mL of methylene chloride.

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 0.86 mg of hyoscyamine, to a separator containing 5 mL of *Buffer*. Add, by pipet, 2.0 mL of *Internal standard solution* and adjust with 1 N sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under nitrogen to dryness. Dissolve the residue in 2.0 mL of methylene chloride.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Column: 1.8-m × 2-mm glass; packed with 3% liquid phase G3 on support S1AB, cured as directed in the chapter for *Gas Chromatography*

Column temperature: 225°

Carrier gas: Nitrogen

Flow rate: 25 mL/min

Injection volume: 1 µL

System suitability

Sample: 6–10 injections of the *Standard solution*

Suitability requirements

Resolution: NLT 5

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for the ratio of the peak areas

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the areas under the peaks for hyoscyamine and homatropine in each chromatogram. Calculate the ratio, R_U, of the area of the hyoscyamine peak to the area of the internal standard peak from the *Sample solution*, and similarly calculate the ratio, R_S, from the *Standard solution*.

Calculate the percentage of the labeled amount of hyoscyamine ($C_{17}H_{23}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio from the *Sample solution*

R_S = peak response ratio from the *Standard solution*

C_S = concentration of USP Hyoscyamine Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hyoscyamine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of hyoscyamine, 289.37

M_{r2} = molecular weight of anhydrous hyoscyamine sulfate, 676.83

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISINTEGRATION** ⟨701⟩: 30 min, the use of disks being omitted

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

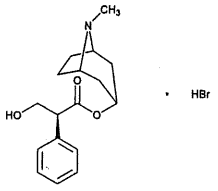
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Hyoscyamine Sulfate RS

Hyoscyamine Hydrobromide



$C_{17}H_{23}NO_3 \cdot HBr$ 370.28

Benzeneacetic acid, α-(hydroxymethyl)-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, hydrobromide [3(*S*)-*endo*]-; 1α*H*,5α*H*-Tropan-3α-ol (–)-tropate (ester) hydrobromide [306-03-6].

DEFINITION

Hyoscyamine Hydrobromide contains NLT 98.5% and NMT 100.5% of hyoscyamine hydrobromide ($C_{17}H_{23}NO_3 \cdot HBr$), calculated on the dried basis.

[**CAUTION**—Handle Hyoscyamine Hydrobromide with exceptional care, since it is highly potent.]

IDENTIFICATION

• **A.**

Standard solution: Transfer 36 mg of USP Hyoscyamine Sulfate RS to a 60-mL separator with the aid of 5 mL of water. Add 1.5 mL of 1 N sodium hydroxide and 10 mL of chloroform to the separator. Shake for 1 min, allow the layers to separate, and pass the chloroform extracts through a filter of about 2 g of anhydrous granular sodium sulfate supported on a pledget of glass wool. Extract the aqueous layer with two additional 10-mL portions of chloroform, filtering and combining with the main extract. Evaporate the chloroform solution, under reduced pressure, to dryness, and dissolve the residue in 10 mL of carbon disulfide.

Sample solution: Prepare as directed in the *Standard solution* using 30 mg of Hyoscyamine Hydrobromide.

Acceptance criteria: The IR absorption spectrum (determined in a 1-mm cell) of the *Sample solution* exhibits maxima only at the same wavelengths as those of the *Standard solution*.

• **B.**

Sample solution: 1 in 20

Analysis: To 1 mL of the *Sample solution*, add gold chloride TS, dropwise with shaking, until a definite precipitate separates. Add a small amount of 3 N hydrochloric acid, dissolve the precipitate with the aid of heat, and then allow the solution to cool.

Acceptance criteria: Lustrous reddish-brown scales that may be accompanied by reddish-brown needles are formed (distinction from atropine and scopolamine).

• **C.**

Sample solution: 1 in 20

www.webofpharma.com

Analysis: To the *Sample solution,* add silver nitrate TS.
Acceptance criteria: A yellowish-white precipitate is formed, and it is insoluble in nitric acid.

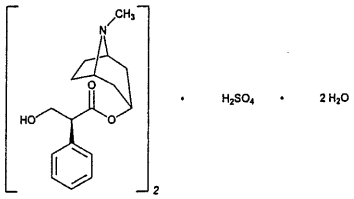
ASSAY
• **PROCEDURE**
Sample: 700 mg
Analysis: Dissolve the *Sample* in a mixture of 50 mL of glacial acetic acid and 10 mL of mercuric acetate TS. Add 1 drop of crystal violet TS. Titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 37.03 mg of hyoscyamine hydrobromide ($C_{17}H_{23}NO_3 \cdot HBr$).
Acceptance criteria: 98.5%–100.5% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
• **OTHER ALKALOIDS**
Sample solution: Dissolve 250 mg of Hyoscyamine Hydrobromide in 1 mL of 0.1 N hydrochloric acid, and dilute with water to 15 mL.
Analysis 1: To 5 mL of the *Sample solution,* add a few drops of platinic chloride TS.
Acceptance criteria 1: No precipitate is formed immediately.
Analysis 2: To another 5-mL portion of the *Sample solution,* add 2 mL of 6 N ammonium hydroxide.
Acceptance criteria 2: The mixture may develop a slight opalescence, but no turbidity or precipitate is formed immediately.

SPECIFIC TESTS
• **MELTING RANGE OR TEMPERATURE** ⟨741⟩: NLT 149°
• **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩
Sample solution: 50 mg/mL in water
Acceptance criteria: NLT –24°
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hyoscyamine Sulfate RS

Hyoscyamine Sulfate



$(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O$ 712.85
$(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$ 676.83
Benzeneacetic acid, α-(hydroxymethyl)-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, [3(*S*)-*endo*]-, sulfate (2:1), dihydrate;
1α*H*,5α*H*-Tropan-3α-ol (–)-tropate (ester) sulfate (2:1) (salt) dihydrate;
(1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl (*S*)-3-hydroxy-2-phenylpropanoate sulfate dihydrate [6835-16-1].

DEFINITION
Hyoscyamine Sulfate contains NLT 98.0% and NMT 102.0% of hyoscyamine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4]$, calculated on the anhydrous basis.
[**CAUTION**—Handle hyoscyamine sulfate with exceptional care because it is highly potent.]

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy,* 197A or 197K:▲ (CN 1-May-2020) Meets the requirements.
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
• **C. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Chemical Identification Tests, Sulfate*
Sample solution: 50 mg/mL
Acceptance criteria: Meets the requirements

ASSAY
• **PROCEDURE**
Buffer: 7.0 g/L of monobasic potassium phosphate, adjusted with 0.05 M phosphoric acid to a pH of 3.3
Solution A: Dissolve 3.5 g of sodium dodecyl sulfate in 606 mL of *Buffer,* add 320 mL of acetonitrile, and mix.
Solution B: Acetonitrile
Mobile phase: See *Table 1.* Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2	95	5
20	70	30

Standard solution: 0.24 mg/mL of USP Hyoscyamine Sulfate RS in *Solution A*
Sample solution: 0.24 mg/mL of Hyoscyamine Sulfate in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of hyoscyamine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4]$ in the portion of Hyoscyamine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hyoscyamine Sulfate RS in the *Standard solution* (mg/mL)

www.webofpharma.com

C_U = concentration of Hyoscyamine Sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 1.2 mg/mL of USP Hyoscyamine Sulfate RS in *Solution A*

Standard solution: 0.24 µg/mL of USP Hyoscyamine Sulfate RS from the *Standard stock solution* in *Solution A*

System suitability stock solution: 2.4 µg/mL of USP Hyoscyamine Related Compound A RS in *Solution A*

System suitability solution: Transfer 5.0 mL of *System suitability stock solution* to a 50-mL volumetric flask, add 10 mL of the *Standard stock solution*, and dilute with *Solution A* to volume.

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between hyoscyamine related compound A and hyoscyamine, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Hyoscyamine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of hyoscyamine from the *Standard solution*

C_S = concentration of USP Hyoscyamine Sulfate RS in the *Standard solution* (µg/mL)

C_U = concentration of Hyoscyamine Sulfate in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
DL-Tropic acid	0.2	2.1	0.2
7-Hydroxyhyoscyamine	0.67	1.0	0.2
6-Hydroxyhyoscyamine	0.72	1.0	0.2
Scopolamine	0.8	1.0	0.2
Norhyoscyamine (hyoscyamine related compound A)	0.9	1.0	0.3
Hyoscyamine	1.0	—	—
Littorine	1.1	1.0	0.2
Apoatropine	1.8	1.7	0.2
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	0.5

SPECIFIC TESTS

- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 50 mg/mL in water

Acceptance criteria: −24° to −29°, measured at 20°

- **WATER DETERMINATION** ⟨921⟩, *Method I, Method Ia*: 2.0%–5.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Hyoscyamine Sulfate RS

USP Hyoscyamine Related Compound A RS

Norhyoscyamine sulfate;
(1*R*,3*r*,5*S*)-8-Azabicyclo[3.2.1]oct-3-yl(2*S*)-3-hydroxy-2-phenylpropanoate sulfate (2:1).
$C_{32}H_{44}N_2O_{10}S$ 648:77

Hyoscyamine Sulfate Elixir

DEFINITION

Hyoscyamine Sulfate Elixir contains NLT 90.0% and NMT 110.0% of the labeled amount of hyoscyamine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: Transfer 13.6 g of monobasic potassium phosphate to a 2000-mL volumetric flask, and dissolve in about 1800 mL of water. Adjust with phosphoric acid to a pH of 3.0 ± 0.1, dilute with water to volume, and filter.

Mobile phase: Methanol and *Buffer* (25:75), degassed

Diluent: 0.01 N hydrochloric acid

Standard stock solution: 0.16 mg/mL of anhydrous hyoscyamine sulfate from USP Hyoscyamine Sulfate RS in *Diluent*. [NOTE—This solution may be stored in a refrigerator for 30 days.]

Standard solution: 4.8 µg/mL of anhydrous hyoscyamine sulfate from the *Standard stock solution* in *Diluent*

Tropic acid solution: 4 µg/mL of tropic acid in *Diluent*

System suitability solution: Transfer 3.0 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 4.0 mL of the *Tropic acid solution*, and dilute with *Diluent* to volume.

Sample solution: 5 µg/mL of hyoscyamine sulfate from an appropriate volume of Elixir in *Diluent*. Pass an aliquot through a suitable filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Columns

Guard: 3-mm × 4-mm; packing L11

Analytical: 4.6-mm × 15-cm; 4-µm packing L11

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 50 µL

System suitability

Sample: *System suitability solution*

[NOTE—The elution order is the tropic acid peak, followed by the hyoscyamine peak.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 1.5 between tropic acid and hyoscyamine
Tailing factor: NMT 2.0 for the hyoscyamine peak
Relative standard deviation: NMT 2.0% for replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hyoscyamine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$ in the portion of Elixir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of anhydrous hyoscyamine sulfate in the *Standard solution* (µg/mL)
C_U = nominal concentration of hyoscyamine sulfate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of hyoscyamine sulfate, 712.85
M_{r2} = molecular weight of anhydrous hyoscyamine sulfate, 676.83

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 3.0–6.5
- **Alcohol Determination** ⟨611⟩**:** 90.0%–110.0% of the labeled amount of alcohol (C_2H_5OH)

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Hyoscyamine Sulfate RS

Hyoscyamine Sulfate Injection

DEFINITION
Hyoscyamine Sulfate Injection is a sterile solution of Hyoscyamine Sulfate in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of hyoscyamine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. Identification Tests—General,** *Sulfate* ⟨191⟩**:** After evaporation to dryness, or appropriate adjustment of concentration, it meets the requirements of the tests.
- **C.** The angular rotation of the Injection is levorotatory.

ASSAY
- **Procedure**
  Buffer: Transfer 13.6 g of monobasic potassium phosphate to a 2000-mL volumetric flask, and dissolve in about 1800 mL of water. Adjust with phosphoric acid to a pH of 3.0 ± 0.1, dilute with water to volume, and filter.
  Mobile phase: With continuous stirring, add 0.3 mL of triethylamine to 1800 mL of *Buffer*. Add 200 mL of acetonitrile, and degas.
  Diluent: 0.01 N hydrochloric acid
  Standard stock solution: 0.16 mg/mL of anhydrous hyoscyamine sulfate from USP Hyoscyamine Sulfate RS in *Diluent*. [Note—This solution may be stored in a refrigerator for 30 days.]
  Standard solution: 4.8 µg/mL of anhydrous hyoscyamine sulfate from the *Standard stock solution* in *Diluent*
  Sample solution: 5.0 µg/mL of hyoscyamine sulfate from an appropriate volume of Injection in *Diluent*. Pass an aliquot through a suitable filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 205 nm
  Columns
  Guard: 3-mm × 4-mm; packing L11
  Analytical: 4.6-mm × 15-cm; 4-µm packing L11
  Column temperature: 30°
  Flow rate: 1.0 mL/min
  Injection volume: 50 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 1.8
  Relative standard deviation: NMT 2.0% for six replicate injections
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of hyoscyamine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$ in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of anhydrous hyoscyamine sulfate in the *Standard solution* (µg/mL)
C_U = nominal concentration of hyoscyamine sulfate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of hyoscyamine sulfate, 712.85
M_{r2} = molecular weight of anhydrous hyoscyamine sulfate, 676.83

Acceptance criteria: 93.0%–107.0%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 3.0–6.5
- **Bacterial Endotoxins Test** ⟨85⟩**:** It contains NMT 714.3 USP Endotoxin Units/mg of hyoscyamine sulfate.
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Hyoscyamine Sulfate RS

Hyoscyamine Sulfate Oral Solution

DEFINITION
Hyoscyamine Sulfate Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of hyoscyamine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$.

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Buffer: Transfer 13.6 g of monobasic potassium phosphate to a 2000-mL volumetric flask, dissolve in about 1800 mL of water, adjust with phosphoric acid to a pH of 3.0 ± 0.1, dilute with water to volume, and filter.

Mobile phase: With continuous stirring, add 0.3 mL of triethylamine to 1800 mL of *Buffer*. Add 200 mL of acetonitrile, and degas.

Diluent: 0.01 N hydrochloric acid

Standard stock solution: 0.16 mg/mL of anhydrous hyoscyamine sulfate from USP Hyoscyamine Sulfate RS in *Diluent*. [NOTE—This solution may be stored in a refrigerator for 30 days.]

Standard solution: 4.8 µg/mL of anhydrous hyoscyamine sulfate from the *Standard stock solution* in *Diluent*

Sample solution: 5 µg/mL of hyoscyamine sulfate from an appropriate volume of Oral Solution in *Diluent*. Pass an aliquot through a suitable filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Columns

Guard: 3-mm × 4-mm; packing L11

Analytical: 4.6-mm × 15-cm; 4-µm packing L11

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.8

Relative standard deviation: NMT 2.0% for six replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hyoscyamine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$ in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of anhydrous hyoscyamine sulfate in the *Standard solution* (µg/mL)

C_U = nominal concentration of hyoscyamine sulfate in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of hyoscyamine sulfate, 712.85

M_{r2} = molecular weight of anhydrous hyoscyamine sulfate, 676.83

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For single-unit containers

Acceptance criteria: Meets the requirements

• **DELIVERABLE VOLUME** ⟨698⟩

For multiple-unit containers

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 3.0–6.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Hyoscyamine Sulfate RS

Hyoscyamine Sulfate Tablets

DEFINITION

Hyoscyamine Sulfate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of hyoscyamine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩**:** A filtered solution of Tablets meets the requirements of the tests.

ASSAY

• **PROCEDURE**

Buffer: Transfer 13.6 g of monobasic potassium phosphate to a 2000-mL volumetric flask, dissolve in about 1800 mL of water, and adjust with phosphoric acid to a pH of 3.0 ± 0.1. Dilute with water to volume, and filter.

Mobile phase: With continuous stirring, add 0.3 mL of triethylamine to 1800 mL of *Buffer*. Add 200 mL of acetonitrile and degas.

Diluent: 0.01 N hydrochloric acid

Standard stock solution: 0.16 mg/mL of anhydrous hyoscyamine sulfate from USP Hyoscyamine Sulfate RS in *Diluent*. [NOTE—This solution may be stored in a refrigerator for 30 days.]

Standard solution: 4.8 µg/mL of anhydrous hyoscyamine sulfate from the *Standard stock solution* in *Diluent*

Tropic acid solution: 3 µg/mL of tropic acid in *Diluent*

System suitability solution: Transfer 3.0 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 4.0 mL of the *Tropic acid solution*, and dilute with *Diluent* to volume.

Sample solution: Finely powder NLT 20 Tablets. Transfer a quantity of the powder equivalent to 0.125 mg of hyoscyamine sulfate to a 25-mL volumetric flask. Add 20 mL of *Diluent*, and sonicate for 15 min with occasional swirling. Allow to cool to room temperature, and dilute with *Diluent* to volume. Pass an aliquot through a suitable filter of 0.45-µm pore size, and discard the first 5 mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Columns

Guard: 3-mm × 4-mm; packing L11

Analytical: 4.6-mm × 15-cm; 4-µm packing L11

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 50 µL

System suitability

Sample: *System suitability solution*

[NOTE—The elution order is the tropic acid peak, followed by the hyoscyamine peak.]

Suitability requirements

Resolution: NLT 1.5 between tropic acid and hyoscyamine

Tailing factor: NMT 1.8 for the hyoscyamine peak

Relative standard deviation: NMT 2.0% for six replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the percentage of the labeled amount of hyoscyamine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O]$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of anhydrous hyoscyamine sulfate in the *Standard solution* (µg/mL)
C_U = nominal concentration of hyoscyamine sulfate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of hyoscyamine sulfate, 712.85
M_{r2} = molecular weight of anhydrous hyoscyamine sulfate, 676.83

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISINTEGRATION** ⟨701⟩**:** 15 min
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Hyoscyamine Sulfate RS

Hypromellose

Portions of this monograph that are national *USP* text, and are not part of the harmonized text, are marked with symbols (• •) to specify this fact.

Cellulose, 2-hydroxypropyl methyl ether;
Cellulose hydroxypropyl methyl ether [9004-65-3].

DEFINITION
Hypromellose is a methyl and hydroxypropyl mixed ether of cellulose. It contains, calculated on the dried basis, methoxy ($-OCH_3$: 31.03) and hydroxypropoxy ($-OC_3H_6OH$: 75.09) groups conforming to the limits for the types of Hypromellose (hydroxypropyl methylcellulose) set forth in the following table.

	Methoxy (%)		Hydroxypropoxy (%)	
Substitution Type	Min.	Max.	Min.	Max.
1828	16.5	20.0	23.0	32.0
2208	19.0	24.0	4.0	12.0
2906	27.0	30.0	4.0	7.5
2910	28.0	30.0	7.0	12.0

IDENTIFICATION
- **A.**
  Sample: 1 g
  Analysis: Gently add the *Sample* to the top of 100 mL of water in a beaker, and allow to disperse over the surface, tapping the top of the container to ensure an even dispersion of the substance. Allow the beaker to stand for 1–2 min.
  Acceptance criteria: The powdered material aggregates on the surface.
- **B.**
  Sample: 1 g
  Analysis: Add the *Sample* to 100 mL of boiling water, and stir the mixture using a magnetic stirrer with a bar 25-mm long.
  Acceptance criteria: A slurry is formed, but the powdered material does not dissolve. Cool the slurry to 10°, and stir using a magnetic stirrer. The resulting liquid is a clear or slightly turbid solution with thickness dependent on the viscosity grade.
- **C.**
  Solution A: Sulfuric acid and water (9 in 10). [NOTE—Carefully add sulfuric acid to water.]
  Sample solution: 0.1 mL of the solution prepared for *Identification B*
  Analysis: To the *Sample solution* add 9 mL of *Solution A*, and shake. Heat in a water bath for exactly 3 min, immediately cool in an ice bath, and add carefully 0.6 mL of ninhydrin TS. Shake, and allow to stand at 25°.
  Acceptance criteria: A red color develops at first that changes to purple within 100 min.
- **D.**
  Sample solution: 2–3 mL of the solution prepared for *Identification B*
  Analysis: Pour the *Sample solution* onto a glass slide as a thin film, and allow the water to evaporate.
  Acceptance criteria: A coherent, clear film forms on the glass slide.
- **E.**
  Sample solution: 50 mL of the solution prepared in *Identification B*
  Analysis: Add the *Sample solution* to exactly 50 mL of water in a beaker. Insert a thermometer into the solution. Stir the solution on a magnetic stirrer/hot plate, and begin heating at a rate of 2°–5°/min. Determine the temperature at which a turbidity increase begins to occur, and designate this temperature as the flocculation temperature.
  Acceptance criteria: The flocculation temperature is higher than 50°.

ASSAY

Change to read:

- **PROCEDURE**
  [**CAUTION**—Hydriodic acid and its reaction byproducts are highly toxic. Perform all steps in the preparation of the *Standard solution* and the *Sample solution* in a properly functioning hood. Specific safety practices to be followed are to be identified to the analyst performing this test.]
  Apparatus: For the reaction vial, use a 5-mL pressure-tight serum vial, 50 mm in height, 20 mm in outside diameter, and 13 mm in inside diameter at the mouth. The vial is equipped with a pressure-tight septum with a polytetrafluoroethylene-faced butyl rubber and an airtight seal using an aluminum crimp or any sealing system that provides sufficient airtightness. Use a heater having a heating module that has a square-shaped aluminum block with holes 20 mm in diameter and 32 mm in depth, into which the reaction vial fits. The heating module is also equipped with a magnetic stirrer capable of mixing the contents of the vial, or use a reciprocal shaker that performs a reciprocating motion of about 100 times/min.
  Hydriodic acid: Use a reagent with a typical concentration of hydrogen iodide (HI) of about 57%.
  Internal standard solution: 30 mg/mL of *n*-octane in *o*-xylene
  Standard solution: Into a suitable serum vial, weigh between 60 and 100 mg of adipic acid, and add 2.0 mL of

www.webofpharma.com

Hydriodic acid and 2.0 mL of *Internal standard solution*. Close the vial securely with a suitable septum stopper. Weigh the vial and contents, add between 15 and 22 µL of isopropyl iodide through the septum with a syringe, weigh again, and calculate the weight of isopropyl iodide added, by difference. Add 45 µL of methyl iodide similarly, weigh again, and calculate the weight of methyl iodide added, by difference. Shake the reaction vial well, and allow the layers to separate. Use the upper layer as the *Standard solution*.

Sample solution: Transfer 0.065 g of Hypromellose to a 5-mL thick-walled reaction vial equipped with a pressure-tight septum-type closure, add between 60 and 100 mg of adipic acid, and pipet 2.0 mL of *Internal standard solution* into the vial. Cautiously pipet 2.0 mL of *Hydriodic acid* into the mixture, immediately cap the vial tightly, and weigh. Using the magnetic stirrer equipped in the heating module, or using a reciprocal shaker, mix the contents of the vial continuously, heating and maintaining the temperature of the contents at 130 ± 2° for 60 min. If a reciprocal shaker or magnetic stirrer cannot be used, shake the vial well by hand at 5-min intervals during the initial 30 min of the heating time. Allow the vial to cool, and weigh. If the weight loss is ▲≥26 mg▲ (USP 1-May-2019) or there is evidence of a leak, discard the mixture, and prepare another *Sample solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Thermal conductivity or hydrogen flame-ionization

Column: ▲0.53-mm × 30-m fused silica capillary, coated with a 3-µm layer of phase G1. Use a guard column if necessary.

Temperatures

Injection port: 250°

Detector: 280°

Column: See the temperature program shown in *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	3
50	10	100	—
100	34.9	250	8▲ (USP 1-May-2019)

Carrier gas: ▲Helium▲ (USP 1-May-2019)

Flow rate: With the *Standard solution*, adjust the flow rate so that the retention time of the internal standard is about 10 min ▲(about 4.3 mL/min).▲ (USP 1-May-2019)

Injection volume: 1–2 µL

▲Injection type: Split ratio, 40:1

Run time: 20.3 min

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: Methyl iodide, isopropyl iodide, and the internal standard are eluted in this order, with resolution NLT 5 between these peaks.

Relative standard deviation: NMT 2.0% for 6 injections of the *Standard solution*, using the ratio of the peak area of methyl iodide, isopropyl iodide to that of the internal standard. ▲ (USP 1-May-2019)

Analysis

Samples: Upper layer of the *Standard solution* and the *Sample solution*

Calculate the percentage of methoxy ($-OCH_3$) in the portion of Hypromellose taken:

$$\text{Result} = 21.864 \times (R_{Ua}/R_{Sa}) \times (W_{Sa}/W_U)$$

R_{Ua} = peak area ratio of methyl iodide to *n*-octane from the *Sample solution*

R_{Sa} = peak area ratio of methyl iodide to *n*-octane from the *Standard solution*

W_{Sa} = weight of methyl iodide in the *Standard solution* (mg)

W_U = weight of Hypromellose, calculated on the dried basis, taken for the *Sample solution* (mg)

Calculate the percentage of hydroxypropoxy ($-OC_3H_6OH$) in the portion of Hypromellose taken:

$$\text{Result} = 44.17 \times (R_{Ub}/R_{Sb}) \times (W_{Sb}/W_U)$$

R_{Ub} = peak area ratio of isopropyl iodide to *n*-octane from the *Sample solution*

R_{Sb} = peak area ratio of isopropyl iodide to *n*-octane from the *Standard solution*

W_{Sb} = weight of isopropyl iodide in the *Standard solution* (mg)

W_U = weight of Hypromellose, calculated on the dried basis, taken for the *Sample solution* (mg)

Acceptance criteria: See the limits, calculated on the dried basis, in the table in the *Definition*.

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩

Sample: 1.0 g

Acceptance criteria: NMT 1.5%

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample: Use the solution prepared in the tests for *Viscosity—Capillary Methods* and *Viscosity—Rotational Methods*.

Analysis: Read the indicated pH value after the probe has been immersed for 5 ± 0.5 min.

Acceptance criteria: 5.0–8.0

• **LOSS ON DRYING** ⟨731⟩

Sample: 1.0 g

Analysis: Dry the *Sample* at 105° for 1 h.

Acceptance criteria: NMT 5.0%

• **VISCOSITY—CAPILLARY METHODS** ⟨911⟩ and **VISCOSITY—ROTATIONAL METHODS** ⟨912⟩

Method 1: This method is applied to Hypromellose samples with a viscosity type of less than 600 mPa · s.

Sample solution: Transfer a quantity of Hypromellose equivalent to 4 g of solids, calculated on the dried basis, to a tared, wide-mouth centrifuge bottle. Add hot water (90°–99°) to obtain a total weight of the sample and water of 200.0 g. Capping the bottle, stir by mechanical means at 400 ± 50 rpm for 10–20 min until the particles are thoroughly dispersed and wetted out. Scrape down the walls of the bottle with a spatula, if necessary, to ensure that there is no undissolved material on the sides of the bottle, and continue the stirring in a cooling water bath equilibrated at a temperature below 10° for another 20–40 min. Adjust the solution weight, if necessary, to 200.0 g, using cold water. Centrifuge the solution, if necessary, to expel any entrapped air. If any foam is present, remove with a spatula.

USP Monographs

www.webofpharma.com

Analysis: Determine the viscosity at 20 ± 0.1° in a suitable viscometer of the Ubbelohde type as directed in *Viscosity —Capillary Methods* ⟨911⟩.

Acceptance criteria: 80%–120% of the viscosity stated on the label

Method 2: This method is applied to Hypromellose samples with a viscosity type of 600 mPa · s or higher.

Sample solution: Transfer a quantity of Hypromellose equivalent to 10 g of solids, calculated on the dried basis, to a tared, wide-mouth centrifuge bottle, and add hot water (90°–99°) to obtain a total weight of the sample and water of 500.0 g. Capping the bottle, stir by mechanical means at 400 ± 50 rpm for 10–20 min until the particles are thoroughly dispersed and wetted out. Scrape down the walls of the bottle with a spatula, if necessary, to ensure that there is no undissolved material on the sides of the bottle, and continue the stirring in a cooling water bath equilibrated at a temperature below 10° for another 20–40 min. Adjust the solution weight, if necessary, to 500.0 g, using cold water. Centrifuge the solution, if necessary, to expel any entrapped air. If any foam is present, remove with a spatula.

Analysis: Equip a suitable single-cylinder type rotational viscometer (Brookfield type LV model, or equivalent), and determine the viscosity of this solution at 20 ± 0.1° under the operating conditions specified in *Table 2*.

Table 2

Labeled Viscosity[a] (mPa · s)	Rotor No.	Revolution (rpm)	Calculation Multiplier
600 or more and less than 1400	3	60	20
1400 or more and less than 3500	3	12	100
3500 or more and less than 9500	4	60	100
9500 or more and less than 99,500	4	6	1000
99,500 or more	4	3	2000

[a] The labeled viscosity is based on the manufacturer's specifications.

Allow the spindle to rotate for 2 min before taking the measurement. Allow a rest period of at least 2 min between subsequent measurements. Repeat the operation twice to rotate the spindle as specified above, and average the three readings.

Acceptance criteria: 75%–140% of the viscosity stated on the label

ADDITIONAL REQUIREMENTS

- ***PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements are specified.◆
- **LABELING:** Label it to indicate its substitution type and its nominal viscosity value in millipascals per second (mPa · s).

Hypromellose Ophthalmic Solution

» Hypromellose Ophthalmic Solution is a sterile solution of Hypromellose. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of Hypromellose (hydroxypropyl methylcellulose). It may contain suitable antimicrobial, buffering, and stabilizing agents.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Hypromellose RS

Identification—

A: Pour a few mL of Ophthalmic Solution onto a glass plate, and allow the water to evaporate: a thin, self-sustaining film results.

B: Heat 5 mL of Ophthalmic Solution in a test tube over a low flame: the warm solution turns cloudy but clears upon chilling.

Sterility Tests ⟨71⟩ **:** meets the requirements.

pH ⟨791⟩**:** between 6.0 and 7.8.

Assay—

Standard preparation—Dissolve a suitable quantity of USP Hypromellose RS, accurately weighed, in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 100 µg per mL.

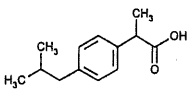
Assay preparation—Dilute an accurately measured volume of Ophthalmic Solution quantitatively with water to obtain a solution having an equivalent concentration of about 100 µg of hypromellose per mL.

Procedure—Pipet 2 mL each of the *Standard preparation*, the *Assay preparation*, and water to provide a blank, into separate, glass-stoppered test tubes. To each tube add 5.0 mL of diphenylamine solution (prepared by dissolving 3.75 g of colorless diphenylamine crystals in 150 mL of glacial acetic acid and diluting the solution with 90 mL of hydrochloric acid), mix, and immediately insert the tubes into an oil bath at 105° to 110° for 30 minutes, the temperature being kept uniform within 0.1° during heating. Remove the tubes, and place them in an ice-water bath for 10 minutes or until thoroughly cool. At room temperature and using a suitable spectrophotometer, concomitantly determine the absorbances of the solutions from the *Standard preparation* and the *Assay preparation* at 635 nm, using the water solution as the blank. Calculate the quantity, in mg, of hypromellose in each mL of the Ophthalmic Solution taken by the formula:

$$0.001\,Cd(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Hypromellose RS in the *Standard preparation; d* is the dilution factor used to obtain the *Assay preparation;* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Ibuprofen



$C_{13}H_{18}O_2$ 206.28

Benzeneacetic acid, α-methyl-4-(2-methylpropyl), (±)-.
(±)-*p*-Isobutylhydratropic acid.
(±)-2-(*p*-Isobutylphenyl)propionic acid [15687-27-1].
(±) Mixture [58560-75-1].

www.webofpharma.com

» Ibuprofen contains not less than 97.0 percent and not more than 103.0 percent of $C_{13}H_{18}O_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Ibuprofen RS
USP Ibuprofen Related Compound C RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020)—Do not dry specimens.

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 250 µg per mL.
Medium: 0.1 N sodium hydroxide.
Respective absorptivities at 264 nm and 273 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

C: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ibuprofen, the retention time of which, relative to that of the internal standard, corresponds to that exhibited in the chromatogram of the *Standard preparation,* obtained as directed in the *Assay.*
Water Determination, *Method I* ⟨921⟩: not more than 1.0%.
Residue on ignition ⟨281⟩: not more than 0.5%.

Chromatographic purity—
Mobile phase—Prepare a suitable filtered mixture of water, previously adjusted with phosphoric acid to a pH of 2.5 and acetonitrile (1340:680). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Test preparation—Prepare a solution of Ibuprofen in acetonitrile containing about 5 mg per mL.
Resolution solution—Prepare a solution in acetonitrile containing in each mL about 5 mg of Ibuprofen and 5 mg of valerophenone.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4-mm × 15-cm column that contains 5-µm packing L1 and is maintained at 30 ± 0.5°. The flow rate is about 2 mL per minute. Chromatograph a series of 5-µL injections of the *Test preparation* to condition the column. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for valerophenone and 1.0 for ibuprofen, and the resolution, *R,* between the valerophenone peak and the ibuprofen peak is not less than 2.0.
Procedure— [NOTE—Use peak areas where peak responses are indicated.] Inject about 5 µL of the *Test preparation* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity taken by the formula:

$$100 r_i / r_t$$

in which r_i is the response of an individual peak, other than the solvent peak and the main ibuprofen peak, and r_t is the sum of the responses of all the peaks, excluding that of the solvent peak: not more than 0.3% of any individual impurity is found, and the sum of all the individual impurities found does not exceed 1.0%.
Limit of ibuprofen related compound C—Using the chromatograms of the *Assay preparation* and the *Ibuprofen related compound C standard solution,* obtained as directed in the *Assay,* calculate the percentage of ibuprofen related compound C ($C_{12}H_{16}O$) in the portion of Ibuprofen taken by the formula:

$$10{,}000(C/W)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Ibuprofen Related Compound C RS in the *Ibuprofen related compound C standard solution; W* is the weight, in mg, of Ibuprofen taken to prepare the *Assay preparation;* and R_U and R_S are the peak response ratios of ibuprofen related compound C to valerophenone obtained from the *Assay preparation* and the *Ibuprofen related compound C standard solution,* respectively: not more than 0.1% is found.

Assay—
Mobile phase—Dissolve 4.0 g of chloroacetic acid in 400 mL of water, and adjust with ammonium hydroxide to a pH of 3.0. Add 600 mL of acetonitrile, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Internal standard solution—Prepare a solution of valerophenone in *Mobile phase* having a concentration of about 0.35 mg per mL.
Standard preparation—Dissolve an accurately weighed quantity of USP Ibuprofen RS in *Internal standard solution* to obtain a solution having a known concentration of about 12 mg per mL.
Ibuprofen related compound C standard solution—Quantitatively dissolve an accurately weighed quantity of USP Ibuprofen Related Compound C RS in acetonitrile to obtain a solution having a known concentration of about 0.6 mg per mL. Add 2.0 mL of this stock solution to 100.0 mL of *Internal standard solution,* and mix to obtain a solution having a known concentration of about 0.012 mg of ibuprofen related compound C per mL.
Assay preparation—Transfer about 1200 mg of Ibuprofen, accurately weighed, to a 100-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.4 for the internal standard and 1.0 for ibuprofen; the resolution, *R,* between ibuprofen and the internal standard is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Ibuprofen related compound C standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for valerophenone and 1.2 for ibuprofen related compound C; the resolution, *R,* between valerophenone and ibuprofen related compound C is not less than 2.5; the tailing factors for the individual peaks are not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation,* the *Assay preparation,* and the *Ibuprofen related compound C standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{13}H_{18}O_2$ in the portion of Ibuprofen taken by the formula:

$$100C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Ibuprofen RS in the *Standard preparation;* and R_U and R_S are

USP Monographs

www.webofpharma.com

the peak response ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ibuprofen Oral Suspension

DEFINITION

Ibuprofen Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$).

IDENTIFICATION

- **A.** The UV absorption spectra of the ibuprofen peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the *Standard solution,* as obtained in the *Assay.*
- **B.** The retention time of the ibuprofen peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 1.52 g of monobasic potassium phosphate in 560 mL of water. Adjust with phosphoric acid to a pH of 2.05 if necessary. Add 440 mL of tetrahydrofuran and mix.

Diluent: Methanol and water (1:1)

Standard solution: 0.4 mg/mL of USP Ibuprofen RS in *Diluent*

Sample solution: Nominally 0.4 mg/mL of ibuprofen in *Diluent* prepared as follows. Transfer a suitable amount of Oral Suspension to a suitable volumetric flask. Add about 60% of the final volume of *Diluent.* Sonicate for 45 min. Cool and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 254 nm. For *Identification A,* use a diode-array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.0 mL/min

Injection volume: 25 µL

Run time: NLT 1.9 times the retention time of ibuprofen

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 3.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ibuprofen from the *Sample solution*

r_S = peak response of ibuprofen from the *Standard solution*

C_S = concentration of USP Ibuprofen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Medium: pH 7.2 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Buffer: Dilute 0.7 mL of phosphoric acid with water to obtain 1000 mL of 0.01 M phosphoric acid.

Mobile phase: Acetonitrile and *Buffer* (37:63)

Internal standard solution: 0.3 mg/mL of benzophenone in acetonitrile

Standard stock solution: Dissolve a quantity of USP Ibuprofen RS in *Medium* to obtain a solution with a known concentration of 0.011*J* mg/mL, *J* being the labeled amount of ibuprofen in the Oral Suspension, in mg/mL.

Standard solution: *Internal standard solution* and *Standard stock solution* (1:1), passed through a suitable filter of 0.5-µm or finer pore size

Sample stock solution: Filter a portion of the solution under test.

Sample solution: *Internal standard solution* and *Sample stock solution* (1:1), passed through a suitable filter of 0.5-µm or finer pore size

Density: Using a tared 50-mL volumetric flask, weigh 50 mL of Oral Suspension that has been previously well shaken to ensure homogeneity. Allow to stand until the entrapped air has risen, and invert carefully just prior to transferring it to the volumetric flask. From the observed weight of 50 mL of Oral Suspension, calculate the density of Oral Suspension in g/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for benzophenone and ibuprofen are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between benzophenone and ibuprofen

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Using an accurately tared syringe, draw about 10 mL of well-mixed Oral Suspension into the syringe, which is connected to tubing, and weigh. [NOTE—The tubing of the syringe is placed into a zone that is between the surface of the *Medium* and the top of the rotating blade.] Express the Oral Suspension into the *Medium.* Promptly reweigh the syringe and determine the weight, in g, of Oral Suspension added to the *Medium.*

Calculate the percentage of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$) dissolved:

$$\text{Result} = (R_U/R_S) \times C_S \times V \times (D/W_U) \times (1/L) \times 100$$

R_U = peak area ratio of ibuprofen to benzophenone from the *Sample solution*

R_S = peak area ratio of ibuprofen to benzophenone from the *Standard solution*

C_S = concentration of USP Ibuprofen RS in the *Standard solution* (mg/mL)

V = volume of *Medium,* 900 mL

D = density of Oral Suspension (g/mL)

www.webofpharma.com

W_U = weight of the portion of Oral Suspension added to the *Medium* (g)
L = label claim (mg/mL)

Tolerances: NLT 80% (Q) of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩
For single-unit containers
Acceptance criteria: Meets the requirements
• **Deliverable Volume** ⟨698⟩
For multiple-unit containers
Acceptance criteria: Meets the requirements

IMPURITIES

• **Organic Impurities**
Mobile phase, Diluent, and **Sample solution:** Proceed as directed in the *Assay*.
System suitability solution: 0.4 mg/mL of USP Ibuprofen RS, 0.004 mg/mL each of USP Ibuprofen Related Compound C RS and USP Ibuprofen Related Compound J RS, and 0.0169 mg/mL of USP Benzoic Acid RS in *Diluent*
Sensitivity solution: 0.0002 mg/mL each of USP Ibuprofen Related Compound J RS and USP Ibuprofen Related Compound C RS in *Diluent*
Standard solution A: 0.0002 mg/mL of USP Ibuprofen RS in *Diluent*
Standard solution B: 0.004 mg/mL each of USP Ibuprofen Related Compound J RS and USP Ibuprofen Related Compound C RS in *Diluent*
Chromatographic system: Proceed as directed in the *Assay*, except for the *Detector*.
Detectors
For the quantitation of unspecified degradation products: UV 220 nm
For the quantitation of ibuprofen related compound C and ibuprofen related compound J: UV 254 nm
System suitability
Samples: *System suitability solution, Sensitivity solution, Standard solution A,* and *Standard solution B*
Suitability requirements
For the quantitation of unspecified degradation products
Resolution: NLT 2.0 between benzoic acid and ibuprofen related compound J; NLT 2.0 between ibuprofen related compound J and ibuprofen related compound C; NLT 2.0 between ibuprofen related compound C and ibuprofen, *System suitability solution*
Relative standard deviation: NMT 10.0%, *Standard solution A*
For the quantitation of ibuprofen related compound J and ibuprofen related compound C
Resolution: NLT 2.0 between benzoic acid and ibuprofen related compound J; NLT 2.0 between ibuprofen related compound J and ibuprofen related compound C; NLT 2.0 between ibuprofen related compound C and ibuprofen, *System suitability solution*
Relative standard deviation: NMT 2.0% for ibuprofen related compound J and ibuprofen related compound C, *Standard solution B*
Signal-to-noise ratio: NLT 10 for ibuprofen related compound J and ibuprofen related compound C, *Sensitivity solution*
Analysis
Samples: *Sample solution, Standard solution A,* and *Standard solution B*
Calculate the percentage of ibuprofen related compound J and ibuprofen related compound C in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ibuprofen related compound J or ibuprofen related compound C from the *Sample solution*
r_S = peak response of ibuprofen related compound J or ibuprofen related compound C from *Standard solution B*
C_S = concentration of USP Ibuprofen Related Compound J RS or USP Ibuprofen Related Compound C RS in *Standard solution B* (mg/mL)
C_U = nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified degradation product from the *Sample solution*
r_S = peak response of ibuprofen from *Standard solution A*
C_S = concentration of USP Ibuprofen RS in *Standard solution A* (mg/mL)
C_U = nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)	
		For 20 mg/mL Strength	For 40 mg/mL Strength
Benzoic acid[a]	0.33	—	—
Ibuprofen related compound J	0.48	0.2	0.2
Ibuprofen related compound C	0.81	0.25	0.10
Ibuprofen	1.00	—	—
Any unspecified degradation product	—	0.2	0.2
Total degradation products	—	0.9	0.7

[a] Excipient, not included in the total degradation products.

SPECIFIC TESTS

• **pH** ⟨791⟩: 3.6–4.6

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Benzoic Acid RS
USP Ibuprofen RS
USP Ibuprofen Related Compound C RS
4-Isobutylacetophenone.
$C_{12}H_{16}O$ 176.25
USP Ibuprofen Related Compound J RS
2-(4-Isobutyrylphenyl)propanoic acid.
$C_{13}H_{16}O_3$ 220.26

USP Monographs

www.webofpharma.com

Ibuprofen Tablets

DEFINITION
Ibuprofen Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$).

IDENTIFICATION
- **A.** The UV absorption spectra of the ibuprofen peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the ibuprofen peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Mobile phase: Dissolve 4.0 g of chloroacetic acid in 400 mL of water, adjust with ammonium hydroxide to a pH of 3.0 if necessary, add 600 mL of acetonitrile, and mix.
Standard solution: 10.0 mg/mL of USP Ibuprofen RS in *Mobile phase*
Sample solution: Nominally 10.0 mg/mL of ibuprofen prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask and add about 50% final volume of *Mobile phase*. Shake on a mechanical shaker for at least 60 min or until the Tablets are disintegrated. Dilute with *Mobile phase* to volume. Centrifuge a portion of the solution at about 3000 rpm for about 10 min or until a clear supernatant is obtained. Use the supernatant for analysis.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
[NOTE—It is suggested to use the mixture of methanol and water (90:10) for needle wash.]
Mode: LC
Detectors
Assay: UV 254 nm
Identification test A: Diode array UV 200–400 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 2.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ibuprofen from the *Sample solution*
r_S = peak response of ibuprofen from the *Standard solution*
C_S = concentration of USP Ibuprofen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩

Medium: pH 7.2 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffers*); 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Standard solution: A known concentration of USP Ibuprofen RS in *Medium*
Sample solution: Filter a portion of the solution under test, and suitably dilute with *Medium* if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 221 nm
Analysis: Determine the amount of ibuprofen ($C_{13}H_{18}O_2$) dissolved by comparing the UV absorbance of the *Sample solution* with that of the *Standard solution*. [NOTE—Where the Tablets are labeled as gelatin-coated, determine the amount of ibuprofen ($C_{13}H_{18}O_2$) dissolved from the UV absorbance at the wavelength of maximum absorbance at about 266 nm, from which is subtracted the absorbance at 280 nm, in comparison with the *Standard solution*, similarly measured.]
Tolerances: NLT 80% (*Q*) of the labeled amount of ibuprofen ($C_{13}H_{18}O_2$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES
- **ORGANIC IMPURITIES**

Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.
Sensitivity solution: 0.005 mg/mL of USP Ibuprofen RS in *Mobile phase*
System suitability solution: 10.0 mg/mL of USP Ibuprofen RS and 0.01 mg/mL each of USP Ibuprofen Related Compound C RS and USP Ibuprofen Related Compound J RS in *Mobile phase*
Standard solution: 0.02 mg/mL of USP Ibuprofen RS and 0.01 mg/mL each of USP Ibuprofen Related Compound C RS and USP Ibuprofen Related Compound J RS in *Mobile phase*
System suitability
Samples: *Sensitivity solution*, *System suitability solution*, and *Standard solution*
Suitability requirements
Resolution: NLT 2.5 between ibuprofen related compound J and ibuprofen; NLT 2.5 between ibuprofen and ibuprofen related compound C, *System suitability solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Relative standard deviation: NMT 6.0% for ibuprofen related compound J, ibuprofen, and ibuprofen related compound C, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ibuprofen related compound J and ibuprofen related compound C in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ibuprofen related compound J or ibuprofen related compound C from the *Sample solution*
r_S = peak response of ibuprofen related compound J or ibuprofen related compound C from the *Standard solution*
C_S = concentration of USP Ibuprofen Related Compound J RS or USP Ibuprofen Related Compound C RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

www.webofpharma.com

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified degradation product from the *Sample solution*
r_S = peak response of ibuprofen from the *Standard solution*
C_S = concentration of USP Ibuprofen RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ibuprofen in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ibuprofen related compound J	0.47	0.2
Ibuprofen	1.00	—
Ibuprofen related compound C	1.62	0.25
Any unspecified degradation product	—	0.2
Total degradation products	—	1.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Where the Tablets are gelatin-coated, the label so states.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Ibuprofen RS
  USP Ibuprofen Related Compound C RS
  4-Isobutylacetophenone.
  $C_{12}H_{16}O$ 176.25
  USP Ibuprofen Related Compound J RS
  2-(4-Isobutyrylphenyl)propanoic acid.
  $C_{13}H_{16}O_3$ 220.26

Ibuprofen and Pseudoephedrine Hydrochloride Tablets

» Ibuprofen and Pseudoephedrine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of Ibuprofen ($C_{13}H_{18}O_2$) and Pseudoephedrine Hydrochloride ($C_{10}H_{15}NO \cdot HCl$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Ibuprofen RS
USP Pseudoephedrine Hydrochloride RS

Identification—

A: Place a Tablet in a small beaker, crack the Tablet coating, add 10 mL of methanol, and stir by mechanical means for about 10 minutes. Allow to settle, and use the clear supernatant as the *Test solution*. Prepare a Standard solution in methanol containing about 20 mg of USP Ibuprofen RS and 20*J* mg of USP Pseudoephedrine Hydrochloride RS per mL, *J* being the ratio of the labeled amount, in mg, of pseudoephedrine hydrochloride to the labeled amount, in mg, of ibuprofen per Tablet. Separately apply 10 µL each of the *Test solution* and the Standard solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) covered with a 0.25-mm layer of chromatographic silica gel mixture and activated by heating the plate at 105° for about 30 minutes. Place the plate in a chromatographic chamber equilibrated with a solvent system consisting of a mixture of chloroform, methanol, and glacial acetic acid (80:15:5). Develop the chromatograms until the solvent has moved about 10 cm from the origin. Remove the plate from the chromatographic chamber, place it in a chamber containing iodine vapors for about 10 minutes, and examine the chromatograms: the principal spots obtained from the *Test solution* correspond in R_F value and appearance to those obtained from the Standard solution.

B: The retention times of the pseudoephedrine and ibuprofen peaks, relative to that of the butylparaben internal standard peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—

Medium: pH 7.2 phosphate buffer (see under *Buffers* in the section *Reagents, Indicators, and Solutions*); 900 mL.

Apparatus 2: 50 rpm.

Times: 30 minutes (ibuprofen); 45 minutes (pseudoephedrine hydrochloride).

Procedure for ibuprofen—Determine the amount of ibuprofen ($C_{13}H_{18}O_2$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 224 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Ibuprofen RS in the same medium.

Procedure for pseudoephedrine hydrochloride—

MOBILE PHASE—Prepare a solution of monobasic potassium phosphate in water containing 500 mg per 1000 mL. Filter through a filter having a porosity of 0.5 µm or finer. Prepare a mixture of this solution and acetonitrile (500:500), and adjust with phosphoric acid to a pH of 3.3 ± 0.1. Make any necessary adjustments (see *System Suitability* under *Chromatography* ⟨621⟩). Increasing the concentration of monobasic potassium phosphate or increasing the pH increases the retention time of pseudoephedrine.

STANDARD PREPARATION —Prepare a solution of USP Pseudoephedrine Hydrochloride RS in *Dissolution Medium* having a known concentration of about *P*/900 mg per mL, *P* being the labeled quantity, in mg, of pseudoephedrine hydrochloride per Tablet.

CHROMATOGRAPHIC SYSTEM (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector, a guard column containing packing L10, and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor for the pseudoephedrine peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

PROCEDURE—Pass a portion of the solution under test through a filter having a porosity of 0.5 µm or finer. Separately inject equal volumes (about 10 µL) of the filtrate and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the areas for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) dissolved by the formula:

USP Monographs

www.webofpharma.com

$$900C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are pseudoephedrine peak responses obtained from the solution under test and the *Standard preparation,* respectively.

Tolerances—Not less than 75% *(Q)* of the labeled amounts of ibuprofen ($C_{13}H_{18}O_2$) and pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) are dissolved in 30 minutes and in 45 minutes, respectively.

Uniformity of dosage units ⟨905⟩—

Procedure for content uniformity—Proceed as directed in the *Assay,* preparing the *Assay preparation* as follows. Transfer 1 Tablet to a glass-stoppered conical flask, add 10.0 mL of *Internal standard solution,* and stir with a magnetic stirrer until the Tablet disintegrates. Add 10.0 mL of acetonitrile, stir for about 15 minutes, and filter.

Assay—

Mobile phase—Dissolve 2.5 g of docusate sodium in a mixture of water and acetonitrile (590:410). Add 1.0 mL of phosphoric acid, and adjust with ammonium hydroxide to a pH of 3.2 ± 0.05. Make any necessary adjustments (see *System Suitability* under *Chromatography* ⟨621⟩). Decreasing the proportion of docusate sodium increases the resolution between pseudoephedrine and ibuprofen.

Internal standard solution—Prepare a solution of butylparaben in *Mobile phase* containing about 0.15 mg per mL.

Standard preparation—Prepare a solution in *Internal standard solution* having known concentrations of about 20 mg of USP Ibuprofen RS and 20*J* mg of USP Pseudoephedrine Hydrochloride RS per mL, *J* being the ratio of the labeled amount, in mg, of pseudoephedrine hydrochloride to the labeled amount, in mg, of ibuprofen per Tablet. To the resulting solution add an equal volume of acetonitrile, accurately measured, and mix. Pass through a filter having 0.5 µm porosity or finer, and use the filtrate as the *Standard preparation.* This solution contains about 10 mg of USP Ibuprofen RS and 10*J* mg of USP Pseudoephedrine Hydrochloride RS per mL.

Assay preparation—Transfer an accurately counted number of Tablets, equivalent to about 2000 mg of ibuprofen, to a glass-stoppered conical flask, add 100 mL of *Internal standard solution,* and stir with a magnetic stirrer until the Tablets disintegrate. Add 100 mL of acetonitrile, and mix. Filter through a filter of 0.5 µm porosity or finer, and use the filtrate as the *Assay preparation.*

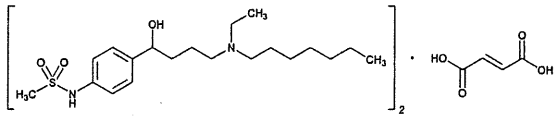
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector, a guard column that contains packing L1, and a 4.6-mm × 10-cm column that contains 5-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.55 for butylparaben, 0.7 for pseudoephedrine, and 1.0 for ibuprofen; the resolution, *R,* between the butylparaben peak and the pseudoephedrine peak and between the pseudoephedrine peak and the ibuprofen peak is not less than 2.0; the tailing factors for the butylparaben peak, the pseudoephedrine peak, and the ibuprofen peak are not more than 3.0; and the relative standard deviation for replicate injections determined from the peak response ratios is not more than 2.0%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of ibuprofen ($C_{13}H_{18}O_2$) and of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$) in each Tablet taken by the formula:

$$200(C/N)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Ibuprofen RS or USP Pseudoephedrine Hydrochloride RS, as appropriate, in the *Standard preparation; N* is the number of Tablets taken; and R_U and R_S are the ratios of the relevant analyte peak response to the butylparaben peak response obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ibutilide Fumarate



$(C_{20}H_{36}N_2O_3S)_2 \cdot C_4H_4O_4$ 885.23

Methanesulfonamide, *N*-[4-[4-(ethylheptylamino)-1-hydroxybutyl]phenyl]-, (±)-, (*E*)-2-butenedioate (2:1) (salt); (±)-4′-[4-(Ethylheptylamino)-1-hydroxybutyl] methanesulfonanilide fumarate (2:1) (salt) [122647-32-9].

Ibutilide free base [122647-31-8].

DEFINITION

Ibutilide Fumarate contains NLT 98.0% and NMT 102.0% of ibutilide fumarate [$(C_{20}H_{36}N_2O_3S)_2 \cdot C_4H_4O_4$], calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy:* **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Solution A: 2 mL/L of triethylamine in water. Adjust with perchloric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Solution A* (40:60)

Standard solution: 0.2 mg/mL of USP Ibutilide Fumarate RS in *Mobile phase.* [NOTE—Sonication may be necessary for complete dissolution.]

Sample solution: 0.2 mg/mL of Ibutilide Fumarate in *Mobile phase.* [NOTE—Sonication may be necessary for complete dissolution.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 3 times the retention time of the ibutilide peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

USP Monographs

www.webofpharma.com

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ibutilide fumarate $[(C_{20}H_{36}N_2O_3S)_2 \cdot C_4H_4O_4]$ in the portion of Ibutilide Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ibutilide from the *Sample solution*

r_S = peak response of ibutilide from the *Standard solution*

C_S = concentration of USP Ibutilide Fumarate RS in the *Standard solution* (mg/mL)

C_U = concentration of Ibutilide Fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.20%
- **CONTENT OF FUMARIC ACID**

Solution A, Mobile phase, and **Sample solution:** Proceed as directed in the *Assay*.

Chromatographic system: Proceed as directed in the *Assay*, except use a UV detector at 207 nm.

Standard stock solution: 0.2 mg/mL of USP Fumaric Acid RS in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution.]

Standard solution: 0.02 mg/mL of USP Fumaric Acid RS in *Mobile phase*

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of fumaric acid content in the portion of Ibutilide Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of fumaric acid from the *Sample solution*

r_S = peak response of fumaric acid from the *Standard solution*

C_S = concentration of USP Fumaric Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Ibutilide Fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 12.7%–13.5%

- **ORGANIC IMPURITIES**

Solution A: 2 mL/L of triethylamine in water. Adjust with perchloric acid to a pH of 2.5.

Solution B: Acetonitrile

Diluent: Acetonitrile and *Solution A* (40:60)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
15	60	40
30	30	70
40	30	70
50	60	40
55	60	40

Standard solution: 2 µg/mL of USP Ibutilide Fumarate RS and 3 µg/mL each of USP Ibutilide Related Compound A RS and USP Ibutilide Related Compound B RS in *Diluent*

Sample solution: 2 mg/mL of Ibutilide Fumarate in *Diluent*

Chromatographic system: Proceed as directed in the *Assay*, except use a run time of NLT 2.5 times the retention time of ibutilide for the *Standard solution* and NLT 6.2 times the retention time of ibutilide for the *Sample solution*.

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 5000 theoretical plates for the ibutilide peak

Relative standard deviation: NMT 3.0% for the ibutilide peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ibutilide related compound A, ibutilide related compound B, and any unspecified impurity in the portion of Ibutilide Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ibutilide related compound A, ibutilide related compound B, or any other unspecified impurity from the *Sample solution*

r_S = peak response of ibutilide related compound A, ibutilide related compound B, or ibutilide (for calculating any other unspecified impurity) from the *Standard solution*

C_S = concentration of USP Ibutilide Related Compound A RS, USP Ibutilide Related Compound B RS, or USP Ibutilide Fumarate RS (for calculating any unspecified impurity) in the *Standard solution* (mg/mL)

C_U = concentration of Ibutilide Fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard the fumaric acid peak and any peak less than 0.01%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fumaric acid[a]	0.33	—
Ibutilide	1.00	—
Ibutilide related compound B[b]	2.66	0.15
Ibutilide related compound A[c]	3.38	0.15
Any unspecified impurity	—	0.10
Total impurities	—	0.35

[a] This impurity is controlled in the test for *Content of Fumaric Acid*.

[b] *N*-Ethyl-*N*-heptyl-4-hydroxy-4-[4-(methylsulfonamido)phenyl]butanamide.

[c] *N*-Ethyl-*N*-heptyl-4-[4-(methylsulfonamido)phenyl]-4-oxobutanamide.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.0%

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in air-tight containers, and protect from light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Fumaric Acid RS
USP Ibutilide Fumarate RS
USP Ibutilide Related Compound A RS
N-Ethyl-*N*-heptyl-4-[4-(methylsulfonamido)phenyl]-4-oxobutanamide.
$C_{20}H_{32}N_2O_4S$ 396.54
USP Ibutilide Related Compound B RS
N-Ethyl-*N*-heptyl-4-hydroxy-4-[4-(methylsulfonamido)phenyl]butanamide.
$C_{20}H_{34}N_2O_4S$ 398.56

Ichthammol

Ichthammol [8029-68-3].

DEFINITION

Ichthammol is obtained by the destructive distillation of certain bituminous schists, sulfonation of the distillate, and neutralization of the product with ammonia. Ichthammol yields NLT 2.5% of ammonia (NH_3) and NLT 10.0% of total sulfur (S).

IDENTIFICATION

- **A.**

Sample solution: Ichthammol and water (10:90). Stir for 5 min with a magnetic stirrer.
Analysis: To the *Sample solution* add hydrochloric acid by 25% of the total volume. A heavy, resinous precipitate is formed. Remove the liquid by decantation, and wash the precipitate with 2 N hydrochloric acid until the last washing is nearly colorless. Transfer the precipitate to absorbent paper, allow it to stand for 10 min, and then transfer 10 mg of the precipitate to a 250-mL conical flask. To the flask add 100 mL of ether, attach an air condenser to the flask, and stir for 30 min with a magnetic stirrer.
Acceptance criteria: The precipitate does not dissolve completely.

- **B.**

Sample solution: 1 in 10
Analysis: To the *Sample solution* add 1 N sodium hydroxide, and heat to the boiling point.
Acceptance criteria: Ammonia is evolved.

ASSAY

- **AMMONIA**

Sample solution: 50 mg/mL of Ichthammol in water
Titrimetric system
Mode: Residual titration
Titrant: 0.5 N sulfuric acid VS
Back-titrant: 0.5 N sodium hydroxide VS
Endpoint detection: Visual
Analysis: Transfer 100 mL of the *Sample solution* to a distillation flask, add 3 g of paraffin, and then add 20 mL of sodium hydroxide solution (4 in 10). Connect the flask to a condenser by means of a spray trap, and immerse the lower outlet tube of the condenser in 30.0 mL of *Titrant*. Distill slowly, collect 50 mL of distillate, and then titrate the excess acid with *Back-titrant*, using methyl red TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.5 N sulfuric acid is equivalent to 8.515 mg of ammonia (NH_3).
Acceptance criteria: NLT 2.5% of ammonia (NH_3)

- **TOTAL SULFUR**

Sample: 500–800 mg of Ichthammol
Analysis: Transfer the *Sample* to a Kjeldahl flask with the aid of 20 mL of water. Add 3 g of potassium chlorate, then slowly add 30 mL of nitric acid, and evaporate the mixture on a hot plate to 5 mL. Cool, repeat the oxidation with 3 g of potassium chlorate and 30 mL of nitric acid, and evaporate to 5 mL. Add 25 mL of hydrochloric acid, and again evaporate to 5 mL. Add 100 mL of water, heat to boiling, filter, and wash well. To the hot filtrate add 25 mL of barium chloride TS, and heat on a steam bath for 1 h. Collect the barium sulfate on a previously ignited and tared filtering crucible. Wash, dry, and ignite. Then cool, and weigh. Each g of barium sulfate is equivalent to 137.4 mg of total sulfur (S).
Acceptance criteria: NLT 10.0% of total sulfur (S)

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.5%
- **Limit of Ammonium Sulfate**

Sample solution: 40 mg/mL of Ichthammol in alcohol
Analysis: To 25 mL of *Sample solution* stir, filter, and wash the filter with a mixture of equal volumes of ether and alcohol until the last washing is clear and colorless. Air-dry the filter and residue, and pass 200 mL of warm water, slightly acidified with hydrochloric acid, through the residue on the filter. Heat the filtrate to boiling, add barium chloride TS in excess, and heat for 1 h on a steam bath. Collect the precipitate of barium sulfate on a filter. Wash it well, dry, and ignite to constant weight. Each g of barium sulfate is equivalent to 566.1 mg of ammonium sulfate $[(NH_4)_2SO_4]$.
Acceptance criteria: NMT 8.0% of ammonium sulfate $[(NH_4)_2SO_4]$

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 80° for 8 h, and continue the drying at 100° for constant weight.
Acceptance criteria: NMT 50.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Ichthammol Ointment

DEFINITION

Ichthammol Ointment contains an amount of ichthammol equivalent to NLT 0.25% of ammonia (NH_3).

Ichthammol	100 g
Lanolin	100 g
Petrolatum	800 g
To make	1000 g

Thoroughly incorporate the *Ichthammol* with the *Lanolin*, and combine this mixture with the *Petrolatum*.

ASSAY

- **AMMONIA**

Sample: Transfer a portion of the Ointment, equivalent to 2 g of ichthammol, to a 250-mL beaker, and add 70 mL of boiling water. Mix with a glass rod, and heat on a steam bath with frequent agitation for 10 min. Cover with a watch glass without removing the stirring rod, and allow to stand at room temperature for 15–20 min. Place in a refrigerator

www.webofpharma.com

to cause the upper layer to congeal, form a second opening through the congealed layer with the glass rod, and transfer the dark-colored aqueous extract to a funnel containing a pledget of cotton, collecting the filtrate in a 500-mL volumetric flask. Repeat the extraction of the portion of the Ointment several times in the same manner until the aqueous extract is practically colorless, passing each extract through the same cotton filter into the flask containing the main extract. Dilute with water to volume, and mix.

Titrimetric system

Mode: Residual titration

Titrant: 0.05 N sulfuric acid VS

Back-titrant: 0.05 N sodium hydroxide VS

Endpoint detection: Colorimetric

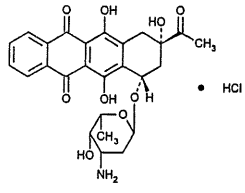
Analysis: Transfer 100.0 mL of the *Sample* to a suitable distillation flask, add 3 g of paraffin, and add 20 mL of sodium hydroxide solution (4 in 10). Connect the flask to a condenser by means of a spray trap, and immerse the lower outlet tube of the condenser in 30.0 mL of *Titrant*. Distill slowly, collect about 50 mL of distillate, and then titrate the excess sulfuric acid with *Back-titrant*, using methyl red TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 0.8515 mg of ammonia (NH_3).

Acceptance criteria: NLT 0.25% of ammonia (NH_3)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in collapsible tubes or in tight containers. Avoid prolonged exposure to temperatures exceeding 30°.

Idarubicin Hydrochloride



$C_{26}H_{27}NO_9 \cdot HCl$ 533.95

5,12-Naphthacenedione, 9-acetyl-7-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxyhydrochloride, (7*S*-*cis*)-.

(1*S*,3*S*)-3-Acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-6,11-dioxo-1-naphthacenyl 3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside, hydrochloride [57852-57-0].

» Idarubicin Hydrochloride contains not less than 960 µg and not more than 1030 µg of $C_{26}H_{27}NO_9 \cdot HCl$ per mg, calculated on the anhydrous basis. [**CAUTION**—Great care should be taken to prevent inhaling particles of Idarubicin Hydrochloride and exposing the skin to it.]

Packaging and storage—Preserve in tight containers.

Labeling—The amorphous form is so labeled.

USP Reference standards ⟨11⟩—

USP Idarubicin Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The chromatogram of the *Assay preparation* obtained in the *Assay* exhibits a major peak for idarubicin, the retention time of which corresponds to that in the chromatogram of the *Standard preparation* obtained in the *Assay*.

Crystallinity ⟨695⟩: meets the requirements, except where it is labeled as amorphous, most of the particles do not exhibit birefringence and extinction positions.

pH ⟨791⟩: between 5.0 and 6.5, in a solution containing 5 mg per mL.

Water Determination, *Method I* ⟨921⟩: not more than 5.0%.

Chromatographic purity—Using the chromatogram of the *Assay preparation* obtained in the *Assay*, and disregarding the solvent peak, calculate the percentage of each impurity taken by the formula:

$$100 r_i / r_S$$

in which r_i is the response of each impurity peak; and r_S is the sum of the responses of all the peaks: not more than 1.0% of any individual impurity is found; and the sum of all impurities is not more than 3.0%.

Assay—

Mobile phase—Prepare a mixture of water, acetonitrile, methanol, and phosphoric acid (540:290:170:2). Dissolve 1 g of sodium lauryl sulfate in 1000 mL of this solution, adjust with 2 N sodium hydroxide to a pH of 3.6 ± 0.1, pass through a filter having a porosity of 0.5 µm or finer, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare as directed for *Mobile phase*, except to omit the sodium lauryl sulfate.

Standard preparation—Dissolve an accurately weighed quantity of USP Idarubicin Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 500 µg of idarubicin hydrochloride per mL.

Assay preparation—Transfer about 50 mg of Idarubicin Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in *Diluent*, dilute with *Diluent* to volume, and mix.

Resolution solution—Prepare an aqueous solution containing 1 mg of Idarubicin Hydrochloride per mL. To 2 mL of this solution in a test tube, add 20 µL of hydrochloric acid, and heat in an oil bath at 95° for about 8 minutes. Mix 1 mL of this solution and 9 mL of *Diluent*. This *Resolution solution* contains a mixture of 4-demethoxydaunorubicinone and idarubicin.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L13. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.5 for 4-demethoxydaunorubicinone and 1.0 for idarubicin; and the resolution, *R*, between the 4-demethoxydaunorubicinone peak and the idarubicin peak is not less than 9.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the capacity factor, *k'*, for the idarubicin peak is not less than 10 and not more than 20; the tailing factor for the idarubicin peak is not less than 0.85 and not more than 1.2; the column efficiency calculated from the idarubicin peak is not less than 3000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

www.webofpharma.com

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of $C_{26}H_{27}NO_9 \cdot HCl$ in each mg of the Idarubicin Hydrochloride taken by the formula:

$$100(C/M)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of idarubicin hydrochloride ($C_{26}H_{27}NO_9 \cdot HCl$) in the *Standard preparation*; M is the quantity, in mg, of Idarubicin Hydrochloride taken to prepare the *Assay preparation*; and r_U and r_S are the responses of the idarubicin peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Idarubicin Hydrochloride for Injection

» Idarubicin Hydrochloride for Injection is a sterile mixture of Idarubicin Hydrochloride and Lactose. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{26}H_{27}NO_9 \cdot HCl$.
[**CAUTION**—*Great care should be taken to prevent inhaling particles of Idarubicin Hydrochloride and exposing the skin to it.*]

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Idarubicin Hydrochloride RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
Identification—The chromatogram of the *Assay preparation* obtained in the *Assay* exhibits a major peak for idarubicin, the retention time of which corresponds to that in the chromatogram of the *Standard preparation* obtained in the *Assay.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 8.9 USP Endotoxin Units per mg of idarubicin hydrochloride, a solution of Idarubicin Hydrochloride for Injection containing 0.07 mg of idarubicin hydrochloride per mL being used in the *Test Procedure.*
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*
pH ⟨791⟩: between 5.0 and 7.0, in a solution constituted as directed in the labeling, water being used as the diluent.
Water Determination, *Method I* ⟨921⟩: not more than 4.0%, the *Test Preparation* being prepared as directed for a hygroscopic specimen.
Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
Mobile phase, Diluent, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Idarubicin Hydrochloride.*
Assay preparation—Dilute the contents of 1 container of Idarubicin Hydrochloride for Injection quantitatively with *Diluent* to obtain a solution containing about 0.5 mg of idarubicin hydrochloride per mL.

Procedure—Proceed as directed for *Procedure*— under *Idarubicin Hydrochloride.* Calculate the quantity, in mg, of $C_{26}H_{27}NO_9 \cdot HCl$ in the container of Idarubicin Hydrochloride for Injection taken by the formula:

$$(C/1000)(L/D)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of idarubicin hydrochloride ($C_{26}H_{27}NO_9 \cdot HCl$) in the *Standard preparation*; L is the labeled quantity, in mg, of idarubicin hydrochloride in the container; D is the concentration, in mg per mL, of idarubicin hydrochloride in the *Assay preparation* on the basis of the labeled quantity in the container and the extent of dilution; and r_U and r_S are the responses of the idarubicin peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Idarubicin Hydrochloride Injection

DEFINITION
Idarubicin Hydrochloride Injection is a sterile solution in water. It contains NLT 90.0% and NMT 110.0% of the labeled amount of idarubicin hydrochloride ($C_{26}H_{27}NO_9 \cdot HCl$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Solution A: 2.9 g/L of sodium lauryl sulfate in water. To each liter of this solution add 1.3 mL of phosphoric acid.
Mobile phase: Acetonitrile and *Solution A* (1:1)
Standard solution: 0.04 mg/mL of USP Idarubicin Hydrochloride RS in water
Sample solution: Nominally 0.04 mg/mL of idarubicin hydrochloride in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 50-mm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of idarubicin hydrochloride ($C_{26}H_{27}NO_9 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Idarubicin Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of idarubicin hydrochloride in the *Sample solution* (mg/mL)
P = potency of USP Idarubicin Hydrochloride RS (µg/mg)

USP Monographs

www.webofpharma.com

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: 2.9 g/L of sodium lauryl sulfate and 2.3 g/L of phosphoric acid in water

Mobile phase: Tetrahydrofuran, methanol, and *Solution A* (25:15:60)

Diluent A: 2.3 g/L of phosphoric acid in water

Diluent B: Tetrahydrofuran, methanol, and *Diluent A* (25:15:60). Adjust with 2 N sodium hydroxide to a pH of 3.6.

Standard solution: 0.01 mg/mL of USP Idarubicin Hydrochloride RS in *Diluent B*

Sample solution: Nominally 0.5 mg/mL of idarubicin hydrochloride in *Diluent B*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F_1 \times (1/F_2) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of idarubicin from the *Standard solution*

C_S = concentration of USP Idarubicin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of idarubicin hydrochloride in the *Sample solution* (mg/mL)

P = potency of USP Idarubicin Hydrochloride RS (µg/mg)

F_1 = conversion factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.04%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Idarubicin aglycone[a]	0.3	1.5	2.5
Dianhydro idarubicinaglycone[b]	0.9	1.3	0.5
Idarubicin hydrochloride	1.0	—	—
Any other individual impurity	—	1.0	0.5
Total impurities	—	—	3.5

[a] (7*S*,9*S*)-9-Acetyl-6,7,9,11-tetrahydroxy-7,8,9,10-tetrahydrotetracene-5,12-dione.

[b] 8-Acetyl-6,11-dihydroxynaphthacene-5,12-dione.

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Contains NMT 8.9 USP Endotoxin Units/mg of idarubicin hydrochloride

• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements

• **pH** ⟨791⟩: 3.0–4.5

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections

ADDITIONAL REQUIREMENTS

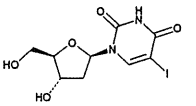
• **PACKAGING AND STORAGE:** Preserve in tight containers as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*. Store at 2°–8°, protected from light.

• **LABELING:** Meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Idarubicin Hydrochloride RS

Idoxuridine



$C_9H_{11}IN_2O_5$ 354.10

Uridine, 2′-deoxy-5-iodo-;

2′-Deoxy-5-iodouridine [54-42-2].

DEFINITION

Idoxuridine contains NLT 98.0% and NMT 101.0% of idoxuridine ($C_9H_{11}IN_2O_5$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M▲** (CN 1-May-2020)

Change to read:

• **B. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U▲** (CN 1-May-2020)

Analytical wavelength: 279 nm

Buffer: pH 12.0 solution prepared as follows. Dissolve 7.46 g of potassium chloride and 24 mL of 1 N sodium hydroxide in 2000 mL of water.

Sample solution: 35 µg/mL in *Buffer*

Acceptance criteria: Absorptivities, calculated on the dried basis for the *Sample solution* only, do not differ by more than 2.0%.

ASSAY

• **PROCEDURE**

Sample: 250 mg

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium methoxide in toluene VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 20 mL of dimethylformamide that has previously been neutralized with *Titrant*, and add 3 mg/mL of thymol blue in methanol as the indicator. Titrate with *Titrant* to a blue endpoint. Take precautions against absorption of atmospheric carbon dioxide. Perform a blank determination, and make any

www.webofpharma.com

necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 35.41 mg of idoxuridine ($C_9H_{11}IN_2O_5$).
Acceptance criteria: 98.0%–101.0% on the dried basis

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨731⟩
Sample: 500 mg
Analysis: Dry the *Sample* under vacuum at 60° for 2 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Idoxuridine RS

Idoxuridine Ophthalmic Ointment

DEFINITION
Idoxuridine Ophthalmic Ointment is Idoxuridine in a petrolatum base. It contains NLT 0.45% and NMT 0.55% of idoxuridine ($C_9H_{11}IN_2O_5$). It is sterile.

IDENTIFICATION
- **A.**
Standard solution and **Sample solution:** Proceed as directed in the *Assay*.
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*.

ASSAY
- **PROCEDURE**
Chromatographic column: Mix 4 g of chromatographic siliceous earth with 4 mL of 0.1 N hydrochloric acid in a glass mortar until the mixture is fluffy. Transfer to a 19- × 250-mm chromatographic tube (see *Chromatography* ⟨621⟩) that contains a pledget of glass wool and is fitted with a stopcock at the bottom. Tamp gently to compress to a uniform mass.
Mobile phase: Butyl alcohol and chloroform (1:5)
Standard stock solution: 0.5 mg/mL of USP Idoxuridine RS in methanol
Standard solution: 25 µg/mL of USP Idoxuridine RS in *Mobile phase* from *Standard stock solution*
Sample solution: Nominally 25 µg/mL of idoxuridine in eluant obtained from the *Chromatographic column* prepared as follows. Mix 4 g of chromatographic siliceous earth with 2 mL of 0.1 N hydrochloric acid in a glass mortar until the mixture is fluffy. Add an equivalent to 5 mg of idoxuridine from Ophthalmic Ointment to the mixture, and transfer to the prepared *Chromatographic column*. Transfer 2 g of chromatographic siliceous earth and 2 mL of 0.1 N hydrochloric acid to the glass mortar, and mix until fluffy. Use this material to rinse the mortar and pick up any remaining Ophthalmic Ointment. Transfer half of this mixture to the tube, and tamp gently until the column appears uniform. Transfer the remaining portion to the *Chromatographic column*, and tamp as before. Wipe the walls of the mortar with a small pledget of glass wool, and insert the pledget in the top of the column. Pass 50 mL of chloroform through the column at a flow rate of approximately 1 mL/min, and discard the chloroform. Elute with 200 mL of *Mobile phase* at the same flow rate, discarding the first 20 mL of the eluate. Collect the remainder of the eluate in a 200-mL volumetric flask, and dilute with *Mobile phase* to volume.
Instrumental conditions
Mode: UV
Analytical wavelengths: 320 and 283 nm
Cell: 1 cm
Blank: *Mobile phase*
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the absorbances of the *Sample solution* and the *Standard solution*.
Calculate the percentage of idoxuridine ($C_9H_{11}IN_2O_5$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (A_{U283} - A_{U320}/A_{S283} - A_{S320}) \times (C_S/C_U) \times 100$$

A_{U283} = absorbance of the *Sample solution* at 283 nm
A_{U320} = absorbance of the *Sample solution* at 320 nm
A_{S283} = absorbance of the *Standard solution* at 283 nm
A_{S320} = absorbance of the *Standard solution* at 320 nm
C_S = concentration of USP Idoxuridine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of idoxuridine in the *Sample solution* (µg/mL)

Acceptance criteria: 0.45%–0.55%

SPECIFIC TESTS
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Particulate and Foreign Matter* in *Ophthalmic Products—Quality Tests* ⟨771⟩, *Drug Product Quality, Universal Tests, Particulate and Foreign Matter.*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes in a cool place.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Idoxuridine RS

Idoxuridine Ophthalmic Solution

DEFINITION
Idoxuridine Ophthalmic Solution is a sterile, aqueous solution of Idoxuridine. It contains NLT 0.09% and NMT 0.11% of idoxuridine ($C_9H_{11}IN_2O_5$). It may contain suitable buffers, stabilizers, and antimicrobial agents.

IDENTIFICATION
- **A.**
Standard solution and **Sample solution:** Use as directed in the *Assay*.
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*.

ASSAY
- **PROCEDURE**
Chromatographic column: Mix 4 g of chromatographic siliceous earth with 4 mL of 0.1 N hydrochloric acid in a glass mortar until the mixture is fluffy. Transfer to a 19- × 250-mm chromatographic tube (see *Chromatography* ⟨621⟩) that contains a pledget of glass wool and is fitted with a stopcock at the bottom. Tamp gently to compress to a uniform mass.
Eluting solvent: Butyl alcohol and chloroform (1:5)
Standard stock solution: 0.5 mg/mL of USP Idoxuridine RS in methanol
Standard solution: Dilute 5.0 mL of *Standard stock solution* with *Eluting solvent* to 100.0 mL.

www.webofpharma.com

Sample solution: Nominally 25 µg/mL of idoxuridine in eluant obtained from the *Chromatographic column*. Mix an equivalent to 5 mg of idoxuridine from Ophthalmic Solution with 3 g of chromatographic siliceous earth in a glass mortar until the mixture is fluffy.

Instrumental conditions

Mode: UV

Analytical wavelengths: 320 and 283 nm

Cell: 1 cm

Blank: *Eluting solvent*

Analysis

Samples: *Standard solution* and *Sample solution*

Transfer the *Sample solution* to the prepared *Chromatographic column*. Transfer 2 g of chromatographic siliceous earth and 2 mL of 0.1 N hydrochloric acid to the glass mortar, and mix until fluffy. Use this material to rinse the mortar and pick up any remaining Ophthalmic Solution. Transfer half of this mixture to the tube, and tamp gently until the column appears uniform. Transfer the remaining portion to the *Chromatographic column*, and tamp as before. Wipe the walls of the mortar with a small pledget of glass wool, and insert the pledget in the top of the column. Elute with 200 mL of *Eluting solvent* at a flow rate of approximately 1 mL/min, discarding the first 20 mL of the eluate. Collect the remainder of the eluate in a 200-mL volumetric flask, and dilute with *Eluting solvent* to volume. Determine the absorbances of this solution and the *Standard solution*.

Calculate the percentage of idoxuridine ($C_9H_{11}IN_2O_5$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = difference in the absorbances of the *Sample solution* at the wavelengths indicated by ($A_{283} - A_{320}$)

A_S = difference in the absorbances of the *Standard solution* at the wavelengths indicated by ($A_{283} - A_{320}$)

C_S = concentration of USP Idoxuridine RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of idoxuridine in the *Sample solution* (µg/mL)

Acceptance criteria: 0.09%–0.11%

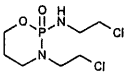
SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **PH** ⟨791⟩**:** 4.5–7.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers in a cold place.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Idoxuridine RS

Ifosfamide



$C_7H_{15}Cl_2N_2O_2P$ 261.09

2*H*-1,3,2-Oxazaphosphorin-2-amine,*N*,3-bis(2-chloroethyl) tetrahydro-, 2-oxide.

3-(2-Chloroethyl)-[(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide [3778-73-2].

» Ifosfamide contains not less than 98.0 percent and not more than 102.0 percent of $C_7H_{15}Cl_2N_2O_2P$.

[**CAUTION**—Great care should be taken in handling Ifosfamide, *as it is a potent cytotoxic agent and suspected carcinogen.*]

Packaging and storage—Preserve in tight containers at a temperature not exceeding 25°.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩—
USP Ifosfamide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

pH ⟨791⟩: between 4.0 and 7.0 in a solution (1 in 10).

Water Determination, *Method I* ⟨921⟩: not more than 0.3%.

Ionic chloride—

Standard sodium chloride solution—Transfer about 118.7 mg of sodium chloride, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with water to volume, and mix. This solution contains 360 ppm of ionic chloride.

Procedure—Pipet 10 mL of *Standard sodium chloride solution* into a beaker, and add 90 mL of water and 10 mL of acetic acid. Titrate with 0.01 N silver nitrate VS (prepared fresh daily), determining the endpoint potentiometrically using silver and silver-silver chloride electrodes. Record the volume, V_1, of 0.01 N silver nitrate VS consumed. Transfer about 2.0 g of Ifosfamide, accurately weighed, into a beaker, and add 90 mL of water and 10 mL of acetic acid. Pipet 10 mL of *Standard sodium chloride solution* into the beaker, and stir, if necessary, until solution is complete. Titrate with 0.01 N silver nitrate VS as directed above, and record the volume, V_2, of 0.01 N silver nitrate VS consumed. Calculate the difference in volume, V, of 0.01 N silver nitrate VS consumed between the two determinations by subtracting V_1 from V_2: a difference of not more than 1.0 mL corresponding to not more than 0.018% of ionic chloride is found.

Chloroform-insoluble phosphorus—

Ammonium molybdate solution— [NOTE—Prepare fresh on the day of use.] Dissolve 25 g of ammonium molybdate in 300 mL of water (*Solution A*). Cautiously add 75 mL of sulfuric acid to 100 mL of water, cool to room temperature, and dilute with water to 200.0 mL (*Solution B*). Mix *Solution A* and *Solution B* to obtain *Ammonium molybdate solution*.

Hydroquinone solution—Dissolve 0.5 g of hydroquinone in 100 mL of water, and add one drop of concentrated sulfuric acid. [NOTE—When this solution darkens, discard it and prepare fresh.]

Sodium sulfite solution—Prepare a solution of sodium sulfite in water having a concentration of 200 mg per mL. [NOTE—Prepare fresh at the time of use.]

Phosphorus stock solution—Transfer 0.1824 g of monobasic potassium phosphate, accurately weighed, to a 1000-mL

www.webofpharma.com

volumetric flask, dissolve in and dilute with water to volume, and mix.

Phosphorus intermediate solution—Transfer 10.0 mL of *Phosphorus stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Prepare this solution fresh on the day of use.

Phosphorus standard solution—Transfer 10.0 mL of *Phosphorus intermediate solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Test preparation—Transfer 1 g of Ifosfamide, accurately weighed, to a 100-mL volumetric flask, dissolve in 50 mL of water, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a separatory funnel, and add 5 mL of water. Add 15 mL of chloroform, shake vigorously for 30 seconds, allow the layers to separate and drain, and discard the lower chloroform layer. Repeat this extraction four times, each time with 15 mL of chloroform, discarding the chloroform layer after each extraction. Transfer the aqueous portion to a conical flask, wash the separatory funnel with two 5-mL portions of water, and collect all the aqueous washings in the same flask. Add 3 mL of sulfuric acid, and heat under a hood until white fumes appear. Remove the flask from the heat, and with swirling, add 0.6 mL of hydrogen peroxide. Heat until white fumes reappear. If the solution is not colorless, repeat additions of hydrogen peroxide followed by heating until all color is gone. Cool to room temperature, add 25 mL of water, and cautiously add 10 mL of ammonium hydroxide. Cool to room temperature, add 2 drops of phenolphthalein TS, and then add hydrochloric acid dropwise until all pink color has disappeared. Transfer the contents of the flask to a 100-mL flask, dilute with water to volume, and mix.

Blank solution—To 3 mL of sulfuric acid in a second conical flask, adding 0.6 mL of hydrogen peroxide, proceed as directed for the *Test preparation*, beginning with "Heat until white fumes reappear."

Procedure—Transfer 15.0 mL each of the *Test preparation*, the *Blank solution*, and the *Phosphorus standard solution* to three separate 25-mL volumetric flasks. Add 2.5 mL of *Ammonium molybdate solution* to each of the flasks, swirl, and allow to stand for about 30 seconds. To each of the three flasks in order, rapidly add 2.5 mL each of *Hydroquinone solution* and *Sodium sulfite solution*. Dilute the contents of each flask with water to volume, mix, and allow the flasks to stand for 30 minutes. Concomitantly determine the absorbances of the solutions obtained from the *Test preparation* and the *Phosphorus standard solution* in 1-cm cells at the wavelength of maximum absorbance at about 730 nm, with a suitable spectrophotometer, using the solution obtained from the *Blank solution* as the blank. Calculate the percentage of chloroform-insoluble phosphorus in the portion of Ifosfamide taken by the formula:

$$100(C/W)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of phosphorus in the *Phosphorus standard solution*; W is the weight, in mg, of Ifosfamide taken; and A_U and A_S are the absorbances from the solutions obtained from the *Test preparation* and the *Phosphorus standard solution*, respectively: not more than 0.0415% is found.

Limit of 2-chloroethylamine hydrochloride—

Standard solution—Dissolve an accurately weighed quantity of 2-chloroethylamine hydrochloride in *N,N*-dimethylacetamide, and dilute quantitatively, and stepwise if necessary, with the same solvent to obtain a solution having a known concentration of about 0.025 mg per mL.

Test solution—Transfer about 100 mg of Ifosfamide, accurately weighed, to a flask, add 10.0 mL of *N,N*-dimethylacetamide, and shake until dissolved.

Chromatographic system—The gas chromatograph is equipped with a flame-ionization detector and contains a 2-mm × 1.8-m column packed with 10% liquid phase G16 containing 2% potassium hydroxide on 80- to 100-mesh support S1A. The injection port is maintained at a temperature of about 200°, the detector is maintained at a temperature of about 300°, the oven is maintained at a temperature of about 140°, and nitrogen is used as the carrier gas at a flow rate of about 25 mL per minute.

Procedure—Separately inject equal volumes (about 1.0 µL) of the *Test solution* and the *Standard solution* into the gas chromatograph, record the chromatograms, and measure the areas of the peaks due to 2-chloroethylamine hydrochloride. Calculate the percentage of 2-chloroethylamine hydrochloride in the portion of Ifosfamide taken by the formula:

$$1000(C/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of 2-chloroethylamine hydrochloride in the *Standard solution*; W is the weight, in mg, of Ifosfamide taken; and r_U and r_S are the areas of the 2-chloroethylamine peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.25% of 2-chloroethylamine hydrochloride is found.

Other requirements—Where the label states that Ifosfamide is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ and for *Bacterial endotoxins* under *Ifosfamide for Injection*. Where the label states that Ifosfamide must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Ifosfamide for Injection*.

Assay— [NOTE—Ifosfamide degrades in solution. Prepare solutions of Ifosfamide fresh daily and do not store for more than 24 hours. Prepare the *Standard preparation* and the *Assay preparation* simultaneously.]

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (70:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer about 50 mg of ethylparaben, accurately weighed, to a 100-mL volumetric flask, and add 25 mL of alcohol to dissolve. Dilute with water to volume, and mix.

Standard preparation—Transfer about 15 mg of USP Ifosfamide RS, accurately weighed, to a 25-mL volumetric flask, add 1.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

Assay preparation—Transfer about 150 mg of Ifosfamide, accurately weighed, to a 250-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 195-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R, between ifosfamide and ethylparaben is not less than 6.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_7H_{15}Cl_2N_2O_2P$ in the portion of Ifosfamide taken by the formula:

www.webofpharma.com

$$250C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Ifosfamide RS in the *Standard preparation;* and R_U and R_S are the ratios of the responses of the ifosfamide peak to the ethylparaben peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ifosfamide for Injection

» Ifosfamide for Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_7H_{15}Cl_2N_2O_2P$.

Caution— Great care should be taken in handling Ifosfamide, as it is a potent cytotoxic agent and suspected carcinogen.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution,* at controlled room temperature.
USP Reference standards ⟨11⟩—
USP Ifosfamide RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

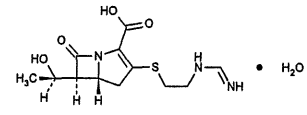
Identification—
A: (See *Thin-layer Chromatographic Identification Tests* ⟨201⟩.)
Developing solvent—Prepare a mixture of isopropyl alcohol and toluene (1:1).
Standard solution—Dissolve 20.0 mg of USP Ifosfamide RS in 1.0 mL of alcohol.
Test solution—Dissolve 20 mg of Ifosfamide for Injection in 1.0 mL of alcohol.
Procedure—Apply separately 10 µL each of the *Standard solution* and the *Test solution* to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture, allow the spots to dry, and develop the plate in a paper-lined chromatographic chamber equilibrated with *Developing solvent* for about 15 minutes prior to use. Allow the chromatogram to develop until the solvent front has moved about 15 cm. Remove the plate, mark the solvent front, and air-dry for 5 minutes. Place the plates into a chromatographic chamber containing iodine crystals, and view the spots that develop. [NOTE—For better detection, overspray the iodine stain with a mixture of alcohol and water (1:1).] The R_F value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution.*
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation,* both relative to the internal standard, as obtained in the *Assay.*
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.125 USP Endotoxin Unit per mg.
pH ⟨791⟩: between 4.0 and 7.0 in a solution prepared as directed for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions,* determined 30 minutes after its preparation.
Water Determination, *Method I* ⟨921⟩: not more than 0.3%.
Other requirements—It meets the requirements for *Sterility Tests* ⟨71⟩, *Uniformity of Dosage Units* ⟨905⟩, and *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

Assay—
Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Ifosfamide.*
Assay preparation—Select an accurately counted number of containers of Ifosfamide for Injection, the combined contents of which are equivalent to about 6 g of Ifosfamide. Dissolve the contents of each container in water and combine all of the solutions in a 1000-mL volumetric flask. Rinse each container with water, and add the rinsings to the volumetric flask. Dilute with water to volume, and mix. Transfer 10.0 mL of the resulting solution to a 100-mL volumetric flask, add 4.0 mL of *Internal standard solution,* dilute with water to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Ifosfamide.* Calculate the quantity, in g, of $C_7H_{15}Cl_2N_2O_2P$ in each container of Ifosfamide for Injection taken by the formula:

$$10(C/N)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Ifosfamide RS in the *Standard preparation; N* is the number of containers selected for the *Assay preparation,* and R_U and R_S are the ratios of the responses of the ifosfamide peak to the ethylparaben peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Imipenem



$C_{12}H_{17}N_3O_4S \cdot H_2O$ 317.36
1-Azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, 6-(1-hydroxyethyl)-3-[[2-(iminomethyl)amino]ethyl]thio]-7-oxo-, monohydrate, [5*R*-[5α,6α(*R**)]]-.
(5*R*,6*S*)-3-[[2-(Formimidoylamino)ethyl]thio]-6-[(*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrate [74431-23-5].
Anhydrous 299.35
[64221-86-9].

» Imipenem contains the equivalent of not less than 98.0 percent and not more than 101.0 percent of imipenem monohydrate ($C_{12}H_{17}N_3O_4S \cdot H_2O$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution,* and store in a cold place.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile.
USP Reference standards ⟨11⟩—
USP Imipenem Monohydrate RS

Change to read:

Identification, ▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197M*** ▲ (CN 1-May-2020)•
Specific rotation ⟨781S⟩: between +84° and +89°.
Test solution: 5 mg per mL, in a pH 7 buffer. Prepare the pH 7 buffer solution as follows. Dissolve 5 g of monobasic potassium phosphate and 11 g of dibasic potassium

USP Monographs

www.webofpharma.com

phosphate in 900 mL of water, adjust with phosphoric acid or 5 N sodium hydroxide to a pH of 7, dilute with water to 1000 mL, and mix.

Crystallinity ⟨695⟩: meets the requirements.

Bacterial Endotoxins Test ⟨85⟩ (where the label states that Imipenem is sterile)—It contains not more than 0.17 USP Endotoxin Unit per mg.

Sterility Tests ⟨71⟩ (where the label states that Imipenem is sterile)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 6 g of specimen dissolved in 200 mL of *Fluid A* being used.

Loss on drying ⟨see *Thermal Analysis* ⟨891⟩⟩— [NOTE—The quantity taken for the determination may be adjusted, if necessary, for instrument sensitivity. Weight loss occurring at temperatures above about 160°, indicative of decomposition, is not to be interpreted as *Loss on drying*.] Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument, using 5 to 10 mg of Imipenem, accurately weighed. Heat the specimen under test at a rate of 20° per minute under vacuum. Record the thermogram to 200°, and calculate the weight loss at the plateau or inflection point at about 150°: it loses not less than 5.0% and not more than 8.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.2%.

Solvents—

Internal standard solution—Add 1 mL of *n*-propyl alcohol to 2000 mL of water, and mix.

Standard preparation—Transfer 1.0 mL of acetone and 2.0 mL of isopropyl alcohol to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 1.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 25-mL volumetric flask, dilute with water to volume, and mix. Each mL of this *Standard preparation* contains 31.6 µg of acetone and 63.2 µg of isopropyl alcohol.

Test preparation—Transfer about 250 mg of Imipenem, accurately weighed, to a 10-mL volumetric flask, add 4.0 mL of 1 N ammonium hydroxide, and dissolve by swirling. Add 2.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 3-mm × 1.8-m column containing 10% phase G16 on support S5. The column temperature is programmed to operate at 70° for 8 minutes, then to increase at a rate of 32° per minute to 170°, and to maintain the temperature at 170° for 8 minutes. The injection port is maintained at 200°, the detector is maintained at 250°, and helium is used as the carrier gas at a flow rate of about 19 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the relative retention times are about 0.3 for acetone, 0.5 for isopropyl alcohol, and 1.0 for *n*-propyl alcohol, and the relative standard deviation of each of the ratios of the response of the respective analyte peak to the response of the *n*-propyl alcohol peak for replicate injections is not more than 5%.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 2 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, using the solvent (water) flush technique, record the chromatograms, and measure the responses for the acetone, isopropyl alcohol, and *n*-propyl alcohol peaks. Calculate the percentages of acetone and isopropyl alcohol in the portion of Imipenem taken by the same formula:

$$(C/W)(R_U/R_S)$$

in which C is the concentration, in µg per mL, of the appropriate analyte in the *Standard preparation*; W is the quantity, in mg, of Imipenem taken to prepare the *Test preparation*; and R_U and R_S are the ratios of the peak response of each of the corresponding analytes to the peak responses of *n*-propyl alcohol obtained from the *Test preparation* and the *Standard preparation*, respectively. Add the percentages of acetone and isopropyl alcohol found: the total is not more than 0.25%.

Assay—

Mobile phase—Dissolve 0.54 g of monobasic potassium phosphate in 3600 mL of water, adjust with 0.5 N sodium hydroxide or 0.5 M phosphoric acid to a pH of 6.8 ± 0.1, dilute with water to make 4000 mL of solution, and mix. Filter this solution through a filter of 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Imipenem Monohydrate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.4 mg per mL. Store this solution in an ice bath, and discard after 8 hours.

Assay preparation—Transfer about 100 mg of Imipenem, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Store this solution in an ice bath, and discard the unused portion after 8 hours.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 300-nm detector and a 4.6-mm × 30-cm column that contains packing L1, and is maintained at a temperature of 30 ± 1.0°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 600 theoretical plates, and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of imipenem monohydrate ($C_{12}H_{17}N_3O_4S \cdot H_2O$) in the portion of Imipenem taken by the formula:

$$(317.36/299.35)(0.25CP)(r_U/r_S)$$

in which 317.36 and 299.35 are the molecular weights of imipenem monohydrate and anhydrous imipenem, respectively; C is the concentration, in mg per mL, of USP Imipenem Monohydrate RS in the *Standard preparation*; P is the content, in µg per mg, of anhydrous imipenem ($C_{12}H_{17}N_3O_4S$) in USP Imipenem Monohydrate RS; and r_U and r_S are the imipenem peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Imipenem and Cilastatin for Injection

» Imipenem and Cilastatin for Injection is a sterile mixture of Imipenem, Cilastatin Sodium, and Sodium Bicarbonate. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of imipenem ($C_{12}H_{17}N_3O_4S$) and cilastatin ($C_{16}H_{26}N_2O_5S$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*,

USP Monographs

www.webofpharma.com

Packaging for constitution, and store at controlled room temperature.

Labeling—Label it to indicate that after constitution it is to be solubilized in a suitable parenteral fluid prior to intravenous infusion.

USP Reference standards ⟨11⟩—
USP Cilastatin Ammonium Salt RS
USP Imipenem Monohydrate RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

Identification—The retention times of the peaks for imipenem and cilastatin in the chromatogram of the *Assay preparation* correspond to those in the chromatograms of the *Imipenem standard preparation* and the *Cilastatin standard preparation,* as obtained in the *Assay.*

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.17 USP Endotoxin Unit per mg of imipenem and not more than 0.17 USP Endotoxin Unit per mg of cilastatin.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined,* the specimen being dissolved in *Fluid A.*

pH ⟨791⟩: between 6.5 and 8.5, when constituted as directed in the labeling.

Uniformity of dosage units ⟨905⟩: meets the requirements.

Loss on drying ⟨731⟩—Dry about 100 mg in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.5% of its weight.

Particulate Matter in Injections ⟨788⟩: meets the requirements for small-volume injections.

Assay—

pH 6.8 Buffer—Dissolve 0.54 g of monobasic potassium phosphate in 3600 mL of water, adjust with 0.5 N sodium hydroxide or 0.5 M phosphoric acid to a pH of 6.8 ± 0.1, dilute with water to make 4000 mL of solution, and mix. Pass this solution through a filter of 0.5-µm or finer porosity.

Mobile phase—Dissolve 2.0 g of sodium 1-hexanesul fonate in 800 mL of *pH 6.8 Buffer,* adjust with 0.5 N sodium hydroxide or 0.5 M phosphoric acid to a pH of 6.8 ± 0.1, and dilute with *pH 6.8 Buffer* to make 1000 mL of solution. Pass this solution through a filter of 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Imipenem standard preparation—Transfer about 13 mg of USP Imipenem Monohydrate RS, accurately weighed, to a 25-mL volumetric flask. Add 5 mL of saline TS, 0.5 mL of a 0.1% solution of sodium bicarbonate, and about 15 mL of *pH 6.8 Buffer,* and dissolve by shaking and sonicating. [NOTE—The duration of sonication should not exceed 1 minute.] Dilute with *pH 6.8 Buffer* to volume, and mix. This solution contains the equivalent of about 500 µg of anhydrous imipenem per mL. Use this solution immediately.

Cilastatin standard preparation—Transfer about 12.5 mg of USP Cilastatin Ammonium Salt RS, accurately weighed, to a 25-mL volumetric flask. Add 5 mL of saline TS, 0.5 mL of a 0.1% solution of sodium bicarbonate, and about 15 mL of *pH 6.8 Buffer,* and dissolve by shaking and sonicating. [NOTE—The duration of sonication should not exceed 1 minute.] Dilute with *pH 6.8 Buffer* to volume, and mix. This solution contains the equivalent of about 500 µg of cilastatin per mL. Use this solution immediately.

Assay preparation—Constitute Imipenem and Cilastatin for Injection in a volume of saline TS, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively transfer this suspension to a 100-mL volumetric flask with the aid of *pH 6.8 Buffer,* dilute with *pH 6.8 Buffer* to volume, and mix. Dilute an accurately measured volume of this solution quantitatively with *pH 6.8 Buffer* to obtain an *Assay preparation* having a concentration of about 500 µg of imipenem per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 30-cm column that contains packing L1, and is maintained at a temperature of 50 ± 1.0°. The flow rate is about 2 mL per minute. Chromatograph the *Imipenem standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the imipenem peak is not less than 600 theoretical plates when calculated by the formula:

$$5.545(t/W_{h/2})^2$$

the tailing factor for the imipenem peak is not more than 1.5 when calculated by the formula:

$$W_{0.1}/2f$$

where $W_{0.1}$ is the width of the peak at 10% height, and the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Cilastatin standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the cilastatin peak is not less than 600 theoretical plates when calculated by the formula:

$$5.545(t/W_{h/2})^2$$

the tailing factor for the cilastatin peak is not more than 1.5 when calculated by the formula:

$$W_{0.1}/2f$$

and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Imipenem standard preparation,* the *Cilastatin standard preparation,* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of anhydrous imipenem ($C_{12}H_{17}N_3O_4S$) and cilastatin ($C_{16}H_{26}N_2O_5S$) in the container, taken by the same formula:

$$(CPL/D)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Imipenem Monohydrate RS or USP Cilastatin Ammonium Salt RS in the appropriate *Standard preparation; P* is the content, in µg per mg, of anhydrous imipenem ($C_{12}H_{17}N_3O_4S$) or cilastatin ($C_{16}H_{26}N_2O_5S$) in the relevant Reference Standard; L is the labeled quantity, in mg, of imipenem or cilastatin in the container; D is the concentration, in µg per mL, of imipenem or cilastatin in the *Assay preparation* based on the labeled quantity in the container and the extent of dilution; and r_U and r_S are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the appropriate *Standard preparation,* respectively.

Imipenem and Cilastatin for Injectable Suspension

DEFINITION

Imipenem and Cilastatin for Injectable Suspension is a sterile mixture of Imipenem and Cilastatin Sodium. It contains NLT

USP Monographs

www.webofpharma.com

90.0% and NMT 115.0% of the labeled amounts of imipenem ($C_{12}H_{17}N_3O_4S$) and cilastatin ($C_{16}H_{26}N_2O_5S$).

IDENTIFICATION

• **A.** The retention times of the peaks for imipenem and cilastatin of the *Sample solution* correspond to those of *Standard solution 1* and *Standard solution 2*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 0.54 g of monobasic potassium phosphate in 3600 mL of water, adjust with 0.5 N sodium hydroxide or 0.5 M phosphoric acid to a pH of 6.8 ± 0.1, dilute with water to make 4000 mL of solution, and mix. Pass this solution through a filter of 0.5-µm or finer pore size.

Solution A: 0.1% solution of sodium bicarbonate in water

Mobile phase: Dissolve 2.0 g of sodium 1-hexanesulfonate in 800 mL of *Buffer*, adjust with 0.5 N sodium hydroxide or 0.5 M phosphoric acid to a pH of 6.8 ± 0.1, and dilute with *Buffer* to make 1000 mL of solution. Pass this solution through a filter of 0.5-µm or finer pore size, and degas.

Standard solution 1: 0.5 mg/mL of imipenem from USP Imipenem Monohydrate RS prepared as follows. Transfer a suitable amount of USP Imipenem Monohydrate RS to a volumetric flask. Add saline TS, *Solution A*, and *Buffer*, using 20%, 2%, and 60% of the final volume, respectively. Dissolve by shaking and sonicating. The duration of sonication should not exceed 1 min. Dilute with *Buffer* to volume. Use this solution immediately.

Standard solution 2: 0.5 mg/mL of cilastatin from USP Cilastatin Ammonium Salt RS prepared as follows. Transfer a suitable amount of USP Cilastatin Ammonium Salt RS to a volumetric flask. Add saline TS, *Solution A*, and *Buffer*, using 20%, 2%, and 60% of the final volume, respectively. Dissolve by shaking and sonicating. The duration of sonication should not exceed 1 min. Dilute with *Buffer* to volume. Use this solution immediately.

Sample stock solution: Nominally 2.5 mg/mL of imipenem prepared as follows. Constitute Imipenem and Cilastatin for Injectable Suspension in a volume of saline TS corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with saline TS to final volume.

Sample solution: Nominally 0.5 mg/mL each of imipenem and cilastatin from *Sample stock solution* in *Buffer*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 30-cm; packing L1

Column temperature: 50 ± 1.0°

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution 1* and *Standard solution 2*

Suitability requirements

Column efficiency: NLT 600 theoretical plates for the imipenem peak, *Standard solution 1*; NLT 600 theoretical plates for the cilastatin peak, *Standard solution 2*. Calculate as follows:

$$\text{Result} = 5.545 \times (t_R / W_{h/2})^2$$

t_R = retention time of the peak in *Standard solution 1* or *Standard solution 2*

$W_{h/2}$ = width of the peak at half-height in *Standard solution 1* or *Standard solution 2*

Tailing factor: NMT 1.5 for the imipenem peak, *Standard solution 1*; NMT 1.5 for the cilastatin peak, *Standard solution 2*. Calculate as follows:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$ = width of the peak at 10% height in *Standard solution 1* or *Standard solution 2*

f = distance from the peak maximum to the leading edge of the peak at 5% of the peak height in *Standard solution 1* or *Standard solution 2*

Relative standard deviation: NMT 2.0% for the imipenem peak, *Standard solution 1*; NMT 2.0% for the cilastatin peak, *Standard solution 2*

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of the labeled amount of anhydrous imipenem ($C_{12}H_{17}N_3O_4S$) in the portion of Imipenem and Cilastatin for Injectable Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from *Standard solution 1*

C_S = concentration of imipenem in *Standard solution 1* (mg/mL)

C_U = nominal concentration of imipenem in the *Sample solution* (mg/mL)

P = potency of imipenem in USP Imipenem Monohydrate RS (mg/mg)

Calculate the percentage of the labeled amount of cilastatin ($C_{16}H_{26}N_2O_5S$) in the portion of Imipenem and Cilastatin for Injectable Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from *Standard solution 2*

C_S = concentration of cilastatin in *Standard solution 2* (mg/mL)

C_U = nominal concentration of cilastatin in the *Sample solution* (mg/mL)

P = potency of cilastatin in USP Cilastatin Ammonium Salt RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0% for each analyte

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.23 USP Endotoxin Unit/mg of imipenem and NMT 0.23 USP Endotoxin Unit/mg of cilastatin

• **PH** ⟨791⟩

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 6.0–7.5

• **STERILITY TESTS** ⟨71⟩

Sample solution: Dissolve the sample in *Fluid A*.

Acceptance criteria: Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*

• **LOSS ON DRYING** ⟨731⟩

Sample: 100 mg

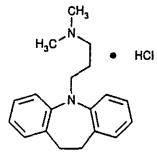
www.webofpharma.com

Analysis: Dry the *Sample* under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.
Acceptance criteria: NMT 3.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, and store at controlled room temperature.
- **LABELING:** Label it to indicate that the suspension obtained when constituted as directed in the labeling is for intramuscular injection only.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Cilastatin Ammonium Salt RS
  USP Imipenem Monohydrate RS

Imipramine Hydrochloride



$C_{19}H_{24}N_2 \cdot HCl$ 316.87
5*H*-Dibenz[*b,f*]azepine-5-propanamine, 10,11-dihydro-*N,N*-dimethyl-, monohydrochloride.
5-3-(Dimethylamino)propyl-10,11-dihydro-5*H*-dibenz[*b,f*]-azepine monohydrochloride [113-52-0].

» Imipramine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{19}H_{24}N_2 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Desipramine Hydrochloride RS
USP Imipramine Hydrochloride RS
USP Iminodibenzyl RS
$C_{14}H_{13}N$ 195.28

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

C: Dissolve 0.10 g in 2 mL of alcohol, and add 1 mL of 2 N nitric acid and 3 drops of silver nitrate TS: a white precipitate is formed, and it dissolves on the dropwise addition of ammonium hydroxide.

Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds— [NOTE—Use low-actinic glassware throughout the following procedure.]

Mobile phase, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay*.

Standard solution—Dissolve accurately weighed quantities of USP Imipramine Hydrochloride RS and USP Iminodibenzyl RS in acetonitrile, and dilute with a mixture of water and acetonitrile (5:3) to obtain a solution having known concentrations of about 2.5 µg of each component per mL.

Test solution—Transfer about 63 mg of Imipramine Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with a mixture of water and acetonitrile (5:3), and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. The relative retention times are about 0.8 for *N*-(dimethylaminopropyl) iminostilbene and 1.0 for imipramine. Calculate the percentage of iminodibenzyl in the portion of Imipramine Hydrochloride taken by the formula:

$$5(C/W)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Iminodibenzyl RS in the *Standard solution; W* is the weight, in mg, of Imipramine Hydrochloride taken to prepare the *Test solution;* and r_U and r_S are the iminodibenzyl peak responses obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.1% of iminodibenzyl is found. Calculate the percentage of each other impurity in the portion of Imipramine Hydrochloride taken by the formula:

$$5(C/W)(r_i/r_S)$$

in which *C* is the concentration, in µg per mL, of USP Imipramine Hydrochloride RS in the *Standard solution; W* is the weight, in mg, of Imipramine Hydrochloride taken to prepare the *Test solution;* r_i is the peak response of each individual impurity, excluding iminodibenzyl, obtained from the *Test solution,* and r_S is the peak response of imipramine obtained from the *Standard solution:* not more than 0.1% of *N*-(dimethylaminopropyl)iminostilbene is found; not more than 0.2% of any other impurity is found; and the total of all impurities found is not more than 1.0%.

Assay— [NOTE—Use low-actinic glassware throughout the following procedure.]

Mobile phase—Prepare a filtered and degassed mixture of 0.06 M sodium perchlorate, acetonitrile, and triethylamine (625:375:1), and adjust with perchloric acid to a pH of 2.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Transfer about 15 mg of USP Imipramine Hydrochloride RS and about 15 mg of USP Desipramine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with a mixture of water and acetonitrile (5:3) to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Imipramine Hydrochloride RS in a mixture of water and acetonitrile (5:3) to obtain a solution having a known concentration of about 0.3 mg per mL.

Assay preparation—Transfer about 30 mg of Imipramine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with a mixture of water and acetonitrile (5:3) to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 269-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The column temperature is maintained at 40°, and the flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the imipramine and desipramine peaks is not less than 1.3. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0% for imipramine.

USP Monographs

www.webofpharma.com

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of $C_{19}H_{24}N_2 \cdot HCl$ in the portion of Imipramine Hydrochloride taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Imipramine Hydrochloride RS in the *Standard preparation*; and r_U and r_S are the imipramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Imipramine Hydrochloride Injection

» Imipramine Hydrochloride Injection is a sterile solution of Imipramine Hydrochloride in Water for Injection. It contains, in each mL, not less than 11.5 mg and not more than 13.5 mg of $C_{19}H_{24}N_2 \cdot HCl$.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Imipramine Hydrochloride RS
Identification—Transfer 10 mL of Injection to a separator, add 2 mL of 2 N hydrochloric acid, extract with 10 mL of chloroform, filter, and evaporate the chloroform solution to about 2 mL. Carefully add ether until the liquid becomes turbid, heat on a steam bath to produce a clear solution, then cool, and allow to stand. Filter the crystalline precipitate, wash with ether, and dry in vacuum at 105° for 30 minutes: the precipitate so obtained responds to *Identification*— test *A:* under *Imipramine Hydrochloride.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 5.0 USP Endotoxin Units per mg of imipramine hydrochloride.
pH ⟨791⟩: between 4.0 and 5.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Transfer an accurately measured volume of Injection, equivalent to about 25 mg of imipramine hydrochloride, to a 100-mL volumetric flask, add 0.5 N hydrochloric acid to volume, and mix. Pipet 10 mL of this solution into a separator, add 10 mL of 1 N sodium hydroxide, and extract with four 20-mL portions of ether, shaking each portion for 2 minutes and collecting the extracts in a second separator. Extract the combined ether extracts with four 20-mL portions of 0.5 N hydrochloric acid, and combine the extracts in a 250-mL beaker. Aerate this solution with nitrogen to remove residual ether, then transfer to a 100-mL volumetric flask, and rinse the beaker with 0.5 N hydrochloric acid, collecting the rinsings in the flask. Add the 0.5 N acid to volume, and mix. Dissolve an accurately weighed quantity of USP Imipramine Hydrochloride RS in 0.5 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 25 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 250 nm, with a suitable spectrophotometer, using 0.5 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{19}H_{24}N_2 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Imipramine Hydrochloride RS in the Standard solution; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Imipramine Hydrochloride Tablets

DEFINITION
Imipramine Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$).

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
  Sample: Powder a suitable number of Tablets, equivalent to 100 mg of imipramine hydrochloride, and macerate the powder with 10 mL of chloroform. Filter the chloroform extract through paper into a wide-mouth test tube, and evaporate the filtrate to 3 mL. Carefully add ether until the liquid becomes turbid, heat on a steam bath to produce a clear solution, cool, and allow to stand. The precipitate that is formed may be recrystallized from acetone. Filter the crystalline precipitate, wash with ether, and dry under vacuum at 105° for 30 min. Use the precipitate.
  Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY
- **PROCEDURE**
  Solution A: 0.02 M ammonium bicarbonate in water. Adjust with 28%–30% ammonia solution to a pH of 8.0.
  Solution B: Acetonitrile and methanol (70:30)
  Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
1.0	90	10
5.0	60	40
7.0	60	40
10.0	48	52
13.0	48	52
14.0	20	80
16.0	20	80
16.1	90	10
18.0	90	10

Diluent: Acetonitrile and water (40:60)
Standard solution: 0.25 mg/mL of USP Imipramine Hydrochloride RS in *Diluent*
Sample stock solution: Nominally 1.0 mg/mL of imipramine hydrochloride from Tablets prepared as follows. Powder NLT 10 Tablets and transfer a sufficient portion of powder to a volumetric flask. Add 75% of the

USP Monographs

www.webofpharma.com

final flask volume of *Diluent*. Sonicate as needed to aid in dissolution. Allow to cool to room temperature, then dilute with *Diluent* to volume. Centrifuge and use the supernatant. [NOTE—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]

Sample solution: Nominally 0.25 mg/mL of imipramine hydrochloride from *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 2.1-mm × 10-cm, 1.7-µm packing L7

Column temperature: 35°

Flow rate: 0.4 mL/min

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Imipramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of imipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Mode: UV-Vis

Analytical wavelength: UV 250 nm

Standard solution: USP Imipramine Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* to a suitable concentration.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Imipramine Hydrochloride RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*, if needed

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

Procedure for content uniformity

Diluent: Hydrochloric acid and water (1:100)

Standard solution: 0.025 mg/mL of USP Imipramine Hydrochloride RS in *Diluent*

Sample stock solution: Transfer 1 finely powdered Tablet to a 100-mL volumetric flask with the aid of 70 mL of *Diluent* and shake by mechanical means for 30 min. Dilute with *Diluent* to volume, and filter, if necessary, discarding the first 20 mL of the filtrate.

Sample solution: Nominally 0.025 mg/mL of imipramine hydrochloride from *Sample stock solution* in *Diluent*

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: UV 250 nm

Cell: 1 cm

Blank: *Diluent*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Imipramine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of imipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.002 mg/mL each of USP Imipramine Hydrochloride RS, USP Depramine RS, USP Desipramine Hydrochloride RS, and USP Iminodibenzyl RS in *Diluent*. Sonicate if necessary to aid dissolution.

Sample solution: Nominally 1.0 mg/mL of imipramine hydrochloride from Tablets prepared as follows. Powder NLT 10 Tablets and transfer a portion to a volumetric flask. Add about 75% of the final flask volume of *Diluent*. Sonicate as necessary to aid dissolution. Allow to cool to room temperature, then dilute with *Diluent* to volume. Centrifuge and use the supernatant. [NOTE—The use of a centrifuge speed of 3000 rpm for 10 min may be suitable.]

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for desipramine, depramine, imipramine, and iminodibenzyl are 0.82, 0.84, 1.0, and 1.18, respectively.]

Suitability requirements

Resolution: NLT 1.5 between the depramine and desipramine peaks

Relative standard deviation: NMT 2.5% each for desipramine, imipramine, and iminodibenzyl

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of desipramine and iminodibenzyl in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of desipramine or iminodibenzyl from the *Sample solution*

r_S = peak response of USP Desipramine Hydrochloride RS or USP Iminodibenzyl RS from the *Standard solution*

www.webofpharma.com

C_S = concentration of USP Desipramine Hydrochloride RS or USP Iminodibenzyl RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of imipramine hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of imipramine from the *Standard solution*
C_S = concentration of USP Imipramine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of imipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

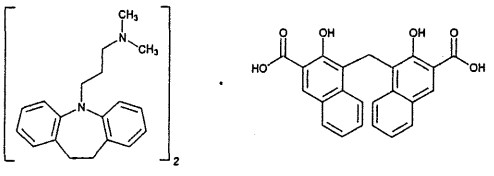
Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desipramine	0.82	0.2
Imipramine	1.0	—
Iminodibenzyl	1.18	0.5
Any individual unspecified degradation product	—	0.2
Total degradation products	—	1.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Depramine RS
  3-(5*H*-Dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine hydrochloride.
  $C_{19}H_{22}N_2 \cdot HCl$ 314.85
  USP Desipramine Hydrochloride RS
  10,11-Dihydro-5*H*-[3-(methylamino)propyl]-5*H*-dibenz[*b,f*]azepine monohydrochloride.
  $C_{18}H_{22}N_2 \cdot HCl$ 302.84
  USP Iminodibenzyl RS
  10,11-Dihydro-5*H*-dibenzo[*b,f*]azepine.
  $C_{14}H_{13}N$ 195.28
  USP Imipramine Hydrochloride RS

Imipramine Pamoate



$(C_{19}H_{24}N_2)_2 \cdot C_{23}H_{16}O_6$ 949.18
Naphthalenecarboxylic acid, 4,4′-methylenebis[3-hydroxy-, compd. with 5*H*-dibenz[*b,f*]azepine-5-propanamine, 10,11-dihydro-*N,N*-dimethyl-, 2- (1:2);
5-[3-(Dimethylamino)propyl]-10,11-dihydro-5*H*-dibenz[*b,f*]azepine compound (2:1) with 4,4′-methylenebis(3-hydroxy-2-naphthoic acid);
3-[10,11-Dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine 4,4′-methylenebis(3-hydroxy-2-naphthoate) salt (2:1) [10075-24-8].

DEFINITION

Imipramine Pamoate contains NLT 98.0% and NMT 102.0% of imipramine pamoate $[(C_{19}H_{24}N_2)_2 \cdot C_{23}H_{16}O_6]$, calculated on anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 5.2 g/L of dibasic potassium phosphate in water
Solution A: Acetonitrile and *Buffer* (15:85). Adjust with phosphoric acid to a pH of 8.0.
Solution B: Acetonitrile and *Buffer* (38:62). Adjust with phosphoric acid to a pH of 8.0.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	70	30
20	35	65
30	35	65
31	90	10
35	90	10

Diluent: Acetonitrile and water (75:25)
Standard stock solution: 2 mg/mL of USP Imipramine Pamoate RS in *Diluent*
Standard solution: 0.2 mg/mL of USP Imipramine Pamoate RS from *Standard stock solution* in *Solution A*
Sample stock solution: 2 mg/mL of Imipramine Pamoate in *Diluent*
Sample solution: 0.2 mg/mL of Imipramine Pamoate from *Sample stock solution* in *Solution A*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 269 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Autosampler temperature: 10°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—Approximate relative retention times for pamoic acid and imipramine are 0.3 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between pamoic acid and imipramine

USP Monographs

www.webofpharma.com

Tailing factor: NMT 1.8 for imipramine
Relative standard deviation: NMT 2.0% for imipramine

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of imipramine pamoate $[(C_{19}H_{24}N_2)_2 \cdot C_{23}H_{16}O_6]$ in the portion of Imipramine Pamoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of imipramine from the *Sample solution*
r_S = peak response of imipramine from the *Standard solution*
C_S = concentration of USP Imipramine Pamoate RS in the *Standard solution* (mg/mL)
C_U = concentration of Imipramine Pamoate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.10%
- **ORGANIC IMPURITIES**

Buffer: 5.2 g/L of dibasic potassium phosphate in water
Solution A: Acetonitrile and *Buffer* (38:62). Adjust with phosphoric acid to a pH of 7.9.
Solution B: Acetonitrile and *Buffer* (70:30). Adjust with phosphoric acid to a pH of 6.0.
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
50	0	100
60	0	100
61	100	0
75	100	0

Diluent: Acetonitrile and water (75:25)
System suitability solution: 0.5 mg/mL of USP Imipramine Pamoate RS, 0.02 mg/mL of USP Depramine RS, and 0.02 mg/mL USP Iminodibenzyl RS in *Diluent*
Standard solution: 0.002 mg/mL of USP Imipramine Pamoate RS in *Diluent*
Sample solution: 2 mg/mL of Imipramine Pamoate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Column: 40°
Autosampler: 10°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 3* for relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between imipramine and depramine, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Imipramine Pamoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of imipramine from the *Standard solution*
C_S = concentration of USP Imipramine Pamoate RS in the *Standard solution* (mg/mL)
C_U = concentration of Imipramine Pamoate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard peaks that are less than 0.05% of the imipramine peak.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pamoic acid (Pamoate)[a]	0.2	—
Desipramine[b]	0.5	0.10
Depramine	0.7	0.10
Imipramine	1.0	—
Iminodibenzyl	3.7	0.10
Any individual, unspecified impurity	—	0.10
Total impurities	—	0.75

[a] Pamoic acid is not an impurity. It is listed for identification purposes only.
[b] 10,11-Dihydro-5-[3-(methylamino)propyl]-5*H*-dibenz[*b,f*]azepine.

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Depramine RS
3-(5*H*-Dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine.
$C_{19}H_{22}N_2$ 278.39
USP Iminodibenzyl RS
10,11-Dihydro-5*H*-dibenzo[*b,f*]azepine.
$C_{14}H_{13}N$ 195.28
USP Imipramine Pamoate RS

Imipramine Pamoate Capsules

DEFINITION

Imipramine Pamoate Capsules contain imipramine pamoate $[(C_{19}H_{24}N_2)_2 \cdot C_{23}H_{16}O_6]$ equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$).

USP Monographs

www.webofpharma.com

IDENTIFICATION

- **A.** The retention time of the imipramine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the imipramine peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Organic Impurities*.

ASSAY

Change to read:

- **PROCEDURE**

Buffer: 5.2 g/L of dibasic potassium phosphate in water
Solution A: ▲Acetonitrile▲ (ERR 1-May-2019) and *Buffer* (15:85). Adjust with phosphoric acid to a pH of 8.0.
Solution B: ▲Acetonitrile▲ (ERR 1-May-2019) and *Buffer* (38:62). Adjust with phosphoric acid to a pH of 8.0.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	70	30
20	35	65
30	35	65
31	90	10
35	90	10

Diluent: ▲Acetonitrile▲ (ERR 1-May-2019) and water (75:25)
Standard stock solution: 0.75 mg/mL of USP Imipramine Pamoate RS in *Diluent*
Standard solution: 0.23 mg/mL of USP Imipramine Pamoate RS (equivalent to 0.15 mg/mL of imipramine hydrochloride) from the *Standard stock solution* in *Solution A*. Pass a portion through a suitable filter of 0.45-µm pore size. Use the filtrate.
Sample stock solution: Transfer the contents of Capsules (NLT 5) into a suitable volumetric flask, and add the corresponding Capsule shells. Add 10% of the final flask volume of acetonitrile, and sonicate for 10 min with intermittent shaking. Add 80% of the final flask volume of *Diluent*, and sonicate for 15 min with intermittent shaking. Allow to cool to room temperature, and dilute with *Diluent* to volume. Allow to stand for 5 min.
Sample solution: Nominally 0.23 mg/mL of imipramine pamoate (equivalent to 0.15 mg/mL of imipramine hydrochloride) from the *Sample stock solution* in *Solution A*. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size. Use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 269 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Autosampler temperature: 10°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for pamoic acid and imipramine are 0.3 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between pamoic acid and imipramine
Tailing factor: NMT 2.0 for imipramine
Relative standard deviation: NMT 2.0% for imipramine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) in the portion of Imipramine Pamoate Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M \times (M_{r1}/M_{r2})] \times 100$$

r_U = peak area of imipramine from the *Sample solution*
r_S = peak area of imipramine from the *Standard solution*
C_S = concentration of USP Imipramine Pamoate RS in the *Standard solution* (mg/mL)
C_U = equivalent concentration of imipramine hydrochloride in the *Sample solution* (mg/mL)
M = number of moles of imipramine hydrochloride equivalent to each mole of imipramine pamoate, 2
M_{r1} = molecular weight of imipramine hydrochloride, 316.87
M_{r2} = molecular weight of imipramine pamoate, 949.18

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** ⟨711⟩

Test 1
Tier 1
Medium 1: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Times: 30 and 90 min
Tier 2
Medium 2: 0.1 N hydrochloric acid with 0.3% purified pepsin; 900 mL
Apparatus 1: 100 rpm
Times: 30 and 90 min
Buffer: 4.4 g/L of dibasic potassium phosphate in water
Mobile phase: Acetonitrile, triethylamine, and *Buffer* (400:5:600). Adjust with phosphoric acid to a pH of 8.0.
Diluent A: Acetonitrile and water (75:25)
Diluent B: 20.4 g/L of monobasic potassium phosphate and 3 g/L of sodium hydroxide. Adjust with 1 N sodium hydroxide or 1 N phosphoric acid to a pH of 7.4.
Standard stock solution: 0.63 mg/mL of USP Imipramine Pamoate RS in *Diluent A*
Standard solution: 0.038 mg/mL of USP Imipramine Pamoate RS from the *Standard stock solution* prepared as follows. Transfer a suitable volume of the *Standard stock solution* to an appropriate flask that already contains 60% of the final flask volume of *Diluent B* and 30% of the final flask volume of *Medium*. Dilute with *Diluent B* to volume.
Sample stock solution: Centrifuge a portion of the solution under test. Use the supernatant. Replace the portion of solution removed from the vessel with the same volume of fresh *Medium 1* or *Medium 2* at 37°. [NOTE—The use of a centrifuge speed of 5000 rpm for 10 min may be suitable.]
Sample solution: Nominally equivalent to about 0.025 mg/mL of imipramine hydrochloride prepared from the *Sample stock solution* in *Diluent B* in a suitable volumetric flask
Dissolution procedure: Perform the test using the conditions under *Tier 1*. In the presence of cross-linking, repeat the test with a new set of Capsules using the conditions under *Tier 2*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)

USP Monographs

www.webofpharma.com

Mode: LC
Detector: UV 252 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 30°
Flow rate: 1.2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_i = (r_i/r_S) \times C_S \times [M \times (M_{r1}/M_{r2})] \times D \times V \times (1/L) \times 100$$

r_i = peak area of imipramine from the *Sample solution* at each time point
r_S = peak area of imipramine from the *Standard solution*
C_S = concentration of USP Imipramine Pamoate RS in the *Standard solution* (mg/mL)
M = number of moles of imipramine hydrochloride equivalent to each mole of imipramine pamoate, 2
M_{r1} = molecular weight of imipramine hydrochloride, 316.87
M_{r2} = molecular weight of imipramine pamoate, 949.18
D = dilution factor of the *Sample solution*
V = volume of *Medium 1* or *Medium 2*, 900 mL
L = label claim (mg/Capsule)

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (min)	Amount Dissolved NLT (%)
1	30	40
2	90	75

The percentage of imipramine pamoate dissolved equivalent to the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) dissolved at the times specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 1*.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Times: 30 and 150 min
Standard solution: A solution containing USP Imipramine Pamoate RS at the concentrations listed in *Table 3* prepared as follows. Transfer a suitable quantity of USP Imipramine Pamoate RS to an appropriate volumetric flask. Add 5% of the final flask volume of methanol and sonicate for 5 min. Add 75% of the final flask volume of *Medium* that has been heated to NLT 60° and stir for 30 min. Allow to cool to room temperature. Dilute with *Medium* to volume and mix. Pass through a suitable filter and use the filtrate.

Table 3

Labeled Amount of Imipramine Hydrochloride (mg/Capsule)	Concentration of USP Imipramine Pamoate RS (mg/mL)	Equivalent Concentration of Imipramine Hydrochloride (mg/mL)
75	0.12	0.08
100	0.17	0.11
125	0.21	0.14
150	0.26	0.17

Sample solution: Pass a portion of the solution under test through a suitable filter. Use the filtrate.
Instrumental conditions
Mode: UV-Vis
Wavelength: 251 nm
Cell: 0.2 cm
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_i = (A_i/A_S) \times C_S \times [M \times (M_{r1}/M_{r2})] \times V \times (1/L) \times 100$$

A_i = absorbance of imipramine from the *Sample solution* at each time point
A_S = absorbance of imipramine from the *Standard solution*
C_S = concentration of USP Imipramine Pamoate RS in the *Standard solution* (mg/mL)
M = number of moles of imipramine hydrochloride equivalent to each mole of imipramine pamoate, 2
M_{r1} = molecular weight of imipramine hydrochloride, 316.87
M_{r2} = molecular weight of imipramine pamoate, 949.18
V = volume of *Medium*, 900 mL
L = label claim (mg/Capsule)

Tolerances: See *Table 4*.

Table 4

Time Point (*i*)	Time (min)	Amount Dissolved NLT (%)
1	30	25
2	150	80

The percentage of imipramine pamoate dissolved equivalent to the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) dissolved at the times specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 1*.

• **Uniformity of Dosage Units** ⟨905⟩: Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**
Protect solutions containing imipramine from light.
Buffer: 5.2 g/L of dibasic potassium phosphate in water
Solution A: ▲Acetonitrile▲ (ERR 1-May-2019) and *Buffer* (3:100). Adjust with phosphoric acid to a pH of 7.2.

www.webofpharma.com

Solution B: Methanol and ▲acetonitrile▲ (ERR 1-May-2019) (70:30)
Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	62	38
12	62	38
25	50	50
65	20	80
70	20	80
75	62	38
95	62	38

System suitability solution: 1.5 mg/mL of USP Imipramine Pamoate RS (equivalent to 1 mg/mL of imipramine hydrochloride), and 0.001 mg/mL each of USP Desipramine Hydrochloride RS and USP Depramine RS in *Solution B*. Pass a portion through a suitable membrane filter of 0.2-µm pore size, and use the filtrate.
Standard solution: 0.015 mg/mL of USP Imipramine Pamoate RS (equivalent to 0.010 mg/mL of imipramine hydrochloride) in *Solution B*. Pass a portion through a suitable membrane filter of 0.2-µm pore size, and use the filtrate.
Sample solution: Nominally 1.5 mg/mL of imipramine pamoate (equivalent to 1.0 mg/mL of imipramine hydrochloride) from NLT 20 Capsules prepared as follows. Transfer a portion of the contents of the Capsules equivalent to 50 mg of imipramine hydrochloride to a 50-mL volumetric flask. Add 30 mL of *Solution B*, and sonicate for 10 min in a cool water bath with intermittent shaking. Dilute with *Solution B* to volume. Pass a portion through a suitable membrane filter of 0.2-µm pore size, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm. For *Identification* test *B*, a diode-array detector may be used in the wavelength range of 200–300 nm.
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Temperatures
Autosampler: 10°
Column: 45°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 6* for relative retention times.]
Suitability requirements
Resolution: NLT 2.0 between the desipramine and depramine peaks; NLT 2.0 between the depramine and imipramine peaks, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [M \times (M_{r1}/M_{r2})] \times (1/F) \times 100$$

r_U = peak response of each imipramine impurity from the *Sample solution*
r_S = peak response of imipramine from the *Standard solution*
C_S = concentration of USP Imipramine Pamoate RS in the *Standard solution* (mg/mL)
C_U = equivalent concentration of imipramine hydrochloride in the *Sample solution* (mg/mL)
M = number of moles of imipramine hydrochloride equivalent to each mole of imipramine pamoate, 2
M_{r1} = molecular weight of imipramine hydrochloride, 316.87
M_{r2} = molecular weight of imipramine pamoate, 949.18
F = relative response factor (see *Table 6*)

Acceptance criteria: See *Table 6*. Disregard any degradation product peaks less than 0.02%.

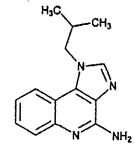
Table 6

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%, w/w)
Pamoic acid[a]	0.1	—	—
Desipramine	0.40	1.0	0.2
Depramine	0.66	0.87	0.10
Imipramine	1.0	—	—
Iminodibenzyl[b]	1.3	1.5	0.2
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	0.75

[a] Included for identification only. This peak is due to the pamoate counterion; hence it is not an impurity.
[b] 10,11-Dihydro-5*H*-dibenzo[*b*,*f*]azepine.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **LABELING:** The labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Depramine RS
3-(5*H*-Dibenzo[*b*,*f*]azepin-5-yl)-*N*,*N*-dimethylpropan-1-amine.
$C_{19}H_{22}N_2$ 278.39
USP Desipramine Hydrochloride RS
USP Imipramine Pamoate RS

Imiquimod



$C_{14}H_{16}N_4$ 240.30
1*H*-Imidazo[4,5-*c*]quinolin-4-amine, 1-(2-methylpropyl)-; 4-Amino-1-isobutyl-1*H*-imidazo[4,5-*c*]quinoline [99011-02-6].

www.webofpharma.com

DEFINITION

Imiquimod contains NLT 98.0% and NMT 102.0% of imiquimod ($C_{14}H_{16}N_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 0.005 M sodium 1-octanesulfonate in water containing 0.1% triethylamine. Adjust with dilute perchloric acid to a pH of 2.0.
Mobile phase: Acetonitrile and *Buffer* (27:73)
Standard solution: 50 µg/mL of USP Imiquimod RS in *Mobile phase*
Sample solution: 50 µg/mL of Imiquimod in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of imiquimod ($C_{14}H_{16}N_4$) in the portion of Imiquimod taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Imiquimod RS in the *Standard solution* (µg/mL)
C_U = concentration of Imiquimod in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**

Buffer: 0.005 M sodium octanesulfonate in water containing 0.1% triethylamine. Adjust with dilute perchloric acid to a pH of 2.0.
Diluent: Acetonitrile and *Buffer* (20:80)
Solution A: *Buffer*
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
20	80	20
45	10	90
50	10	90
55	80	20
65	80	20

Standard solution: 1.25 µg/mL each of USP Imiquimod Related Compound A RS, USP Imiquimod Related Compound B RS, USP Imiquimod Related Compound C RS, and USP Imiquimod RS in *Diluent*
Sample solution: 0.25 mg/mL of Imiquimod in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 226 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 45°
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3 between imiquimod and imiquimod related compound C
Relative standard deviation: NMT 3.0% for imiquimod
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each known impurity in the portion of Imiquimod taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each known impurity from the *Sample solution*
r_S = peak response of the corresponding related compound from the *Standard solution*
C_S = concentration of the corresponding Reference Standard in the *Standard solution* (µg/mL)
C_U = concentration of Imiquimod in the *Sample solution* (µg/mL)

Calculate the percentage of any other impurity in the portion of Imiquimod taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of imiquimod from the *Standard solution*
C_S = concentration of USP Imiquimod RS in the *Standard solution* (µg/mL)
C_U = concentration of Imiquimod in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Imiquimod related compound B	0.30	0.15
Imiquimod related compound A	0.43	0.15
Imiquimod	1.0	—
Imiquimod related compound C	1.12	0.15

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any unspecified individual impurity	—	0.10
Total impurities	—	0.50

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩
  Analysis: Dry a sample at 100°–105° for 3 h.
  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Imiquimod RS
  USP Imiquimod Related Compound A RS
  1-Isobutyl-1*H*-imidazo[4,5-*c*]quinoline.
  $C_{14}H_{15}N_3$ 225.29
  USP Imiquimod Related Compound B RS
  1-Isobutyl-1*H*-imidazo[4,5-*c*]quinoline 5-oxide.
  $C_{14}H_{15}N_3O$ 241.29
  USP Imiquimod Related Compound C RS
  4-Chloro-1-isobutyl-1*H*-imidazo[4,5-*c*]quinoline.
  $C_{14}H_{14}ClN_3$ 259.73

Imiquimod Cream

DEFINITION

Imiquimod Cream contains NLT 90% and NMT 110% of the labeled amount of imiquimod ($C_{14}H_{16}N_4$).

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
  Wavelength range: 220–400 nm
  Diluent: Acetonitrile and 0.1 N hydrochloric acid (30:70)
  Standard solution: 2 µg/mL of USP Imiquimod RS in *Diluent*. Sonicate, if necessary, to dissolve.
  Sample stock solution: Nominally 20 µg/mL of imiquimod in *Diluent* prepared as follows. Transfer a portion of Cream equivalent to 5 mg of imiquimod into a 250-mL volumetric flask. Add about 60% of the flask volume of *Diluent* and sonicate for 30 min with occasional swirling to dissolve, if necessary. Dilute with *Diluent* to volume.
  Sample solution: Nominally 2 µg/mL of imiquimod from *Sample stock solution* in *Diluent*. Pass through a suitable filter.
  Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**
  Buffer: 1.17 g/L of sodium 1-octanesulfonate in water. Add 1 mL of triethylamine for each liter of solution and adjust with perchloric acid to a pH of 2.5.
  Mobile phase: Acetonitrile and *Buffer* (270:730)
  Diluent: Acetonitrile and 0.1 N hydrochloric acid (30:70)
  Standard stock solution: 0.2 mg/mL of USP Imiquimod RS in *Diluent*. Sonicate to dissolve, if necessary.
  Standard solution: 0.01 mg/mL of USP Imiquimod RS in *Mobile phase* from *Standard stock solution*
  Sample stock solution: Nominally 0.2 mg/mL of imiquimod in *Diluent* prepared as follows. Transfer a portion of Cream equivalent to about 50 mg of imiquimod into a 250-mL volumetric flask. Add about 60% of the flask volume of *Diluent*, sonicate for 30 min with occasional swirling to dissolve, and cool if necessary. Dilute with *Diluent* to volume.
  Sample solution: Nominally 0.01 mg/mL of imiquimod from *Sample stock solution* in *Mobile phase*. Pass through a suitable filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 226 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L7
  Column temperature: 30°
  Flow rate: 1.5 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of imiquimod ($C_{14}H_{16}N_4$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of imiquimod from the *Sample solution*
r_S = peak response of imiquimod from the *Standard solution*
C_S = concentration of USP Imiquimod RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of imiquimod in the *Sample solution* (mg/mL)

Acceptance criteria: 90%–110%

PERFORMANCE TESTS

- **Minimum Fill** ⟨755⟩: Meets the requirement

IMPURITIES

- **Organic Impurities**
  Solution A: 1.17 g/L of sodium 1-octanesulfonate in water. Add 1 mL of triethylamine for each liter of solution and adjust with perchloric acid to a pH of 2.5.
  Solution B: Acetonitrile and methanol (90:10)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
30	70	30
45	40	60
55	40	60
60	90	10
70	90	10

Diluent: Acetonitrile and 0.1 N hydrochloric acid (30:70)

USP Monographs

www.webofpharma.com

Standard stock solution: 0.5 mg/mL of USP Imiquimod RS in *Diluent*. Sonicate to dissolve, if necessary.
Standard solution: 0.5 µg/mL of USP Imiquimod RS from *Standard stock solution* in *Diluent*
Sample solution: Nominally 0.5 mg/mL of imiquimod prepared as follows. Transfer a portion of Cream equivalent to 25 mg of imiquimod into a 50-mL volumetric flask. Add about 30 mL of *Diluent*, and sonicate for 40 min with occasional swirling. Dilute with *Diluent* to final volume. Pass through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 50°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard stock solution* and *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0, *Standard stock solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of imiquimod from the *Standard solution*
C_S = concentration of USP Imiquimod RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of imiquimod in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

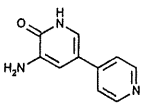
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Imiquimod related compound B[a]	0.57	1.15	0.2
Imiquimod related compound A[b]	0.77	1.5	—[c]
Imiquimod	1.0	—	—
Imiquimod related compound C[d]	1.2	1.85	—[c]
Any unspecified individual impurity	—	1.0	0.5
Total impurities	—	—	1.0

[a] 1-Isobutyl-1*H*-imidazo[4,5-*c*]quinoline 5-oxide.
[b] 1-Isobutyl-1*H*-imidazo[4,5-*c*]quinoline.
[c] Process impurities monitored in the drug substance and not included in the calculation of total impurities.
[d] 4-Chloro-1-isobutyl-1*H*-imidazo[4,5-*c*]quinoline.

SPECIFIC TESTS
- **pH** ⟨791⟩
Sample: Nominally 2.5 mg/mL of imiquimod from Cream in water. Sonicate to disperse with constant swirling.
Acceptance criteria: 4.5–7.0
- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count is NMT 10^2 cfu/g, and the total yeasts and molds count is NMT 10^1 cfu/g. It meets the requirements for absence of *S. aureus* and *P. aeruginosa*.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers, and store at 4°–25°. Protect from freezing.
- **USP Reference Standards** ⟨11⟩
USP Imiquimod RS

Inamrinone



$C_{10}H_9N_3O$ 187.20
[3,4′-Bipyridin]-6(1*H*)-one, 5-amino-.
5-Amino[3,4′-bipyridin]-6(1*H*)-one [60719-84-8].

» Inamrinone contains not less than 98.0 percent and not more than 102.0 percent of $C_{10}H_9N_3O$, calculated on the anhydrous basis.
[**CAUTION**—Inamrinone is a cardiotonic agent.]

Packaging and storage—Preserve in well-closed containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.
USP Reference standards ⟨11⟩—
USP Inamrinone RS
USP Inamrinone Related Compound A RS
5-Carboxamide[3,4′-bipyridin]-6(1*H*)-one.
$C_{11}H_9N_3O_2$ 215.21

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
pH 8.9 Buffer—Dissolve 107 g of dibasic sodium phosphate in water, adjust, if necessary, with 0.1 M sodium hydroxide or 0.1 M phosphoric acid to a pH of 8.9 ± 0.1, dilute with water to 1000 mL, and mix.
Solution: 6 µg per mL, prepared as follows. Dissolve 100 mg in 20 mL of water and 1.0 mL of 1 N hydrochloric acid in a 100-mL volumetric flask, dilute with water to volume, and mix. Dilute 5.0 mL of this solution to 50.0 mL with 0.01 N hydrochloric acid, mix, and transfer 3.0 mL to a 50-mL volumetric flask. Dilute with *pH 8.9 Buffer* to volume, and mix.
Ratio: A_{237}/A_{318} do not differ by more than 3.0%.
Water Determination, *Method I* ⟨921⟩**:** not more than 1.0%.
Residue on ignition ⟨281⟩**:** not more than 0.2%.

USP Monographs

www.webofpharma.com

Chromatographic purity—

Solution A—Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water, add 2 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.5. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Solution B—Prepare a mixture of *Solution A* and acetonitrile (85:15).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.*

Diluting solution—Dissolve 0.25 g of sodium metabisulfite in 1000 mL of *Solution A.*

Standard stock solution—Dissolve an accurately weighed quantity of USP Inamrinone RS in *Diluting solution* to obtain a solution having a known concentration of about 2 mg per mL.

Standard solution—Dilute a suitable volume of *Standard stock solution* quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of 4 µg of USP Inamrinone RS per mL.

System suitability solution—Prepare a solution of USP Inamrinone Related Compound A RS in *Diluting solution* having a concentration of 2 mg per mL. Transfer 5.0 mL of this solution and 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, dilute with *Diluting solution* to volume, and mix.

Test solution—Transfer about 100 mg of Inamrinone, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix. [NOTE—Use this solution within 1 hour after preparation.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 315-nm detector and a 4.0-mm × 25-cm analytical column that contains packing L1 and is fitted with a guard column that contains packing L1. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	87	13	equilibration
0–1	87	13	isocratic
1–29	87→0	13→100	linear gradient
29–30	0	100	isocratic

Allow the system to equilibrate at the original conditions before making subsequent injections. Chromatograph 15 µL of the *System suitability solution,* record the chromatograms, and measure the peak responses as described for *Procedure:* the relative retention times are 0.6 for inamrinone and 1.0 for inamrinone related compound A; and the resolution, *R,* between inamrinone and inamrinone related compound A is not less than 4.0. Chromatograph about 15 µL of the *Standard solution,* and record the peak responses for inamrinone as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 15 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms allowing the *Test solution* to elute for not less than five times the retention times of inamrinone, and measure the areas of all the peaks observed in the chromatogram of the *Test solution.* Calculate the percentage of each impurity in the portion of Inamrinone taken by the formula:

$$5000(C/W)(r_i/r_s)$$

in which C is the concentration, in mg per mL, of USP Inamrinone RS in the *Standard solution; W* is the weight, in mg, of inamrinone taken for the *Test solution; r_i* is the response of each impurity peak; and r_s is the mean response for the *Standard solution:* not more than 0.2% of any individual impurity is found; and the sum of all impurities is not more than 1.0%.

Assay—Weigh accurately about 500 mg of Inamrinone, and proceed as directed under *Nitrite Titration* ⟨451⟩. Each mL of 0.1 M sodium nitrite is equivalent to 18.72 mg of $C_{10}H_9N_3O$.

Inamrinone Injection

DEFINITION

Inamrinone Injection is a sterile solution of Inamrinone in Water for Injection, prepared with the aid of Lactic Acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of inamrinone ($C_{10}H_9N_3O$).

[CAUTION—Inamrinone is a cardiotonic agent.]

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution,* as obtained in the *Assay.*▲ (USP 1-Aug-2019)

Change to read:

• ▲**C.**▲ (USP 1-Aug-2019)

Indicator solution: Freshly prepare. Dissolve 250 mg of sodium nitroferricyanide in sufficient water to make 9 mL and mix with 1 mL of morpholine.

Sample solution: Transfer a volume of Injection, equivalent to about 50 mg of inamrinone, to a glass-stoppered container. Add about 2 g of 50–100 mesh sulfonic acid cation-exchange resin, and shake for about 2 min or until the supernatant becomes essentially colorless. Filter, and collect the filtrate in an arsine generator flask (see *Arsenic* ⟨211⟩, *Procedures, Apparatus*).

Analysis: Add 5 mL of diluted sulfuric acid to the *Sample solution,* and boil gently on a hot plate for 5–10 min. Cool to room temperature. Add 10 mL of ▲0.1 N potassium permanganate VS,▲ (USP 1-Aug-2019)attach the scrubber unit and absorber tube, and place the apparatus on a warm hot plate. Add 1 mL of *Indicator solution* to the absorber tube. Heat gently, allowing the vapors to bubble through the indicator solution.

Acceptance criteria: The indicator solution turns blue within 5 min (presence of lactate).

ASSAY

Change to read:

• **PROCEDURE**

▲Freshly prepare all inamrinone-containing solutions before injection.▲ (USP 1-Aug-2019)

Solution A: 0.5 M of borate buffer prepared as follows. Transfer 31 g of boric acid to a beaker containing approximately 800 mL of water. Slowly add ▲5 N sodium hydroxide▲ (USP 1-Aug-2019) in small quantities, stirring well after each addition, until all the boric acid is dissolved and

USP Monographs

www.webofpharma.com

the pH is constant at 7.0 ± 0.3. Transfer this solution to a 1000-mL volumetric flask, and dilute with water to volume.
Mobile phase: Methanol, *Solution A*, and water (48:2:50)
System suitability solution: 50 µg/mL each of USP Inamrinone RS and USP Inamrinone Related Compound C RS in *Mobile phase*
Standard solution: 50 µg/mL of USP Inamrinone RS in *Mobile phase*
Sample solution: Nominally 50 µg/mL of inamrinone prepared as follows. Transfer a volume of Injection, equivalent to about 5 mg of inamrinone, to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Aug-2019)
Column: 3.9-mm × 30-cm; ▲10-µm▲ (USP 1-Aug-2019) packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for inamrinone related compound C and inamrinone are about 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3 between inamrinone related compound C and inamrinone, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of inamrinone ($C_{10}H_9N_3O$) in the portion of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of inamrinone from the *Sample solution*
r_S = peak response of inamrinone from the *Standard solution*
C_S = concentration of USP Inamrinone RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of inamrinone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

• **LACTIC ACID CONTENT**
Ion–exchange column: Place a small pledget of glass wool at the bottom of a 100- × 6-mm glass column equipped with a stopcock and a 25-mL reservoir. Soak a suitable quantity of 50- to 100-mesh sulfonic acid cation-exchange resin in 6 N hydrochloric acid for several minutes. Wash with water until the wash is neutral to wide-range pH indicator paper. Fill the column with the prepared resin to the base of the reservoir. Wash the column with 50 mL of water in several portions, draining each wash to the top of the resin before adding the next portion. Discard the washes.
Sample solution: Place a 125-mL conical flask below the ion-exchange column. Pipet a volume of Injection equivalent to about 50 mg of inamrinone onto the column. Allow the specimen to pass through the column at the rate of about 0.5–1 mL/min, draining the specimen to the top of the column and collecting the eluate in the flask. Wash the column with five 5-mL portions of water, collecting the washings in the flask. Add several small glass beads to the solution in the flask, and boil on a hot plate for 10 min.
▲Blank: Proceed as in *Sample solution*, substituting the volume of Injection used with the same volume of water.▲ (USP 1-Aug-2019)
Titrimetric system
(See *Titrimetry* ⟨541⟩, *Residual Titrations*.)
Mode: Residual titration
Titrant: ▲0.1 N sodium hydroxide VS▲ (USP 1-Aug-2019)
Back-titrant: 0.1 N hydrochloric acid VS
Endpoint detection: Visual
Analysis: Add 10.0 mL of the *Titrant* to the *Sample solution*, and boil for 20 min. Add phenolphthalein TS, and titrate the warm solution with the *Back-titrant*. Perform a blank determination with the *Blank*.
▲Calculate the content of lactic acid ($C_3H_6O_3$) in the portion of the Injection taken:

$$\text{Result} = (V_B - V_S) \times M \times F \times (1/V)$$

V_B = volume of the *Back-titrant* consumed by the *Blank* (mL)
V_S = volume of the *Back-titrant* consumed by the *Sample solution* (mL)
M = actual molarity of the *Back-titrant* (mM/mL)
F = equivalency factor, 90.08 mg/mM
V = volume of Injection taken from the *Sample solution* (mL)▲ (USP 1-Aug-2019)

Acceptance criteria: 5.0–7.5 mg/mL of Injection

Change to read:

• **ORGANIC IMPURITIES**
▲Freshly prepare all inamrinone-containing solutions before injection.▲ (USP 1-Aug-2019)
Solution A: Phosphoric acid and water (11.4: 978.6)
Mobile phase: Acetonitrile and *Solution A* (1:99)
Lactic acid solution: 11.8 mg/mL of lactic acid in water
Standard stock solution: 0.1 mg/mL of USP Inamrinone RS and 0.25 mg/mL of USP Inamrinone Related Compound B RS, prepared as follows. Transfer suitable quantities of USP Inamrinone RS and USP Inamrinone Related Compound B RS to a suitable volumetric flask. Add 60% of the flask volume of *Lactic acid solution*, and sonicate for 2 min to effect solution. Cool, and dilute with *Lactic acid solution* to volume.
Standard solution: 4 µg/mL of USP Inamrinone RS and 10 µg/mL of USP Inamrinone Related Compound B RS, prepared as follows. ▲Transfer a volume▲ (USP 1-Aug-2019) of *Standard stock solution* into a ▲suitable▲ (USP 1-Aug-2019) volumetric flask. Dilute with *Mobile phase* to volume.
Sample solution: ▲Nominally 2 mg/mL of inamrinone prepared as follows.▲ (USP 1-Aug-2019) Transfer a volume of Injection, equivalent to about 100 mg of inamrinone into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.▲▲ (USP 1-Aug-2019)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 313 nm
Column: ▲4.6-mm × 15-cm; 5-µm base-deactivated packing L7▲ (USP 1-Aug-2019)
Column temperature: 30°–35°
Flow rate: 2 mL/min

www.webofpharma.com

Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 10 between inamrinone and inamrinone related compound B
Relative standard deviation: NMT 10% for inamrinone related compound B
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of inamrinone related compound B and any unspecified impurity in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for inamrinone related compound B or any unspecified impurity from the *Sample solution*
r_S = peak response for inamrinone related compound B RS from the *Standard solution*
C_S = concentration of USP Inamrinone Related Compound B RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of inamrinone in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Inamrinone	1.0	—
Inamrinone related compound B	3.7	2.0
Any unspecified impurity	—	0.5
Total impurities	—	3.0

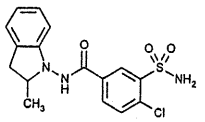
SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.5 USP Endotoxin Unit/mg of inamrinone
- **pH** ⟨791⟩: 3.2–4.0
- **OTHER REQUIREMENTS:** Meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, protected from light. Store at room temperature.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
USP Inamrinone RS
USP Inamrinone Related Compound B RS
▲2-Hydroxy-*N*-(6-oxo-1,6-dihydro-[3,4′-bipyridin]-5-yl) propanamide.▲ (USP 1-Aug-2019)
$C_{13}H_{13}N_3O_3$ 259.27
USP Inamrinone Related Compound C RS
▲6-Oxo-1,6-dihydro-[3,4′-bipyridine]-5-carbonitrile.▲ (USP 1-Aug-2019)
$C_{11}H_7N_3O$ 197.20

Indapamide



$C_{16}H_{16}ClN_3O_3S$ 365.83
Benzamide, 3-(aminosulfonyl)-4-chloro-*N*-(2,3-dihydro-2-methyl-1*H*-indol-1-yl)-.
4-Chloro-*N*-(2-methyl-1-indolinyl)-3-sulfamoylbenzamide [26807-65-8].

» Indapamide contains not less than 98.0 percent and not more than 101.0 percent of $C_{16}H_{16}ClN_3O_3S$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Indapamide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 5 µg per mL.
Medium: methanol.
Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 3.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.
Chromatographic purity— [CAUTION—*Minimize exposure to light while weighing the samples and spotting on the thin-layer chromatographic plate. Use low-actinic glassware or wrap the glassware with aluminum foil and protect all the chromatographic solutions from light. Place the chromatographic tanks in a dark room or cover them with aluminum foil during the development. The paperlined chamber should be saturated with solvent vapor for 1 hour before development of the plates.*]
Standard preparations—Dissolve USP Indapamide RS in methanol, and mix to obtain *Standard preparation A* having a known concentration of 0.30 mg per mL. Dilute quantitatively with methanol to obtain *Standard preparation B* and *Standard preparation C* containing 0.15 mg and 0.075 mg of USP Indapamide RS per mL, respectively.
Test preparation—Dissolve an accurately weighed quantity of Indapamide in methanol, and dilute quantitatively with methanol to obtain a solution containing 30 mg per mL.
Procedure—Apply separately 10 µL of the *Test preparation* and 10 µL of each *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of toluene, ethyl acetate, and glacial acetic acid (70:30:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry under a current of air. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots observed in the

www.webofpharma.com

chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations:* no secondary spot from the chromatograms of the *Test preparation* is larger or more intense than the principal spot obtained from *Standard preparation B* (0.5%), and the sum of the intensities of the secondary spots obtained from the *Test preparation* corresponds to not more than 2.0%.

Assay— [NOTE—Where peak responses are indicated, use peak areas.]

Mobile phase—Prepare a filtered and degassed mixture consisting of water, acetonitrile, methanol, and glacial acetic acid (650:175:175:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve a suitable quantity of *p*-chloroacetanilide in methanol to obtain a solution having a concentration of about 5.0 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Indapamide RS in *Internal standard solution*, and dilute quantitatively with *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL of the Reference Standard and about 0.25 mg per mL of the internal standard.

Assay preparation—Transfer about 100 mg of Indapamide, accurately weighed, to a 100-mL volumetric flask, dissolve in 5.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the resolution, *R*, between any peak of interest and any adjacent peak is not less than 2.0, the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention time, relative to indapamide, is about 0.65 for *p*-chloroacetanilide. Calculate the quantity, in mg, of $C_{16}H_{16}ClN_3O_3S$ in the portion of Indapamide taken by the formula:

$$100C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Indapamide RS in the *Standard preparation*; and R_U and R_S are the ratios of the peak area of indapamide to the peak area of internal standard in the *Assay preparation* and the *Standard preparation*, respectively.

Indapamide Tablets

» Indapamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{16}H_{16}ClN_3O_3S$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Indapamide RS

Identification—

A: Crush a quantity of Tablets, equivalent to about 15 mg of indapamide, remove and discard any coating material, and finely powder the remaining tablet cores. Agitate the powdered tablets with two 30-mL portions of 0.2 N sodium hydroxide in a centrifuge tube for 10 minutes. Centrifuge each mixture, and combine the supernatants in a 250-mL separator. Acidify the liquid with about 12 mL of dilute hydrochloric acid (1 in 10). Extract the acidic solution with two 4.0-mL portions of ether, filter the extracts through anhydrous sodium sulfate contained in a filter paper, and evaporate the ether, with the aid of a current of dry air, on a water bath. Dry the crystals at 105° for 1 hour: the crystals so obtained respond to *Identification* test *A* under *Indapamide*

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay.*

Dissolution ⟨711⟩—

Medium: 0.05 M pH 6.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Determine the amount of $C_{16}H_{16}ClN_3O_3S$ dissolved by employing the following method.

Mobile phase—Proceed as directed in the *Assay.*

Standard solution—Dissolve an accurately weighed quantity of USP Indapamide RS in methanol, and dilute quantitatively, and stepwise if necessary, with a mixture of *Medium* and methanol (99:1) to obtain a solution having a known concentration equivalent to the solution under test.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 242-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of a filtered portion of the solution under test and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Determine the amount of $C_{16}H_{16}ClN_3O_3S$ dissolved.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{16}H_{16}ClN_3O_3S$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase—Dissolve 1.08 g of sodium 1-octanesulfonate in 700 mL of water, add 10 mL of glacial acetic acid, and mix. Add 300 mL of acetonitrile, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Prepare a solution of 2′-chloroacetophenone in acetonitrile having a concentration of about 0.25 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Indapamide RS in acetonitrile to obtain a solution having a known concentration of about 0.1 mg per mL. Transfer 5.0 mL of this solution and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with a mixture of water and acetonitrile (50:10) to volume, and mix.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of powder, equivalent to about 2.5 mg of indapamide, to a 50-mL volumetric flask, add about 25 mL of acetonitrile, and sonicate for about 20 minutes. Cool, dilute with acetonitrile to volume, and mix. Transfer this solution to a 50-mL centrifuge tube, and centrifuge at 2000 rpm for about 10 minutes. Transfer 10.0

USP Monographs

www.webofpharma.com

mL of the supernatant to a 50-mL volumetric flask, add 3.0 mL of *Internal standard solution*, dilute with a mixture of water and acetonitrile (70:4) to volume, and mix.

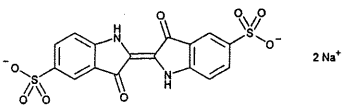
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 242-nm detector and a 4.5-mm × 10-cm column that contains a 3-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between the analyte peak and the internal standard peak is not less than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention time, relative to indapamide, is about 1.18 for the internal standard. Calculate the quantity, in mg, of $C_{16}H_{16}ClN_3O_3S$ in the portion of Tablets taken by the formula:

$$250C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Indapamide RS in the *Standard preparation;* and R_U and R_S are the ratios of the peak responses of indapamide to the internal standard obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Indigotindisulfonate Sodium



$C_{16}H_8N_2Na_2O_8S_2$ 466.35

1*H*-Indole-5-sulfonic acid,2-(1,3-dihydro-3-oxo-5-sulfo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-, disodium salt;
Disodium 3,3′-dioxo-[$\Delta^{2,2'}$-biindoline]-5,5′-disulfonate [860-22-0].

DEFINITION

Indigotindisulfonate Sodium contains NLT 96.0% and NMT 102.0% of sodium indigotinsulfonates, calculated on the dried basis as indigotindisulfonate sodium ($C_{16}H_8N_2Na_2O_8S_2$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨191⟩ and *Sulfate* ⟨191⟩
  Sample: Incinerate a portion of it.
  Acceptance criteria: The residue meets the requirements.
- **B.** The addition of hydrochloric acid to a solution of it changes the color to bluish violet, and further dilution with water restores the original color.
- **C.** The addition of 1 N sodium hydroxide to a solution of it changes the color to yellow or olive-brown.
- **D.** The addition of sodium chloride to a solution of it produces a blue precipitate.

ASSAY

- **PROCEDURE**
  Standard solution: 10 µg/mL in USP Indigotindisulfonate Sodium RS in dilute hydrochloric acid (1 in 100)
  Sample solution: 10 µg/mL of Indigotindisulfonate Sodium in dilute hydrochloric acid (1 in 100)
  Blank: Dilute hydrochloric acid (1 in 100)

Instrumental conditions
Mode: Vis
Analytical wavelength: 610 nm
Cell: 1 cm

Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of indigotindisulfonate sodium ($C_{16}H_8N_2Na_2O_8S_2$) in the portion of Indigotindisulfonate Sodium taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Indigotindisulfonate Sodium RS in the *Standard solution* (µg/mL)
C_U = concentration of Indigotindisulfonate Sodium in the *Sample solution* (µg/mL)

Acceptance criteria: 96.0%–102.0% on the dried basis

OTHER COMPONENTS

- **SULFUR CONTENT**
  Sample solution: Place 25 mg in halide-free filter paper measuring 4 cm square, and fold the paper to enclose it.
  Analysis: Proceed as directed in *Oxygen Flask Combustion* ⟨471⟩, using a 1-L flask and a mixture of 25 mL of water and 5 mL of hydrogen peroxide TS as the absorbing liquid. When the combustion is complete, place a few mL of water in the cup, loosen the stopper, and rinse the stopper, the specimen holder, and the sides of the flask with 20 mL of water. Add 2 mL of hydrochloric acid, dilute with water to 250 mL, heat to boiling, and slowly add 10 mL of barium chloride TS. Heat the mixture on a steam bath for 1 h, and collect the precipitate of barium sulfate on a filter. Wash until it is free from chloride, dry, ignite, and weigh. Each g of residue is equivalent to 137.4 mg of sulfur.
  Acceptance criteria: 13.0%–14.0% on the dried basis

IMPURITIES

- **ARSENIC,** *Method II* ⟨211⟩**:** NMT 8 ppm
- **LIMIT OF LEAD** ⟨251⟩
  Sample solution: Place 4.0 g in a Kjeldahl flask, moisten with water, and add 10 mL of sulfuric acid and 5 mL of nitric acid. As soon as the first violent reaction subsides, heat until most of the brown fumes are expelled. Repeat the addition of nitric acid, 1–3 mL at a time, and heat until the Indigotindisulfonate Sodium is practically decomposed and most of the organic matter is in solution. Then add, cautiously and in small portions, 5 mL of perchloric acid. When the violent reaction subsides, continue the addition of small amounts of nitric acid, and heat as before until a colorless solution is obtained. (If the solution fails to become clear in 10–20 min after the addition of the perchloric acid, add 1–3 mL more of this acid, and continue the nitric acid treatment until the solution is colorless.) Boil for 10–15 min, cool, and neutralize with 1 N sodium hydroxide. Transfer to a 100-mL volumetric flask, and dilute with water to volume.
  Analysis: Test 5 mL of the *Sample solution* according to the limit test for *Lead* ⟨251⟩ using 3 mL of Ammonium Citrate Solution, 1 mL of Potassium Cyanide Solution, and 0.5 mL of Hydroxylamine Hydrochloride Solution.
  Acceptance criteria: 5 mL of the *Sample solution* contains NMT 2 µg of lead (corresponding to NMT 0.001%).

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 5.0%

www.webofpharma.com

• **WATER-INSOLUBLE SUBSTANCES**
Sample solution: 10.0 mg/mL in water
Analysis: Pass 100 mL of the *Sample solution* through a tared filtering crucible, wash with water until the filtrate is practically colorless, and dry the residue at 105° for 1 h.
Acceptance criteria: The weight of the residue does not exceed 5 mg.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Indigotindisulfonate Sodium RS

Indigotindisulfonate Sodium Injection

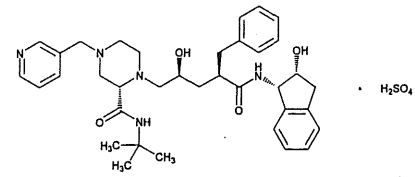
» Indigotindisulfonate Sodium Injection is a sterile solution of Indigotindisulfonate Sodium in Water for Injection. It contains not less than 90.0 percent and not more than 105.0 percent of the labeled amount of $C_{16}H_8N_2Na_2O_8S_2$.

Packaging and storage—Preserve in single-dose, light-resistant containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Indigotindisulfonate Sodium RS
Identification—It responds to *Identification* tests *B*, *C*, and *D* under *Indigotindisulfonate Sodium*.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 5.0 USP Endotoxin Units per mg of indigotindisulfonate sodium.
pH ⟨791⟩: between 3.0 and 6.5.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Quantitatively dilute a portion of Injection, equivalent to about 40 mg of indigotindisulfonate sodium, with dilute hydrochloric acid (1 in 100) to obtain a solution having a known concentration of about 10 µg of indigotindisulfonate sodium per mL. Proceed as directed in the *Assay* under *Indigotindisulfonate Sodium*, beginning with "Concomitantly determine the absorbances." Calculate the quantity, in mg, of $C_{16}H_8N_2Na_2O_8S_2$ in each mL of the Injection taken by the formula:

$$4(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Indigotindisulfonate Sodium RS in the Standard solution; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Indinavir Sulfate



$C_{36}H_{47}N_5O_4 \cdot H_2SO_4$ 711.87
D-*erythro*-Pentonamide, 2,3,5-trideoxy-*N*-(2,3-dihydro-2-hydroxy-1*H*-inden-1-yl)-5-[2-[[(1,1-dimethylethyl)amino]carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-(phenylmethyl)-, [1(1*S*,2*R*),5(*S*)]-, sulfate (1:1) (salt); (α*R*,γ*S*,2*S*)-α-Benzyl-2-(*tert*-butylcarbamoyl)-γ-hydroxy-*N*-[(1*S*,2*R*)-2-hydroxy-1-indanyl]-4-(3-pyridylmethyl)-1-piperazinevaleramide sulfate (1:1) (salt) [157810-81-6].

DEFINITION
Indinavir Sulfate contains NLT 98.5% and NMT 101.5% of $C_{36}H_{47}N_5O_4 \cdot H_2SO_4$, calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)**:** Maxima at about 3.0–3.1, 5.9, 6.2, and 13.6 µm
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **C. IDENTIFICATION TESTS—GENERAL,** *Sulfate* ⟨191⟩**:** Meets the requirements
Sample solution: A solution of 10 mg/mL in water

ASSAY
• **PROCEDURE**
Solution A: Dibutyl ammonium phosphate and water (1:50). Adjust with sodium hydroxide TS to a pH of 6.5 ± 0.5.
Mobile phase: Acetonitrile and *Solution A* (9:11)
Standard solution: 0.5 mg/mL of USP Indinavir RS in *Mobile phase*
Sample solution: 0.6 mg/mL of Indinavir Sulfate in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 40°
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 4000 theoretical plates
Tailing factor: Less than 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{36}H_{47}N_5O_4 \cdot H_2SO_4$ in the portion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

www.webofpharma.com

r_S = peak response from the *Standard solution*
C_S = concentration of USP Indinavir RS in the *Standard solution* (mg/mL)
C_U = concentration of Indinavir Sulfate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of indinavir sulfate, 711.87
M_{r2} = molecular weight of indinavir, 613.79

Acceptance criteria: 98.5%–101.5% on the anhydrous, solvent-free basis

OTHER COMPONENTS

• **PROCEDURE 1: CONTENT OF SULFATE**

Solution A: Methanol and formaldehyde (1000: 0.3)
Diluent: *Solution A* and water (1:1)
Sample solution: 6.25 mg/mL of Indinavir Sulfate in *Diluent*
Analysis: Titrate with 0.1 M lead perchlorate VS, using a lead-specific electrode in conjunction with a suitable reference electrode. Each mL of 0.1 M lead perchlorate VS is equivalent to 9.604 mg of sulfate.
Acceptance criteria: 13.2%–14.4%, calculated on the anhydrous and solvent-free basis

• **PROCEDURE 2: CONTENT OF ALCOHOL**

Standard solution: 0.001 mL/mL of dehydrated alcohol, in water. [NOTE—Dehydrated alcohol is at 20°.]
Sample solution: 4 mg/mL of Indinavir Sulfate in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.53-mm × 30-m capillary column with a 1.0-µm film of phase G16
Temperature
Column: 35°
Injector: 140°
Detector: 220°
[NOTE—At the end of each 5-min isothermal run, increase the oven temperature to 200° before adjusting the column temperature to 35° for the next injection.]
Flow rate: 10 mL/min
Carrier gas: Helium
Injection size: 0.1 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of alcohol in the portion of Indinavir Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times D \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of dehydrated alcohol in the *Standard solution* (mL/mL)
C_U = concentration of Indinavir Sulfate in the *Sample solution* (mg/mL)
D = density of alcohol at 20°, 790 mg/mL

Acceptance criteria: 5.0%–8.0%

IMPURITIES

INORGANIC IMPURITIES

• **Residue on Ignition** ⟨281⟩: NMT 0.1%

ORGANIC IMPURITIES

• **Procedure**

Solution A: 0.27 g/L of monobasic potassium phosphate and 1.395 g/L of dibasic potassium phosphate, in water
Solution B: Acetronitrile
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	80	20
40	30	70
45	30	70
47	80	20
52	80	20

Diluent: *Solution A* and *Solution B* (1:1)
System suitability solution: 0.4 mg/mL of USP Indinavir System Suitability RS in *Diluent*
Sample solution: 0.5 mg/mL of Indinavir Sulfate in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.8 between indinavir and indinavir related compound C
Tailing factor: More than 0.95 and less than 2.0, determined from the indinavir peak
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Indinavir Sulfate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area response for each impurity
r_T = sum of the responses of all the peaks

Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
cis-Aminoindanol[a]	0.18	0.1
Desnicotinyl indinavir[b]	0.80	0.1
threo-Indinavir[c]	0.98	0.1
Indinavir lactone[d]	1.14	0.1

USP Monographs

www.webofpharma.com

Impurity Table 1 *(continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Diindanyl indinavir[e]	1.30	0.1

[a] (1*S*,2*R*)-1-Aminoindan-2-ol.
[b] (*S*)-1-{(2*S*,4*R*)-4-Benzyl-2-hydroxy-5-[(1*S*,2*R*)-2-hydroxyindan-1-ylamino]-5-oxopentyl}-*N*-*tert*-butylpiperazine-2-carboxamide.
[c] (*S*)-1-{(2*R*,4*R*)-4-Benzyl-2-hydroxy-5-[(1*S*,2*R*)-2-hydroxyindan-1-ylamino]-5-oxopentyl}-*N*-*tert*-butyl-4-(pyridin-3-ylmethyl)piperazine-2-carboxamide.
[d] (*S*)-1-{[(2*S*,4*R*)-4-Benzyl-5-oxotetrahydrofuran-2-yl]methyl}-*N*-*tert*-butyl-4-(pyridin-3-ylmethyl)piperazine-2-carboxamide.
[e] (2*R*,2′*R*,4*S*,4′*S*)-5,5′-[(*S*)-2-(*tert*-Butylcarbamoyl)piperazine-1,4-diyl]bis{2-benzyl-4-hydroxy-*N*-[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]pentanamide}.

SPECIFIC TESTS
- **OPTICAL ROTATION,** *Specific Rotation* ⟨781S⟩: +122° to +129°, at 365 nm, determined on the anhydrous, solvent-free basis
  Sample solution: 10 mg/mL in water
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 1.5%, using 0.25 g

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from moisture. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Indinavir RS
  USP Indinavir System Suitability RS

Indium In 111 Capromab Pendetide Injection

» Indium In 111 Capromab Pendetide Injection is a sterile, nonpyrogenic, murine monoclonal antibody, 7E11-C 5.3, (CYT-351), an immunoconjugate prepared by specific modification of the carbohydrate groups and covalent binding to the tripeptide linker chelator, glycyltyrosyl-(N, *E*-diethylenetriaminepentaacetic acid)-lysine hydrochloride that is complexed with ^{111}In. It contains not less than 90.0 percent and not more than 110.0 percent of the specified amount of ^{111}In capromab pendetide, expressed in megabexquerels (or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity. Immediately prior to use, the radiolabeling is performed with Indium In 111 Chloride Solution in the presence of a sodium acetate buffer. It contains sodium chloride and buffering agents as stabilizers. The immuno-reactive fraction, determined by a validated method, is not less than 70 percent. The monomer content is not less than 95 percent determined by a validated electrophoretic mobility method.

Packaging and storage—Preserve in adequately shielded single-dose containers at controlled room temperature for not more than 8 hours.
Labeling—Label it to include the following in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{111}In capromab pendetide as total MBq (or mCi) and concentration of MBq (or mCi) per mL, at the time of calibration; the expiration date and time; and the storage temperature and the statement, "Caution—Radioactive Material." The labeling indicates that, in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{111}In is 67.2 hours.
Bacterial Endotoxins Test ⟨85⟩—The limit of endotoxin content is not more than 175/*V* USP Endotoxin Units per mL of the Injection, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.
pH ⟨791⟩: between 5.0 and 7.0.

Radiochemical purity—
Absorbent: 1-cm × 8-cm instant silica gel strip.
Test solution: a mixture of the Injection and 0.05 M pentetic acid (1:1).
Application volume: 10 µL.
Developing solvent system: 0.9% sodium chloride solution.
Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩ by ascending chromatography. Determine the distribution of radioactivity on the chromatogram by scanning with a suitable collimated radiochromatogram strip scanner, and determine the percentage of radiochemical purity of the test specimen. Not less than 90% of the In 111 activity is present as a band between the R_F values of 0 and 0.1.
Other requirements—It meets the requirements for *Radionuclide identification* and *Radionuclidic purity (see Radioactivity 821)*— under *Indium In 111 Chloride Solution*. It meets also the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the radioactive component may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the date of manufacture.
Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the total radioactivity, in MBq (or µCi), of the unshielded Injection by use of a calibrated system.

Indium In 111 Chloride Solution

Indium Chloride ($^{111}InCl_3$).
Indium (^{111}In) trichloride [10025-82-8].

» Indium In 111 Chloride Solution is a sterile, nonpyrogenic solution of radioactive indium (^{111}In) in dilute hydrochloric acid suitable for the radiolabeling of proteins such as monoclonal antibodies, peptides, or small biologically active organic molecules. The concentration of acid and ^{111}In per mL of Indium In 111 Chloride Solution may require adjustment for the specific antibody or peptide being labeled. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{111}In expressed as

www.webofpharma.com

megabecquerels (or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity. [NOTE—Indium In 111 Chloride Solution is generally recommended for use with specific antibodies or peptides. Consult the product labeling for recommendations and applications for radiolabeling.]

Specific activity: not less than 1.85 gigabecquerels (50 millicuries) per µg of indium at the date and time of calibration.

Packaging and storage—Preserve in single-unit containers at controlled room temperature.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{111}In as labeled chloride expressed as total megabecquerels (or millicuries) and the concentration as megabecquerels per mL (or as millicuries per mL) on the date and time of calibration; the expiration date; the statement, "Not for direct administration. Use only as an ingredient for radiolabeling;" and the statement, "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{111}In is 67.3 hours.

Identification—Add 1 drop of it to 2 drops of 0.1 M silver nitrate in a glass test tube: a white precipitate is formed (presence of chloride).

Bacterial Endotoxins Test ⟨85⟩—It contains not more than $175/V$ USP Endotoxin Unit per mL, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

Acidity—Pipet 20 µL of Solution into a plastic tube containing 1 drop of bromocresol green, and titrate with 0.0025 N sodium carbonate to a blue endpoint. Calculate the acidity of the Solution by the formula:

$$0.0025V_T/20$$

in which V_T is the volume of titrant consumed: the molarity of the Solution is between 0.035 and 0.045.

Radionuclide identification ⟨821⟩—Its gamma-ray spectrum is identical to that of a specimen of ^{111}In that exhibits major photopeaks having energies of 0.171 and 0.245 MeV.

Radionuclidic purity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly , determine the radioactivity of each radionuclidic impurity, in kBq per MBq (µCi per mCi) of ^{111}In, in the Solution by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

INDIUM 110M—The limit of ^{110m}In is 3 kBq per Mbq (or 3 µCi per mCi) of ^{111}In. The presence of ^{110m}In in the Solution is demonstrated by a characteristic gamma-ray spectrum with prominent photopeaks having energies of 0.66 and 0.91 MeV. ^{110m}In decays with a half-life of 4.9 hours.

INDIUM 114M—The limit of ^{114m}In is 3 kBq per MBq (or 3 µCi per mCi) of ^{111}In. ^{114m}In is quantified by counting the beta emissions of ground state ^{114}In with a beta-liquid scintillation counter having a high-energy channel set to discriminate against all counts arising from ^{111}In.

Z INC 65—The limit of ^{65}Zn is 3 kBq per MBq (or 3 µCi per mCi) of ^{111}In. The presence of ^{65}Zn in the Solution is demonstrated by a characteristic gamma-ray spectrum with a prominent photopeak at 1.116 MeV. ^{65}Zn decays with a radioactive half-life of 243.9 days.

Radiochemical purity—

Adsorbent: instant thin-layer chromatography (ITLC) strips (2.5 cm × 10 cm).[1]

Test solution—Dispense about 50 µL of Solution into 1 mL of 0.05 M hydrochloric acid, taking care to use polypropylene tips prewashed in 0.05 M hydrochloric acid for all dispensings.

Application volume: 2 µL. The amount of ^{111}In spotted should be between 0.5 µCi and 30 µCi as of the day of the test.

Developing solvent system: a mixture of a 1 in 10 solution of ammonium acetate and methanol (1:1).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. Examine the plate with an appropriate scanner, and determine the percentage of radiochemical purity of the *Test solution*. The indium chloride will remain at the origin. Not less than 95% of indium is present as ionic indium.

Chemical purity—

Copper—Determine the copper, in µg per mL, in the Solution by atomic absorption spectrometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), using a graphite furnace to volatilize the copper, as directed by the manufacturer of the instrument used, and measuring the absorbance at 324.8 nm against a standard.

Nickel—Determine the nickel, in µg per mL, in the Solution by atomic absorption spectrometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), using a graphite furnace to volatilize the nickel, as directed by the manufacturer of the instrument used, and measuring the absorbance at 232.0 nm against a standard.

Cadmium—Determine the cadmium, in µg per mL, in the Solution by atomic absorption spectrometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), using a graphite furnace to volatilize the cadmium, as directed by the manufacturer of the instrument used, and measuring the absorbance at 228.8 nm against a standard.

Lead—Determine the lead, in µg per mL, in the Solution by atomic absorption spectrometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), using a graphite furnace to volatilize the lead, as directed by the manufacturer of the instrument used, and measuring the absorbance at 217.0 nm against a standard.

Mercury—Determine the mercury, in µg per mL, in the Solution by atomic absorption spectrometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), using a graphite furnace to volatilize the mercury, as directed by the manufacturer of the instrument used, and measuring the absorbance at 253.7 nm against a standard.

Iron—Determine the iron, in µg per mL, in the Solution by atomic absorption spectrometry (see *Atomic Absorption Spectroscopy* ⟨852⟩), using a graphite furnace to volatilize the iron, as directed by the manufacturer of the instrument used, and measuring the absorbance at 248.3 nm against a standard.

Zinc—Prepare a zinc stock solution in dilute hydrochloric acid (1 in 100) having a concentration of 1 µg of zinc per mL. Pipet 10 mL of the zinc stock solution into a 100-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a concentration of 0.1 µg of zinc per mL (*Standard solution A*). Pipet 20 mL of the zinc stock solution into a 100-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a concentration of 0.2 µg of zinc per mL (*Standard solution B*). Pipet 0.1 mL of Indium Chloride In 111 Solution into a 10-mL volumetric flask, dilute with water to volume, and mix to obtain the test solution. Determine the absorbances of the *Standard solutions* and the test solution at

[1] Type SG impregnated glass microfiber sheet (Gelman Sciences, Ann Arbor, MI).

USP Monographs

www.webofpharma.com

the zinc emission line at 213.9 nm with an atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* ⟨852⟩) equipped with a zinc hollow-cathode lamp and an air–acetylene flame, using water as the blank. Determine the quantity of zinc, in µg per mL, in the Solution.

The composite total metal ion content is not greater than 1.0 µg per mL.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the Solution may be distributed or dispensed prior to completion of the test for *Sterility Tests* ⟨71⟩, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container content*.

Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly , determine the radioactivity, in MBq (or in microcuries or millicuries) per mL, of the Solution, by the use of a calibrated system.

Indium In 111 Oxyquinoline Solution

» Indium In 111 Oxyquinoline Solution is a sterile, nonpyrogenic, isotonic aqueous solution suitable for the radiolabeling of blood cells, especially leukocytes and platelets, containing radioactive indium (^{111}In) in the form of a complex with 8-hydroxyquinoline, the latter being present in excess. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{111}In as the 8-hydroxyquinoline complex expressed as megabecquerels (millicuries) per mL at the time indicated in the labeling. It may contain sodium chloride, surfactants, and buffers. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

Specific activity: not less than 1.85 GBq (50 millicuries) per µg of indium.

Packaging and storage—Preserve in single-unit containers at a temperature between 15° and 25°.

Labeling—Label it to contain the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{111}In as the 8-hydroxyquinoline complex expressed as total megabecquerels (millicuries) and concentration as megabecquerels (millicuries) per mL on the date and time of calibration; the expiration date; the statement "Not for direct administration. Use only for radiolabeling of leukocytes in vitro. Administer radiolabeled cells subsequently by intravenous injection," and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{111}In is 67.9 hours.

Pyrogen—It meets the requirements of the *Pyrogen Test* ⟨151⟩.

pH ⟨791⟩: between 6.5 and 7.5.

Radionuclide identification (see *Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of ^{111}In that exhibits major photopeaks having energies of 0.171 and 0.245 MeV.

Radiochemical purity—Place a suitable volume, about 100 µL, of Solution, dilute with 3 mL of 0.9 percent sodium chloride solution in a separator, and extract with 6 mL of *n*-octanol by vigorous shaking. Allow the phases to separate and then drain the lower, aqueous layer into a suitable stoppered counting tube. Drain the residual, organic layer into a similar counting tube. Rinse the separator with 1 mL of *n*-octanol, and drain this rinse into the counting tube containing the organic layer. Rinse the separator with 5 mL of 2 N hydrochloric acid, and drain this rinse into a third counting tube. Insert the stopper and measure the radioactivity in each of the three tubes in a suitable gamma counter or ionization chamber calibrated for ^{111}In. The radiochemical purity is calculated by the formula:

$$(A/B)$$

where A is the radioactivity measured in the organic layer and B is the sum of the radioactivity measured in the organic, aqueous, and acid solutions. The radioactivity of the 8-hydroxyquinoline complex is not less than 90.0% of the total radioactivity and is found in the organic layer.

Radionuclidic purity—Using a suitable counting assembly, determine the radioactivity of each radionuclidic impurity, in kBq per MBq (µCi per mCi) of ^{111}In, in the Solution by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

INDIUM 114m—The limit of ^{114m}In is 3 kBq per MBq (3 µCi per mCi) of ^{111}In. ^{114m}In is quantified by counting the beta emissions of ground state ^{114}In using a beta-liquid scintillation counter with a high-energy channel set to discriminate against all counts arising from ^{111}In.

ZINC 65—The limit of ^{65}Zn is 3 kBq per MBq (3 µCi per mCi) of ^{111}In. The presence of ^{65}Zn in the Solution is demonstrated by a characteristic gamma-ray spectrum with a prominent photopeak at 1.116 MeV. ^{65}Zn decays with a radioactive half-life of 243.9 days.

Assay for radioactivity—Using a suitable counting assembly, determine the radioactivity, in MBq (mCi) per mL, of Solution by the use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

Indium In 111 Pentetate Injection

» Indium In 111 Pentetate Injection is a sterile, isotonic solution suitable for intrathecal administration, containing radioactive indium (^{111}In) in the form of a chelate of pentetic acid. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{111}In as the pentetic acid complex expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. It may contain sodium chloride and buffers. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose containers.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{111}In as labeled pentetic acid complex expressed as total megabecquerels (millicuries or microcuries) and concentration as megabecquerels (microcuries or millicuries) per mL on the date and time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The

USP Monographs

www.webofpharma.com

labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{111}In is 2.83 days.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 14/*V* USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.
pH ⟨791⟩: between 7.0 and 8.0.
Radionuclide identification (see *Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of ^{111}In that exhibits major photopeaks having energies of 0.173 and 0.247 MeV.
Radiochemical purity—Place 2 µL to 5 µL of Injection about 17 mm from one end of a 65- × 97-mm piece of silica gel-impregnated glass microfiber sheet (see under *Reagents* in the section *Reagents, Indicators, and Solutions*) (see also *Chromatography* ⟨621⟩), and allow to dry. Repeat applications may be made to obtain a suitable count rate. Develop the chromatogram over a suitable period of time by ascending chromatography, using dilute methanol (8.5 in 10), and dry in an oven at 105 ± 5° for 5 minutes. Determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. The radioactivity of the indium pentetic acid complex band is not less than 90.0% of the total radioactivity, and the R_f value is between 0.8 and 1.0.
Radionuclidic purity—Using a suitable counting assembly, determine the radioactivity of each radionuclidic impurity, in kBq per MBq (µCi per mCi) of ^{111}In, in the Injection by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.
I *NDIUM* 114m—The presence of ^{114m}In in the Injection is demonstrated by a characteristic gamma-ray spectrum with prominent photopeaks having energies of 0.192, 0.558, and 0.724 MeV. ^{114m}In decays with a radioactive half-life of 49.5 days. The amount of ^{114m}In is not greater than 3 kBq per MBq (3 µCi per mCi) of ^{111}In.
Z *INC* 65—The presence of ^{65}Zn in the Injection is demonstrated by a characteristic gamma-ray spectrum with a prominent photopeak at 1.115 MeV. ^{65}Zn decays with a radioactive half-life of 243.9 days. The amount of ^{65}Zn is not greater than 3 kBq per MBq (3 µCi per mCi) of ^{111}In.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container content*.
Assay for radioactivity—Using a suitable counting assembly, determine the radioactivity, in MBq per mL, of Injection by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

Indium In 111 Pentetreotide Injection

» Indium In 111 Pentetreotide Injection is a sterile solution, suitable for intravenous administration, containing radioactive indium (^{111}In) in the form of a chelate of pentetreotide. It contains not less than 90.0 percent and not more than 110 percent of the labeled amount of ^{111}In as the pentetreotide complex expressed in megabecquerels (or in millicuries) per mL at the time indicated in the labeling. It may contain sodium chloride, stabilizers, and buffers. Other forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose containers.
Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{111}In as labeled pentetreotide complex expressed as total megabecquerels (or millicuries) and the concentration expressed as megabecquerels (or millicuries) per mL on the date and time of calibration; the expiration date; and the statement, "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and states that the radioactive half-life of ^{111}In is 67.3 hours.
Radionuclide identification (see *Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of ^{111}In that exhibits major photopeaks having energies of 0.171 and 0.245 MeV.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 175/*V* USP Endotoxin Unit per mL, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.
pH ⟨791⟩: between 3.8 and 4.3.

Radiochemical purity—
Solution A—Dissolve 6.8 g of sodium acetate in 500 mL of water. Adjust with glacial acetic acid to a pH of 5.5, dilute with water to 1000 mL, and mix. Filter through a filter having a porosity of 0.5 µm or less, and degas.
Solution B—Use methanol.
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed under *Chromatographic system.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 3.9-mm × 30-cm stainless steel column that contains 10-µm packing L1. It is also equipped with a flow-through gamma-ray detector having a cell volume of about 50 µL and calibrated to provide a linear response within the range of 0.5 to 15 MBq (14 to 400 µCi). The column temperature is maintained at 35°. The chromatograph is programmed to provide variable mixtures of *Solution A* and *Solution B*, and the initial flow rate is about 1 mL per minute. The column is equilibrated for at least 15 minutes with a mobile phase consisting of 60% *Solution A* and 40% *Solution B*. After injection, the composition of the mobile phase is changed linearly to 20% *Solution A* and 80% *Solution B* at 20 minutes, then changed to 100% *Solution B* over the next 0.1 minute, and is held at that percentage while the flow rate is increased linearly from 1 to 2 mL over the next 5 minutes, which is the end of the run. Counts are recorded and charted for 25 minutes at about 2-second intervals.
Procedure—Constitute the Injection, and allow to stand for 30 minutes. Inject a volume of Injection having an activity of 0.5 to 15 MBq (14 to 400 µCi) into the chromatograph, and record the chromatogram. The retention time of the ^{111}In pentetreotide peak (which should elute as a double peak) is between 4 and 5 relative to that of unbound ^{111}In. Record the counts for the ^{111}In pentetreotide, unbound ^{111}In, other impurity peaks, and a representative baseline segment, and calculate the percentage of radioactivity from ^{111}In pentetreotide by the formula:

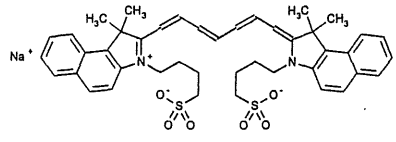
$$100P/(P + O)$$

in which *P* is the count of the ^{111}In pentetreotide peak, and *O* is the count for all other peaks, each being corrected for its

www.webofpharma.com

corresponding baseline count. The radioactivity of ^{111}In pentetreotide is not less than 90% of the total radioactivity.
Radionuclidic purity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radioactivity of each radionuclidic impurity, in kBq per MBq (or µCi per mCi) of ^{111}In, in the Injection by use of a calibrated system.
I *NDIUM* 114m—The presence of ^{114m}In in the Injection is demonstrated by a characteristic gamma-ray spectrum with prominent photopeaks having energies of 0.192, 0.558, and 0.724 MeV. ^{114m}In decays with a radioactive half-life of 49.5 days. The amount of ^{114m}In is not greater than 3 kBq per MBq (3 µCi per mCi) of ^{111}In.
Z *INC* 65—The presence of ^{65}Zn in the Injection is demonstrated by a characteristic gamma-ray spectrum with a prominent photopeak at 1.115 MeV. ^{65}Zn decays with a radioactive half-life of 243.9 days. The amount of ^{65}Zn is not greater than 3 kBq per MBq (3 µCi per mCi) of ^{111}In.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility Tests* ⟨71⟩, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.
Assay for radioactivity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly , determine the radioactivity, in MBq per mL of Injection, by use of a calibrated system.

Indocyanine Green



$C_{43}H_{47}N_2NaO_6S_2$ 774.96
1*H*-Benz[*e*]indolium, 2-[7-[1,3-dihydro-1,1-dimethyl-3-(4-sulfobutyl)-2*H*-benz[*e*]indol-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-(4-sulfobutyl)-, hydroxide, inner salt, sodium salt.
2-[7-[1,1-Dimethyl-3-(4-sulfobutyl)benz[*e*]indolin-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-(4-sulfobutyl)-1*H*benz[*e*] indolium hydroxide, inner salt, sodium salt [3599-32-4].

» Indocyanine Green contains not less than 89.0 percent and not more than 100.0 percent of $C_{43}H_{47}N_2NaO_6S_2$, calculated on the dried basis. It contains not more than 5.0 percent of sodium iodide, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
USP Reference standards ⟨11⟩—
USP Indocyanine Green RS

Identification—
A: Incinerate a portion of it: the residue responds to the tests for *Sodium* ⟨191⟩ and for *Sulfate* ⟨191⟩.
B: To a solution (1 in 20,000) add 10 drops of 1 N sodium hydroxide, and heat to about 60°. Add 10 drops of hydrogen peroxide TS, and mix: a red color develops within about 4 minutes and, on standing, fades to a pale orange.
Loss on drying ⟨731⟩—Dry it in vacuum at 50° for 3 hours: it loses not more than 6.0% of its weight.
Arsenic, *Method II* ⟨211⟩: 8 ppm.
Lead—A 5-mL portion of the solution prepared for the test for *Arsenic* ⟨211⟩ contains not more than 2 µg of lead (corresponding to not more than 0.001%) when tested by the limit test for *Lead* ⟨251⟩, 3 mL of *Ammonium Citrate Solution,* 1 mL of *Potassium Cyanide Solution,* and 0.5 mL of *Hydroxylamine Hydrochloride Solution* being used.
Sodium iodide—Dissolve about 200 mg, accurately weighed, in 100 mL of water, add 1 mL of nitric acid, mix, and titrate with 0.01 N silver nitrate VS, determining the endpoint potentiometrically, using silver and glass electrodes. Each mL of 0.01 N silver nitrate is equivalent to 1.499 mg of sodium iodide. Not more than 5.0% of sodium iodide, calculated on the dried basis, is found.
Other requirements—Where the label states that Indocyanine Green is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ and for *Bacterial endotoxins* under *Indocyanine Green for Injection.* Where the label states that Indocyanine Green must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Indocyanine Green for Injection.*
Assay—Dissolve a quantity of Indocyanine Green, equivalent to about 100 mg of dried indocyanine green and accurately weighed, in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution containing about 2 µg per mL. Dissolve a quantity of USP Indocyanine Green RS, accurately weighed, in methanol, and dilute quantitatively and stepwise with methanol to obtain a Standard solution having a known concentration of about 2 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 785 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_{43}H_{47}N_2NaO_6S_2$ in the Indocyanine Green taken by the formula:

$$50C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Indocyanine Green RS in the Standard solution; and A_U and A_S are the absorbances of the solution of Indocyanine Green and the Standard solution, respectively.

Indocyanine Green for Injection

» Indocyanine Green for Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{43}H_{47}N_2NaO_6S_2$.

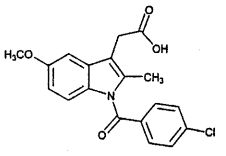
Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
USP Reference standards ⟨11⟩—
USP Indocyanine Green RS
Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

USP Monographs

www.webofpharma.com

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 7.1 USP Endotoxin Units per mg of indocyanine green.
pH ⟨791⟩: between 5.5 and 7.5, in a solution (1 in 200).
Content variation—Transfer the contents of each of 5 containers individually to separate 100-mL volumetric flasks with the aid of methanol. To each flask add methanol to volume. Dilute the solutions quantitatively and stepwise with methanol, to obtain a concentration of about 2.5 µg per mL. Proceed as directed in the *Assay*— under *Indocyanine Green*, beginning with "Concomitantly determine the absorbances." The requirements are met if the content of each of not less than 4 of the containers tested is within the limits specified under *Uniformity of Dosage Units* ⟨905⟩.
Other requirements—It responds to the *Identification* tests, and meets the requirements for *Arsenic*, *Lead*, *Sodium iodide*, and *Assay*— under *Indocyanine Green*. It meets also the requirements for *Sterility Tests* ⟨71⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Indomethacin



$C_{19}H_{16}ClNO_4$ 357.79
1*H*-Indole-3-acetic acid, 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-;
1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid [53-86-1].

DEFINITION
Indomethacin contains NLT 98.0% and NMT 102.0% of indomethacin ($C_{19}H_{16}ClNO_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197M▲ (CN 1-May-2020)
- **B.**
The retention time of the indomethacin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Solution A: 0.1% formic acid
Solution B: Acetonitrile
Mobile phase: *Solution A* and *Solution B* (55:45)
Diluent: *Solution A* and *Solution B* (55:45), adjusted with 0.2 M sodium hydroxide to a pH of 8.0
System suitability solution: 2.0 mg/mL of USP Indomethacin RS, 0.002 mg/mL of USP Indomethacin Related Compound A RS, and 0.01 mg/mL of USP Indomethacin Related Compound B RS, prepared as follows. Transfer a quantity of USP Indomethacin RS to a suitable volumetric flask. Add 50% of the final volume of acetonitrile and sonicate to dissolve. Add suitable amounts of USP Indomethacin Related Compound A RS and USP Indomethacin Related Compound B RS to the resulting solution, sonicate to dissolve if necessary, and dilute with *Diluent* to volume.
Standard solution: 0.5 mg/mL of USP Indomethacin RS in *Diluent*. Sonicate to dissolve.
Sample solution: 0.5 mg/mL of Indomethacin in *Diluent*. Sonicate to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 4°
Column: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for indomethacin related compound A and indomethacin related compound B are 0.24 and 0.37, respectively.]
Suitability requirements
Resolution: NLT 2.0 between indomethacin related compound B and indomethacin related compound A, *System suitability solution*
Tailing factor: NMT 2.0 for indomethacin, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of indomethacin ($C_{19}H_{16}ClNO_4$) in the portion of Indomethacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of indomethacin from the *Sample solution*
r_S = peak response of indomethacin from the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (mg/mL)
C_U = concentration of Indomethacin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.2%
- **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.002 mg/mL of USP Indomethacin RS, 0.002 mg/mL of USP Indomethacin Related Compound A RS, and 0.01 mg/mL of USP Indomethacin Related Compound B RS in *Diluent*. Sonicate to dissolve.
Sample solution: 2.0 mg/mL of Indomethacin, prepared as follows. Transfer a quantity of Indomethacin to a suitable volumetric flask. Add 50% of the final volume of acetonitrile and sonicate to dissolve. Dilute with *Diluent* to volume.
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between indomethacin related compound A and indomethacin related compound B
Tailing factor: NMT 2.0 for indomethacin related compound A, indomethacin related compound B, and indomethacin
Relative standard deviation: NMT 5.0% for indomethacin related compound A, indomethacin related compound B, and indomethacin

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of indomethacin related compound A and indomethacin related compound B in the portion of Indomethacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of indomethacin related compound A or indomethacin related compound B from the *Sample solution*
r_S = peak response of indomethacin related compound A or indomethacin related compound B from the *Standard solution*
C_S = concentration of USP Indomethacin Related Compound A RS or USP Indomethacin Related Compound B RS in the *Standard solution* (mg/mL)
C_U = concentration of Indomethacin in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Indomethacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*
r_S = peak response of indomethacin from the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (mg/mL)
C_U = concentration of Indomethacin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Indomethacin related compound A	0.24	0.15
Indomethacin related compound B	0.37	0.5
Indomethacin	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

SPECIFIC TESTS
• **Loss on Drying** ⟨731⟩
Analysis: Dry at a pressure below 5 mm of mercury at 100° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in well-closed, light-resistant containers.
• **USP Reference Standards** ⟨11⟩
USP Indomethacin RS
USP Indomethacin Related Compound A RS
(5-Methoxy-2-methylindol-3-yl)acetic acid.
$C_{12}H_{13}NO_3$ 219.24
USP Indomethacin Related Compound B RS
4-Chlorobenzoic acid.
$C_7H_5ClO_2$ 156.57

Indomethacin Capsules

DEFINITION
Indomethacin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$).

IDENTIFICATION
• **A.**
Standard: A solution of 25 mg of USP Indomethacin RS in 5 mL of acetone recrystallized and prepared similarly as for the *Sample*.
Sample: Shake a portion of the contents of Capsules, nominally equivalent to about 50 mg of indomethacin, with 10 mL of acetone for about 2 min, and filter. Transfer 5 mL of the filtrate to a stoppered flask, add 20 mL of water, and shake for about 2 min until a precipitate forms and crystallizes. Filter, and collect the crystals. Dry the crystals in air, then dry at a pressure below 5 mm of mercury at 100° for 2 h.
Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the *Sample* exhibits maxima only at the same wavelengths as those of the *Standard*.
• **B.** The retention time of the indomethacin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**
Solution A: Phosphoric acid and water (2:1000)
Mobile phase: Acetonitrile and *Solution A* (45:55)
Diluent: *Mobile phase* adjusted with 0.2 N sodium hydroxide to a pH of 6.0
Standard stock solution: 1 mg/mL of USP Indomethacin RS prepared as follows. Transfer a suitable quantity of USP Indomethacin RS to an adequate volumetric flask, add 50% of the final volume of acetonitrile, and dilute with *Diluent* to final volume.
Standard solution: 0.04 mg/mL of USP Indomethacin RS in *Diluent* from *Standard stock solution*
Sample stock solution: Nominally 1 mg/mL of indomethacin prepared as follows. Transfer a suitable portion of the contents from NLT 20 Capsules to an adequate volumetric flask, add 50% of the final volume of acetonitrile, and sonicate for about 10 min with intermittent shaking. Add about 25% of the final volume of *Diluent* and sonicate for about 10 min with intermittent shaking. Dilute with *Diluent* to final volume and mix. Allow the resulting mixture to stand until a clear supernatant is obtained. Use the clear supernatant.
Sample solution: Nominally 0.04 mg/mL of indomethacin in *Diluent* from *Sample stock solution*. Pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 25 µL
Run time: NLT 2 times the retention time of indomethacin
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*

USP Monographs

www.webofpharma.com

Calculate the percentage of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of indomethacin from the *Sample solution*
r_S = peak response of indomethacin from the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of indomethacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: pH 7.2 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*) and water (1:4); 750 mL
Apparatus 1: 100 rpm
Time: 20 min
Standard solution: USP Indomethacin RS at a known concentration in *Medium*
Sample solution: Filter a portion of the solution under test and suitably dilute with *Medium*, if necessary.
Instrumental conditions
Mode: UV
Analytical wavelength: Maximum absorbance at about 318 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 80% (*Q*) of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Mobile phase, Diluent, Standard stock solution, Sample stock solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability stock solution: 0.2 mg/mL each of USP Indomethacin Related Compound A RS and USP Indomethacin Related Compound B RS prepared as follows. Transfer suitable quantities of USP Indomethacin Related Compound A RS and USP Indomethacin Related Compound B RS to an adequate volumetric flask, add 40% of the final volume of acetonitrile, and sonicate to dissolve. Dilute with *Diluent* to final volume.
System suitability solution: 0.002 mg/mL each of USP Indomethacin Related Compound A RS and USP Indomethacin Related Compound B RS, and 0.4 mg/mL of USP Indomethacin RS in *Diluent* from *System suitability stock solution* and *Standard stock solution*
Sensitivity solution: 0.2 µg/mL of USP Indomethacin RS in *Diluent* from *Standard stock solution*
Standard solution: 0.002 mg/mL of USP Indomethacin RS in *Diluent* from *Standard stock solution*
Sample solution: Nominally 0.4 mg/mL of indomethacin in *Diluent* from *Sample stock solution*
System suitability
Samples: *System suitability solution, Sensitivity solution,* and *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between indomethacin related compound A and indomethacin related compound B, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of indomethacin related compound A, indomethacin related compound B, and any unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of indomethacin related compound A, or indomethacin related compound B, or any unspecified degradation product from the *Sample solution*
r_S = peak response of indomethacin from the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of indomethacin in the *Sample solution* (mg/mL)
F = relative response factor of each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Indomethacin related compound A	0.3	0.8	0.20
Indomethacin related compound B	0.4	2.1	0.20
Indomethacin	1.0	1.0	—
Any unspecified degradation product	—	1.0	0.20
Total degradation products	—	—	1.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Indomethacin RS
USP Indomethacin Related Compound A RS
2-(5-Methoxy-2-methyl-1*H*-indol-3-yl)acetic acid.
$C_{12}H_{13}NO_3$ 219.24
USP Indomethacin Related Compound B RS
4-Chlorobenzoic acid.
$C_7H_5ClO_2$ 156.57

Indomethacin Extended-Release Capsules

DEFINITION

Indomethacin Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$).

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: ⟨197K⟩▲ (USP 1-May-2020)

Standard:▲ (USP 1-May-2020) Prepare a solution of 5 mg/mL of USP Indomethacin RS in acetone. ▲Transfer 5 mL of this solution to a stoppered flask, add 20 mL of water, and shake for 2 min until a precipitate forms and crystallizes. Filter and collect the crystals. Dry the crystals in air, then dry at a pressure below 5 mm of mercury at 100° for 2 h.▲ (USP 1-May-2020)

Sample:▲ (USP 1-May-2020) Shake a portion of Capsule contents, nominally equivalent to 50 mg of indomethacin, with 10 mL of acetone for about 2 min, and filter. ▲Transfer 5 mL of the filtrate to a stoppered flask, add 20 mL of water, and shake for 2 min until a precipitate forms and crystallizes. Filter and collect the crystals. Dry the crystals in air, then dry at a pressure below 5 mm of mercury at 100° for 2 h.▲ (USP 1-May-2020)

Acceptance criteria: ▲The IR absorption spectrum of the *Sample* exhibits maxima only at the same wavelengths as that of the *Standard*.▲ (USP 1-May-2020)

Change to read:

• **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

Delete the following:

▲• **C.**

Sample solution: Equivalent to 1 mg/mL of indomethacin in sodium hydroxide solution (0.4 mg/mL) from powdered Capsule contents

Analysis: Shake the *Sample solution* for 5 min, and filter. To 1 mL of the clear filtrate add 1 mL of 1 mg/mL sodium nitrite solution, mix, and allow to stand for 5 min. Add 0.5 mL of sulfuric acid.

Acceptance criteria: A golden yellow color develops.▲ (USP 1-May-2020)

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: Methanol, water, and phosphoric acid (600: 400: 0.8)

Diluent: Phosphoric acid and water (1:99)

▲Standard solution:▲ (USP 1-May-2020) 0.8 mg/mL of USP Indomethacin RS, prepared as follows. Transfer a suitable quantity of USP Indomethacin RS to a suitable volumetric flask, dissolve with 60% of the flask volume of acetonitrile, and dilute with *Diluent* to volume.

▲▲ (USP 1-May-2020)

Sample solution: ▲Nominally 0.75 mg/mL of indomethacin prepared as follows.▲ (USP 1-May-2020) Weigh and finely powder the contents of NLT 20 Capsules. Transfer a portion of the powder, nominally equivalent to 75 mg of indomethacin, to a 100-mL volumetric flask, add 40 mL of *Diluent*, and shake for 1 h. Sonicate for 15 min, add 40 mL of acetonitrile, sonicate for 15 min, and dilute with acetonitrile to volume. Centrifuge a portion of this solution, and use the ▲supernatant.▲ (USP 1-May-2020)

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm
Column: 3.9-mm × 30-cm; ▲10-µm▲ (USP 1-May-2020) packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*▲▲ (USP 1-May-2020)
Suitability requirements
▲▲ (USP 1-May-2020)
Tailing factor: NMT 2.0▲▲ (USP 1-May-2020)
Relative standard deviation: NMT 2.0%▲▲ (USP 1-May-2020)
Analysis
Samples: ▲*Standard solution*▲ (USP 1-May-2020) and *Sample solution*
Calculate the percentage of ▲the labeled amount of▲ (USP 1-May-2020) indomethacin ($C_{19}H_{16}ClNO_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response ▲of indomethacin▲ (USP 1-May-2020) from the *Sample solution*
r_S = peak response ▲of indomethacin from the *Standard solution*▲ (USP 1-May-2020)
C_S = concentration of USP Indomethacin RS in ▲the *Standard solution*▲ (USP 1-May-2020) (mg/mL)
C_U = nominal concentration of indomethacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
▲▲ (USP 1-May-2020)
Medium: pH 6.2 phosphate buffer (see *Reagents and Reference Tables, Solutions, Buffer Solutions*); 750 mL
Apparatus 1: 75 rpm
Times: 1, 2, 4, 6, 12, and 24 h
Standard solution: USP Indomethacin RS at a known concentration in *Medium*
Sample solution: ▲Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.▲ (USP 1-May-2020)
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 318 nm
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved
1	10%–25%
2	20%–40%
4	35%–55%
6	45%–65%
12	60%–80%

www.webofpharma.com

Table 1 *(continued)*

Time (h)	Amount Dissolved
24	NLT 80%

The percentages of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: pH 6.2 phosphate buffer (see *Reagents and Reference Tables, Solutions, Buffer Solutions*); 900 mL
▲Apparatus 1: 75 rpm▲ (USP 1-May-2020)
Standard solution, Sample solution, and **Analysis:** Proceed as directed in *Test 1*.
Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved
1	12%–32%
2	27%–52%
4	50%–80%
12	NLT 80%

The percentages of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.
Medium: pH 6.8 phosphate buffer (see *Reagents and Reference Tables, Solutions, Buffer Solutions*); 750 mL
▲Apparatus 1: 75 rpm▲ (USP 1-May-2020)
Standard solution, Sample solution, and **Analysis:** Proceed as directed in *Test 1*.
Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved
1	15%–40%
2	35%–55%
4	55%–75%
6	65%–85%
12	NLT 75%
24	NLT 85%

The percentages of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: pH 6.2 phosphate buffer (see *Reagents and Reference Tables, Solutions, Buffer Solutions*); 900 mL
Apparatus 1: 75 rpm
Times: 1, 2, 4, 12, and 24 h
Mobile phase: Acetonitrile and 0.1% phosphoric acid (60:40)
Standard stock solution: 0.4 mg/mL of USP Indomethacin RS▲ (USP 1-May-2020) prepared as follows. Transfer a suitable amount of USP Indomethacin RS into a suitable volumetric flask. Add 10% of the flask volume of acetonitrile, and sonicate to promote dissolution, if necessary. Dilute with *Medium* to volume.
Standard solution: (L/900) mg/mL of USP Indomethacin RS in *Medium* from the *Standard stock solution*, where L is the label claim, in mg
Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 235 nm
Column: 4.6-mm × 100-mm; 3.5-µm packing L1
Column temperature: 40°
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of indomethacin ($C_{19}H_{16}ClNO_4$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of indomethacin from the *Sample solution*
r_S = peak response of indomethacin from the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* ▲(mg/mL)▲ (USP 1-May-2020)

Calculate the percentages of the labeled amount (Q_{ti}) of indomethacin ($C_{19}H_{16}ClNO_4$) dissolved at each time point i:

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + [C_1 \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_i = \{[C_i \times (V - \{[i - 1] \times V_S\})] + [(C_{[i-1]} + C_{[i-2]} + \ldots + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of indomethacin in the portion of sample withdrawn at time point i (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim of indomethacin (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time (h)	Time Point (i)	Amount Dissolved
1	1	10%–30%
2	2	20%–40%
4	3	35%–55%
12	4	60%–80%
24	5	NLT 75%

The percentages of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

www.webofpharma.com

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: pH 6.2 phosphate buffer (see *Reagents and Reference Tables, Solutions, Buffer Solutions*); 750 mL
Apparatus 1: 75 rpm
Times: 1, 2, 4, 6, 12, and 24 h
Standard stock solution: 0.5 mg/mL of USP Indomethacin RS in methanol. Sonicate, if needed, to dissolve.
Standard solution: 0.025 mg/mL of USP Indomethacin RS from the *Standard stock solution* diluted in *Medium*. Pass through a suitable filter of 0.45-µm pore size.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size. Dilute with *Medium*, if necessary.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 318 nm
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.0%
Analysis: Replace the volume of medium withdrawn for analysis with an equal volume of fresh *Medium* after each sampling.
Samples: *Standard solution* and *Sample solution*
Calculate the concentration, C_i, of indomethacin ($C_{19}H_{16}ClNO_4$) in the sample withdrawn from the vessel at each time point (*i*):

$$C_i = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of the *Sample solution* at time point (*i*)
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*

Calculate the percentage of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) dissolved at each time point (*i*):

$$\text{Result}_1 = (C_1 \times V) \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_i = \{(C_i \times V) + [(C_{[i-1]} + C_{[i-2]} + \ldots + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of indomethacin in the portion of sample withdrawn at time point (*i*) (mg/mL)
V = volume of *Medium*, 750 mL
L = label claim (mg/Capsule)
V_S = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved (%)
1	10–25
2	20–40
4	35–55
6	45–65
12	65–85
24	NLT 80

The percentages of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Change to read:

• **Uniformity of Dosage Units** ⟨905⟩: ▲Meets the requirements▲ (USP 1-May-2020)
Procedure for content uniformity
Solution A: Dissolve 17.42 g of dibasic potassium phosphate in 800 mL of water, adjusting with phosphoric acid to a pH of 7.5, and diluting with water to 1000 mL (pH 7.5 phosphate buffer).
Diluent: Methanol and *Solution A* (1:1)
Standard solution: 25 µg/mL of USP Indomethacin RS in *Diluent*
Sample solution: Nominally 25 µg/mL of indomethacin in *Diluent*, prepared as follows. Transfer the contents of 1 Capsule to a 200-mL volumetric flask, and add 100 mL of *Diluent*. Sonicate until the contents are dispersed, dilute with *Diluent* to volume, and centrifuge. Dilute a portion of the clear solution with *Diluent* to obtain the above concentration.
Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV
Analytical wavelength: 318 nm
Cell: 1 cm
Blank: Methanol and *Solution A* (1:1)
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage ▲of the labeled amount▲ (USP 1-May-2020) of indomethacin ($C_{19}H_{16}ClNO_4$) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of indomethacin in the *Sample solution* (µg/mL)

▲▲ (USP 1-May-2020)

IMPURITIES

Delete the following:

▲• **Limit of 4-Chlorobenzoic Acid**
Mobile phase, Diluent, Standard solution A, Standard solution B, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution B* and *Sample solution*
Using the peak responses measured and recorded in the *Assay*, calculate the percentage of 4-chlorobenzoic acid ($C_7H_5ClO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

www.webofpharma.com

r_S = peak response from the *Standard solution*
C_S = concentration of 4-chlorobenzoic acid in *Standard solution B* (mg/mL)
C_U = measured concentration of indomethacin in the *Sample solution* as determined from the *Assay* (mg/mL)

Acceptance criteria: NMT 0.44%▲ (USP 1-May-2020)

Add the following:

▲• **ORGANIC IMPURITIES**

Solution A: Dilute 1 mL of phosphoric acid with water to 1000 mL.
Solution B: Acetonitrile
Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	80	20
40	30	70
45	30	70
50	80	20
60	80	20

Diluent: Acetonitrile and water (60:40)
Sensitivity solution: 0.4 µg/mL of USP Indomethacin RS in *Diluent*. Sonicate to dissolve if needed.
Standard solution: 0.8 µg/mL of USP Indomethacin RS, 1.1 µg/mL of USP Indomethacin Related Compound A RS, and 3.3 µg/mL of USP Indomethacin Related Compound B RS in *Diluent*. Sonicate to dissolve if needed.
Sample solution: Nominally 750 µg/mL of indomethacin in *Diluent*, prepared as follows. Transfer a suitable quantity of the contents of Capsules (NLT 20), equivalent to about 75 mg of indomethacin, to a 100-mL volumetric flask. Add about 60 mL of *Diluent*, shake gently for 5 min, then sonicate for about 10 min with intermittent shaking. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
[NOTE—Rinsing with 2 mL of methanol and water (80:20) may be used before and after injection.]
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Temperatures
Autosampler: 6°
Column: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Relative standard deviation: NMT 5.0% for indomethacin, indomethacin related compound A, and indomethacin related compound B, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of indomethacin related compound A and indomethacin related compound B in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of indomethacin related compound A or indomethacin related compound B from the *Sample solution*
r_S = peak response of indomethacin related compound A or indomethacin related compound B from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (µg/mL)
C_U = nominal concentration of indomethacin in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified degradation product from the *Sample solution*
r_S = peak response of indomethacin from the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of indomethacin in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 7*.

Table 7

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Indomethacin related compound A	0.38	0.15
Indomethacin related compound B	0.59	0.44
Indomethacin benzamide impurity[a, b]	0.86	—
Indomethacin	1.0	—
Indomethacin dibenzylate impurity[a, c]	1.07	—
Indomethacin diamide impurity[a, d]	1.34	—
Any unspecified degradation product	—	0.2
Total degradation products	—	1.2

[a] Process impurity listed for peak identification only, and not to be reported or to be included in the total degradation products.
[b] 4-Chloro-*N*-(4-methoxyphenyl)benzamide.
[c] 1-(4-Chlorobenzoyl)-1-(4-methoxyphenyl)-2-(4-chlorobenzoyl)hydrazide.
[d] 4-Chloro-*N*′-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl) acetyl)-*N*-(4-methoxyphenyl)benzohydrazide.▲ (USP 1-May-2020)

ADDITIONAL REQUIREMENTS

Change to read:

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. ▲Store at controlled room temperature.▲ (USP 1-May-2020)

www.webofpharma.com

Change to read:

• **LABELING:** ▲When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.▲ (USP 1-May-2020)

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲USP Indomethacin RS
USP Indomethacin Related Compound A RS
2-(5-Methoxy-2-methyl-1*H*-indol-3-yl)acetic acid.
$C_{12}H_{13}NO_3$ 219.24
USP Indomethacin Related Compound B RS
4-Chlorobenzoic acid.
$C_7H_5ClO_2$ 156.57▲ (USP 1-May-2020)

Indomethacin Compounded Topical Gel

DEFINITION
Indomethacin Compounded Topical Gel contains NLT 0.90 g and NMT 1.10 g of Indomethacin in 100 mL of gel.
Prepare Indomethacin Compounded Topical Gel as follows.

Indomethacin	1.0 g
Carbomer 941	2.0 g
Purified Water	10 mL
Alcohol (95% ethyl alcohol), a sufficient quantity to make	100 mL

Transfer the *Indomethacin* to a suitable beaker, and dissolve it in 55 mL of *Alcohol*. Transfer this solution to a glass mortar, and slowly add the *Carbomer 941* so that it is thoroughly distributed. Press out any white lumps until a smooth gel is formed. Slowly add the *Purified Water* with mixing. Add a sufficient quantity of *Alcohol* to bring to final volume, and mix. Transfer the Topical Gel to a wide-mouth container or ointment jar.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Package in tight, light-resistant, wide-mouth containers or ointment jars. Store at controlled room temperature.
• **BEYOND-USE DATE:** NMT 30 days after the date on which it was compounded when stored at controlled room temperature
• **LABELING:** Label it to state that it is for topical, external use only. Label it to indicate that the container should be kept tightly closed. Label it to state the *Beyond-Use Date*.

Indomethacin for Injection

DEFINITION
Indomethacin for Injection contains an amount of Indomethacin Sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
• **B.** The UV absorption spectrum of the indomethacin peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol, phosphoric acid, and water (600:1:400)
Diluent: Acetonitrile, phosphoric acid, and water (300:1:700)
Standard solution: 0.1 mg/mL of USP Indomethacin RS prepared as follows. Dissolve a suitable amount of USP Indomethacin RS with 30% of the final volume of acetonitrile, and dilute with water to volume.
Sample stock solution: Nominally 0.5 mg/mL of indomethacin in *Diluent* from NLT 10 containers of Indomethacin for Injection
Sample solution: Nominally 0.1 mg/mL of indomethacin in *Diluent* from the *Sample stock solution*. Pass through a filter of 0.5-µm or finer pore size. Use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 240 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) in the portion of Indomethacin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of indomethacin from the *Sample solution*
r_S = peak area of indomethacin from the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of indomethacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES
• **CONTENT OF INDOMETHACIN RELATED COMPOUND B**
Mobile phase, Diluent, Sample stock solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 0.22 mg/mL of USP Indomethacin Related Compound B RS in acetonitrile
Standard solution: 0.00044 mg/mL of USP Indomethacin Related Compound B RS prepared as follows. Transfer an adequate volume of the *Standard stock solution* to a suitable volumetric flask, and add 30% of the final volume of acetonitrile. Dilute with water to the final volume.
System suitability
Sample: *Standard solution*

www.webofpharma.com

Suitability requirements
Relative standard deviation: NMT 5%
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of indomethacin related compound B in the portion of Indomethacin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of indomethacin related compound B from the *Sample solution*
r_S = peak area of indomethacin related compound B from the *Standard solution*
C_S = concentration of USP Indomethacin Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of indomethacin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.2%

SPECIFIC TESTS
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩
Sample solution: Nominally 1.0 mg/mL of indomethacin in LAL Reagent Water from Indomethacin for Injection
Acceptance criteria: NMT 20.0 USP Endotoxin Units/mg of indomethacin
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **pH** ⟨791⟩
Sample solution: A solution in water (1 in 2000) containing 0.3 mL of saturated potassium chloride solution per 100 mL
Acceptance criteria: 5.0–7.0
- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products.*

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Store at controlled room temperature. Protect from light. Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution.*
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Indomethacin RS
USP Indomethacin Related Compound B RS
4-Chlorobenzoic acid.
$C_7H_5ClO_2$ 156.57

Indomethacin Suppositories

DEFINITION
Indomethacin Suppositories contain NLT 90.0% and NMT 110.0% of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$).

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the indomethacin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
Solution A: Prepare 0.1% of formic acid by diluting 1 mL of formic acid with water to 1 L.
Mobile phase: Acetonitrile and *Solution A* (45:55)
Diluent: *Mobile phase* adjusted with 0.2 M sodium hydroxide (NaOH) to a pH of 8.0
System suitability solution: 0.002 mg/mL of USP Indomethacin RS, 0.002 mg/mL of USP Indomethacin Related Compound A RS, and 0.01 mg/mL of USP Indomethacin Related Compound B RS in *Diluent*
Standard solution: 0.5 mg/mL of USP Indomethacin RS in *Diluent*. Sonicate if necessary.
Sample solution: Prepare a solution nominally equivalent to 0.5 mg/mL of indomethacin in *Diluent* as follows. Mash NLT 10 Suppositories in a beaker, and heat (at about 50°) on a water bath until melted. Mix well and cool. Transfer a portion of the mass equivalent to 10 mg of indomethacin into a 20-mL volumetric flask and add 10 mL of acetonitrile. Heat in a water bath at 50° to dissolve, and dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: PDA (scan 200–600). Calculations should be based on the chromatograms collected at 240 nm. For *Identification* test *B*, use spectra at the scanned range.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 4 between indomethacin related compound A and indomethacin related compound B, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution*
Relative standard deviation: NMT 1.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) in the portion of Suppositories taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of indomethacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **DISSOLUTION** ⟨711⟩
Medium: 0.1 M, pH 7.2 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL
Apparatus 2: 50 rpm
Time: 60 min
Standard solution: USP Indomethacin RS at a known concentration in *Medium*
Sample solution: Proceed as directed for sample per *Dissolution* ⟨711⟩. Dilute with *Medium* as needed.
Instrumental conditions
Mode: UV
Analytical wavelength: 320 nm

www.webofpharma.com

Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 75% (*Q*) of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Diluent: Methanol and glacial acetic acid (199:1)
Standard solution: 25 µg/mL of USP Indomethacin RS in *Diluent*
Sample solution: Place 1 Suppository into a 100-mL volumetric flask containing 80 mL of *Diluent*, shake by mechanical means until the Suppository is dissolved, and dilute with *Diluent* to volume. Filter a portion of the solution, discarding the first 15 mL of the filtrate, and dilute a volume of the clear filtrate with the *Diluent* to obtain a solution having a concentration of 25 µg/mL of indomethacin.
Instrumental conditions
Mode: UV
Detector: 320 nm
Blank: *Diluent*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) in the Suppository taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of indomethacin in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements in *Uniformity of Dosage Units* ⟨905⟩

IMPURITIES

• **ORGANIC IMPURITIES**
Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.002 mg/mL of USP Indomethacin RS, 0.002 mg/mL of USP Indomethacin Related Compound A RS, and 0.01 mg/mL of USP Indomethacin Related Compound B RS in *Diluent*
Sample solution: Prepare a solution nominally equivalent to 2.0 mg/mL of indomethacin in *Diluent* as follows. Mash NLT 10 Suppositories in a beaker, and heat (at about 50°) on a water bath until melted. Mix well and cool. Transfer a portion of the mass equivalent to 40 mg of indomethacin into a 20-mL volumetric flask and add 10 mL of acetonitrile. Heat in a water bath at 50° to dissolve, and dilute with *Diluent* to volume.
System suitability
Sample: *Standard solution*
[NOTE—See *Table 1* for the relative retention times.]
Suitability requirements
Resolution: NLT 4 between indomethacin related compound A and indomethacin related compound B
Relative standard deviation: NMT 2.8%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of indomethacin related compound A and indomethacin related compound B in the portion of Suppositories taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of indomethacin related compound A or indomethacin related compound B from the *Sample solution*
r_S = peak response of indomethacin related compound A or indomethacin related compound B from the *Standard solution*
C_S = concentration of the corresponding USP Indomethacin Related Compound A RS or USP Indomethacin Related Compound B RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of indomethacin in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Suppositories taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of unspecified impurity from the *Sample solution*
r_S = peak response of indomethacin from the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of indomethacin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Indomethacin related compound A	0.15	0.1
Indomethacin related compound B	0.25	0.5
Indomethacin	1	—
Any other individual, unidentified impurity	—	0.1
Total impurities	—	2.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Indomethacin RS
USP Indomethacin Related Compound A RS
5-Methoxy-2-methyl-3-indoleacetic acid.
$C_{12}H_{13}NO_3$ 219.24
USP Indomethacin Related Compound B RS
4-Chlorobenzoic acid.
$C_7H_5ClO_2$ 156.57

Indomethacin Oral Suspension

DEFINITION

Indomethacin Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$).

IDENTIFICATION

• **A.** The UV absorption spectrum of the indomethacin peak of the *Sample solution* exhibits maxima and minima at the

www.webofpharma.com

same wavelengths as those of the *Standard solution*, as obtained in the *Assay*.

• **B.** The retention time of the indomethacin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 0.2% (v/v) phosphoric acid in water

Solution B: Dehydrated alcohol and butyl alcohol (80:50)

Mobile phase: *Solution B* and *Solution A* (39:61). Pass through a suitable filter of 0.5-µm or finer pore size.

Standard solution: 0.8 mg/mL of USP Indomethacin RS and 0.16 mg/mL of sorbic acid prepared as follows. Transfer appropriate amounts of USP Indomethacin RS and sorbic acid to a suitable volumetric flask. Add 20% of the total volume of *Solution A* and 30% of the total volume of *Solution B*, and sonicate for 5 min. Dilute with *Solution A* to the total volume.

Sample solution: Nominally 0.8 mg/mL of indomethacin prepared as follows. Transfer an appropriate volume of Oral Suspension, freshly mixed and free from air bubbles, to a suitable volumetric flask. Add 30% of *Solution B*, and sonicate for 10 min. Dilute with *Solution A* to volume, and pass through a suitable filter of 0.5-µm or finer pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.

Column: 8-mm × 10-cm; packing L1

Flow rate: 3 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between sorbic acid and indomethacin

Tailing factor: NMT 2.0 for indomethacin

Relative standard deviation: NMT 2.0% for indomethacin

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of indomethacin from the *Sample solution*

r_S = peak response of indomethacin from the *Standard solution*

C_S = concentration of USP Indomethacin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of indomethacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Buffer solution: 0.01 M pH 7.2 phosphate buffer prepared by dissolving 1.36 g of monobasic potassium phosphate in 1 L of water and adjusting with 0.1 N sodium hydroxide to a pH of 7.2 ± 0.1

Medium: *Buffer solution*; 900 mL

Apparatus 2: 50 rpm

Time: 20 min

Standard solution: USP Indomethacin RS at a known concentration in *Medium*. [NOTE—A quantity of methanol not to exceed 1.0% of the volume of the *Standard solution* may be used to bring the USP Reference Standard into solution before dilution with *Medium*, and the solution may be sonicated to effect complete dissolution of the USP Reference Standard.]

Sample solution: Transfer to the surface of the *Medium* in the dissolution vessel a volume of Oral Suspension, freshly mixed and free from air bubbles, nominally equivalent to 25 mg of indomethacin. Filter portions of the solution under test, and suitably dilute with *Medium* if necessary.

Instrumental conditions

Mode: UV

Analytical wavelength: 320 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 80% (*Q*) of the labeled amount of indomethacin ($C_{19}H_{16}ClNO_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩

For single-unit containers

Acceptance criteria: Meets the requirements

• **DELIVERABLE VOLUME** ⟨698⟩

For multiple-unit containers

Acceptance criteria: Meets the requirements

IMPURITIES

• **CONTENT OF INDOMETHACIN RELATED COMPOUND B**

Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 0.09 mg/mL of USP Indomethacin Related Compound B RS in *Solution B*

Standard solution: 0.0018 mg/mL of USP Indomethacin Related Compound B RS prepared as follows. Transfer an adequate amount of *Standard stock solution* to a suitable volumetric flask containing 30% of the total volume of *Solution B*, and dilute with *Solution A* to the total volume.

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor, *k'*: NLT 1.0

Relative standard deviation: NMT 2.5%

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of indomethacin related compound B in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of indomethacin related compound B from the *Sample solution*

r_S = peak response of indomethacin related compound B from the *Standard solution*

C_S = concentration of USP Indomethacin Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of indomethacin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.44%

SPECIFIC TESTS

• **PH** ⟨791⟩**:** 2.5–5.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store below 30° and avoid temperatures above 50°. Preserve in tight, light-resistant containers and protect from freezing.

• **USP REFERENCE STANDARDS** ⟨11⟩

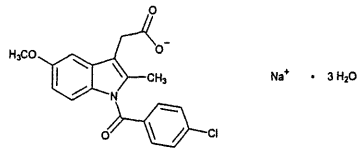
USP Indomethacin RS

USP Monographs

www.webofpharma.com

USP Indomethacin Related Compound B RS
4-Chlorobenzoic acid.
$C_7H_5ClO_2$ 156.57

Indomethacin Sodium



$C_{19}H_{15}ClNNaO_4 \cdot 3H_2O$ 433.82
1*H*-Indole-3-acetic acid, 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-, sodium salt, trihydrate;
Sodium 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate, trihydrate [74252-25-8].
Anhydrous 379.78

DEFINITION
Indomethacin Sodium contains NLT 98.0% and NMT 102.0% of indomethacin sodium ($C_{19}H_{15}ClNNaO_4$), calculated on the dried basis.

IDENTIFICATION
- **A.**
  Sample: A small quantity
  Analysis: Ignite the *Sample* on a platinum wire in a nonluminous flame.
  Acceptance criteria: An intense yellow flame is produced.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.** The UV-Vis spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Solution A: 0.1% formic acid in water
  Solution B: 0.025% formic acid in acetonitrile
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
2	70	30
20	15	85
25	15	85
26	70	30
30	70	30

Diluent: *Solution B* and *Solution A* (45:55) adjusted with 0.2 M sodium hydroxide to a pH of 8.0
System suitability solution: 0.82 mg/mL of USP Indomethacin RS, 0.002 mg/mL of USP Indomethacin Related Compound A RS, and 0.002 mg/mL of USP Indomethacin Related Compound B RS in *Diluent*. Sonicate to dissolve.
Standard solution: 0.82 mg/mL of USP Indomethacin RS dissolved in a minimum quantity of acetonitrile. Dilute with *Diluent* to volume. Sonicate to dissolve.
Sample solution: 1.0 mg/mL of Indomethacin Sodium trihydrate in *Diluent*. Sonicate to dissolve.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: 240 nm. For *Identification C*, use a diode array detector in the range of 190–400 nm.
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for indomethacin related compound A and indomethacin related compound B are 0.50 and 0.67, respectively.]
Suitability requirements
Resolution: NLT 10 between indomethacin related compound A and indomethacin related compound B, *System suitability solution*
Tailing factor: NMT 2.0 for indomethacin, *Standard solution*
Relative standard deviation: NMT 0.73%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of indomethacin sodium ($C_{19}H_{15}ClNNaO_4$) in the portion of Indomethacin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (mg/mL)
C_U = concentration of Indomethacin Sodium in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of anhydrous indomethacin sodium, 379.78
M_{r2} = molecular weight of indomethacin, 357.79

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES
- **LIMIT OF ACETONE**
  Standard solution: Transfer 1.0 mL of acetone to a 100-mL volumetric flask, and dilute with water to volume. Transfer 1.0 mL of this solution to a 200-mL volumetric flask, dilute with water to volume, insert a stopper, and cool in an ice bath.
  Sample solution: Transfer 100 mg of Indomethacin Sodium to a 15-mL centrifuge tube, and dissolve in 1.0 mL of cool water. While vortexing this solution, add 1.0 mL of 0.24 N hydrochloric acid, centrifuge promptly, and filter the supernatant. Collect the filtrate in a suitable tube, cap, and cool in an ice bath.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: GC
  Detector: Flame ionization
  Column: 3-mm × 1.8-m; support S3
  Column temperature: 165°
  Carrier gas: Nitrogen
  Injection volume: 3 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Capacity factor, k': 4–7 for acetone

USP Monographs

www.webofpharma.com

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Use the solvent flush technique, with water as the flushing agent, and record the chromatograms for 6 min.]

Calculate the percentage of acetone in the portion of Indomethacin Sodium taken:

$$\text{Result} = S_G\,(10/W_U)(r_U/r_S)$$

S_G = specific gravity of acetone, 0.79
W_U = quantity of Indomethacin Sodium taken to prepare the *Sample solution* (mg)
r_U = peak area of acetone from the *Sample solution*
r_S = peak area of acetone from the *Standard solution*

Acceptance criteria: NMT 0.1%

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.002 mg/mL each of USP Indomethacin RS, USP Indomethacin Related Compound A RS, and USP Indomethacin Related Compound B RS, dissolved in a minimum quantity of acetonitrile. Dilute with *Diluent* to volume. Sonicate to dissolve.

Sample solution: 1.0 mg/mL of Indomethacin Sodium in *Diluent*. Sonicate to dissolve.

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 10 between indomethacin related compound A and indomethacin related compound B

Relative standard deviation: NMT 2.5% for indomethacin related compound A, indomethacin related compound B, and indomethacin

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of indomethacin related compound A or indomethacin related compound B in the portion of Indomethacin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of indomethacin related compound A or indomethacin related compound B from the *Sample solution*
r_S = peak response of the corresponding related compound from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = concentration of Indomethacin Sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Indomethacin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*
r_S = peak response of indomethacin from the *Standard solution*
C_S = concentration of USP Indomethacin RS in the *Standard solution* (mg/mL)
C_U = concentration of Indomethacin Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Indomethacin related compound A	0.50	0.2[a]
Indomethacin related compound B	0.67	—
Indomethacin	1.0	—
Any individual unspecified impurity	—	0.5
Total impurities	—	1.0[b]

[a] The sum of the percentages of indomethacin related compound A and indomethacin related compound B is NMT 0.2%.
[b] Excluding the percentages for indomethacin related compound A and indomethacin related compound B.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 100° for 2 h at a pressure not exceeding 5 mm of mercury.

Acceptance criteria: 11.5%–13.5%

• **OTHER REQUIREMENTS:** Where the label states that Indomethacin Sodium is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ and for the *Bacterial Endotoxins Test* under *Indomethacin for Injection*. Where the label states that Indomethacin Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for the *Bacterial Endotoxins Test* under *Indomethacin for Injection*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP REFERENCE STANDARDS** ⟨11⟩

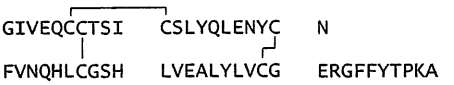
USP Indomethacin RS

USP Indomethacin Related Compound A RS
2-(5-Methoxy-2-methyl-1*H*-indol-3-yl)acetic acid.
($C_{12}H_{13}NO_3$) 219.24

USP Indomethacin Related Compound B RS
4-Chlorobenzoic acid.
($C_7H_5ClO_2$) 156.57

Insulin

Change to read:



$C_{256}H_{381}N_{65}O_{76}S_6$ 5777.54

Insulin (pig) [12584-58-6].

▲ ▲ (RB 1-May-2019)

DEFINITION

Change to read:

▲Insulin is a two-chain peptide hormone consisting of 51 amino acids, and its structure corresponds to native insulin

www.webofpharma.com

produced in vivo by the beta cells of the pancreas. The A-chain is composed of 21 amino acids, and the B-chain is composed of 30 amino acids.▲ (USP 1-May-2019) It is obtained from the pancreas of healthy ▲▲ (RB 1-May-2019) porcine animals, ▲▲ (RB 1-May-2019) used for food by humans. Its potency is NLT 26.5 USP Insulin Units/mg, calculated on the dried basis; Insulin labeled as purified contains NLT 27.0 USP Insulin Units/mg, calculated on the dried basis. ▲▲ (USP 1-May-2019)

[NOTE—1 USP Insulin Unit is equivalent to ▲▲ (RB 1-May-2019) 0.0345 mg of pure Insulin derived from pork.]

IDENTIFICATION

Change to read:

• **A.** The retention time of the major peak in the *Sample solution* corresponds to that ▲▲ (RB 1-May-2019) of the *Identification solution*, as obtained in the *Assay*▲ and no other significant peaks are observed.▲ (RB 1-May-2019)

[NOTE—It may be necessary to inject a mixture of *Sample solution* and *Identification solution*.]

Delete the following:

▲B. PEPTIDE MAPPING

Sulfate buffer: 2.0 M ammonium sulfate and 0.5 M sulfuric acid (1:1)

Enzyme solution: 500 units/mL of *Staphylococcus aureus* V-8 protease activity in water

HEPES buffer: 0.1 M HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid). Adjust with 5 M sodium hydroxide to a pH of 7.5 before diluting with water to a final volume.

Solution A: Acetonitrile, water, and *Sulfate buffer* (100:700:200)

Solution B: Acetonitrile, water, and *Sulfate buffer* (400:400:200)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
60	30	70
65	0	100
70	0	100
71	90	10
86	90	10

Standard digest solution: 2 mg/mL of USP Insulin RS of the appropriate species in 0.01 N hydrochloric acid. Transfer 500 µL of the resulting solution to a clean vial. Add 2.0 mL of *HEPES buffer* and 400 µL of *Enzyme solution*, and incubate at 25° for 6 h. Quench the digestion by adding 2.9 mL of *Sulfate buffer*.

Sample digest solution: 2 mg/mL of Insulin in 0.01 N hydrochloric acid, mix to dissolve. Transfer 500 µL of the resulting solution to a clean vial. Add 2.0 mL of *HEPES buffer* and 400 µL of *Enzyme solution*, and incubate at 25° for 6 h. Quench the digestion by adding 2.9 mL of *Sulfate buffer*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 10-cm; packing L1

Column temperature: 40°

Flow rate: 1 mL/min

System suitability

Sample: *Standard digest solution*

Suitability requirements

Chromatogram comparability: The chromatogram of the *Standard digest solution* corresponds to that of the reference chromatogram provided with USP Insulin RS of the appropriate species.

Resolution: NLT 1.9 between digest fragments II and III.

[NOTE—Fragment I elutes at the same time in insulin derived from pork and Insulin Human; Fragment II elutes at the same time in Insulin Human and insulin derived from beef and pork; and Fragment III elutes at the same time in insulin derived from beef and pork.]

Tailing factor: NMT 1.5

Analysis

Samples: *Standard digest solution* and *Sample digest solution*

Using the gradient program, run a blank. Separately inject equal volumes of the *Standard digest solution* and the *Sample digest solution*, and record the responses of each peak.

Acceptance criteria: The chromatographic profile of the *Sample digest solution* corresponds to that of the *Standard digest solution*.▲ (USP 1-May-2019)

Add the following:

▲• B. PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS ⟨121.1⟩, *Peptide Mapping*: Proceed as directed in the chapter, except for the *Mobile phase* and *System suitability*.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
60	30	70
65	0	100
70	0	100
71	90	10
86	90	10

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.9 between digest fragments II and III

[NOTE—Fragment I elutes at the same time in insulin derived from pork and Insulin Human; Fragment II elutes at the same time in Insulin Human and insulin derived from beef and pork; and Fragment III elutes at the same time in insulin derived from beef and pork.]

Tailing factor: NMT 1.5 for digest fragments II and III

Chromatogram similarity: The chromatogram of the *Standard solution* corresponds to that of the reference chromatogram provided with USP Insulin Pork RS.

Acceptance criteria: Meets the requirements▲ (USP 1-May-2019)

www.webofpharma.com

Add the following:

▲• **C. Insulin Assays** ⟨121⟩, *Assay, Bioidentity Test:* Meets the requirements ▲(USP 1-May-2019)

ASSAY

Change to read:

• **Procedure**

Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.

Mobile phase: Acetonitrile and *Solution A* (26:74).
[Note—The acetonitrile is warmed to a temperature of NLT 20° to avoid precipitation.]

System suitability solution: 1.5 mg/mL of Insulin in 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.
[Note—The *Identification solution*, *Standard solution*, and *Sample solution* may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Identification solution: 0.6 mg/mL of USP Insulin Pork RS▲▲(RB 1-May-2019) in 0.01 N hydrochloric acid

Standard solution: 1.5 mg/mL of ▲USP Insulin Pork RS▲(RB 1-May-2019) in 0.01 N hydrochloric acid▲▲(RB 1-May-2019)

Sample solution: 1.5 mg/mL of Insulin in 0.01 N hydrochloric acid

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between insulin and A-21 desamido insulin, *System suitability solution*
Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*
Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis
Samples: *Identification solution*, *Standard solution*, and *Sample solution*
Measure the peak responses for insulin and A-21 desamido insulin, using the chromatogram of the *Identification solution* to identify the insulin peaks.
▲▲(RB 1-May-2019) Calculate the potency on the undried basis, in USP Insulin Units/mg, of Insulin in the *Sample solution*:

$$\text{Result} = (\Sigma r_U/\Sigma r_S) \times (C_S/C_U)$$

r_U = sum of the peak responses of insulin and A-21 desamido insulin from the *Sample solution*
r_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*
C_S = concentration of ▲▲(RB 1-May-2019)USP Insulin Pork RS in the *Standard solution* (USP Insulin Units/mL)
C_U = concentration of Insulin in the *Sample solution* (mg/mL)

▲▲(RB 1-May-2019)
Acceptance criteria: NLT 26.5 USP Insulin Units/mg on the dried basis; Insulin labeled as purified contains NLT 27.0 USP Insulin Units/mg on the dried basis.

OTHER COMPONENTS

Change to read:

• **Zinc Determination** ⟨591⟩
▲**Acceptance criteria:** NMT 1.0% on the dried basis▲(USP 1-May-2019)

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

Change to read:

• ▲**Product-Related Substances**▲(USP 1-May-2019)

Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.

Solution B: Acetonitrile and *Solution A* (18:82)

Solution C: Acetonitrile and *Solution A* (50:50)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution B (%)	Solution C (%)
0	81	19
60	81	19
85	36	64
91	36	64
92	81	19

System suitability solution: 1.5 mg/mL of Insulin in 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.
[Note—*Standard solutions A–C* may be stored at room temperature for up to 12 h and in a refrigerator for up to 48 h.]

Standard solution A: 3.75 mg/mL of ▲USP Insulin Pork RS▲(RB 1-May-2019) in 0.01 N hydrochloric acid▲▲(RB 1-May-2019)

Standard solution B: 0.375 mg/mL of ▲▲(RB 1-May-2019)USP Insulin Pork RS in 0.01 N hydrochloric acid prepared as follows. Pipet 1 mL of *Standard solution A* into a 10-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Standard solution C: 0.0375 mg/mL of ▲▲(RB 1-May-2019)USP Insulin Pork RS in 0.01 N hydrochloric acid prepared as follows. Pipet 1 mL of *Standard solution B* into a 10-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Sample solution: 3.75 mg/mL of Insulin in 0.01 N hydrochloric acid. Prepare the solution in a capped vial, cap the vial, and shake gently to dissolve. Store the solution for NMT 2 h at room temperature or for NMT 12 h in a refrigerator.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL

www.webofpharma.com

System suitability
Samples: *System suitability solution, Standard solution A, Standard solution B,* and *Standard solution C*
[NOTE—Adjust the *Mobile phase* composition and the duration of the isocratic elution to obtain a retention time of about 31 min for insulin, with the A-21 desamido insulin eluting just prior to the start of the gradient elution phase.]
Suitability requirements for the System suitability solution
Resolution: NLT 2.0 between insulin and A-21 desamido insulin
Tailing factor: NMT 1.8 for the insulin peak
Suitability requirements for the Standard solutions
Calculate the factor X_1:

$$X_1 = (r_B/r_A) \times D$$

r_B = peak response from *Standard solution B*
r_A = peak response from *Standard solution A*
D = dilution factor, 10

Result: Between 0.91 and 1.09
Calculate the factor X_2:

$$X_2 = (r_C/r_A) \times D$$

r_C = peak response from *Standard solution C*
r_A = peak response from *Standard solution A*
D = dilution factor, 100

Result: Between 0.7 and 1.3
Analysis
Sample: *Sample solution*
Calculate the percentage of insulin, A-21 desamido insulin, and other ▲insulin-related substances▲ (USP 1-May-2019) in the portion of Insulin taken:
Calculate the percentage of Insulin (%I):

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = peak response of insulin from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Calculate the percentage of A-21 desamido insulin (%D):

$$\text{Result} = (r_D/r_T) \times 100$$

r_D = peak response of A-21 desamido insulin from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Calculate the percentage of other insulin-related ▲substances:▲ (USP 1-May-2019)

$$\text{Result} = 100 - (\%I + \%D)$$

Acceptance criteria: NMT 10.0% of A-21 desamido insulin, and NMT 5.0% of other insulin-related ▲substances▲ (USP 1-May-2019)
▲▲ (RB 1-May-2019)

Change to read:

- ▲**PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Limit of High Molecular Weight Proteins:* Meets the requirements▲ (USP 1-May-2019)

Acceptance criteria: NMT 1.0%

PROCESS-RELATED IMPURITIES

Add the following:

- ▲• **PROINSULIN CONTENT:** NMT 10 ng/mg, determined by a validated method▲ (USP 1-May-2019)

SPECIFIC TESTS

Delete the following:

- ▲• **INSULIN ASSAYS** ⟨121⟩, *Assay, Bioidentity Test:* Meets the requirements▲ (USP 1-May-2019)
- **LOSS ON DRYING** ⟨731⟩
Sample: 200 mg
Analysis: Dry the *Sample* at 105° for 16 h.
Acceptance criteria: NMT 10.0%

Delete the following:

- ▲• **ZINC DETERMINATION** ⟨591⟩, *Procedure, Dithizone Method*
Sample: 10 mg
Acceptance criteria: NMT 1.0% on the dried basis▲ (USP 1-May-2019)
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 10 USP Endotoxin Units/mg of insulin
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total bacterial count does not exceed 3×10^2 cfu/g, the test being performed on a portion of about 0.2 g, accurately weighed.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store, protected from light, in a freezer.

Change to read:

- **LABELING:** Label it ▲▲ (RB 1-May-2019) as pork▲▲ (RB 1-May-2019). If the Insulin is purified, label it as such.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
▲▲ (RB 1-May-2019)
USP Insulin Pork RS

Insulin Injection

DEFINITION
Insulin Injection is an isotonic, sterile solution of Insulin. Its potency, based on the sum of the insulin and desamido insulin components, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units/mL.

IDENTIFICATION

Change to read:

- **A.** The retention time of the insulin ▲pork▲ (RB 1-May-2019) peak of *Sample solution A* or *Sample solution B* corresponds to that ▲▲ (RB 1-May-2019) of the *Identification solution,* as obtained in the *Assay* ▲and no other significant peaks are observed.▲ (RB 1-May-2019)[NOTE—It may be necessary to inject a mixture of *Sample solution* and *Identification solution.*]

www.webofpharma.com

ASSAY

Change to read:

• **PROCEDURE**

Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.

Mobile phase: Acetonitrile and *Solution A* (26:74). [NOTE—The acetonitrile is warmed to NLT 20° to avoid precipitation.]

System suitability solution: 1.5 mg/mL of ▲▲ (RB 1-May-2019) insulin pork in 0.01 N hydrochloric acid. ▲▲ (RB 1-May-2019) Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin. [NOTE—The *Identification solution*, *Standard solution*, and *Sample solutions* may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Identification solution: 0.6 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid

Standard solution: 1.5 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid. ▲▲ (RB 1-May-2019)

Sample solution A (for Injection labeled as containing 40 USP Insulin Units/mL): Add 2.5 µL of 9.6 N hydrochloric acid for each milliliter of an accurately measured volume of Injection. Allow the suspension, if present, to clarify, and mix.

Sample solution B (for Injection labeled as containing 100 USP Insulin Units/mL): Add 2.5 µL of 9.6 N hydrochloric acid for each milliliter of an accurately measured volume of Injection. Allow the suspension, if present, to clarify, and mix. [NOTE—Pooling several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between insulin and A-21 desamido insulin, *System suitability solution*
Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*
Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis
Samples: *Identification solution*, *Standard solution*, and either *Sample solution A* or *Sample solution B*
Measure the peak responses for insulin and A-21 desamido insulin using the chromatogram of the *Identification solution* to identify the insulin peaks.
▲▲ (RB 1-May-2019) Calculate the potency, in USP Insulin Units/mL, in the portion of Injection taken:

$$\text{Result} = (\Sigma r_U/\Sigma r_S) \times C_S \times D$$

r_U = sum of the peak responses of insulin and A-21 desamido insulin from the *Sample solution*
r_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*
C_S = concentration of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in the *Standard solution* (USP Insulin Units/mL)
D = dilution factor used to prepare the *Sample solution*

▲▲ (RB 1-May-2019)
Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Units/mL

OTHER COMPONENTS

Change to read:

• **ZINC DETERMINATION** ⟨591⟩**:** 10–40 µg for every 100 USP Insulin Units ▲▲ (RB 1-May-2019)

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Limit of High Molecular Weight Proteins***:** Proceed as directed in the chapter, except for the *Sample solution*. It meets the requirements.
Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each milliliter of an accurately measured volume of Injection, and mix.
Acceptance criteria: NMT 2.0%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 7.0–7.8
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 80 USP Endotoxin Units per 100 USP Insulin Units
• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** Meets the requirements
• **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

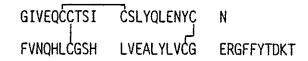
Change to read:

• **LABELING:** Label it ▲▲ (RB 1-May-2019) as porcine ▲▲ (RB 1-May-2019). If the Insulin Injection is made from insulin that is purified, label it as such. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units/mL.

Change to read:

• **USP REFERENCE STANDARDS** ⟨11⟩
▲▲ (RB 1-May-2019)
USP Insulin Pork RS

Insulin Aspart



$C_{256}H_{381}N_{65}O_{79}S_6$ 5825.54
28^B-L-Aspartic acid-insulin (human) [116094-23-6].

DEFINITION

Insulin Aspart is a two-chain peptide containing 51 amino acids. The A-chain is composed of 21 amino acids, and the

USP Monographs

www.webofpharma.com

B-chain is composed of 30 amino acids. It is identical in primary structure to Insulin Human, except that it has an aspartic acid instead of proline at position 28 of the B-chain. As in human insulin, insulin aspart contains two interchain disulfide bonds, and one intrachain disulfide bond.

Insulin Aspart is produced by methods based on recombinant DNA technology. The presence of host cell DNA in Insulin Aspart is process specific. The capability of the process to clear host-derived DNA requires validation and is determined by validated methods. The host cell-derived protein content is below the limit approved by the competent authority. The content of single-chain precursor is determined by a suitably sensitive method and is below the limit approved by the competent authority. Insulin Aspart contains NLT 90.0% and NMT 104.0% of insulin aspart ($C_{256}H_{381}N_{65}O_{79}S_6$) plus A21Asp insulin aspart, B3Asp insulin aspart, B3isoAsp insulin aspart, and B28isoAsp insulin aspart, on the dried basis. [NOTE—1 USP Insulin Aspart Unit is equivalent to 0.0350 mg of pure insulin aspart.]

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Peptide Mapping*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
60	30	70
65	0	100
70	0	100

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 8.0 between the peaks indicated as fragments II and III
Tailing factor: NMT 1.5 for the peaks indicated as fragments II and III
Chromatogram similarity: In the chromatogram from the *Standard solution*, identify the peaks due to digest fragments I, II, III, and IV. The chromatogram of the *Standard solution* corresponds to that of the typical chromatogram provided with USP Insulin Aspart RS.
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Solution A: Dissolve 142.0 g of anhydrous sodium sulfate in water, add 13.5 mL of phosphoric acid, and dilute with water to 5 L. Adjust, if necessary, with 10 N sodium hydroxide solution to a pH of 3.6. Mix 9 volumes of this solution with 1 volume of acetonitrile.
Solution B: Acetonitrile and water (1:1)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	58	42
35	58	42
40	20	80
45	20	80
46	58	42
60	58	42

[NOTE—Adjust the *Mobile phase* composition and the duration of the isocratic elution to obtain a retention time of about 22 min for the insulin aspart and to ensure that B3isoAsp insulin aspart is eluted before the gradient starts.]
System suitability solution: Use an appropriate solution with a content of B3Asp insulin aspart and A21Asp insulin aspart of NLT 1.0%. This may be achieved by storing the *Standard solution* at room temperature for about 1–3 days.
Standard solution: Dissolve the contents of a vial of USP Insulin Aspart RS in 0.01 N hydrochloric acid to obtain a concentration of 4.0 mg/mL.
Sample solution: Dissolve the sample to be examined in 0.01 N hydrochloric acid to obtain a concentration of 4.0 mg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.0-mm × 25.0-cm; 5-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for B28isoAsp insulin aspart, insulin aspart, B3Asp insulin aspart plus A21Asp insulin aspart (generally coelute), and B3isoAsp insulin aspart are about 0.9, 1.0, 1.3, and 1.5 min, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the peak for insulin aspart and the peak for A21Asp insulin aspart and B3Asp insulin aspart, *System suitability solution*
Tailing factor: NMT 1.5 for the insulin aspart peak, *System suitability solution*
Relative standard deviation: NMT 1.4% for five replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of insulin aspart ($C_{256}H_{381}N_{65}O_{79}S_6$) plus B28isoAsp insulin aspart, A21Asp insulin aspart, B3Asp insulin aspart, and B3isoAsp insulin aspart using the areas of the corresponding peaks in the chromatograms from the *Sample solution* and the *Standard solution* and the declared content of insulin aspart plus B28isoAsp insulin aspart, A21Asp insulin aspart, B3Asp insulin aspart, and B3isoAsp insulin aspart in the portion of Insulin Aspart taken:

$$\text{Result} = (r_{IA(U)} + r_{B28iso(U)} + r_{A21(U)} + r_{B3(U)} + r_{B3iso(U)})/(r_{IA(S)} + r_{B28iso(S)} + r_{A21(S)} + r_{B3(S)} + r_{B3iso(S)}) \times (C_S/C_U) \times 100$$

$r_{IA(U)}$ = peak response of insulin aspart from the *Sample solution*

Stop following USP

www.webofpharma.com

$r_{B28iso(U)}$ = peak response of B28isoAsp insulin aspart from the *Sample solution*
$r_{A21(U)}$ = peak response of A21Asp insulin aspart from the *Sample solution*
$r_{B3(U)}$ = peak response of B3Asp insulin aspart from the *Sample solution*
$r_{B3iso(U)}$ = peak response of B3isoAsp insulin aspart from the *Sample solution*
$r_{IA(S)}$ = peak response of insulin aspart from the *Standard solution*
$r_{B28iso(S)}$ = peak response of B28isoAsp insulin aspart from the *Standard solution*
$r_{A21(S)}$ = peak response of A21Asp insulin aspart from the *Standard solution*
$r_{B3(S)}$ = peak response of B3Asp insulin aspart from the *Standard solution*
$r_{B3iso(S)}$ = peak response of B3isoAsp insulin aspart from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–104.0% on the dried basis

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 6.0% determined on 0.2 g (dried substance)
- **PRODUCT-RELATED SUBSTANCES**

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay* using the normalization procedure.

Acceptance criteria

Individual impurities: NMT 1.0% of B28isoAsp insulin aspart; NMT 2.0% total of the peaks due to A21Asp insulin aspart, B3Asp insulin aspart, and B3isoAsp insulin aspart

Total of other impurities: NMT 1.5%

- **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Limit of High Molecular Weight Proteins***:** Meets the requirements; NMT 0.5%

SPECIFIC TESTS

- **INSULIN ASSAYS** ⟨121⟩, *Assay, Bioidentity Test***:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** It contains NMT 10 USP Endotoxin Units/mg of Insulin Aspart.
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic count does not exceed 300 cfu/g, the test being performed on a portion of about 0.2 g.
- **LOSS ON DRYING** ⟨731⟩

Sample: About 200 mg
Analysis: Dry the *Sample* at 105° for 24 h.
Acceptance criteria: NMT 10.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store in a freezer and protect from light.
- **LABELING:** Label it to indicate that it has been produced by methods based on recombinant DNA technology.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Insulin Aspart RS

Insulin Aspart Injection

DEFINITION

Insulin Aspart Injection is an isotonic, sterile solution of Insulin Aspart in Water for Injection. It has a potency of NLT 95% and NMT 105% of the potency stated on the label, expressed in USP Insulin Aspart Units/mL. [NOTE—1 USP Insulin Aspart Unit is equivalent to 0.0350 mg of pure insulin aspart.]

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: Dissolve 70 g of anhydrous sodium sulfate in approximately 4500 mL of water, add 6.5 mL of phosphoric acid, and adjust with sodium hydroxide TS to a pH of 3.4. Dilute with water to 5000 mL. Mix 900 mL of this solution with 100 mL of acetonitrile.
Solution B: Acetonitrile and water (1:1)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	56	44
35	56	44
40	20	80
45	20	80
46	56	44
60	56	44

[NOTE—If necessary, adjust the *Mobile phase* composition to obtain a retention time of insulin aspart of 20–26 min and to ensure that the preservatives are well separated from B28isoAsp insulin aspart peak. If necessary, adjust the start time of the gradient to ensure that B3isoAsp insulin aspart is eluted before the gradient starts.]

System suitability solution: Use an appropriate solution with a content of B3Asp insulin aspart and A21Asp insulin aspart of NLT 1%. This may be achieved by storing the *Standard solution* at room temperature for about 1–3 days.
Standard solution: Dissolve the contents of 1 vial of USP Insulin Aspart RS in 0.01 N hydrochloric acid to obtain a known concentration of 100 USP Insulin Aspart Units/mL. Add 4 µL of 6 N hydrochloric acid per milliliter, and mix.
Sample solution: Acidify each milliliter of Injection with 4 µL of 6 N hydrochloric acid. Dilute, if necessary, a portion of the acidified solution with 0.01 N hydrochloric acid to obtain a solution containing about 100 USP Insulin Aspart Units/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.0-mm × 25.0-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for B28isoAsp insulin aspart, insulin aspart, B3Asp insulin aspart plus A21Asp insulin aspart (generally coelute), and B3isoAsp insulin aspart are about 0.9, 1.0, 1.3, and 1.5 min, respectively.]

USP Monographs

www.webofpharma.com

Suitability requirements
Resolution: NLT 1.6 between the peak for insulin aspart and the peak for A21Asp insulin aspart and for B3Asp insulin aspart, *System suitability solution*
Relative standard deviation: NMT 1.4% for 5 replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the potency, in USP Insulin Aspart Units/mL, of Injection taken:

$$\text{Result} = C_S \times D \times (r_U/r_S)$$

C_S = concentration of insulin aspart in the *Standard solution* (USP Insulin Aspart Units/mL)
D = dilution factor used to prepare the *Sample solution*
r_U = peak area of insulin aspart (sum of B28isoAsp insulin aspart, insulin aspart, B3Asp insulin aspart, A21Asp insulin aspart, and B3isoAsp insulin aspart peak areas) from the *Sample solution*
r_S = peak area of insulin aspart (sum of B28isoAsp insulin aspart, insulin aspart, B3Asp insulin aspart, A21Asp insulin aspart, and B3isoAsp insulin aspart peak areas) from the *Standard solution*

Acceptance criteria: 95%–105% of the potency stated on the label, expressed in USP Insulin Aspart Units/mL

IMPURITIES

- **Related Proteins**

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay* using the normalization procedure.
Acceptance criteria
Individual impurities: NMT 2.5% of B28isoAsp insulin aspart; NMT 5.0% total of the peaks due to A21Asp insulin aspart, B3Asp insulin aspart, and B3isoAsp insulin aspart
Total of other impurities: NMT 3.5%

- **Physicochemical Analytical Procedures for Insulins** ⟨121.1⟩, *Limit of High Molecular Weight Proteins*: Meets the requirements

Sample solution: Acidify each milliliter of Injection with 4 µL of 6 N hydrochloric acid. Dilute, if necessary, a portion of the acidified solution with 0.01 N hydrochloric acid to obtain a solution containing about 100 USP Insulin Aspart Units/mL.
Acceptance criteria: NMT 1.5%

SPECIFIC TESTS

- **Bacterial Endotoxins Test** ⟨85⟩: NMT 80 USP Endotoxin Units per 100 USP Insulin Aspart Units
- **Sterility Tests** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **Particulate Matter in Injections** ⟨788⟩: Meets the requirements for small-volume injections
- **pH** ⟨791⟩: 7.0–7.8, determined potentiometrically

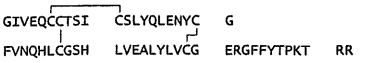
Change to read:

- **▲Zinc Determination** ⟨591⟩:▲ (IRA 1-Jan-2019) 10–40 µg for every 100 USP Insulin Aspart Units
- **Injections and Implanted Drug Products** ⟨1⟩: It meets the requirements.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in the unopened, multi-dose container provided by the manufacturer. Store in a refrigerator, protect from sunlight, and avoid freezing.
- **Labeling:** The label states that it has been prepared with insulin aspart obtained from microbial synthesis; it is to be stored in a refrigerator and that freezing is to be avoided; the potency, expressed in USP Insulin Aspart Units/mL.
- **USP Reference Standards** ⟨11⟩
USP Insulin Aspart RS

Insulin Glargine



$C_{267}H_{404}N_{72}O_{78}S_6$ 6062.89
Insulin (human), 21^A-glycine-30^{B}a-L-arginine-30^{B}b-L-arginine [160337-95-1].

DEFINITION

Insulin Glargine is a two-chain peptide containing 53 amino acids. The A-chain is composed of 21 amino acids, and the B-chain is composed of 32 amino acids. It is identical to the primary structure of Human Insulin except for position A21 which has Gly rather than Asn as in Human Insulin and two additional amino acids at the C terminal of the B-chain Arg (B31) and Arg (B32). Insulin Glargine is produced by methods based on recombinant DNA technology. Residual host cell protein (HCP) content is determined by a validated method and is NMT 10 ppm (ng HCP per mg of Insulin Glargine). Insulin Glargine contains NLT 94.0% and NMT 105.0% of insulin glargine ($C_{267}H_{404}N_{72}O_{78}S_6$), calculated on the anhydrous basis, or on the dried basis when other volatile solvents in addition to water are present.
[NOTE—One USP Insulin Glargine Unit is equivalent to 0.0364 mg of pure Insulin Glargine.]

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. Peptide Mapping**

Phosphate/perchlorate buffer: Dissolve 11.6 g of phosphoric acid and 42.1 g of sodium perchlorate in 1600 mL of water. Adjust with triethylamine to a pH of 2.3, and dilute with water to a final volume of 2000 mL.
Solution A: Prepare a filtered and degassed mixture of acetonitrile and *Phosphate/perchlorate buffer* (7:93).
Solution B: Prepare a filtered and degassed mixture of acetonitrile and *Phosphate/perchlorate buffer* (57:43).
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
30	20	80
35	20	80
36	90	10

Tris buffer solution: Dissolve 12.11 g of tris(hydroxymethyl)aminomethane in 90 mL of water. Adjust with hydrochloric acid to a pH of 7.5, and dilute with water to a final volume of 100 mL.
Enzyme solution: Prepare a solution of *Staphylococcus aureus* V-8 protease in *Tris buffer solution* having an activity of 20 Units/mL (using Z-Phe-Leu-Glu-4-nitroanilide as the substrate).
Standard solution: Transfer to a vial 35 µL of the *Standard solution* from the *Assay*. To this vial, add 1.0 mL of *Tris buffer*

USP Monographs

www.webofpharma.com

solution and 100 µL of *Enzyme solution*, and incubate at 45° for 2–3 h. Quench the digestion by adding 2 µL of phosphoric acid.

Sample solution: Transfer to a vial 35 µL of the *Sample solution* from the *Assay*. To this vial, add 1.0 mL of *Tris buffer solution* and 100 µL of *Enzyme solution*, and incubate at 45° for 2–3 h. Quench the digestion by adding 2 µL of phosphoric acid.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 3.0-mm × 12.5-cm; 4-µm packing L1
Column temperature: 35°
Flow rate: 0.6 mL/min
Injection volume: 50 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.4 between the peaks indicated as fragments II and III
Tailing factor: NMT 1.5 for the peaks indicated as fragments II and III
Chromatogram similarity: In the chromatogram from the *Standard solution*, identify the peaks due to digest fragments I, II, III, and IV. The chromatogram of the *Standard solution* corresponds to that of the typical chromatogram provided with USP Insulin Glargine RS.

Analysis
Samples: *Standard solution* and *Sample solution*
Run a blank, and record the chromatograms.

Acceptance criteria: The chromatographic profile of the *Sample solution* corresponds to that of the *Standard solution*. All four fragments, I, II, III, and IV must be present.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 20.7 g of anhydrous monobasic sodium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 2.5, and dilute with water to a final volume of 1000 mL.

Solution A: Dissolve 18.4 g of sodium chloride in 250 mL of *Buffer*, add 250 mL of acetonitrile, and mix. Dilute the solution with water to a final volume of 1000 mL.

Solution B: Dissolve 3.2 g of sodium chloride in 250 mL of *Buffer*, add 650 mL of acetonitrile, and mix. Dilute the solution with water to a final volume of 1000 mL.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	96	4
20	83	17
30	63	37
33	96	4

[NOTE—Adjust the *Mobile phase* composition and the gradient by a parallel shift to obtain a retention time of 18–23 min for the insulin glargine main peak.]

System suitability solution: Dissolve the contents of 1 vial of USP Insulin Glargine for Peak Identification RS in 0.3 mL of 0.01 N hydrochloric acid, and add 1.7 mL of water.

Standard solution: Dissolve the contents of 1 vial of USP Insulin Glargine RS in 1.5 mL of 0.01 N hydrochloric acid, transfer the solution to a 10-mL volumetric flask, and dilute with water to volume.

Sample solution: Dissolve 15 mg of Insulin Glargine in 1.5 mL of 0.01 N hydrochloric acid, and dilute with water to a final volume of 10 mL.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 3.0-mm × 25.0-cm; 4-µm packing L1
Column temperature: 35°
Flow rate: 0.6 mL/min
Injection volume: 5 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 for the ratio of the height of the 0 ^{A}-Arg-insulin glargine peak to the height of the valley between the 0 ^{A}-Arg-insulin glargine peak and the insulin glargine peak, *System suitability solution*
Tailing factor: NMT 1.8 for the insulin glargine peak, *System suitability solution*
Relative standard deviation: NMT 2.0% for six replicate injections, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the potency, in percent, of insulin glargine ($C_{267}H_{404}N_{72}O_{78}S_6$) in the portion of Insulin Glargine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of insulin glargine from the *Sample solution*
r_S = peak response of insulin glargine from the *Standard solution*
C_S = concentration of USP Insulin Glargine RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (corrected for the water content or loss on drying) (mg/mL)

Acceptance criteria: 94.0%–105.0% on the anhydrous basis or dried basis

OTHER COMPONENTS

• **ZINC DETERMINATION**

Standard stock solution: 10 µg/mL of zinc in 0.01 N hydrochloric acid, from a commercially available zinc standard solution for atomic absorption

Standard solutions: 0.2, 0.4, and 0.6 µg/mL of zinc from the *Standard stock solution* diluted with 0.01 N hydrochloric acid

Sample solution: Dissolve 45 mg of Insulin Glargine, accurately weighed, in 50 mL of 0.01 N hydrochloric acid. Dilute 10 mL of the solution with 0.01 N hydrochloric acid to a final volume of 100 mL.

Blank: 0.01 N hydrochloric acid

Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Zinc absorption line at 213.9 nm
Flame: Air–acetylene flame of suitable composition (for example, 11 L of air and 2 L of acetylene per min)
Lamp: Suitable radiation source such as zinc hollow-cathode or electrodeless-discharge-lamp (EDL)

System suitability
Samples: *Standard solutions* and *Blank*
Using the *Standard solutions* and *Blank*, construct a calibration curve by plotting the absorbances of the *Standard solutions* versus their concentrations, and draw the straight line best fitting the three plotted points.

USP Monographs

www.webofpharma.com

Suitability requirements
Correlation coefficient: NLT 0.999
Analysis
Samples: *Standard solutions*, *Sample solution*, and *Blank*
Determine the concentration, C, in µg/mL of zinc in the *Sample solution* using the calibration curve.
Calculate the percentage of zinc in the portion of Insulin Glargine taken:

$$\text{Result} = [C \times F_1 \times V \times (F_2/W)] \times 100$$

C = concentration of zinc in the *Sample solution* (µg/mL)
F_1 = conversion factor from µg/mL to mg/mL, 0.001
V = volume of the *Sample solution*, 100 mL
F_2 = sampling factor, 5
W = weight of Insulin Glargine taken (mg)

Acceptance criteria: NMT 0.80%

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **PRODUCT-RELATED SUBSTANCES**
Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Sample: *Sample solution*
Calculate the percentage of each individual insulin glargine related substance ($\%i_x$) in the portion of Insulin Glargine taken:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = peak response of the insulin glargine related substance from the *Sample solution*
r_T = sum of the responses for all of the peaks from the *Sample solution*

Calculate the total percentage of insulin glargine related substances in the portion of Insulin Glargine taken:

$$\text{Result} = \Sigma\%i_x$$

$\Sigma\%i_x$ = total percentage of insulin glargine related substances from the *Sample solution*

Acceptance criteria
Any individual insulin glargine related substance: NMT 0.5%
Total insulin glargine related substances: NMT 1.5%

• **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**
Mobile phase: Prepare a mixture of acetonitrile, water, and glacial acetic acid (300:400:200). Adjust with concentrated ammonia (25%–30%) to a pH of 3.0, and dilute with water to a final volume of 1000 mL.
System suitability solution: Dissolve 15 mg of Insulin Glargine containing more than 0.4% high molecular weight proteins in 1.5 mL of 0.01 N hydrochloric acid. Dilute with water to a final volume of 10 mL. [NOTE—Insulin Glargine containing the indicated percentage of high molecular weight proteins may be prepared by incubating Insulin Glargine at 100° for 1.5–3 h.]
Sample solution: Dissolve 15 mg of Insulin Glargine in 1.5 mL of 0.01 N hydrochloric acid. Dilute with water to a final volume of 10 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 276 nm
Column: Two 8.0-mm × 30-cm in series; 5-µm packing L20
Column temperature: Ambient
Flow rate: 0.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *System suitability solution*
[NOTE—The retention time for the insulin monomer is about 35 min, and the high molecular weight proteins elute earlier.]
Suitability requirements
Resolution: The ratio of the height of the high molecular weight proteins peak to the height of the valley between the high molecular weight proteins peak and the insulin glargine peak is NLT 2.
Tailing factor: NMT 2.0 for the insulin glargine peak
Analysis
Sample: *Sample solution*
Measure the areas of the peak responses, disregarding any peaks having retention times greater than that of the insulin monomer.
Calculate the percentage of high molecular weight proteins in the portion of Insulin Glargine taken:

$$\text{Result} = [\Sigma r_H/(\Sigma r_H + r_U)] \times 100$$

Σr_H = sum of the responses for all peaks having retention times less than that of insulin glargine from the *Sample solution*
r_U = peak response of insulin glargine from the *Sample solution*

Acceptance criteria: NMT 0.3%

SPECIFIC TESTS

• **INSULIN ASSAYS** ⟨121⟩, *Bioidentity Test*: Meets the requirements
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 10 USP Endotoxin Units/mg of Insulin Glargine
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total bacterial count does not exceed 300 cfu/g, the test being performed on a portion of about 0.2 g, accurately weighed.
• **WATER DETERMINATION** ⟨921⟩, *Method Ic*: NMT 8.0%. [NOTE—Use this test when the drug substance predominantly contains water.]
• **LOSS ON DRYING** ⟨731⟩: NMT 10.0%. [NOTE—Use this test when the drug substance contains water and other volatile solvents.]

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and store in a freezer.
• **LABELING:** Label it to indicate that the material is produced by methods based on recombinant DNA technology. Where it is a dried basis, the label so indicates.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Insulin Glargine RS
USP Insulin Glargine for Peak Identification RS
Contains insulin glargine and 0 ^{A}-Arg-insulin glargine.

Insulin Glargine Injection

DEFINITION

Insulin Glargine Injection is a sterile solution of Insulin Glargine in Water for Injection. It has a potency of NLT 95.0 and NMT 105.0 USP Insulin Glargine Units/mL.

www.webofpharma.com

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solutions*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 20.7 g of anhydrous monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 2.5, and dilute with water to a final volume of 1000 mL.

Solution A: Dissolve 18.4 g of sodium chloride in 250 mL of *Buffer*, add 250 mL of acetonitrile, and mix. Dilute the solution with water to a final volume of 1000 mL.

Solution B: Dissolve 3.2 g of sodium chloride in 250 mL of *Buffer*, add 650 mL of acetonitrile, and mix. Dilute the solution with water to a final volume of 1000 mL.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	96	4
20	83	17
30	63	37
40	96	4

[NOTE—Adjust the *Mobile phase* composition and the gradient by a parallel shift to obtain a retention time of 18–23 min for the insulin glargine main peak.]

System suitability solution: Dissolve the contents of 1 vial of USP Insulin Glargine for Peak Identification RS in 0.3 mL of 0.01 N hydrochloric acid, and add 1.7 mL of water.

Standard solution 1: Dissolve the contents of 1 vial of USP Insulin Glargine RS in 1.5 mL of 0.01 N hydrochloric acid, transfer the solution to a 5-mL volumetric flask, and dilute with water to volume. Dilute 4 mL of this solution with water to 10 mL in a volumetric flask.

Standard solution 2: Dissolve the contents of 1 vial of USP Insulin Glargine RS in 1.5 mL of 0.01 N hydrochloric acid, transfer the solution to a 10-mL volumetric flask, and dilute with water to volume.

Standard solution 3: Dissolve the contents of 1 vial of USP Insulin Glargine RS in 1.5 mL of 0.01 N hydrochloric acid, transfer the solution to a 5-mL volumetric flask, and dilute with water to volume. Dilute 3 mL of this solution with water to 5 mL in a volumetric flask.

Sample solution: Quantitatively dilute a portion of Injection with water to obtain a solution containing about 40 USP Insulin Glargine Units/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 3.0-mm × 25.0-cm; 4-µm packing L1

Column temperature: 35°

Flow rate: 0.55 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solutions*

Suitability requirements

Resolution: NLT 2.0 for the ratio of the height of the 0 ^{A}-Arg-insulin glargine peak to the height of the valley between the 0 ^{A}-Arg-insulin glargine peak and the insulin glargine peak, *System suitability solution*

Tailing factor: NMT 1.8 for the insulin glargine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, calculated from six response factors from two duplicate injections of each *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*

Analysis

Samples: *Standard solutions* and *Sample solution*

Measure the responses of the major peaks. Prepare a calibration curve based on the peak responses from the *Standard solutions* versus the concentrations (USP Insulin Glargine Units/mL) using linear regression.

Calculate the potency, in USP Insulin Glargine Units/mL, of the portion of Injection taken:

$$\text{Result} = [(r_U - b)/a] \times D$$

r_U = peak response of insulin glargine from the *Sample solution*

b = y-intercept of the calibration curve

a = slope of the calibration curve

D = dilution factor used to prepare the *Sample solution*

Acceptance criteria: 95.0–105.0 USP Insulin Glargine Units/mL

OTHER COMPONENTS

• **ZINC DETERMINATION**

Standard stock solution: 10 µg/mL of zinc in 0.01 N hydrochloric acid, from a commercially available zinc standard solution for atomic absorption

Standard solutions: 0.2, 0.4, and 0.6 µg/mL of zinc from the *Standard stock solution* diluted with 0.01 N hydrochloric acid

Sample solution: Dilute 1 mL of Injection with 0.01 N hydrochloric acid to 100 mL.

Blank: 0.01 N hydrochloric acid

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Zinc absorption line at 213.9 nm

Flame: Air–acetylene flame of suitable composition (for example, 11 L of air and 2 L of acetylene per min)

Lamp: Suitable radiation source, such as zinc hollow-cathode or electrodeless-discharge-lamp (EDL)

System suitability

Samples: *Standard solutions* and *Blank*

Using the *Standard solutions* and *Blank*, construct a calibration curve by plotting the absorbances of the *Standard solutions* versus their concentrations, and draw the straight line best fitting the three plotted points.

Suitability requirements

Correlation coefficient: NLT 0.999

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Determine the concentration, C, in µg/mL of zinc in the *Sample solution* using the calibration curve.

Calculate the quantity of zinc in the portion of Injection taken:

$$\text{Result} = C \times D$$

C = concentration of zinc in the *Sample solution* (µg/mL)

D = dilution factor, 100

Acceptance criteria: 20–40 µg/mL

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **PRODUCT-RELATED SUBSTANCES**

Mobile phase, System suitability solution, Standard solutions, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

www.webofpharma.com

Analysis
Sample: *Sample solution*
Calculate the percentage of each individual insulin glargine related substance ($\%i_X$) in the portion of Injection taken:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = peak response of the insulin glargine related substance from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Calculate the total percentage of insulin glargine related substances in the portion of Injection taken:

$$\text{Result} = \Sigma\%i_X$$

$\Sigma\%i_X$ = total percentage of insulin glargine related substances from the *Sample solution*

Acceptance criteria
Any individual insulin glargine related substance: NMT 0.5%
Total insulin glargine related substances: NMT 2.0%

- **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**

Mobile phase: Prepare a mixture of acetonitrile, water, and glacial acetic acid (300:400:200). Adjust with concentrated ammonia (25%–30%) to a pH of 3.0, and dilute with water to a final volume of 1000 mL.
System suitability solution: Dissolve 15 mg of insulin glargine containing more than 0.4% high molecular weight proteins in 1.5 mL of 0.01 N hydrochloric acid. Dilute with water to a final volume of 10 mL. [NOTE—Insulin glargine containing the indicated percentage of high molecular weight proteins may be prepared by incubating insulin glargine at 100° for 1.5–3 h.]
Sample solution: Quantitatively dilute a portion of Injection with water to obtain a solution containing about 40 USP Insulin Glargine Units/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 276 nm
Column: Two 8.0-mm × 30-cm in series; 5-µm packing L20
Column temperature: Ambient
Flow rate: 0.5 mL/min
Injection volume: 100 µL
System suitability
Sample: *System suitability solution*
[NOTE—The retention time for the insulin monomer is about 35 min, and the high molecular weight proteins elute earlier.]
Suitability requirements
Resolution: The ratio of the height of the high molecular weight proteins peak to the height of the valley between the high molecular weight proteins peak and the insulin glargine peak is NLT 2.
Tailing factor: NMT 2.0 for the insulin glargine peak
Analysis
Sample: *Sample solution*
Measure the areas of the peak responses, disregarding any peaks having retention times greater than that of the insulin monomer.
Calculate the percentage of high molecular weight proteins in the portion of Injection taken:

$$\text{Result} = [\Sigma r_H/(\Sigma r_H + r_U)] \times 100$$

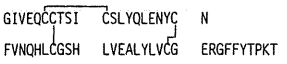
Σr_H = sum of the responses for all peaks having retention times less than that of insulin glargine from the *Sample solution*
r_U = peak response of insulin glargine from the *Sample solution*

Acceptance criteria: NMT 0.5%

SPECIFIC TESTS
- **pH** ⟨791⟩: 3.5–4.5
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 80 USP Endotoxin Units per 100 USP Insulin Glargine Units
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protected from sunlight, and avoid freezing.
- **LABELING:** States that it has been prepared with Insulin Glargine produced by methods based on recombinant DNA technology. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Glargine Units/mL.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Insulin Glargine RS
USP Insulin Glargine for Peak Identification RS
Contains insulin glargine and 0 ^{A}-Arg-insulin glargine.

Insulin Human



$C_{257}H_{383}N_{65}O_{77}S_6$ 5807.57
Insulin (human) [11061-68-0].

DEFINITION
Insulin Human is a two-chain peptide hormone consisting of 51 amino acids, and its structure corresponds to native insulin produced *in vivo* by the beta cells of the pancreas. The A-chain is composed of 21 amino acids, and the B-chain is composed of 30 amino acids. It is either produced by methods based on recombinant DNA technology or derived by enzymatic modification of insulin from porcine pancreas to change the amino acid sequence appropriately. The presence of host cell DNA in Insulin Human is process-specific. The capability of the process to clear host-derived DNA requires validation and is determined by validated methods. Its potency is NLT 27.5 USP Insulin Human Units/mg, calculated on the dried basis.
[NOTE—One USP Insulin Human Unit is equivalent to 0.0347 mg of pure Insulin Human.]

IDENTIFICATION
- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Peptide Mapping*

Proceed as directed, except use the following *Mobile phase* and *System suitability*. It meets the requirements.
Mobile phase: See *Table 1*.

USP Monographs

www.webofpharma.com

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
60	30	70
65	0	100
70	0	100
71	90	10
86	90	10

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.4 between digest fragments II and III
Tailing factor: NMT 1.5 for digest fragments II and III
Chromatogram similarity: Identify the peaks due to digest fragments I, II, III, and IV in the *Standard solution*. The chromatogram of the *Standard solution* corresponds to that of the typical chromatogram provided with USP Insulin Human RS.

ASSAY

• **PROCEDURE**

Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.
Mobile phase: Acetonitrile and *Solution A* (26:74). [NOTE—The acetonitrile is warmed to NLT 20° to avoid precipitation.]
System suitability solution: 1.5 mg/mL of Insulin Human in 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin human.
Standard solution: 1.5 mg/mL of USP Insulin Human RS in 0.01 N hydrochloric acid
Sample solution: 1.5 mg/mL of Insulin Human in 0.01 N hydrochloric acid
[NOTE—The *Standard solution* and *Sample solution* may be stored at room temperature for up to 12 h, or in a refrigerator for up to 48 h.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between insulin human and A-21 desamido insulin human, *System suitability solution*
Tailing factor: NMT 1.8 for the insulin human peak, *System suitability solution*
Relative standard deviation: NMT 1.6%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Measure the peak responses for insulin human and A-21 desamido insulin human.
Calculate the potency on the undried basis, in USP Insulin Human Units/mg, of Insulin Human in the *Sample solution*:

$$\text{Result} = (\Sigma r_U/\Sigma r_S) \times (C_S/C_U)$$

Σr_U = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Sample solution*
Σr_S = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Standard solution*
C_S = concentration of USP Insulin Human RS in the *Standard solution* (USP Insulin Human Units/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NLT 27.5 USP Insulin Human Units/mg, on the dried basis

OTHER COMPONENTS

• **ZINC DETERMINATION** ⟨591⟩
Sample: 10 mg
Acceptance criteria: NMT 1.0% on the dried basis

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **RELATED SUBSTANCES**
Solvent: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.
Solution A: Acetonitrile and *Solvent* (18:82)
Solution B: Acetonitrile and *Solvent* (50:50)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	78	22
36	78	22
61	36	64
67	36	64
68	78	22
78	78	22

System suitability solution: 1.5 mg/mL of Insulin Human in 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin human.
Standard solution A: 3.75 mg/mL of USP Insulin Human RS in 0.01 N hydrochloric acid
Standard solution B: Pipet 1 mL of *Standard solution A* into a 10-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix (0.375 mg/mL).
Standard solution C: Pipet 1 mL of *Standard solution B* into a 10-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix (0.0375 mg/mL).
[NOTE—The three Standard solutions may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]
Sample solution: 3.75 mg/mL of Insulin Human in 0.01 N hydrochloric acid. Prepare the solution in a capped vial, cap the vial, and shake gently to dissolve. Store the solution at room temperature for NMT 2 h, or in a refrigerator for NMT 12 h.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL

USP Monographs

www.webofpharma.com

System suitability
Adjust the *Mobile phase* composition and the duration of the isocratic elution to obtain a retention time between 15 and 25 min for the main insulin human peak, with A-21 desamido insulin human eluting just before the start of the gradient elution phase.
Samples: *System suitability solution, Standard solution A, Standard solution B,* and *Standard solution C*
Suitability requirements for the *System suitability solution*
Resolution: NLT 2.0 between insulin human and A-21 desamido insulin human
Tailing factor: NMT 1.8 for the insulin human peak
Suitability requirements for the Standard solutions
Calculate the factor X_1:

$$X_1 = (r_B/r_A) \times D$$

r_B = peak response from *Standard solution B*
r_A = peak response from *Standard solution A*
D = dilution factor, 10

Result: Between 0.91 and 1.09
Calculate the factor X_2:

$$X_2 = (r_C/r_A) \times D$$

r_C = peak response from *Standard solution C*
r_A = peak response from *Standard solution A*
D = dilution factor, 100

Result: Between 0.7 and 1.3
Analysis
Sample: *Sample solution*
Calculate the percentage of insulin human, A-21 desamido insulin human, and other impurities in the portion of Insulin Human taken.
Calculate the percentage of insulin human (%I):

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = peak response of insulin human
r_T = sum of the responses of all the peaks

Calculate the percentage of A-21 desamido insulin human (%D):

$$\text{Result} = (r_D/r_T) \times 100$$

r_D = peak response of A-21 desamido insulin human
r_T = sum of the responses of all the peaks

Calculate the percentage of other insulin human related substances:

$$\text{Result} = 100 - (\%I + \%D)$$

Acceptance criteria
Individual impurities: NMT 2.0% of A-21 desamido insulin human
Total impurities: NMT 2.0%, excluding A-21 desamido insulin human
• **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Limit of High Molecular Weight Proteins*: Meets the requirements
Acceptance criteria: NMT 1.0%

PROCESS-RELATED IMPURITIES
• **SINGLE-CHAIN PRECURSOR CONTENT:** The single-chain precursor content of Insulin Human produced by recombinant DNA technology or the proinsulin content of Insulin Human derived from porcine is NMT 10 ng/mg, determined by a validated method.
• **HOST CELL PROTEIN:** The residual host cell protein content is NMT 10 ng/mg, determined by a validated method or demonstrated by a validated process.

SPECIFIC TESTS
• **INSULIN ASSAYS** ⟨121⟩, *Bioidentity Test*: Meets the requirements
• **LOSS ON DRYING** ⟨731⟩
Sample: 200 mg
Analysis: Dry the *Sample* at 105° for 16 h.
Acceptance criteria: NMT 10.0%
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 10 USP Endotoxin Units/mg of Insulin Human
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total bacterial count does not exceed 300 cfu/g, the test being performed on a portion of 0.2 g, accurately weighed.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers. Store in a freezer and protect from light.
• **LABELING:** Label it to indicate that it has been produced by methods based on recombinant DNA technology or that it is derived by enzymatic modification of insulin from porcine pancreas.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Insulin Human RS

Insulin Human Injection

DEFINITION
Insulin Human Injection is an isotonic, sterile solution of Insulin Human in Water for Injection. It has a potency of NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Human Units/mL.

IDENTIFICATION
• **A.** The retention time of the major peak of *Sample solution A* or *Sample solution B* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.
Mobile phase: Acetonitrile and *Solution A* (26:74). [NOTE—The acetonitrile is warmed to NLT 20° to avoid precipitation.]
System suitability solution: 1.5 mg/mL of insulin human in 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin human.
Standard solution: 1.5 mg/mL of USP Insulin Human RS in 0.01 N hydrochloric acid
Sample solution A (for Injection labeled as containing 40 USP Insulin Human Units/mL): Add 2.5 µL of 9.6 N hydrochloric acid for each mL of an accurately measured volume of Injection. Allow the suspension, if present, to clarify, and mix.
Sample solution B (for Injection labeled as containing 100 USP Insulin Human Units/mL): Add 2.5 µL of 9.6 N hydrochloric acid for each mL of an accurately measured volume of Injection. Allow the suspension, if present, to

USP Monographs

www.webofpharma.com

clarify, and mix. [NOTE—Pooling several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between insulin human and A-21 desamido insulin human, *System suitability solution*
Tailing factor: NMT 1.8 for the insulin human peak, *System suitability solution*
Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis
Samples: *Standard solution* and either *Sample solution A* or *Sample solution B*
Measure the peak responses for insulin human and A-21 desamido insulin human. Calculate the potency, in USP Insulin Human Units/mL, of the Injection taken:

$$\text{Result} = (\Sigma r_U / \Sigma r_S) \times C_S \times D$$

Σr_U = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Sample solution*
Σr_S = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Standard solution*
C_S = concentration of USP Insulin Human RS in the *Standard solution* (USP Insulin Human Units/mL)
D = dilution factor used to prepare the *Sample solution*

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Human Units/mL

OTHER COMPONENTS
- **ZINC DETERMINATION** ⟨591⟩**:** 10–40 µg for every 100 USP Insulin Human Units

PRODUCT-RELATED SUBSTANCES AND IMPURITIES
- **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS,** *Limit of High Molecular Weight Proteins* ⟨121.1⟩

Proceed as directed in *Limit of High Molecular Weight Proteins*, except prepare the following *Sample solution*. It meets the requirements.
Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each mL of an accurately measured volume of Injection, and mix.
Acceptance criteria: NMT 1.7%

SPECIFIC TESTS
- **pH** ⟨791⟩**:** 7.0–7.8
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 80 USP Endotoxin Units/100 USP Insulin Human Units
- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve and dispense in the unopened, multiple-dose container provided by the manufacturer. Store in a refrigerator, protect from sunlight, and avoid freezing.
- **LABELING:** Label it to indicate that it has been prepared with Insulin Human produced by methods based on recombinant DNA technology or that it is derived by enzymatic modification of insulin from porcine pancreas. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Human Units/mL.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Insulin Human RS

Human Insulin Isophane Suspension and Human Insulin Injection

DEFINITION
Human Insulin Isophane Suspension and Human Insulin Injection is a sterile buffered suspension of Insulin Human, complexed with Protamine Sulfate, in a solution of Insulin Human. Its potency, based on the sum of its insulin and desamido insulin components as determined in the *Assay*, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Human Units/mL.

IDENTIFICATION
- **A.** The retention time of the major peak of *Sample solution A* or *Sample solution B* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**

Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into this solution, and adjust with ethanolamine to a pH of 2.3, if necessary.
Mobile phase: Acetonitrile and *Solution A* (26:74). [NOTE—The acetonitrile is warmed to NLT 20° to avoid precipitation.]
System suitability solution: 1.5 mg/mL of insulin human in 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin human.
Standard solution: 1.5 mg/mL of USP Insulin Human RS in 0.01 N hydrochloric acid
Sample solution A (for Injection labeled as containing 40 USP Insulin Human Units/mL)**:** Add 2.5 µL of 9.6 N hydrochloric acid to each milliliter of an accurately measured volume of Injection. Allow the suspension to clarify, and mix.
Sample solution B (for Injection labeled as containing 100 USP Insulin Human Units/mL)**:** Add 2.5 µL of 9.6 N hydrochloric acid to each milliliter of an accurately measured volume of Injection. Allow the suspension to clarify, and mix. [NOTE—Pooling of several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 40°

USP Monographs

www.webofpharma.com

Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between insulin human and A-21 desamido insulin human, *System suitability solution*
Tailing factor: NMT 1.8 for the insulin human peak, *System suitability solution*
Relative standard deviation: NMT 1.6%, *Standard solution*
Analysis
Samples: *Standard solution* and either *Sample solution A* or *Sample solution B*
Calculate the potency, in USP Insulin Human Units/mL, of Injection taken:

$$\text{Result} = (\Sigma r_U/\Sigma r_S) \times C_S \times D$$

r_U = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Sample solution*
r_S = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Standard solution*
C_S = concentration of USP Insulin Human RS in the *Standard solution* (USP Insulin Human Units/mL)
D = dilution factor used to prepare the *Sample solution*

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Human Units/mL

OTHER COMPONENTS

Change to read:

• **▲ZINC DETERMINATION** ⟨591⟩:▲ (IRA 1-Jan-2019) 0.02–0.04 mg for every 100 USP Insulin Human Units

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Limit of High Molecular Weight Proteins*
Proceed as directed in the chapter, except for the *Sample solution*. It meets the requirements.
Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each milliliter of an accurately measured volume of Injection, and mix.
Acceptance criteria: NMT 3.0%

SPECIFIC TESTS

• **SOLUBLE INSULIN HUMAN CONTENT**
[NOTE—Use one of the two methods listed below.]
Method 1
Mobile phase, System suitability solution, Standard solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Soluble insulin sample solution: Maintain the temperature at 25 ± 1° throughout the *Analysis*. Transfer 5.0 mL of Injection to a centrifuge tube. Add 20 µL of 1 N sodium hydroxide, and adjust with 0.05 N hydrochloric acid or 0.05 N sodium hydroxide to a pH of 8.20 ± 0.02 if the total zinc concentration is approximately 20 µg/mL, or adjust to a pH of 8.35 ± 0.02 if the total zinc concentration is approximately 30 µg/mL. Record the volume (V_A), in µL, of acid or base needed to adjust the pH. Allow to stand for 1 h. Centrifuge, transfer the supernatant to another centrifuge tube, and repeat the centrifugation. Transfer 2 mL of the supernatant to another tube, add 5 µL of 9.6 N hydrochloric acid, and mix.
Total insulin sample solution: Transfer 2 mL of Injection to a suitable vessel, add 5 µL of 9.6 N hydrochloric acid, and allow the suspension to clarify. Dilute the resulting solution with 0.01 N hydrochloric acid to the same theoretical concentration of insulin as the *Soluble insulin sample solution*. [NOTE—For example, if Injection is labeled to contain 20% soluble insulin, the dilution factor is 100/20 = 5.]
Analysis
Samples: *Soluble insulin sample solution* and *Total insulin sample solution*
Calculate the quantity of soluble insulin human as a percentage of the total insulin content of Injection:

$$\text{Result} = (\Sigma r_S/\Sigma r_T) \times [(V_T + V_A)/V_I] \times (100/D)$$

r_S = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Soluble insulin sample solution*
r_T = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Total insulin sample solution*
V_T = sum of initial volume (5000 µL) of Injection to be transferred to a centrifuge tube and 20 µL of 1 N sodium hydroxide to be added to Injection for the *Soluble insulin sample solution*, 5020 µL
V_A = volume added to adjust the pH of the *Soluble insulin sample solution* (µL)
V_I = initial volume of Injection to be transferred to a centrifuge tube for the *Soluble insulin sample solution*, 5000 µL
D = dilution factor used to prepare the *Total insulin sample solution*

Acceptance criteria: The percentage of soluble insulin human is in the range $L \pm 5$, where L is the percentage of soluble insulin human stated on the product label.
Method 2
Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*, except use 50 µL for *Injection volume*.
0.1 M tris buffer: Dissolve 3.54 ± 0.01 g of tris(hydroxymethyl)aminomethane hydrochloride and 3.34 ± 0.01 g of tris(hydroxymethyl)aminomethane in 500 mL of water. The pH of this solution must be between 8.15 and 8.35. If the pH is outside of this range, discard the solution and prepare fresh; do not adjust the pH.
System suitability solution: Dissolve about 0.14 mg of insulin human in 1.0 mL of 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin human.
Soluble insulin sample solution: Dilute a suitable volume of Injection with *0.1 M tris buffer* to obtain a solution containing about 6 USP Insulin Human Units/mL of soluble insulin (e.g., 2 mL of 70/30 Injection containing 100 USP Insulin Human Units/mL would be diluted with 8 mL of *0.1 M tris buffer* to obtain a filtrate that contains 6 USP Insulin Human Units/mL of soluble insulin). Immerse the container in a water bath at 25 ± 1° for 30 ± 2 min. Immediately pass this solution through a filter of 0.2-µm pore size using a disposable syringe. Transfer 2 parts of the filtrate to a suitable vessel, and add 1 part 0.2 N hydrochloric acid. [NOTE—For example, the dilution factor for the *Soluble insulin sample solution* that contains 30% soluble insulin is (5 × 3)/2 = 7.5.]
Total insulin sample solution: For each milliliter of Injection add 3.0 µL of 9.6 N hydrochloric acid, mix, and allow the suspension to clarify. Dilute the resulting solution with 0.01 N hydrochloric acid to 4 USP Insulin

USP Monographs

www.webofpharma.com

Human Units/mL (e.g., if the product is labeled to contain a total of 100 USP Insulin Human Units/mL, the dilution factor is 25).

System suitability

Make adjustments as necessary to obtain a retention time for insulin human between 10 and 17 min.

Samples: *System suitability solution* (5 replicate injections)

[NOTE—The retention time for human insulin is between 10 and 17 min.]

Suitability requirements

Resolution: NLT 2.0 between insulin human and A-21 desamido insulin human

Tailing factor: Between 0.8 and 1.5 for the insulin human peak

Relative standard deviation: NMT 1.6%

Analysis

Samples: *Soluble insulin sample solution* and *Total insulin sample solution*

Calculate the quantity of soluble insulin human as a percentage of the total human insulin content of Injection:

$$\text{Result} = (\Sigma r_S/\Sigma r_T) \times (D_S/D_T) \times 100$$

r_S = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Soluble insulin sample solution*

r_T = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Total insulin sample solution*

D_S = dilution factor used to prepare the *Soluble insulin sample solution*

D_T = dilution factor used to prepare the *Total insulin sample solution*

Acceptance criteria: The percentage of soluble insulin human is in the range $L \pm 5$, where L is the percentage of soluble insulin human stated on the product label.

- **pH** ⟨791⟩**:** 7.0–7.8
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 80 USP Endotoxin Units per 100 USP Insulin Human Units
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements when tested as directed in the chapter and the Injection being filtered immediately after it has been put into a solution using a validated suitable solvent.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Store in a refrigerator, protect from sunlight, and avoid freezing.
- **LABELING:** The Injection container label states that the Injection is to be properly resuspended before use. Label it to indicate that it has been prepared with Insulin Human produced by methods based on recombinant DNA technology or that it is derived by enzymatic modification of insulin from porcine pancreas. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Human Units/mL and the percent ratio of Human Insulin Isophane Suspension to soluble Human Insulin Injection.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Insulin Human RS

Isophane Insulin Suspension

DEFINITION

Isophane Insulin Suspension is a sterile suspension of zinc–insulin crystals and Protamine Sulfate in buffered Water for Injection, combined in a manner such that the solid phase of the suspension consists of crystals composed of insulin, protamine, and zinc. The Protamine Sulfate is prepared from the sperm or from the mature testes of fish belonging to the genus *Oncorhynchus* Suckley, or *Salmo* L. (Fam. Salmonidae). Its potency, based on the sum of its insulin and desamido insulin components, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units/mL.

IDENTIFICATION

Change to read:

- **A.** The retention time of the insulin ▲pork▲ (RB 1-May-2019) peak of *Sample solution A* or *Sample solution B* corresponds to that of the ▲main peak▲ (RB 1-May-2019) of the *Identification solution*, as obtained in the *Assay*▲, and no other significant peaks are observed.▲ (RB 1-May-2019)[NOTE—It may be necessary to inject a mixture of *Sample solution* and *Identification solution*.]

ASSAY

Change to read:

- **PROCEDURE**

Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.

Mobile phase: Acetonitrile and *Solution A* (26:74). [NOTE—The acetonitrile is warmed to NLT 20° to avoid precipitation.]

System suitability solution: 1.5 mg/mL of ▲▲ (RB 1-May-2019) insulin pork in 0.01 N hydrochloric acid. ▲▲ (RB 1-May-2019) Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.

Identification solution: 0.6 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid. [NOTE—The *Identification solution* may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Standard solution: 1.5 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid. ▲▲ (RB 1-May-2019)

Sample solution A (for Suspension labeled as containing 40 USP Insulin Units/mL)**:** Add 2.5 µL of 9.6 N hydrochloric acid for each milliliter of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix.

Sample solution B (for Suspension labeled as containing 100 USP Insulin Units/mL)**:** Add 2.5 µL of 9.6 N hydrochloric acid for each milliliter of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix. [NOTE—Pooling several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; packing L1

Column temperature: 40°

Flow rate: 1 mL/min

USP Monographs

www.webofpharma.com

Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between insulin and A-21 desamido insulin, *System suitability solution*
Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*
Relative standard deviation: NMT 1.6%, *Standard solution*
Analysis
Samples: *Identification solution*, *Standard solution*, and either *Sample solution A* or *Sample solution B*
Measure the peak responses for insulin and A-21 desamido insulin ▲▲ (RB 1-May-2019), using the chromatogram of the *Identification solution* to identify the insulin peaks. ▲▲ (RB 1-May-2019) Calculate the potency, in USP Insulin Units/mL, in the portion of Suspension taken:

$$\text{Result} = (\Sigma r_U/\Sigma r_S) \times C_S \times D$$

r_U = sum of the peak responses of insulin and A-21 desamido insulin from the *Sample solution*
r_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*
C_S = concentration of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in the *Standard solution* (USP Insulin Units/mL)
D = dilution factor used to prepare the *Sample solution*

▲▲ (RB 1-May-2019)
Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Units/mL

OTHER COMPONENTS

- **ZINC DETERMINATION** ⟨591⟩**:** 10–40 µg for every 100 USP Insulin Units

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

- **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Limit of High Molecular Weight Proteins***:** Proceed as directed in the chapter, except for the *Sample solution*. It meets the requirements.

Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each milliliter of an accurately measured volume of Suspension, and mix.
Acceptance criteria: NMT 3.0%

SPECIFIC TESTS

- **INSULIN IN THE SUPERNATANT**

Sample solution: Centrifuge 10 mL of the Suspension at 1500 × g for 10 min. Use the supernatant.
Analysis: Determine the insulin content of the *Sample solution* by a suitable method.
Acceptance criteria: NMT 1.0 USP Insulin Unit/mL

- **pH** ⟨791⟩**:** 7.0–7.8
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 80 USP Endotoxin Units per 100 USP Insulin Units
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** Meets the requirements when tested as directed in the chapter and the Suspension being filtered immediately after it has been put into a solution using a validated suitable solvent

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

Change to read:

- **LABELING:** Label it as ▲▲ (RB 1-May-2019) porcine ▲▲ (RB 1-May-2019). If the Isophane Insulin Suspension is made from insulin that is purified, label it as such. The Suspension container label states that the Suspension is to be shaken carefully before use. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units/mL.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

▲▲ (RB 1-May-2019)
USP Insulin Pork RS

Isophane Insulin Human Suspension

DEFINITION

Isophane Insulin Human Suspension is a sterile suspension of zinc–insulin human crystals and Protamine Sulfate in buffered Water for Injection, combined in a manner such that the solid phase of the suspension consists of crystals composed of insulin human, protamine, and zinc. Protamine Sulfate is prepared from the sperm or from the mature testes of fish belonging to the genus *Oncorhynchus* Suckley, or *Salmo* L. (Fam. Salmonidae). Its potency, based on the sum of its insulin and desamido insulin components as determined in the *Assay*, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Human Units/mL.

IDENTIFICATION

- **A.** The retention time of the major peak of *Sample solution A* or *Sample solution B* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **PROCEDURE**

Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into this solution, and adjust with ethanolamine to a pH of 2.3, if necessary.
Mobile phase: Acetonitrile and *Solution A* (26:74). [NOTE—The acetonitrile is warmed to NLT 20° to avoid precipitation.]
System suitability solution: 1.5 mg/mL of insulin human in 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin human.
Standard solution: 1.5 mg/mL of ▲USP Insulin Human RS▲ (ERR 1-Apr-2019) in 0.01 N hydrochloric acid
Sample solution A (for Suspension labeled as containing 40 USP Insulin Human Units/mL)**:** Add 2.5 µL of 9.6 N hydrochloric acid to each milliliter of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix.
Sample solution B (for Suspension labeled as containing 100 USP Insulin Human Units/mL)**:** Add 2.5 µL of 9.6 N hydrochloric acid to each milliliter of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix. [NOTE—Pooling of several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric

USP Monographs

www.webofpharma.com

flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between insulin human and A-21 desamido insulin human, *System suitability solution*
Tailing factor: NMT 1.8 for the insulin human peak, *System suitability solution*
Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis
Samples: *Standard solution* and either *Sample solution A* or *Sample solution B*
Calculate the potency, in USP Insulin Human Units/mL, of Suspension taken:

$$\text{Result} = (\Sigma r_U/\Sigma r_S) \times C_S \times D$$

r_U = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Sample solution*
r_S = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Standard solution*
C_S = concentration of USP Insulin Human RS in the *Standard solution* (USP Insulin Human Units/mL)
D = dilution factor used to prepare the *Sample solution*

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Human Units/mL

OTHER COMPONENTS

- **Zinc Determination** ⟨591⟩**:** 0.021–0.04 mg for every 100 USP Insulin Human Units

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

- **Physicochemical Analytical Procedures for Insulins** ⟨121.1⟩, *Limit of High Molecular Weight Proteins*

Proceed as directed in the chapter, except for the *Sample solution*. It meets the requirements.
Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each milliliter of an accurately measured volume of Suspension, and mix.
Acceptance criteria: NMT 3.0%

SPECIFIC TESTS

- **Insulin in the Supernatant**

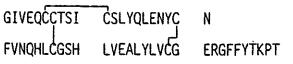
Sample solution: Centrifuge 10 mL of Suspension at 1500 × g for 10 min. Use the supernatant.
Analysis: Determine the insulin content of the *Sample solution* by a suitable method.
Acceptance criteria: NMT 1.0 USP Insulin Human Unit/mL

- **pH** ⟨791⟩**:** 7.0–7.5
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 80 USP Endotoxin Units per 100 USP Insulin Human Units
- **Sterility Tests** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements when tested as directed and the Suspension being filtered immediately after it has been put into a solution using a validated suitable solvent.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.
- **Labeling:** The Suspension container label states that the Suspension is to be shaken carefully before use. The labeling states that it has been prepared with Insulin Human produced by methods based on recombinant DNA technology or that it is derived by enzymatic modification of insulin from porcine pancreas. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Human Units/mL.
- **USP Reference Standards** ⟨11⟩
USP Insulin Human RS

Insulin Lispro



$C_{257}H_{383}N_{65}O_{77}S_6$ 5807.57
Insulin (human), 28^B-L-lysine-29^B-L-proline-; 28^B-L-Lysine-29^B-L-prolineinsulin (human) [133107-64-9].

DEFINITION

Insulin Lispro is identical in structure to Insulin Human, except that it has lysine and proline at positions 28 and 29, respectively, of the B-chain, whereas this sequence is reversed in Insulin Human. Insulin Lispro is produced by methods based on recombinant DNA technology. The presence of host cell DNA in Insulin Lispro is process-specific. The capability of the process to clear host-derived DNA requires validation and is determined by validated methods. Its potency is NLT 27.0 USP Insulin Lispro Units/mg, calculated on the dried basis.
[NOTE—1 USP Insulin Lispro Unit is equivalent to 0.0347 mg of pure Insulin Lispro.]

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. Physicochemical Analytical Procedures for Insulins** ⟨121.1⟩, *Peptide Mapping*

Proceed as directed in the chapter, except for the *Flow rate* in the *Chromatographic system* and *System suitability*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Flow rate: 0.8 mL/min
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.4 between digest fragments II and III
Tailing factor: NMT 1.5 for digest fragments II and III
Chromatogram similarity: Identify the peaks due to digest fragments I, II, III, and IV in the *Standard solution*. The chromatogram of the *Standard solution* corresponds to that of the typical chromatogram provided with USP Insulin Lispro RS.
Acceptance criteria: Meets the requirements

ASSAY

- **Procedure**

Solution A: 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.3.
Mobile phase: Acetonitrile and *Solution A* (51:149)

USP Monographs

www.webofpharma.com

System suitability solution: 1 mg/mL of Insulin Lispro in 0.01 N hydrochloric acid. Allow to stand at room temperature to obtain a solution containing 0.8%–11% of A-21 desamido insulin lispro.
Standard solution: About 0.7 mg/mL of USP Insulin Lispro RS in 0.01 N hydrochloric acid
Sample solution: About 0.8 mg/mL of Insulin Lispro in 0.01 N hydrochloric acid
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 10-cm; packing L1
Column temperature: 40°
Flow rate: 0.8 mL/min
Injection volume: 20 µL
System suitability
Adjust the *Mobile phase* to obtain a retention time of about 24 min for the main insulin lispro peak.
Sample: *System suitability solution* (3 replicate injections)
Suitability requirements
Resolution: NLT 3.0 between insulin lispro and A-21 desamido insulin lispro
Tailing factor: NMT 1.5 for the insulin lispro peak
Relative standard deviation: NMT 1.1% for the insulin lispro peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the potency on the undried basis, in USP Insulin Lispro Units/mg, of insulin lispro in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U)$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Insulin Lispro RS in the *Standard solution* (USP Insulin Lispro Units/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NLT 27.0 USP Insulin Lispro Units/mg on the dried basis

OTHER COMPONENTS

Change to read:

• **▲ZINC DETERMINATION** ⟨591⟩▲ (IRA 1-Jan-2019)
Acceptance criteria: 0.30%–0.60% on the dried basis

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **RELATED SUBSTANCES**
Solvent: 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.3.
Solution A: Acetonitrile and *Solvent* (18:82)
Solution B: Acetonitrile and *Solvent* (50:50)
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	81	19
60	81	19
83	51	49
84	81	19
94	81	19

System suitability solution: 3.5 mg/mL of Insulin Lispro in 0.01 N hydrochloric acid. Allow to stand at room temperature to obtain a solution containing 0.8%–11% of A-21 desamido insulin lispro.
Sample solution: 3.5 mg/mL of Insulin Lispro in 0.01 N hydrochloric acid. [NOTE—Store this solution for NMT 56 h in a refrigerator.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Adjust the *Mobile phase* composition and the duration of the isocratic elution to obtain a retention time of about 41 min for the main insulin lispro peak, with A-21 desamido insulin lispro eluting just before the start of the gradient elution phase.
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.5 between insulin lispro and A-21 desamido insulin lispro
Tailing factor: NMT 2.0 for the insulin lispro peak
Analysis
Sample: *Sample solution*
Calculate the percentage of insulin lispro, A-21 desamido insulin lispro, and other impurities in the portion of Insulin Lispro taken.
Calculate the percentage of insulin lispro (%*I*):

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = peak response of insulin lispro from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Calculate the percentage of A-21 desamido insulin lispro (%*D*):

$$\text{Result} = (r_D/r_T) \times 100$$

r_D = peak response of A-21 desamido insulin lispro from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Calculate the percentage of other insulin lispro-related substances:

$$\text{Result} = 100 - (\%I + \%D)$$

Acceptance criteria
Individual impurities: NMT 1.00% of A-21 desamido insulin lispro
Other individual impurities: NMT 0.50% of any insulin lispro-related substance
Total impurities: NMT 2.00%, excluding A-21 desamido insulin lispro
• **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Limit of High Molecular Weight Proteins*: Meets the requirements
Acceptance criteria: NMT 0.25%

www.webofpharma.com

PROCESS-RELATED IMPURITIES

- **SINGLE-CHAIN PRECURSOR CONTENT:** The single-chain precursor content of Insulin Lispro is NMT 10 ng/mg, determined by a validated method.
- **HOST CELL PROTEIN:** The residual host cell protein content is NMT 10 ng/mg, determined by a validated method or demonstrated by a validated process.

SPECIFIC TESTS

- **INSULIN ASSAYS** ⟨121⟩, *Assay, Bioidentity Test*
  Analysis: Proceed as directed in the chapter, except obtain the first blood specimen at 45 min, instead of 1 h, after the time of injection.
  Acceptance criteria: Meets the requirements
- **LOSS ON DRYING** ⟨731⟩
  Sample: 300 mg
  Analysis: Dry the *Sample* at 105° for 16 h.
  Acceptance criteria: NMT 10.0%
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩, *Photometric Quantitative Techniques, Chromogenic Technique*: NMT 10 USP Endotoxin Units/mg of Insulin Lispro, using the kinetic-chromogenic assay
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 10^2 cfu/g, the test being performed on a portion of about 0.3 g, accurately weighed.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store in a freezer and protect from light.
- **LABELING:** Label it to indicate that it has been produced by methods based on recombinant DNA technology.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Insulin Lispro RS

Insulin Lispro Injection

DEFINITION

Insulin Lispro Injection is an isotonic, sterile solution of Insulin Lispro in Water for Injection. It has a potency of NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Lispro Units/mL.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
  Solution A: 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.3.
  Mobile phase: Acetonitrile and *Solution A* (51:149)
  System suitability solution: 1 mg/mL of insulin lispro in 0.01 N hydrochloric acid. Allow to stand at room temperature to obtain a solution containing 0.8%–11% of A-21 desamido insulin lispro.
  Standard solution: 0.7 mg/mL of USP Insulin Lispro RS in 0.01 N hydrochloric acid
  Sample solution: Acidify each milliliter of Injection with 3 µL of 9.6 N hydrochloric acid. Quantitatively dilute a portion of the acidified solution with 0.01 N hydrochloric acid to obtain a solution containing 20 USP Insulin Lispro Units/mL.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 214 nm
  Column: 4.6-mm × 10-cm; packing L1
  Column temperature: 40°
  Flow rate: 0.8 mL/min
  Injection volume: 20 µL
  System suitability
  Adjust the *Mobile phase* to obtain a retention time of about 24 min for the main insulin lispro peak.
  Sample: *System suitability solution* (3 replicate injections)
  Suitability requirements
  Resolution: NLT 3.0 between insulin lispro and A-21 desamido insulin lispro
  Tailing factor: NMT 1.5 for the insulin lispro peak
  Relative standard deviation: NMT 1.1% for the insulin lispro peak
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the potency, in USP Insulin Lispro Units/mL, of Injection taken:

$$\text{Result} = (r_U/r_S) \times C_S \times D$$

r_U	= peak response of insulin lispro from the *Sample solution*
r_S	= peak response of insulin lispro from the *Standard solution*
C_S	= concentration of USP Insulin Lispro RS in the *Standard solution* (USP Insulin Lispro Units/mL)
D	= dilution factor used to prepare the *Sample solution*

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Lispro Units/mL

OTHER COMPONENTS

Change to read:

- **▲ZINC DETERMINATION** ⟨591⟩:▲ (IRA 1-Jan-2019) 14–35 µg for every 100 USP Insulin Lispro Units

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

Change to read:

- **RELATED SUBSTANCES**
  Solvent: 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.3.
  Solution A: Acetonitrile and *Solvent* (18:82)
  Solution B: Acetonitrile and *Solvent* (50:50)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	81	19
60	81	19
83	51	49
84	81	19
94	81	19

System suitability solution: 3.5 mg/mL of insulin lispro in 0.01 N hydrochloric acid. Allow to stand at room temperature to obtain a solution containing 0.8%–11% of A-21 desamido insulin lispro.
Sample solution: Acidify each milliliter of Injection with 3 µL of 9.6 N hydrochloric acid.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

www.webofpharma.com

Detector: UV 214 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Adjust the *Mobile phase* composition and the duration of the isocratic elution to obtain a retention time of about 41 min for the main insulin lispro peak, with A-21 desamido insulin lispro eluting just prior to the start of the gradient elution phase.
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.5 between insulin lispro and A-21 desamido insulin lispro
Tailing factor: NMT 2.0 for the insulin lispro peak
Analysis
Sample: *Sample solution*
Calculate the percentage of insulin lispro, A-21 desamido insulin lispro, and other impurities in the portion of ▲Injection▲ (ERR 1-Sep-2018) taken.
Calculate the percentage of insulin lispro (%*I*):

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = peak response of insulin lispro from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Calculate the percentage of A-21 desamido insulin lispro (%*D*):

$$\text{Result} = (r_D/r_T) \times 100$$

r_D = peak response of A-21 desamido insulin lispro from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Calculate the percentage of other insulin lispro-related substances:

$$\text{Result} = 100 - (\%I + \%D)$$

Acceptance criteria
Individual impurities: NMT 1.50% of A-21 desamido insulin lispro
Total impurities: NMT 4.00%, excluding A-21 desamido insulin lispro
• **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Limit of High Molecular Weight Proteins*
Proceed as directed in the chapter, except for the *Sample solution*. It meets the requirements.
Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each milliliter of an accurately measured volume of Injection, and mix.
Acceptance criteria: NMT 1.50%

SPECIFIC TESTS
• **pH** ⟨791⟩: 7.0–7.8
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩, *Photometric Quantitative Techniques, Chromogenic Technique*: NMT 80 USP Endotoxin Units per 100 USP Insulin Lispro Units, using the kinetic-chromogenic assay
• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
• **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩: Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, multiple-dose containers. Dispense in the unopened, multiple-dose container provided by the manufacturer. Store in a refrigerator, protect from sunlight, and avoid freezing.
• **LABELING:** Label it to indicate that it has been prepared with Insulin Lispro produced by methods based on recombinant DNA technology. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Lispro Units/mL.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Insulin Lispro RS

Insulin Zinc Suspension

Insulin zinc [8049-62-5].

DEFINITION
Insulin Zinc Suspension is a sterile suspension of Insulin in buffered Water for Injection, modified by the addition of a suitable zinc salt in a manner such that the solid phase of the suspension consists of a mixture of crystalline and amorphous insulin in a ratio of approximately 7 parts of crystals to 3 parts of amorphous material. Its potency, based on the sum of its insulin and desamido insulin components, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units/mL.

IDENTIFICATION

Change to read:

• **A.** The retention time of the insulin ▲pork▲ (RB 1-May-2019) peak of *Sample solution A* or *Sample solution B* corresponds to that of ▲▲ (RB 1-May-2019) of the *Identification solution*, as obtained in the *Assay* ▲and no other significant peaks are observed.▲ (RB 1-May-2019) [NOTE—It may be necessary to inject a mixture of *Sample solution* and *Identification solution*.]

ASSAY

Change to read:

• **PROCEDURE**
Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.
Mobile phase: Acetonitrile and *Solution A* (26:74). [NOTE—The acetonitrile is warmed to NLT 20° to avoid precipitation.]
System suitability solution: 1.5 mg/mL of ▲▲ (RB 1-May-2019) insulin pork ▲▲ (RB 1-May-2019) in 0.01 N hydrochloric acid. ▲▲ (RB 1-May-2019) Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.
Identification solution: 0.6 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid. [NOTE—The *Identification solution* may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]
Standard solution: 1.5 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid. ▲▲ (RB 1-May-2019)
Sample solution A (for Suspension labeled as containing 40 USP Insulin Units/mL): Add 2.5 µL of 9.6 N hydrochloric

Supplement 3

www.webofpharma.com

acid for each milliliter of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix.

Sample solution B (for Suspension labeled as containing 100 USP Insulin Units/mL): Add 2.5 µL of 9.6 N hydrochloric acid for each milliliter of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix. [NOTE—Pooling several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between insulin and A-21 desamido insulin, *System suitability solution*
Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*
Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis
Samples: *Identification solution*, *Standard solution*, and either *Sample solution A* or *Sample solution B*
Measure the peak responses for insulin and A-21 desamido insulin using the chromatogram of the *Identification solution* to identify the insulin peaks.
▲▲ (RB 1-May-2019) Calculate the potency, in USP Insulin Units/mL, in the portion of Suspension taken:

$$\text{Result} = (\Sigma r_U/\Sigma r_S) \times C_S \times D$$

r_U = sum of the peak responses of insulin and A-21 desamido insulin from the *Sample solution*
r_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*
C_S = concentration of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in the *Standard solution* (USP Insulin Units/mL)
D = dilution factor used to prepare the *Sample solution*

▲▲ (RB 1-May-2019)
Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Units/mL

OTHER COMPONENTS

- **ZINC DETERMINATION** ⟨591⟩: 0.12–0.25 mg for every 100 USP Insulin Units
- **ZINC IN THE SUPERNATANT**

Analysis: Centrifuge a portion of Suspension sufficient for the test and determine the zinc content in the clear supernatant as directed in *Zinc Determination* ⟨591⟩.
Acceptance criteria: Concentration of zinc (mg/mL) is 20%–65% of the zinc concentration in Suspension.

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

- **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Limit of High Molecular Weight Proteins*: Proceed as directed in the chapter, except for the *Sample solution*. It meets the requirements.

Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each milliliter of an accurately measured volume of Suspension, and mix.
Acceptance criteria: NMT 1.5%

SPECIFIC TESTS

- **INSULIN NOT EXTRACTED BY BUFFERED ACETONE SOLUTION**

Sample solution: Centrifuge a quantity of Suspension representing 1000 USP Insulin Units, and discard the supernatant. Suspend the residue in 8.4 mL of water, quickly add 16.6 mL of buffered acetone TS, shake or stir vigorously, and centrifuge within 3 min after the addition of the buffered acetone TS. Discard the supernatant, repeat the treatment with water and buffered acetone TS, centrifuge, and discard the supernatant. Dissolve the crystalline residue in 5 mL of dilute hydrochloric acid (1 in 100), transfer to a 25-mL flask, and dilute with water to volume.
Analysis: Use an appropriate method to determine the insulin concentration.
Acceptance criteria: Insulin concentration is 63%–77% of the insulin content of an equal amount of Suspension.

- **INSULIN IN THE SUPERNATANT**

Sample solution: Centrifuge 10 mL of Suspension at 1500 × g for 10 min. Use the supernatant.
Analysis: Determine the insulin content of the *Sample solution* by a suitable method.
Acceptance criteria: NMT 1.0 USP Insulin Unit/mL

- **pH** ⟨791⟩: 7.0–7.8
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 80 USP Endotoxin Units per 100 USP Insulin Units
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements when tested as directed and the Suspension being filtered immediately after it has been put into a solution using a validated suitable solvent

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

Change to read:

- **LABELING:** Label it ▲▲ (RB 1-May-2019) as porcine ▲▲ (RB 1-May-2019). If the Insulin Zinc Suspension is made from insulin that is purified, label it as such. The Suspension container label states that the Suspension is to be shaken carefully before use. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units/mL.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

▲▲ (RB 1-May-2019)
USP Insulin Pork RS

Extended Insulin Zinc Suspension

DEFINITION

Extended Insulin Zinc Suspension is a sterile suspension of Insulin in buffered Water for Injection, modified by the addition of a suitable zinc salt in a manner such that the solid phase of the suspension is predominantly crystalline. Its potency, based on the sum of its insulin and desamido insulin components, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units/mL.

www.webofpharma.com

IDENTIFICATION

Change to read:

• **A.** The retention time of the insulin ▲pork▲ (RB 1-May-2019) peak of *Sample solution A* or *Sample solution B* corresponds to that of ▲▲ (RB 1-May-2019) the *Identification solution*, as obtained in the *Assay* ▲and no other significant peaks are observed.▲ (RB 1-May-2019) [NOTE—It may be necessary to inject a mixture of *Sample solution* and *Identification solution*.]

ASSAY

Change to read:

• **PROCEDURE**

Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.

Mobile phase: Acetonitrile and *Solution A* (26:74). [NOTE—The acetonitrile is warmed to NLT 20° to avoid precipitation.]

System suitability solution: 1.5 mg/mL of ▲▲ (RB 1-May-2019) insulin pork in 0.01 N hydrochloric acid. ▲▲ (RB 1-May-2019) Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.

Identification solution: ▲▲ (RB 1-May-2019) 0.6 mg/mL of USP Insulin Pork RS in 0.01 N hydrochloric acid. [NOTE—The *Identification solution* may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Standard solution: 1.5 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid ▲▲ (RB 1-May-2019)

Sample solution A (for Suspension labeled as containing 40 USP Insulin Units/mL): Add 2.5 µL of 9.6 N hydrochloric acid for each mL of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix.

Sample solution B (for Suspension labeled as containing 100 USP Insulin Units/mL): Add 2.5 µL of 9.6 N hydrochloric acid for each mL of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix. [NOTE—Pooling several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between insulin and A-21 desamido insulin, *System suitability solution*
Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*
Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis
Samples: *Identification solution*, *Standard solution*, and either *Sample solution A* or *Sample solution B*
Measure the peak responses for insulin and A-21 desamido insulin using the chromatogram of the *Identification solution* to identify the insulin peaks.

▲▲ (RB 1-May-2019) Calculate the potency, in USP Insulin Units/mL, of the portion of Suspension taken:

$$\text{Result} = (\Sigma r_U/\Sigma r_S) \times C_S \times D$$

Σr_U = sum of the peak responses of insulin and A-21 desamido insulin from the *Sample solution*
Σr_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*
C_S = concentration of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in the *Standard solution* (USP Insulin Units/mL)
D = dilution factor used to prepare the *Sample solution*

▲▲ (RB 1-May-2019)
Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Units/mL

OTHER COMPONENTS

• **ZINC DETERMINATION** ⟨591⟩**:** 0.12–0.25 mg for every 100 USP Insulin Units

• **ZINC IN THE SUPERNATANT**
Analysis: Centrifuge a portion of Suspension sufficient for the test and determine the zinc content of the clear supernatant as directed in *Zinc Determination* ⟨591⟩.
Acceptance criteria: Concentration of zinc (mg/mL) is 20%–65% of the zinc concentration of the Suspension.

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Limit of High Molecular Weight Proteins***:** Proceed as directed in *Limit of High Molecular Weight Proteins*, except prepare the following *Sample solution*. It meets the requirements.
Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each mL of an accurately measured volume of Suspension, and mix.
Acceptance criteria: NMT 1.5%

SPECIFIC TESTS

• **INSULIN NOT EXTRACTED BY BUFFERED ACETONE SOLUTION**
Sample solution: Centrifuge a quantity of Suspension representing 1000 USP Insulin Units, and discard the supernatant. Suspend the residue in 8.4 mL of water, quickly add 16.6 mL of buffered acetone TS, shake or stir vigorously, and centrifuge within 3 min after the addition of the buffered acetone TS. Discard the supernatant, repeat the treatment with water and buffered acetone TS, centrifuge, and discard the supernatant. Dissolve the crystalline residue in 5 mL of dilute hydrochloric acid (1 in 100), transfer to a 25-mL flask, and dilute with water to volume.
Analysis: Use an appropriate method to determine the insulin concentration.
Acceptance criteria: The insulin concentration is NLT 90% of the insulin content of an equal amount of the Suspension.

• **INSULIN IN THE SUPERNATANT**
Sample solution: Centrifuge 10 mL of the Suspension at 1500 × g for 10 min. Use the supernatant.
Analysis: Determine the insulin content of the *Sample solution* by a suitable method.
Acceptance criteria: NMT 1.0 USP Insulin Unit/mL

• **pH** ⟨791⟩**:** 7.0–7.8

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 80 USP Endotoxin Units/100 USP Insulin Units

• **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** Meets the requirements when tested as directed, and the Suspension being filtered

USP Monographs

www.webofpharma.com

immediately after it has been put into solution using a validated suitable solvent

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

Change to read:

- **LABELING:** Label it ▲▲ (RB 1-May-2019) as porcine ▲▲ (RB 1-May-2019). If the Extended Insulin Zinc Suspension is made from insulin that is purified, label it as such. The container label states that the Suspension is to be shaken carefully before use. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units/mL.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

▲▲ (RB 1-May-2019)
USP Insulin Pork RS

Prompt Insulin Zinc Suspension

DEFINITION

Prompt Insulin Zinc Suspension is a sterile suspension of Insulin in buffered Water for Injection, modified by the addition of a suitable zinc salt in a manner such that the solid phase of the suspension is amorphous. Its potency, based on the sum of its insulin and desamido insulin components, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units/mL.

IDENTIFICATION

Change to read:

- **A.** The retention time of the insulin ▲pork▲ (RB 1-May-2019) peak of *Sample solution A* or *Sample solution B* corresponds to that ▲▲ (RB 1-May-2019) of the *Identification solution*, as obtained in the *Assay* ▲and no other significant peaks are observed.▲ (RB 1-May-2019) [NOTE—It may be necessary to inject a mixture of *Sample solution* and *Identification solution*.]

ASSAY

Change to read:

- **PROCEDURE**

Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.

Mobile phase: Acetonitrile and *Solution A* (26:74). [NOTE—The acetonitrile is warmed to NLT 20° to avoid precipitation.]

System suitability solution: 1.5 mg/mL of ▲▲ (RB 1-May-2019) insulin pork in 0.01 N hydrochloric acid. ▲▲ (RB 1-May-2019) Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.

Identification solution: 0.6 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid. [NOTE—The *Identification solution* may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Standard solution: 1.5 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid. ▲▲ (RB 1-May-2019)

Sample solution A (for Suspension labeled as containing 40 USP Insulin Units/mL): Add 2.5 µL of 9.6 N hydrochloric acid for each mL of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix.

Sample solution B (for Suspension labeled as containing 100 USP Insulin Units/mL): Add 2.5 µL of 9.6 N hydrochloric acid for each mL of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix. [NOTE—Pooling several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between insulin and A-21 desamido insulin, *System suitability solution*

Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*

Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis

Samples: *Identification solution*, *Standard solution*, and either *Sample solution A* or *Sample solution B*

Measure the peak responses for insulin and A-21 desamido insulin using the chromatogram of the *Identification solution* to identify the insulin peaks.

▲▲ (RB 1-May-2019) Calculate the potency, in USP Insulin Units/mL, of the portion of Suspension taken:

$$\text{Result} = (\Sigma r_U / \Sigma r_S) \times C_S \times D$$

Σr_U = sum of the peak responses of insulin and A-21 desamido insulin from the *Sample solution*

Σr_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*

C_S = concentration of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in the *Standard solution* (USP Insulin Units/mL)

D = dilution factor used to prepare the *Sample solution*

▲▲ (RB 1-May-2019)

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Units/mL

OTHER COMPONENTS

- **ZINC DETERMINATION** ⟨591⟩**:** 0.12–0.25 mg for every 100 USP Insulin Units
- **ZINC IN THE SUPERNATANT**

Analysis: Centrifuge a portion of Suspension sufficient for the test and determine the zinc content of the clear supernatant as directed in *Zinc Determination* ⟨591⟩.

Acceptance criteria: Concentration of zinc (mg/mL) is 20%–65% of the zinc concentration of the Suspension.

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

- **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩, *Limit of High Molecular Weight Proteins*: Proceed as

www.webofpharma.com

directed in *Limit of High Molecular Weight Proteins*, except prepare the following *Sample solution*. It meets the requirements.

Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each mL of an accurately measured volume of Suspension, and mix.

Acceptance criteria: NMT 1.5%

SPECIFIC TESTS

- **INSULIN NOT EXTRACTED BY BUFFERED ACETONE SOLUTION**

Sample solution: Centrifuge 15 mL (40-Unit), 8 mL (80-Unit), or 6 mL (100-Unit) of Suspension, and discard the supernatant. Suspend the residue in 8.4 mL of water, quickly add 16.6 mL of buffered acetone TS, shake or stir vigorously, and centrifuge within 3 min after the addition of the buffered acetone TS. Discard the supernatant, repeat the treatment with water and buffered acetone TS, centrifuge, and discard the supernatant.

Acceptance criteria: No crystalline residue remains.

- **INSULIN IN THE SUPERNATANT**

Sample solution: Centrifuge 10 mL of the Suspension at 1500 × g for 10 min. Use the supernatant.

Analysis: Determine the insulin content of the *Sample solution* by a suitable method.

Acceptance criteria: NMT 1.0 USP Insulin Unit/mL

- **pH** ⟨791⟩**:** 7.0–7.8
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 80 USP Endotoxin Units/100 USP Insulin Units
- **STERILITY TESTS** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** Meets the requirements when tested as directed, and the Suspension being filtered immediately after it has been put into solution using a validated suitable solvent

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

Change to read:

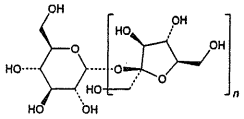
- **LABELING:** Label it ▲▲ (RB 1-May-2019) as porcine ▲▲ (RB 1-May-2019). If the Prompt Insulin Zinc Suspension has been made from insulin that is purified, label it as such. The container label states that the Suspension is to be shaken carefully before use. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units/mL.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩

▲▲ (RB 1-May-2019)
USP Insulin Pork RS

Inulin



$C_6H_{11}O_5(C_6H_{10}O_5)_n OH$
Inulin.
Inulin [9005-80-5].

» Inulin is a polysaccharide which, on hydrolysis, yields mainly fructose. It contains not less than 94.0 percent and not more than 102.0 percent of $C_6H_{11}O_5(C_6H_{10}O_5)_n OH$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Dextrose RS
USP Fructose RS

Completeness of solution—Dissolve 10 g in 20 mL of boiling water in a 200-mL volumetric flask, add 150 mL of water, allow to cool, dilute with water to volume, and mix: the solution is clear.

Specific rotation ⟨781S⟩**:** between –32.0° and –40.0°.
Test solution: 100 mg per mL, in 0.012 N ammonium hydroxide.

Microbial enumeration tests ⟨61⟩ and ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli* and for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa;* the total aerobic microbial count is less than 1000 per g.

Loss on drying ⟨731⟩**:** not more than 10.0% after it has been dried at 105° for 2 hours, 2 g of the finely ground powder being used for the test.

Residue on ignition ⟨281⟩—Multiply the percentage of *Calcium* found by 3.4. The residue on ignition does not exceed this percentage by more than 0.05%.

pH, Chloride, Iron, and Reducing sugars—Dissolve 10.0 g in 20 mL of boiling water in a 100-mL volumetric flask, allow to cool, dilute with water to volume, and mix. Use the solution for the following tests.

pH ⟨791⟩—The pH of the solution is between 4.5 and 7.0.

Chloride ⟨221⟩—A 10-mL portion of the solution shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.014%).

Iron—To 10 mL of the solution add 0.5 mL of hydrochloric acid and 3 drops of potassium ferrocyanide TS: the solution does not become blue within 1 minute.

Reducing sugars—To 2 mL of the solution add 5 mL of alkaline cupric tartrate TS: no reduction occurs at room temperature, and only slight reduction occurs after 1 minute of boiling.

Calcium—Heat 10.0 g in 100 mL of water to dissolve. Cool to room temperature, add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Not more than 5.0 mL is required: not more than 0.10% calcium is found.

Sulfate ⟨221⟩—A 1.0 g portion shows no more sulfate than corresponds to 0.5 mL of 0.020 N sulfuric acid (0.05%).
[NOTE—Inulin should be dissolved in 30 to 40 mL of water with gentle warming, prior to dilution to final volume.]

Free fructose—
Blue tetrazolium solution—Dissolve 50 mg of blue tetrazolium in 10 mL of alcohol, and mix.
Tetramethylammonium hydroxide solution—Prepare a mixture of 1 volume of tetramethylammonium hydroxide TS and 9 volumes of alcohol.
Standard stock solution—Prepare an aqueous solution having a known concentration of about 250 µg of USP Fructose RS per mL. Store at about 4°.
Standard preparation—On the day of use, quantitatively dilute a portion of the *Standard stock solution* with alcohol to obtain a solution having a known concentration of about 2.5 µg per mL. Store at about 4°.

www.webofpharma.com

Test preparation—Transfer about 2.5 g of Inulin, accurately weighed, to a 100-mL volumetric flask, add about 75 mL of water, heat on a steam bath until solution is complete, cool to room temperature, dilute with water to volume, and mix. Pipet 1 mL into a 100-mL volumetric flask, dilute with alcohol to volume, and mix. If the solution is turbid, pass through fine-porosity filter paper.

Procedure—Pipet 10 mL of the *Test preparation* and 10 mL of the *Standard preparation* into separate glass-stoppered centrifuge tubes. Into each of the tubes, and into a similar tube containing 10.0 mL of alcohol to provide the blank, pipet 1 mL of *Blue tetrazolium solution,* and mix. Then into each tube pipet 1 mL of *Tetramethylammonium hydroxide solution,* mix, and allow to stand in the dark for 60 minutes. Without delay, concomitantly determine the absorbances of the solutions from the *Test preparation* and the *Standard preparation* at 530 nm, with a suitable spectrophotometer, against the blank. Calculate the percentage, *F,* of free fructose in the Inulin taken by the formula:

$$F = (C/W)(A_U/A_S)$$

in which *C* is the concentration, in µg per mL, of USP Fructose RS in the *Standard preparation; W* is the quantity, in g, of Inulin taken; and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation,* respectively. The limit is 2.0%, calculated on the dried basis.

Content of combined glucose—

Standard stock solution—Transfer about 50 mg of USP Dextrose RS, accurately weighed, to a 100-mL volumetric flask, dissolve in a solution of benzoic acid (1.7 in 1000), dilute with the same solution to volume, and mix. Allow to stand at room temperature for not less than 3 hours before using. This solution is stable for 1 month at about 4°.

Standard preparation—Pipet 7 mL of *Standard stock solution* into a 100-mL volumetric flask, dilute with water to volume, mix, and use at once.

Assay preparation—Transfer about 0.5 g of Inulin, accurately weighed, to a 100-mL volumetric flask, add 5.0 mL of water, dissolve by heating on a steam bath, cool to room temperature, add 0.5 mL of 8 N hydrochloric acid, and mix. Place the flask in a boiling water bath for 5 minutes, cool, dilute with water to volume, and mix. Pipet 2 mL of this solution into a 10-mL volumetric flask, dilute with water to volume, and mix. [NOTE—This solution is used also for preparing the *Assay preparation* in the *Assay for inulin.*]

Procedure—Pipet 3-mL portions of glucose oxidase–chromogen TS into 3 separate test tubes, and bring to a temperature of 37 ± 0.5° in a water bath. Pipet 2 mL of *Standard preparation* into one of the tubes, pipet 2 mL of *Assay preparation* into another, and pipet 2 mL of water into the third tube to provide the blank. Maintain at 37 ± 0.5° for an additional 10 minutes, then remove the tubes, and allow them to cool. Determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* at about 505 nm, with a suitable spectrophotometer, using the reagent blank as a reference. Calculate the percentage of combined glucose, *G,* in the Inulin taken by the formula:

$$G = 50(C/W)(A_U/A_S)$$

in which *C* is the concentration, in mg per mL, of USP Dextrose RS in the *Standard preparation; W* is the amount, in g, of Inulin taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively. Not less than 2.0% and not more than 5.0%, calculated on the dried basis, is found.

Assay for inulin—

Thiobarbituric acid solution—Dissolve 250 mg of thiobarbituric acid in 100 mL of 8 N hydrochloric acid, and mix. This solution is stable for 2 weeks at a temperature of about 4°.

Standard stock solution—Quantitatively dissolve an accurately weighed quantity of USP Fructose RS in an aqueous solution of benzoic acid (1.7 in 1000) to obtain a solution having a known concentration of about 1 mg of USP Fructose RS per mL. [NOTE—This solution is stable for 1 month at about 4°.]

Standard preparation—Quantitatively dilute the *Standard stock solution* with water to one-fiftieth of its concentration. Use immediately.

Assay preparation—Pipet 4 mL of the *Assay preparation* from the *Content of combined glucose* into a 200-mL volumetric flask, add water to volume, and mix.

Procedure—Pipet 1-mL portions of the *Standard preparation* and the *Assay preparation* into separate glass-stoppered tubes. Pipet 1 mL of water into a third tube to provide the blank. Pipet 5-mL portions of *Thiobarbituric acid solution* into each tube, and mix. Place all of the tubes simultaneously in a water bath maintained at a temperature of about 83°, and allow them to stay immersed for 5 minutes, accurately timed. Remove the tubes simultaneously, and allow them to cool in a dark place for 30 minutes. Determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* at about 435 nm, with a suitable spectrophotometer, using the reagent blank as a reference. Calculate the percentage of $C_6H_{11}O_5(C_6H_{10}O_5)_n OH$ in the Inulin taken by the formula:

$$0.900[2.5(C/W)(A_U/A_S) - F] + G$$

in which 0.900 is the ratio of the formula weight of an anhydrofructose unit of inulin to that of fructose; *C* is the concentration, in µg per mL, of USP Fructose RS in the *Standard preparation; W* is the quantity, in g, of Inulin weighed for the *Content of combined glucose;* A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively; *F* is the percentage of free fructose; and *G* is the percentage of combined glucose.

Inulin in Sodium Chloride Injection

» Inulin in Sodium Chloride Injection is a sterile solution, which may be supersaturated, of Inulin and Sodium Chloride in Water for Injection. It may require heating before use if crystallization has occurred. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $(C_6H_{12}O_6)_n$ and not less than 95.0 percent and not more than 105.0 percent of the labeled amount of NaCl. It contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose containers, preferably of Type I or Type II glass.

USP Reference standards ⟨11⟩—
USP Dextrose RS
USP Fructose RS

Clarity—If particulate matter is present, heat at 100° for 30 minutes: the resulting solution is free from turbidity and particulate matter.

USP Monographs

www.webofpharma.com

[NOTE—Before applying the following tests, dissolve any solid matter by heating, and cool to room temperature.]
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.1 USP Endotoxin Unit per mg of inulin.
pH ⟨791⟩: between 4.0 and 7.0.

Free fructose—
Blue tetrazolium solution, Tetramethylammonium hydroxide solution, Standard stock solution, and *Standard preparation*—Proceed as directed in the test for *Free fructose* under *Inulin.*
Test preparation—Transfer an accurately measured volume of Injection, equivalent to about 2.5 g of inulin, to a 100-mL volumetric flask, add water to volume, and mix. Just prior to use, pipet 1 mL of this solution into a 100-mL volumetric flask, dilute with alcohol to volume, mix, and, if the solution is turbid, pass through fine-porosity filter paper.
Procedure—Proceed as directed for *Procedure* in the test for *Free fructose* under *Inulin.* Calculate the quantity, *F,* in mg, of free fructose in each mL of the Injection taken by the formula:

$$10(C/V)(A_U/A_S)$$

in which *C* is the concentration, in µg per mL, of USP Fructose RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation,* respectively. The limit is 2.2 mg per mL.
Other requirements—It meets the other requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for inulin—
Content of combined glucose—Proceed as directed in the *Content of combined glucose* under *Inulin,* but in preparing the *Assay preparation* use, instead of 0.5 g of inulin, an accurately measured volume of the Injection equivalent to about 0.5 g of inulin. Calculate the quantity, in mg, of combined glucose, *G,* in each mL of the Injection taken by the formula:

$$500(C/V)(A_U/A_S)$$

in which *C* is the concentration, in mg per mL, of USP Dextrose RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.
Procedure—Proceed as directed in the *Assay for inulin* under *Inulin.* Calculate the quantity, in mg, of $(C_6H_{12}O_6)_n$ in each mL of the Injection taken by the formula:

$$0.900[25(C/V)(A_U/A_S) - F] + G$$

in which 0.900 is the ratio of the formula weight of an anhydrofructose unit of inulin to that of fructose; *C* is the concentration, in µg per mL, of USP Fructose RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively; and *F* is the quantity, in mg, of free fructose in each mL of Injection, and the other terms are as defined therein.
Assay for sodium chloride—Pipet a volume of Injection, equivalent to about 90 mg of sodium chloride, into a porcelain casserole, and add 140 mL of water and 1 mL of dichlorofluorescein TS. Mix, and titrate with 0.1 N silver nitrate VS until the silver chloride flocculates and the mixture acquires a faint pink color. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl.

Iodine

I_2 253.81
Iodine [7553-56-2].

DEFINITION
Iodine contains NLT 99.8% and NMT 100.5% of I.

IDENTIFICATION
- **A.** Solutions (1 in 1000) in chloroform and in carbon disulfide have a violet color.
- **B.**
  Analysis: To a saturated solution add starch–potassium iodide TS.
  Acceptance criteria: A blue color is produced. When the mixture is boiled, the color vanishes but reappears as the mixture cools, unless it has been subjected to prolonged boiling.

ASSAY
- **PROCEDURE**
  Sample: 500 mg of powdered Iodine
  Analysis: Place the *Sample* in a tared, glass-stoppered flask, insert the stopper, and add 1 g of potassium iodide dissolved in 5 mL of water. Dilute with water to 50 mL, add 1 mL of 3 N hydrochloric acid, and titrate with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 12.69 mg of Iodine (I).
  Acceptance criteria: 99.8%–100.5%

IMPURITIES
- **LIMIT OF CHLORIDE OR BROMIDE**
  Sample solution: Triturate 250 mg of finely powdered Iodine with 10 mL of water, and filter the solution.
  Analysis: To the *Sample solution* add, dropwise, sulfurous acid (free from chloride), previously diluted with several volumes of water, until the iodine color just disappears. Add 5 mL of 6 N ammonium hydroxide, followed by 5 mL of silver nitrate TS in small portions. Filter, and acidify the filtrate with nitric acid.
  Acceptance criteria: The resulting liquid is not more turbid than a control made with the same quantities of the same reagents to which 0.10 mL of 0.020 N hydrochloric acid has been added, the sulfurous acid being omitted (0.028% as chloride).
- **LIMIT OF NONVOLATILE RESIDUE**
  Analysis: Place 5.0 g in a tared porcelain dish, heat on a steam bath until the iodine has been driven off, and dry at 105° for 1 h.
  Acceptance criteria: NMT 0.05% of the residue remains.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.

Iodine Topical Solution

DEFINITION
Iodine Topical Solution contains NLT 1.8 g and NMT 2.2 g of iodine (I) and NLT 2.1 g and NMT 2.6 g of sodium iodide (NaI) in each 100 mL.

Iodine	20 g
Sodium Iodide	24 g
Purified Water, a sufficient quantity to make	1000 mL

www.webofpharma.com

Dissolve *Iodine* and *Sodium Iodide* in 50 mL of *Purified Water*, then add sufficient *Purified Water* to bring to final volume.

IDENTIFICATION

• **A.**

Analysis: Add 1 drop to a mixture of 1 mL of starch TS and 9 mL of water.

Acceptance criteria: A deep blue color is produced.

• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Iodide*

Sample: Evaporate a few milliliters on a steam bath to dryness.

Acceptance criteria: The residue meets the requirements of the test for *Iodide*.

• **C. SODIUM:** The *Sample* obtained in *Identification B* imparts an intense yellow color to a nonluminous flame.

ASSAY

• **IODINE**

Sample: 10.0 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a glass-stoppered 500-mL flask, and dilute with 10 mL of water. Titrate with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Each milliliter of *Titrant* is equivalent to 12.69 mg of iodine (I).

Acceptance criteria: 1.8–2.2 g in 100 mL of Topical Solution

• **SODIUM IODIDE**

Sample: 10.0 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M potassium iodate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a glass-stoppered 500-mL flask, add 30 mL of water and 50 mL of hydrochloric acid, cool to room temperature, and titrate with *Titrant* until the dark brown solution that is produced becomes pale brown. Add 1 mL of amaranth TS, and continue the titration slowly until the red color just changes to yellow. The difference in volume, in milliliters, between the *Titrant* used and half the volume of 0.1 N sodium thiosulfate VS used in the *Assay* for *Iodine*, multiplied by 14.99, represents the number of milligrams of sodium iodide (NaI) in the portion of Topical Solution taken.

Acceptance criteria: 2.1–2.6 g in 100 mL of Topical Solution

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at a temperature not exceeding 35°.

Strong Iodine Solution

DEFINITION

Strong Iodine Solution contains, in each 100 mL, NLT 4.5 g and NMT 5.5 g of iodine (I), and NLT 9.5 g and NMT 10.5 g of potassium iodide (KI).

Strong Iodine Solution may be prepared by dissolving 50 g of Iodine and 100 g of Potassium Iodide in 100 mL of Purified Water, then adding Purified Water to make the product measure 1000 mL.

IDENTIFICATION

• **A.**

Analysis: Dilute 1 drop with 10 mL of water, and add 1 mL of starch TS.

Acceptance criteria: A deep blue color is produced.

• **B. IDENTIFICATION TESTS—GENERAL,** *Potassium* ⟨191⟩ and *Iodide* ⟨191⟩

Analysis: Evaporate a few mL on a steam bath to dryness, and ignite gently to volatilize any free iodine.

Acceptance criteria: Meets the requirements

ASSAY

• **IODINE**

Sample solution: Transfer 10.0 mL of Solution into a glass-stoppered 500-mL flask, and add 10 mL of water.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 12.69 mg of iodine (I).

Acceptance criteria: 4.5–5.5 g of iodine (I) in each 100 mL

• **POTASSIUM IODIDE**

Sample solution: Transfer 10.0 mL of Solution to a glass-stoppered 500-mL flask, add 30 mL of water and 50 mL of hydrochloric acid, and cool to room temperature.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M potassium iodate VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with *Titrant* until the dark brown solution that is produced becomes pale brown. Add 1 mL of amaranth TS, and continue the titration slowly until the red color just changes to yellow. The difference between the number of mL of 0.05 M potassium iodate used and half the number of mL of *Titrant* used in the *Assay* for *Iodine*, multiplied by 16.60, represents the number of mg of KI in the volume of the Solution taken.

Acceptance criteria: 9.5–10.5 g of potassium iodide (KI) in each 100 mL

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, preferably at a temperature not exceeding 35°.

Iodine Tincture

DEFINITION

Iodine Tincture contains NLT 1.8 g and NMT 2.2 g of iodine (I) and NLT 2.1 g and NMT 2.6 g of sodium iodide (NaI) in each 100 mL.

Prepare Iodine Tincture as follows.

Iodine	20 g
Sodium Iodide	24 g
Alcohol	500 mL
Purified Water, a sufficient quantity to make	1000 mL

Dissolve *Iodine* and *Sodium Iodide* in *Alcohol*. Add *Purified Water* to bring to final volume.

USP Monographs

www.webofpharma.com

IDENTIFICATION

• **A.**

Analysis: Add 1 drop to a mixture of 1 mL of starch TS and 9 mL of water.

Acceptance criteria: A deep blue color is produced.

• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Iodide*

Sample: Evaporate a few milliliters on a steam bath to dryness.

Acceptance criteria: The residue meets the requirements of the test for *Iodide*.

• **C. SODIUM:** The *Sample* obtained in *Identification B* imparts an intense yellow color to a nonluminous flame.

ASSAY

• **IODINE**

Sample: 10 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a glass-stoppered 500-mL flask, and dilute with 10 mL of water. Titrate with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Each milliliter of *Titrant* is equivalent to 12.69 mg of iodine (I).

Acceptance criteria: 1.8–2.2 g in 100 mL of Tincture

• **SODIUM IODIDE**

Sample: 10 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M potassium iodate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a glass-stoppered 500-mL flask, add 30 mL of water and 50 mL of hydrochloric acid, cool to room temperature, and titrate with *Titrant* until the dark brown solution that is produced becomes pale brown. Add 1 mL of amaranth TS, and continue the titration slowly until the red color just changes to yellow. The difference in volume, in milliliters, between the *Titrant* used and half the volume of the 0.1 N sodium thiosulfate used in the *Assay* for *Iodine*, multiplied by 14.99, represents the number of milligrams of sodium iodide (NaI) in the portion of Tincture taken.

Acceptance criteria: 2.1–2.6 g in 100 mL of Tincture

OTHER COMPONENTS

• **ALCOHOL DETERMINATION** ⟨611⟩**:** 44.0%–50.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight containers.

Strong Iodine Tincture

DEFINITION

Strong Iodine Tincture contains NLT 6.8 g and NMT 7.5 g of iodine (I) and NLT 4.7 g and NMT 5.5 g of potassium iodide (KI) in each 100 mL.

Prepare Strong Iodine Tincture as follows.

Potassium Iodide	50 g
Iodine	70 g
Purified Water	50 mL
Alcohol, a sufficient quantity to make	1000 mL

Dissolve *Potassium Iodide* in *Purified Water*. Add *Iodine* and agitate until the solution is effected. Add *Alcohol* to bring to final volume.

IDENTIFICATION

• **A.**

Analysis: Add 1 drop to a mixture of 1 mL of starch TS and 9 mL of water.

Acceptance criteria: A deep blue color is produced.

• **B. IDENTIFICATION TESTS—GENERAL** ⟨191⟩, *Iodide*

Analysis: Evaporate a few milliliters on a steam bath to dryness.

Acceptance criteria: The residue meets the requirements of the test for *Iodide*.

• **C. POTASSIUM:** The *Sample* obtained in *Identification B* imparts a violet color to a nonluminous flame. The presence of small quantities of sodium masks the color unless the yellow color produced by sodium is screened out by viewing through a blue filter that blocks the emission at 589 nm (sodium). It is transparent to the emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

ASSAY

• **IODINE**

Sample: 10 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a glass-stoppered 500-mL flask and add 10 mL of water. Titrate with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Each milliliter of *Titrant* is equivalent to 12.69 mg of iodine (I).

Acceptance criteria: 6.8–7.5 g of iodine (I) in 100 mL

• **POTASSIUM IODIDE**

Sample: 10 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M potassium iodate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a glass-stoppered 500-mL flask, add 30 mL of water and 50 mL of hydrochloric acid, cool to room temperature, and titrate with *Titrant* until the dark brown solution that is produced becomes pale brown. Add 1 mL of amaranth TS, and continue the titration slowly until the red color just changes to yellow. The difference between the volume of *Titrant* used and half the volume of 0.1 N sodium thiosulfate used in the *Assay* for *Iodine*, in milliliters, multiplied by 16.60, represents the number of milligrams of potassium iodide (KI) in the portion of Tincture taken.

Acceptance criteria: 4.7–5.5 g of potassium iodide (KI) in 100 mL

OTHER COMPONENTS

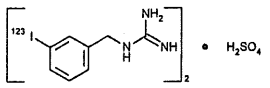
• **ALCOHOL DETERMINATION** ⟨611⟩**:** 82.5%–88.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers.

www.webofpharma.com

Iobenguane I 123 Injection



$(C_8H_{10}{}^{123}IN_3)_2 \cdot H_2SO_4$
[[3-(Iodo-^{123}I)-phenyl]methyl]guanidine sulfate (2:1).
(*m*-Iodo-^{123}I)-benzyl)guanidine sulfate (2:1) [139755-80-9].

» Iobenguane I 123 Injection is a sterile solution containing iobenguane sulfate in which a portion of the molecules contain radioactive iodine (^{123}I) in the molecular structure. Iobenguane I 123 Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of I 123 as iobenguane expressed in megabecquerels (or in millicuries) per mL at the time indicated in the labeling. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of iobenguane. It may contain preservatives or stabilizers.

Packaging and storage—Preserve in single-dose or in multiple-dose containers that are adequately shielded. Store in a freezer.
Labeling—Label it to include the following: the time and date of calibration; the amount of ^{123}I as iobenguane expressed as total megabecquerels (or millicuries) per mL at the time of calibration; the name and quantity of any added preservative or stabilizer; the expiration time; and the statement "Caution —Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{123}I is 13.2 hours.
Radionuclidic identification (see *Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of ^{123}I of known purity that exhibits a major photopeak having an energy of 159 KeV.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 175/*V* USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration time.
pH ⟨791⟩: between 6.0 and 7.5.
Radionuclidic purity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radionuclidic purity of the Injection: not less than 97% of the total radioactivity is present as ^{123}I.

Radiochemical purity—
METHOD 1 (High-pressure liquid chromatographic method) —
Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay for iobenguane sulfate,* except that the liquid chromatograph is also equipped with a collimated radiation detector (see *Radioactivity* ⟨821⟩).
Test preparation—Use *Iobenguane I 123 Injection.*
Procedure—Inject a volume (about 20 µL) of Injection, equivalent to 1.6 to 2.7 MBq (44 to 64 µCi) of the Injection into the chromatograph, record the chromatogram, and measure the areas for the major peaks. The radioactivity of the iobenguane ^{123}I peak is not less than 90% of the total radioactivity measured, and its retention time is within 10% of that in the chromatogram of the *Standard preparation* obtained in the *Assay for iobenguane sulfate.*

METHOD 2 (Thin-layer chromatographic method)—Apply 0.2 to 0.4 µL of the Injection 10 mm from one end of a 20- × 200-mm silica gel glass plate (see *Chromatography* ⟨621⟩), and allow to dry. Develop the chromatogram by ascending chromatography until the solvent front has moved 100 mm from the origin (about 20 minutes), using a solvent system consisting of a mixture of alcohol, ethyl acetate, and ammonium hydroxide (20:20:1). Air-dry the chromatogram, and determine the radioactivity distribution by scanning the chromatogram with a collimated radiation detector: not less than 90% of the total radioactivity is found as ^{123}I at the origin.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the Injection may be distributed or dispensed prior to the completion of the test for *Sterility Tests* ⟨71⟩, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content.*

Assay for iobenguane sulfate—
Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (900:100). Add 3.04 g of triethylamine per liter, and adjust with phosphoric acid to a pH of 4. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Using an accurately weighed quantity of iobenguane sulfate, prepare a solution in water having a known concentration of about 1 mg per mL.
Assay preparation—Use the Injection, which has not yet been brought to full volume with bacteriostatic saline.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 229-nm detector and a 4.6-mm × 25-cm column that contains 10-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed under *Procedure:* the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 1.2, and the relative standard deviation for replicate injections is not more than 1.5%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of iobenguane sulfate in each mL of the Injection taken by the formula:

$$C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of iobenguane sulfate in the *Standard preparation;* and r_U and r_S are the iobenguane peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.
Assay for radioactivity—Using a counting assembly, determine the radioactivity, in MBq (or µCi), per mL, of the Injection by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

Sodium Iodide I 123 Capsules

Sodium iodide ($Na^{123}I$).
Sodium iodide ($Na^{123}I$) [41927-88-2].

» Sodium Iodide I 123 Capsules contain radioactive iodine (^{123}I) processed in the form of Sodium Iodide obtained from the bombardment of enriched tellurium 124 with protons or of enriched tellurium

www.webofpharma.com

122 with deuterons, or by proton irradiation of enriched xenon 124, or by the decay of xenon 123 in such manner that it is carrier-free. Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{123}I as iodide expressed in megabecquerels (microcuries or millicuries) at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 5 percent of the total radioactivity. The Capsules may contain a stabilizer.

Packaging and storage—Preserve in well-closed containers that are adequately shielded.
Labeling—Label the Capsules to include the following: the name of the Capsules; the name, address, and batch or lot number of the manufacturer; the time and date of calibration; the amount of ^{123}I as iodide expressed in megabecquerels (microcuries or millicuries) per Capsule at the time of calibration; the name and quantity of any added preservative or stabilizer; a statement indicating that the Capsules are for oral use only; the expiration date and time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{123}I is 13.2 hours.
Radionuclide identification—A solution or suspension of 1 or more Capsules in water responds to the test for *Radionuclide identification (see Radioactivity 821)*— under *Sodium Iodide I 123 Solution.*
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Determine the instrument response of each of 20 Capsules by measurement in a suitable counting assembly and under identical geometric conditions. Calculate the mean radioactivity value per Capsule: the requirements of the test are met if not less than 19 of the Capsules are within the limits of 96.5% and 103.5% of the mean radioactivity value.
Radionuclidic purity—A solution or suspension of 1 or more Capsules in water responds to the test for *Radionuclidic purity (see Radioactivity 821)*— under *Sodium Iodide I 123 Solution.*
Radiochemical purity—Homogenize 1 Capsule in 3 mL of water, add 3 mL of methanol, and centrifuge: the supernatant so obtained meets the requirements of the test for *Radiochemical purity*— under *Sodium Iodide I 123 Solution.*
Other requirements—A solution or suspension prepared by homogenizing 1 or more Capsules in water to yield a concentration of not less than 1 MBq (25 µCi) per mL meets the requirements of the *Assay for radioactivity*— under *Sodium Iodide I 123 Solution.*

Sodium Iodide I 123 Solution

Sodium iodide ($Na^{123}I$).
Sodium iodide ($Na^{123}I$) [41927-88-2].

» Sodium Iodide I 123 Solution is a solution, suitable for oral or for intravenous administration, containing radioactive iodine (^{123}I) processed in the form of Sodium Iodide, obtained from the bombardment of enriched tellurium 124 with protons or of enriched tellurium 122 with deuterons, or by proton irradiation of enriched xenon 124, or by the decay of xenon 123 in such manner that it is carrier-free.

Sodium Iodide I 123 Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{123}I as iodide expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 5 percent of the total radioactivity. The Solution may contain a preservative or stabilizer.

Packaging and storage—Preserve in single-dose or multiple-dose containers that previously have been treated to prevent adsorption, if necessary.
Labeling—Label it to include the following: the time and date of calibration; the amount of ^{123}I as iodide expressed as total megabecquerels (microcuries or millicuries) per mL at the time of calibration; the name and quantity of any added preservative or stabilizer; a statement to indicate whether the contents are intended for oral or for intravenous use; the expiration date and time; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{123}I is 13.2 hours.
Radionuclide identification (see *Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of ^{123}I of known purity that exhibits a major photoelectric peak having an energy of 0.159 MeV.
Radionuclidic purity (see *Radioactivity* ⟨821⟩)—Using a suitable counting assembly, determine the radionuclidic purity of the Solution: not less than 90% of the total radioactivity is present as I 123.
Bacterial Endotoxins Test ⟨85⟩—Solution intended for intravenous use meets the requirements of the *Bacterial Endotoxins Test* ⟨85⟩; and the limit of endotoxin content is not more than $175/V$ USP Endotoxin Unit per mL of the Injection, when compared with USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.
pH ⟨791⟩**:** between 7.5 and 9.0 for solutions intended for intravenous administration; between 7.5 and 10.0 for solutions intended for oral administration.
Radiochemical purity—Place a measured volume of a solution, containing 100 mg of potassium iodide, 200 mg of potassium iodate, and 1 g of sodium bicarbonate in each 100 mL, 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* ⟨621⟩), and allow to dry. To the same area add a similar volume of appropriately diluted Solution such that it provides a count rate of about 20,000 counts per minute, and allow to dry. Develop the chromatogram over a period of about 4 hours by ascending chromatography, using dilute methanol (7 in 10). Dry the chromatogram in air, and determine the radioactivity distribution by scanning with a suitable collimated radiation detector: the radioactivity of the iodide ^{123}I band is not less than 95.0% of the total radioactivity, and its R_F value falls within ±5.0% of the value found for sodium iodide when determined under similar conditions. Confirmation of the identity of the iodide band is made by the addition to the suspected iodide band of 6 drops of acidified hydrogen peroxide solution (prepared by adding 6 drops of 1 N hydrochloric acid to 10 mL of hydrogen peroxide solution) followed by the dropwise addition of starch TS: the development of a blue color indicates the presence of iodide.

www.webofpharma.com

Other requirements—Solution intended for intravenous use meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.
Assay for radioactivity—Using a suitable counting assembly, determine the radioactivity, in MBq (μCi) per mL, of Solution by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

Iodinated I 125 Albumin Injection

Albumin labeled with iodine-125.

» Iodinated I 125 Albumin Injection is a sterile, buffered, isotonic solution containing normal human albumin adjusted to provide not more than 37 megabecquerels (1 millicurie) of radioactivity per mL. It is derived by mild iodination of normal human albumin with the use of radioactive iodine (^{125}I) to introduce not more than one gram-atom of iodine for each gram-molecule (60,000 g) of albumin.

Iodinated I 125 Albumin Injection contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of ^{125}I as iodinated albumin, expressed in megabecquerels (microcuries or in millicuries) per mL at the time indicated in the labeling. Other forms of radioactivity do not exceed 3 percent of the total radioactivity. Its production and distribution are subject to federal regulations (see *Biologics* ⟨1041⟩ and *Radioactivity* ⟨821⟩).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, at a temperature between 2° and 8°.
Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the date of calibration; the amount of ^{125}I as iodinated albumin, expressed as total megabecquerels (microcuries or millicuries), and concentration as megabecquerels (microcuries or millicuries) per mL on the date of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{125}I is 60 days.
Radionuclide identification (see *Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of ^{125}I of known purity that exhibits a major photopeak having an energy of 0.0355 MeV.
Bacterial Endotoxins Test ⟨85⟩—The limit of endotoxin content is not more than 175/*V* USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.
pH ⟨791⟩: between 7.0 and 8.5.
Radiochemical purity—Place a measured volume, diluted with a suitable diluent so that it provides a count rate of about 20,000 counts per minute, about 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* ⟨621⟩), and allow to dry. Develop the chromatogram over a period of about 4 hours by ascending chromatography, using dilute methanol (7 in 10), and air-dry. Determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector: not less than 97.0% of the total activity is found as albumin (at the point of application).
Other requirements—It meets the requirements under *Biologics* ⟨1041⟩ and under *Injections and Implanted Drug Products* ⟨1⟩, except that it is not subject to the recommendation on *Container Content*. It meets all other applicable requirements of the FDA.
Assay for radioactivity—Using a suitable counting assembly, determine the radioactivity, in MBq (μCi) per mL, of Injection by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

Iothalamate Sodium I 125 Injection

» Iothalamate Sodium I 125 Injection is a sterile solution of Iothalamic Acid in Water for Injection prepared with the aid of Sodium Bicarbonate. A portion of the molecules contain radioactive iodine (^{125}I) in the molecular structure. It may contain small amounts of suitable buffers or a stabilizer.

Iothalamate Sodium I 125 Injection contains not less than 90.0 percent and not more than 110.0 percent of the concentration of Iothalamate Sodium and of the labeled amount of ^{125}I as Iothalamate Sodium expressed in kilobecquerels (or in microcuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 2.0 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose or in multiple-dose containers that are adequately shielded.
Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{125}I as iothalamate sodium expressed as total megabecquerels (microcuries or millicuries equivalent) per mL at the time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{125}I is 60 days.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 175/*V* USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.
pH ⟨791⟩: between 7.0 and 8.5.
Radionuclide identification (see*Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of ^{125}I of known purity that exhibits a major photoelectric peak having an energy of 0.035 MeV.
Radiochemical purity—Place a measured volume of Injection, diluted with a suitable diluent so that it provides a count rate of about 20,000 counts per minute, about 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* ⟨621⟩), and allow to dry. Develop

www.webofpharma.com

the chromatogram over a period of about 4 hours by ascending chromatography, using methanol and ammonium hydroxide (100: 1.5) adjusted with 2 N sulfuric acid to a pH of 3 to 6, and air-dry. Determine the radioactivity distribution with a suitable collimated radiation detector. The radioactivity under the free radioiodide peak is not more than 2% of the total area of all peaks: not less than 98% of the total activity is found at the point of application (as iothalamate sodium).
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it is not subject to the recommendation in *Container Content*.
Assay for radioactivity—Using a suitable counting assembly, determine the radioactivity, in kilobecquerels (or μCi) per mL, of the Injection by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

Iodinated I 131 Albumin Injection

Albumin labeled with iodine-131.

» Iodinated I 131 Albumin Injection is a sterile, buffered, isotonic solution containing normal human albumin adjusted to provide not more than 37 MBq (1 mCi) of radioactivity per mL. It is derived by mild iodination of normal human albumin with the use of radioactive iodine (^{131}I) to introduce not more than one gram-atom of iodine for each gram-molecule (60,000 g) of albumin.

Iodinated I 131 Albumin Injection contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of ^{131}I as iodinated albumin, expressed in megabecquerels (microcuries or in millicuries) per mL at the time indicated in the labeling. Other forms of radioactivity do not exceed 3 percent of the total radioactivity. Its production and distribution are subject to federal regulations (see *Biologics* ⟨1041⟩ and *Radioactivity* ⟨821⟩).

Labeling—Label it to include the following, in addition to the information specified for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*: the date of calibration; the amount of ^{131}I as iodinated albumin, expressed as total megabecquerels (millicuries or microcuries), and concentration as megabecquerels (microcuries or millicuries) per mL on the date of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{131}I is 8.08 days.
Radionuclide identification (see *Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of ^{131}I of known purity that exhibits a major photopeak having an energy of 0.364 MeV.
Other requirements—It meets the requirements for *Packaging and storage*—, *Bacterial Endotoxins Test 85*—, *pH 791:*, *Radiochemical purity*—, and *Assay for radioactivity*—under *Iodinated I 125 Albumin Injection*. It meets also the requirements under *Biologics* ⟨1041⟩, and the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that it is not subject to the recommendation on *Container Content*. It meets all other applicable requirements of the FDA.

Sodium Iodide I 131 Capsules

Sodium iodide ($Na^{131}I$).
Sodium iodide ($Na^{131}I$) [7790-26-3].

» Sodium Iodide I 131 Capsules contain radioactive iodine (^{131}I) processed in the form of Sodium Iodide from products of uranium fission or the neutron bombardment of tellurium in such a manner that it is essentially carrier-free and contains only minute amounts of naturally occurring iodine 127. Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{131}I as iodide expressed in megabecquerels (microcuries or millicuries) at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 5 percent of the total radioactivity. The Capsules may contain a stabilizing agent.

Packaging and storage—Preserve in well-closed containers.
Labeling—Label the Capsules to include the following: the date of calibration; the amount of ^{131}I as iodide expressed in megabecquerels (microcuries or millicuries) per Capsule at the time of calibration; a statement of whether the contents are intended for diagnostic or therapeutic use; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{131}I is 8.08 days.
Radionuclide identification—A solution or suspension of 1 or more Capsules in water responds to the test for *Radionuclide identification (see Radioactivity 821)*— under *Sodium Iodide I 131 Solution*.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Determine the instrument response of each of 20 Capsules by measurement in a suitable counting assembly and under identical geometric conditions. Calculate the mean radioactivity value per Capsule. The requirements are met if not fewer than 19 of the Capsules are within the limits of 96.5% and 103.5% of the mean radioactivity value.
Radiochemical purity—Homogenize 1 Capsule in 3 mL of water, add 3 mL of methanol, and centrifuge. The supernatant meets the requirements of the test for *Radiochemical purity* under *Sodium Iodide I 131 Solution*.
Other requirements—A solution or suspension prepared by homogenizing 1 or more Capsules in sufficient water to yield a concentration of not less than 1 MBq (25 μCi) per mL meets the requirements of the *Assay for radioactivity*— under *Sodium Iodide I 131 Solution*.

Sodium Iodide I 131 Solution

Sodium iodide ($Na^{131}I$).
Sodium iodide ($Na^{131}I$) [7790-26-3].

» Sodium Iodide I 131 Solution is a solution suitable for either oral or intravenous administration, containing radioactive iodine (^{131}I) processed in the

USP Monographs

www.webofpharma.com

form of Sodium Iodide from the products of uranium fission or the neutron bombardment of tellurium in such a manner that it is essentially carrier-free and contains only minute amounts of naturally occurring iodine 127.

Sodium Iodide I 131 Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{131}I as iodide expressed in megabecquerels (microcuries or in millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 5 percent of the total radioactivity. The Solution may contain a preservative or stabilizer.

Packaging and storage—Preserve in single-dose or multiple-dose containers that previously have been treated to prevent adsorption.

Labeling—Label it to include the following: the time and date of calibration; the amount of ^{131}I as iodide expressed as total megabecquerels (microcuries or millicuries) and as megabecquerels (microcuries or millicuries) per mL at the time of calibration; the name and quantity of any added preservative or stabilizer; a statement of the intended use, whether oral or intravenous; a statement of whether the contents are intended for diagnostic or therapeutic use; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{131}I is 8.08 days.

Radionuclide identification (see *Radioactivity* ⟨821⟩)—Its gamma-ray spectrum is identical to that of a specimen of iodine 131 of known purity that exhibits a major photopeak having an energy of 0.364 MeV.

Bacterial Endotoxins Test ⟨85⟩—Solution intended for intravenous use meets the requirements of the *Bacterial Endotoxins Test* ⟨85⟩, the limit of endotoxin content being not more than 175/*V* USP Endotoxin Unit per mL of the Solution, when compared with the USP Endotoxin RS, in which *V* is the maximum recommended total dose, in mL, at the expiration date or time.

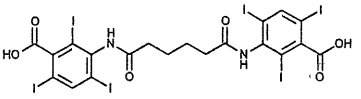
pH ⟨791⟩: between 7.5 and 9.0 for solutions intended for intravenous administration; between 7.5 and 10.0 for solutions intended for oral administration.

Radiochemical purity—Place a measured volume of a solution containing 100 mg of potassium iodide, 200 mg of potassium iodate, and 1 g of sodium bicarbonate in each 100 mL, 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* ⟨621⟩), and allow to dry. To the same area add a similar volume of appropriately diluted Solution such that it provides a count rate of about 20,000 counts per minute, and allow to dry. Develop the chromatogram over a period of about 4 hours by ascending chromatography, using dilute methanol (7.0 in 10). Dry the chromatogram in air, and determine the radioactivity distribution by scanning with a suitable collimated radiation detector: the radioactivity of the iodide ^{131}I band is not less than 95% of the total radioactivity, and its R_F value falls within ±5% of the value found for sodium iodide when determined under parallel conditions. Confirmation of the identity of the iodide band is made by the addition to the suspected iodide band of 6 drops of acidified hydrogen peroxide solution (prepared by adding 6 drops of 1 N hydrochloric acid to 10 mL of hydrogen peroxide solution) followed by the dropwise addition of starch TS; the development of a blue color indicates presence of iodide.

Other requirements—Solution intended for intravenous use meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, except that the Solution may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

Assay for radioactivity—Using a suitable counting assembly, determine the radioactivity, in MBq (μCi) per mL, of Solution by use of a calibrated system as directed under *Radioactivity* ⟨821⟩.

Iodipamide



$C_{20}H_{14}I_6N_2O_6$ 1139.76

Benzoic acid, 3,3′-[(1,6-dioxo-1,6-hexanediyl)diimino] bis[2,4,6-triiodo-.

3,3′-(Adipoyldiimino)bis[2,4,6-triiodobenzoic acid] [606-17-7].

» Iodipamide contains not less than 98.0 percent and not more than 102.0 percent of $C_{20}H_{14}I_6N_2O_6$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—

USP 3-Amino-2,4,6-triiodobenzoic Acid RS

$C_7H_4I_3NO_2$ 514.83

USP Iodipamide RS

Identification—

A: It responds to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, the test solution and the Standard solution being prepared at a concentration of 1 mg per mL in an 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

B: Heat about 500 mg in a suitable crucible: violet vapors are evolved.

Water Determination, *Method I* ⟨921⟩: not more than 1.0%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Free aromatic amine—Transfer 1.0 g to a 50-mL volumetric flask, and add 12.5 mL of water and 2.5 mL of 1 N sodium hydroxide. To a second 50-mL volumetric flask transfer 4 mL of water, 10 mL of 0.1 N sodium hydroxide, and 1.0 mL of a Standard solution prepared by dissolving a suitable quantity of USP 3-Amino-2,4,6-triiodobenzoic Acid RS in 0.1 N sodium hydroxide (use 0.2 mL of the 0.1 N sodium hydroxide for each 5.0 mg of the Reference standard) and by diluting with water to obtain a solution having a known concentration of 500 μg per mL. Proceed as directed in the test for *Free aromatic amine* under *Diatrizoate Meglumine* beginning with "To a third 50-mL volumetric flask add 5 mL of water."

Other requirements—It meets the requirements of the tests for *Iodine and iodide* under *Diatrizoic Acid*.

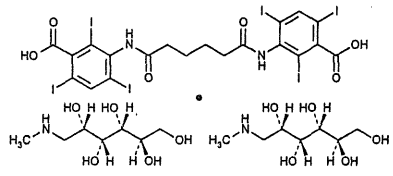
Assay—Transfer about 300 mg of Iodipamide, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 30 mL of 1.25 N sodium hydroxide and 500 mg of powdered

USP Monographs

www.webofpharma.com

zinc, connect the flask to a reflux condenser, and reflux the mixture for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the filter and the flask thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid and 1 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.05 N silver nitrate VS until the yellow precipitate just turns green. Each mL of 0.05 N silver nitrate is equivalent to 9.498 mg of $C_{20}H_{14}I_6N_2O_6$.

Iodipamide Meglumine Injection



$C_{20}H_{14}I_6N_2O_6 \cdot 2C_7H_{17}NO_5$ 1530.19

Benzoic acid, 3,3′-[(1,6-dioxo-1,6-hexanediyl)diimino] bis[2,4,6-triiodo-, compd. with 1-deoxy-1-(methylamino)-D-glucitol(1:2).

1-Deoxy-1-(methylamino)-D-glucitol 3,3′-(adipoyldiimino) bis[2,4,6-triiodobenzoate] (2:1) (salt) [3521-84-4].

» Iodipamide Meglumine Injection is a sterile solution of Iodipamide in Water for Injection, prepared with the aid of Meglumine. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of iodipamide meglumine ($C_{20}H_{14}I_6N_2O_6 \cdot 2C_7H_{17}NO_5$). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Iodipamide Meglumine Injection intended for intravascular use contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose containers, preferably of Type I or Type III glass, protected from light.

Labeling—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container. Label containers of Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.

USP Reference standards ⟨11⟩—

USP 3-Amino-2,4,6-triiodobenzoic Acid RS
$C_7H_4I_3NO_2$ 514.83

USP Iodipamide RS

Identification—

A: Dilute a volume of the Injection, if necessary, with a 0.8 in 1000 solution of sodium hydroxide in methanol to obtain a test solution having a concentration of 1 mg per mL. The test solution responds to the *Thin-layer Chromatographic Identification Test* ⟨201⟩, the Standard solution being prepared at a concentration of 1 mg of USP Iodipamide RS per mL in a 0.8 in 1000 solution of sodium hydroxide in methanol, the solvent mixture being a mixture of chloroform, methanol, and ammonium hydroxide (20:10:2), and short-wavelength UV light being used to locate the spots.

B: Evaporate a volume of Injection, equivalent to about 500 mg of iodipamide, to dryness, and heat the residue so obtained in a suitable crucible: violet vapors are evolved.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 3.6 USP Endotoxin Units per mL.

pH ⟨791⟩: between 6.5 and 7.7.

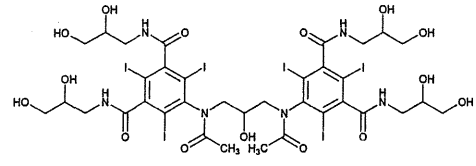
Free aromatic amine—Transfer an accurately measured volume of Injection, equivalent to 1 g of iodipamide meglumine, to a 50-mL volumetric flask, dilute with water to 5 mL, and add 10 mL of 0.1 N sodium hydroxide. To a second 50-mL volumetric flask add 10 mL of 0.1 N sodium hydroxide, 4 mL of water, and 1.0 mL of a Standard solution prepared by dissolving a suitable quantity of USP 3-Amino-2,4,6-triiodobenzoic Acid RS in 0.1 N sodium hydroxide (use 0.2 mL of the 0.1 N sodium hydroxide for each 5.0 mg of the Reference Standard) and diluting with water to obtain a solution having a known concentration of 500 µg per mL. Proceed as directed in the test for *Free aromatic amine*— under *Diatrizoate Meglumine*, beginning with "To a third 50-mL volumetric flask add 5 mL of water."

Meglumine content—Proceed as directed in the test for *Meglumine content*— under *Diatrizoate Meglumine Injection*. The meglumine content is not less than 23.5% and not more than 26.8% of the labeled amount of iodipamide meglumine.

Other requirements—It meets the requirements of the tests for *Iodine and iodide*— under *Diatrizoate Meglumine Injection*. It meets also the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—Pipet a volume of Injection, equivalent to about 5 g of iodipamide meglumine, into a 100-mL volumetric flask, add 1.25 N sodium hydroxide to volume, and mix. Pipet 10 mL of the solution into a glass-stoppered, 250-mL flask, add 20 mL of the sodium hydroxide solution and 500 mg of powdered zinc, and proceed as directed in the *Assay*— under *Diatrizoate Meglumine Injection*, beginning with "connect the flask to a reflux condenser." Each mL of 0.05 N silver nitrate is equivalent to 12.75 mg of $C_{20}H_{14}I_6N_2O_6 \cdot 2C_7H_{17}NO_5$.

Iodixanol



$C_{35}H_{44}I_6N_6O_{15}$ 1550.18

1,3-Benzenedicarboxamide, 5,5′-[(2-hydroxy-1,3-propanediyl)bis(acetylimino)]bis[*N,N′*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-;

5,5′-[(2-Hydroxytrimethylene)bis(acetylimino)]bis[*N,N′*-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide] [92339-11-2].

DEFINITION

Iodixanol contains NLT 98.6% and NMT 101.0% of iodixanol ($C_{35}H_{44}I_6N_6O_{15}$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

www.webofpharma.com

• **B.** The retention times of the three principal peaks of the *Sample solution* correspond to those of the *Identification solution*, as obtained in the test for *Limit of Iodixanol Related Compound E and Iodixanol Impurity H*. [NOTE—A third isomer may appear as a minor peak.]

ASSAY

• **PROCEDURE**

Sample solution: Transfer 500 mg of Iodixanol to a glass-stoppered, 125-mL conical flask. Add 25 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc. Connect the flask to a reflux condenser, and reflux for 1 h. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and pass the mixture through a filter. Rinse the flask and the filter thoroughly with small portions of water, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Potentiometric

Analysis

Sample: *Sample solution*

Titrate the *Sample solution* with the *Titrant*.

Calculate the percentage of iodixanol ($C_{35}H_{44}I_6N_6O_{15}$) in the portion of Iodixanol taken:

$$\text{Result} = [(V \times N \times F)/W] \times 100$$

V = sample titrant volume (mL)
N = *Titrant* normality (meq/mL)
F = equivalent weight of iodixanol, 258.4 mg/meq
W = weight of iodixanol (mg)

Acceptance criteria: 98.6%–101.0% on the anhydrous basis

IMPURITIES

• **LIMIT OF FREE IODIDE**

Sample solution: 5 g of Iodixanol in 30 mL of water

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.001 N silver nitrate VS

Endpoint detection: Potentiometric

Analysis

Calculate the percentage of free iodide in the portion of Iodixanol taken:

$$\text{Result} = [(V \times N \times F)/W] \times 100$$

V = sample titrant volume (mL)
N = *Titrant* normality (meq/mL)
F = equivalent weight of iodide, 0.1269 mg/meq
W = weight of iodixanol (mg)

Acceptance criteria: NMT 0.001%

• **LIMIT OF IONIC COMPOUNDS**

[NOTE—Rinse all glassware with water.]

Standard solution: 4 µg/mL of sodium chloride in water

Sample solution: 2 g of Iodixanol in 100 mL of water

Acceptance criteria: The specific conductance in the *Sample solution* is NMT that of the *Standard solution* (equivalent to NMT 0.02% of ionic compounds, as sodium chloride).

• **LIMIT OF FREE AROMATIC AMINE**

Solution A: 3 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in a mixture of propylene glycol and water (70:30)

Blank solution: Add 15 mL of water to a 25-mL volumetric flask.

Standard stock solution: 10 µg/mL of USP Iohexol Related Compound B RS in water

Standard solution: Transfer 10.0 mL of the *Standard stock solution* and 5 mL of water to a 25-mL volumetric flask.

Sample solution: Transfer 200 mg of Iodixanol to a 25-mL volumetric flask, and add 15 mL of water.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 495 nm

Cell: 5 cm

Analysis

Samples: *Blank solution*, *Standard solution*, and *Sample solution*

Treat the *Samples* as follows. Place the flask in an ice bath for 5 min. Add 1.5 mL of 6 N hydrochloric acid, and mix by swirling. Add 1.0 mL of sodium nitrite solution (20 mg/mL), and allow to stand in the ice bath for 4 min. Remove the flask from the ice bath, add 1.0 mL of sulfamic acid solution (40 mg/mL), and swirl gently until gas evolution ceases. [**CAUTION**—Considerable pressure is produced.] Add 1.0 mL of *Solution A*, dilute with water to volume, and allow to stand for 5 min.

Measure the absorbance of the *Standard solution* and the *Sample solution* against the *Blank solution*.

Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (NMT 0.05% of free aromatic amine).

• **LIMIT OF 2-METHOXYETHANOL**

Internal standard solution: 0.01 mg/mL of secondary butyl alcohol in water

Standard stock solution: 0.005 mg/mL of methanol and 0.01 mg each of isopropyl alcohol, secondary butyl alcohol, and 2-methoxyethanol in *Internal standard solution*

Standard solution: Transfer about 0.25 g of USP Iodixanol RS and 1.0 mL of *Standard stock solution* to a headspace vial and seal the vial with a septum and crimp cap.

Sample solution: Transfer about 0.25 g of Iodixanol and 1.0 mL of *Internal standard solution* to a headspace vial and seal the vial with a septum and crimp cap.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC with suitable headspace autosampler

Detector: Flame ionization

Column: 0.53-mm × 30-m fused-silica; coated with a 1-µm phase G16

Temperatures

Autosampler: 105°

Needle: 130°–140°

Injection port: 150°

Detector: 200°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	3
40	8	100	1

Carrier gas: Helium

Flow rate: 11 mL/min

Injection volume: 1 mL of the headspace

System suitability

Sample: *Standard solution*

www.webofpharma.com

[NOTE—The typical relative retention times for methanol, isopropyl alcohol, secondary butyl alcohol, and 2-methoxyethanol are 0.5, 0.6, 1.0 and 1.9 respectively.]

Suitability requirements

Resolution: NLT 1.0 between methanol and isopropyl alcohol

Relative standard deviation: NMT 10.0% for the ratio of 2-methoxyethanol to internal standard

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount of 2-methoxyethanol in the portion of Iodixanol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak response ratio of 2-methoxyethanol to the internal standard from the *Sample solution*

R_S = peak response ratio of 2-methoxyethanol to the internal standard from the *Standard solution*

C_S = concentration of 2-methoxyethanol in the *Standard solution* (µg/mL)

C_U = concentration of Iodixanol in *Sample solution* (g/mL)

Acceptance criteria: NMT 10 µg/g of 2-methoxyethanol

• **ORGANIC IMPURITIES**

Solution A: Water

Solution B: Acetonitrile and water (50:50)

System suitability solution: 0.25 mg/mL of USP Iodixanol RS, 0.0025 mg/mL of USP Iodixanol Related Compound C RS, and 0.005 mg/mL of USP Iodixanol Related Compound D RS in water

Sample solution: 2.5 mg/mL of Iodixanol in water

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	94	6
2	94	6
32	80	20
72	0	100
82	0	100

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

Use the chromatogram from the *System suitability solution* to identify the peaks based on the relative retention times given in *Table 3*.

Suitability requirements

Resolution: NLT 1.5 between the two peaks due to iodixanol related compound D

Peak-to-valley ratio: NLT 1.3 between the first iodixanol peak and iodixanol related compound C (first peak)

Analysis

Sample: *Sample solution*

[NOTE—If iodixanol related compound C is present, only the first and larger peak with a retention time of 1.04 relative to the main iodixanol peak is seen between the two principal iodixanol peaks; the second iodixanol related compound C peak co-elutes with iodixanol. The area of the first and larger peak corresponds to approximately 80% of the total area of iodixanol related compound C. Determine the peak area of the first peak by drawing a vertical line through the minimum before the peak and a horizontal baseline at the minimum after the peak.]

Calculate the percentage of each impurity in the *Sample solution*:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity in the *Sample solution*

r_T = sum of all peak responses greater than 0.05% of the principal peaks in the *Sample solution*

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any impurity less than or equal to 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Iodixanol related compound D (first peak)	0.8	1.0	Sum of both peaks 0.1% if present
Iodixanol related compound D (second peak)	0.9	1.0	
Iodixanol (first peak)	1.0	—	—
Iodixanol related compound C (first peak)	1.04	0.76	0.4
Overalkylated impurities	1.3–1.7	1.0	(Sum of all) 1.0
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.5

• **LIMIT OF IODIXANOL RELATED COMPOUND E AND IODIXANOL IMPURITY H**

Solution A: Acetonitrile and water (50:50)

Solution B: Acetonitrile

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	30	70
2	30	70
27	68	32

System suitability solution: 0.25 mg/mL of USP Iodixanol RS and 0.025 mg/mL of USP Iodixanol Related Compound E RS in water

Identification solution: 2.5 mg/mL of USP Iodixanol RS in water

Sample solution: 2.5 mg/mL of Iodixanol in water

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L8

Flow rate: 1.7 mL/min

www.webofpharma.com

Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—See *Table 5* for relative retention times.]
Suitability requirements
Resolution: NLT 5.0 between iodixanol related compound E (first peak) and iodixanol (first peak)
Analysis
Samples: *Identification solution* and *Sample solution*
[NOTE—Iodixanol related compound E exhibits two peaks, the second of which may partly overlap with one of iodixanol peaks; use only the area of the first and larger peak of iodixanol related compound E, which corresponds to approximately 60% of the total area of iodixanol related compound E.]
Use the chromatograms obtained from the *Identification solution* and *Sample solution* for *Identification* test *B*.
Calculate the percentage of iodixanol related compound E and iodixanol impurity H in the *Sample solution*:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity in the *Sample solution*
r_T = sum of all peak responses greater than 0.05% of the principal peaks in the *Sample solution*
F = relative response factor (see *Table 5*)

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Iodixanol related compound E (first peak)	0.7	0.58	Sum of both peaks 0.3
Iodixanol related compound E (second peak)	0.8	1.0	
Iodixanol (first peak)	1.0	—	—
Iodixanol impurity H[a]	1.4	1.0	0.6

[a] 5-[[3-[[3-[[3-[[3-[[3,5-bis-[[[2,3-Dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl](acetylimino)]-2-hydroxypropyl](acetylimino)]-5-[[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl]carbonyl]amino]-2-hydroxypropyl]oxy]-2-hydroxypropyl](acetylimino)]-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzendicarboxamide.

SPECIFIC TESTS
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 4.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Iodixanol RS
USP Iodixanol Related Compound C RS
5-[Acetyl[3-[[3,5-bis[[(2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]-2-hydroxypropyl]amino]*N,N'*-bis-(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
$C_{33}H_{42}I_6N_6O_{14}$ 1508.15
USP Iodixanol Related Compound D RS
5-[Acetyl(2-hydroxy-3-methoxypropyl)amino]-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
$C_{20}H_{28}I_3N_3O_9$ 835.16
USP Iodixanol Related Compound E RS
(5-{*N*-[3-(*N*-{3-Carbamoyl-5-[(2,3-dihydroxypropyl)carbamoyl]-2,4,6-triiodophenyl}acetamido)-2-hydroxypropyl]acetamido}-*N*1,*N*3-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide.
USP Iohexol Related Compound B RS
5-Amino-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
$C_{14}H_{18}I_3N_3O_6$ 705.02

Iodixanol Injection

DEFINITION
Iodixanol Injection is a sterile solution of Iodixanol in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of iodixanol ($C_{35}H_{44}I_6N_6O_{15}$), as organically bound iodine. It may contain stabilizers and buffers. It contains no antimicrobial agents.

IDENTIFICATION
• **A.** The retention times of the two major peaks of the *Sample solution* correspond to those of *Standard solution B*, as obtained in *Organic Impurities, Procedure 2*.

ASSAY
• **PROCEDURE**
Sample solution: Transfer 2 mL of the Injection to a glass-stoppered, 125-mL conical flask, add 50 mL of 5% sodium hydroxide solution and 1.0 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 1 h. Cool the flask to room temperature, and rinse the condenser with 20 mL of water, adding the rinsing to the refluxed solution. Filter the mixture, rinsing the flask and the filter with several small portions of water, and adding the filtered rinsings to the filtrate. Add 20 mL of glacial acetic acid, dilute with water to 200.0 mL, and transfer 100.0 mL of this solution to a 250-mL conical flask.
Analysis: Titrate with 0.1 N silver nitrate VS using autotitration. Each mL of 0.1 N silver nitrate is equivalent to 25.84 mg of $C_{35}H_{44}I_6N_6O_{15}$. [NOTE—The result must be corrected for any inorganic halides that may be present due to added stabilizers or buffers.]
Acceptance criteria: 95.0%–105.0%

IMPURITIES
• **ORGANIC IMPURITIES, PROCEDURE 1**
Solution A: Acetonitrile and water (1:1)
Solution B: Water
Blank solution: Water
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	6	94
30	20	80
70	100	0
80	100	0
81	6	94
90	6	94

Standard stock solution A: 12.5 mg/mL of anhydrous iodixanol from USP Iodixanol RS in water

www.webofpharma.com

Standard stock solution B: 0.25 mg/mL of anhydrous iodixanol related compound C from USP Iodixanol Related Compound C RS in water
Standard stock solution C: 0.025 mg/mL of anhydrous iodixanol related compound D from USP Iodixanol Related Compound D RS in water
Standard solution A: 2.5 mg/mL of *Standard stock solution A* in water
Standard solution B: *Standard stock solution A*, *Standard stock solution B*, *Standard stock solution C*, and water (5: 2.5: 2.5: 15)
Sample solution A: 25 mg/mL of iodixanol obtained from a volume of Injection in water
Sample solution B: 2.5 mg/mL of iodixanol obtained from a volume of Injection in water
Control solution: *Sample solution A*, *Standard stock solution B*, and water (5: 2.5: 42.5)
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Blank solution*, *Standard solution A*, at least three replicates of *Standard solution B*, and *Control solution*
Chromatograph the solutions as mentioned above. The chromatogram from *Standard solution A* exhibits two or three principal unresolved peaks. If the chromatogram exhibits two principal peaks, their relative areas are 60% and 40%. If the chromatogram exhibits three principal peaks, their relative areas are 60%, 38%, and 2%. The chromatogram from *Standard solution B* exhibits two resolved peaks due to iodixanol related compound D that elute before the iodixanol peaks and one iodixanol related compound C peak between the two principal iodixanol peaks. The area of the two iodixanol related compound D peaks is between 0.075% and 0.125% of the total area. Add the areas of the two isomer peaks for iodixanol related compound D from each of the three injections of *Standard solution B*, and calculate the relative standard deviation for the three summed areas: the relative standard deviation is NMT 5%. Measure the height of the iodixanol related compound C peak, and, if necessary, adjust the sensitivity of the amplifier to obtain a peak height between 80% and 100% of the full scale. Measure the height, *A*, above the baseline of the iodixanol related compound C peak and the height, *B*, above the baseline of the lowest part of the curve separating this peak from the first principal iodixanol peak: *A* is NLT 1.3*B*. In the chromatogram from the *Control solution*, iodixanol related compound C exhibits a measurable peak.
Analysis
Samples: *Blank solution*, *Sample solution A*, and *Sample solution B*
High-low chromatography: Where it is specified to proceed as directed for *High-low chromatography*, for the chromatogram from *Sample solution A*, calculate the percentage of each specified related compound in the portion of Injection taken:

$$\text{Result} = (10X)/(0.1Y + Z)$$

X = peak area for each of the specified related compounds from *Sample solution A*
Y = total area of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent
Z = sum of peak areas of iodixanol and any related compounds that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

Iohexol: If iohexol is present, it exhibits two peaks, with retention times of 0.37 and 0.39 relative to the main iodixanol peak, in the chromatogram from *Sample solution A*. Draw a baseline at the height of the baseline from the *Blank solution*.
Calculate the total area of the two peaks and the percentage of iohexol in the portion of Injection taken as directed for *High-low chromatography*.
Iohexol related compound A[1]**:** If iohexol related compound A is present, it elutes as a single peak with a retention time of 0.34 relative to the main iodixanol peak, in the chromatogram from *Sample solution A*. Draw a baseline at the height of the baseline from the *Blank solution*.
Calculate the area of the peak and the percentage of iohexol related compound A in the portion of Injection taken as directed for *High-low chromatography*.
Iodixanol related compound C: If iodixanol related compound C is present, only the first and larger peak, with a retention time of 1.07 relative to the main iodixanol peak, is seen between the two principal iodixanol peaks in the chromatogram from *Sample solution A*; the second iodixanol related compound C peak co-elutes with iodixanol. The area of the first and larger peak corresponds to 80% of the total area of iodixanol related compound C. Draw a vertical line through the minimum before the first and larger peak. Draw a horizontal baseline at the minimum after the first and larger peak.
Calculate the percentage of iodixanol related compound C in the portion of Injection taken:

$$\text{Result} = 12.5X_2/(0.1Y + Z)$$

X_2 = iodixanol related compound C peak area
Y = total area of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent
Z = sum of peak areas of iodixanol and any related compounds that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

Iodixanol related coumpound F[2]**:** If iodixanol related compound F is present, only the first and smaller peak with a retention time of 0.8 relative to the main iodixanol peak can be seen in the chromatogram from *Sample solution A*; the second peak co-elutes with iodixanol. The area of the first and smaller peak corresponds to 25% of the total area of iodixanol related compound F. Draw the baseline at the height of the baseline from the *Blank solution*.
Calculate the percentage of iodixanol related compound F in the portion of Injection taken:

$$\text{Result} = 40X_1/(0.1Y + Z)$$

X_1 = actual observed area of the peak of iodixanol related compound F from *Sample solution A*

[1] 5-(Acetylamino)-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
[2] 2-[[Acetyl[3,5-bis[[(2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4*H*-1,4-benzoxazine-6,8-dicarboxamide.

USP Monographs

www.webofpharma.com

Y = total area of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent
Z = sum of peak areas of iodixanol and any related compounds that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

Iodixanol related compound G[3]**:** If iodixanol related compound G is present, the second and larger peak, with a retention time of 1.18 relative to the last iodixanol peak, is seen in the chromatogram from *Sample solution A*; the first peak co-elutes with iodixanol. The area of the second peak corresponds to 85% of the total area of iodixanol related compound G. Draw the baseline at the height of the baseline from the *Blank solution*.
Calculate the percentage of iodixanol related compound G in the portion of Injection taken:

$$\text{Result} = 10X_3/[0.85(0.1Y + Z)]$$

X_3 = peak area of iodixanol related compound G
Y = total area of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent
Z = sum of peak areas of iodixanol and any related compounds that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

Overalkylated related compounds: These compounds elute after iodixanol related compound G, with a retention time greater than 1.18 relative to the last iodixanol peak. Draw the baseline at the height of the baseline from the *Blank solution*, and determine the peak areas.
Calculate the percentage of overalkylated related compounds as directed for *High-low chromatography*.
Unspecified related compounds: Examine the chromatograms from *Sample solution A* and the area of each peak eluting before or after iodixanol, other than those of iodixanol, specified related compounds, specified impurities, and overalkylated related compounds. Draw the baseline at the height of the baseline from the *Blank solution*.
Calculate the percentage of the largest of these peaks as directed for *High-low chromatography*.
Other unspecified related compounds: Determine the area of any unspecified peak eluting between those of iodixanol. Draw the baseline between minima, and calculate the percentage as directed for *High-low chromatography*.
Individual impurities: See *Table 2*.

Table 2

Name	Acceptance Criteria, NMT (%)
Iohexol related compound A	0.2
Iodixanol related compound C	0.4
Iodixanol related compound F	0.2
Iodixanol related compound G	0.2
Iohexol	0.6
Overalkylated related compounds	1.0
Any individual unspecified related compound	0.2
Total unspecified related compounds	0.5

[3] 4-Acetyl-2-[[acetyl[3,5-bis[[(2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4*H*-1,4-benzoxazine-6,8-dicarboxamide.

Total related compounds: From each of the chromatograms from *Sample solution A*, calculate the percentage of all related compounds as the sum of the results for the peaks appearing between the two principal iodixanol peaks, and the area percent:

$$\text{Result} = [100(Y - X_1 - X_3 + X_1/0.25 + X_2/0.8 + X_3/0.85)]/10(0.1Y + Z)$$

Y = total area of all the peaks eluted before and after iodixanol from *Sample solution A*, disregarding any peaks due to injection noise or solvent
X_1 = actual observed area of the peak of iodixanol related compound F from *Sample solution A*
X_3 = peak area of iodixanol related compound G
X_2 = iodixanol related compound C peak area
Z = sum of peak areas of iodixanol and any related compounds that are eluted together with, and between, the principal iodixanol peaks from *Sample solution B*

Total impurities: NMT 1.5%

• **ORGANIC IMPURITIES, PROCEDURE 2**
Solution A: Acetonitrile
Solution B: Water
Blank solution: Water
Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	85	15
25	66	34

Standard stock solution A: 12.5 mg/mL of anhydrous iodixanol from USP Iodixanol RS
Standard stock solution B: 0.025 mg/mL of anhydrous iodixanol related compound D from USP Iodixanol Related Compound D RS
Standard stock solution C: 2.5 mg/mL of anhydrous iodixanol related compound E from USP Iodixanol Related Compound E RS
Standard solution A: 2.5 mg/mL of anhydrous iodixanol from *Standard stock solution A* diluted with water
Standard solution B: 2.5 mg/mL of anhydrous iodixanol and 0.0025 mg/mL of anhydrous iodixanol related compound D from *Standard stock solution A* and *Standard stock solution B*, respectively, diluted with water
Standard solution C: 2.5 mg/mL of anhydrous iodixanol and 0.25 mg/mL of anhydrous iodixanol related compound E from *Standard stock solution A* and *Standard stock solution C*, respectively, diluted with water
Sample solution: 2.5 mg/mL of iodixanol from a volume of Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L8
Flow rate: 2.5 mL/min

www.webofpharma.com

Injection volume: 10 µL
System suitability
Samples: *Standard solution A*, three times of *Standard solution B*, *Standard solution C*, and *Sample solution*
The chromatogram from *Standard solution A* exhibits three principal unresolved peaks: the relative areas are 62%, 35%, and 3%; and the retention time of the last iodixanol peak is NMT 14 min. The chromatogram from *Standard solution B* exhibits two partially unresolved peaks due to iodixanol related compound D, with relative retention times of 0.33 and 0.39, that elute before the iodixanol peaks: the peak area of iodixanol related compound D is between 0.075% and 0.125% of the total area. Disregard any peak due to the solvent.
Determine the sum of the peak areas of the two isomers of iodixanol related compound D for each of the three chromatograms from *Standard solution B*.
Relative standard deviation: NMT 5%
The chromatogram from *Standard solution C* exhibits two unresolved peaks due to iodixanol related compound E, with relative retention times of 0.67 and 0.72, that elute before the iodixanol peaks. Adjust the sensitivity of the amplifier so that the peak heights are between 90% and 100% of full scale of the highest peak.
Resolution, R: NLT 5.0 between the first and largest iodixanol related compound E peak and the first principal iodixanol peak
Analysis
Samples: *Standard solution A*, three times of *Standard solution B*, *Standard solution C*, and *Sample solution*
For the first chromatogram from *Standard solution B*, adjust the sensitivity of the amplifier to obtain a peak height of 15% of the first and larger peak that corresponds to iodixanol related compound D. Use this sensitivity setting for the subsequent injections.
Compare the retention times of the peaks from *Standard solution C* to those from the *Sample solution*. Iodixanol related compound E exhibits two peaks, the second of which may partly overlap with another peak; use only the area of the first and larger peak, which corresponds to 60% of the total area of iodixanol related compound E. Draw a baseline at the height of the baseline from the *Blank solution*.
Calculate the percentage of iodixanol related compound E by dividing the area from the *Sample solution* by 0.6 and using area percent.
Iodixanol related compound H[4]: Appears as a single peak, with a shoulder, on the tail of the iodixanol peak. Calculate the percentage of iodixanol related compound H by using area percent.
Acceptance criteria: NMT 0.3% of iodixanol related compound E is found and NMT 0.6% of iodixanol related compound H is found.

SPECIFIC TESTS
- **pH** ⟨791⟩: 6.8–7.7
- **Bacterial Endotoxins Test** ⟨85⟩: NMT 0.2 USP Endotoxin Unit/50 mg of iodine
- **Osmolality and Osmolarity,** *Osmolality* ⟨785⟩: 270–310 mOsmol/kg
- **Injections and Implanted Drug Products** ⟨1⟩: Meets the requirements
- **Limit of Free Iodide**
Sample: 5.0 mL of Injection
Analysis: Transfer *Sample* to a suitable container, add 2.0 mL of acetic acid solution (1:5 glacial acetic acid in water) and 30 mL of water, and titrate with 0.001 N silver nitrate VS. Each mL of 0.001 N silver nitrate is equivalent to 0.1269 mg of iodine. NMT 15.0 mL of 0.001 N silver nitrate is required.
Acceptance criteria: NMT 0.02% based on the content of iodixanol

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in single-dose or multi-dose plastic or Type I glass containers. Store at controlled room temperature, protected from light. Do not freeze.
- **Labeling:** Label containers of Injection to direct the user to discard any unused portion. The labeling states also that it is not to be used if it is discolored or contains a precipitate. Label it to state its routes of administration. Label it to indicate "not approved for intrathecal use".
- **USP Reference Standards** ⟨11⟩
USP Iodixanol RS
USP Iodixanol Related Compound C RS
5-[Acetyl[3-[[3,5-bis[[(2,3-dihydroxyprop yl)amino] carbonyl]-2,4,6-triiodophenyl]amino]-2-hydroxypropyl] amino]*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
USP Iodixanol Related Compound D RS
5-[Acetyl(2-hydroxy-3-methoxypropyl)amino]-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
USP Iodixanol Related Compound E RS
5-[[3-[[3-[[(2,3-Dihydroxypropyl)amino]carbonyl]-5-[[amino]carbonyl]-2,4,6-triiodophenyl](acetylimino)]-2-hydroxypropyl]-(acetylimino)]-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.

Iodoform

CHI_3 393.73
Triiodomethane [75-47-8].

DEFINITION
Iodoform, previously dried over silica gel for 24 h, contains NLT 99.0% and NMT 100.5% of iodoform (CHI_3).

IDENTIFICATION
- **A.**
Sample: 0.1 g
Analysis: Heat the *Sample*.
Acceptance criteria: A purple gas is evolved.

ASSAY
- **Procedure**
Sample: 200 mg of Iodoform, previously dried over silica gel for 24 h
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Residual titration
Titrant: 0.1 N silver nitrate VS
Back-titrant: 0.1 N ammonium thiocyanate VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 500-mL glass-stoppered conical flask. Add 20 mL of alcohol, and swirl to dissolve. Add 30.0 mL of *Titrant* and 10 mL of nitric acid, insert the stopper, and allow it to stand in a dark place for 16 h. Add 150 mL of water and 5 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with *Back-titrant*.

[4] 5-[[3-[[3-[[[3-[[3-[[3,5-Bis-[[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl](acetylimino)]-2-hydroxypropyl](acetylimino)]-5-[[[2,3-dihydroxypropyl]amino]carbonyl]-2,4,6-triiodophenyl]carbonyl] amino]-2-hy droxypropyl]oxy]-2-hydroxypropyl](acetylimino)]-*N*,*N*′-bis(2,3-dihy droxypropyl)-2,4,6-triiodo-1,3-benzendicarboxamide.

www.webofpharma.com

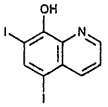
Perform a blank determination. Each mL of 0.1 N silver nitrate is equivalent to 13.124 mg of iodoform (CHI_3).
Acceptance criteria: 99.0%–100.5% on the previously dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
• **CHLORIDE AND SULFATE,** *Chloride* ⟨221⟩
Sample solution: 3.0 g of powdered Iodoform in 75 mL of water. Shake this solution for 1 min, allow to stand, and filter the supernatant. Use the filtrate for the analysis of Chloride and Sulfate.
Analysis: To 25 mL of the *Sample solution* add 6 mL of 3 N nitric acid, and dilute with water to 40 mL.
Acceptance criteria: 0.011%; this solution shows no more chloride than corresponds to 0.15 mL of 0.020 N hydrochloric acid.
• **CHLORIDE AND SULFATE,** *Sulfate* ⟨221⟩
Sample solution: Use as directed in the analysis of Chloride.
Analysis: To 25 mL of the *Sample solution* obtained in the test for *Chloride* add 1 mL of 3 N hydrochloric acid, and dilute with water to 40 mL.
Acceptance criteria: 0.017%; this solution shows no more sulfate than corresponds to 0.17 mL of 0.020 N sulfuric acid.

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry over silica gel for 24 h.
Acceptance criteria: NMT 0.5%
• **COLORING MATTER, ACIDS, AND ALKALIES**
Sample: 2 g
Analysis: Shake the *Sample* with 5 mL of water for 1 min, and filter.
Acceptance criteria: The filtrate is colorless and is neutral to litmus.

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, store at controlled room temperature, and prevent exposure to excessive heat.
• **LABELING:** Label it to indicate that it is intended for use in compounding dosage forms for topical, periodontal, nasal, and intracavitary use only.

Iodoquinol



$C_9H_5I_2NO$ 396.95
8-Quinolinol, 5,7-diiodo-;
5,7-Diiodo-8-quinolinol [83-73-8].

DEFINITION
Iodoquinol contains NLT 96.0% and NMT 100.5% of iodoquinol ($C_9H_5I_2NO$), calculated on the dried basis.

IDENTIFICATION
• **A. INFRARED ABSORPTION**
Standard solution: 5 mg/mL of USP Iodoquinol RS in carbon disulfide. Warm slightly, if necessary, to effect a complete solution.
Sample solution: 5 mg/mL of Iodoquinol in carbon disulfide. Warm slightly, if necessary, to effect a complete solution.
Instrumental conditions
Mode: IR
Wavelength range: 7–11 µm
Cell: 3-mm sodium chloride
Blank: Carbon disulfide
Acceptance criteria: The spectrum of the *Sample solution* exhibits absorption maxima and minima only at the same wavelengths as those of the *Standard solution.*
• **B.**
Analysis: Warm a small quantity of Iodoquinol with 1 mL of sulfuric acid.
Acceptance criteria: Violet vapors of iodine are evolved.

ASSAY
• **PROCEDURE**
Sample solution: 14 mg of Iodoquinol. Proceed as directed in *Oxygen Flask Combustion* ⟨471⟩ using a mixture of 10 mL of sodium hydroxide solution (1 in 100) and 1 mL of freshly prepared sodium bisulfite solution (1 in 100) as the absorbing liquid. When the combustion is complete, place a few mL of water around the stopper of the flask. Loosen the stopper. Then rinse the stopper, the specimen holder, and the sides of the flask with 20 mL of water, added in small portions. Add 1 mL of an oxidizing solution prepared by adding 5 mL of bromine to 100 mL of a solution (1 in 10) of sodium acetate in glacial acetic acid. Insert the stopper in the flask, and shake vigorously for 1 min. Add 0.5 mL of formic acid, replace the stopper, and shake vigorously for 1 min. Remove the stopper. Then rinse the stopper, the specimen holder, and the sides of the flask with several small portions of water. Bubble nitrogen through the flask to remove the oxygen and excess bromine. Add 500 mg of potassium iodide, swirl to dissolve, add 3 mL of 2 N sulfuric acid, and allow to stand for 2 min.
Titrimetric system
Titrant: 0.02 N sodium thiosulfate VS
Endpoint detection: Visual
Analysis: Titrate with *Titrant,* adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.02 N sodium thiosulfate is equivalent to 0.6616 mg of iodoquinol ($C_9H_5I_2NO$).
Acceptance criteria: 96.0%–100.5% on the dried basis

IMPURITIES
• **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.5%

SPECIFIC TESTS
• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry over silica gel for 4 h.
Acceptance criteria: NMT 0.5%
• **FREE IODINE AND IODIDE**
Sample solution: 50 mg/mL of Iodoquinol in water. Shake the *Sample solution* for 30 s, allow to stand for 5 min, and filter.
Control solution: Dilute 2 mL of potassium iodide solution (1 in 6000) with water to 10 mL. Add 6 mL of 2 N sulfuric acid, 1 mL of potassium dichromate TS, and 2 mL of chloroform; and shake for 15 s.
Analysis: To 10 mL of the filtrate from the *Sample solution* add 1 mL of 2 N sulfuric acid, then add 2 mL of chloroform, and shake; no violet color appears in the chloroform (free iodine). To the mixture add 5 mL of 2 N sulfuric acid and 1 mL of potassium dichromate TS, and shake for 15 s.
Acceptance criteria: The color in the chloroform layer is not deeper than that produced for the *Control solution* (0.05% of iodide).

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

USP Monographs

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Iodoquinol RS

Iodoquinol Tablets

DEFINITION
Iodoquinol Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of iodoquinol ($C_9H_5I_2NO$).

IDENTIFICATION
• **A. INFRARED ABSORPTION**
Blank: Carbon disulfide
Standard solution: 0.5 mg/mL of USP Iodoquinol RS in the *Blank*. Warm slightly, if necessary, to effect a complete solution.
Sample solution: Shake a portion of finely powdered Tablets, equivalent to 0.5 mg/mL of iodoquinol, with the *Blank*, and filter. Warm slightly, if necessary, to effect complete solution.
Instrumental conditions
Mode: IR
Wavelength range: 7–11 µm
Cell: 3-mm sodium chloride
Acceptance criteria: The spectrum of the *Sample solution* exhibits absorption maxima and minima only at the same wavelengths as those of the *Standard solution*.
• **B.**
Sample: Powdered Tablets prepared for the *Assay*, nominally 50 mg of iodoquinol
Analysis: Place a portion of the *Sample* in a dry test tube, add 1 mL of sulfuric acid, and warm gently.
Acceptance criteria: Violet vapors of iodine are evolved.

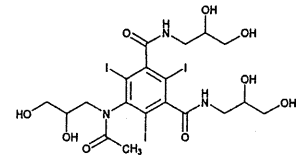
ASSAY
• **PROCEDURE**
Sample solution: Finely powder NLT 20 Tablets. Using a portion of the powder, nominally 14 mg of iodoquinol, and using a mixture of 10 mL of sodium hydroxide solution (1 in 100) and 1 mL of freshly prepared sodium bisulfite solution (1 in 100) as the absorbing liquid, proceed as directed in *Oxygen Flask Combustion* ⟨471⟩. When the combustion is complete, place a few mL of water around the stopper of the flask, loosen the stopper, then rinse the stopper, the specimen holder, and the sides of the flask with 20 mL of water, added in small portions. Add 1 mL of an oxidizing solution prepared by adding 5 mL of bromine to 100 mL of a solution (1 in 10) of sodium acetate in glacial acetic acid. Insert the stopper in the flask, and shake vigorously for 1 min. Add 0.5 mL of formic acid, replace the stopper, and shake vigorously for 1 min. Remove the stopper. Then rinse the stopper, the specimen holder, and the sides of the flask with several small portions of water. Bubble nitrogen through the flask to remove the oxygen and excess bromine. Add 500 mg of potassium iodide, swirl to dissolve, add 3 mL of 2 N sulfuric acid, and allow to stand for 2 min.
Titrimetric system
Titrant: 0.02 N sodium thiosulfate VS
Endpoint detection: Visual
Analysis: Titrate with *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.02 N sodium thiosulfate is equivalent to 0.6616 mg of iodoquinol ($C_9H_5I_2NO$).
Acceptance criteria: 95.0%–105.0% of the labeled amount

IMPURITIES
• **SOLUBLE IODIDES**
Sample solution: Digest a quantity of powdered Tablets, nominally 100 mg of iodoquinol, with 5 mL of water for 10 min, cool, and filter.
Analysis: To the filtered *Sample solution* add 1 mL of 3 N hydrochloric acid, 2 drops of ferric chloride TS, and 2 mL of chloroform. Shake gently, and allow to separate.
Acceptance criteria: Any violet color in the chloroform is not more intense than that in a blank to which 0.2 mg of potassium iodide has been added.

PERFORMANCE TESTS
• **DISINTEGRATION** ⟨701⟩**:** 1 h
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Iodoquinol RS

Iohexol



$C_{19}H_{26}I_3N_3O_9$ 821.14
1,3-Benzenedicarboxamide, 5-[acetyl(2,3-dihydroxypropyl) amino]-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo;
N,*N*′-Bis(2,3-dihydroxypropyl)-5-[*N*-(2,3-dihydroxypropyl) acetamido]-2,4,6-triiodoisophthalamide [66108-95-0].

DEFINITION
Iohexol contains NLT 98.0% and NMT 102.0% of iohexol ($C_{19}H_{26}I_3N_3O_9$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
• **B.** The retention times of the two principal peaks of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Organic Impurities*.

ASSAY
• **PROCEDURE**
Sample: 500 mg of Iohexol
Sample solution: Transfer the *Sample* to a glass-stoppered, 125-mL conical flask. Add 25 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc. Connect the flask to a reflux condenser, and reflux for 1 h. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and pass the mixture through a filter. Rinse the flask and the filter thoroughly with small portions of water, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 N silver nitrate VS

USP Monographs

www.webofpharma.com

Endpoint detection: Potentiometric
Analysis: Titrate the *Sample solution* with 0.1 N silver nitrate VS.
Calculate the percentage of iohexol ($C_{19}H_{26}I_3N_3O_9$) in the portion of Iohexol taken:

$$\text{Result} = [(V \times N \times F)/W] \times 100$$

V = *Titrant* volume consumed by the *Sample* (mL)
N = *Titrant* normality (mEq/mL)
F = equivalent weight of iohexol, 273.7 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **LIMIT OF IONIC COMPOUNDS**
Rinse all glassware five times with distilled water.
Standard solution: 0.002 mg/mL of sodium chloride in water
Sample solution: 1 g of Iohexol in 50 mL of water
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The specific conductance of the *Sample solution* is NMT that of the *Standard solution* (equivalent to 0.01% ionic compounds as sodium chloride).

• **LIMIT OF FREE IODIDE**
Sample: 5 g of Iohexol
Sample solution: Dissolve the *Sample* in 20 mL of water.
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.001 N silver nitrate VS
Endpoint detection: Potentiometric
Analysis
Calculate the percentage of free iodide in the portion of the *Sample* taken:

$$\text{Result} = [(V \times N \times F)/W] \times 100$$

V = *Titrant* volume consumed by the *Sample* (mL)
N = *Titrant* normality (mEq/mL)
F = equivalent weight of iodide, 126.9 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NMT 0.001%

• **ORGANIC IMPURITIES**
Solution A: Acetonitrile
Solution B: Water
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	1	99
60	13	87

System suitability solution: 1.5 mg/mL of USP Iohexol RS and 0.0075 mg/mL of USP Iohexol Related Compound A RS in water
Sample solution: 1.5 mg/mL of Iohexol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—Iohexol may give two nonresolved peaks due to exo–endo isomerism. In addition, a small peak due to iohexol usually appears at the leading edge of the first principal peak. This small peak has a retention time about 1.2 min less than the first principal peak. The relative retention times for the iohexol related compound A, iohexol endo-isomer, iohexol exo-isomer, and *O*-alkylated compounds peaks are 0.85, 0.96, 1.0, and 1.1–1.4, respectively.]
Suitability requirements
Resolution: NLT 5.0 between iohexol related compound A and the exo-isomer (the second and greater peak) of iohexol
Analysis
Sample: *Sample solution*
Calculate the percentage of *O*-alkylated compounds and any other individual impurity in the portion of Iohexol taken. Disregard any peak less than or equal to 0.03% of the principal peaks.

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity
r_T = sum of all of the peak responses

Acceptance criteria
***O*-alkylated compounds:** NMT 0.6%
Any individual impurity: NMT 0.1%
Total impurities excluding *O*-alkylated compounds: NMT 0.3%

• **LIMIT OF 2-METHOXYETHANOL**
Internal standard solution: 0.01 mg/mL of secondary butyl alcohol in water
Standard stock solution: 0.005 mg/mL of methanol and 0.01 mg/mL each of isopropyl alcohol, secondary butyl alcohol, and 2-methoxyethanol in *Internal standard solution*
Standard solution: Transfer about 0.25 g of USP Iohexol RS and 1.0 mL of *Standard stock solution* to a headspace vial, and seal the vial with a septum and crimp cap.
Sample solution: Transfer about 0.25 g of Iohexol and 1.0 mL of *Internal standard solution* to a headspace vial, and seal the vial with a septum and crimp cap.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC with suitable headspace autosampler
Detector: Flame ionization
Column: 0.53-mm × 30-m fused-silica coated with a 1-µm phase G16
Temperatures
Autosampler: 105°
Needle: 130°–140°
Injection port: 150°
Detector: 200°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	3
40	8	100	1

Carrier gas: Helium
Flow rate: 11 mL/min

www.webofpharma.com

Injection volume: 1 mL of the headspace
System suitability
Sample: *Standard solution*
[NOTE—The typical relative retention times for methanol, isopropyl alcohol, secondary butyl alcohol, and 2-methoxyethanol are 0.5, 0.6, 1.0, and 1.9, respectively.]
Suitability requirements
Resolution: NLT 1.0 between methanol and isopropyl alcohol
Relative standard deviation: NMT 10.0% for the ratio of 2-methoxyethanol to the internal standard
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of 2-methoxyethanol in the portion of Iohexol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak response ratio of 2-methoxyethanol to the internal standard from the *Sample solution*
R_S = peak response ratio of 2-methoxyethanol to the internal standard from the *Standard solution*
C_S = concentration of 2-methoxyethanol in the *Standard solution* (µg/mL)
C_U = concentration of Iohexol in the *Sample solution* (g/mL)

Acceptance criteria: NMT 20 µg/g of 2-methoxyethanol

• **LIMIT OF 3-CHLOROPROPANE-1,2-DIOL**
Standard solution: 0.025 mg/mL of 3-chloropropane-1,2-diol in ethyl acetate
Sample solution: Transfer 1 g of Iohexol to a separator. Dissolve in 1 mL of water. Extract 4 times with 2 mL of ethyl acetate, and combine the extracts. Dry the combined extracts with anhydrous sodium sulfate. Filter, and wash the filter with a small amount of ethyl acetate. Combine the wash with the filtrate, and concentrate to a volume of 0.7 mL, using a warm water bath and a stream of nitrogen. Dilute with ethyl acetate to 2 mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m fused-silica capillary bonded with a 1-µm layer of phase G46
Temperatures
Injection port: 230°
Detector: 250°
Column: See *Table 3*.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	—	80	2
80	15	275	—
275	—	275	2

Carrier gas: Helium at 1 mL/min
Injection volume: 2 µL
System suitability
Sample: *Standard solution*
[NOTE—The retention time of the 3-chloropropane-1,2-diol peak is about 8 min.]
Suitability requirements
Relative standard deviation: NMT 10.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The area of the principal peak from the *Sample solution* is NMT the area of the principal peak from the *Standard solution* (NMT 0.0025%).

• **LIMIT OF FREE AROMATIC AMINE**
Solution A: 3 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in a mixture of propylene glycol and water (70:30)
Standard stock solution: 10 µg/mL of USP Iohexol Related Compound B RS in water
Standard solution: Transfer 5 mL of water and 10.0 mL of the *Standard stock solution* to a 25-mL volumetric flask.
Sample solution: Transfer 200 mg of Iohexol to a 25-mL volumetric flask, add 15 mL of water, and mix to dissolve.
Blank: Add 15 mL of water to a 25-mL volumetric flask.
Instrumental conditions
Mode: Vis
Analytical wavelength: 495 nm
Cell: 5 cm
Analysis
Samples: *Standard solution*, *Sample solution*, and *Blank*
In conducting the following steps, keep the flasks in iced water and protected as much as possible from light until all of the reagents have been added.
Treat the *Samples* as follows. Place the flask in an ice bath for 5 min. Add 1.5 mL of 6 N hydrochloric acid, and mix by swirling. Add 1.0 mL of sodium nitrite solution (20 mg/mL), and allow to stand in the ice bath for 4 min. Remove the flask from the ice bath, add 1.0 mL of sulfamic acid solution (40 mg/mL), and swirl gently until gas evolution ceases. [**CAUTION**—Considerable pressure is produced.] Add 1.0 mL of *Solution A*, dilute with water to volume, and allow to stand for 5 min. Measure the absorbance of the *Standard solution* and *Sample solution* against the *Blank solution*.
Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (NMT 0.05% of free aromatic amine).

SPECIFIC TESTS

• **COLOR OF SOLUTION**
Sample solution: 647.2 mg/mL
Blank: Water
Instrumental conditions
Mode: UV-Vis
Analytical wavelengths: 400, 420, and 450 nm
Cell: 1 cm
Analysis
Samples: *Sample solution* and *Blank*
Pass the *Sample solution* through a filter of 0.22-µm pore size.
Determine the absorbances of the *Sample solution* against the *Blank*.
Acceptance criteria: See *Table 4*.

Table 4

Wavelength (nm)	NMT (au)
400	0.180
420	0.030
450	0.015

• **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 4.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at room temperature.

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Iohexol RS
USP Iohexol Related Compound A RS
5-(Acetylamino)-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
$C_{16}H_{20}I_3N_3O_7$ 747.06
USP Iohexol Related Compound B RS
5-Amino-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
$C_{14}H_{18}I_3N_3O_6$ 705.02

Iohexol Injection

DEFINITION
Iohexol Injection is a sterile solution of Iohexol in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of iohexol ($C_{19}H_{26}I_3N_3O_9$) as organically bound iodine. It may contain small amounts of suitable buffers and Edetate Calcium Disodium as a stabilizer. Iohexol Injection intended for intravascular or intrathecal use contains no antimicrobial agents.

IDENTIFICATION
• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Organic Impurities*.

ASSAY
• **PROCEDURE**
Sample: A volume of Injection equivalent to 300 mg of iodine
Analysis: Transfer the *Sample* to a glass-stoppered, 250-mL conical flask. Add 25 mL of 1.25 N sodium hydroxide and 500 mg of powdered zinc, connect the flask to a reflux condenser, and reflux the solution for 1 h. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly with small portions of water, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid, and titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 27.37 mg of $C_{19}H_{26}I_3N_3O_9$.
Acceptance criteria: 95.0%–105.0%

IMPURITIES
• **ORGANIC IMPURITIES**
Solution A: Acetonitrile
Solution B: Water
Mobile phase: The percentage of *Solution A* increases from 1% to 13% at a rate of 0.2%/min.
System suitability solution: 1.5 mg/mL, 0.0075 mg/mL, and 0.0069 mg/mL each of USP Iohexol RS, USP Iohexol Related Compound A RS, and USP Iohexol Related Compound C RS in water
Sample solution: 1.5 mg/mL of Iohexol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm stainless steel column; packing L1
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for the *exo*-isomer of iohexol and the *O*-alkylated compounds are 1.0 and between 1.1 and 1.4, respectively.]
[NOTE—The peak area of iohexol related compound C is 0.5% ± 0.1% compared to the total area of all the peaks in the chromatogram.]
Suitability requirements
Resolution: NLT 20.0 between iohexol related compound A and iohexol related compound C
Analysis
Sample: *Sample solution*
Excluding peaks with retention times between 0.84 (relative to the *endo*-isomer of iohexol, which is the first main peak) and 1.0, calculate the percentage of *O*-alkylated compounds and any other individual impurity peak, in the portion of Iohexol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity
r_T = sum of all the peak responses

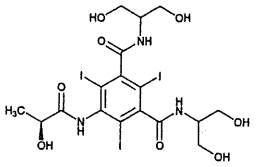
Acceptance criteria
Individual impurity: NMT 0.6% of *O*-alkylated compounds; NMT 0.1% of any other individual impurity
Total impurities: NMT 0.3%, excluding *O*-alkylated compounds

SPECIFIC TESTS
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.2 USP Endotoxin Unit per 50 mg of iodine
• **pH** ⟨791⟩**:** 6.8–7.7
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** The Injection labeled for intrathecal use meets the requirements for small-volume injections.
• **FREE IODIDE:** Transfer 5.0 mL of Injection to a suitable container, add 20 mL of water, and titrate with 0.001 N silver nitrate VS using a silver electrode in combination with an appropriate reference electrode. Each mL of 0.001 N silver nitrate is equivalent to 0.1269 mg of iodine.
Acceptance criteria: NMT 0.02%, based on the content of iohexol
• **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve Injection intended for intravascular or intrathecal use in single-dose or multiple-dose plastic or Type I glass containers. Store at controlled room temperature, protected from light. Do not freeze.
• **LABELING:** Label containers of Injection to direct the user to discard any unused portion. The labeling states also that it is not to be used if it is discolored or contains a precipitate. Label it also to state its routes of administration. When the specific dose strength is not intended for intrathecal use, label it to indicate "serious injury can occur if given by intrathecal route".
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Iohexol RS
USP Iohexol Related Compound A RS
5-(Acetylamino)-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide.
USP Iohexol Related Compound C RS
N,*N*′-Bis(2,3-dihydroxypropyl)-5-nitro-1,3-benzenedicarboxamide.

www.webofpharma.com

Iopamidol



$C_{17}H_{22}I_3N_3O_8$ 777.09

1,3-Benzenedicarboxamide, *N,N′*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[(2-hydroxy-1-oxopropyl)amino]-2,4,6-triiodo-, (*S*)-.

(*S*)-*N,N′*-Bis[2-hydroxy-1-(hydroxymethyl)ethyl]-2,4,6-triiodo-5-lactamidoisophthalamide [60166-93-0].

» Iopamidol contains not less than 98.0 percent and not more than 101.0 percent of iopamidol, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Iopamidol RS
USP Iopamidol Related Compound A RS
N,N′-Bis-(1,3-dihydroxy-2-propyl)-5-amino-2,4,6-triiodoisophthalamide.
$C_{14}H_{18}I_3N_3O_6$ 705.03
USP Iopamidol Related Compound C RS
4-Chloro-*N*¹, *N*³-bis(1,3-dihydroxypropan-2-yl)-5-*(S)*-lactamido-2,6-diiodoisophthalamide.
$C_{17}H_{22}ClI_2N_3O_8$ 685.63

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Heat about 500 mg in a suitable crucible: violet vapors are evolved.

C: The retention time of the major peak in the chromatogram of the *Identification solution* corresponds to that of the iopamidol peak observed in the chromatogram of the *System suitability solution*, as obtained in the test for *Related compounds*.

Specific rotation ⟨781S⟩: between −4.6° and −5.2° (*t* = 20°; λ = 436 nm).

Test solution: 400 mg per mL, in water, heating on a water bath, if necessary to effect solution, and passing through a membrane filter having a 3-µm or finer porosity.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Free aromatic amine—Transfer 500 mg to a 25-mL volumetric flask, and add 20 mL of water, heating on a water bath, if necessary, to effect solution. To a second 25-mL volumetric flask transfer 18.4 mL of water and 1.6 mL of a Standard solution prepared by dissolving a suitable quantity of USP Iopamidol Related Compound A RS in water and diluting with water to obtain a solution having a concentration of 62.5 µg per mL. To a third 25-mL volumetric flask add 20 mL of water to provide a blank. Treat each flask as follows. Place the flasks in an ice bath, protected from light, for 5 minutes. [NOTE—In conducting the following steps, keep the flasks in the ice bath and protected from light as much as possible until all of the reagents have been added.] Add slowly 1 mL of hydrochloric acid, mix, and allow to stand for 5 minutes. Add 1 mL of sodium nitrite solution (1 in 50), mix, and allow to stand for 5 minutes. Add 1 mL of ammonium sulfamate solution (3 in 25), shake, and allow to stand for 5 minutes. [**CAUTION**—Considerable pressure is produced.] Add 1 mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000), and mix. Remove the flasks from the ice bath, and allow to stand in a water bath at about 25° for 10 minutes. Dilute with water to volume, mix, and without delay (about 5 minutes from final dilution), concomitantly determine the absorbances of the solution from the substance under test and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 500 nm, with a suitable spectrophotometer, against the prepared blank. The absorbance of the solution from the Iopamidol is not greater than that of the Standard solution (0.02%).

Free iodine—Transfer 2.0 g to a stoppered, 50-mL centrifuge tube, add sufficient water to dissolve, heating on a water bath, if necessary, to effect solution, and dilute with water to 25 mL. Add 5 mL of toluene and 5 mL of 2 N sulfuric acid, shake well, and centrifuge: the toluene layer shows no red color.

Limit of free iodide—Transfer about 6.0 g, accurately weighed, to a suitable container, dissolve in 50 mL of water, and add 2.0 mL of 0.001 M potassium iodide. Titrate with 0.001 N silver nitrate VS, determining the endpoint potentiometrically, using a silver indicator electrode and an appropriate reference electrode. Perform a blank determination, and make any necessary correction. Each mL of 0.001 N silver nitrate is equivalent to 126.9 µg of iodide. Not more than 0.001% is found.

Free acid or alkali—Dissolve 10.0 g in 100 mL of freshly boiled and cooled water. Using a pH meter and a glass–calomel electrode system, determine the volume of 0.01 N hydrochloric acid VS or 0.01 N sodium hydroxide VS to bring the pH of the test solution to 7.0: not more than 1.37 mL of 0.01 N sodium hydroxide, equivalent to a free acid content of 5 mg of hydrochloric acid per 100 g, or not more than 0.75 mL of 0.01 N hydrochloric acid, equivalent to a free alkali content of 3 mg of sodium hydroxide per 100 g, is required.

Related compounds—

Solution A—Use water.

Solution B—Prepare a filtered and degassed mixture of water and acetonitrile (1:1).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve accurately weighed quantities of USP Iopamidol RS and USP Iopamidol Related Compound C RS in water, and dilute with water to obtain a solution having concentration of about 20 µg per mL of each.

Standard solution—Dissolve accurately weighed quantities of USP Iopamidol RS and USP Iopamidol Related Compound C RS in water, and dilute with water to obtain a solution having concentrations of about 20 µg per mL and 50 µg per mL, respectively.

Test solution—Transfer about 0.5 g of Iopamidol, accurately weighed, to a 50-mL volumetric flask, add water to volume, and mix.

Identification solution—Dilute a suitable volume of the *Test solution* with water to obtain a solution with a concentration of iopamidol of about 20 µg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and two 4.6-mm × 25-cm columns that contain packing L11, connected in series. The column temperature is maintained at

USP Monographs

www.webofpharma.com

60°, and the flow rate is about 2.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–18	100	0	isocratic
18–40	100→62	0→38	linear gradient
40–45	62→50	38→50	linear gradient
45–50	50→100	50→0	linear gradient
50–60	100	0	isocratic

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between iopamidol related compound C and iopamidol is not less than 2.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the tailing factor for each peak is between 0.7 and 1.5, and the relative standard deviation for replicate injections for either of the two peaks is not more than 2.0%. Chromatograph the *Identification solution*, and record the peak responses as directed for *Procedure* to obtain a chromatogram for *Identification* test *C*.

Procedure—Separately inject equal volumes (about 20 µL) of the *Identification solution*, the *System suitability solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses.

Calculate the total percentage of iopamidol related compound C and 2-chloro derivative in the portion of Iopamidol taken by the formula:

$$100(C_1V/W)(r_i/r_S)$$

in which C_1 is the concentration, in mg per mL, of iopamidol related compound C in the *Standard solution*; *V* is the volume of the *Test solution*; *W* is the weight of Iopamidol used to prepare the *Test solution*; r_i is the total peak response for the iopamidol related compound C and 2-chloro derivative obtained from the *Test solution*; and r_S is the peak response for iopamidol related compound C obtained from the *Standard solution*.

Calculate the total percentage of any other impurity in the portion of Iopamidol taken by the formula:

$$100(C_2V/W)(r_i/r_S)$$

in which C_2 is the concentration of iopamidol, in mg per mL, in the *Standard solution*; *V* and *W* are as previously defined; r_i is the peak response for the individual impurity obtained from the *Test solution*; and r_S is the peak response for iopamidol obtained from the *Standard solution*. In addition to not exceeding the limits for each impurity shown in *Table 1*, not more than 0.1% of any other individual impurity is found; and not more than 0.20% of total impurities, other than iopamidol related compound C and 2-chloro derivative, is found.

Table 1

Name	Relative Retention Time	Limit (%)
Monocarboxylic acid[1]	0.1	0.1
Iopamidol related compound B[2]	0.6	0.1
Iopamidol related compound C[3] and 2-chloro derivative[4]	0.9	0.5*
Iopamidol	1.0	—
2,3-Dihydroxypropyl isomer[5]	1.1	0.1
Diiodo derivative[6]	1.2	0.1
Acetyl analog[7]	1.3	0.1
Hydroxyethyl derivative[8]	1.5	0.1
O-Acetyl iopamidol[9]	2.2	0.1
N,N-Dimethylamino derivative[10]	2.3	0.1

* These peaks, appearing at a relative retention time of 0.9, are integrated together to determine conformance.

[1] 3-(1,3-Dihydroxypropan-2-ylcarbamoyl)-5-(*S*)-lactamido-2,4,6-triiodobenzoic acid.

[2] 5-Glycolamido-*N*¹,*N*³-bis(1,3-dihydroxy-2-propyl)-2,4,6-triiodoisophthalamide.

[3] 4-Chloro-*N*¹,*N*³-bis(1,3-dihydroxypropan-2-yl)-5-(*S*)-lactamido-2,6-diiodoisophthalamide.

[4] 2-Chloro-*N*¹,*N*³-bis(1,3-dihydroxypropan-2-yl)-5-(*S*)-lactamido-4,6-diiodoisophthalamide.

[5] *N*¹-(1,3-Dihydroxypropan-2-yl)-*N*³-(2,3-dihydroxypropyl)-5-(*S*)-lactamido-2,4,6-triiodoisophthalamide.

[6] *N*¹,*N*³-Bis(1,3-dihydroxypropan-2-yl)-5-(*S*)-lactamido-2,4-diiodoisophthalamide.

[7] 5-Acetamido-*N*¹,*N*³-bis(1,3-dihydroxy-2-propyl)-2,4,6-triiodoisophthalamide.

[8] *N*¹-(1,3-Dihydroxypropan-2-yl)-*N*³-(2-hydroxyethyl)-5-(*S*)-lactamido-2,4,6-triiodoisophthalamide.

[9] (*S*)-5-(2-Acetoxypropanamido)-*N*¹,*N*³-bis(1,3-dihydroxypropan-2-ylcarbamoyl)-2,4,6-triiodoisophthalamide.

[10] *N*¹-(1,3-Dihydroxypropan-2-yl)-5-(*S*)-lactamido-2,4,6-triiodo-*N*³,*N*³-dimethylisophthalamide.

Assay—Transfer about 300 mg of Iopamidol, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 40 mL of 1.25 N sodium hydroxide and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly, adding the rinsings to the filtrate. Add 5 mL of glacial acetic acid, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 25.90 mg of $C_{17}H_{22}I_3N_3O_8$.

Iopamidol Injection

» Iopamidol Injection is a sterile solution of Iopamidol in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of iopamidol ($C_{17}H_{22}I_3N_3O_8$). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium as a stabilizer. Iopamidol Injection intended for intravascular or intrathecal use contains no antimicrobial agents.

Packaging and storage—Preserve Injection intended for intravascular or intrathecal use in single-dose containers, preferably of Type I glass, and protected from light.

Labeling—Label containers of Injection to direct the user to discard any unused portion remaining in the container and to

www.webofpharma.com

check for the presence of particulate matter before using. Label it also to state its routes of administration.

USP Reference standards ⟨11⟩—
USP Iopamidol RS
USP Iopamidol Related Compound A RS
N,N'-Bis-(1,3-dihydroxy-2-propyl)-5-amino-2,4,6-triiodoisophthalamide.
$C_{14}H_{18}I_3N_3O_6$ 705.03
USP Iopamidol Related Compound B RS
5-Glycolamido-*N,N'*-bis[2-hydroxy-1-(hydroxymeth yl) ethyl]-2,4,6-triiodoisophthalamide.
$C_{16}H_{20}I_3N_3O_7$ 747.07

Identification—
A: Evaporate a volume of Injection, equivalent to about 500 mg of iopamidol, to dryness, and heat the residue so obtained in a suitable crucible: violet vapors are evolved.
B: It responds to the *Thin-Layer Chromatographic Identification Test* ⟨201⟩, the test solution and the Standard solution being prepared at a concentration of 0.5 mg per mL in a mixture of methanol and water (9:1), the solvent mixture being chloroform, methanol, ammonium hydroxide, and water (60:30:9:1), and short-wavelength UV light being used to locate the spots.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.6 USP Endotoxin Unit per mg of iodine.

pH ⟨791⟩: between 6.5 and 7.5.

Particulate Matter in Injections ⟨788⟩ —The Injection labeled for intrathecal use meets the requirements for small-volume injections.

Free aromatic amine—Transfer an accurately measured volume of Injection, equivalent to about 500 mg of iopamidol, to a 25-mL volumetric flask, dilute with water to 20 mL, and mix. To a second 25-mL volumetric flask transfer 16 mL of water and 4.0 mL of Standard solution prepared by dissolving a suitable quantity of USP Iopamidol Related Compound A RS in water and diluting with water to obtain a solution having a concentration of 62.5 µg per mL. Proceed as directed in the test for *Free aromatic amine*— under *Iopamidol*, beginning with "to the third 25-mL volumetric flask add 20 mL of water." The absorbance of the solution from the iopamidol is not greater than that of the Standard solution (0.05%).

Free iodine—Transfer a volume of Injection, equivalent to 2.0 g of iopamidol, to a glass-stoppered test tube. Add 2 mL of 2 N sulfuric acid and 1.0 mL of toluene, shake, and allow the layers to separate: the toluene layer shows no red color.

Limit of free iodide—Transfer 10.0 mL of Injection to a beaker, add 40 mL of water, and mix. Proceed as directed in the *Limit of free iodide* test under *Iopamidol* beginning with "add 2.0 mL of 0.001 M potassium iodide." Not more than 3.1 mL of 0.001 N silver nitrate is required (0.04 mg of iodide per mL).

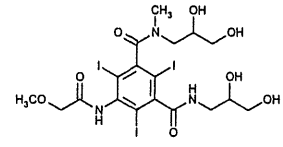
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Solution A—Use water.
Solution B—Prepare a filtered and degassed mixture of water and methanol (3:1).
Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Resolution solution—Transfer 10.0 mg of USP Iopamidol Related Compound B RS and 10.0 mg of USP Iopamidol RS to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume, and mix.
Standard preparation—Dissolve about 20 mg of USP Iopamidol RS, accurately weighed, in about 10 mL of water, and dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 80 µg of USP Iopamidol RS per mL.
Assay preparation—Dilute an accurately measured volume of Injection, equivalent to about 1000 mg of iopamidol, quantitatively and stepwise with water to obtain a solution having a concentration of about 80 µg of iopamidol per mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 25-cm stainless steel column that contains 5-µm packing L1. The column temperature is maintained at 35°, and the flow rate is about 1.5 mL per minute. The chromatograph is programmed to provide variable mixtures of *Solution A* and *Solution B,* the percentage of *Solution B* being 8.0% at the time of injection, and is held at that percentage for 6 minutes, then increased linearly to 35.0% at 18 minutes, after which it is changed to increase linearly to 92.0% at 30 minutes, maintained at that percentage for 4 minutes, and decreased linearly to 8.0% at 36 minutes, where it is held to the end of the run at 40 minutes. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between iopamidol related compound B and iopamidol is not less than 7.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of iopamidol ($C_{17}H_{22}I_3N_3O_8$) in the portion of Injection taken by the formula:

$$12.5C(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Iopamidol RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Iopromide



$C_{18}H_{24}I_3N_3O_8$ 791.11
1,3-Benzenedicarboxamide, *N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-[(methoxyacetyl)amino]-*N*-methyl-.
N,N'-Bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-(2-methoxyacetamido)-*N*-methylisophthalamide [73334-07-3].

» Iopromide contains not less than 97.0 percent and not more than 102.5 percent of $C_{18}H_{24}I_3N_3O_8$, calculated on the anhydrous and solvent-free basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Iopromide RS
USP Iopromide Related Compound A RS
USP Iopromide Related Compound B RS
5-(Acetylamino)-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-*N*-methyl-1,3-benzenedicarboxamide.

www.webofpharma.com

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: The R_F value of the principal spot in the chromatogram, developed with the *Basic eluant*, obtained from the *Test solution* corresponds to that obtained from the *Standard solution* in the *Ordinary impurities* test.

Water Determination, *Method I* ⟨921⟩: not more than 1.5%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Free iodine—Transfer 2.0 g of Iopromide to a glass-stoppered test tube, and dissolve in 20 mL of water. Add 2 mL of toluene and 2 mL of diluted sulfuric acid, and shake vigorously: the toluene layer shows no red color.

Limit of free iodide—Transfer 10.0 g of Iopromide to a 150-mL conical flask, and dissolve in 70 mL of water. Adjust with 0.1 N sulfuric acid to a pH of 3.5 ± 0.5. Titrate with 0.001 N silver nitrate VS, determining the endpoint potentiometrically, using a silver or platinum electrode in combination with an appropriate reference electrode (see *Titrimetry* ⟨541⟩). Each mL of 0.001 N silver nitrate is equivalent to 126.9 µg of I: the limit is 0.002%.

Limit of free aromatic amine—

Test solution—Transfer 500 mg of Iopromide to a 25-mL volumetric flask, add 20 mL of water, and mix.

Standard solution—Dissolve a suitable quantity of USP Iopromide Related Compound A RS in water, and dilute with water to obtain a stock solution having a known concentration of 0.25 mg per mL. Transfer 2.0 mL of this stock solution to a 25-mL volumetric flask, add 18.0 mL of water, and mix.

Blank solution—Transfer 20 mL of water to a 25-mL volumetric flask.

Procedure—Treat each flask as follows. Place the flasks in an ice bath, and protect from light. Add slowly 1.0 mL of 8 N hydrochloric acid, mix, and allow to stand for 5 minutes. Add 1.0 mL of sodium nitrite solution (1 in 50), mix, and allow to stand for 5 minutes. Add 0.50 mL of freshly prepared sulfamic acid solution, (8 in 100). Shake each flask vigorously several times within the next 5 minutes, venting off the gas that evolves. [**CAUTION**—Considerable pressure is produced.] Add 1.0 mL of freshly prepared *N*-(1-naphthyl) ethylenediamine dihydrochloride solution, (1 in 1000) in a mixture of propylene glycol and water (70:30), and shake. Remove the flasks from the ice bath, and allow to stand in a water bath at about 25° for 10 minutes. Dilute with water to volume, mix, and degas with the aid of sonication for 1 minute. Concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells at the wavelength of maximum absorbance at about 495 nm, with a suitable spectrophotometer, using the *Blank solution,* treated in the same manner. The absorbance of the *Standard solution* is not less than 0.40. Calculate the percentage of free aromatic amine in the portion of Iopromide taken by the formula:

$$10(W_S/W_U)(A_U/A_S)$$

in which W_S is the quantity, in mg, of USP Iopromide Related Compound A RS taken to prepare the *Standard solution*; W_U is the quantity, in mg, of the Iopromide taken to prepare the *Test solution*; and A_U and A_S are the absorbances of the *Test solution* and the *Standard solution*, respectively: not more than 0.1% is found.

Limit of alcohol—

Standard solution—Prepare a solution of alcohol in dimethylformamide to obtain a solution having a known concentration of about 0.050 mg of alcohol (C_2H_5OH) per mL.

Test solution—Dissolve an accurately weighed portion of Iopromide in dimethylformamide to obtain a concentration of about 50 mg per mL.

Blank solution—Use dimethylformamide.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a headspace injector, a flame-ionization detector, and a 0.25-mm × 30-m capillary column, the internal wall of which is coated with a 1.4-µm film of liquid phase G43. The column temperature is programmed according to the following steps: it is held at 40° for 10 minutes, then increased at a rate of 5° per minute to 70°; it is then increased at a rate of 30° per minute to 220°. The injector port is maintained at 160°; the headspace sampler is maintained at 80°; and the detector is maintained at 250°. Helium is used as the carrier gas at a flow rate of about 27 cm per second. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*:the retention time for alcohol is about 3 minutes; and the relative standard deviation for three injections of the *Standard solution* is not more than 4.0%. Chromatograph the *Blank solution*, and record the peak responses as directed for *Procedure:* the chromatogram shows no peak at the retention time for alcohol.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Transfer 2.0 mL each of the *Test solution*, the *Standard solution*, and the *Blank solution* to separate headspace vials, add 10 µL of 1 N hydrochloric acid to each vial, then seal the vials using a flanged cap so that the cap can no longer be turned. Record the chromatograms, and measure the responses for the alcohol peak. Calculate the concentration of alcohol in the portion of Iopromide taken by the formula:

$$(C/I)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of alcohol (C_2H_5OH) in the *Standard solution*; I is the quantity, in mg per mL, of Iopromide in the *Test solution*; and r_U and r_S are the alcohol peak responses in the chromatograms obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.4% of alcohol (C_2H_5OH) is found. Use the percentage obtained to calculate the *Assay* result on the solvent-free basis.

Limit of *N*-acetyl compound (iopromide related compound B)—Using the chromatograms obtained in the *Assay*, calculate the percentage of N-acetyl compound in the Iopromide taken by the formula:

$$20(W_B/W_I)[(A_{Y1} + A_{Y2})/(R_{Y1} + R_{Y2})]$$

in which W_B is the quantity, in mg, of USP Iopromide Related Compound B RS taken to prepare the *Related compound B standard solution*; W_I is the quantity, in mg, of Iopromide taken to prepare the *Assay preparation*; A_{Y1} and A_{Y2} are the peak responses for the iopromide related compound B *Y*1- and *Y*2-isomers, respectively, in the chromatogram obtained from the *Assay preparation*; and R_{Y1} and R_{Y2} are the peak responses for the iopromide related compound B *Y*1- and *Y*2-isomers, respectively, in the chromatogram obtained from the *Related compound B standard solution:* not more than 1.5% of N-acetyl compound is found.

Ordinary impurities ⟨466⟩—

Test solution: a mixture of methanol and water (1:1).

Standard solutions: a mixture of methanol and water (1:1).

Visualization solution—

SOLUTION A—Dissolve 2.7 g of ferric chloride in 100 mL of 2.4 N hydrochloric acid. Store this solution in a refrigerator.

USP Monographs

www.webofpharma.com

SOLUTION B—Dissolve 3.5 g of potassium ferricyanide in 100 mL of water. Store this solution in a refrigerator.

SOLUTION C—Dissolve 5.0 g of sodium arsenite in 30 mL of 1 N sodium hydroxide solution that has been cooled to 0°. While stirring, mix with 65 mL of 2.4 N hydrochloric acid, and store at room temperature. Use the clear supernatant.

Procedure—Mix 10 mL of *Solution A*, 10 mL of *Solution B*, and 2.0 mL of *Solution C*. Use within 30 minutes.

Basic eluant: a mixture of dioxane, water, and ammonium hydroxide (85:15:4).

Acidic eluant: a mixture of chloroform, methanol, water, and 96 percent formic acid (62:32:6:2).

Procedure—Apply 1 µL and 2 µL of the *Test solution* and 1 µL of each of the *Standard solutions* to two separate thin-layer chromatographic plates. Place one plate in a development chamber containing the *Acidic eluant*, and the second plate in a development chamber containing the *Basic eluant*. After the chromatograms have developed, remove the plates from the chambers, and allow to dry at room temperature.

Visualization—

DETECTION 1—Observe both plates under 254-nm UV light.

DETECTION 2—The plate developed with the *Acidic eluant* is exposed to ammonia vapors for 10 to 30 minutes and is air dried. Both plates are exposed to unfiltered 254-nm UV light for several minutes until the principal spots appear yellow. Overspray with *Visualization solution*, and examine the plates under ambient light. Determine the percentage of all secondary spots, except those due to free aromatic amine and to the *N*-acetyl compound.

Limit—The sum of all secondary spots observed in the chromatograms of the *Test solution*, except those due to the free aromatic amine and to the *N*-acetyl compound in addition to the percentage of *N*-acetyl compound obtained in the test for *Limit of N-Acetyl compound*, corresponds to not more than 3.0%.

Isomer distribution—Using the chromatogram of the *Assay preparation* obtained in the *Assay*, calculate the percentage of iopromide *E*1- and *Z*1-isomers in the Iopromide taken by the formula:

$$100(r_{E1} + r_{Z1})/(r_{E1} + r_{E2} + r_{Z1} + r_{Z2})$$

in which r_{E1}, r_{E2}, r_{Z1}, and r_{Z2} are the peak responses for iopromide *E*1-, *E*2-, *Z*1-, and *Z*2-isomers, respectively, in the chromatogram obtained from the *Assay preparation:* between 40.0% and 51.0% of *E*1- and *Z*1-isomers is found. Calculate the percentages of iopromide *E*2- and *Z*2-isomers in the Iopromide taken by the formula:

$$100(r_{E2} + r_{Z2})/(r_{E1} + r_{E2} + r_{Z1} + r_{Z2})$$:

between 49.0% and 60.0% *E*2- and *Z*2-isomers is found.

Assay—

Diluent—Prepare a mixture of methanol and water (1:1).

Mobile phase—

[NOTE—Use chloroform, methanol, and water that have been filtered and degassed.]

Mix 6 g of chloroform with 59 g of methanol, then add 900 g of water. Store in a sealed container, and do not stir or sparge the *Mobile phase* during use.

Standard preparation—Transfer an accurately weighed quantity of about 38 mg of USP Iopromide RS, to a 20-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

Related compound B standard solution—Transfer about 1.9 mg of USP Iopromide Related Compound B RS, accurately weighed, to a 100-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

Assay preparation—Transfer about 38 mg of Iopromide, accurately weighed, to a 20-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.2 mL per minute. The temperature is maintained at a constant temperature of about 20°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times for iopromide *E*1-isomer, iopromide *E*2-isomer, iopromide *Z*1-isomer, and iopromide *Z*2-isomer are about 0.70, 0.75, 0.85, and 1.0, respectively; the resolution, *R*, between iopromide isomers *Z*1 and *Z*2 is not less than 2.0; and the relative standard deviation for replicate injections for total iopromide area is not more than 2.0%. Chromatograph the *Related compound B standard solution*, and measure the area of the peak responses: the relative retention times for the iopromide related compound B *Y*1- and *Y*2-isomers are about 0.28 and 0.31, respectively; and the signal-to-noise ratio for the iopromide related compound B *Y*2-isomer is not less than 20.

Determine which peaks in the chromatograms correspond to the *E*-isomers as follows. Transfer a portion of the *Standard preparation* to a vial, seal with a crimp-top, and heat to 121° for 15 minutes. Inject the cooled solution. Compare the chromatogram obtained with that of the unheated *Standard preparation*, and note the retention times of the two *E*-isomer peaks, which increase in size after heating.

Procedure— [NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard preparation*, the *Related compound B standard solution*, and the *Assay preparation* into the chromatograph, and measure the responses for the major peaks. Allow the *Mobile phase* to flow for not less than 60 minutes between each injection to prevent interference from late-eluting amine peaks. Calculate the quantity of $C_{18}H_{24}I_3N_3O_8$ in the portion of Iopromide taken by the formula:

$$C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Iopromide RS in the *Standard preparation*; and r_U and r_S are the sums of the peak responses for iopromide *E*1-isomer, iopromide *E*2-isomer, iopromide *Z*1-isomer, and iopromide *Z*2-isomer in the chromatograms obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Iopromide Injection

» Iopromide Injection is a sterile solution of Iopromide in Water for Injection. It contains not less than 94.0 percent and not more than 105.0 percent of the labeled amount of iopromide ($C_{18}H_{24}I_3N_3O_8$). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium as a stabilizer. It contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose glass containers as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging*, and protect from light. Store at controlled room temperature.

Labeling—Label Injection to state that it is not to be used if it contains particulate matter and that after use any unused

www.webofpharma.com

portion remaining in the container is to be discarded. It is labeled also to state that it is not for intrathecal use.

USP Reference standards ⟨11⟩—
USP Iopromide RS
USP Iopromide Related Compound A RS
USP Iopromide Related Compound B RS
5-(Acetylamino)-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-*N*-methyl-1,3-benzenedicarboxamide.

Identification—

A: Evaporate 3 mL of Injection to dryness, and heat the residue so obtained in a crucible in a hood: violet vapors are evolved.

B: The R_F value of the principal spot in the chromatogram obtained from the *Test solution*, developed with the *Basic eluant*, in the test for *Ordinary impurities* corresponds to that obtained from the *Standard solution* similarly tested.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.25 USP Endotoxin Units per mL of Injection.

pH ⟨791⟩: between 6.5 and 8.0.

Free iodine—Transfer a volume of Injection, equivalent to 2 g of iopromide, to a 50-mL centrifuge tube. Dilute with water to 24 mL. Add 2 mL of toluene and 2 mL of diluted sulfuric acid solution, and shake: the toluene layer shows no red color.

Limit of free iodide—Transfer 10.0 mL of Injection and 50 mL of water to a 150-mL titration vessel, and titrate with 0.001 N silver nitrate VS using a silver or platinum electrode in combination with a reference electrode, determining the endpoint potentiometrically. Each mL of 0.001 N silver nitrate is equivalent to 126.9 µg of I. The limit is 80 µg of iodide per g of iopromide, based on the labeled content of iopromide.

Limit of free aromatic amine—Proceed as directed in the test for *Limit of free aromatic amine* under *Iopromide*, except to prepare the *Test solution* as follows. Transfer an accurately measured volume of Injection, equivalent to about 500 mg of iopromide, to a 25-mL volumetric flask, dilute with water to 20 mL, and mix. Calculate the percentage of free aromatic amine based on the labeled amount of iopromide in the Injection taken by the formula:

$$10(W_S/CV)(A_U/A_S)$$

in which W_S is the quantity, in mg, of USP Iopromide Related Compound A RS taken to prepare the *Standard solution*; *C* is the labeled concentration, in mg per mL, of iopromide in the Injection used to prepare the *Test solution*; *V* is the volume, in mL, of Injection to prepare the *Test solution;* and A_U and A_S are the absorbances of the *Test solution* and the *Standard solution*, respectively: not more than 0.2% is found.

Limit of *N*-acetyl compound (iopromide related compound B)—Using the chromatogram of the *Assay preparation* obtained in the *Assay*, calculate the percentage of *N*-acetyl compound in the iopromide in the Injection taken by the formula:

$$(W_B/C)[(A_{Y1} + A_{Y2})/(R_{Y1} + R_{Y2})]$$

in which W_B is the quantity, in mg, of USP Iopromide Related Compound B RS taken to prepare the *Related compound B standard solution*; *C* is the concentration, in mg of iopromide per mL, in the *Assay preparation* based on the labeled amount and the extent of dilution; A_{Y1} and A_{Y2} are the peak responses for iopromide related compound B *Y*1- and *Y*2-isomers, respectively, from the *Assay preparation*; and R_{Y1} and R_{Y2} are the peak responses for iopromide related compound B *Y*1- and *Y*2-isomers, respectively, from the *Related compound B standard solution:* not more than 1.5% is found.

Isomer distribution—Using the chromatogram of the *Assay preparation* obtained in the *Assay*, calculate the percentage of iopromide isomers in the iopromide in the Injection taken by the formula:

$$100(r_i)/(r_{E1} + r_{E2} + r_{Z1} + r_{Z2})$$

in which r_i is the peak response of each individual iopromide isomer; and r_{E1}, r_{E2}, r_{Z1}, and r_{Z2}, are the peak responses for the iopromide *E*1-, *E*2-, *Z*1-, and *Z*2-isomers, respectively, from the *Assay preparation:* between 8.0% and 12.0% of the *E*1-isomer, between 9.0% and 14.0% of the *E*2-isomer, between 32.0% and 40.0% of the *Z*1-isomer, and between 38.0% and 46.0% of the *Z*2-isomer are found.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩, and meets the requirements for *Ordinary impurities* under *Iopromide*.

Assay—

Diluent, Mobile phase, Standard preparation, Related compound B standard solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Iopromide*.

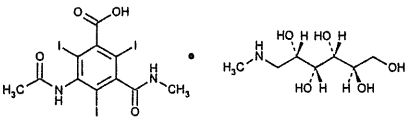
Assay preparation—Dilute an accurately measured volume of Injection, quantitatively and stepwise, with *Diluent* to obtain a solution having a final nominal concentration of 1.9 mg of iopromide per mL.

Procedure—Proceed as directed in the *Assay* under *Iopromide*. Calculate the quantity, in mg, of iopromide ($C_{18}H_{24}I_3N_3O_8$) in each mL of the Injection taken by the formula:

$$(CL/D)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Iopromide RS in the *Standard preparation*; *L* is the labeled quantity, in mg, of iopromide in each mL of Injection; *D* is the concentration, in mg per mL, of iopromide in the *Assay preparation*, based on the volume of Injection taken and the extent of dilution; and the other factors are as defined therein.

Iothalamate Meglumine Injection



$C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$ 809.13

Benzoic acid, 3-(acetylamino)-2,4,6-triiodo-5-[(methylamino)carbonyl]-, compd. with 1-deoxy-1-(methylamino)-D-glucitol (1:1).

1-Deoxy-1-(methylamino)-D-glucitol 5-acetamido-2,4,6-triiodo-*N*-methylisophthalamate (salt) [13087-53-1].

» Iothalamate Meglumine Injection is a sterile solution of Iothalamic Acid in Water for Injection, prepared with the aid of Meglumine. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of iothalamate meglumine ($C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Iothalamate Meglumine Injection intended for intravascular use contains no antimicrobial agents.

www.webofpharma.com

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light.
Labeling—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container. Label containers of Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.
USP Reference standards ⟨11⟩—
USP 5-Amino-2,4,6-triiodo-N-methylisophthalamic Acid RS
$C_9H_7I_3N_2O_3$ 571.88
USP Iothalamic Acid RS
Identification—Dilute 3 mL of Injection with water to 100 mL, add an excess of 3 N hydrochloric acid, and filter. Wash the precipitated iothalamic acid on the filter with four 10-mL portions of water, and dry at 105° for 4 hours: the dried iothalamic acid responds to the following tests.
A: The IR absorption spectrum of a 0.5% potassium bromide dispersion of the dried acid exhibits maxima only at the same wavelengths as that of a similar preparation of USP Iothalamic Acid RS.
B: Heat about 500 mg of the dried acid in a suitable crucible: violet vapors are evolved.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.9 USP Endotoxin Unit per mL.
pH ⟨791⟩: between 6.5 and 7.7.
Free aromatic amine—Dilute a suitable volume of Injection with water to yield a solution containing 100 mg of iothalamate meglumine per mL. Proceed as directed in the test for *Free aromatic amine*— under *Iothalamic Acid*, beginning with "Pipet 5 mL of this solution into a 50-mL volumetric flask."
Iodine and iodide—Dilute a volume of Injection, equivalent to 2 g of iothalamate meglumine, with 20 mL of water in a 50-mL beaker, add 5 mL of 2 N sulfuric acid, stir, and filter into a glass-stoppered, 50-mL cylinder. Proceed as directed for *Procedure* in the test for *Iodine and iodide*— under *Iothalamic Acid*, beginning with "To the filtrate add 5 mL of toluene."
Meglumine content—Proceed as directed in the test for *Meglumine content*— under *Diatrizoate Meglumine Injection*. The meglumine content is not less than 22.9% and not more than 25.3% of the labeled amount of iothalamate meglumine.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay—Pipet a volume of Injection, equivalent to about 4 g of iothalamate meglumine, into a 250-mL volumetric flask, dilute with water to volume, and mix. Pipet 25 mL of this solution into a glass-stoppered, 125-mL conical flask, add 12 mL of 5 N sodium hydroxide and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 30 minutes. Cool to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the filter and the flask thoroughly, adding the rinsings to the filtrate. Add 40 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using silver-calomel electrodes and an agar-potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 13.49 mg of $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$.

Iothalamate Meglumine and Iothalamate Sodium Injection

» Iothalamate Meglumine and Iothalamate Sodium Injection is a sterile solution of Iothalamic Acid in Water for Injection, prepared with the aid of Meglumine and Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of iothalamate meglumine ($C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$) and iothalamate sodium ($C_{11}H_8I_3N_2NaO_4$). It may contain small amounts of suitable buffers and of Edetate Calcium Disodium or Edetate Disodium as a stabilizer. Iothalamate Meglumine and Iothalamate Sodium Injection intended for intravascular use contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light.
Labeling—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container. Label containers of Injection intended for other than intravascular injection to show that the contents are not intended for intravascular injection.
USP Reference standards ⟨11⟩—
USP 5-Amino-2,4,6-triiodo-N-methylisophthalamic Acid RS
$C_9H_7I_3N_2O_3$ 571.88
USP Iothalamic Acid RS
Identification—Dilute 3 mL of Injection with water to 100 mL, add an excess of 3 N hydrochloric acid, mix, and filter. Wash the precipitate of iothalamic acid so obtained with four 10-mL portions of water, and dry at 105° for 4 hours: the dried iothalamic acid so obtained responds to the following tests.
A: The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Iothalamic Acid RS.
B: Heat about 500 mg in a suitable crucible: violet vapors are evolved.
Bacterial Endotoxins Test ⟨85⟩—It contains not more than 3.35 USP Endotoxin Units per mL.
pH ⟨791⟩: between 6.5 and 7.7.
Free aromatic amine—Dilute a suitable volume of Injection with water to yield a solution containing 100 mg of the total of iothalamate meglumine and iothalamate sodium per mL. Pipet 5 mL of this solution into a 50-mL volumetric flask, and add 10 mL of water. Proceed as directed in the test for *Free aromatic amine*— under *Iothalamic Acid*, beginning with "In another flask place 15 mL of water. The absorbance of the solution from the Injection is not greater than that of the Standard solution (0.05%).
Iodine and iodide—Dilute a volume of Injection, equivalent to 2 g of the total of iothalamate meglumine and iothalamate sodium, with 20 mL of water in a 50-mL beaker, and proceed as directed for *Procedure* in the test for *Iodine and iodide*— under *Iothalamic Acid*, beginning with "add 5 mL of 2 N sulfuric acid." The limit of *Iodine and Iodide* is 0.02% of iodide.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.
Assay for iothalamate meglumine—Pipet 5 mL of Injection into a 10-mL volumetric flask, add water to volume, and mix. Determine the angular rotation (see *Optical Rotation* ⟨781⟩) of the diluted Injection, using a 10-cm cell and a suitable polarimeter. Calculate the quantity, in mg per mL, of iothalamate meglumine in the Injection taken by the formula:

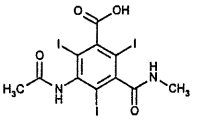
$$2000a/6.01$$

in which a is the observed angular rotation, in degrees, corrected for the blank; and the factor 6.01 is the specific rotation, in degrees, of iothalamate meglumine.

www.webofpharma.com

Assay for iothalamate sodium—Transfer an accurately measured volume of Injection, equivalent to about 4 g of iothalamate meglumine and iothalamate sodium, to a 250-mL volumetric flask, dilute with water to volume, and mix. Pipet 25 mL of this solution into a glass-stoppered, 125-mL conical flask, add 12 mL of 5 N sodium hydroxide and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux the mixture for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly, adding the rinsings to the filtrate. Add 40 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N silver nitrate VS, determining the end-point potentiometrically, using silver–calomel electrodes and an agar–potassium nitrate salt bridge. Calculate the volume, in mL, consumed by the iothalamate meglumine in the portion of solution taken, using the value found in the *Assay for iothalamate meglumine*. Each mL of 0.05 N silver nitrate is equivalent to 13.49 mg of $C_{11}H_9I_3N_2O_4 \cdot C_7H_{17}NO_5$. Subtract this volume from the total volume of 0.05 N silver nitrate consumed. Use the resulting volume to calculate the amount, in mg per mL, of iothalamate sodium in the Injection. Each mL of 0.05 N silver nitrate is equivalent to 10.60 mg of $C_{11}H_8I_3N_2NaO_4$.

Iothalamic Acid



$C_{11}H_9I_3N_2O_4$ 613.91

Benzoic acid, 3-(acetylamino)-2,4,6-triiodo-5-[(methylamino)carbonyl]-.

5-Acetamido-2,4,6-triiodo-*N*-methylisophthalamic acid [2276-90-6].

» Iothalamic Acid contains not less than 98.0 percent and not more than 102.0 percent of $C_{11}H_9I_3N_2O_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—

USP 5-Amino-2,4,6-triiodo-N-methylisophthalamic Acid RS
$C_9H_7I_3N_2O_3$ 571.88

USP Iothalamic Acid RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Heat about 500 mg in a suitable crucible: violet vapors are evolved.

Water Determination, *Method I* ⟨921⟩: not more than 1.0%.

Residue on ignition ⟨281⟩: not more than 0.1%.

Free aromatic amine—Dissolve 10.0 g of Iothalamic Acid in a minimal amount of 1 N sodium hydroxide in a 150-mL beaker, add 75 mL of water, and adjust with 1 N sulfuric acid to a pH of 7 ± 0.1. Transfer the solution to a 100-mL cylinder, dilute with water to 100 mL, and mix. Pipet 5 mL of this solution into a 50-mL volumetric flask, and add 10 mL of water. In another flask place 15 mL of water to provide a blank, and to a third flask add 12.5 mL of water and 2.5 mL of a Standard solution prepared as follows. Dissolve 25.0 mg of USP 5-Amino-2,4,6-triiodo-N-methylisophthalamic Acid RS, accurately weighed, in a mixture of 0.5 mL of 1 N sodium hydroxide and 2.5 mL of water in a 250-mL beaker, swirling to effect solution, then add 225 mL of water, mix, adjust with 1 N sulfuric acid to a pH of 7 ± 0.1, transfer to a 250-mL volumetric flask, add water to volume, and mix. Place the three flasks containing the solutions from the substance under test, the Standard solution, and the blank, respectively, in an ice bath. [NOTE—In conducting the following steps, keep the flasks in the ice bath and in the dark as much as possible, until all of the reagents have been added. Chill all reagents and the diluting water to about 5° prior to addition.] Treat each flask as follows. Add 5 mL of freshly prepared sodium nitrite solution (1 in 200), immediately add 10 mL of 1 N hydrochloric acid, and swirl gently to mix. [NOTE—Disregard any precipitate that may be formed at this point.] Allow to stand for 2 minutes, accurately timed. Add 10 mL of ammonium sulfamate solution (1 in 50), and shake frequently during 5 minutes. Five minutes after the addition of the ammonium sulfamate solution, add 3 drops of a 1 in 10 solution of 1-naphthol in alcohol. Mix, and allow to stand for 1 minute. Add 3.5 mL of a pH 10 buffer (made by dissolving 67.5 g of ammonium chloride in 300 mL of water, adding 570 mL of ammonium hydroxide, and diluting with water to 1 L). Mix, remove from the ice bath, and immediately dilute, with water that has been chilled to 5°, to volume. Within 20 minutes of diluting the contents of all three flasks to 50 mL, concomitantly determine the absorbances of the test solution and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 485 nm, with a suitable spectrophotometer, versus the prepared blank. The absorbance of the solution from the Iothalamic Acid is not greater than that of the Standard solution (0.05%).

Iodine and iodide—

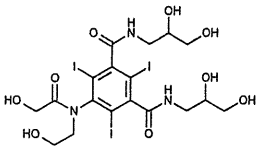
Test solution—To 10.0 g in a 50-mL beaker add 16 mL of 1 N sodium hydroxide, and stir until solution is complete. Dilute with water to about 35 mL, and adjust the solution to a pH of between 7.0 and 7.5 with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid. Dilute with water to 50 mL.

Procedure—Dilute 10 mL of *Test solution* with 20 mL of water in a 50-mL beaker, add 5 mL of 2 N sulfuric acid, stir, and filter into a glass-stoppered, 50-mL cylinder. To the filtrate add 5 mL of toluene, and shake: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), and shake: any red color in the toluene layer is not darker than that obtained when a mixture of 2 mL of potassium iodide solution (1 in 4000) and 22 mL of water is substituted for the solution under test (0.02% of iodide).

Assay—Transfer about 400 mg of Iothalamic Acid, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 12 mL of 5 N sodium hydroxide, 20 mL of water, and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly, adding the rinsings to the filtrate. Add 40 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using silver-calomel electrodes and an agar-potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 10.23 mg of $C_{11}H_9I_3N_2O_4$.

www.webofpharma.com

Ioversol



$C_{18}H_{24}I_3N_3O_9$ 807.11

1,3-Benzenedicarboxamide, *N,N'*-bis(2,3-dihydroxypropyl)-5-[(hydroxyacetyl)(2-hydroxyethyl)amino]-2,4,6-triiodo-. *N,N'*-Bis(2,3-dihydroxypropyl)-5-*N*-(2-hydroxyethyl)glycol amido]-2,4,6-triiodoisophthalamide. [87771-40-2].

» Ioversol contains not less than 97.0 percent and not more than 101.0 percent of $C_{18}H_{24}I_3N_3O_9$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Ioversol RS
USP Ioversol Related Compound A RS
USP Ioversol Related Compound B RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: Heat about 500 mg in a crucible: violet vapors are evolved.

Water Determination, *Method I* ⟨921⟩ **:** not more than 5%.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Related compounds—

Mobile phase—Prepare a degassed mixture of water and acetonitrile (99.5: 0.5).

Standard solution—Dissolve accurately weighed quantities of USP Ioversol Related Compound A RS and USP Ioversol Related Compound B RS in water to obtain a solution having known concentrations of about 1.0 µg per mL of USP Ioversol Related Compound A RS and 5.0 µg per mL of USP Ioversol Related Compound B RS.

Test solution—Transfer about 100 mg of Ioversol, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm stainless steel column containing packing L7. The flow rate is about 1 mL per minute. The column temperature is maintained at 35 ± 0.5°. The system is capable of operating at a column pressure of up to 3000 psi. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between ioversol related compound A and ioversol related compound B is not less than 2.0; and the relative standard deviation for replicate injections is not more than 5%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each ioversol related compound in the portion of Ioversol taken by the formula:

$$100(C/W)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ioversol Related Compound A RS or USP Ioversol Related Compound B RS in the *Standard solution; W* is the concentration, in mg per mL, of Ioversol in the *Test solution;* r_U is the peak response obtained from the *Test solution;* and r_S is the average peak response from the *Standard solution.* Not more than 0.10% of ioversol related compound A and not more than 0.50% of ioversol related compound B are found.

Iodine and iodide—Dissolve 2.0 g of Ioversol in water in a 50-mL glass-stoppered cylinder, and dilute with water to 15 mL. To this solution add 5 mL each of diluted sulfuric acid and toluene. Shake vigorously, and allow the layers to separate: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), and shake: any red color in the toluene layer is not darker than that obtained from a mixture of 2 mL of potassium iodide solution (1 in 4000) and 13 mL of water, similarly treated (0.02% of iodide).

Assay—Transfer about 500 mg of Ioversol, accurately weighed, to a glass-stoppered 125-mL conical flask, add 12 mL of 5 N sodium hydroxide, 20 mL of water, and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 40 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-silver chloride double junction reference electrode and a silver billet electrode. Each mL of 0.05 N silver nitrate is equivalent to 13.45 mg of $C_{18}H_{24}I_3N_3O_9$.

Ioversol Injection

» Ioversol Injection is a sterile solution of Ioversol in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of ioversol ($C_{18}H_{24}I_3N_3O_9$) and iodine (I). It may contain small amounts of suitable buffers and Edetate Calcium Disodium as a stabilizer. Ioversol Injection intended for intravascular use contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light.

Labeling—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container.

USP Reference standards ⟨11⟩—
USP Ioversol RS
USP Ioversol Related Compound A RS
USP Ioversol Related Compound B RS

Identification—

A: The IR absorption spectrum of the test specimen, a zinc sulfide cell having a thickness of 0.01 to 0.02 mm being used, exhibits maxima only at the same wavelength as that of a similar preparation of USP Ioversol RS.

B: Heat about 1 mL in a crucible: violet vapors are evolved.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1.4 USP Endotoxin Units per mL of Injection.

pH ⟨791⟩: between 6.0 and 7.4.

USP Monographs

www.webofpharma.com

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Related compounds—

Mobile phase and *Chromatographic system*—Proceed as directed in the test for *Related compounds*— under *Ioversol.*

Standard solution—Dissolve accurately weighed quantities of USP Ioversol Related Compound A RS and USP Ioversol Related Compound B RS in water to obtain a solution having known concentrations of 1.5 and 15 µg per mL, respectively.

Test solution—Dilute an accurately measured volume of Injection with water to obtain a solution containing 1 mg of ioversol per mL.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each ioversol related compound in the volume of Injection taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Ioversol Related Compound A RS or USP Ioversol Related Compound B RS in the *Standard solution;* C_U is the concentration, in mg per mL, of Ioversol in the *Assay preparation;* r_U is the peak response obtained from the *Test solution;* and r_S is the average peak response obtained from the *Standard solution.* Not more than 0.15% of ioversol related compound A and not more than 1.5% of ioversol related compound B are found.

Assay—Transfer an accurately measured volume of Injection, equivalent to about 0.5 g of ioversol, to a glass-stoppered, 125-mL conical flask, add 12 mL of 5 N sodium hydroxide, 20 mL of water, and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and filter thoroughly, adding the rinsings to the filtrate. Add 40 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-silver chloride double junction reference electrode and a silver billet electrode. Each mL of 0.05 N silver nitrate is equivalent to 13.45 mg of $C_{18}H_{24}I_3N_3O_9$.

Ioxaglate Meglumine and Ioxaglate Sodium Injection

» Ioxaglate Meglumine and Ioxaglate Sodium Injection is a sterile solution of Ioxaglic Acid in Water for Injection, prepared with the aid of Meglumine and Sodium Hydroxide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of ioxaglate meglumine ($C_{24}H_{21}I_6N_5O_8 \cdot C_7H_{17}NO_5$) and iodine (I). It may contain small amounts of Edetate Calcium Disodium as a stabilizer. Ioxaglate Meglumine and Ioxaglate Sodium Injection intended for intravascular use contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light.

Labeling—Label containers of Injection intended for intravascular injection to direct the user to discard any unused portion remaining in the container. Label containers of Injection intended for other than intravascular injection to indicate that the contents are not intended for intravascular injection.

USP Reference standards ⟨11⟩—
USP Ioxaglic Acid RS

Identification—

A: It responds to *A: Thin-Layer Chromatographic Identification Test 201*— test *A: Thin-Layer Chromatographic Identification Test 201*— under *Ioxaglic Acid,* a solution of 1.7 mL of Injection in 100 mL of water being used as the test solution.

B: Evaporate a volume of Injection, equivalent to about 500 mg of ioxaglate meglumine and ioxaglate sodium, to dryness, and heat the residue so obtained in a crucible: violet vapors are evolved.

pH ⟨791⟩: between 6.0 and 7.6.

Free iodine and iodide—

Test solution—Transfer a volume of Injection, equivalent to 2 g of the total of ioxaglate meglumine and ioxaglate sodium, to a 50-mL centrifuge tube, add 25 mL of water and 15 mL of 2 N sulfuric acid, and mix thoroughly. Centrifuge for 15 minutes, and decant the supernatant layer into a glass-stoppered 50-mL graduated cylinder. Repeat the sulfuric acid washing and centrifugation once more, adding the supernatant layer into the 50-mL graduated cylinder.

Procedure—Proceed as directed for *Procedure* in the test for *Free iodine and iodide* under *Ioxaglic Acid* (0.02% of iodide).

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

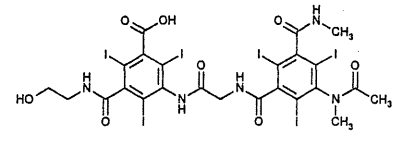
Assay for ioxaglate meglumine—Determine the angular rotation (see *Optical Rotation* ⟨781⟩) of the Injection, using a 10-cm cell and a suitable polarimeter. Calculate the percentage of ioxaglate meglumine in the Injection taken by the formula:

$$100a/3.32$$

in which a is the observed angular rotation, in degrees, corrected for a water blank; and 3.32 is the specific rotation, in degrees, of ioxaglate meglumine.

Assay for iodine—Transfer an accurately measured volume of Injection, equivalent to about 5 g (total) of ioxaglate meglumine and ioxaglate sodium, to a 250-mL volumetric flask, dilute with water to volume, and mix. Pipet 25 mL of this solution into a 125-mL conical flask, add 12 mL of 5 N sodium hydroxide and 1 g of powdered zinc, connect the flask to a reflux condenser, and reflux for 30 minutes. Proceed as directed in the *Assay for iodine* under *Ioxaglic Acid.* Each mL of 0.05 N silver nitrate is equivalent to 6.345 mg of iodine (I).

Ioxaglic Acid



$C_{24}H_{21}I_6N_5O_8$ 1268.88

Benzoic acid, 3-[[[[3-(acetylmethylamino)-2,4,6-triiodo-5-[(methylamino)carbonyl]benzoyl]amino]acetyl]amino]-5-[[(2-hydroxyethyl)amino]carbonyl]-2,4,6-triiodo-.

www.webofpharma.com

N-(2-Hydroxyethyl)-2,4,6-triiodo-5[2-[2,4,6-triiodo-3-(*N*-methylacetamido)-5-(methylcarbamoyl)benz amido] acetamido]isophthalamic acid [59017-64-0].

» Ioxaglic Acid contains not less than 98.5 percent and not more than 101.5 percent of $C_{24}H_{21}I_6N_5O_8$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Ioxaglic Acid RS

Identification—

A: Thin-Layer Chromatographic Identification Test ⟨201⟩—
Test solution—Dissolve 50 mg in 5 mL of methanol.
Application volume: 5 µL.
Developing solvent system: butyl alcohol, water, and acetic acid (50:25:11).
Procedure—Proceed as directed in the chapter. The R_F values of the two spots obtained from the *Test solution* correspond to those obtained from the Standard solution.

B: Heat about 500 mg in a crucible: violet vapors are evolved.

Water Determination, *Method I* ⟨921⟩ **:** not more than 5%.

Residue on ignition ⟨281⟩**:** not more than 0.1%, the residue being moistened with 2 mL of 35% sulfuric acid and ignited at 600°.

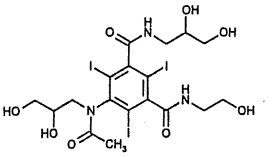
Free iodine and iodide—

Test solution—Dissolve 2 g of Ioxaglic Acid in 20 mL of 0.1 N sodium hydroxide in a 50-mL centrifuge tube. Add 15 mL of 2 N sulfuric acid, mix on a vortex mixer, centrifuge for 15 minutes, and decant the supernatant layer into a glass-stoppered 100-mL graduated cylinder. Repeat the sulfuric acid washing and centrifugation twice more, decanting each supernatant layer into the 100-mL graduated cylinder.

Procedure—Add 5 mL of toluene, shake vigorously, and allow the layers to separate: the toluene layer shows no red color. Add 1 mL of sodium nitrite solution (1 in 50), shake, and allow the layers to separate: any red color in the toluene layer is not darker than that obtained when a mixture of 2.0 mL of potassium iodide solution (1 in 4000), 25 mL of water, and 15 mL of 2 N sulfuric acid is substituted for the *Test solution* (0.02% of iodide).

Assay—Transfer about 500 mg of Ioxaglic Acid, accurately weighed, to a glass-stoppered 125-mL conical flask, and add 12 mL of 5 N sodium hydroxide, 20 mL of water, and 1 g of powdered zinc. Connect the flask to a reflux condenser, and reflux for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 mL of water, disconnect the flask from the condenser, and filter the mixture. Rinse the flask and the filter thoroughly, adding the rinsing to the filtrate. Add 40 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using silver-calomel electrodes and an agar–potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 10.57 mg of $C_{24}H_{21}I_6N_5O_8$.

Ioxilan



» Ioxilan contains not less than 98.0 percent and not more than 102.0 percent of $C_{18}H_{24}I_3N_3O_8$, calculated on the anhydrous and methanol-free basis.

Packaging and storage—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩—
USP Ioxilan RS
USP Ioxilan Related Compound A RS
5-Amino-2,4,6-triiodo-3 *N*-(2-hydroxyethyl)carbamoyl benzoic acid.
$C_{10}H_9I_3N_2O_4$ 601.90

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 10 µg per mL.
Medium: water.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.2 USP Endotoxin Unit per 50 mg of iodine.

pH ⟨791⟩**:** between 5.0 and 7.5, in a solution (1 in 10). [NOTE—Heat gently, if necessary, to dissolve. Cool to room temperature before proceeding.]

Water Determination, *Method I* ⟨921⟩ **:** not more than 4.0%.

Free iodine—Transfer 2 g to a stoppered flask, add 12 mL of water, and swirl to dissolve. [NOTE—Heat gently, if necessary, to dissolve. Cool to room temperature before proceeding.] Add 2.5 mL of 2 N sulfuric acid and 4 mL of toluene, stopper the flask, and shake for 1 minute. Allow the layers to separate: the toluene layer shows no red color. Reserve the contents of the flask for use as the test solution in the test for *Free iodide.*

Free iodide—Prepare a solution of potassium iodide in water containing 1000 µg of iodide per mL. Quantitatively dilute portions of this solution with water to obtain standard solutions having concentrations of 100, 50, 25, and 10 µg of iodide per mL. Transfer 2 mL of each standard solution to separate stoppered flasks, and add 12 mL of water to each flask. Add 14 mL of water to an additional flask to serve as the blank. Add 2.5 mL of 2 N sulfuric acid and 4 mL of toluene to the flasks containing the standard solutions and the blank, stopper the flasks, and shake for 1 minute. To the test solution obtained in the test for *Free iodine,* each of the standard solutions, and the blank, add 0.5 mL of sodium nitrite solution (2 in 100), stopper the flasks, and shake vigorously for 1 minute. Allow the layers to separate, transfer the toluene layers to separate centrifuge tubes, and centrifuge for 1 minute. Concomitantly determine the absorbances of the test solution and the standard solutions against the blank. From a linear

USP Monographs

www.webofpharma.com

regression equation calculated from the concentrations and absorbances of the iodide standard solutions, determine the content of iodide in the Ioxilan: the limit is 30 µg of iodide per g of Ioxilan.

Free aromatic amine— [NOTE—Protect the Standard solution, the test solution, and the blank from light throughout the test.]

pH 10 Buffer—To 33.7 g of ammonium chloride add 285 mL of ammonium hydroxide, dilute with water to 500 mL, and mix.

Procedure—Transfer 20 mg of USP Ioxilan Related Compound A RS, accurately weighed, to a 200-mL volumetric flask, dissolve in a small volume of hot water, cool, dilute with water to volume, and mix. Transfer 2.5 mL of this solution to a 50-mL volumetric flask, add 12 mL of water, and mix. Transfer 0.5 g of Ioxilan to a second 50-mL volumetric flask, add 12 mL of water, and shake to dissolve. [NOTE—Heat gently, if necessary, to dissolve. Cool to room temperature before proceeding.] To a third 50-mL volumetric flask, add 12 mL of water to serve as the blank, and place the flasks containing the Standard solution, the test solution, and the blank in an ice bath. [NOTE—In conducting the following steps, keep the flasks in the ice bath as much as possible until all of the reagents have been added.] Treat each of the flasks as follows. Add 5 mL of sodium nitrite solution (0.5 in 100), and shake.

[**CAUTION**—Considerable pressure is produced with the addition of each reagent.]

Add 10 mL of 1 N hydrochloric acid, swirl, and allow to stand for 2 minutes. Add 3 drops of a 1 in 10 solution of α-naphthol in alcohol, swirl, and allow to stand for 2 minutes. Add 3.5 mL of *pH 10 Buffer*, swirl, and allow to stand out of the ice bath for 2 minutes. Degas all solutions in a water bath at 25° for 10 minutes, dilute with water to 50 mL, and mix. Within 15 minutes of this final dilution, concomitantly determine the absorbances of the test solution and the Standard solution at the wavelength of maximum absorbance at about 485 nm, with a suitable spectrophotometer, against the blank. The absorbance of the test solution is not greater than that of the Standard solution (0.05%).

Residual methanol—

Standard stock solution—Transfer 200 mg of methanol to a tared 200-mL volumetric flask containing 20 mL of water, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask containing 20 mL of water, dilute with water to volume, and mix.

Internal standard solution—Transfer 200 mg of dehydrated alcohol to a tared 200-mL volumetric flask containing 20 mL of water, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard solutions A, B, C, and *D*—Transfer 0.5, 1.0, 2.0, and 4.0 mL of *Standard stock solution* to separate 10-mL volumetric flasks, each containing 2 mL of water. Add 1.0 mL of *Internal standard solution* to each flask, dilute with water to volume, and mix to obtain *Standard solutions A, B, C,* and *D* having concentrations of 0.5, 1.0, 2.0, and 4.0 mg of methanol per L, respectively.

Test solution—Transfer about 1 g of Ioxilan to a tared 10-mL ampul, and add water to the ampul to a final weight of 10.45 g, taking care to avoid leaving water in the neck of the ampul. Seal the ampul, and immerse it in a water bath at 90° until the Ioxilan is dissolved. Mix, and allow to cool to room temperature. Mix again, open the ampul, and dilute the contents with a volume of water suitable to obtain a methanol concentration within the range of the standard curve (between 0.5 and 4 mg per L), allowing for the addition of 1.0 mL of *Internal standard solution.*

Chromatographic system (see *System Suitability* under *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 10-m × 0.53-mm fused silica capillary column coated with support S2. The column temperature is programmed to increase from 45° to 80° at a rate of 5° per minute. The injection port temperature is maintained at 180°, and the detector temperature is maintained at 200°. Helium is used as the carrier gas at a flow rate of about 30 mL per minute. Chromatograph *Standard solution D,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the methanol and alcohol peaks is not less than 10; the tailing factor for the methanol peak is not more than 3.0; and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 1 µL) of each *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the peak responses. From a linear regression equation calculated from the ratios of the peak responses of methanol to those of alcohol for each of the *Standard solutions* versus the methanol concentrations, in mg per L, determine the methanol content in the *Test solution:* not more than 2.0% is found.

Limit of serinol impurity—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (91:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Transfer about 60 mg of USP Ioxilan RS to a 10-mL volumetric flask, add 1 mL of water, and heat at not more than 80° to dissolve. Cool to room temperature, dilute with acetonitrile to volume, and mix.

Test solution—Using about 60 mg of Ioxilan, proceed as directed for *Standard solution.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 245-nm detector and a 25-cm × 4.6-mm stainless steel column that contains 5-µm packing L18. The flow rate is about 2 mL per minute, and the column temperature is maintained at 30°. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the retention time of the serinol peak is about 0.9 relative to that of the larger of the Ioxilan peaks; the resolution, *R,* between the larger of the Ioxilan peaks and the serinol peak is not less than 1.0; and the relative standard deviation of the response of the serinol peak for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Test solution* and the *Standard solution* into the chromatograph, and allow the chromatogram to run for about 35 minutes. Measure the areas of the peak responses, and determine the percentage of serinol in the portion of Ioxilan taken: not more than 0.5% is found.

Chromatographic purity—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (87:13). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Transfer about 60 mg of USP Ioxilan RS to a 10-mL volumetric flask, and add 1 mL of water. [NOTE—Heat gently, if necessary, to dissolve. Cool to room temperature before proceeding.] Dilute with acetonitrile to volume, and mix.

Test solution—Using about 60 mg of Ioxilan, proceed as directed for *Standard solution.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 245-nm detector and a 25-cm × 4.6-mm stainless steel column that contains 5-µm packing L8. The column is maintained at 30°, and the flow rate is about 1 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the two largest

www.webofpharma.com

impurity peaks eluting immediately after the second ioxilan isomer peak is not less than 0.3.

Procedure—Separately inject equal volumes (about 10 µL) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the areas of the peak responses. Determine the percentage of each impurity (excluding the serinol impurity, which has a retention time of 0.9 relative to the larger ioxilan isomer) in the portion of Ioxilan taken: not more than 0.5% of any individual impurity is found; and the total of all impurities is not more than 1.5%.

Assay—Transfer about 500 mg of Ioxilan, accurately weighed, to a 100-mL round-bottom flask. Add 1 g of powdered zinc and 40 mL of 1.25 N sodium hydroxide, connect the flask to a reflux condenser that is cooled with water at 5° to 10°, add a few glass boiling beads, and gently reflux the mixture for 1 hour. Allow the flask to cool to room temperature, and rinse the condenser with 10 to 20 mL of water. Disconnect the flask from the condenser, add 5 mL of glacial acetic acid, mix, and allow the mixture to cool. Pass through general purpose fast-flowing filter paper mixture. Rinse the flask and the filter with 1 N acetic acid, and add the rinsings to the filtrate. Add 5 mL of tetrabromophenolphthalein ethyl ester TS, and titrate with 0.1 N silver nitrate VS, with continuous stirring, until the precipitate turns green. Each mL of 0.1 N silver nitrate is equivalent to 26.37 mg of $C_{18}H_{24}I_3N_3O_8$.

Ioxilan Injection

» Ioxilan Injection is a sterile solution of Ioxilan in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of ioxilan ($C_{18}H_{24}I_3N_3O_8$) as organically bound iodine. It may contain small amounts of suitable buffers and of Edetate Calcium Disodium as a stabilizer. Ioxilan Injection contains no antimicrobial agents.

Packaging and storage—Preserve injection in single-dose containers of Type I glass, protected from light.

Labeling—Label containers of the Injection to direct the user to discard any unused portion. The label states that it is not to be used if it is discolored or contains a precipitate and states also that it is not for intrathecal use.

USP Reference standards ⟨11⟩—
USP Ioxilan RS

Identification—

A: Evaporate a volume of Injection, equivalent to about 500 mg of ioxilan, to dryness. Char the residue so obtained in a suitable crucible: violet vapors are evolved (*presence of iodine*).

B: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those of the major peaks in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 0.2 USP Endotoxin Unit per 50 mg of iodine.

pH ⟨791⟩: between 6.0 and 7.5.

Free iodine—Transfer an accurately measured volume of Injection, equivalent to about 1 g of Ioxilan, to a tared 25-mL volumetric flask, add 13 mL of water, and swirl to dissolve. Add 2.5 mL of 2 N sulfuric acid and 4 mL of toluene. Stopper the flask, and shake vigorously for 1 minute. Allow layers to separate: the toluene layer shows no red color. Reserve the contents of the flask for use as the test solution in the test for *Free iodide*.

Free iodide—Proceed as directed in the test for *Free iodine*— under *Ioxilan*. The limit is 200 µg of iodide per mL.

Residual methanol—

Standard stock solution, Internal standard solution, Standard solutions A, B, C, D, and *Chromatographic system*—Proceed as directed in the test for *Residual methanol*— under *Ioxilan*.

Test solution—Transfer an accurately measured volume of Injection, equivalent to about 1 g of ioxilan, to a 10-mL volumetric flask. Add 1.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the test for *Residual methanol*— under *Ioxilan*: not more than 0.005% is found.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (87:13). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation A—Transfer about 60 mg of USP Ioxilan RS, to a 10-mL volumetric flask, and add 1 mL of water. [NOTE—Heat gently, if necessary, to effect dissolution. Cool to room temperature before proceeding.] Dilute with acetonitrile to volume, and mix.

Standard preparation B—Prepare a 1 in 10 dilution of *Standard preparation A* in *Mobile phase*.

Assay preparation A—Transfer an accurately measured volume of Injection, equivalent to about 60 mg of ioxilan, to a 10-mL volumetric flask, add 1 mL of water, and swirl to mix. Dilute with acetonitrile to volume, and mix.

Assay preparation B—Prepare a 1 in 10 dilution of *Assay preparation A* in *Mobile phase*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 245-nm detector and a 25-cm × 4.6-mm stainless steel column that contains packing L8. The column is maintained at 30°, and the flow rate is about 1 mL per minute. Chromatograph *Standard preparation A*, and record the peak responses as directed under *Procedure*:the resolution, *R*, between the two largest impurity peaks eluting immediately after the second ioxilan isomer peak is not less than 0.3. Chromatograph *Standard preparation B*, and record the peak responses as directed under *Procedure*:the resolution, *R*, between the two ioxilan isomer peaks is not less than 2.2, the column efficiency, based on the larger ioxilan isomer peak, is not less than 4000 theoretical plates, and the relative standard deviation of the sums of the responses of the two ioxilan isomer peaks from replicate injections is not less than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of *Assay preparation A, Assay preparation B, Standard preparation A,* and *Standard preparation B* into the chromatograph, record the chromatograms, and measure the areas of the responses for the major peaks. Record the integrated results, summing the areas of the two ioxilan peaks. From the chromatogram of *Assay preparation A* in comparison to that of *Standard preparation B*, determine the quantity of ioxilan in the Injection taken. Determine the percentage of impurities (excluding the serinol impurity with a relative retention time of 0.9 relative to the larger ioxilan isomer peak) by area percent, using the chromatogram of *Assay preparation A*. Not more than 0.5% of any individual impurity is found, and the total of all impurities is not more than 1.5%. [NOTE—The impurities eluting on the tail of the second ioxilan isomer peak should be integrated by skimming the peaks.]

www.webofpharma.com

Ipecac

DEFINITION

Ipecac consists of the dried rhizome and roots of *Cephaëlis acuminata* Karsten, or of *Cephaëlis ipecacuanha* (Brotero) A.Richard (Fam. Rubiaceae). Ipecac yields NLT 2.0% of the total ether-soluble alkaloids of ipecac. Its content of emetine ($C_{29}H_{40}N_2O_4$) and cephaeline ($C_{28}H_{38}N_2O_4$) together is NLT 90.0% of the amount of the total ether-soluble alkaloids. The content of cephaeline varies from an amount equal to, to an amount NMT 2.5 times, the content of emetine.

COMPOSITION

• **TOTAL ETHER-SOLUBLE ALKALOIDS**

[NOTE—It is important that the ether used in this *Assay* must have been shown by test to be free from peroxides within 24 h before use.]

The alkaloids may be extracted by either of the methods given below.

Method 1

Sample solution: To 10 g of finely powdered Ipecac, in a suitable container, add 100 mL of ether, accurately measured at 25°, insert the stopper in the container tightly, shake the mixture thoroughly, and allow to stand for 5 min. Add 10 mL of 6 N ammonium hydroxide, close again tightly, shake it for 1 h in a mechanical shaker or intermittently during 2 h, and allow to stand overnight at a temperature not exceeding 25°. Again shake the mixture intermittently during 30 min, and allow the drug to settle at 25°. Transfer to a separator a 50.0-mL aliquot of the clear supernatant, representing 5 g of Ipecac.

Method 2

Sample solution: Place 5 g of the finely powdered Ipecac in a continuous-extraction thimble. Add enough ether to cover the powder, and allow to stand for 10 min with occasional stirring. Add 3 mL of ammonium hydroxide, mix, and allow to stand overnight. Cover the drug with a pledget of cotton, pack well, and extract with ether for 5 h. Transfer the ether extract to a separator.

Analysis: Extract the alkaloids from the ether with 2 N sulfuric acid, using at first 15 mL, or more if necessary, to ensure an acid reaction, then successive 10-mL portions until extraction is complete, and filtering all extracts through the same filter into a second separator. To the combined acid solutions add an equal volume of ether, render the mixture distinctly alkaline with 6 N ammonium hydroxide (at least pH 10, by test paper), and extract with successive portions of ether until the last extract shows NMT a slight turbidity when treated as follows: Evaporate 1 mL of the last extraction, dissolve the residue in 0.5 mL of 0.5 N hydrochloric acid, and add 1 drop of mercuric iodide TS.

Filter each portion of the ether extract into a flask or beaker, and carefully evaporate the combined ether extracts on a steam bath almost to dryness. Add 5 mL of ether and 10.0 mL of 0.1 N sulfuric acid VS, and heat on a steam bath to dissolve the alkaloids and to remove all of the ether. Cool, add 15 mL of water, then add methyl red TS, and titrate the excess acid with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sulfuric acid is equivalent to 24.0 mg of the total ether-soluble alkaloids of ipecac, calculated as emetine ($C_{29}H_{40}N_2O_4$).

Acceptance criteria: NLT 2.0%

• **EMETINE AND CEPHAELINE**

Solution A: Prepare 0.5 M monobasic potassium phosphate (containing 5.1 g/75 mL) and 0.5 M dibasic potassium phosphate (containing 2.2 g/25 mL). Mix three volumes of 0.5 M monobasic potassium phosphate with one volume of 0.5 M dibasic potassium phosphate, and adjust by the addition of one or the other of the solutions to a pH of 6.0 ± 0.05. Dissolve 7.5 g of potassium chloride in 100 mL of the resulting solution.

Solution B: 0.5 M sodium citrate (containing 6.5 g/50 mL) and citric acid (containing 4.8 g/50 mL). Mix equal volumes of these solutions, and adjust by the addition of one or the other of the solutions to a pH of 4.0 ± 0.05.

Standard solution: 50 µg/mL of emetine from USP Emetine Hydrochloride RS in 0.5 N sulfuric acid

Sample solution: Transfer 200 mg of the fine powder to a 150-mL beaker. Add 2 mL of dimethyl sulfoxide, mix with a flattened stirring rod to ensure complete wetting of the powder, and allow to stand for 30 min. Add 2 mL of water and 1 g of sodium bicarbonate.

Chromatographic columns: For each column, pack a pledget of fine glass wool in the base of a chromatographic tube (25- × 200-mm test tube to which is fused a 5-cm length of 7-mm tubing) with the aid of a tamping rod having a disk with a diameter 1 mm less than that of the tube.

Column I: Prepare *Column I* as follows. To the *Sample solution* add 6 g of purified siliceous earth, mix, transfer the mixture to the column, scrub the beaker with 1 g of the purified siliceous earth, transfer this to the top of the column, and tamp.

Column II: Prepare *Column II* using 3 g of the purified siliceous earth and 2 mL of *Solution A*.

Column III: Prepare *Column III* using 2 mL of *Solution B* and 3 g of the purified siliceous earth.

Column IV: Prepare *Column IV* using 2 mL of sodium hydroxide solution (1 in 50) and 3 g of the purified siliceous earth.

Pack a pledget of glass wool on the top of each column.

Analysis

[NOTE—Use water-saturated solvents throughout this *Analysis*. Rinse the tips of the chromatographic columns before discarding them.]

Emetine solution: Mount *Columns I* and *II* so that the effluent from *Column I* flows onto *Column II*. Pass three 50-mL portions of ether through the columns, and discard *Column I* and the eluate. Mount *Column III* below *Column II* and pass three 50-mL portions of a mixture of one volume of ether and three volumes of chloroform through the columns. Discard *Column II* and the eluate. Wash *Column III* with 25 mL of the ether–chloroform mixture, followed by 25 mL of a mixture of equal volumes of ether and isooctane, and discard the washings. Wash *Column IV* with 20 mL of a 1-in-50 solution of triethylamine in the ether–isooctane mixture, and discard the washing. Mount *Column IV* below *Column III*, and place as a receiver under *Column IV* a 125-mL separator containing 15 mL of 4 N sulfuric acid. Pass through the columns 10 mL of a 1-in-5 solution of triethylamine in the ether–isooctane mixture, followed by three 10-mL portions of a 1-in-50 solution of triethylamine in the ether–isooctane mixture. Discard *Column III*, and pass through *Column IV* 20 mL of the 1-in-50 solution of triethylamine in the ether–isooctane mixture. Shake the separator, allow the phases to separate, and transfer the aqueous extract to a 50-mL volumetric flask. Extract with two additional 10-mL portions of 0.5 N sulfuric acid, combining the extracts in the volumetric flask. Add 0.5 N sulfuric acid to volume.

Cephaeline solution: Elute *Column IV* with 75 mL of chloroform, collecting the eluate in a 250-mL separator containing 150 mL of ether. Discard *Column IV*. Extract with one 20-mL and then with two 10-mL portions of 0.5 N sulfuric acid, collecting the extracts in a 50-mL volumetric flask. Rinse the stem of the separator, and add the acid to volume.

www.webofpharma.com

Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: UV-Vis
Analytical wavelengths: 283 and 350 nm
Cell: 1 cm
Blank: 0.5 N sulfuric acid
Analysis
Samples: *Standard solution, Emetine solution,* and *Cephaeline solution*
Calculate the percentage of emetine in the portion of Ipecac taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = difference in the absorbances of the emetine solution from the *Sample solution* at the wavelengths indicated by $(A_{283} - A_{350})$
A_S = difference in the absorbances of the emetine solution from the *Standard solution* at the wavelengths indicated by $(A_{283} - A_{350})$
C_S = concentration of emetine in the *Standard solution* (µg/mL)
C_U = nominal concentration of emetine in the *Sample solution* (mg/mL)

Calculate the percentage of cephaeline in the portion of Ipecac taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = difference in the absorbances of the cephaeline solution from the *Sample solution* at the wavelengths indicated by $(A_{283} - A_{350})$
A_S = difference in the absorbances of the cephaeline solution from the *Standard solution* at the wavelengths indicated by $(A_{283} - A_{350})$
C_S = concentration of emetine in the *Standard solution* (µg/mL)
C_U = nominal concentration of cephaeline in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of cephaeline, 466.61
M_{r2} = molecular weight of emetine, 480.64

Acceptance criteria: The content of emetine ($C_{29}H_{40}N_2O_4$) and cephaeline ($C_{28}H_{38}N_2O_4$) together is NLT 90.0% of the total amount of the ether-soluble alkaloids. The content of cephaeline varies from an amount equal to, to an amount NMT 2.5 times, the content of emetine.

CONTAMINANTS
• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Pesticide Residue Analysis*: Meets the requirements

SPECIFIC TESTS
• **BOTANICAL CHARACTERISTICS**
Macroscopic: A mixture of segments of the roots and rhizomes. The root segments are mostly curved and flexuous, occasionally branched, up to 15 cm in length and usually 3–6.5 mm in diameter, but may be up to 9 mm in diameter, grayish, grayish brown, or reddish brown, the reddish brown type often having light-colored abrasions, transverse ridges 0.5–1.0 mm wide that extend halfway around the circumference of the root and fade at their tapering extremities into the general surface, with from one to six of these ridges per centimeter, and annulations sometimes seen at irregular intervals. The rhizomes are cylindrical, 2 mm thick, finely longitudinally wrinkled, with a few elliptical scars. The odor is distinctive; the dust is sternutatory.

Microscopic: At the center of the root is a well-defined primary xylem but no pith. Surrounding this is a dense wood of secondary xylem crossed by medullary rays. These elements are all lignified. External to the wood is a narrow band of secondary phloem and a wide parenchymatous phelloderm surrounded by a narrow layer of cork a few cells thick. The secondary xylem consists of narrow, bordered-pitted tracheidal vessels and tracheids in combination with xylem parenchyma. The latter have simple pits and contain starch grains. Starch is present also in the medullary rays. The phloem occurs as small groups of sieve tissue embedded in parenchyma. The wide phelloderm consists of round-celled cellulose parenchyma filled with starch grains and a few idioblasts, each of which contains a bundle of acicular raphides of calcium oxalate crystals 30- to 80-µm long. The starch grains are rarely single but usually occur as 2–4 and sometimes 8 in a clump. Individual grains measure up to 22 µm in diameter.
The rhizome differs from the root in having a ring of xylem around a large pith. The pericycle contains characteristic sclerenchymatous cells. Spiral vessels are found in the protoxylem. The pith is composed of pitted parenchyma, which is somewhat lignified.
• **OVERGROUND STEMS:** The proportion of overground stems is NMT 5%.
• **ARTICLES OF BOTANICAL ORIGIN** ⟨561⟩, *Methods of Analysis, Foreign Organic Matter*: The proportion of foreign organic matter is NMT 2.0%.

ADDITIONAL REQUIREMENTS
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Emetine Hydrochloride RS

Powdered Ipecac

» Powdered Ipecac is Ipecac reduced to a fine or a very fine powder and adjusted to a potency of not less than 1.9 percent and not more than 2.1 percent of the total ether-soluble alkaloids of ipecac, by the addition of exhausted marc of ipecac or of other suitable inert diluent or by the addition of powdered ipecac of either a lower or a higher potency.

The content of emetine ($C_{29}H_{40}N_2O_4$) and cephaeline ($C_{28}H_{38}N_2O_4$) together is not less than 90.0 percent of the total amount of the ether-soluble alkaloids. The content of cephaeline varies from an amount equal to, to an amount not more than 2.5 times, the content of emetine.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Emetine Hydrochloride RS
Botanic characteristics—Thin-walled, fairly small cork cells, the starch grains rarely simple and usually 2- to 8-compound, the single grains up to 22 µm in diameter; raphides of calcium oxalate 30 to 80 µm in length; tracheids and tracheidal vessels found in groups having very numerous, small, bordered pits; parenchyma of phelloderm filled with starch or acicular crystals of calcium oxalate, having cells thin-walled, oval with intercellular spaces; parenchyma of the xylem composed of small rectangular and longitudinally elongated cells with moderately thick walls and scattered

USP Monographs

www.webofpharma.com

bordered or simple pits; rhizome parenchyma cells larger than root parenchyma cells, with slightly thicker walls and lignified with fairly numerous simple pits; sclereids from the rhizome large, rectangular, with uneven walls and large, conspicuous pits.

Assay for total ether-soluble alkaloids—Proceed with Powdered Ipecac as directed in the *Assay for total ether-soluble alkaloids* under *Ipecac*.

Assay for emetine and cephaeline—

Standard preparation, Phosphate buffer, Citric acid buffer, and *Chromatographic columns*—Prepare as directed in the *Assay for emetine and cephaeline* under *Ipecac*.

Assay preparation—Transfer to a 150-mL beaker about 200 mg, accurately weighed, of Powdered Ipecac. Add 2 mL of dimethyl sulfoxide, mix with a flattened stirring rod to ensure complete wetting of the powder, and allow to stand for about 30 minutes. Add 2 mL of water and about 1 g of sodium bicarbonate, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay for emetine and cephaeline* under *Ipecac*. Calculate the quantity, in mg, of emetine in the portion of Powdered Ipecac taken by the formula:

$$0.05C(A_{283} - A_{350})_U/(A_{283} - A_{350})_S$$

in which the parenthetic expressions are the differences in the absorbances of the solution of emetine from the *Assay preparation (U)* and the *Standard preparation (S),* respectively, at the wavelengths indicated by the subscripts; and C is as defined for *Procedure* in the *Assay for emetine and cephaeline* under *Ipecac*. Calculate the quantity, in mg, of cephaeline in the portion of Powdered Ipecac taken by the formula:

$$0.971(0.05C)(A_{283} - A_{350})_U/(A_{283} - A_{350})_S$$

in which 0.971 is the ratio of the molecular weight of cephaeline to that of emetine; the parenthetic expressions are the differences in the absorbances of the solution of cephaeline from the *Assay preparation (U)* and the *Standard preparation (S),* respectively, at the wavelengths indicated by the subscripts; and C is as defined above.

Ipecac Oral Solution

» Ipecac Oral Solution yields, from each 100 mL, not less than 123 mg and not more than 157 mg of the total ether-soluble alkaloids of ipecac.

The content of emetine ($C_{29}H_{40}N_2O_4$) and cephaeline ($C_{28}H_{38}N_2O_4$) together is not less than 90.0 percent of the amount of the total ether-soluble alkaloids. The content of cephaeline varies from an amount equal to, to an amount not more than 2.5 times, the content of emetine.

Powdered Ipecac	70 g
Glycerin	100 mL
Syrup, a sufficient quantity, to make	1000 mL

Exhaust the powdered Ipecac by percolation, using a mixture of 3 volumes of alcohol and 1 volume of water as the menstruum, macerating for 72 hours, and percolating slowly. Reduce the entire percolate to a volume of 70 mL by evaporation at a temperature not exceeding 60° and preferably in vacuum, and add 140 mL of water. Allow the mixture to stand overnight, filter, and wash the residue on the filter with water. Evaporate the filtrate and washings to 40 mL, and to this add 2.5 mL of hydrochloric acid and 20 mL of alcohol, mix, and filter. Wash the filter with a mixture of 30 volumes of alcohol, 3.5 volumes of hydrochloric acid, and 66.5 volumes of water, using a volume sufficient to produce 70 mL of the filtrate. Add 100 mL of Glycerin and enough Syrup to make the product measure 1000 mL, and mix.

Packaging and storage—Preserve in tight containers, preferably at a temperature not exceeding 25°. Containers intended for sale to the public without prescription contain not more than 30 mL of Oral Solution.

USP Reference standards ⟨11⟩—
USP Emetine Hydrochloride RS

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Escherichia coli*.

Alcohol Determination ⟨611⟩: between 1.0% and 2.5% of C_2H_5OH.

Assay for total ether-soluble alkaloids— [NOTE—It is important that the ether used in this assay shall have been shown by test to be free from peroxides within 24 hours prior to use.] Transfer about 50 mL, accurately measured, of Oral Solution to a liquid-liquid automatic extractor, add water, if necessary, to reduce the viscosity, render the liquid distinctly alkaline with ammonium hydroxide, and extract with ether for at least 4 hours or until the extraction is complete. Use a water bath to boil the ether. Frequently disconnect the extractor from the condenser, and agitate the lower layer by raising and lowering the center tube or by other suitable manipulation. At the conclusion of the extraction period, transfer the ether extract to a separator, and rinse the extraction flask with 2 or more small volumes of ether, adding the rinsings to the separator. Complete the assay as directed in the *Assay for total ether-soluble alkaloids* under *Ipecac*, beginning with "Extract the alkaloids from the ether."

Assay for emetine and cephaeline—

Standard preparation, Phosphate buffer, and *Citric acid buffer*—Prepare as directed in the *Assay for emetine and cephaeline* under *Ipecac*.

Assay preparation—Pipet 10 mL of water into a 25-mL volumetric flask. With the aid of a 20-mL pipet, add Oral Solution to volume, taking care to prevent contact of the Oral Solution with the neck of the flask above the graduation line. Insert the stopper, and mix.

Chromatographic columns—Pack a pledget of fine glass wool in the base of a chromatographic tube (25-mm × 200-mm test tube to which is fused a 5-cm length of 7-mm tubing) with the aid of a tamping rod having a disk with a diameter about 1 mm less than that of the tube.

To prepare *Column I*, transfer 4.0 mL of the *Assay preparation* to a 150-mL beaker, add about 1 g of sodium bicarbonate, and mix. Then proceed as directed for *Chromatographic columns* in the *Assay for emetine and cephaeline* under *Ipecac*, beginning with "add 6 g of purified siliceous earth," and prepare *Columns II, III,* and *IV* as directed therein.

USP Monographs

www.webofpharma.com

Procedure—Proceed as directed for *Procedure* in the *Assay for emetine and cephaeline* under *Ipecac*.

Calculate the quantity, in mg, of emetine in each 100 mL of Oral Solution taken by the formula:

$$2.08C(A_{283} - A_{350})_U/(A_{283} - A_{350})_S$$

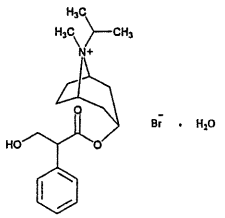
in which the parenthetic expressions are the differences in the absorbances of the solution of emetine from the *Assay preparation* (*U*) and the *Standard preparation* (*S*), respectively, at the wavelengths indicated by the subscripts, and *C* is as defined in the *Procedure*.

Calculate the quantity, in mg, of cephaeline in each 100 mL of Oral Solution taken by the formula:

$$0.971(2.08C)(A_{283} - A_{350})_U/(A_{283} - A_{350})_S$$

in which 0.971 is the ratio of the molecular weight of cephaeline to that of emetine, the parenthetic expressions are the differences in the absorbances of the solution of cephaeline from the *Assay preparation* (*U*) and the *Standard preparation* (*S*), respectively, at the wavelengths indicated by the subscripts, and *C* is as defined in the *Procedure*.

Ipratropium Bromide



$C_{20}H_{30}BrNO_3 \cdot H_2O$ 430.38

8-Azoniabicyclo[3.2.1]octane-3-(3-hydroxy-1-oxo-2-phenylpropoxy)-8-methyl-8-(1-methylethyl)-, bromide, monohydrate(*endo,syn*)-, (±)-;

(8*r*)-3α-Hydroxy-8-isopropyl-1αH,5αH-tropanium bromide (±)-tropate monohydrate [66985-17-9].

DEFINITION

Ipratropium Bromide contains NLT 98.0% and NMT 102.0% of ipratropium bromide ($C_{20}H_{30}BrNO_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B. IDENTIFICATION TESTS—GENERAL,** *Bromide* ⟨191⟩
  Sample solution: 10 mg/mL
  Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 89 mg/mL of dibasic sodium phosphate dihydrate in water

Buffer: 14.3 g/L of monobasic sodium phosphate dihydrate and 2.0 g/L of tetrapropylammonium chloride in water. Adjust with *Solution A* to a pH of 5.5 ± 0.2. Pass through a nylon membrane filter of 0.45-µm or finer pore size.

Mobile phase: Methanol and *Buffer* (13:87). [NOTE—Do not use the *Mobile phase* after 36 h.]

System suitability solution: 0.5 mg/mL of USP Ipratropium Bromide RS and 0.1 mg/mL of USP Ipratropium Bromide Related Compound C RS in *Mobile phase*

Standard solution: 0.5 mg/mL of USP Ipratropium Bromide RS in *Mobile phase*

Sample solution: 0.5 mg/mL of Ipratropium Bromide in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 5 µL

System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for ipratropium related compound C and ipratropium are about 0.7 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 4 between ipratropium related compound C and ipratropium
Tailing factor: NMT 2.5 for the ipratropium peak
Relative standard deviation: NMT 1% for ipratropium

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ipratropium bromide ($C_{20}H_{30}BrNO_3$) in the portion of Ipratropium Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Ipratropium Bromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Ipratropium Bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **LIMIT OF IPRATROPIUM RELATED COMPOUND A**

Standard stock solution: 0.05 mg/mL of USP Ipratropium Bromide Related Compound A RS in methanol

Standard solution A: 0.02 mg/mL of USP Ipratropium Bromide Related Compound A RS in methanol from the *Standard stock solution*

Standard solution B: 0.01 mg/mL of USP Ipratropium Bromide Related Compound A RS in methanol from the *Standard stock solution*

Standard solution C: 0.005 mg/mL of USP Ipratropium Bromide Related Compound A RS in methanol from the *Standard stock solution*
[NOTE—These solutions correspond to 0.1% (*Standard solution A*), 0.05% (*Standard solution B*), and 0.025% (*Standard solution C*), respectively, of the *Sample solution* concentration.]

Sample solution: 20 mg/mL of Ipratropium Bromide in methanol

Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Mode: TLC

www.webofpharma.com

Adsorbent: A suitable HPTLC silica gel mixture
Application volume: 1 µL
Developing solvent system: Methylene chloride, dehydrated alcohol, water, and formic acid (18:18:3:1)
Spray reagent 1: Dragendorff's TS
Spray reagent 2: Sodium nitrite solution (5 in 100)

Analysis
Samples: *Standard solutions* and *Sample solution*
Develop the plate for 15 min, then remove from the tank and dry at 60° for 15 min. Spray the plate with *Spray reagent 1*, and allow to dry briefly. Spray the plate with *Spray reagent 2* and immediately cover with a glass plate. The R_F values for ipratropium bromide related compound A and ipratropium bromide are about 0.15 and 0.36, respectively.

Acceptance criteria: Any spot of the *Sample solution*, except for the principal spot, is not more intense than the spot from *Standard solution A* (0.1%).

• **ORGANIC IMPURITIES**
Solution A, Buffer, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
System suitability solution: 0.03 mg/mL of USP Ipratropium Bromide RS and 0.01 mg/mL of USP Ipratropium Bromide Related Compound B RS in *Mobile phase*
Standard solution: 0.03 mg/mL of USP Ipratropium Bromide RS in *Mobile phase*
Sample solution: 10 mg/mL of Ipratropium Bromide in *Mobile phase*
System suitability
[NOTE—See *Table 1* for the relative retention times.]
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 4 between ipratropium and ipratropium related compound B
Tailing factor: NMT 2.5 for the ipratropium peak
Relative standard deviation: NMT 5% for ipratropium
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Ipratropium Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of ipratropium from the *Standard solution*
C_S = concentration of USP Ipratropium Bromide RS in the *Standard solution* (mg/mL)
C_U = concentration of Ipratropium Bromide in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard the bromide counterion peak eluting at a relative retention time of about 0.1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ipratropium related compound C	0.7	3.8	0.10
Ipratropium bromide	1.0	1.0	—
Ipratropium related compound B [(8*s*)-ipratropium bromide]	1.3	1.0	0.10
N-Isopropylnoratropinium bromide[a]	2.3	1.0	0.10
Apo-ipratropium bromide[b]	5.1	2.0	0.10
Any unspecified impurity	—	—	0.10
Total impurities	—	—	0.25

[a] (1*R*,3*r*,5*S*)-8-(1-Methylethyl)-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-3-hydroxy-2-phenylpropanoate.
[b] (1*R*,3*r*,5*S*,8*r*)-8-Methyl-8-(1-methylethyl)-3-[(2-phenylpropenoyl)oxy]-8-azoniabicyclo[3.2.1]octane.

SPECIFIC TESTS
• **pH** ⟨791⟩
Sample solution: 100 mg/mL in carbon dioxide-free water prepared as follows. Dissolve 2.0 g in carbon dioxide-free water while heating to about 50°. Cool, and dilute with carbon dioxide-free water to 20.0 mL.
Acceptance criteria: 5.0–7.0
• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** 3.9%–4.4%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ipratropium Bromide RS
USP Ipratropium Bromide Related Compound A RS
(1*R*,3*r*,5*S*,8*r*)-3-Hydroxy-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane, bromide.
$C_{11}H_{22}BrNO$ 264.20
USP Ipratropium Bromide Related Compound B RS
(1*R*,3*r*,5*S*,8*s*)-3-[[(2*RS*)-3-Hydroxy-2-phenylpropanoyl]oxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane, bromide.
$C_{20}H_{30}BrNO_3$ 412.36
USP Ipratropium Bromide Related Compound C RS
(2*RS*)-3-Hydroxy-2-phenylpropanoic acid.
$C_9H_{10}O_3$ 166.17

Ipratropium Bromide and Albuterol Sulfate Inhalation Solution

DEFINITION
Ipratropium Bromide and Albuterol Sulfate Inhalation Solution is an isotonic sterile solution of ipratropium bromide and albuterol sulfate. It may contain chelating agents, isotonicity agents and pH adjusting agents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ipratropium bromide ($C_{20}H_{30}BrNO_3$) and albuterol ($C_{13}H_{21}NO_3$) as albuterol sulfate.

IDENTIFICATION
• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

USP Monographs

www.webofpharma.com

• **B.** The UV spectra of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 2.7 g/L of monobasic potassium phosphate in water

Solution A: Acetonitrile and *Buffer* (5:95). [NOTE—The pH of the solution is about 4.7.]

Solution B: Acetonitrile and *Buffer* (40:60). [NOTE—The pH of the solution is about 5.4.]

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
30	50	50
35	100	0
40	100	0

System suitability solution: 0.1 mg/mL of USP Ipratropium Bromide RS, 0.5 mg/mL of USP Albuterol Sulfate RS, and 0.05 mg/mL each of USP Ipratropium Bromide Related Compound B RS and USP Ipratropium Bromide Related Compound C RS in water

Standard solution: 0.04 mg/mL of USP Ipratropium Bromide RS and 0.25 mg/mL of USP Albuterol Sulfate RS in water

Sample solution: Nominally 0.04 mg/mL of ipratropium bromide and 0.25 mg/mL of albuterol sulfate from the pooled contents of NLT 20 vials of Inhalation Solution prepared as follows. Transfer a suitable volume of the pooled composite Inhalation Solution to a suitable volumetric flask. Dilute with water to obtain the final nominal concentration.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm. For *Identification B*, use a diode array detector in the range of 200–300 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 15°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times of the components relative to ipratropium are shown in *Table 2*.]

Table 2

Component	Relative Retention Time
Albuterol	0.3
Ipratropium related compound C	0.7
Ipratropium	1.0
Ipratropium related compound B	1.05

Suitability requirements

Resolution: NLT 2.0 between ipratropium and ipratropium related compound B, *System suitability solution*

Tailing factor: NMT 2.0 for the albuterol and ipratropium peaks, *Standard solution*

Relative standard deviation: NMT 1.0% for the albuterol and ipratropium peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ipratropium bromide ($C_{20}H_{30}BrNO_3$) in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ipratropium from the *Sample solution*

r_S = peak response of ipratropium from the *Standard solution*

C_S = concentration of USP Ipratropium Bromide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ipratropium bromide in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of albuterol ($C_{13}H_{21}NO_3$) in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times N \times 100$$

r_U = peak response of albuterol from the *Sample solution*

r_S = peak response of albuterol from the *Standard solution*

C_S = concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of albuterol in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of albuterol, 239.31

M_{r2} = molecular weight of albuterol sulfate, 576.70

N = number of moles of albuterol per mole of albuterol sulfate, 2

Acceptance criteria

Ipratropium bromide: 90.0%–110.0%

Albuterol: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A: Dissolve 2.7 g of monobasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 3.9.

Solution B: Acetonitrile

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	95	5
3	95	5
40	84	16
60	60	40
75	60	40
77	95	5
85	95	5

www.webofpharma.com

System suitability solution: 0.18 mg/mL of USP Ipratropium Bromide RS, 1 mg/mL of USP Albuterol Sulfate RS, and 0.005 mg/mL each of USP Levalbuterol Related Compound D RS, USP Ipratropium Bromide Related Compound B RS, and USP Ipratropium Bromide Related Compound C RS in water

Standard solution: 0.9 µg/mL of USP Ipratropium Bromide RS and 5 µg/mL of USP Albuterol Sulfate RS in water

Sample solution: Nominally 170 µg/mL of ipratropium bromide and 833 µg/mL of albuterol prepared by pooling the contents of 10 vials of Inhalation Solution into a suitable vessel. Shake well and inject as such.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 50 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 4* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between ipratropium related compound C and ipratropium; NLT 2.0 between ipratropium and ipratropium related compound B, *System suitability solution*
Tailing factor: NMT 2.0 for the albuterol and ipratropium peaks, *Standard solution*
Relative standard deviation: NMT 5.0% for albuterol and ipratropium, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levalbuterol related compound D in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times N \times 100$$

r_U = peak response of levalbuterol related compound D from the *Sample solution*
r_S = peak response of albuterol from the *Standard solution*
C_S = concentration of USP Albuterol Sulfate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of albuterol in the *Sample solution* (µg/mL)
F = relative response factor of the corresponding degradation product from *Table 4*
M_{r1} = molecular weight of albuterol, 239.31
M_{r2} = molecular weight of albuterol sulfate, 576.70
N = number of moles of albuterol per mole of albuterol sulfate, 2

Calculate the percentage of atropic acid and ipratropium related compound C in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*
r_S = peak response of ipratropium from the *Standard solution*
C_S = concentration of USP Ipratropium Bromide RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of ipratropium bromide in the *Sample solution* (µg/mL)
F = relative response factor of the corresponding degradation product from *Table 4*

Calculate the percentage of each unspecified degradation product in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 4*. The reporting threshold is 0.1%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bromide[a]	0.07	—	—
Albuterol	0.24	—	—
Albuterol related compound A[b, c]	0.59	—	—
Levalbuterol related compound D	0.70	2.5	0.1
Albuterol related compound E[c, d]	0.83	—	—
Ipratropium related compound C	0.91	2.5	0.50
Ipratropium	1.0	—	—
Ipratropium related compound B[c]	1.10	—	—
Apo-ipratropium[c, e]	1.58	—	—
Levalbuterol related compound F[c, f]	1.60	—	—
Atropic acid[g]	1.64	3.7	0.50
Any individual unspecified degradation product	—	—	0.20
Total degradation products	—	—	1.0

[a] Counter ion of ipratropium bromide; not to be included in total degradation products.
[b] 4-{2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl}-2-methylphenol.
[c] Process impurity; controlled in the drug substance.
[d] 2,2′-Oxybis(methylene)bis{4-[2-(*tert*-butylamino)-1-hydroxyethyl]phenol}; also known as 1,1′[Oxybis[methylene(4-hydroxy-1,3-phenylene)]]bis[2-[(1,1-dimethylethyl)amino]ethanol].
[e] (1*R*,3*r*,5*S*,8*r*)-8-Isopropyl-8-methyl-3-[(2-phenylacryloyl)oxy]-8-azabicyclo[3.2.1]octan-8-ium; also known as (1*R*,3*r*,5*S*,8*r*)-8-Methyl-8-(1-methylethyl)-3-[(2-phenylpropenoyl)oxy]-8-azoniabicyclo[3.2.1]octane.
[f] 1-[4-(Benzyloxy)-3-(hydroxymethyl)phenyl]-2-(*tert*-butylamino)ethanol; also known as α-{[(1,1-Dimethylethyl)amino]methyl}-4-(phenylmethoxy)-1,3-benzenedimethanol or (1*RS*)-2-[(1,1-Dimethylethyl)amino]-1-[4-(benzyloxy)-3-(hydroxymethyl)phenyl]ethanol.
[g] 2-Phenylacrylic acid.

SPECIFIC TESTS

- **STERILITY TESTS** ⟨71⟩: Meets the requirements
- **pH** ⟨791⟩: 3.4–4.5
- **PARTICULATE MATTER**
(See *Particulate Matter in Injections* ⟨788⟩, *Method 1 Light Obscuration Particle Count Test.*)
Sample: Pool the contents of NLT 20 units.
Acceptance criteria: See *Table 5*.

www.webofpharma.com

Table 5

Particle Size (µm)	Limit, NMT (particles/container)
≥10	6000
≥25	600

• **OSMOLALITY AND OSMOLARITY** ⟨785⟩: 278–349 mOsmol/kg

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Protect from light. Store in pouch until the time of use. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Albuterol Sulfate RS

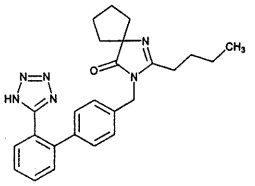
USP Ipratropium Bromide RS

USP Ipratropium Bromide Related Compound B RS
(1*R*,3*r*,5*S*,8*s*)-3-[[(2*RS*)-3-Hydroxy-2-phenylpropanoyl]oxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane bromide;
Also known as (±)-(*endo,anti*)-3-(3-Hydroxy-1-oxo-2-phenylpropoxy)-8-isopropyl-8-methyl-8-azoniabicyclo[3.2.1]octane bromide.
$C_{20}H_{30}BrNO_3$ 412.36

USP Ipratropium Bromide Related Compound C RS
(2*RS*)-3-Hydroxy-2-phenylpropanoic acid.
$C_9H_{10}O_3$ 166.17

USP Levalbuterol Related Compound D RS
5-[2-{(1,1-Dimethylethyl)amino}-1-hydroxyethyl]-2-hydroxy-benzaldehyde sulfate;
Also known as 5-(2-(*tert*-Butylamino)-1-hydroxyethyl)-2-hydroxybenzaldehyde sulfate.
$(C_{13}H_{19}NO_3)_2 \cdot H_2SO_4$ 572.67

Irbesartan



$C_{25}H_{28}N_6O$ 428.53

1,3-Diazaspiro[4.4]non-1-en-4-one, 2-butyl-3-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-;
2-Butyl-3-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one [138402-11-6].

DEFINITION

Irbesartan contains NLT 98.0% and NMT 102.0% of irbesartan ($C_{25}H_{28}N_6O$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Phosphoric acid and water (v/v) (5.5: 950). Adjust with triethylamine to a pH of 3.2.

Mobile phase: Acetonitrile and *Buffer* (330:670)

System suitability solution: 0.05 mg/mL each of USP Irbesartan RS and USP Irbesartan Related Compound A RS in methanol

Standard solution: 0.5 mg/mL of USP Irbesartan RS in methanol

Sample solution: 0.5 mg/mL of Irbesartan in methanol

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.0-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for irbesartan related compound A and irbesartan are 0.8 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between irbesartan and irbesartan related compound A, *System suitability solution*
Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of irbesartan ($C_{25}H_{28}N_6O$) in the portion of Irbesartan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of irbesartan from the *Sample solution*
r_S = peak response of irbesartan from the *Standard solution*
C_S = concentration of USP Irbesartan RS in the *Standard solution* (mg/mL)
C_U = concentration of Irbesartan in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **LIMIT OF AZIDE**

Mobile phase: 0.1 N sodium hydroxide solution
Standard stock solution: 0.25 mg/mL of sodium azide in *Mobile phase*
Standard solution: 0.312 µg/mL of sodium azide in *Mobile phase*, from the *Standard stock solution*
Sample solution: 20 mg/mL of Irbesartan in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Conductimetric with a suitable background suppressor unit
Column: 4.0-mm × 25-cm; L31 packing
Flow rate: 1 mL/min
Injection volume: 200 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Signal-to-noise ratio: NLT 10 for the azide peak
Analysis
Samples: *Standard solution* and *Sample solution*

www.webofpharma.com

Calculate the amount of azide, in ppm, in the portion of Irbesartan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times F$$

r_U = peak area of azide from the *Sample solution*
r_S = peak area of azide from the *Standard solution*
C_S = concentration of sodium azide in the *Standard solution* (µg/mL)
C_U = concentration of Irbesartan in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of azide, 42.02
M_{r2} = molecular weight of sodium azide, 65.01
F = unit conversion factor, 1000

Acceptance criteria: NMT 10 ppm

• **ORGANIC IMPURITIES**

Buffer and **Mobile phase:** Prepare as directed in the *Assay*.
Standard solution: Use the *System suitability solution*, prepared as directed in the *Assay*.
Sample solution: 1 mg/mL of Irbesartan in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.0-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of irbesartan related compound A in the portion of Irbesartan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of irbesartan related compound A from the *Sample solution*
r_S = peak response of irbesartan related compound A from the *Standard solution*
C_S = concentration of USP Irbesartan Related Compound A RS in the *Standard solution* (mg/mL)
C_U = concentration of Irbesartan in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Irbesartan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other impurity from the *Sample solution*
r_S = peak response of Irbesartan from the *Standard solution*
C_S = concentration of USP Irbesartan RS in the *Standard solution* (mg/mL)
C_U = concentration of Irbesartan in the *Sample solution* (mg/mL)

Acceptance criteria
Irbesartan related compound A: NMT 0.2%
Any other impurity: NMT 0.1%
Total impurities: NMT 0.5%

SPECIFIC TESTS

• **WATER DETERMINATION,** *Method I* ⟨921⟩**:** NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature below 30°.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Irbesartan RS
USP Irbesartan Related Compound A RS
1-Pentanoylamino-cyclopentanecarboxylic acid [2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amide.
$C_{25}H_{30}N_6O_2$ 446.54

Irbesartan Tablets

DEFINITION

Irbesartan Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of irbesartan ($C_{25}H_{28}N_6O$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
Sample: Add 10 mL of methanol to 1 Tablet, and sonicate for 10 min. Pass the solution through a glass microfiber membrane filter of 0.45-µm pore size. Evaporate to dryness, using a stream of nitrogen. Mix approximately 1 mg of the residue and approximately 250 mg of potassium bromide to obtain a homogenous mixture.
Acceptance criteria: The IR absorption spectrum of the *Sample* exhibits maxima only at the same wavelengths as that of a similarly prepared standard using USP Irbesartan RS.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dilute 5.5 mL of phosphoric acid in approximately 950 mL of water. Adjust by adding triethylamine, slowly and dropwise, to a pH of 3.0, and dilute with water to 1 L.
Mobile phase: Acetonitrile and *Buffer* (400:600)
System suitability solution: 0.1 mg/mL each of USP Irbesartan RS and USP Irbesartan Related Compound A RS in methanol
Standard solution: 0.15 mg/mL of USP Irbesartan RS in methanol
Sample solution: Nominally 0.15 mg/mL of irbesartan in methanol prepared as follows. To a suitable amount of irbesartan from NLT 5 powdered Tablets add methanol to fill about 75% of the volume of the flask. Sonicate for 15 min, stirring at 5-min intervals. Dilute with methanol to volume, and pass through a glass microfiber membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 15 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between irbesartan and irbesartan related compound A, *System suitability solution*

USP Monographs

www.webofpharma.com

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of irbesartan ($C_{25}H_{28}N_6O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of irbesartan from the *Sample solution*

r_S = peak response of irbesartan from the *Standard solution*

C_S = concentration of USP Irbesartan RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of irbesartan in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 1000 mL

Apparatus 2: 50 rpm

Time: 20 min

Standard solution: USP Irbesartan RS in *Medium*

Sample solution: Sample per *Dissolution* ⟨711⟩. Pass the *Sample solution* through a suitable acrylic copolymer on a nylon support filter[1] of 0.45-µm pore size, and dilute with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 244 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of irbesartan ($C_{25}H_{28}N_6O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of irbesartan from the *Sample solution*

A_S = absorbance of irbesartan from the *Standard solution*

C_S = concentration of USP Irbesartan RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (Q) of the labeled amount of irbesartan ($C_{25}H_{28}N_6O$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity

r_T = sum of the responses of all the peaks

Acceptance criteria

Irbesartan related compound A: NMT 0.2%

Any other impurity: NMT 0.2%

Total impurities: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Irbesartan RS

USP Irbesartan Related Compound A RS

1-Pentanoylamino-cyclopentanecarboxylic acid [2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amide.

$C_{25}H_{30}N_6O_2$ 446.54

Irbesartan and Hydrochlorothiazide Tablets

DEFINITION

Irbesartan and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of irbesartan ($C_{25}H_{28}N_6O$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)

Sample solution: Transfer the contents of 1 Tablet, previously crushed with a mortar and pestle, to a suitable vial. Add 5 mL of acetone, sonicate for 10 min, and pass through a membrane filter of 0.45-µm pore size. Evaporate the filtrate to dryness.

• **B.** The relative retention times of the major peaks from the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 1.36 g of monobasic potassium phosphate in 900 mL of water, add 2 mL of triethylamine, and adjust with phosphoric acid to a pH of 3.0 ± 0.1. Dilute further with water to 1 L.

Mobile phase: Acetonitrile, methanol, and *Buffer* (13:20:67)

Acidified water: Adjust with phosphoric acid (or sodium hydroxide, if necessary) to a pH of 2.0 ± 0.1.

Extraction solution: Methanol and *Acidified water* (7:3)

Irbesartan standard stock solution: Dissolve USP Irbesartan RS in a suitable volumetric flask in methanol (1/5 of the volume of the flask), and dilute with *Extraction solution* to prepare a 0.6 mg/mL solution. Sonicate for 2 min.

Hydrochlorothiazide standard stock solution: Dissolve USP Hydrochlorothiazide RS in methanol (1/20 of the volume of the flask), and dilute with *Extraction solution* to prepare a 0.1 mg/mL solution. Sonicate for 2 min.

Irbesartan related compound A standard stock solution: 0.1 mg/mL of USP Irbesartan Related Compound A RS in methanol. Sonicate for 2 min.

[1] A suitable filter is Acrodisc, manufactured by Gelman Sciences and distributed by Pall Corp. (www.pall.com).

www.webofpharma.com

Benzothiadiazine related compound A standard stock solution: 0.05 mg/mL of USP Benzothiadiazine Related Compound A RS in methanol. Sonicate for 2 min.
Standard solution: 0.24 mg/mL of irbesartan and 0.02 mg/mL of hydrochlorothiazide from the *Irbesartan standard stock solution* and the *Hydrochlorothiazide standard stock solution* in the *Extraction solution*
System suitability solution: Prepare 0.05 mg/mL of USP Irbesartan RS, 0.005 mg/mL of USP Hydrochlorothiazide RS, 1.0 µg/mL of USP Irbesartan Related Compound A RS, and 3.0 µg/mL of USP Benzothiadiazine Related Compound A RS in the *Extraction solution* from the respective Standard stock solutions
Sample stock solution: 0.75 mg/mL of irbesartan from NLT 5 Tablets in a suitable volumetric flask. Add *Acidified water* up to 30% of the volume of the flask, and sonicate until the Tablets disintegrate. Add methanol to fill the flask up to 90% of the total volume. Sonicate for 5 min, and stir. Dilute with methanol to volume, and pass through a filter of 0.45-µm pore size.
Sample solution: 0.225 mg/mL of irbesartan in the *Extraction solution* from the *Sample stock solution*. [NOTE—The hydrochlorothiazide concentration may vary depending on the ratio of irbesartan to hydrochlorothiazide in the Tablet.]
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L10
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.7 between irbesartan and irbesartan related compound A; NLT 1.7 between hydrochlorothiazide and benzothiadiazine related compound A, *System suitability solution*
Relative standard deviation: NMT 1.5% for both the irbesartan and hydrochlorothiazide peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{25}H_{28}N_6O$ and $C_7H_8ClN_3O_4S_2$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of irbesartan or hydrochlorothiazide from the *Sample solution*
r_S = peak area of irbesartan or hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Irbesartan RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of irbesartan or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: 0.1 N hydrochloric acid; 1000 mL
Apparatus 2: 50 rpm
Time: 30 min
pH 3.0 phosphate buffer: 1.36 g/L of monobasic potassium phosphate in water. Adjust with 10% phosphoric acid to a pH of 3.0 ± 0.1. This solution is stable for 3 months.
Mobile phase: *pH 3.0 phosphate buffer*, methanol, and acetonitrile (45:35:20)
Irbesartan standard stock solution: Transfer 50 mg of USP Irbesartan RS to a 100-mL volumetric flask. Add 15 mL of methanol, and sonicate for 5 min. Dilute with *Medium* to volume. This solution is stable for 14 days when stored at 4°.
Hydrochlorothiazide standard stock solution: Transfer 20 mg of USP Hydrochlorothiazide RS to a 200-mL volumetric flask. Add 5 mL of methanol, and sonicate for 5 min. Dilute with *Medium* to volume. This solution is stable for 14 days when stored at 4°.
Standard solution: Prepare on day of use dilutions of the *Irbesartan standard stock solution* and *Hydrochlorothiazide standard stock solution* in *Medium* as directed in the table below:

Label Claim of Irbesartan/Hydrochlorothiazide (mg/Tablet)	Volume of the Irbesartan standard stock solution (mL)	Volume of the Hydrochlorothiazide standard stock solution (mL)	Final Volume (mL)
75/12.5	15	12.5	100
150/12.5	30	12.5	100
300/12.5	60	12.5	100
300/25	60	25.0	100

System suitability solution: Transfer 10 mg of USP Irbesartan Related Compound A RS to a 100-mL volumetric flask. Add 5 mL of methanol, and sonicate to dissolve. Dilute with *Medium* to volume. Transfer 10.0 mL of this solution, 5.0 mL of the *Irbesartan standard stock solution*, and 12.5 mL of the *Hydrochlorothiazide standard stock solution* to a 100-mL volumetric flask. Dilute with *Medium* to volume.
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first few mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 272 nm
Column: 4.6-mm × 25-cm; 5-µm packing L10
Column temperature: 40°
Flow rate: 1.4 mL/min
Injection volume: 25 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between irbesartan and irbesartan related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0% for both the irbesartan and hydrochlorothiazide peaks, *Standard solution*
Calculate the percentage of irbesartan and hydrochlorothiazide dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response for irbesartan or hydrochlorothiazide from the *Sample solution*
r_S = peak response for irbesartan or hydrochlorothiazide from the *Standard solution*
C_S = concentration of irbesartan or hydrochlorothiazide in the *Standard solution*

www.webofpharma.com

L = label claim for irbesartan or hydrochlorothiazide (mg/Tablet)
V = volume of *Medium* (mL), 1000

Tolerances: NLT 80% (Q) of the labeled amounts of irbesartan ($C_{25}H_{28}N_6O$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

IMPURITIES

ORGANIC IMPURITIES

• **Procedure**

Buffer, Mobile phase, Acidified water, Extraction solution, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of irbesartan related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of irbesartan related compound A from the *Sample solution*
r_S = peak response of irbesartan from the *Standard solution*
C_S = concentration of USP Irbesartan RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of irbesartan in the *Sample solution* (mg/mL)

Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 1/F \times 100$$

r_U = peak response of benzothiadiazine related compound A from the *Sample solution*
r_S = peak response of hydrochlorothiazide from the *Standard solution*
C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)
F = relative response factor (see *Impurity Table 1*)

Calculate the percentage of any other individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each other impurity in the *Sample solution*
r_T = sum of the peak responses excluding hydrochlorothiazide and benzothiadiazine related compound A from the *Sample solution*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 1.5% (sum of all the individual unknown impurities, irbesartan related compound A, and benzothiadiazine related compound A)

Impurity Table 1

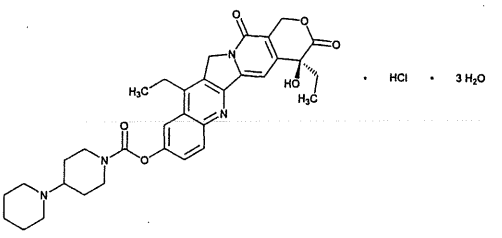
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A	0.15	1.3	1.0
Hydrochlorothiazide	0.18	—	—
Irbesartan related compound A	0.86	1.0	0.3
Irbesartan	1.00	—	—
Any other individual, unidentified impurity	—	1.0	0.2

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Hydrochlorothiazide RS
USP Irbesartan RS
USP Irbesartan Related Compound A RS
1-Pentanoylamino-cyclopentanecarboxylic acid [2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amide.
$C_{25}H_{30}N_6O_2$ 446.54
USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
$C_6H_8ClN_3O_4S_2$ 285.73

Irinotecan Hydrochloride



$C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$ Anhydrous: 623.14 Trihydrate: 677.18
[1,4′-Bipiperidine]-1′-carboxylic acid, 4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1*H*-pyrano[3′,4′:6,7] indolizino[1,2-*b*]quinolin-9-yl ester, monohydrochloride, trihydrate, (*S*)-;
(+)-7-Ethyl-10-hydroxycamptothecine 10-[1,4′-bipiperidine]-1′-carboxylate, monohydrochloride, trihydrate [136572-09-3].

DEFINITION

Irinotecan Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{33}H_{38}N_4O_6 \cdot HCl$, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to the irinotecan (*S*-enantiomer) peak in the *Identification solution*, as obtained in the test for *Limit of Irinotecan Hydrochloride Enantiomer*.

• **C. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩: A 2-mg/mL solution meets the requirements of the tests.

USP Monographs

www.webofpharma.com

ASSAY

• **PROCEDURE**

Solution A: 2.8 g/L of monobasic sodium phosphate monohydrate and 1.8 g/L of 1-octanesulfonic acid sodium salt monohydrate in water

Mobile phase: Acetonitrile, methanol, and *Solution A* (17:24:59)

Diluent: Use *Mobile phase* adjusted with diluted hydrochloric acid to a pH of 3.65 ± 0.15.

Standard solution: 1 mg/mL of USP Irinotecan Hydrochloride RS in *Diluent*

Sample solution: 1 mg/mL of Irinotecan Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 255 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection size: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of irinotecan hydrochloride ($C_{33}H_{38}N_4O_6 \cdot HCl$) in the portion of Irinotecan Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Irinotecan Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Irinotecan Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%

• **LIMIT OF IRINOTECAN HYDROCHLORIDE ENANTIOMER**

Mobile phase: Hexane, dehydrated alcohol, and diethylamine (250:250:1)

Diluent: Dehydrated alcohol and diethylamine (250:1)

System suitability solution: 0.1 mg/mL each of USP Irinotecan Hydrochloride RS and USP Irinotecan Related Compound D RS in *Diluent*

Identification solution: 1 mg/mL of USP Irinotecan Hydrochloride RS in *Diluent*. [NOTE—This solution is used for *Identification* test *B*.]

Standard solution: 1.5 µg/mL of USP Irinotecan Related Compound D RS in *Diluent*

Sensitivity solution: 0.5 µg/mL of USP Irinotecan Related Compound D RS in *Diluent*, from the *Standard solution*

Sample solution: 1 mg/mL of Irinotecan Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 370 nm

Column: 4.6-mm × 25-cm; 10-µm packing L40

Flow rate: 1.0 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—The relative retention times for irinotecan related compound D (*R*-enantiomer) and irinotecan (*S*-enantiomer) are 0.7 and 1.00, respectively.]

Suitability requirements

Resolution: NLT 2.5 between irinotecan related compound D and irinotecan, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Sensitivity: The irinotecan related compound D peak should be visible, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of irinotecan hydrochloride *R*-enantiomer in the portion of Irinotecan Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of irinotecan related compound D from the *Sample solution*

r_S = peak area of irinotecan related compound D from the *Standard solution*

C_S = concentration of USP Irinotecan Related Compound D RS in the *Standard solution* (mg/mL)

C_U = concentration of Irinotecan Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria

***R*-enantiomer:** NMT 0.15%

[NOTE—On the basis of the synthetic route, perform either *Organic Impurities Procedure 1* or *Organic Impurities Procedure 2*.]

• **ORGANIC IMPURITIES PROCEDURE 1 (FOR MATERIAL LABELED AS PRODUCED BY A SYNTHETIC PROCESS)**

Mobile phase, Diluent, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability stock solution: 0.01 mg/mL each of USP Irinotecan Related Compound B RS and USP Irinotecan Related Compound C RS in methanol

System suitability solution: 1.0 µg/mL each of USP Irinotecan Related Compound B RS and USP Irinotecan Related Compound C RS in *Diluent*, from *System suitability stock solution*

Standard solution: 2.0 µg/mL of USP Irinotecan Hydrochloride RS in *Diluent*

Sensitivity solution: 0.5 µg/mL of USP Irinotecan Hydrochloride RS in *Diluent*

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.1 between irinotecan related compound B and irinotecan related compound C, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Irinotecan Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

www.webofpharma.com

r_U = peak area of each impurity from the *Sample solution*
r_S = peak area of irinotecan from the *Standard solution*
C_S = concentration of irinotecan hydrochloride in the *Standard solution* (mg/mL)
C_U = concentration of Irinotecan Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria
Individual impurities: See *Table 1*. [NOTE—Disregard any impurity peaks less than 0.05%.]

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Irinotecan related compound B	0.55	0.15
Irinotecan related compound C	0.60	0.10
Irinotecan hydrochloride	1.0	—
Any unspecified impurity	—	0.10
Total impurities	—	0.5

• **ORGANIC IMPURITIES PROCEDURE 2 (FOR MATERIAL LABELED AS PRODUCED BY A SEMI-SYNTHETIC PROCESS)**
Solution A: 2.72 g/L of monobasic potassium phosphate in water. Adjust with dilute phosphoric acid (1 in 20) to a pH of 3.5 ± 0.05.
Solution B: Acetonitrile and methanol (3:2)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
40	30	70
45	30	70
50	80	20
55	80	20

Diluent: Acetonitrile, methanol, and *Solution A* (1:1:2)
System suitability solution: 0.1 mg/mL each of USP Irinotecan Hydrochloride RS and USP Irinotecan Related Compound A RS in *Diluent*
Standard solution: 1 µg/mL of USP Irinotecan Hydrochloride RS in *Diluent*
Sample solution: 1 mg/mL of Irinotecan Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between irinotecan and irinotecan related compound A, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Irinotecan Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*
r_S = peak area of irinotecan from the *Standard solution*
C_S = concentration of USP Irinotecan Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Irinotecan Hydrochloride in the *Sample solution* (mg/mL)
F = relative response factor for each individual impurity (see *Table 3*)

Acceptance criteria
Individual impurities: See *Table 3*. [NOTE—Disregard any unspecified impurity peaks less than 0.05%.]

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
7-Desethyl irinotecan[a]	0.82	0.77	0.15
Irinotecan	1.00	—	—
Irinotecan related compound A[b]	1.15	1.4	0.15
11-Ethyl irinotecan[c]	1.27	0.63	0.15
Camptothecin[d]	1.35	1.4	0.15
Irinotecan related compound B[e]	1.50	1.3	0.15
7-Ethylcamptothecin[f]	1.76	1.2	0.15
7,11-Diethyl-10-hydroxy-camptothecin[g]	2.05	0.65	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.50

[a] (*S*)-4-Ethyl-4-hydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione-9-yl (1,4′-bipiperidine)-1′-carboxylate.
[b] (*S*)-4-Ethyl-4,9-dihydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione.
[c] (*S*)-4,8,11-Triethyl-4-hydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione-9-yl (1,4′-bipiperidine)-1′-carboxylate.
[d] (*S*)-4-Ethyl-4-hydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione.
[e] (*S*)-4,11-Diethyl-4,9-dihydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione.
[f] (*S*)-4,11-Diethyl-4-hydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione.
[g] (*S*)-4,8,11-Triethyl-4,9-dihydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione.

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩: The total aerobic microbial count does not exceed 1000 cfu/g, and the total combined molds and yeasts count does not exceed 100 cfu/g.
• **WATER DETERMINATION,** *Method I* ⟨921⟩: Between 7.0% and 9.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.

USP Monographs

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Irinotecan Hydrochloride RS
USP Irinotecan Related Compound A RS
(*S*)-4-Ethyl-4,9-dihydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-b]quinoline-3,14(4*H*,12*H*)-dione.
$C_{20}H_{16}N_2O_5$ 364.35
USP Irinotecan Related Compound B RS
(*S*)-4,11-Diethyl-4,9-dihydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione.
$C_{22}H_{20}N_2O_5$ 392.40
USP Irinotecan Related Compound C RS
11-Ethyl-4-hydroxy-4-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinolin-9-yl (1,4′-bipiperidine)-1′-carboxylate hydrochloride.
$C_{32}H_{36}N_4O_6 \cdot HCl$ 609.11
USP Irinotecan Related Compound D RS
(*R*)-9-[(1,4′-Bipiperidine)-1′-carbonyloxy]-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline hydrochloride, trihydrate.
$C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3\,H_2O$ 677.18

Irinotecan Hydrochloride Injection

DEFINITION
Irinotecan Hydrochloride Injection is a sterile solution of Irinotecan Hydrochloride in Water for Injection. It contains NTL 90.0% and NMT 110.0% of the labeled amount of irinotecan hydrochloride ($C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$).

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)
Sample solution: 4 µg/mL
Medium: Methanol
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **PROCEDURE**
Buffer: Dissolve 2 g of sodium 1-hexanesulfonate and 2 mL of triethylamine in 1 L of water.
Mobile phase: Acetonitrile and *Buffer* (34:66). Adjust with phosphoric acid to a pH of 2.5.
Standard solution: 0.04 mg/mL of USP Irinotecan Hydrochloride RS in *Mobile phase*. Sonication and shaking may be used to aid dissolution.
Sample solution: 0.04 mg/mL of irinotecan hydrochloride in *Mobile phase* from Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of irinotecan hydrochloride ($C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Irinotecan Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of irinotecan hydrochloride in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of irinotecan hydrochloride ($C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$), 677.18
M_{r2} = molecular weight of irinotecan hydrochloride, anhydrous ($C_{33}H_{38}N_4O_6 \cdot HCl$), 623.14

Acceptance criteria: 90.0%–110.0%

IMPURITIES
• **ORGANIC IMPURITIES**
Solution A: Dissolve 2 g of sodium 1-hexanesulfonate and 1 mL of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 2.5.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
20	80	20
50	65	35
63	50	50
64	80	20
70	80	20

Diluent: Acetonitrile, phosphoric acid, and *Solution A* (500:15:500)
System suitability solution: 0.2 mg/mL of USP Irinotecan Hydrochloride RS and 0.4 µg/mL of irinotecan related compound E in *Diluent*, added stepwise, if necessary. Sonication may be used to aid dissolution.
Sample solution: 0.2 mg/mL of irinotecan hydrochloride in *Diluent* from Injection
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Temperatures
Column: 55°
Sample: 15°
Injection volume: 25 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 4.0 between irinotecan and irinotecan related compound E
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Irinotecan Hydrochloride Injection taken:

www.webofpharma.com

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_T = sum of the peak areas from the *Sample solution*
F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Irinotecan related compound B[a]	0.53	0.74	0.2
Camptothecin[b, d]	0.65	—	—
Irinotecan	1.00	—	—
7-Ethylcamptothecin[c, d]	1.16	—	—
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	1.0

[a] (*S*)-4,11-Diethyl-4,9-dihydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione.
[b] (*S*)-4-Ethyl-4-hydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione. It is a process impurity and is controlled in the API monograph.
[c] Irinotecan related compound E. It is a process impurity and is controlled in the API monograph.
[d] These process impurities are included in the table for identification only and are not included in the *Total impurities*.

SPECIFIC TESTS
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.83 USP Endotoxin Units/mg of irinotecan hydrochloride
- **STERILITY TESTS** ⟨71⟩: Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **pH** ⟨791⟩: 3.0–3.8
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩: Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose vials, protected from light. Store at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be diluted with either 5% dextrose solution (USP) or 0.9% Sodium Chloride Injection (USP) prior to intravenous infusion.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Irinotecan Hydrochloride RS
  USP Irinotecan Related Compound E RS
  (*S*)-4,11-Diethyl-4-hydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione.
  $C_{22}H_{20}N_2O_4$ 376.41

Iron, Carbonyl

Fe 55.85
[7439-89-6].

DEFINITION
Carbonyl Iron is elemental iron produced by chemical decomposition of purified iron pentacarbonyl. It is a powder, composed of spherical microparticles. It contains NLT 98.0% of iron (Fe), calculated on the as-is basis.

IDENTIFICATION
- **A.**
  Analysis: Dissolve a sample in a dilute mineral acid.
  Acceptance criteria: Hydrogen is evolved, and the resulting solutions give a positive test for *Identification Tests—General* ⟨191⟩, *Iron, Ferrous Salts*.
- **B.**
  Analysis: View a sample under a microscope having a magnifying power of 500 or greater.
  Acceptance criteria: It appears as spheres built up with concentric shells. Its particle size is 45–75 µm.

ASSAY
- **PROCEDURE**
  Sample: 200 mg
  Blank: Proceed as directed in the *Analysis*, omitting use of the *Sample*.
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N ceric sulfate VS
  Endpoint detection: Potentiometric
  Electrode system: Platinum indicating electrode and a silver–silver chloride reference electrode (or an equivalent combination electrode)
  Analysis: Transfer the *Sample* into a 300-mL Erlenmeyer flask. Add 50 mL of 2 N sulfuric acid, and close the flask with a stopper containing a Bunsen valve (made by inserting a glass tube connected to a short piece of rubber tubing with a slit on the side and a glass rod inserted in the other end and arranged so that gases can escape but air cannot enter). Heat on a steam bath to completely dissolve the *Sample*. [NOTE—The solution should be clear.] Remove the flask from the steam bath, and allow the solution to cool at room temperature with the stopper in place.
  Add a stir bar and 50 mL of recently boiled and cooled water to the flask. Titrate the solution with the *Titrant* through the inflection point. Perform a blank determination.
  Calculate the percentage of iron (Fe) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual *Titrant* normality (mEq/mL)
F = equivalency factor, 55.85 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: NLT 98.0% on the as-is basis

IMPURITIES
- **ACID-INSOLUBLE SUBSTANCES**
  Sample: 1 g of Carbonyl Iron
  Analysis: Dissolve the *Sample* in 25 mL of 2 N sulfuric acid, and heat on a steam bath until the evolution of hydrogen ceases. Filter through a tared filter crucible, wash the residue with water until free from sulfate, dry at 105° for 1 h, cool to room temperature, and weigh.
  Acceptance criteria: NMT 0.2%. The residue weighs NMT 2 mg.
- **ELEMENTAL IMPURITIES,** *Procedures* ⟨233⟩
  Acceptance criteria
  Arsenic: NMT 3 µg/g
  Lead: NMT 4 µg/g
  Mercury: NMT 2 µg/g

USP Monographs

www.webofpharma.com

SPECIFIC TESTS

- **PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING,** *Test Sieves* ⟨786⟩: NLT 100% passes through a 200-mesh sieve and NLT 95% passes through a 325-mesh sieve.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Iron Dextran Injection

DEFINITION

Iron Dextran Injection is a sterile, colloidal solution of ferric hydroxide in complex with partially hydrolyzed Dextran of low molecular weight, in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of iron. It may contain NMT 0.5% of phenol as a preservative.

IDENTIFICATION

- **A.** To 1 mL of Injection on a watch glass add 2 drops of ammonium hydroxide. No precipitate is formed. Add 2 mL of hydrochloric acid, and add 2 mL of ammonium hydroxide. A brown precipitate is formed.

ASSAY

- **PROCEDURE FOR IRON**

Solution A: Transfer 2.64 g of calcium chloride dihydrate to a 1000-mL volumetric flask. Add 500 mL of water, and swirl to dissolve. Add 5.0 mL of hydrochloric acid, and dilute with water to volume.

Standard stock solution: 50 µg/mL of iron prepared as follows. Transfer 350 mg of ferrous ammonium sulfate hexahydrate to a 1000-mL volumetric flask. Add water to dissolve, dilute with water to volume, and mix.

Standard solutions: 1.0, 2.0, 3.0, 4.0, and 5.0 µg/mL of iron from *Standard stock solution* prepared as follows. To separate 100-mL volumetric flasks transfer 2.0, 4.0, 6.0, 8.0, and 10.0 mL, respectively, of *Standard stock solution*. Dilute the contents in each flask with *Solution A* to volume, and mix.

Sample stock solution: Nominally equivalent to 0.5 mg/mL of iron prepared as follows. Using a "to contain" pipet, transfer a volume of Injection, nominally equivalent to 100 mg of iron, to a 200-mL volumetric flask. Dilute with *Solution A* to volume, and mix.

Sample solution: Nominally equivalent to 4 µg/mL of iron in *Solution A* from *Sample stock solution* prepared as follows. Transfer 2.0 mL of *Sample stock solution* to a 250-mL volumetric flask, and dilute with *Solution A* to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)

Mode: Atomic absorption

Analytical wavelength: Iron emission line of 248.3 nm

Lamp: Iron hollow-cathode

Flame: Air–acetylene

Blank: *Solution A*

Analysis

Samples: *Standard solutions* and *Sample solution*

Plot the absorbance of each of the *Standard solutions* versus concentration, in µg/mL, of iron, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration of iron, in µg/mL, in the *Sample solution*, C_A.

Calculate the percentage of the labeled amount of iron in the portion of Injection taken:

$$\text{Result} = (C_A/C_T) \times 100$$

C_A = concentration of iron in the *Sample solution*, determined from the standard calibration graph (µg/mL)

C_T = nominal concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

OTHER COMPONENTS

- **ANTIMICROBIAL AGENTS—CONTENT,** *Phenol* ⟨341⟩: NMT 0.5%

IMPURITIES

- **CONTENT OF CHLORIDE**

Sample: Using a "to contain" pipet, transfer 10.0 mL of Injection to a 150-mL beaker, rinsing the pipet into the beaker with several small portions of water. Add 50 mL of water and 2 mL of nitric acid.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Potentiometric

Analysis: Titrate with *Titrant* determining the endpoint potentiometrically using silver–glass electrodes. Each mL of *Titrant* consumed is equivalent to 3.545 mg of chloride (Cl).

Acceptance criteria

For products labeled to contain 50 mg/mL of iron: 0.48%–0.68%

For products labeled to contain 75 or 100 mg/mL of iron: 0.8%–1.1%

- **NONVOLATILE RESIDUE**

Sample solution: Using a "to contain" pipet, transfer 1.0 mL of Injection onto 3–5 g of sand spread in a shallow layer in a stainless steel dish, the dish and sand having been previously dried and weighed. Rinse the pipet, with several small portions of water, onto the sand.

Analysis: Evaporate the *Sample solution* on a steam bath to dryness, continue the drying in an oven at 105° for 15 h, and weigh.

Acceptance criteria

For products labeled to contain 50 mg/mL of iron: 28.0%–32.0%

For products labeled to contain 75 mg/mL of iron: 35.0%–40.0%

For products labeled to contain 100 mg/mL of iron: 37.0%–43.0%

SPECIFIC TESTS

- **pH** ⟨791⟩: 4.5–7.0
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 0.50 USP Endotoxin Units/mg of iron
- **ACUTE TOXICITY**

Analysis: Select five mice, each weighing 18–25 g, maintained on an adequately balanced diet. Inject a dose of Injection, equivalent to 200 mg of iron/kg of body weight, into a tail vein at a rate of NMT 0.1 mL/s. Keep the mice under observation for 48 h after the injection. If none of the mice show outward symptoms of toxicity, the requirements of the test are met. If any of the mice die within the observation period, select four groups of 10 mice, each weighing 18–25 g. Inject, intravenously, all mice of one group with one of the following doses of Injection: 375, 500, 750, or 1000 mg of iron/kg of body weight. Observe the mice for 7 days, and record the number of deaths in each group. If more than 16 mice die, calculate the LD_{50} with log-doses and probits. The four doses of Injection, in mg of iron per kg of body weight, are transformed to $x_1 = 2.574$, $x_2 = 2.699$, $x_3 = 2.875$, and $x_4 = 3.000$. The probits corresponding to the number of deaths observed in each group of 10 mice are designated

www.webofpharma.com

y_1, y_2, y_3, and y_4, respectively, and are given in *Table 1* for mortalities from 10% to 90%. For observed deaths of 0 and 10 adjacent to doses giving an intermediate mortality, use the approximate probits 3.02 and 6.98, respectively; omit the end value (at x_1 or x_4) if not adjacent to an intermediate mortality. Since the information in a probit varies with its expectation, assign each probit an approximate relative weight (w) for computing the LD_{50} of the Injection, as shown in *Table 2*.

Table 2

No. of Deaths	0 or 10	1 or 9	2 or 8	3 or 7	4 to 6
Weight (w)	0.3	0.7	1.0	1.2	1.3

Calculate the weighted means:

$$\bar{x} = \Sigma(wx)/\Sigma w$$

and

$$\bar{y} = \Sigma(wy)/\Sigma w$$

from the sum of the weights, Σw, of the four (or three) acceptable responses and the corresponding weighted sums of the log-doses, $\Sigma(wx)$, and of the probits, $\Sigma(wy)$. From the sums of the weighted products, $\Sigma(wxy)$, and of the weighted squares, $\Sigma(wx^2)$, compute the slope b of the log-dose-probit line as:

$$b = [\Sigma(wxy) - \bar{x}\,\Sigma(wy)]/[\Sigma(wx^2) - \bar{x}\,\Sigma(wx)]$$

The LD_{50} for this safety test, in mg of iron per kg of body weight, is calculated as:

$$LD_{50} = \text{antilog}[\bar{x} + (5 - \bar{y})/b]$$

Acceptance criteria: NLT 500 mg of iron/kg of body weight

- **ABSORPTION FROM INJECTION SITE**

Analysis: Prepare a site over the semitendinosus muscle of one leg of each of two rabbits by clipping the fur and disinfecting the exposed skin. Inject each site with a dose of 0.4 mL/kg of body weight in the following manner. Place the needle in the distal end of the semitendinosus muscle at an angle such as to ensure that the full length of the needle is used, then pass it through the sartorius and vastus medialis muscles. House the rabbits separately. Seven days after the injection, sacrifice the rabbits, and dissect the treated legs to examine the muscles.

Acceptance criteria: No heavy black deposit of unabsorbed iron compounds is observed, and the tissue is only lightly colored.

- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.

Iron Sorbitex Injection

» Iron Sorbitex Injection is a sterile solution of a complex of iron, Sorbitol, and Citric Acid that is stabilized with the aid of Dextrin and an excess of Sorbitol. It contains not less than 94.0 percent and not more than 104.0 percent of the labeled amount of iron.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.

Labeling—Label it to indicate its expiration date, which is not more than 24 months after date of manufacture.

Identification—

A: To 1 mL of Injection add 5 mL of water and 1 mL of ammonium hydroxide: no precipitate is formed.

B: To 1 mL of Injection add 0.1 mL of 3 N hydrochloric acid and 0.5 mL of potassium ferrocyanide TS: a dark blue precipitate is formed.

C: To 1 mL of Injection in a separator add 4 mL of water and 10 mL of hydrochloric acid, and extract with three 15-mL portions of isopropyl ether. Dilute the aqueous layer with water to 25 mL, and mix. To 0.5 mL of this solution add 10 mL of water, 2 mL of 2 N sulfuric acid, and 10 mg of potassium

Table 1 (Iron Dextran Injection)

Probits (normal deviates + 5) Corresponding to Percentages in the Margins										
	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
—	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

USP Monographs

www.webofpharma.com

periodate, allow to stand for 10 minutes, and then add 10 mL of 0.1 N sodium arsenite. When the solution is colorless, add 10 mL of a 1 in 250 solution of phenylhydrazine hydrochloride in 0.5 N hydrochloric acid. Allow to stand for 10 minutes, add 1 mL of potassium ferricyanide solution (1 in 20), allow to stand for 15 minutes, and then add 3 mL of hydrochloric acid: a wine-red color is produced (*presence of sorbitol*).

D: Dilute 1 mL of Injection with 50 mL of water, and to 4 mL of this solution add 1 mL of 6 N sulfuric acid and 0.5 mL of phosphoric acid, mix, and allow to stand for about 5 minutes or until decolorized. Add 1 mL of potassium bromide solution (1 in 10) and 1 mL of potassium permanganate solution (1 in 20). After 10 minutes, add hydrogen peroxide TS, dropwise, to discharge the pink color. Transfer the solution to a small separator, shake with 20 mL of solvent hexane, discard the water layer, and wash the hexane layer with 20 mL of water. To the washed hexane solution add 5 mL of a 1 in 25 solution of thiourea in sodium borate solution (1 in 50), and shake the mixture: the aqueous layer that separates shows a yellow color (*presence of citric acid*).

Specific gravity ⟨841⟩: between 1.17 and 1.19 at 20°.

Viscosity—Capillary Methods ⟨911⟩**:** between 8 and 13 centipoises, determined at 20° with a capillary tube viscometer.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 10.0 USP Endotoxin Units per mL.

pH ⟨791⟩: between 7.2 and 7.9.

Limit of ferrous iron—Using a "to contain" pipet, transfer 10 mL of Injection to a glass-stoppered, 125-mL flask, rinsing the pipet into the flask with several small portions of water. Add 5 mL of sulfuric acid, shake vigorously until decolorization is effected, add 2 drops of orthophenanthroline TS, and immediately titrate with 0.1 N ceric sulfate VS. Each mL of 0.1 N ceric sulfate is equivalent to 5.585 mg of ferrous iron. The ferrous iron content is not more than 8.5 mg per mL of Injection.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for iron—

Standard preparation—Transfer an accurately weighed portion of ferrous ammonium sulfate, equivalent to about 100 mg of iron (Fe), to a 300-mL Kjeldahl flask, and proceed as directed under *Assay preparation*, beginning with "Add 10 mL of nitric acid," to obtain a *Standard preparation* having a known concentration of about 2 µg of iron per mL.

Assay preparation—Using a "to contain" pipet, transfer an accurately measured volume of Injection, equivalent to about 100 mg of iron, to a 300-mL Kjeldahl flask, rinsing the pipet into the flask with several small portions of water. Add 10 mL of nitric acid, 10 mL of sulfuric acid, and a few glass beads, and boil the solution gently until fumes of sulfur trioxide appear. Cool, add 3 mL of nitric acid, and heat gently again until fumes of sulfur trioxide appear. Continue the addition of nitric acid, followed by gentle boiling, until the solution is clear and light yellow or green in color, and then boil for an additional 30 minutes. Cool, cautiously add 100 mL of water, and boil gently until solution is complete. Cool, transfer the solution to a 500-mL volumetric flask, rinse the Kjeldahl flask with several small portions of water, dilute with water to volume, and mix. Transfer 5.0 mL of the solution to a second 500-mL volumetric flask, add 100 mL of water and 1 g of ascorbic acid, dilute with water to volume, and mix.

Procedure—Transfer 5.0-mL portions of the *Standard preparation*, of the *Assay preparation*, and of water to serve as the blank to separate, clean, dry test tubes, and to each add 3.0 mL of a 1 in 1500 solution of 2,2′-bipyridine in 0.6 N glacial acetic acid, mix, and allow to stand for 15 minutes. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 510 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of iron in each mL of the Injection taken by the formula:

$$(50C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of the *Standard preparation*; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and *Standard preparation*, respectively.

Iron Sucrose Injection

DEFINITION

Iron Sucrose Injection is a sterile, colloidal solution of ferric hydroxide in complex with Sucrose in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of iron. Sodium Hydroxide may be added to adjust the pH. It contains no antimicrobial agent, chelating agent, dextran, gluconate, or other added substances.

IDENTIFICATION

• **A. Iron**

To 2.5 mL of Injection add 17.5 mL of water and 5 mL of hydrochloric acid. Mix and heat the solution for 5 min in a boiling water bath. Cool, add dropwise 13.5 N ammonium hydroxide until no further precipitation of ferric hydroxide occurs, and filter. Wash the precipitate with water to remove excess ammonium hydroxide, dissolve the precipitate in a minimum volume of 2 N hydrochloric acid, and add sufficient water to make a volume of 20 mL. To 3 mL of the solution add 1 mL of 2 N hydrochloric acid and 1 mL of potassium thiocyanate TS: the resulting solution (Solution 1) is red. To 1 mL of Solution 1 add 5 mL of amyl alcohol or ethyl ether, shake, and allow to stand: the organic layer is pink. To a separate 1-mL aliquot of Solution 1 add 2 mL of mercuric chloride TS: a red color is discharged [iron (III) salts].

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Sucrose*.

• **C. Molecular Weight Determination**

Mobile phase: Dissolve 7.12 g of dibasic sodium phosphate dihydrate, 5.52 g of monobasic sodium phosphate, and 0.40 g of sodium azide in 2 L of water.

System suitability solution: Dissolve 200 mg of high molecular weight dextran and 100 mg of glucose in 20 mL of *Mobile phase*.

Standard solutions: Transfer about 20 mg of each polysaccharide molecular weight standard (5,000–400,000 Da) to separate 5-mL volumetric flasks. Add 4 mL of *Mobile phase* to each flask, and allow each aliquot to stand at or below 25° for a minimum of 12 h. After the agglomerate particles of each *Standard solution* have swelled to their fullest extent, gently swirl each *Standard solution* until dissolved.

[Note—The chromatograms of freshly prepared *Standard solutions* regularly show a small, unidentified secondary peak following the main peak. Discard the *Standard solutions* if the secondary peak reaches half the height of the main peak.]

Sample solution: Transfer 5.0 mL of Injection to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

USP Monographs

www.webofpharma.com

Detector: Refractive index, maintained at a constant temperature of 45°
Columns: Two 7.8-mm × 30-cm; packing L39 with pore sizes of 1000 and 120 Å, respectively
Column temperature: 45 ± 2°
Flow rate: 0.5 mL/min
Injection volume: 25 µL

System suitability
Samples: *System suitability solution* and *Standard solutions*
Suitability requirements
Resolution: NLT 4.0 between dextran and glucose, *System suitability solution*
Correlation coefficient: NLT 0.98 for the calibration curve generated using a suitable program, plotting the retention times of the *Standard solutions* and their molecular weights to generate a third order (cubic) calibration curve

Analysis
Samples: *System suitability solution*, *Standard solutions*, and *Sample solution*
The molecular weight of the complex is calculated from the calibration curve. The molecular weight distribution curve of the sample is sliced into fractions.
Calculate the weight-average molecular weight (M_W) as follows:

$$\text{Result} = \Sigma(A_T M_T)/\Sigma A_T$$

Calculate the number-average molecular weight (M_N) as follows:

$$\text{Result} = \Sigma(A_T)/\Sigma(A_T/M_T)$$

A_T = area of each fraction of the sample distribution
M_T = corresponding mean molecular weight of each fraction as determined from its retention time on the calibration curve

Acceptance criteria: The molecular weight distribution curve of the Injection conforms to the following parameters.
M_W: 34,000–60,000 Da
M_N: NLT 24,000 Da
M_W/M_N: NMT 1.7

ASSAY

• **SUCROSE**

Mobile phase: Acetonitrile and water (79:21)
Standard solutions: Individual solutions of 13, 16, 18, 21, and 23 mg/mL of sucrose from USP Sucrose RS, in water
Sample solution: Transfer about 1.875 g of Injection to a 25-mL flask. Add 1.25 mL of water and mix. Add 1.25 mL of a monobasic sodium phosphate solution, prepared by dissolving 30 g in 50 mL, and mix. Allow the resulting solution to stand for 10 min to precipitate the colloidal ferric hydroxide. Dilute with water to volume. Centrifuge this solution at 3000 rpm for 15 min. Pass the resulting solution through a filter, discarding the first 2 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 4-mm × 25-cm; packing L8
Temperatures
Detector: 20–25° (±2°)
Column: 20–25° (±2°)
Flow rate: 2 mL/min
Injection volume: 20 µL

System suitability
Samples: *Standard solutions*
[NOTE—The retention time for sucrose is about 8 min.]
Suitability requirements
Correlation coefficient: NLT 0.998 from the linear regression of the *Standard solutions*

Analysis
Samples: *Standard solutions* and *Sample solution*
Plot the peak area for each *Standard solution* versus concentration of sucrose in mg/mL, and draw the straight line best fitting the five plotted points. From the graph, determine the concentration of sucrose, in mg/mL, in the *Sample solution*.
Calculate the quantity of sucrose, in mg, in each mL of Injection taken:

$$\text{Result} = (C_U \times D \times G)/W$$

C_U = concentration of sucrose in the *Sample solution* (mg/mL)
D = dilution volume of the *Sample solution* (mL)
G = density of Injection taken (g/mL)
W = weight of Injection taken (g)

Acceptance criteria: 260–340 mg/mL

• **IRON**

Solution A: Transfer 2.64 g of calcium chloride to a 1000-mL volumetric flask, add 500 mL of water, and swirl to dissolve. Add 5.0 mL of hydrochloric acid, and dilute with water to volume.
Standard stock solution: 50 µg/mL of iron prepared as follows. Transfer about 350 mg of ferrous ammonium sulfate to a 1000-mL volumetric flask. Add water to dissolve, dilute with water to volume, and mix.
Standard solutions: Individual solutions containing 2.0, 4.0, 6.0, 8.0, and 10.0 µg/mL of iron in *Solution A* from the *Standard stock solution*
Sample stock solution: Using a "to contain" pipet, transfer 2.0 mL of Injection to a 100-mL volumetric flask. Rinse the pipet several times with *Solution A*. Add 5 mL of hydrochloric acid, and swirl until the solution turns yellow. After the solution has cooled to room temperature, dilute with *Solution A* to volume, and mix.
Sample solution: Nominally 8.0 µg/mL of iron prepared as follows. Pipet 2.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with *Solution A* to volume.
Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: 248.3 nm iron emission line
Lamp: Iron hollow-cathode
Flame: Air–acetylene
Blank: *Solution A*

Analysis
Samples: *Standard solutions* and *Sample solution*
Plot the absorbance of each *Standard solution* versus concentration, in µg/mL, of iron, and draw the straight line best fitting the five plotted points. From the graph, determine the concentration, in µg/mL, of iron in the *Sample solution*.
Calculate the percentage of the labeled amount of iron in each mL of Injection taken:

$$\text{Result} = (C_A/C_U) \times 100$$

C_A = actual concentration of iron in the *Sample solution* determined from the calibration curve (µg/mL)

www.webofpharma.com

C_U = nominal concentration of iron in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

OTHER COMPONENTS

- **CONTENT OF CHLORIDE**

Sample: About 12 g of Injection

Analysis: Transfer the *Sample* to a 50-mL beaker. Add 40 mL of water and 0.3 mL of 65% nitric acid, and, while stirring, titrate with 0.01 N silver nitrate VS, determining the endpoint potentiometrically with silver-glass electrodes. Calculate the content of chloride, in mg, of Injection taken. Each mL of 0.01 N silver nitrate consumed is equal to 0.3545 mg of chloride (Cl).

Acceptance criteria: 0.012%–0.025%

IMPURITIES

- **LIMIT OF IRON [Fe(II)]**

Supplementary electrolyte solution: Dissolve 15.0 g of sodium acetate in 100 mL of water and adjust with 0.1 N acetic acid to a pH of 7.0.

Sample solution: Volume of Injection equivalent to 20–120 µg/mL of elemental iron

Analysis: Transfer a suitable amount of *Supplementary electrolyte solution* to a polarographic cell equipped with a mercury drop electrode. With the electrode submerged in the liquid, bubble nitrogen through the liquid for 5 min. Avoiding any undue exposure to air, immediately transfer the *Sample solution* to the polarographic cell. The sample must be analyzed immediately upon opening the container.

Record the polarogram from 0 mV and –1700 mV. The iron [Fe(III)/Fe(II)] peak is detected at –750 ± 50 mV and the iron [Fe(II)/Fe(0)] peak is detected at –1400 ± 50 mV. Measure the iron [Fe(II)/Fe(III)] peak responses obtained from the polarogram, and perform a blank determination.

Calculate the content of iron [Fe(II)], in % w/v, in the volume of Injection taken:

$$\text{Result} = [1 - (2/R)] \times C_T$$

R = peak response ratio of iron [Fe(II)] to iron [Fe(III)]

C_T = total iron concentration of the Injection (% w/v)

Acceptance criteria: NMT 0.4%

SPECIFIC TESTS

- **pH** ⟨791⟩: 10.5–11.1 at 20°
- **TURBIDITY**

Sample solution: Transfer 0.5 g of Injection to a 150-mL beaker. Add 100 mL of water and, with constant stirring, adjust with 0.1 N hydrochloric acid VS to a pH of 6.0.

Analysis: Remove the pH electrode from the solution. Adjust a light source such that the beam hits the beaker at a parallel angle 2 cm below the surface of the liquid. The light must shine through to the surface, and the solution must not have any turbidity. Measurement must be carried out in a room as dark as possible. Slowly add 0.1 N hydrochloric acid VS, dropwise, until a slight but lasting turbidity develops. Record the pH of the solution as the turbidity point of the Injection.

Acceptance criteria: 4.4–5.3

- **ABSENCE OF LOW-MOLECULAR WEIGHT IRON [Fe(II) AND Fe(III)] COMPLEXES:** In the polarograms obtained in the test for *Limit of Iron [Fe(II)]*, no additional peaks are found.
- **ALKALINITY**

Sample solution: 5 mL of Injection

Analysis: Titrate the *Sample solution* with 0.1 N hydrochloric acid VS with constant stirring to a pH of 7.4. Record the volume of 0.1 N hydrochloric acid VS consumed, and calculate the alkalinity of the Injection as the volume of acid, in mL, consumed per mL of Injection.

Acceptance criteria: 0.5–0.8 mL of 0.1 N hydrochloric acid VS is consumed per mL of Injection.

- **OSMOLALITY AND OSMOLARITY** ⟨785⟩, *Osmolarity*

Sample solution: Dilute Injection in water (1 in 10).

Acceptance criteria: 1150–1350 mOsmol/L

- **SPECIFIC GRAVITY** ⟨841⟩: 1.135–1.165 at 20°
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩, *Method 1 Light Obscuration Particle Count Test*

Sample solution: Prepare a solution of Injection (1 in 40) using water that has been passed through a filter having a 1.2-µm or finer pore size.

Acceptance criteria: Meets the requirements for small-volume injections

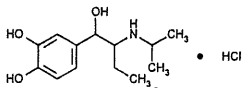
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: NMT 3.7 USP Endotoxin Units/mg of iron contained in Injection
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers of Type I glass. Store at controlled room temperature. Do not freeze.
- **LABELING:** Label it to indicate that it is for intravenous use only, and that when administered by intravenous infusion, the Injection must be diluted with 0.9% Sodium Chloride Injection to a concentration of 1.0–2.0 mg/mL of elemental iron. Label it also to state the total osmolarity of the solution expressed in mOsmol/L.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Sucrose RS

Isoetharine Hydrochloride



$C_{13}H_{21}NO_3 \cdot HCl$ 275.77

1,2-Benzenediol, 4-[1-hydroxy-2-[(1-methylethyl)amino] butyl]-, hydrochloride.

3,4-Dihydroxy-α-[1-(isopropylamino)propyl]benzyl alcohol hydrochloride [2576-92-3].

» Isoetharine Hydrochloride contains not less than 97.0 percent and not more than 102.0 percent of $C_{13}H_{21}NO_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—

USP Isoetharine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: A solution (1 in 100) responds to the tests for *Chloride* ⟨191⟩.

pH ⟨791⟩: between 4.0 and 5.6, in a solution (1 in 100).

Loss on drying ⟨731⟩—Dry it at 100° for 4 hours: it loses not more than 1.0% of its weight.

USP Monographs

www.webofpharma.com

Aromatic ketones—Its absorptivity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) at 312 nm, determined in a solution in 0.01 N hydrochloric acid containing 2.0 mg per mL, is not more than 0.20.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Isoetharine Hydrochloride RS in freshly prepared sodium bisulfite solution (3 in 1000) to obtain a solution having a concentration of about 5 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.17 N acetic acid to volume, and mix to obtain a solution having a known concentration of about 500 µg per mL.

Assay preparation—Transfer about 125 mg of Isoetharine Hydrochloride, accurately weighed, to a 25-mL volumetric flask, dissolve in sodium bisulfite solution (3 in 1000), dilute with sodium bisulfite solution to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.17 N acetic acid to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 278-nm detector and a 4-mm × 30-cm column that contains packing L1. The mobile phase is 0.17 N acetic acid, having a flow rate of about 2.2 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 3.0%.

Procedure—Using a microsyringe or sampling valve, chromatograph 10 µL of the *Standard preparation*, and adjust the specimen size and other operating parameters, if necessary, until satisfactory chromatography and peak responses are obtained. Chromatograph equal volumes of the *Standard preparation* and the *Assay preparation*, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of $C_{13}H_{21}NO_3 \cdot HCl$ in the portion of Isoetharine Hydrochloride taken by the formula:

$$0.25C(h_U/h_S)$$

in which C is the concentration, in µg per mL, of USP Isoetharine Hydrochloride RS in the *Standard preparation;* and h_U and h_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Isoetharine Inhalation Solution

» Isoetharine Inhalation Solution is a sterile solution of Isoetharine Hydrochloride in Purified Water. It may contain Sodium Chloride. It contains not less than 92.0 percent and not more than 108.0 percent of the labeled amount of isoetharine hydrochloride ($C_{13}H_{21}NO_3 \cdot HCl$).

Packaging and storage—Preserve in small, tight containers that are well-filled or otherwise protected from oxidation. Protect from light.

Labeling—The label indicates that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

USP Reference standards ⟨11⟩—
USP Isoetharine Hydrochloride RS

Color and clarity—

Standard solution—Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Visually examine a portion of the Inhalation Solution *(Test solution)* in a suitable clear glass test tube against a white background: it is not pinkish and it contains no precipitate. If any yellow color is observed in the *Test solution*, concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm: the absorbance of the *Test solution* does not exceed that of the *Standard solution*.

Identification—Dilute the Inhalation Solution with water to obtain a solution containing about 2.5 mg of isoetharine hydrochloride per mL. Apply 10-µL portions of this solution and a solution of USP Isoetharine Hydrochloride RS containing 2.5 mg per mL to a thin-layer chromatographic plate coated with silica gel mixture. Develop the plate in a mixture consisting of *n*-butyl alcohol, water, and formic acid (70:20:10) to a height of 12 to 14 cm above the point of application. Remove the plate, and evaporate the solvents with the aid of warm, circulating air. Examine under short-wavelength UV light. Spray the plate with Folin–Ciocalteu phenol TS, and then expose to ammonia vapor until the isoetharine spots develop an intense blue color: the R_F value and color of the principal spot obtained from the solution under test correspond to those obtained from the *Standard solution*.

Sterility Tests ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 2.5 and 5.5.

Assay—

Standard preparation—Prepare as directed in the *Assay* under *Isoetharine Hydrochloride*.

Assay preparation—Transfer an accurately measured volume of Inhalation Solution, equivalent to about 50 mg of isoetharine hydrochloride, to a 100-mL volumetric flask, dilute with 0.17 N acetic acid solution to volume, and mix.

Chromatographic system—Proceed as directed in the *Assay* under *Isoetharine Hydrochloride*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Isoetharine Hydrochloride*. Calculate the quantity, in mg, of $C_{13}H_{21}NO_3 \cdot HCl$ in each mL of the Inhalation Solution taken by the formula:

$$0.1(C/V)(h_U/h_S)$$

in which V is the volume, in mL, of Inhalation Solution taken; and C, h_U, and h_S are as defined therein.

Isoetharine Mesylate

$C_{13}H_{21}NO_3 \cdot CH_4O_3S$ 335.42

1,2-Benzenediol, 4-[1-hydroxy-2-[(1-methylethyl)amino] butyl]-, methanesulfonate (salt).

3,4-Dihydroxy-α-[1-(isopropylamino)propyl]benzyl alcohol methanesulfonate (salt) [7279-75-6].

» Isoetharine Mesylate contains not less than 97.0 percent and not more than 102.0 percent of $C_{13}H_{21}NO_3 \cdot CH_4O_3S$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Isoetharine Hydrochloride RS

USP Monographs

www.webofpharma.com

Identification—

A: It responds to the *Thin-layer Chromatographic Identification Test* ⟨201⟩, the test solution and the Standard solution of USP Isoetharine Hydrochloride RS being prepared at a concentration of 2.5 mg per mL in methanol, the solvent mixture being *n*-butanol, water, and formic acid (64:25:11), and the spots being located by spraying with sodium hydroxide solution (1 in 10).

B: Mix about 50 mg with about 200 mg of powdered sodium hydroxide, transfer the mixture to a small test tube, heat in a small flame to fusion, and continue the heating for a few minutes longer. Cool, add about 0.5 mL of water, then add a moderate excess of hydrochloric acid, and warm: starch iodate paper placed over the mouth of the test tube turns blue.

Melting range ⟨741⟩: between 162° and 168°.

pH ⟨791⟩: between 4.5 and 5.5, in a solution (1 in 100).

Loss on drying ⟨731⟩—Dry it at 80° under vacuum at a pressure of not more than 5 mm of mercury for 4 hours: it loses not more than 1.0% of its weight.

Limit of keto precursor—Its absorptivity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) at 312 nm, determined in a solution in 0.01 N hydrochloric acid containing 2.0 mg per mL, is not more than 0.20.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 0.1 M sodium sulfate in 0.8% acetic acid. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 60 mg of USP Isoetharine Hydrochloride RS, accurately weighed, to a 25-mL volumetric flask, add 4.0 mL of alcohol, and mix. Add 3 drops of 1 N hydrochloric acid, dilute with water to volume, and mix.

Assay preparation—Transfer about 75 mg of Isoetharine Mesylate, accurately weighed, to a 25-mL volumetric flask, add 4.0 mL of alcohol, and mix. Add 3 drops of 1 N hydrochloric acid, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L9. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of $C_{13}H_{21}NO_3 \cdot CH_4O_3S$ in the portion of Isoetharine Mesylate taken by the formula:

$$0.025C(335.42/275.77)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Isoetharine Hydrochloride RS in the *Standard preparation;* 335.42 and 275.77 are the molecular weights of isoetharine mesylate and isoetharine hydrochloride, respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Delete the following:

▲Isoetharine Mesylate Inhalation Aerosol

» Isoetharine Mesylate Inhalation Aerosol is a solution of Isoetharine Mesylate in Alcohol in an inert propellant base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isoetharine mesylate ($C_{13}H_{21}NO_3 \cdot CH_4O_3S$).

Packaging and storage—Preserve in small, nonreactive, light-resistant, aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

USP Reference standards ⟨ 11 ⟩ —
USP Isoetharine Hydrochloride RS

Identification—

A: Expel a quantity of Inhalation Aerosol, equivalent to about 12 mg of isoetharine mesylate, into 2 mL of methanol, dilute with methanol to 5 mL, and mix: this solution responds to *Identification* test *A* under *Isoetharine Inhalation Solution.*

B: Expel a quantity of Inhalation Aerosol, equivalent to about 12 mg of isoetharine mesylate, into a test tube, evaporate on a steam bath just to dryness, and add 50 mg of powdered sodium hydroxide. Heat in a small flame to fusion, and continue heating for a few seconds longer. Cool, add about 0.5 mL of water, then add a moderate excess of 3 N hydrochloric acid: starch iodate paper placed over the mouth of the test tube turns blue.

Alcohol Determination ⟨611⟩ —Weigh accurately a filled Inhalation Aerosol container, and record the weight. Invert the container, and place the outlet tip against the bottom of a 50-mL beaker containing 5 mL of water. Slowly actuate the valve 10 times. Raise the unit above the contents of the beaker, and wash the outlet with 1 mL of water. Collect the washings in the beaker. Dip the outlet stem in alcohol, shake to remove the solvent completely, air-dry the valve, and again weigh the Inhalation Aerosol container. Record the weight of the expelled specimen. Transfer the contents of the beaker, with the aid of 4 mL of water, to a glass-stoppered graduated cylinder. Determine the alcohol content of the test solution thus prepared by the gas-liquid chromatographic procedure, 2 mL of dilute isopropyl alcohol (15 in 100) being used as the internal standard. Calculate the alcohol content of the Inhalation Aerosol taken by the formula:

$$SV/W$$

in which S is the percentage (w/v) of alcohol in the test solution; V is the total volume, in mL, of the test solution; and W is the weight, in g, of the expelled specimen taken: between 25.9% and 35.0% (w/w) of C_2H_5OH is found.

Dose uniformity over the entire contents: meets the requirements for *Metered-Dose Inhalers* under *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* ⟨601⟩.

PROCEDURE FOR DOSE UNIFORMITY—

Ferro-citrate solution and *Buffer solution*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Standard preparation—Dissolve an accurately weighed quantity of USP Isoetharine Hydrochloride RS in a freshly prepared sodium bisulfite solution (1 in 1000), and dilute

www.webofpharma.com

quantitatively and stepwise with the same sodium bisulfite solution as necessary to obtain a solution having a known concentration of about 34 µg per mL.

Test preparation—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with two 5.0-mL portions of a freshly prepared sodium bisulfite solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform, insert the stopper, shake vigorously for 5 minutes and centrifuge. Use the clear supernatant as directed in the *Procedure*.

Procedure—Into three separate 25-mL volumetric flasks transfer the *Test preparation*, 10.0 mL of the *Standard preparation*, and 10.0 mL of a freshly prepared sodium bisulfite solution (1 in 1000) to provide the blank. To each flask add 0.5 mL of *Ferro-citrate solution* followed by 5 mL of *Buffer solution*. Dilute with the sodium bisulfite solution (1 in 1000) to volume, mix, and allow the color to develop for 10 minutes. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation* relative to the blank at the wavelength of maximum absorbance at about 530 nm. Calculate the quantity, in µg, of $C_{13}H_{21}NO_3 \cdot CH_4O_3S$ contained in the minimum dose taken by the formula:

$$10CN(335.42 / 275.77)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Isoetharine Hydrochloride RS in the *Standard preparation*; N is the number of sprays discharged to obtain the minimum recommended dose; 335.42 and 275.77 are the molecular weights of isoetharine mesylate and isoetharine hydrochloride, respectively; and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

Assay—

Mobile phase and *Standard preparation*—Proceed as directed in the *Assay* under *Isoetharine Mesylate*.

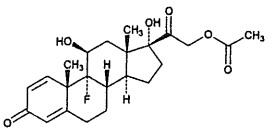
Assay preparation—Weigh 1 Inhalation Aerosol container with contents. Invert the container, and place the outlet tip against the bottom of a 50-mL beaker containing 2.5 mL of 0.01 N hydrochloric acid. Slowly actuate the valve about 90 times (the weight of the assay specimen is approximately 5 g). Raise the unit above the contents of the beaker, and wash the outlet with a few mL of water. Collect the washings in the beaker. Dip the outlet stem in alcohol, shake to remove the solvent completely, air-dry the valve, and then again weigh the Inhalation Aerosol container. Record the weight of the expelled specimen. Transfer the contents of the beaker to a 100-mL volumetric flask with the aid of water, dilute with water to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Isoetharine Mesylate*. Calculate the percentage of $C_{13}H_{21}NO_3 \cdot CH_4O_3S$ in the portion of Inhalation Aerosol taken by the formula:

$$0.01(335.42 / 275.77)(C / W)(r_U / r_S)$$

in which W is the weight, in g, of Inhalation Aerosol taken; and the other terms are as defined therein. ▲2S (USP41)

Isoflupredone Acetate



$C_{23}H_{29}FO_6$ 420.47

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-9-fluoro-11,17-dihydroxy-, (11β)-.

9-Fluoro-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione 21-acetate [338-98-7].

» Isoflupredone Acetate contains not less than 97.0 percent and not more than 103.0 percent of $C_{23}H_{29}FO_6$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

Labeling—Label it to indicate that it is intended for veterinary use only. Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩—
USP Isoflupredone Acetate RS
USP Prednisolone Acetate RS

Change to read:

▲ ***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197M***▲ (CN 1-May-2020)

Absorptivity—

Test preparation: 25 mg in 2000 mL of alcohol.

Procedure—Proceed as directed under *Ultraviolet-Visible Spectroscopy* ⟨857⟩, and measure the absorbance at 240 nm: the absorptivity is between 35.0 and 38.0.

Specific rotation ⟨781S⟩: between +110° and +120°.

Test solution: 10 mg per mL, in dioxane.

Bacterial Endotoxins Test ⟨85⟩ —Where the label states that Isoflupredone Acetate is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains not more than 125 USP Endotoxin Units per mg of isoflupredone acetate.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.5%.

Chromatographic purity—

Solution A—Prepare a mixture of water, methanol, acetonitrile, and glacial acetic acid (500:350:150:3), and degas.

Solution B—Prepare a mixture of acetonitrile, methanol, and water (550:500:3), and degas.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve accurately weighed quantities of USP Isoflupredone Acetate RS and USP Prednisolone Acetate RS in *Solution A* to obtain a solution having known concentrations of about 0.03 mg of each per mL. Sonicate, if necessary, to dissolve.

Test solution—Dissolve an accurately weighed quantity of Isoflupredone Acetate in *Solution A* to obtain a solution having

www.webofpharma.com

a concentration of about 0.3 mg per mL. Sonicate, if necessary, to dissolve. Use this solution within 16 hours.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1 mL per minute. Protect the column from temperature fluctuations. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–32.5	100	0	isocratic
32.5–47.5	100→0	0→100	linear gradient
47.5–50.5	0	100	isocratic
50.5–51.5	0→100	100→0	linear gradient
51.5–61.5	100	0	isocratic

Chromatograph the *System suitability solution,* and record the peak areas as directed for *Procedure:* the retention time for isoflupredone acetate is between 21 and 26 minutes; the relative retention times are about 1.1 for prednisolone acetate and 1.0 for isoflupredone acetate; the resolution, *R,* between isoflupredone acetate and prednisolone acetate is not less than 1.2; and the column efficiency determined from isoflupredone is not less than 6000 theoretical plates.

Procedure—Inject a volume (about 50 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Isoflupredone Acetate taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found, excluding those that are present in amounts less than 0.05%.

Other requirements—Where the label states that it is sterile, it meets the requirements for *Sterility Tests* ⟨71⟩ when tested as directed for *Direct Transfer Method* under *Test Procedures.*

Assay—

Mobile phase—Prepare a mixture of *n*-butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Use water-saturated chloroform.

Internal standard solution—Dissolve an accurately weighed quantity of fluoxymesterone in *Diluent* to obtain a solution having a known concentration of about 0.9 mg per mL.

Standard preparation—Dissolve about 4 mg of USP Isoflupredone Acetate RS, accurately weighed, in 8.0 mL of *Internal standard solution* and 32.0 mL of *Diluent.*

Assay preparation—Transfer about 4 mg of Isoflupredone Acetate, accurately weighed, to a suitable container. Dissolve in 8.0 mL of *Internal standard solution* and 32.0 mL of *Diluent,* centrifuge, and use the clear chloroform portion.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L3. The flow rate is about 0.7 mL per minute. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the relative retention times are about 1.0 for isoflupredone acetate and 1.2 for fluoxymesterone; the resolution, *R,* between isoflupredone acetate and fluoxymesterone is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 12 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{23}H_{29}FO_6$ in the portion of Isoflupredone Acetate taken by the formula:

$$W_S(R_U/R_S)$$

in which W_S is the weight, in mg, of USP Isoflupredone Acetate RS taken to prepare the *Standard preparation;* and R_U and R_S are the peak area ratios of isoflupredone acetate to fluoxymesterone obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Isoflupredone Acetate Injectable Suspension

» Isoflupredone Acetate Injectable Suspension contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of isoflupredone acetate ($C_{23}H_{29}FO_6$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

Labeling—Label it to indicate that it is intended for veterinary use only.

USP Reference standards ⟨11⟩—
USP Isoflupredone Acetate RS
USP Prednisolone Acetate RS

Change to read:

▲***Spectroscopic Identification Tests*** ⟨197⟩, *Infrared Spectroscopy:* ***197M***▲ (CN 1-May-2020).

Test specimen—Transfer about 25 mg of Injectable Suspension to a centrifuge tube, add 20 mL of water, and shake well. Centrifuge, and discard the liquid layer. Repeat this washing step with three additional 20-mL portions of water. Dry the material so obtained at 105° for 3 hours.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 125 USP Endotoxin Units per mg of isoflupredone acetate.

Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 5.0 and 7.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase, Diluent, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Isoflupredone Acetate*

Assay preparation—Transfer an accurately measured volume of Injectable Suspension, equivalent to about 4 mg of isoflupredone acetate, to a suitable container. Add 8.0 mL of *Internal standard solution* and 32.0 mL of *Diluent,* and swirl to dissolve.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Isoflupredone Acetate* Calculate the quantity, in mg, of

USP Monographs

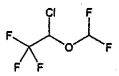
www.webofpharma.com

isoflupredone acetate ($C_{23}H_{29}FO_6$) in each mL of Injectable Suspension taken by the formula:

$$(W_S/V)(R_U/R_S)$$

in which V is the volume, in mL, of Injectable Suspension taken to prepare the *Assay preparation;* and the other terms are as defined therein.

Isoflurane



$C_3H_2ClF_5O$ 184.49

Ethane, 2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-; 1-Chloro-2,2,2-trifluoroethyl difluoromethyl ether [26675-46-7].

DEFINITION

Isoflurane contains NLT 99.9% and NMT 100.0% of isoflurane ($C_3H_2ClF_5O$).

IDENTIFICATION

- **A.** The IR absorption spectrum of Isoflurane, obtained using a gas cell, exhibits maxima only at the same wavelengths as those of a similar preparation of USP Isoflurane RS.

ASSAY

- **PROCEDURE**

Analysis: Using the results from the test for *Organic Impurities,* calculate the percentage of isoflurane ($C_3H_2ClF_5O$) in the sample of Isoflurane taken by subtracting the sum of percentages for the impurities found from 100.0%.

Acceptance criteria: 99.9%–100.0%

IMPURITIES

- **CHLORIDE**

Sample solution: Pipet 10 mL of Isoflurane into a suitable vessel containing 60 mL of isopropyl alcohol and 4 drops of dilute nitric acid (1:1), and stir to dissolve.

Analysis: Titrate potentiometrically with 0.002 N silver nitrate in isopropyl alcohol VS.

Acceptance criteria: NMT 2.11 mL is required (NMT 10 ppm)

- **LIMIT OF FLUORIDE**

Use plasticware throughout this test.

Buffer: Dissolve 110 g of sodium chloride and 1 g of sodium citrate in 700 mL of water in a 2-L volumetric flask. Cautiously add 150 g of sodium hydroxide, and dissolve with shaking. Cool to room temperature, and, while stirring, cautiously add 450 mL of glacial acetic acid to the cooled solution. Cool, add 600 mL of isopropyl alcohol, dilute with water to volume, and mix. The pH of this solution is between 5.0 and 5.5. [NOTE—This solution may be used for 6 weeks if stored at room temperature.]

Solution A: Transfer 55 mg of USP Sodium Fluoride RS, previously dried at 150° for 4 h, to a 25-mL volumetric flask. Add 5 mL of water, and mix to dissolve. Add 1.0 mL of 0.0025 N sodium hydroxide, dilute with water to volume, and mix. Each mL of this solution contains 1 mg of fluoride ion. Store in a tightly closed plastic container. [NOTE—This solution may be used for 2 weeks if stored in a refrigerator.]

Standard stock solution 1: 2.0 µg/mL of fluoride in water from *Solution A*

Standard stock solution 2: 6.0 µg/mL of fluoride in water from *Solution A*

Standard stock solution 3: 10.0 µg/mL of fluoride in water from *Solution A*

Standard stock solution 4: 20.0 µg/mL of fluoride in water from *Solution A*

Standard solution 1: 1.0 µg/mL of fluoride in *Buffer* from *Standard stock solution 1*

Standard solution 2: 3.0 µg/mL of fluoride in *Buffer* from *Standard stock solution 2*

Standard solution 3: 5.0 µg/mL of fluoride in *Buffer* from *Standard stock solution 3*

Standard solution 4: 10.0 µg/mL of fluoride in *Buffer* from *Standard stock solution 4*

Sample solution: Shake 50.0 mL of Isoflurane with 50.0 mL of water for 5 min, and allow the liquids to separate completely. Transfer 25.0 mL of the water layer to a 50-mL volumetric flask, dilute with *Buffer* to volume, and mix.

Analysis

Samples: *Standard solutions 1–4* and *Sample solution* (See *pH* ⟨791⟩.)

Concomitantly measure the potentials in mV, of *Standard solutions 1–4* and the *Sample solution* with a pH meter capable of a minimum reproducibility of ±0.2 mV and equipped with a fluoride ion electrode and a glass-sleeved calomel reference electrode or a double-junction fluoride ion-selective combination electrode. When taking measurements, immerse the electrodes in the solution under test, which has been transferred to a 150-mL beaker containing a polytef-coated stirring bar. Allow to stir on a magnetic stirrer with an insulated top until equilibrium is attained (1–2 min), and record the potential. Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the fluoride ion electrode.

A satisfactory response is achieved if the difference in potential between the potentials obtained with *Standard solution 1* and *Standard solution 4,* having fluoride concentrations of 1.0 and 10.0 µg/mL, respectively, is in the range of 50–60 mV. Plot the logarithm of the fluoride ion concentrations, in µg/mL, of *Standard solutions 1–4* versus potential, in mV. From the measured potential of the *Sample solution* and the standard response line, determine the concentration, in µg/mL, of fluoride in the *Sample solution.*

Acceptance criteria: NMT 5 µg/mL of fluoride in the *Sample solution* [NMT 0.001% (w/v) fluoride in Isoflurane]

- **NONVOLATILE RESIDUE**

Analysis: Transfer 10.0 mL of Isoflurane to a suitable weighed evaporating dish, evaporate with the aid of a current of air or stream of nitrogen to dryness, and dry the residue at 50° for 2 h.

Acceptance criteria: The weight of the residue does not exceed 2.0 mg.

- **ORGANIC IMPURITIES**

Standard stock solution: To a suitable tared vial, fitted with a septum, add 20 mL (29.8 g) of Isoflurane. Seal and reweigh the vial to determine the weight of the Isoflurane added. To this vial sequentially add 20 µL (30 mg) of USP Isoflurane Related Compound A RS, 21 µL (30 mg) of USP Isoflurane Related Compound B RS, and 38 µL (30 mg) of USP Acetone RS. Record the weight after the addition of each impurity and determine the total weight.

Calculate the percentage of each impurity in the *Standard stock solution:*

$$\text{Result} = (W_I/W_T) \times P_I$$

W_I = weight of each impurity added (g)

W_T = total weight of the *Standard stock solution* (g)

www.webofpharma.com

P_I = purity of each impurity added (%)

Standard solution: To a suitable tared vial, fitted with a septum, add 10 mL (15 g) of Isoflurane. Seal and reweigh the vial to determine the weight of the Isoflurane added. To this vial add 300 µL of the *Standard stock solution*, and record the weight to determine the weight of the *Standard stock solution* added and the final weight of the *Standard solution*.

Calculate the spiked concentration (C_S) of each impurity in the *Standard solution*:

$$\text{Result} = (W_I/W_T) \times C_I$$

W_I = weight of the *Standard stock solution* added (g)
W_T = total weight of the *Standard solution* (g)
C_I = concentration of each impurity in the *Standard stock solution* (%)

System suitability solution: To a suitable vial, fitted with a septum, add 10 mL (15 g) of Isoflurane. Seal the vial. To this vial add 100 µL of the *Standard stock solution*.

Sample solution: Isoflurane

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 60-m; fused-silica capillary coated with a 1.0-µm film of G16

Carrier gas: Helium

Flow rate: 1.7 mL/min (constant flow mode)

Temperatures

Injection port: 175°

Detector: 200°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	8
40	10	170	4

Injection volume: 2 µL

Injection type: Split; split ratio, 23:1

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Tailing factor: NMT 1.5 for acetone, *Standard solution*

Relative standard deviation: NMT 5% each for isoflurane related compound A, isoflurane related compound B, and acetone, *Standard solution*

Signal-to-noise ratio: NLT 15 for isoflurane related compound B, *System suitability solution*

Analysis: Injections of Isoflurane used to prepare the *Standard solution* must be made to estimate the amount of known impurities that may be present in the solvent. The final concentration of each impurity is equal to the concentration of the impurity added plus the concentration inherent in the matrix.

Samples: *Standard solution* and *Sample solution*

Calculate the final concentration (C_F) of each impurity in the *Standard solution*:

$$\text{Result} = [r_U/(r_S - r_U) \times C_S] + C_S$$

r_U = peak response of each impurity from the Isoflurane used as the solvent
r_S = peak response of each impurity from the *Standard solution*
C_S = spiked concentration of each impurity in the *Standard solution* (%)

Calculate the percentage of isoflurane related compound A, isoflurane related compound B, and acetone observed in the *Sample solution*, which are also present in the *Standard solution*:

$$\text{Result} = (r_U/r_S) \times C_F$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of each impurity from the *Standard solution*
C_F = final concentration of each impurity in the *Standard solution* (%)

Calculate the percentage of all other impurities:

$$\text{Result} = (r_U/r_S) \times C_S \times (1/F)$$

r_U = peak response of the impurity from the *Sample solution*
r_S = peak response of isoflurane related compound B from the *Standard solution*
C_S = final concentration of USP Isoflurane Related Compound B RS in the *Standard solution* (%)
F = relative response factor relative to isoflurane related compound B (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time[a]	Relative Response Factor[b]	Acceptance Criteria, NMT (%)
Dichloroisoflurane[c]	0.41	0.28	0.003
Isoflurane isomer[d]	0.43	0.87	0.003
Isoflurane related compound A	0.46	—	0.01
Isoflurane related compound B	0.56	1.00	0.007
Chloroisoflurane[e]	0.59	0.35	0.003
Acetone	0.79	—	0.008
Isoflurane	1.00	—	—
Any individual unspecified impurity	—	1.00	0.003
Total impurities	—	—	0.1

[a] Relative to Isoflurane.
[b] Relative to isoflurane related compound B.
[c] 1,1-Dichloro-1-(chlorodifluoromethoxy)-2,2,2-trifluoroethane.
[d] 2-(Chlorodifluoromethoxy)-1,1,1-trifluoroethane.
[e] 1,1-Dichloro-1-(difluoromethoxy)-2,2,2-trifluoroethane.

SPECIFIC TESTS

- **Refractive Index** ⟨831⟩: 1.2990–1.3005 at 20°
- **Water Determination** ⟨921⟩, *Method I*: NMT 0.10%
- **Acidity or Alkalinity**

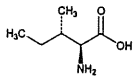
Sample: Transfer 5 mL of Isoflurane and 2 mL of carbon dioxide-free water to a 10-mL glass-stoppered graduated cylinder, shake for 3 min, and allow the layers to separate.

www.webofpharma.com

Analysis: Test the aqueous layer (upper) with both red litmus paper and blue litmus paper.
Acceptance criteria: The aqueous layer is neutral to litmus paper. The aqueous phase should not turn the red litmus paper blue, nor should it turn the blue litmus paper red.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature. Replace the cap securely after each use.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Acetone RS
  USP Isoflurane RS
  USP Isoflurane Related Compound A RS
  1-Chloro-2,2,2-trifluoroethyl chlorodifluoromethyl ether.
  $C_3HCl_2F_5O$ 218.94
  USP Isoflurane Related Compound B RS
  2,2,2-Trifluoroethyl difluoromethyl ether.
  $C_3H_3F_5O$ 150.05
  USP Sodium Fluoride RS

Isoleucine



$C_6H_{13}NO_2$ 131.17
L-Isoleucine [73-32-5].

DEFINITION
Isoleucine contains NLT 98.5% and NMT 101.5% of L-isoleucine ($C_6H_{13}NO_2$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
- **PROCEDURE**
  Sample: 130 mg of Isoleucine
  Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Direct titration
  Titrant: 0.1 N perchloric acid VS
  Endpoint detection: Potentiometric
  Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with the *Titrant*. Perform the blank determination.
  Calculate the percentage of L-isoleucine ($C_6H_{13}NO_2$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N_A \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N_A = actual normality of the *Titrant* (mEq/mL)
F = equivalency factor, 131.2 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.3%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Chloride*
  Standard solution: 0.50 mL of 0.020 N hydrochloric acid
  Sample: 0.73 g of Isoleucine
  Acceptance criteria: NMT 0.05%
- **CHLORIDE AND SULFATE** ⟨221⟩, *Sulfate*
  Standard solution: 0.10 mL of 0.020 N sulfuric acid
  Sample: 0.33 g of Isoleucine
  Acceptance criteria: NMT 0.03%
- **IRON** ⟨241⟩: NMT 30 ppm

Delete the following:

- **▲HEAVY METALS,** *Method I* ⟨231⟩: NMT 15 ppm▲ (Official 1-Jan-2018)

Change to read:

- **RELATED COMPOUNDS**
  ▲▲ (RB 1-Aug-2017)
  System suitability solution: 0.4 mg/mL each of USP L-Isoleucine RS and USP L-Valine RS in 0.1 N hydrochloric acid
  Standard solution: 0.05 mg/mL of ▲USP L-Isoleucine RS▲ (ERR 1-Oct-2018) in 0.1 N hydrochloric acid. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]
  Sample solution: 10 mg/mL of Isoleucine in 0.1 N hydrochloric acid
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
  Mode: TLC
  Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
  Application volume: 5 µL
  Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)
  Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)
  System suitability
  Sample: *System suitability solution*
  Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.
  Analysis
  Samples: *System suitability solution*, *Standard solution*, and *Sample solution*
  After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.
  Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.
  Individual impurities: NMT 0.5%
  Total impurities: NMT 2.0%

SPECIFIC TESTS
- **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*
  Sample solution: 40 mg/mL in 6 N hydrochloric acid
  Acceptance criteria: +38.9° to +41.8°
- **pH** ⟨791⟩
  Sample solution: 10 mg/mL in water
  Acceptance criteria: 5.5–7.0
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 0.3%

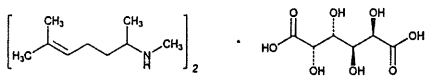
www.webofpharma.com

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP L-Isoleucine RS
  ▲ (RB 1-Aug-2017)
  USP L-Valine RS

Isometheptene Mucate



$(C_9H_{19}N)_2 \cdot C_6H_{10}O_8$ 492.65
Isometheptene, galactarate (2:1) (salt);
6-Methylamino-2-methylheptene, tetrahydroxyadipic acid (2:1) (salt) [7492-31-1].

DEFINITION
Isometheptene Mucate contains NLT 99.0% and NMT 103.0% of isometheptene mucate $[(C_9H_{19}N)_2 \cdot C_6H_{10}O_8]$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY
- **PROCEDURE**
  Sample: 500 mg of Isometheptene Mucate
  Titrimetric system
  (See *Titrimetry* ⟨541⟩.)
  Mode: Residual titration
  Titrant: 0.1 N bromine VS
  Back-titrant: 0.1 N sodium thiosulfate VS
  Endpoint detection: Visual
  Analysis:
  Sample: *Sample*
  Transfer the *Sample* to a 125-mL conical flask. Add 25 mL of water, and swirl to dissolve. Add 45.0 mL of *Titrant*. Attach a 30-mL separator to the conical flask, and evacuate the system. Stir with a magnetic stirrer for 1 h. Equilibrate the system to atmospheric pressure. Add 5 mL of hydrochloric acid to the separator, and allow 4 mL of it to enter the conical flask. Add 10 mL of potassium iodide TS to the separator, and allow the contents to pass into the conical flask.
  Immediately titrate the liberated iodine with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a blank titration. Each mL of 0.1 N sodium thiosulfate is equivalent to 12.32 mg of isometheptene mucate $[(C_9H_{19}N)_2 \cdot C_6H_{10}O_8]$.
  Acceptance criteria: 99.0%–103.0% on the dried basis

IMPURITIES
- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

SPECIFIC TESTS
- **PH** ⟨791⟩
  Sample solution: A solution of Isometheptene Mucate in water (1 in 20)
  Acceptance criteria: 6.0–7.5
- **LOSS ON DRYING** ⟨731⟩
  Analysis: Dry at 60° for 18 h.
  Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Isometheptene Mucate RS

Isometheptene Mucate, Dichloralphenazone, and Acetaminophen Capsules

DEFINITION
Isometheptene Mucate, Dichloralphenazone, and Acetaminophen Capsules contain NLT 85.0% and NMT 110.0% of the labeled amounts of isometheptene mucate $[(C_9H_{19}N)_2 \cdot C_6H_{10}O_8]$ and dichloralphenazone $(C_{15}H_{18}Cl_6N_2O_5)$, and NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen $(C_8H_9NO_2)$.

IDENTIFICATION
- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  Mobile phase: Acetonitrile, 0.07 M monobasic potassium phosphate, 0.007 M sodium 1-decanesulfonate, and diethylamine (250:750:25:15). Adjust with phosphoric acid to a pH of 3.5.
  Sensitivity solution: Empty the contents of 1 Capsule into a 100-mL volumetric flask. Add 80 mL of water, and swirl to dissolve. Dilute with water to volume. Pass a portion of this solution through a glass-fiber filter, discarding the first 5 mL of the filtrate. [NOTE—Prepare this solution, chromatograph it, and evaluate it as directed for *System suitability* before preparing the *Standard solution* and the *Sample solution*.]
  Standard solution: USP Reference Standards in water as listed below
  Acetaminophen: 3.25 mg/mL of USP Acetaminophen RS
  Dichloralphenazone: 3.25*J* mg/mL of USP Dichloralphenazone RS, where *J* is the ratio of the labeled amount, in mg, of dichloralphenazone to the labeled amount of acetaminophen in mg/Capsule
  Isometheptene mucate: 3.25*J′* mg/mL of USP Isometheptene Mucate RS, where *J′* is the ratio of the labeled amount, in mg, of isometheptene mucate to the labeled amount of acetaminophen in mg/Capsule
  Sample solution: Transfer 20 Capsules to a 2000-mL volumetric flask. Add 1900 mL of water, and heat on a steam bath until the Capsules disintegrate. While still warm, shake by mechanical means for 15 min, sonicate for 15 min, and allow to cool to ambient temperature. Dilute with water to volume. Pass a portion of this mixture through a glass-fiber filter, discarding the first 5 mL of the filtrate.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV
  Analytical wavelength 1: 280 nm until the acetaminophen peak has been recorded

www.webofpharma.com

Analytical wavelength 2: 243 nm until the dichloralphenazone peak has been recorded
Analytical wavelength 3: 190 nm until the isometheptene mucate peak has been recorded
Column: 4.6-mm × 25-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Samples: *Sensitivity solution* and *Standard solution*
Suitability requirements
Sensitivity: Acetaminophen, dichloralphenazone, and isometheptene mucate detected at acceptable levels of sensitivity, *Sensitivity solution*
Relative standard deviation: NMT 2.0% each for acetaminophen, dichloralphenazone, and isometheptene mucate, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amounts of acetaminophen ($C_8H_9NO_2$), dichloralphenazone ($C_{15}H_{18}Cl_6N_2O_5$), and isometheptene mucate [$(C_9H_{19}N)_2 \cdot C_6H_{10}O_8$] in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen, dichloralphenazone, or isometheptene mucate from the *Sample solution*
r_S = peak response of the corresponding peak from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 85.0%–110.0% of the labeled amounts of isometheptene mucate [$(C_9H_{19}N)_2 \cdot C_6H_{10}O_8$] and dichloralphenazone ($C_{15}H_{18}Cl_6N_2O_5$), and 90.0%–110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$)

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩
Medium: Water; 900 mL
Apparatus 1: 100 rpm
Time: 60 min
Mobile phase, Sensitivity solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay* except use an *Injection volume* of 100 µL.
Standard solution: USP Reference Standards in water as listed below. Pass through a suitable filter, and use the filtrate.
Acetaminophen: 0.0011*A* mg/mL of USP Acetaminophen RS, where *A* is the labeled amount of acetaminophen in mg/Capsule
Dichloralphenazone: 0.0011*AJ* mg/mL of USP Dichloralphenazone RS, where *A* is the labeled amount of acetaminophen in mg/Capsule and *J* is the ratio of the labeled amount, in mg, of dichloralphenazone to the labeled amount of acetaminophen in mg/Capsule
Isometheptene mucate: 0.0011*AJ′* mg/mL of USP Isometheptene Mucate RS, where *A* is the labeled amount of acetaminophen in mg/Capsule and *J′* is the ratio of the labeled amount, in mg, of isometheptene mucate to the labeled amount of acetaminophen in mg/Capsule
Sample solution: Pass 20 mL of the solution under test through a glass-fiber filter, and discard the first 15 mL of the filtrate.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentages of the labeled amounts of acetaminophen ($C_8H_9NO_2$), dichloralphenazone ($C_{15}H_{18}Cl_6N_2O_5$), and isometheptene mucate [$(C_9H_{19}N)_2 \cdot C_6H_{10}O_8$] dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

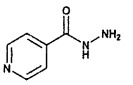
r_U = peak response of acetaminophen, dichloralphenazone, or isometheptene mucate from the *Sample solution*
r_S = peak response of the corresponding peak from the *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
V = volume of the *Medium*, 900 mL
L = label claim of acetaminophen, dichloralphenazone, or isometheptene mucate (mg/Capsule)

Tolerances: NLT 65% (*Q*) of the labeled amounts of acetaminophen ($C_8H_9NO_2$), dichloralphenazone ($C_{15}H_{18}Cl_6N_2O_5$), and isometheptene mucate [$(C_9H_{19}N)_2 \cdot C_6H_{10}O_8$] is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Acetaminophen RS
USP Dichloralphenazone RS
USP Isometheptene Mucate RS

Isoniazid



$C_6H_7N_3O$ 137.14
4-Pyridinecarboxylic acid, hydrazide;
Isonicotinic acid hydrazide [54-85-3].

DEFINITION

Isoniazid contains NLT 98.0% and NMT 102.0% of isoniazid ($C_6H_7N_3O$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A. ▲SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K▲** (CN 1-May-2020)
• **B.** The retention time of the isoniazid peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**
Buffer: 13.6 g/L of monobasic potassium phosphate adjusted with 10 N sodium hydroxide to a pH of 6.9. Add 30 mg/L of triethanolamine.
Mobile phase: Methanol and *Buffer* (5:95)

www.webofpharma.com

Standard solution: 0.32 mg/mL of USP Isoniazid RS in *Mobile phase*
Sample solution: 0.32 mg/mL of Isoniazid in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing: NMT 2.0 for the isoniazid peak
Relative standard deviation: NMT 0.73%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of isoniazid ($C_6H_7N_3O$) in the portion of Isoniazid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of isoniazid from the *Sample solution*
r_S = peak response of isoniazid from the *Standard solution*
C_S = concentration of USP Isoniazid RS in the *Standard solution* (mg/mL)
C_U = concentration of Isoniazid in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.2%
- **ORGANIC IMPURITIES**

Buffer and **Mobile phase:** Proceed as directed in the *Assay*.
System suitability solution: 1.0 µg/mL each of USP Isoniazid RS, isoniacin, isonicotinamide, picolinohydrazide, and isonicotinonitrile in *Mobile phase*
Standard solution: 1.0 µg/mL of USP Isoniazid RS in *Mobile phase*
Sample solution: 1.0 mg/mL of Isoniazid in *Mobile phase*
Chromatographic system: Proceed as directed in the *Assay*, except for the following.
Detector: UV 266 nm
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between any adjacent peak pair, *System suitability solution*
Relative standard deviation: NMT 5.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Isoniazid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of isoniazid from the *Standard solution*
C_S = concentration of USP Isoniazid RS in the *Standard solution* (mg/mL)
C_U = concentration of Isoniazid in the *Sample solution* (mg/mL)
F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Isoniacin[a]	0.50	0.69	0.1
Isoniazid	1.0	1.0	—
Isonicotinamide	1.4	0.70	0.1
Picolinohydrazide[b]	2.1	1.2	0.1
Isonicotinonitrile[c]	3.9	0.74	0.1
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	2.0

[a] Isonicotinic acid.
[b] 2-Isoniazid.
[c] 4-Cyanopyridine.

SPECIFIC TESTS

- **pH** ⟨791⟩
Sample solution: In a solution (1 in 10)
Acceptance criteria: 6.0–7.5
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Isoniazid RS

Isoniazid Injection

DEFINITION

Isoniazid Injection is a sterile solution of Isoniazid in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of isoniazid ($C_6H_7N_3O$).

IDENTIFICATION

- **A.** The retention time exhibited by isoniazid in the *Sample solution* corresponds to that of isoniazid in the *Standard solution*, as obtained in the *Assay*.
- **B.**
Sample stock solution: Nominally 0.1 mg/mL of isoniazid in water prepared as follows. Transfer an equivalent to 50 mg of isoniazid from a volume of Injection to a 500-mL volumetric flask, and dilute with water to volume.
Sample solution: 0.01 mg/mL of isoniazid in water prepared as follows. Transfer 10.0 mL of *Sample stock solution* to a 100-mL volumetric flask, add 2.0 mL of 0.1 N hydrochloric acid, and dilute with water to volume.
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima only at the same wavelengths as that of a similar solution of USP Isoniazid RS, concomitantly measured.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Mobile phase: 4.4 g/L of docusate sodium in a mixture of methanol and water (600:400), and adjust with 2 N sulfuric acid to a pH of 2.5.

Standard solution: 0.32 mg/mL of USP Isoniazid RS in *Mobile phase*

Sample solution: Nominally 0.32 mg/mL of isoniazid in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1800 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of isoniazid ($C_6H_7N_3O$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of isoniazid from the *Sample solution*

r_S = peak response of isoniazid from the *Standard solution*

C_S = concentration of USP Isoniazid RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of isoniazid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 6.0–7.0

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩: Contains NMT 0.3 USP Endotoxin Unit/mg of isoniazid

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

• **LABELING:** Its package label states that if crystallization has occurred, the Injection should be warmed to redissolve the crystals before use.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Isoniazid RS

Isoniazid Oral Solution

DEFINITION

Isoniazid Oral Solution contains, in each 100 mL, NLT 0.93 g and NMT 1.10 g of isoniazid ($C_6H_7N_3O$).

IDENTIFICATION

• **A.**

Sample stock solution: Nominally 0.1 mg/mL of isoniazid in water prepared as follows. Transfer an equivalent to 50 mg of isoniazid from a volume of Oral Solution to a 500-mL volumetric flask, and dilute with water to volume.

Sample solution: 0.01 mg/mL of isoniazid in water prepared as follows. Transfer 10.0 mL of *Sample stock solution* to a 100-mL volumetric flask, add 2.0 mL of 0.1 N hydrochloric acid, and dilute with water to volume.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima only at the same wavelengths as that of a similar solution of USP Isoniazid RS, concomitantly measured.

ASSAY

• **NITRITE TITRATION** ⟨451⟩

Diluent: Dilute hydrochloric acid (1 in 6)

Sample solution: Nominally 2 mg/mL of isonizid prepared as follows. To a volume of Oral Solution, equivalent to 100 mg of isoniazid, add 50 mL of a mixture of 1 part of potassium bromide in 10 parts of *Diluent*.

Analysis: Proceed as directed in the chapter, beginning with "cool to about 15°". Each mL of 0.1 M sodium nitrite is equivalent to 13.71 mg of isoniazid ($C_6H_7N_3O$).

Acceptance criteria: 0.93–1.10 g in each 100 mL

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Isoniazid RS

Isoniazid Tablets

DEFINITION

Isoniazid Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of isoniazid ($C_6H_7N_3O$).

IDENTIFICATION

• **A.** The retention time of the isoniazid peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. ULTRAVIOLET ABSORPTION**

Sample stock solution: Nominally 0.1 mg/mL of isoniazid in water prepared from a portion of finely powdered Tablets

Sample solution: Nominally 0.01 mg/mL of isoniazid in water prepared as follows. Transfer 10.0 mL of *Sample stock solution* to a 100-mL volumetric flask, add 2.0 mL of 0.1 N hydrochloric acid, and dilute with water to volume.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima only at the same wavelengths as that of a similar solution of USP Isoniazid RS, concomitantly measured.

ASSAY

• **PROCEDURE**

Buffer: To 0.1 M monobasic potassium phosphate solution, adjusted with 10 N sodium hydroxide to a pH of 6.9, add sufficient triethanolamine to obtain a solution of 0.2 mM of triethanolamine.

Mobile phase: *Buffer* and methanol (95:5)

Standard solution: 0.32 mg/mL of USP Isoniazid RS in *Mobile phase*

Sample solution: Nominally 0.32 mg/mL of isoniazid in *Mobile phase* prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 32 mg of isoniazid, to a 100-mL volumetric flask. Add 40 mL of *Mobile phase*, and sonicate for 10 min. Cool to room temperature, dilute with *Mobile phase* to volume, and centrifuge for 5 min.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Capacity factor, k′: NLT 2.35
Column efficiency: NLT 1800 theoretical plates
Tailing factor: NMT 1.5
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of isoniazid ($C_6H_7N_3O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Isoniazid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of isoniazid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
Medium: 0.01 N hydrochloric acid; 900 mL
Apparatus 1: 100 rpm
Time: 45 min
Detector: UV 263 nm
Standard solution: USP Isoniazid RS in *Medium*
Sample solution: Dilute with *Medium* to a nominal concentration that is similar to that of the *Standard solution*.
Analysis: Determine the percentage of the labeled amount of isoniazid ($C_6H_7N_3O$) dissolved by using UV absorption on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with the *Standard solution*.
Tolerances: NLT 80% (*Q*) of the labeled amount of isoniazid ($C_6H_7N_3O$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
Procedure for content uniformity
Diluent: 0.1 N hydrochloric acid and water (3 in 100)
Standard solution: 10 µg/mL of USP Isoniazid RS prepared as follows. Dissolve a quantity of USP Isoniazid RS in a volume of water corresponding to that used to dissolve a similar amount of isoniazid from the Tablet, and dilute with *Diluent*.
Sample solution: Nominally 10 µg/mL of isoniazid prepared as follows. Transfer 1 finely powdered Tablet to a 500-mL volumetric flask with the aid of 200 mL of water. Shake by mechanical means for 30 min, and add water to volume. Filter, and discard the first 20 mL of the filtrate. Dilute a portion of the filtrate with *Diluent*.
Instrumental conditions
Mode: UV
Analytical wavelength: 263 nm
Cell: 1 cm
Blank: Water
Calculate the percentage of the labeled amount of isoniazid ($C_6H_7N_3O$) in the Tablet taken:

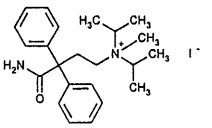
$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Isoniazid RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of isoniazid in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Isoniazid RS

Isopropamide Iodide



$C_{23}H_{33}IN_2O$ 480.43
Benzenepropanaminium, γ-(aminocarbonyl)-*N*-methyl-*N*,*N*-bis(1-methylethyl)-γ-phenyl-, iodide.
(3-Carbamoyl-3,3-diphenylpropyl) diisopropylmethylammonium iodide [71-81-8].

» Isopropamide Iodide, dried in vacuum at 60° for 2 hours, contains not less than 98.0 percent and not more than 101.0 percent of $C_{23}H_{33}IN_2O$.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Isopropamide Iodide RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

B: To 5 mL of a solution (1 in 1000) add 5 mL of sodium carbonate solution (1 in 100), 0.5 mL of bromophenol blue TS, and 10 mL of chloroform, and shake for several minutes: the chloroform layer becomes an intense blue in color.

C: A solution (1 in 1000) responds to the tests for *Iodide* ⟨191⟩.

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 2 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.5%, after ignition at 550 ± 25° for 4 hours.

Ordinary impurities ⟨466⟩—
Test solution: methanol.
Standard solution: methanol.
Eluant: a mixture of methanol, glacial acetic acid, and water (8:1:1).
Visualization: 2.

Assay—Dissolve about 750 mg of Isopropamide Iodide, previously dried and accurately weighed, in 60 mL of glacial acetic acid, add 15 mL of mercuric acetate TS and crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any

USP Monographs

www.webofpharma.com

necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 48.04 mg of $C_{23}H_{33}IN_2O$.

Isopropamide Iodide Tablets

» Isopropamide Iodide Tablets contain an amount of isopropamide iodide ($C_{23}H_{33}IN_2O$) equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of isopropamide ($C_{23}H_{33}N_2O$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Isopropamide Iodide RS

Identification—Triturate a portion of powdered Tablets, equivalent to about 10 mg of isopropamide, with 10 mL of water, and filter: the filtrate responds to *Identification*— tests *B:* and *C:* under *Isopropamide Iodide.*

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 2: 100 rpm.
Time: 60 minutes.
Procedure—Determine the amount of isopropamide ($C_{23}H_{33}N_2O$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 258 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Isopropamide Iodide RS in the same medium.
Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{23}H_{33}N_2O$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Crush and transfer 1 Tablet to a 100-mL volumetric flask with the aid of about 50 mL of water, and shake by mechanical means for 30 minutes. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Concomitantly determine the absorbances of this solution and a Standard solution of USP Isopropamide Iodide RS in the same medium having a known concentration of about 70 µg per mL, in 5-cm cells at 280 nm and at the wavelength of maximum absorbance at about 258 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of isopropamide ($C_{23}H_{33}N_2O$) in the Tablet taken by the formula:

$$(353.53/480.43)(TC/D)[(A_{U258} - A_{U280})/(A_{S258} - A_{S280})]$$

in which 353.53 and 480.43 are the molecular weights of isopropamide and isopropamide iodide, respectively; T is the labeled quantity, in mg, of isopropamide in the Tablet; C is the concentration, in µg per mL, of USP Isopropamide Iodide RS in the Standard solution; D is the concentration, in µg per mL, of isopropamide in the test solution, based upon the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the test solution and the Standard solution, respectively, at the wavelengths indicated by the subscripts.

Assay—
Ion-exchange column—Insert a small pledget of glass wool in the bottom of a 6-mm × 240-mm glass tube fitted with a stopcock, fill the tube with water, and add an aqueous slurry of a suitable anion-exchange resin, chloride form (soaked in water for not less than 24 hours prior to use), until a height of about 200 mm is reached. Wash the column with 50 mL of water, and use without delay.
Standard preparation—Transfer about 135 mg of USP Isopropamide Iodide RS, accurately weighed, to a 250-mL volumetric flask, and dissolve in 150 mL of water. Add 5 mL of aluminum chloride solution (1 in 10) and 2 mL of ammonium hydroxide, then dilute with water to volume, mix, and filter, discarding the first 15 mL of the filtrate. Use the subsequent filtrate as directed for *Procedure.*
Assay preparation—Weigh and finely powder not less than 25 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of isopropamide, to a 250-mL volumetric flask, add 150 mL of water, and shake by mechanical means for 60 minutes. Add 5 mL of aluminum chloride solution (1 in 10) and 2 mL of ammonium hydroxide, dilute with water to volume, mix, and filter, discarding the first 15 mL of the filtrate. Use the subsequent filtrate as directed for *Procedure.*
Procedure—Pipet 50.0 mL each of the *Standard preparation* and the *Assay preparation*, respectively, onto separate ion-exchange columns, and collect the eluates in separate 200-mL volumetric flasks. Regulate the flow of effluent so that it does not exceed 40 drops per minute, and when the liquid level reaches the top of each column, add successively two 5-mL portions and one 10-mL portion of water to each column, allowing each portion just to enter the column before adding the next portion of water. After the eluates have been collected, dilute the contents of each flask with water to volume, and mix. Concomitantly determine the absorbances of the solutions in 5-cm cells at 280 nm and at the maximum at about 258 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of isopropamide ($C_{23}H_{33}N_2O$) in the portion of Tablets taken by the formula:

$$(353.53/480.43)(0.25C)[(A_{U258} - A_{U280})/(A_{S258} - A_{S280})]$$

in which 353.53 and 480.43 are the molecular weights of isopropamide and isopropamide iodide, respectively; C is the concentration, in µg per mL, of USP Isopropamide Iodide RS in the *Standard preparation*; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively, at the wavelengths indicated by the subscripts.

Isopropyl Alcohol



C_3H_8O 60.10
2-Propanol;
Isopropanol [67-63-0].

DEFINITION
Isopropyl Alcohol contains NLT 99.0% of isopropyl alcohol (C_3H_8O).

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197F**▲ (CN 1-May-2020)

www.webofpharma.com

• **B.** The retention time of the major peak of the *Sample solution* corresponds to the 2-propanol peak of the *System suitability solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

System suitability solution: USP 2-Propanol System Suitability RS

Sample solution: Isopropyl Alcohol (neat)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 60-m fused silica column, coated with a 1.4-µm film of phase G43

Temperatures

Detector: 200°

Injection port: 150°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
35	—	35	5
35	1	45	—
45	10	100	1

Carrier gas: Helium

Flow rate: 2.3 mL/min

Injection volume: 1 µL

Injection type: Split injection; split ratio is about 50:1. [NOTE—A 4-mm straight liner is suitable.]

Run time: 22 min

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 2*.]

Table 2

Name	Relative Retention Time
Ethyl ether	0.7
Acetone	0.9
Isopropyl alcohol	1.0
Diisopropyl ether	1.4
n-Propyl alcohol (1-propanol)	1.5
2-Butanol	2.0

Suitability requirements

Resolution: NLT 1.5 between acetone and isopropyl alcohol

Relative standard deviation: NMT 2.0% for the isopropyl alcohol peak

Tailing factor: NMT 2.0 for the isopropyl alcohol peak

Signal-to-noise ratio: NLT 10 for any of the following peaks: ethyl ether, acetone, isopropyl alcohol, diisopropyl ether, 1-propanol, and 2-butanol

Analysis

Sample: *Sample solution*

Calculate the percentage of isopropyl alcohol (C_3H_8O) in the portion of Isopropyl Alcohol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of isopropyl alcohol

r_T = sum of all the peak responses

Acceptance criteria: NLT 99.0%

IMPURITIES

• **LIMIT OF VOLATILE IMPURITIES**

System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *System suitability solution* and *Sample solution*

Identify each individual impurity peak in the *Sample solution* based on that in the *System suitability solution*.

Calculate the percentage of each individual impurity in the portion of Isopropyl Alcohol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity in the *Sample solution*

r_T = sum of all the peaks in the *Sample solution*

Acceptance criteria: See *Table 3*.

Table 3

Impurity	Percentage (%)
Each individual	NMT 0.1
Total	NMT 1.0

• **LIMIT OF NONVOLATILE RESIDUE**

Sample: 50 mL

Analysis: Evaporate the *Sample* in a tared porcelain dish on a steam bath to dryness, and heat at 105° for 1 h.

Acceptance criteria: NMT 2.5 mg (0.005%)

SPECIFIC TESTS

• **SPECIFIC GRAVITY** ⟨841⟩: 0.783–0.787

• **REFRACTIVE INDEX** ⟨831⟩: 1.376–1.378 at 20°

• **ACIDITY**

Sample solution: To 50 mL of Isopropyl Alcohol add 100 mL of carbon dioxide-free water.

Analysis: To the *Sample solution* add 2 drops of phenolphthalein TS, and titrate with 0.020 N sodium hydroxide to a pink color that persists for 30 s.

Acceptance criteria: NMT 0.70 mL of 0.020 N sodium hydroxide is required for neutralization.

• **WATER DETERMINATION,** *Method I* ⟨921⟩

Sample: 5.0 g

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and prevent exposure to excessive heat. Protect from light.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP 2-Propanol RS

USP 2-Propanol System Suitability RS

It contains isopropyl alcohol with 0.1% each of ethyl ether, acetone, diisopropyl ether, 1-propanol, and 2-butanol.

www.webofpharma.com

Azeotropic Isopropyl Alcohol

DEFINITION

Azeotropic Isopropyl Alcohol contains NLT 91.0% and NMT 93.0% of isopropyl alcohol, by volume, the remainder consisting of water.

IDENTIFICATION

• **A. Infrared Absorption:** The IR absorption spectrum of a thin film of it exhibits a strong broad band at 3.0 µm; a strong region of absorption between 3.35 and 3.5 µm, with its highest peak at 3.36 µm, and others at 3.41 and 3.47 µm; many weak peaks between 3.6 and 6.0 µm, among the most noticeable being those at 3.68, 3.77, 3.97, 4.17, and 5.26 µm; a broad band at 6.2 µm; a strong region of absorption between 6.7 and 7.8 µm, the most prominent features being the peaks at 6.80, 7.09, 7.25 (the highest), 7.46, and 7.63 µm; a strong region of absorption between 8.5 and 9.2 µm, peaking at 8.6, 8.85, and 9.0 µm; and strong peaks at 10.5 and 12.3 µm.

IMPURITIES

• **Limit of Nonvolatile Residue**

Sample: 50 mL

Analysis: Evaporate the *Sample* in a tared porcelain dish on a steam bath to dryness, and heat at 105° for 1 h.

Acceptance criteria: The weight of the residue does not exceed 2.5 mg (0.005%).

• **Volatile Impurities**

System suitability solution: USP 2-Propanol System Suitability RS

Sample solution: Azeotropic Isopropyl Alcohol (Neat)

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Thermal conductivity

Column: 0.25-mm × 60-m, coated with a 1.4-µm film of phase G43

Temperature

Injector: 150°

Detector: 200°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
35	0	35	5
35	1	45	2
45	10	100	1

Carrier gas: Helium

Linear velocity: 35 cm/s

Injection size: 1 µL

Split ratio: 10:1

Run time: 30 min

System suitability

Sample: *System suitability solution*

[NOTE—Approximate relative retention times for ethyl ether, acetone, isopropyl alcohol, diisopropyl ether, 1-propanol, and 2-butanol are 0.6, 0.7, 1.0, 1.1, 1.3, and 1.5, respectively.]

Suitability requirements

Resolution: NLT 1.5 between acetone and isopropyl alcohol

Signal-to-noise ratio: NLT 10 for any of the following peaks: ethyl ether, acetone, isopropyl alcohol, diisopropyl ether, 1-propanol, and 2-butanol

Tailing factor: NMT 2.0 for the isopropyl alcohol peak

Relative standard deviation: NMT 2.0% for the isopropyl alcohol peak

Analysis

Samples: *Sample solution*

Calculate the ratio of isopropyl alcohol (C_3H_8O) in the portion of Azeotropic Isopropyl Alcohol taken:

$$\text{Result} = (r_I/r_T)$$

r_I = peak area for isopropyl alcohol

r_T = sum of all of the peak areas excluding the peak for water

Acceptance criteria: NLT 0.99

SPECIFIC TESTS

• **Specific Gravity** ⟨841⟩**:** 0.815–0.810, indicating 91.0%–93.0% of isopropyl alcohol (C_3H_8O) by volume

• **Refractive Index** ⟨831⟩**:** 1.376–1.378 at 20°

• **Acidity**

Sample: 50 mL

Analysis: Place the *Sample* in a suitable flask, and add 100 mL of carbon dioxide-free water. Add 2 drops of phenolphthalein TS, and titrate with 0.020 N sodium hydroxide to a pink color that persists for 30 s.

Acceptance criteria: NMT 0.70 mL of 0.020 N sodium hydroxide is required for neutralization.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, remote from heat.

Change to read:

• **USP Reference Standards** ⟨11⟩

USP 2-Propanol System Suitability RS

▲It is a mixture of the following: ethyl ether (0.1%), acetone (0.1%), diisopropyl ether (0.1%), 1-propanol (0.1%), 2-butanol (0.1%), and isopropyl alcohol (99.5%).▲ (ERR 1-Dec-2018)

Isopropyl Rubbing Alcohol

DEFINITION

Isopropyl Rubbing Alcohol contains NLT 68.0% and NMT 72.0% of isopropyl alcohol by volume, with the remainder consisting of water, with or without suitable stabilizers, perfume oils, and color additives certified by the FDA for use in drugs.

ASSAY

• **Procedure**

Sample: 50.0 mL of Isopropyl Rubbing Alcohol

Analysis: Transfer the *Sample* to a 250-mL distilling flask, and add 100 mL of water. Arrange the flask for distillation, distill, and collect 95 mL of distillate in a 100-mL volumetric flask. Dilute with water to volume, and determine the specific gravity of the distillate at 25° (see *Specific Gravity* ⟨841⟩).

Acceptance criteria: The specific gravity is 0.955–0.950, corresponding to 68.0%–72.0% of isopropyl alcohol.

SPECIFIC TESTS

• **Specific Gravity** ⟨841⟩**:** 0.872–0.883 at 20°

www.webofpharma.com

- **LIMIT OF NONVOLATILE RESIDUE**
  Sample: 50 mL of Isopropyl Rubbing Alcohol
  Analysis: Evaporate the *Sample* in a tared porcelain dish on a steam bath to dryness, and dry at 105° for 1 h.
  Acceptance criteria: 0.01%; NMT 5 mg
- **ACIDITY**
  Sample solution: To 50 mL of Isopropyl Rubbing Alcohol add about 75 mL of carbon dioxide-free water.
  Analysis: Transfer the *Sample solution* to a suitable flask, and titrate potentiometrically to a pH of 8.5.
  Acceptance criteria: NMT 1.0 mL of 0.020 N sodium hydroxide is required for neutralization.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, remote from heat.
- **LABELING:** Label it to indicate that it is flammable.

Isoproterenol Inhalation Solution

» Isoproterenol Inhalation Solution is a sterile solution of Isoproterenol Hydrochloride in Purified Water. It may contain Sodium Chloride. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of isoproterenol hydrochloride ($C_{11}H_{17}NO_3 \cdot HCl$).

Packaging and storage—Preserve in small, tight containers that are well-filled or otherwise protected from oxidation. Protect from light.
Labeling—Label it to indicate that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.
USP Reference standards ⟨11⟩—
USP Isoproterenol Hydrochloride RS

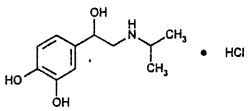
Color and clarity—
Standard solution—Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, dilute with water to volume, and mix.
Procedure—Visually examine a portion of the Inhalation Solution *(Test solution)* in a suitable clear glass test tube against a white background: it is not pinkish and it contains no precipitate. If any yellow color is observed in the *Test solution*, concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm: the absorbance of the *Test solution* does not exceed that of the *Standard solution*.
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay*.
Sterility Tests ⟨71⟩: meets the requirements.
pH ⟨791⟩: between 2.5 and 5.5.

Assay—
Standard preparation—Prepare as directed in the *Assay* under *Isoproterenol Hydrochloride*.
Assay preparation—Transfer an accurately measured volume of Inhalation Solution, equivalent to about 25 mg of isoproterenol hydrochloride, to a 100-mL volumetric flask, dilute with 0.17 N acetic acid solution to volume, and mix.
Chromatographic system—Proceed as directed in the *Assay* under *Isoproterenol Hydrochloride*.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Isoproterenol Hydrochloride*. Calculate the quantity, in mg, of $C_{11}H_{17}NO_3 \cdot HCl$ in each mL of the Inhalation Solution taken by the formula:

$$0.1(C/V)(h_U/h_S)$$

in which V is the volume, in mL, of Inhalation Solution taken; and C, h_U, and h_S are as defined therein.

Isoproterenol Hydrochloride



$C_{11}H_{17}NO_3 \cdot HCl$ 247.72
1,2-Benzenediol, 4-[1-hydroxy-2-[(1-methylethyl)amino] ethyl]-, hydrochloride.
3,4-Dihydroxy-α-[(isopropylamino)methyl]benzyl alcohol hydrochloride [51-30-9].

» Isoproterenol Hydrochloride contains not less than 97.0 percent and not more than 101.5 percent of $C_{11}H_{17}NO_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Isoproterenol Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 50 µg per mL.
Medium: water.
Melting range ⟨741⟩: between 165° and 170°.
Loss on drying ⟨731⟩—Dry about 1 g, accurately weighed, in vacuum over phosphorus pentoxide for 4 hours: it loses not more than 1.0% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.
Sulfate ⟨221⟩—A 0.10-g portion shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.2%).
Limit of isoproterenone—Its absorptivity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) at 310 nm, determined in a solution containing 2 mg per mL, is not more than 0.2.
Chloride content—Dissolve about 500 mg, accurately weighed, in 5 mL of water. Add 5 mL of glacial acetic acid and 40 mL of methanol. Add eosin Y TS, and titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Between 13.9% and 14.6% of Cl is found, calculated on the dried basis.

Assay—
Standard preparation—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in freshly prepared sodium bisulfite solution (3 in 1000) to obtain a solution having a concentration of about 2.5 mg per mL.

www.webofpharma.com

Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.17 N acetic acid to volume, and mix to obtain a solution having a known concentration of about 250 µg per mL.

Assay preparation—Transfer about 125 mg of Isoproterenol Hydrochloride, accurately weighed, to a 25-mL volumetric flask, dissolve in sodium bisulfite solution (3 in 1000), dilute with sodium bisulfite solution to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.17 N acetic acid to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 278-nm detector and a 30-cm × 4-mm stainless steel column that contains packing L1. The mobile phase is 0.17 N acetic acid having a flow rate of about 1.5 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 3.0%.

Procedure—Using a microsyringe or sampling valve, chromatograph 10 µL of the *Standard preparation*, and adjust the specimen size and other operating parameters, if necessary, until satisfactory chromatography and peak responses are obtained. Chromatograph equal volumes of the *Standard preparation* and the *Assay preparation*, and measure the peak responses. Calculate the quantity, in mg, of $C_{11}H_{17}NO_3 \cdot HCl$ in the portion of Isoproterenol Hydrochloride taken by the formula:

$$0.5C(h_U/h_S)$$

in which C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation;* and h_U and h_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Delete the following:

▲Isoproterenol Hydrochloride Inhalation Aerosol

» Isoproterenol Hydrochloride Inhalation Aerosol is a solution of Isoproterenol Hydrochloride in Alcohol in an inert propellant base. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of isoproterenol hydrochloride ($C_{11}H_{17}NO_3 \cdot HCl$).

Packaging and storage—Preserve in small, nonreactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

USP Reference standards ⟨ 11 ⟩ —
USP Isoproterenol Hydrochloride RS

Identification—

A: Place 10 mL of water in a small beaker, and deliver 10 sprays from the Inhalation Aerosol under the surface of the water, actuating the valve by pressing the stem tip against the bottom of the beaker. Filter, place 5 mL of the filtrate in a test tube, and retain the remainder of the solution for *Identification* test *B.* Add 1 drop of 0.2 N sulfuric acid and 0.5 mL of 0.1 N iodine, allow to stand for 5 minutes, and add 1 mL of 0.1 N sodium thiosulfate: a salmon-pink color is produced.

B: Dilute the remainder of the filtrate obtained in *Identification* test *A* with an equal volume of water. Add a few drops of 6 N ammonium hydroxide, filter, acidify the filtrate with nitric acid, and divide into two equal portions. To each portion add a few drops of silver nitrate TS: white precipitates are formed. To one portion add a slight excess of nitric acid: the white precipitate remains. To the other portion add a slight excess of 6 N ammonium hydroxide, and shake: the precipitate dissolves.

Alcohol Determination ⟨611⟩ —Weigh accurately a filled Inhalation Aerosol container, and record the weight. Place the container in a dry ice-alcohol bath, and cool for 60 minutes. Remove the container from the bath, and carefully remove the valve with wire cutters, taking precautions to save all pieces of the valve and cap. With the aid of three 5-mL portions of water, transfer the contents of the container to a beaker previously chilled in the bath. Dry the rinsed empty container and all of its parts in an oven at 105° for 2 hours, cool, and weigh. Calculate the weight of the container contents. Add a few boiling chips to the beaker, and carefully stir to help evaporate the propellant. After the bulk of the propellant has evaporated, transfer the contents of the beaker, with the aid of several mL of water, to a glass-stoppered graduated cylinder, measure the volume, and determine the alcohol content of the *Test solution* thus prepared by the gas-liquid chromatographic procedure, methyl ethyl ketone being used as the internal standard. Calculate the alcohol content of the Inhalation Aerosol taken by the formula:

$$SV/W$$

in which S is the percentage of alcohol (w/v) in the *Test solution;* V is the volume, in mL, of the *Test solution;* and W is the weight, in g, of the container contents: between 28.5% and 38.5% (w/w) of C_2H_5OH is found.

Dose uniformity over the entire contents: meets the requirements for *Metered-Dose Inhalers* under *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* ⟨601⟩.

PROCEDURE FOR DOSE UNIFORMITY—

Ferro-citrate solution and *Buffer solution*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Standard preparation—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in a freshly prepared sodium bisulfite solution (1 in 500), and dilute quantitatively and stepwise with the same sodium bisulfite solution as necessary to obtain a solution having a known concentration of about 6 µg per mL.

Test preparation—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of a freshly prepared sodium bisulfite solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform, insert the stopper, shake vigorously for 1 minute, and centrifuge. Use the clear supernatant as directed for *Procedure.*

Procedure—Into three separate flasks, transfer the *Test preparation,* 20.0 mL of the *Standard preparation,* and 20.0 mL of water to provide the blank. To each flask add 100 µL of *Ferro-citrate solution* followed by 1.0 mL of *Buffer solution,* and mix. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation,* at the wavelength of maximum absorbance at about 530 nm, against the blank. Calculate the quantity, in µg, of $C_{11}H_{17}NO_3 \cdot HCl$ contained in the minimum dose taken by the formula:

$$20CN\,(A_U/A_S)$$

www.webofpharma.com

in which C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; N is the number of sprays discharged to obtain the minimum dose; and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

Assay—

Ferro-citrate Solution, *Buffer Solution*, and *Standard preparation*—Prepare as directed under *Unit spray content*.

Assay preparation—Fit the Inhalation Aerosol container with its inhalation actuator, and prime the unit by firing it 10 times. Remove the actuator, and accurately weigh the container with its remaining contents. Invert the container, place the valve stem tip against the bottom of a 100-mL beaker containing 20 mL of chloroform, and deliver under the surface of the chloroform a number of sprays equivalent to about 500 µg of isoproterenol hydrochloride. Raise the unit above the contents of the beaker, and wash the valve stem with about 2 mL of chloroform. Collect the washings in the beaker. Allow the valve stem to dry, and again accurately weigh the Inhalation Aerosol container with its remaining contents. Record the weight of the expelled specimen. Transfer the contents of the beaker to a centrifuge tube with the aid of two 3-mL portions of chloroform, add 10.0 mL of freshly prepared sodium bisulfite solution (1 in 500), and shake vigorously for 1 minute. Centrifuge, and use the clear supernatant as directed for *Procedure*.

Procedure—Into three separate test tubes, pipet 5 mL each of the *Assay preparation*, *Standard preparation*, and water to provide the blank, respectively. To each tube add 100 µL of *Ferro-citrate Solution*, followed by 1.0 mL of *Buffer Solution*, and mix. Determine the absorbances of the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 530 nm, against the blank. Calculate the percentage (w/w) of $C_{11}H_{17}NO_3 \cdot HCl$ in the portion of Inhalation Aerosol taken by the formula:

$$0.001(C / W)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; W is the weight, in g, of Inhalation Aerosol taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively. ▲ 2S(USP41)

Isoproterenol Hydrochloride Injection

» Isoproterenol Hydrochloride Injection is a sterile solution of Isoproterenol Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of isoproterenol hydrochloride ($C_{11}H_{17}NO_3 \cdot HCl$).

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.

Labeling—Label it to indicate that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

USP Reference standards ⟨11⟩—
USP Isoproterenol Hydrochloride RS

Color and clarity—Using the Injection as the *Test solution*, proceed as directed for *Color and clarity* under *Isoproterenol Inhalation Solution*.

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 1250.0 USP Endotoxin Units per mg of isoproterenol hydrochloride.

pH ⟨791⟩**:** between 2.5 and 4.5.

Particulate Matter in Injections ⟨788⟩ **:** meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase—Dissolve 1.76 g of sodium 1-heptanesulfonate in 800 mL of water. Add 200 mL of methanol, and adjust with 1 M phosphoric acid to a pH of 3.0 ± 0.1. Pass the solution through a membrane filter having a 1-µm or finer porosity.

Standard preparation—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in freshly prepared sodium bisulfite solution (1 in 1000) to obtain a solution having a known concentration of about 20 µg per mL.

Resolution solution—Prepare a solution of epinephrine bitartrate in freshly prepared *Mobile phase* containing 1.0% of sodium bisulfite, having a concentration of about 200 µg per mL. Mix 2.0 mL of this solution and 18.0 mL of the *Standard preparation*.

Assay preparation—Quantitatively dilute an accurately measured volume of Injection with freshly prepared sodium bisulfite solution (1 in 1000) to obtain a solution having a concentration of about 20 µg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.5%. Chromatograph the *Resolution solution:* the relative retention times are about 0.55 for epinephrine and 1.0 for isoproterenol; the resolution, R, for epinephrine and isoproterenol is not less than 3.5; and the tailing factors for the epinephrine and isoproterenol peaks are not more than 2.5.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation*, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of isoproterenol hydrochloride ($C_{11}H_{17}NO_3 \cdot HCl$) in each mL of the Injection taken by the formula:

$$C(L/D)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; L is the labeled quantity, in µg per mL, of isoproterenol hydrochloride in the Injection; D is the concentration, in µg per mL, of isoproterenol hydrochloride in the *Assay preparation*, on the basis of the labeled quantity in each mL and the extent of dilution; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

www.webofpharma.com

Isoproterenol Hydrochloride Tablets

» Isoproterenol Hydrochloride Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{11}H_{17}NO_3 \cdot HCl$.

Packaging and storage—Preserve in well-closed, light-resistant containers.
USP Reference standards ⟨11⟩—
USP Isoproterenol Hydrochloride RS
Identification—Powder a number of Tablets, equivalent to about 50 mg of isoproterenol hydrochloride, digest with 15 mL of hot dehydrated alcohol for 20 minutes, cool, filter, and evaporate the filtrate on a steam bath to dryness: the residue responds to the *Identification*— tests under *Isoproterenol Hydrochloride*.

Dissolution ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{11}H_{17}NO_3 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 279 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Isoproterenol Hydrochloride RS in the same medium.
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{11}H_{17}NO_3 \cdot HCl$ is dissolved in 45 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements.
Procedure for content uniformity—Crush 1 Tablet, and transfer it with the aid of 25 mL of water to a 50-mL volumetric flask. Shake gently until no more dissolves, add water to volume, and mix. Filter through a dry filter into a dry flask, rejecting the first 20 mL of the filtrate. Transfer a portion of the filtrate, equivalent to about 2.5 mg of isoproterenol hydrochloride and accurately measured, to a 50-mL volumetric flask, dilute with water to volume, and mix. Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in water, and dilute quantitatively and stepwise with water to obtain a Standard solution having a known concentration of about 50 µg per mL. Concomitantly and without delay, determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 279 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{11}H_{17}NO_3 \cdot HCl$ in the Tablet taken by the formula:

$$2.5(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the Standard solution; V is the volume, in mL, of the filtrate taken; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—
Mobile phase—Adjust 0.1 M sodium sulfate with phosphoric acid to a pH of 3.0. Mix 90 parts of this solution with 10 parts of methanol.
Standard preparation—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in 0.1 N sulfuric acid, and dilute quantitatively and stepwise with the same solvent to obtain a solution having a known concentration of about 0.1 mg per mL.
Assay preparation—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of isoproterenol hydrochloride, and transfer with the aid of 50 mL of 0.1 N sulfuric acid to a 100-mL volumetric flask. Shake gently until no more dissolves, dilute with the same solvent to volume, and mix. Filter through a dry filter into a dry flask, rejecting the first 20 mL of the filtrate.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph three replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. The retention time is about 3.5 minutes for isoproterenol. Calculate the quantity, in mg, of $C_{11}H_{17}NO_3 \cdot HCl$ in the portion of Tablets taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Delete the following:

▲Isoproterenol Hydrochloride and Phenylephrine Bitartrate Inhalation Aerosol

» Isoproterenol Hydrochloride and Phenylephrine Bitartrate Inhalation Aerosol is a suspension of microfine Isoproterenol Hydrochloride and Phenylephrine Bitartrate in suitable propellants in a pressurized container. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of isoproterenol hydrochloride ($C_{11}H_{17}NO_3 \cdot HCl$) and phenylephrine bitartrate ($C_9H_{13}NO_2 \cdot C_4H_6O_6$).

Packaging and storage—Preserve in small, nonreactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.
USP Reference standards ⟨11⟩—
USP Isoproterenol Hydrochloride RS
USP Phenylephrine Hydrochloride RS

Identification—
A: Place 10 mL of water in a small beaker, and deliver 20 sprays from the Aerosol under the surface of the water, actuating the valve by pressing the tip against the bottom of the beaker. To 5 mL of the solution add 1 drop of dilute sulfuric acid (1 in 200), add 0.5 mL of 0.1 N iodine, allow to stand for 5 minutes, and add 1 mL of 0.1 N sodium thiosulfate: a red-brown color is produced.
B: To the balance of the solution obtained in *Identification* test *A* add 3 mL of *Mercuric sulfate solution*, prepared as

Stop following this

www.webofpharma.com

directed in the test for *Dose uniformity over the entire contents*. Heat on a steam bath for 5 minutes, cool, and add 3 mL of sodium nitrite solution (1 in 80): a deep red color is produced.

Dose uniformity over the entire contents: meets the requirements for *Metered-Dose Inhalers* under *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* ⟨601⟩.

PROCEDURE FOR DOSE UNIFORMITY—

Ferro-citrate solution and *Buffer solution*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Mercuric sulfate solution—While stirring a mixture of 15 g of yellow mercuric oxide and 80 mL of water, slowly add 20 mL of sulfuric acid, and stir until completely dissolved.

Isoproterenol hydrochloride standard preparation—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in dilute sulfuric acid (1 in 1000), and dilute quantitatively and stepwise with the same dilute sulfuric acid as necessary to obtain a solution having a known concentration of about 8 µg per mL.

Phenylephrine hydrochloride standard preparation—Dissolve an accurately weighed quantity of USP Phenylephrine Hydrochloride RS in dilute sulfuric acid (1 in 1000), and dilute quantitatively and stepwise with the same dilute sulfuric acid as necessary to obtain a solution having a known concentration of about 12 µg per mL.

Test preparation for isoproterenol hydrochloride—Discharge the minimum recommended dose into the sampling apparatus, and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of dilute sulfuric acid (1 in 1000), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform, insert the stopper, shake vigorously for 1 minute, and centrifuge for 20 minutes. Use the clear supernatant as directed in the *Procedure for isoproterenol hydrochloride*.

Test preparation for phenylephrine bitartrate—Proceed as directed for *Test preparation for isoproterenol hydrochloride* and use the clear supernatant as directed in the *Procedure for phenylephrine bitartrate*.

Procedure for isoproterenol hydrochloride—Into three separate flasks transfer the *Test preparation for isoproterenol hydrochloride*, 20.0 mL of the *Isoproterenol hydrochloride standard preparation*, and 20.0 mL of dilute sulfuric acid (1 in 1000) to provide the blank. To each flask add 100 µL of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances of the *Test preparation* and the *Standard preparation* against the blank at the wavelength of maximum absorbance at about 530 nm, in 5-cm cells, with a suitable spectrophotometer. Calculate the quantity, in µg, of $C_{11}H_{17}NO_3 \cdot HCl$ contained in the minimum dose taken by the formula:

$$20CN(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Isoproterenol hydrochloride standard preparation*; N is the number of sprays discharged to obtain the minimum dose; and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

Procedure for phenylephrine bitartrate—Into three separate flasks transfer the *Test preparation for phenylephrine bitartrate*, 20.0 mL of the *Phenylephrine hydrochloride standard preparation*, and 20.0 mL of dilute sulfuric acid (1 in 1000) to provide the blank. To each flask add 3.0 mL of *Mercuric sulfate solution*, and mix. Immerse in a water bath maintained between 35° and 40° for 10 minutes, remove, and allow to stand at room temperature for 30 minutes. Add 0.25 mL of a sodium nitrite solution (1 in 80), mix, and allow to stand, with occasional swirling, for 30 minutes. Concomitantly determine the absorbances of the *Test preparation* and the *Standard preparation* against the blank at the wavelength of maximum absorbance at about 495 nm, in 5-cm cells, with a suitable spectrophotometer. Calculate the quantity, in µg, of $C_9H_{13}NO_2 \cdot C_4H_6O_6$ in the minimum dose taken by the formula:

$$(317.30 / 203.67)(20CN)(A_U / A_S)$$

in which 317.30 and 203.67 are the molecular weights of phenylephrine bitartrate and phenylephrine hydrochloride, respectively; C is the concentration, in µg per mL, of USP Phenylephrine Hydrochloride RS in the *Phenylephrine hydrochloride standard preparation*; N is the number of sprays discharged to obtain the minimum dose; and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

Particle size—Prime the valve of the Aerosol by alternately shaking and firing it several times through its oral inhalation actuator, and then actuate one measured spray onto a clean, dry microscope slide held 5 cm from the end of the actuator, perpendicular to the direction of spray. Carefully rinse the slide with about 2 mL of carbon tetrachloride, and allow to dry. Examine the slide under a microscope, equipped with a calibrated ocular micrometer, using 450× magnification. Focus on the particles of 25 fields of view near the center of the sample pattern, and note the size of the great majority of individual particles: they are less than 5 µm in diameter. Record the size of all individual crystalline particles (not agglomerates) more than 10 µm in length measured along the longest axis: not more than 10 such particles are observed.

Assay—

Ferro-citrate solution, *Buffer solution*, and *Mercuric sulfate solution*—Prepare as directed in the test for *Dose uniformity over the entire contents*.

Isoproterenol hydrochloride standard preparation—Transfer about 50 mg of USP Isoproterenol Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add dilute sulfuric acid (1 in 1000) to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, add dilute sulfuric acid (1 in 1000) to volume, and mix. The concentration of USP Isoproterenol Hydrochloride RS in the *Isoproterenol hydrochloride standard preparation* is about 100 µg per mL.

Phenylephrine hydrochloride standard preparation—Transfer about 50 mg of USP Phenylephrine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add dilute sulfuric acid (1 in 1000) to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, add dilute sulfuric acid (1 in 1000) to volume, and mix. The concentration of USP Phenylephrine Hydrochloride RS in the *Phenylephrine hydrochloride standard preparation* is about 100 µg per mL.

Assay preparation— [NOTE—Valve actuation during sampling is to be performed in a manner that will deliver freely the spray into the solvent in the bottom of the sampling beaker, but with minimal lateral pressure on the valve stem to avoid possible side-of-stem leakage. Any devices and techniques designed to accomplish these objectives may be used (e.g., actuating the valve by pressing the tip into an indentation in the bottom of the beaker or using a special adapter to spray at right angles to the valve held at the bottom of the beaker).] Place 20 mL of chloroform in a suitable 100-mL beaker. Prime the valve of the Aerosol by alternately shaking and firing it 10 times through its oral inhalation actuator. Accurately weigh the Aerosol, shake it, and immediately deliver a single spray under the surface of the chloroform. Raise the Aerosol above the surface of the chloroform, and shake it

www.webofpharma.com

gently preparatory to delivering another spray similarly under the surface of the chloroform. Deliver a total of 6 sprays in this manner. Rinse the valve stem and ferrule with about 2 mL of chloroform, collecting the rinsing with the sample in the beaker. Allow the Aerosol to dry, weigh it, and determine the total weight of the 6 sprays. Transfer the solution to a centrifuge tube with the aid of two 3-mL portions of chloroform, and add 10.0 mL of dilute sulfuric acid (1 in 1000). Insert the stopper, shake vigorously for 1 minute, centrifuge for 20 minutes, and use the clear supernatant.

Procedure for isoproterenol hydrochloride—Transfer 3.0 mL each of the *Isoproterenol hydrochloride standard preparation* and the *Assay preparation* to separate test tubes. To each tube add 0.10 mL of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 530 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of isoproterenol hydrochloride ($C_{11}H_{17}NO_3 \cdot HCl$) in each mL of the Aerosol taken by the formula:

$$(0.01Cd / W)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Isoproterenol hydrochloride standard preparation*; d is the density, in g per mL, of Aerosol taken; W is the weight, in g, of the sample taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Isoproterenol hydrochloride standard preparation*, respectively. [The density, d, is determined as follows. Weigh a known volume (v) of the Aerosol in a suitable 5-mL gas-tight syringe equipped with a linear valve. Calibrate the volume of the syringe by filling to the 5-mL mark with dichlorotetrafluoroethane withdrawn from a plastic-coated glass vial sealed with a neoprene multiple-dose rubber stopper and an aluminum seal, using 1.456 g per mL as the density of the calibrating liquid. Maintain the dichlorotetrafluoroethane, the Aerosol sample, and the syringe (protected from becoming wet) at 25° in a water bath. Obtain the sample, equivalent to the same volume as that obtained during the sampling procedure, from the Aerosol by means of a sampling device consisting of a replaceable rubber septum engaged in the plate threads at one end of a threaded fitting, the opposite end of which contains a sharpened tube capable of puncturing the aerosol container, and a rubber gasket around the tube to prevent leakage of the container contents after puncture.* Calculate the density taken by the formula:

$$w / v$$

in which w is the weight of the volume, v, of Aerosol taken.]

Procedure for phenylephrine bitartrate—Transfer 5.0 mL each of the *Phenylephrine hydrochloride standard preparation*, the *Assay preparation*, and a blank consisting of dilute sulfuric acid (1 in 1000), to separate test tubes. To each tube add 3.0 mL of *Mercuric sulfate solution*, and mix. Immerse in a water bath maintained between 35° and 40° for 10 minutes, remove, and allow to stand at room temperature for 30 minutes. Add 0.25 mL of sodium nitrite solution (1 in 80), mix, and allow to stand, with occasional swirling, for 30 minutes. Concomitantly determine the absorbances of the solutions against the blank in 1-cm cells at the wavelength of maximum absorbance at about 495 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of phenylephrine bitartrate ($C_9H_{13}NO_2 \cdot C_4H_6O_6$) in each mL of the Aerosol taken by the formula:

$$(317.30 / 203.67)(0.01Cd / W)(A_U / A_S)$$

in which 317.30 and 203.67 are the molecular weights of phenylephrine bitartrate and phenylephrine hydrochloride, respectively; C is the concentration, in µg per mL, of USP Phenylephrine Hydrochloride RS in the *Phenylephrine hydrochloride standard preparation*; d is the density, in g per mL, of Aerosol taken; W is the weight, in g, of the sample taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Phenylephrine hydrochloride standard preparation*, respectively. ▲2S(USP41)

*A suitable sampling system is available from Alltech Associates, 2051 Waukegan Rd., Deerfield, IL 60015.

Isoproterenol Sulfate

$(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4 \cdot 2H_2O$ 556.62
1,2-Benzenediol, 4-[1-hydroxy-2-[(1-methylethyl)amino] ethyl]-, sulfate (2:1) (salt), dihydrate.
3,4-Dihydroxy-α-[(isopropylamino)methyl]benzyl alcohol sulfate (2:1) (salt) dihydrate [6700-39-6].
Anhydrous 520.60
[299-95-6].

» Isoproterenol Sulfate contains not less than 97.0 percent and not more than 103.0 percent of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—
USP Isoproterenol Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Solution: 50 µg per mL.
Medium: 0.1 N hydrochloric acid.

B: To a solution of 10 mg in 5 mL of water add 1 drop of ferric chloride TS: an intense green color is produced, and it becomes olive-green on standing.

C: To a solution of 10 mg in 1 mL of water add 1 drop of phosphotungstic acid TS: a white precipitate is formed immediately and it becomes brown on standing (*distinction from epinephrine, which forms no precipitate*).

D: Dilute 1.0 mL of a solution (1 in 1000) with water to 10 mL, add 0.1 mL of dilute hydrochloric acid (1 in 120), then add 1.0 mL of 0.10 N iodine. Allow to stand for 5 minutes, and add 2.0 mL of 0.10 N sodium thiosulfate: a salmon pink color is produced (*distinction from norepinephrine, which, at the same pH, about 3, produces no color or at most only a slight pink color*).

E: It responds to the tests for *Sulfate* ⟨191⟩.

Water Determination, *Method I* ⟨921⟩: not more than 7.0%.

Residue on ignition ⟨281⟩: not more than 0.2%.

Chloride ⟨221⟩—A 0.10-g portion shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.14%).

Limit of isoproterenone—It meets the requirements of the test for *Isoproterenone* under *Isoproterenol Hydrochloride.*

www.webofpharma.com

Assay—

Standard preparation—Prepare as directed in the *Assay*—under *Isoproterenol Hydrochloride.*

Assay preparation—Transfer about 125 mg of Isoproterenol Sulfate, accurately weighed, to a 25-mL volumetric flask, dissolve in sodium bisulfite solution (3 in 1000), dilute with sodium bisulfite solution to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.17 N acetic acid to volume, and mix.

Chromatographic system—Proceed as directed for *Procedure* in the *Assay*—under *Isoproterenol Hydrochloride.* Calculate the quantity, in mg, of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ in the portion of Isoproterenol Sulfate taken by the formula:

$$(260.30/247.72)(0.5C)(h_U/h_S)$$

in which 260.30 is one-half of the molecular weight of anhydrous isoproterenol sulfate, 247.72 is the molecular weight of isoproterenol hydrochloride; and C, h_U, and h_S are as defined therein.

Isoproterenol Sulfate Inhalation Aerosol

» Isoproterenol Sulfate Inhalation Aerosol is a suspension of microfine Isoproterenol Sulfate in fluorochlorohydrocarbon propellants in a pressurized container. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isoproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$.

Packaging and storage—Preserve in small, nonreactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

USP Reference standards ⟨11⟩—
USP Isoproterenol Hydrochloride RS

Identification—

A: Place 10 mL of water in a small beaker, and deliver 10 sprays from the Aerosol under the surface of the water, actuating the valve by pressing the tip against the bottom of the beaker. Filter, and to 5 mL of the filtrate add 1 drop of dilute hydrochloric acid (1 in 120). Add 0.50 mL of 0.10 N iodine, allow to stand for 5 minutes, and add 1.0 mL of 0.10 N sodium thiosulfate: a red-brown color is produced.

B: A portion of the filtrate obtained in *Identification* test *A* responds to the tests for *Sulfate* ⟨191⟩.

Microbial enumeration tests ⟨61⟩ **and** ***Tests for specified microorganisms*** ⟨62⟩—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Dose uniformity over the entire contents: meets the requirements for *Metered-Dose Inhalers* under *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* ⟨601⟩.

P*ROCEDURE FOR DOSE UNIFORMITY*—

Ferro-citrate solution and *Buffer solution*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Standard preparation—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in a freshly prepared sodium bisulfite solution (1 in 500), and dilute quantitatively and stepwise with the same sodium bisulfite solution as necessary to obtain a solution having a known concentration of about 4 µg per mL.

Test preparation—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of a freshly prepared sodium bisulfite solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform, insert the stopper, shake vigorously for 1 minute, and centrifuge for 5 minutes. Use the clear supernatant as directed in the *Procedure.*

Procedure—Into three separate flasks transfer the *Test preparation,* 20.0 mL of the *Standard preparation,* and 20.0 mL of water to provide the blank. To each flask add 100 µL of *Ferro-citrate solution* and 1.0 mL of *Buffer solution,* and mix. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 530 nm, against the blank. Calculate the quantity, in µg, of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ contained in the minimum dose taken by the formula:

$$(260.30/247.72)(20CN)(A_U/A_S)$$

in which 260.30 is one-half of the molecular weight of isoproterenol sulfate (anhydrous), and 247.72 is the molecular weight of isoproterenol hydrochloride; C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation; N* is the number of sprays discharged to obtain the minimum recommended dose; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation,* respectively.

Particle size—Prime the valve of the Aerosol by alternately shaking and firing it several times through its oral inhalation actuator, and then actuate one measured spray onto a clean, dry microscope slide held 5 cm from the end of the actuator, perpendicular to the direction of the spray. Carefully rinse the slide with about 2 mL of carbon tetrachloride, and allow to dry. Examine the slide under a microscope, equipped with a calibrated ocular micrometer, using 450 × magnification. Focus on the particles of 25 fields of view near the center of the test specimen pattern, and note the size of the great majority of individual particles: they are less than 5 µm in diameter. Record the number and size of all individual crystalline particles (not agglomerates) more than 10 µm in length measured along the longest axis: not more than 10 such particles are observed.

Assay—

Ferro-citrate solution and *Buffer solution*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Standard preparation—Transfer about 50 mg of USP Isoproterenol Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add a freshly prepared solution of sodium bisulfite (1 in 500) to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with the sodium bisulfite solution to volume, and mix. The concentration of USP Isoproterenol Hydrochloride RS in the *Standard preparation* is about 50 µg per mL.

Assay preparation— [NOTE—A suitable sampling beaker is one having a small indentation formed in its inside bottom surface having dimensions adequate to accept the aerosol valve stem during actuation, thereby preventing particle entrapment and side-of-stem leakage during the sample delivery.] Place 20 mL of chloroform in a suitable 100-mL beaker. Prime the valve of the Aerosol by alternately shaking and firing it 10 times through its oral inhalation actuator. Accurately weigh the Aerosol, shake it, and immediately deliver a single spray under the surface of the chloroform, actuating the valve by pressing the tip into the indentation in the bottom of the beaker. Raise the Aerosol above the surface of the chloroform, and shake it gently preparatory to delivering another spray similarly under the surface of the

USP Monographs

www.webofpharma.com

chloroform. Deliver a total of 5 sprays in this manner. Rinse the valve stem and ferrule with about 2 mL of chloroform, collecting the rinsing with the sample in the beaker. Allow the Aerosol to dry, weigh it, and determine the total weight of the 5 sprays. Transfer the solution to a centrifuge tube with the aid of two 3-mL portions of chloroform, and add 10.0 mL of freshly prepared sodium bisulfite solution (1 in 500). Insert the stopper, shake vigorously for 1 minute, centrifuge for 5 minutes, and use the clear supernatant as directed in the *Procedure*.

Procedure—Transfer 5.0 mL each of the *Standard preparation* and the *Assay preparation* to separate test tubes. To each tube add 100 µL of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 530 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ in each mL of the Aerosol taken by the formula:

$$(260.30/247.72)(0.01Cd/W)(A_U/A_S)$$

in which 260.30 is one-half of the molecular weight of isoproterenol sulfate (anhydrous); 247.72 is the molecular weight of isoproterenol hydrochloride; C is the concentration, in µg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; d is the density, in g per mL, of the Aerosol; W is the weight, in g, of the portion of Inhalation Aerosol taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively. [The density, d, is determined as follows. Weigh a known volume (v) of the Inhalation Aerosol in a suitable, gas-tight, 5-mL syringe equipped with a linear valve. Calibrate the volume of the syringe by filling to the 5-mL mark with dichlorotetrafluoroethane withdrawn from a plastic-coated glass vial sealed with a neoprene multiple-dose rubber stopper and an aluminum seal, using 1.456 g per mL as the density of the calibrating liquid. Maintain the dichlorotetrafluoroethane, the Aerosol assay specimen, and the syringe (protected from becoming wet) in a water bath at 25°. Obtain the specimen equivalent to the same volume as that obtained during the sampling procedure, from the Aerosol by means of a sampling device consisting of a replaceable rubber septum engaged in the plate threads at one end of a threaded fitting, the opposite end of which contains a sharpened tube capable of puncturing the aerosol container, and a rubber gasket around the tube to prevent leakage of the container contents after puncture.* Record the weight of the volume (v) of the Aerosol as w, and calculate the density by the formula w/v.]

Isoproterenol Sulfate Inhalation Solution

» Isoproterenol Sulfate Inhalation Solution is a sterile solution of Isoproterenol Sulfate in Purified Water. It may contain Sodium Chloride. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of isoproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$.

* A suitable sampling system is available from Alltech Associates, 2051 Waukegan Rd., Deerfield, IL 60015.

Packaging and storage—Store in small, tight containers that are well-filled or otherwise protected from oxidation. Protect from light.

Labeling—Label it to indicate that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

USP Reference standards ⟨11⟩—
USP Isoproterenol Hydrochloride RS

Color and clarity—Using the Inhalation Solution as the *Test solution*, proceed as directed for *Color and clarity* under *Isoproterenol Inhalation Solution*.

Identification—It meets the requirements for *Identification* tests *C*, *D*, and *E* under *Isoproterenol Sulfate*.

Sterility Tests ⟨71⟩: meets the requirements.

Assay—

Standard preparation—Prepare as directed in the *Assay* under *Isoproterenol Hydrochloride*.

Assay preparation—Transfer an accurately measured volume of Inhalation Solution, equivalent to about 25 mg of isoproterenol sulfate, to a 100-mL volumetric flask, add 50.0 mL of 0.30 N acetic acid, dilute with water to volume, and mix.

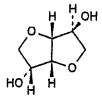
Chromatographic system—Proceed as directed in the *Assay* under *Isoproterenol Hydrochloride*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Isoproterenol Hydrochloride*. Calculate the quantity, in mg, of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ in each mL of the Inhalation Solution taken by the formula:

$$(260.30/247.72)(0.1)(C/V)(h_U/h_S)$$

in which 260.30 is one-half of the molecular weight of anhydrous isoproterenol sulfate; 247.72 is the molecular weight of isoproterenol hydrochloride; V is the volume, in mL, of Inhalation Solution taken; and C, h_U, and h_S are as defined therein.

Isosorbide Concentrate



$C_6H_{10}O_4$ 146.14
D-Glucitol, 1,4:3,6-dianhydro-.
1,4:3,6-Dianhydro-D-glucitol [652-67-5].

» Isosorbide Concentrate is an aqueous solution containing, in each 100 g, not less than 70.0 g and not more than 80.0 g of $C_6H_{10}O_4$.

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—The label states that this article is not intended for direct administration to humans or animals.

USP Reference standards ⟨11⟩—
USP Isosorbide RS

Identification—

[NOTE—Isosorbide is hygroscopic. Take precautions to protect isolated isosorbide crystals from atmospheric moisture.]

A: Dry a portion of it in an evaporating dish over phosphorus pentoxide at 70° and at a pressure of 50 mm of mercury for 48 hours, changing the phosphorus pentoxide

www.webofpharma.com

after 24 hours. Scratch the bottom of the dish with a glass rod or seed with a crystal of isosorbide, if necessary, to initiate crystallization: the crystals so obtained melt between 60° and 63° when tested by the procedure for *Class I* substances (see *Melting Range or Temperature* ⟨741⟩).

B: The IR absorption spectrum of a potassium bromide dispersion of the crystals obtained as directed in *Identification* test *A* exhibits maxima and minima only at the same wavelengths as that of a similar preparation of USP Isosorbide RS.

Specific rotation ⟨781S⟩: between +44.5° and +47.0°.

Test solution: 80 mg of isosorbide per mL, in water.

Water Determination, *Method I* ⟨921⟩: between 24.0% and 26.0%.

Residue on ignition ⟨281⟩: not more than 0.01%.

Periodate consumption—Dilute about 15 g, accurately weighed, with 25 mL of water, and add 50.0 mL of a solution prepared by dissolving 5.4 g of periodic acid in 100 mL of water and adding 1900 mL of glacial acetic acid. Allow to stand for 1 hour. Add 20 mL of potassium iodide TS, and titrate with 0.1 N sodium thiosulfate VS to the disappearance of the brown color. Add 3 mL of starch TS, and complete the titration. Perform a blank determination, and note the difference in volumes required. If the volume required for the specimen is less than 0.8 of that required for the blank, repeat the procedure with a smaller specimen. The difference in volume corresponds to not more than 0.20 mL of 0.1 N sodium thiosulfate for each g of Concentrate taken.

Acid value—Dilute about 15 g, accurately weighed, with 50 mL of water, and titrate with 0.02 N potassium hydroxide VS to a phenolphthalein endpoint. Perform a blank determination, and make any necessary correction. Calculate the acid value taken by the formula:

$$56.11(AN/W)$$

in which A is the number of mL of potassium hydroxide VS consumed; N is its normality; and W is the weight, in g, of Concentrate taken. The limit is 0.5, calculated on the anhydrous basis.

Methyl ethyl ketone—

Internal standard solution—Prepare a solution in water containing about 1 mg per mL of methyl isobutyl ketone.

Standard preparation—Prepare a solution in water containing an accurately known concentration of methyl ethyl ketone equivalent to about 1 mg per mL. Pipet 5 mL of this solution into a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, add water to volume, and mix.

Test preparation—Pipet 5 mL of *Internal standard solution* into a 100-mL volumetric flask, add Concentrate to volume, and mix.

Support—Place about 90 g of unsilanized support S1A in a crystallizing dish, and cover it with chloroform. Stir the mixture thoroughly, and carefully remove the supernatant chloroform with an aspirator. Spread the moist support on a clean surface, and allow it to air-dry. Place the dried support in the crystallizing dish, and cover it with 0.5 N alcoholic potassium hydroxide TS. Allow it to stand for one-half hour with occasional stirring. Carefully pour off the supernatant alcoholic potassium hydroxide solution, and wash the moist support with water until the washing is neutral to phenolphthalein indicator. Spread the moist support on a clean surface, and allow it to air-dry.

Chromatographic system—The gas chromatograph is equipped with a flame-ionization detector and a 0.6-m × 2-mm column packed with 25% liquid phase G16 on unsilanized acid- and base-washed *Support* which has been washed with chloroform, and conditioned as directed (see *Chromatography* ⟨621⟩). The column is maintained at 70°, and nitrogen is used as the carrier gas at a flow rate of about 30 mL per minute. In a suitable chromatogram, the relative standard deviation of five replicate injections is not more than 3.0%, and the resolution is not less than 2.0.

Procedure—Inject about 3 µL of the *Standard preparation* into the gas chromatograph, record the chromatogram, and measure the peak response of each component. [NOTE—Clean the syringe after each injection with pentane. Do not use acetone.] Similarly inject about 3 µL of the *Test preparation*, record the chromatogram, and measure the peak response of each component. Calculate the quantity, in mg, of methyl ethyl ketone in each mL of the Concentrate taken by the formula:

$$(1/0.95)C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of methyl ethyl ketone in the *Standard preparation,* and R_U and R_S are the ratios of the response of the methyl ethyl ketone to the response of the internal standard obtained from the *Test preparation* and the *Standard preparation,* respectively. The limit is 0.05 mg per mL.

Assay—

Internal standard solution—Dissolve a suitable quantity of triethylene glycol in water to obtain a solution containing about 15 mg per mL.

Standard solution—Prepare a solution of USP Isosorbide RS in water containing an accurately known concentration equivalent to about 25 mg of $C_6H_{10}O_4$ per mL.

Standard preparations—Pipet 2-, 3-, 4-, and 5-mL quantities of *Standard solution* into separate 50-mL volumetric flasks, add 5.0 mL of *Internal standard solution* to each, add water to volume, and mix.

Assay preparation—Transfer about 200 mg of Concentrate, accurately weighed, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, add water to volume, and mix.

Chromatographic system—The gas chromatograph is equipped with a flame-ionization detector and a 3-mm × 0.6-m glass column packed with support S9. The column is maintained at 230°, and nitrogen is used as the carrier gas. The retention time of the isosorbide peak is about 1.5, relative to that of triethylene glycol.

System suitability and *standard curve*—Inject 1-µL portions of each *Standard preparation,* and record each peak response. Plot the ratio of the peak response of isosorbide to that of triethylene glycol versus the concentration, in mg per mL, of isosorbide in the respective *Standard preparation.* The analytical system is suitable for conducting the assay if the correlation coefficient for the Standard curve is greater than 0.980, the resolution, R, is not less than 1.5, and neither tailing factor exceeds 2.0.

Procedure—Inject a 1-µL portion of the *Assay preparation,* record the peak responses for the two major peaks, calculate the ratio of the peak responses, and determine the concentration, C, in mg per mL, of isosorbide in the *Assay preparation* by reference to the *Standard curve.* Calculate the quantity, in mg, of $C_6H_{10}O_4$ in the Concentrate taken by the formula:

$$100C.$$

www.webofpharma.com

Isosorbide Oral Solution

» Isosorbide Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isosorbide ($C_6H_{10}O_4$).

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Isosorbide RS
Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatograms of the *Standard preparations,* as obtained in the *Assay.*

Uniformity of dosage units ⟨905⟩—
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

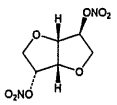
Deliverable volume ⟨698⟩—
FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.
pH ⟨791⟩**:** between 3.2 and 3.8.

Assay—
Internal standard solution, Standard solution, Standard preparations, Chromatographic system, and *System suitability* and *standard curve*—Proceed as directed in the *Assay* under *Isosorbide Concentrate.*
Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 450 mg of isosorbide, to a 250-mL volumetric flask, add 25.0 mL of *Internal standard solution,* then add water to volume, and mix.
Procedure—Proceed as directed for *Procedure* in the *Assay* under *Isosorbide Concentrate.* Calculate the quantity, in mg, of isosorbide ($C_6H_{10}O_4$) in each mL of the Oral Solution taken by the formula:

$$250(C/V)$$

in which C is the concentration, in mg per mL, of isosorbide in the *Assay preparation* found by reference to the *Standard curve;* and V is the volume, in mL, of Oral Solution taken.

Diluted Isosorbide Dinitrate



$C_6H_8N_2O_8$ 236.14
D-Glucitol, 1,4:3,6-dianhydro-, dinitrate;
1,4:3,6-Dianhydro-D-glucitol dinitrate [87-33-2].

DEFINITION
Diluted Isosorbide Dinitrate is a dry mixture of isosorbide dinitrate ($C_6H_8N_2O_8$) with Lactose, Mannitol, or suitable inert excipients to permit safe handling. It may contain up to 1.0% of a suitable stabilizer, such as Ammonium Phosphate. It contains NLT 95.0% and NMT 105.0% of the labeled amount of isosorbide dinitrate ($C_6H_8N_2O_8$). It usually contains approximately 25% of isosorbide dinitrate.
[**CAUTION**—Exercise proper precautions in handling undiluted isosorbide dinitrate, which is a powerful explosive and can be exploded by percussion or excessive heat. Only exceedingly small amounts should be isolated.]

IDENTIFICATION
• **A.**
Sample solution: Transfer to a medium-porosity, sintered-glass filtering crucible a quantity of Diluted Isosorbide Dinitrate, equivalent to about 50 mg of isosorbide dinitrate, and pass three 5-mL portions of acetone through it. Evaporate the combined extracts at a temperature not exceeding 35°, with the aid of a gentle current of air, and dry the residue under vacuum over calcium chloride at room temperature for 16 h. Prepare a solution (1 in 40) of the residue so obtained, in chloroform.
Standard solution: A similar preparation from the residue obtained from USP Diluted Isosorbide Dinitrate RS
Acceptance criteria: The IR absorption spectrum of the *Sample solution,* determined in a 0.1-mm cell, exhibits maxima only at the same wavelengths as that of the *Standard solution.*
• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY
• **PROCEDURE**
Solution A: Methanol and water (6:94)
Solution B: Methanol and water (50:50)
Mobile phase: See *Table 1.*

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2.5	100	0
18.0	60	40
18.1	0	100
20.5	0	100
21.0	100	0
26	100	0

Diluent: Methanol and water (15:85)
Standard solution: 0.25 mg/mL of isosorbide dinitrate prepared as follows. Transfer a suitable portion of USP Diluted Isosorbide Dinitrate RS, equivalent to 25 mg of isosorbide dinitrate, to a 100-mL volumetric flask. Add 10 mL of methanol and sonicate. Dilute with *Diluent* to 60% of the flask volume, and sonicate with occasional shaking until all solids dissolve. Dilute with *Diluent* to volume.
Sample solution: 0.25 mg/mL of isosorbide dinitrate prepared as follows. Transfer a suitable portion of Diluted Isosorbide Dinitrate, equivalent to 25 mg of isosorbide dinitrate, to a 100-mL volumetric flask. Add 10 mL of methanol and sonicate. Dilute with *Diluent* to 60% of the flask volume, and sonicate with occasional shaking until all solids are dissolved. Dilute with *Diluent* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 5-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 3 mL/min
Injection volume: 75 µL
System suitability
Sample: *Standard solution*

www.webofpharma.com

Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 1.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of isosorbide dinitrate ($C_6H_8N_2O_8$) in the portion of Diluted Isosorbide Dinitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of isosorbide dinitrate from the *Sample solution*
r_S = peak response of isosorbide dinitrate from the *Standard solution*
C_S = concentration of isosorbide dinitrate from USP Diluted Isosorbide Dinitrate RS in the *Standard solution* (mg/mL)
C_U = concentration of isosorbide dinitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• **ORGANIC IMPURITIES**
Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard stock solution: 0.3 mg/mL of isosorbide dinitrate prepared as follows. Transfer a suitable portion of USP Diluted Isosorbide Dinitrate RS, equivalent to 30 mg of isosorbide dinitrate, to a 100-mL volumetric flask. Add 10 mL of methanol and sonicate. Dilute with *Diluent* to 60% of the flask volume, sonicate with occasional shaking, and dilute with *Diluent* to volume.
Standard solution: 7.5 µg/mL of isosorbide dinitrate from the *Standard stock solution* in *Diluent*
Sensitivity solution: 0.375 µg/mL of isosorbide dinitrate from the *Standard solution* in *Diluent*
Sample solution: 750 µg/mL of isosorbide dinitrate prepared as follows. Transfer a suitable quantity of Diluted Isosorbide Dinitrate, equivalent to 37.5 mg of isosorbide dinitrate, to a 50-mL volumetric flask. Add 5 mL of methanol, mix, and sonicate. Add *Diluent* to fill 60% of the flask volume, and sonicate with occasional shaking. Allow the solution to equilibrate to room temperature and dilute with *Diluent* to volume.
System suitability
Samples: *Standard solution* and *Sensitivity solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Diluted Isosorbide Dinitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of isosorbide dinitrate from the *Standard solution*
C_S = concentration of isosorbide dinitrate from USP Diluted Isosorbide Dinitrate RS in the *Standard solution* (µg/mL)
C_U = concentration of isosorbide dinitrate in the *Sample solution* (µg/mL)
F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Reporting threshold: 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Isosorbide mononitrate related compound A[a]	0.15	0.61	0.15
Isosorbide mononitrate[b]	0.21	0.61	0.15
Isosorbide dinitrate	1.0	—	—
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

[a] 1,4:3,6-Dianhydro-D-glucitol 2-nitrate.
[b] 1,4:3,6-Dianhydro-D-glucitol 5-nitrate.

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨731⟩
Analysis: Dry under vacuum over calcium chloride at room temperature for 16 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Diluted Isosorbide Dinitrate RS

Isosorbide Dinitrate Extended-Release Capsules

» Isosorbide Dinitrate Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_6H_8N_2O_8$.

Packaging and storage—Preserve in well-closed containers.
USP Reference standards ⟨11⟩—
USP Diluted Isosorbide Dinitrate RS
Identification—The finely powdered contents of the Capsules respond to the *IDENTIFICATION* test under *Isosorbide Dinitrate Tablets*. If separation of interferences is required, transfer a quantity of the finely powdered contents of the Capsules, equivalent to about 20 mg of isosorbide dinitrate, to a glass-stoppered centrifuge tube, add 10 mL of sodium hydroxide solution (1 in 250), shake to wet the powder, add 15 mL of solvent hexane, and shake again. Centrifuge the mixture, and transfer the upper phase to a beaker. Place in a freezer, at a temperature of about −14°, the beaker and a short-stem funnel fitted with a cotton plug that previously has been chloroform-washed and dried. After 30 minutes, filter the solution while still in the freezer. Evaporate the solvent, and dry the residue in vacuum over calcium chloride for 16 hours: the IR absorption spectrum of the residue so obtained, dissolved in 0.4 mL of chloroform and determined with the use of matched 0.1-mm cells, shows all of the significant absorption bands present in the spectrum obtained for a similar preparation from the residue obtained from USP Diluted Isosorbide Dinitrate RS. The major peaks are at about

www.webofpharma.com

1650 cm^{-1}, 1284 cm^{-1} and 1275 cm^{-1} (a doublet), 1106 cm^{-1}, and 844 cm^{-1}.

Dissolution ⟨711⟩—Proceed as directed for *Method B* in *Delayed-Release Dosage Forms* in *Procedure, Apparatus 1 and Apparatus 2,* except to operate the apparatus in the acid medium for 1 hour instead of 2 hours and to use *Acceptance Table 2* in *Extended-Release Dosage Forms* in *Interpretation.*

Apparatus 2: 50 rpm.

Times: 2, 4, and 8 hours.

Determine the amount of $C_6H_8N_2O_8$ dissolved employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of 0.05 M monobasic potassium phosphate and acetonitrile (52:48). Make adjustments, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 224-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph a Standard solution of USP Diluted Isosorbide Dinitrate RS in the same medium, and record the chromatograms as directed for *Procedure:* the tailing factor is not more than 2.5; and the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of a filtered portion of the solution under test, and record the chromatograms. Determine the amount of $C_6H_8N_2O_8$ dissolved in comparison with a Standard solution of USP Diluted Isosorbide Dinitrate RS in the same medium and similarly chromatographed.

Tolerances—The percentages of the labeled amount of $C_6H_8N_2O_8$ dissolved at the times specified conform to *Acceptance Table 2.* [NOTE—The test times given are cumulative, beginning with the 1 hour in the acid medium.]

Time (hours)	Amount dissolved
2	between 10% and 30%
4	between 40% and 75%
8	not less than 75%

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Buffer solution—Dissolve 15.4 g of ammonium acetate in water, add 11.5 mL of glacial acetic acid, dilute with water to 1000 mL, and mix to obtain a solution having a pH of about 4.7.

Mobile phase—Mix 350 mL of water, 100 mL of *Buffer solution,* and 550 mL of methanol. Cool to room temperature, dilute with water to 1000 mL, mix, degas, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer a quantity of diluted nitroglycerin to a suitable volumetric flask, add about 60% of the flask volume of methanol, sonicate for 5 minutes, and shake for 30 minutes. Dilute with methanol to volume to obtain a solution having a concentration of about 3 mg of nitroglycerin per mL, and mix. Allow any undissolved material to settle, filter, and store the filtrate in an airtight container.

Standard preparation—Transfer about 125 mg of recently mixed USP Diluted Isosorbide Dinitrate RS, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of *Mobile phase,* shake for 30 minutes, dilute with *Mobile phase* to volume, and mix. Pipet 10 mL of the resulting solution into a 25-mL volumetric flask, and add 4.0 mL of *Internal standard solution* and 4 mL of dilute *Buffer solution* (1 in 10). Cool to room temperature, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.25 mg of isosorbide dinitrate per mL, based on the quantity of USP Diluted Isosorbide Dinitrate RS weighed and the labeled content of isosorbide dinitrate. Pass a portion of this solution through a 0.45-µm filter.

Assay preparation—Weigh and finely powder the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 12.5 mg of isosorbide dinitrate, to a dry, 50-mL volumetric flask, add about 30 mL of *Mobile phase,* and shake the mixture by hand immediately, to prevent clumping. If clumping persists, disperse with the aid of sonication, or break the aggregates with a stirring rod, or warm on a steam bath while keeping the flask stoppered, or allow the flask to stand until the clumps dissipate. [NOTE—If clumping still continues, discard the mixture, and instead disperse an accurately weighed test portion in 15 mL of a 1 in 10 dilution of *Buffer solution* in water by heating on a steam bath for 1 hour with frequent shaking, then add 15 mL of methanol.] Shake for 30 minutes. Add 8.0 mL of *Internal standard solution,* cool to room temperature, add 8 mL of a 1 in 10 dilution of *Buffer solution* in water, dilute with *Mobile phase* to volume, and mix. Pass a portion through a microporous membrane filter.

Chromatographic system (see Chromatography ⟨621⟩*)*—The liquid chromatograph is equipped with a 220-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure*: the resolution, *R*, between isosorbide dinitrate and nitroglycerin is not less than 2.0; and the relative standard deviation for replicate injections determined from the peak response ratios is not more than 2%. [NOTE—The relative retention times are about 0.75 for isosorbide dinitrate and 1.0 for nitroglycerin. The relative retention times for isosorbide mononitrates, if present, are about 0.38.]

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_6H_8N_2O_8$ in the portion of Capsules taken by the formula:

$$50C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of isosorbide dinitrate from the USP Diluted Isosorbide Dinitrate RS taken for the *Standard preparation;* and R_U and R_S are the ratios of the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Isosorbide Dinitrate Tablets

DEFINITION

Isosorbide Dinitrate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of isosorbide dinitrate ($C_6H_8N_2O_8$).

IDENTIFICATION

Change to read:

- **▲A.▲** (USP 1-May-2019)

Sample: Transfer a suitable quantity of finely powdered Tablets to a glass-stoppered centrifuge tube. Add 10 mL of sodium hydroxide solution (1 in 250), shake to wet the powder, add 15 mL of hexane, and again shake. Centrifuge the mixture, and transfer the upper phase to a beaker. Evaporate the solvent, and dry the residue under vacuum over calcium chloride at room temperature for 16 h.

www.webofpharma.com

Acceptance criteria: The IR absorption spectrum of a suitable solution in chloroform of the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation from the residue obtained from USP Diluted Isosorbide Dinitrate RS.

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **PROCEDURE**

▲**Solution A:** Methanol and water (6:94)

Solution B: Methanol and water (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2.5	100	0
18.0	60	40
18.1	0	100
20.5	0	100
21.0	100	0
26	100	0

Diluent: Methanol and water (15:85)

Standard solution: 0.25 mg/mL of isosorbide dinitrate prepared as follows. Transfer a suitable portion of USP Diluted Isosorbide Dinitrate RS, equivalent to an appropriate amount of isosorbide dinitrate, to a suitable volumetric flask. Add methanol to 10% of the flask volume and sonicate. Dilute with *Diluent* to 60% of the flask volume and sonicate with occasional shaking until the solids are dissolved. Cool to room temperature and dilute with *Diluent* to volume.

Sample solution: Nominally 0.25 mg/mL of isosorbide dinitrate prepared as follows. Transfer a suitable portion of finely powdered Tablets (NLT 10), equivalent to 50 mg of isosorbide dinitrate, to a 200-mL volumetric flask. Add 10 mL of methanol and sonicate. Dilute with *Diluent* to 60% of the flask volume and sonicate with occasional shaking until solids are dissolved. Cool to room temperature and dilute with *Diluent* to volume. Pass the solution through a suitable filter of 0.45-µm pore size and use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 5-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 3 mL/min

Injection volume: 75 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of isosorbide dinitrate ($C_6H_8N_2O_8$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of isosorbide dinitrate from the *Sample solution*

r_S = peak response of isosorbide dinitrate from the *Standard solution*

C_S = concentration of isosorbide dinitrate from USP Diluted Isosorbide Dinitrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of isosorbide dinitrate in the *Sample solution* (mg/mL)▲ (USP 1-May-2019)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

Medium: Water; 1000 mL

Apparatus 2: 75 rpm

Time: 45 min

Mobile phase: Methanol and pH 3.0, 0.1 M ammonium sulfate (1:1). [NOTE—Make adjustments if necessary (see *Chromatography* ⟨621⟩, *System Suitability*), using sulfuric acid for any necessary pH adjustment.]

Standard solution: A known concentration of USP Diluted Isosorbide Dinitrate RS in *Medium*

Sample solutions: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × ▲5▲ (USP 1-May-2019)-cm; packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of isosorbide dinitrate ($C_6H_8N_2O_8$) dissolved.

Tolerances: NLT 70% (Q) of the labeled amount of isosorbide dinitrate ($C_6H_8N_2O_8$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Add the following:

▲• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 0.3 mg/mL of isosorbide dinitrate prepared as follows. Transfer a suitable portion of USP Diluted Isosorbide Dinitrate RS, equivalent to an appropriate amount of isosorbide dinitrate, to a suitable

www.webofpharma.com

volumetric flask. Add methanol to 10% of the flask volume and sonicate. Dilute with *Diluent* to 60% of the flask volume and sonicate with occasional shaking until the solids are dissolved. Cool to room temperature and dilute with *Diluent* to volume.

Standard solution: 7.5 µg/mL of isosorbide dinitrate in *Diluent* from *Standard stock solution*

Sensitivity solution: 0.375 µg/mL of isosorbide dinitrate in *Diluent* from *Standard solution*

Sample solution: Nominally 750 µg/mL of isosorbide dinitrate prepared as follows. Transfer a suitable portion of finely powdered Tablets (NLT 20) equivalent to 75 mg of isosorbide dinitrate, to a 100-mL volumetric flask. Add 10 mL of methanol and sonicate. Dilute with *Diluent* to 60% of the flask volume and sonicate with occasional shaking. Cool to room temperature and dilute with *Diluent* to volume. Pass the solution through a suitable filter of 0.45-µm pore size and use the filtrate.

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of isosorbide dinitrate from the *Standard solution*

C_S = concentration of isosorbide dinitrate from USP Diluted Isosorbide Dinitrate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of isosorbide dinitrate in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Isosorbide mononitrate related compound A[a]	0.15	0.61	0.2
Isosorbide mononitrate[b]	0.21	0.61	0.2
Isosorbide dinitrate	1.0	—	—
Any unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	2.0

[a] 1,4:3,6-Dianhydro-D-glucitol 2-nitrate.

[b] 1,4:3,6-Dianhydro-D-glucitol 5-nitrate. ▲ (USP 1-May-2019)

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. ▲Store at controlled room temperature.▲ (USP 1-May-2019)
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Diluted Isosorbide Dinitrate RS

Isosorbide Dinitrate Chewable Tablets

» Isosorbide Dinitrate Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_6H_8N_2O_8$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Diluted Isosorbide Dinitrate RS

Change to read:

Identification—▲Transfer a suitable quantity of finely powdered Tablets to a glass-stoppered centrifuge tube. Add 10 mL of sodium hydroxide solution (1 in 250), shake to wet the powder, then add 15 mL of solvent hexane, and again shake. Centrifuge the mixture, and transfer the upper phase to a beaker. Evaporate the solvent, and dry the residue in vacuum over anhydrous calcium chloride at room temperature for 16 hours: the IR absorption spectrum of a suitable solution in chloroform of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation from the residue obtained from USP Diluted Isosorbide Dinitrate RS.▲ (ERR 1-May-2018)Where separation of interferences is required, use the technique given under the *Identification* test for *Isosorbide Dinitrate Extended-Release Capsules*.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Change to read:

Assay—

▲*Buffer solution*—Dissolve 15.4 g of ammonium acetate in water, add 11.5 mL of glacial acetic acid, dilute with water to 1000 mL, and mix to obtain a solution having a pH of about 4.7.

Mobile phase—Mix 350 mL of water, 100 mL of *Buffer solution*, and 550 mL of methanol. Cool to room temperature, dilute with water to 1000 mL, mix, degas, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer a quantity of diluted nitroglycerin to a suitable volumetric flask, add about 60% of the flask volume of methanol, sonicate for 5 minutes, and shake for 30 minutes. Dilute with methanol to volume to obtain a solution having a concentration of about 3 mg of nitroglycerin per mL, and mix. Allow any undissolved material to settle, filter, and store the filtrate in an airtight container.

Standard preparation—Transfer about 125 mg of recently mixed USP Diluted Isosorbide Dinitrate RS, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of *Mobile phase*, shake for 30 minutes, dilute with *Mobile phase* to volume, and mix. Pipet 10 mL of the resulting solution into

www.webofpharma.com

a 25-mL volumetric flask, and add 4.0 mL of *Internal standard solution* and 4 mL of dilute *Buffer solution* (1 in 10). Cool to room temperature, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.25 mg of isosorbide dinitrate per mL, based on the quantity of USP Diluted Isosorbide Dinitrate RS weighed and the labeled content of isosorbide dinitrate. Pass a portion of this solution through a 0.45-µm filter. ▲(ERR 1-May-2018)

Assay preparation—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 12.5 mg of isosorbide dinitrate, to a dry, 50-mL volumetric flask, add about 30 mL of *Mobile phase,* and shake the mixture by hand immediately, to prevent clumping. If clumping persists, disperse with the aid of sonication, or break the aggregates with a stirring rod, or warm on a steam bath while keeping the flask stoppered, or allow the flask to stand until the clumps dissipate. [NOTE—If clumping still continues, discard the mixture, and instead disperse an accurately weighed test portion in 15 mL of a 1 in 10 dilution of *Buffer solution* in water by heating on a steam bath for 1 hour with frequent shaking, then add 15 mL of methanol.] Shake for 30 minutes. Add 8.0 mL of *Internal standard solution,* cool to room temperature, add 8 mL of a 1 in 10 dilution of *Buffer solution* in water, dilute with *Mobile phase* to volume, and mix. Pass a portion through a microporous membrane filter.

▲*Chromatographic system* (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure*: the resolution, *R,* between isosorbide dinitrate and nitroglycerin is not less than 2.0; and the relative standard deviation for replicate injections determined from the peak response ratios is not more than 2%. [NOTE—The relative retention times are about 0.75 for isosorbide dinitrate and 1.0 for nitroglycerin. The relative retention times for isosorbide mononitrates, if present, are about 0.38.]▲(ERR 1-May-2018)

Procedure—▲Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.▲(ERR 1-May-2018) Calculate the quantity, in mg, of $C_6H_8N_2O_8$ in the portion of Chewable Tablets taken by the formula:

$$50C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of isosorbide dinitrate from the USP Diluted Isosorbide Dinitrate RS taken for the *Standard preparation;* and R_U and R_S are the ratios of the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Isosorbide Dinitrate Extended-Release Tablets

» Isosorbide Dinitrate Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_6H_8N_2O_8$.

Packaging and storage—Preserve in well-closed containers.

Labeling—When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

USP Reference standards ⟨11⟩—
USP Diluted Isosorbide Dinitrate RS

Identification—Tablets respond to the *Identification* test under *Isosorbide Dinitrate Tablets.* Where separation of interferences is required, use the technique given under the *Identification* test for *Isosorbide Dinitrate Extended-Release Capsules.*

Dissolution ⟨711⟩—

TEST 1—
Medium: water; 500 mL.
Apparatus 2: 50 rpm.
Times: 1, 2, 4, and 6 hours.

Determine the amount of $C_6H_8N_2O_8$ dissolved, using the following method.

pH 3.0 Buffer solution—Add 6.6 g of ammonium sulfate, accurately weighed, to 500 mL of water. Adjust with 1 N sulfuric acid to a pH of 3.0.

Mobile phase—Prepare a filtered and degassed mixture of methanol and *pH 3.0 Buffer solution* (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a UV wavelength detector and a 5-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph a Standard solution of USP Diluted Isosorbide Dinitrate RS in the same *Medium,* and record the chromatograms as directed for *Procedure:* the tailing factor is not more than 2.5; and the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of a filtered portion of the solution under test, and record the chromatograms. Determine the amount of $C_6H_8N_2O_8$ dissolved in comparison with a Standard solution of USP Diluted Isosorbide Dinitrate RS in the same *Medium,* similarly chromatographed.

Tolerances—The percentages of the labeled amount of $C_6H_8N_2O_8$ dissolved at the times specified conform to *Acceptance Table 2.*

Time (hours)	Amount dissolved
1	between 15% and 30%
2	between 50% and 70%
4	between 65% and 85%
6	not less than 75%

TEST 2—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2.*
Medium: pH 1.2 simulated gastric fluid (without pepsin) for the first hour, 900 mL; pH 7.5 simulated intestinal fluid (without enzymes) for the subsequent hours, 900 mL.
Apparatus 2: 50 rpm, with helix sinkers.
Times: 1, 3, 6, and 12 hours.

Determine the amount of isosorbide dinitrate ($C_6H_9NO_6$) dissolved by employing the following method.

Buffer solution and *Mobile phase*—Prepare as directed in the *Assay.*

Standard solution—Prepare two solutions, one in each *Medium.* Dissolve an accurately weighed quantity of USP Diluted Isosorbide Dinitrate RS in *Medium,* and dilute quantitatively, and stepwise if necessary, with *Medium* to

www.webofpharma.com

obtain a solution having a known concentration of about 40 µg per mL.

Test solution—Pass 5 mL of the solution under test through a suitable 10-µm filter. Replace the *Medium* withdrawn at the 3- and 6-hour timepoints.

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay*. Chromatograph the *Standard solution,* and record the chromatogram as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the cumulative percentage of isosorbide dinitrate dissolved at each collection point, corrected for the quantities removed at previous collection points (not applicable for the first hour), as follows:

$$C_U = r_U \times \frac{C_S}{r_S}$$

Percentage released at first hour (see *Formula 1*).
Percentage released at third hour (see *Formula 2*).
Percentage released at sixth hour (see *Formula 3*).
Percentage released at twelfth hour (see *Formula 4*).

in which r_U and r_S are the peak responses for the *Test solution* and the *Standard solution,* respectively; C_U is the sample concentration, in µg per mL, at the indicated collection time point; C_S is the concentration, in µg per mL, of the *Standard solution;* 900 is the volume, in mL, of *Medium;* 1000 is the conversion factor from µg to mg; 100 is the conversion factor to percentage; *LC* is the Tablet label claim, in mg; and 5 is the volume, in mL, of sample withdrawn and of the *Medium* replaced.

Tolerances—The percentages of the labeled amount of $C_6H_8N_2O_8$ dissolved at the times specified conform to *Acceptance Table 2.*

Time (hours)	Amount dissolved
1	between 5% and 25%
3	between 30% and 50%
6	between 50% and 80%
12	not less than 75%

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Buffer solution—Dissolve 15.4 g of ammonium acetate in water, add 11.5 mL of glacial acetic acid, dilute with water to 1000 mL, and mix to obtain a solution having a pH of about 4.7.

Mobile phase—Mix 350 mL of water, 100 mL of *Buffer solution,* and 550 mL of methanol. Cool to room temperature, dilute with water to 1000 mL, mix, degas, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer a quantity of diluted nitroglycerin to a suitable volumetric flask, add about 60% of the flask volume of methanol, sonicate for 5 minutes, and shake for 30 minutes. Dilute with methanol to volume to obtain a solution having a concentration of about 3 mg of nitroglycerin per mL, and mix. Allow any undissolved material to settle, filter, and store the filtrate in an airtight container.

Standard preparation—Transfer about 125 mg of recently mixed USP Diluted Isosorbide Dinitrate RS, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of *Mobile phase,* shake for 30 minutes, dilute with *Mobile phase* to volume, and mix. Pipet 10 mL of the resulting solution into a 25-mL volumetric flask, and add 4.0 mL of *Internal standard solution* and 4 mL of dilute *Buffer solution* (1 in 10). Cool to room temperature, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.25 mg of isosorbide dinitrate per mL, based on the quantity of USP Diluted Isosorbide Dinitrate RS weighed and the labeled content of isosorbide dinitrate. Pass a portion of this solution through a 0.45-µm filter.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the

$$\%\text{released} = C_1 \times \frac{900 \times 100}{1000 \times LC}$$

Formula 1 (Isosorbide Dinitrate Extended-Release Tablets)

$$\%\text{released} = \left[C_3 \times \frac{900 \times 100}{1000 \times LC}\right] + \%\text{released at 1st hour}$$

Formula 2 (Isosorbide Dinitrate Extended-Release Tablets)

$$\%\text{released} = \frac{900 \times 100}{1000 \times LC} \times \left[C_6 + \left(\frac{5 \times C_3}{900}\right)\right] + \%\text{released at 1st hour}$$

Formula 3 (Isosorbide Dinitrate Extended-Release Tablets)

$$\%\text{released} = \frac{900 \times 100}{1000 \times LC} \times \left[C_{12} + \left(\frac{5}{900} \times (C_3 + C_6)\right)\right] + \%\text{released at 1st hour}$$

Formula 4 (Isosorbide Dinitrate Extended-Release Tablets)

Stop Printing Here

www.webofpharma.com

powder, equivalent to about 12.5 mg of isosorbide dinitrate, to a dry, 50-mL volumetric flask, add about 30 mL of *Mobile phase,* and shake the mixture by hand immediately to prevent clumping. If clumping persists, disperse with the aid of sonication, or break the aggregates with a stirring rod, or warm on a steam bath while keeping the flask stoppered, or allow the flask to stand until the clumps dissipate. [NOTE—If clumping still continues, discard the mixture, and instead disperse an accurately weighed test portion in 15 mL of a 1 in 10 dilution of *Buffer solution* in water by heating on a steam bath for 1 hour with frequent shaking, then add 15 mL of methanol.] Shake for 30 minutes. Add 8.0 mL of *Internal standard solution,* cool to room temperature, add 8 mL of a 1 in 10 dilution of *Buffer solution* in water, dilute with *Mobile phase* to volume, and mix. Pass a portion through a microporous membrane filter.

Chromatographic system (see Chromatography ⟨621⟩ *)*—The liquid chromatograph is equipped with a 220-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure*: the resolution, *R,* between isosorbide dinitrate and nitroglycerin is not less than 2.0; and the relative standard deviation for replicate injections determined from the peak response ratios is not more than 2%. [NOTE—The relative retention times are about 0.75 for isosorbide dinitrate and 1.0 for nitroglycerin. The relative retention times for isosorbide mononitrates, if present, are about 0.38.]

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_6H_8N_2O_8$ in the portion of Tablets taken by the formula:

$$50C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of isosorbide dinitrate from the USP Diluted Isosorbide Dinitrate RS taken for the *Standard preparation;* and R_U and R_S are the ratios of the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Isosorbide Dinitrate Sublingual Tablets

» Isosorbide Dinitrate Sublingual Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_6H_8N_2O_8$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—
USP Diluted Isosorbide Dinitrate RS

Change to read:

Identification—▲Transfer a suitable quantity of finely powdered Tablets to a glass-stoppered centrifuge tube. Add 10 mL of sodium hydroxide solution (1 in 250), shake to wet the powder, then add 15 mL of solvent hexane, and again shake. Centrifuge the mixture, and transfer the upper phase to a beaker. Evaporate the solvent, and dry the residue in vacuum over anhydrous calcium chloride at room temperature for 16 hours: the IR absorption spectrum of a suitable solution in chloroform of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation from the residue obtained from USP Diluted Isosorbide Dinitrate RS. ▲ (ERR 1-May-2018)

Disintegration ⟨701⟩: 2 minutes, determined as directed for *Sublingual Tablets.*

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩—
Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 20 minutes.

Mobile phase—Prepare a suitable degassed and filtered mixture of pH 3.0, 0.1 M ammonium sulfate and methanol (50:50).

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 5-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed under *Procedure:* the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and a filtered aliquot of the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of $C_6H_8N_2O_8$ dissolved in comparison with a *Standard solution* having a known concentration of USP Diluted Isosorbide Dinitrate RS, similarly prepared and chromatographed.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_6H_8N_2O_8$ is dissolved in 20 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Change to read:

Assay—

▲*Buffer solution*—Dissolve 15.4 g of ammonium acetate in water, add 11.5 mL of glacial acetic acid, dilute with water to 1000 mL, and mix to obtain a solution having a pH of about 4.7.

Mobile phase—Mix 350 mL of water, 100 mL of *Buffer solution,* and 550 mL of methanol. Cool to room temperature, dilute with water to 1000 mL, mix, degas, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer a quantity of diluted nitroglycerin to a suitable volumetric flask, add about 60% of the flask volume of methanol, sonicate for 5 minutes, and shake for 30 minutes. Dilute with methanol to volume to obtain a solution having a concentration of about 3 mg of nitroglycerin per mL, and mix. Allow any undissolved material to settle, filter, and store the filtrate in an airtight container.

Standard preparation—Transfer about 125 mg of recently mixed USP Diluted Isosorbide Dinitrate RS, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of *Mobile phase,* shake for 30 minutes, dilute with *Mobile phase* to volume, and mix. Pipet 10 mL of the resulting solution into a 25-mL volumetric flask, and add 4.0 mL of *Internal standard solution* and 4 mL of dilute *Buffer solution* (1 in 10). Cool to room temperature, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.25 mg of isosorbide dinitrate per mL, based on the quantity of USP Diluted Isosorbide Dinitrate RS weighed and the labeled content of isosorbide dinitrate. Pass a portion of this solution through a 0.45-µm filter. ▲ (ERR 1-May-2018)

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 12.5 mg of isosorbide dinitrate, to a 50-mL volumetric flask, add about 30 mL of *Mobile*

USP Monographs

www.webofpharma.com

phase, and shake for 30 minutes. Add 8.0 mL of *Internal standard solution,* cool to room temperature, add 8 mL of a 1 in 10 dilution of *Buffer solution* in water, dilute with *Mobile phase* to volume, and mix. Pass a portion through a 0.45-µm filter.

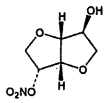
▲*Chromatographic system* (see *Chromatography* ⟨621⟩)— The liquid chromatograph is equipped with a 220-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between isosorbide dinitrate and nitroglycerin is not less than 2.0; and the relative standard deviation for replicate injections determined from the peak response ratios is not more than 2%. [NOTE—The relative retention times are about 0.75 for isosorbide dinitrate and 1.0 for nitroglycerin. The relative retention times for isosorbide mononitrates, if present, are about 0.38.]▲ (ERR 1-May-2018)

Procedure—▲Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.▲ (ERR 1-May-2018) Calculate the quantity, in mg, of $C_6H_8N_2O_8$ in the portion of Tablets taken by the formula:

$$50C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of isosorbide dinitrate from the USP Diluted Isosorbide Dinitrate RS taken for the *Standard preparation;* and R_U and R_S are the ratios of the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Diluted Isosorbide Mononitrate



$C_6H_9NO_6$ 191.14

D-Glucitol, 1,4:3,6-dianhydro-, 5-nitrate.

1,4:3,6-Dianhydro-D-glucitol 5-nitrate [16051-77-7].

» Diluted Isosorbide Mononitrate is a dry mixture of isosorbide mononitrate ($C_6H_9NO_6$) with lactose or other suitable excipients to permit safe handling. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$). [**CAUTION**—Exercise proper precautions in handling undiluted isosorbide mononitrate, which is a powerful explosive and can be exploded by percussion or excessive heat. Only exceedingly small amounts should be isolated.]

Packaging and storage—Preserve in tight containers. Store at a temperature between 20° and 30°.

USP Reference standards ⟨11⟩—

USP Isosorbide RS

[NOTE—The following Reference Standards are dry mixtures of an active component and suitable excipients to permit safe handling. For quantitative applications, calculate the concentration of the active component based on the content stated on the label.]

USP Diluted Isosorbide Dinitrate RS

USP Diluted Isosorbide Mononitrate RS

USP Diluted Isosorbide Mononitrate Related Compound A RS
1,4:3,6-Dianhydro-D-glucitol 2-nitrate.
$C_6H_9NO_6$ 191.14

Identification—

A: Shake a quantity of it, equivalent to about 25 mg of isosorbide mononitrate, with 10 mL of acetone for 5 minutes. Filter, evaporate to dryness at a temperature below 40°, and dry the residue in a vacuum over phosphorus pentoxide for 16 hours: the IR absorption spectrum of a potassium bromide dispersion prepared from the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation from the residue obtained from USP Diluted Isosorbide Mononitrate RS.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Related compounds—

TEST 1—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Standard solution 1—Weigh accurately a quantity of USP Isosorbide RS, and dilute quantitatively, and stepwise if necessary, with absolute alcohol to obtain a solution having a known concentration of about 0.0125 mg of isosorbide per mL.

Standard solution 2—Weigh accurately a quantity of USP Isosorbide RS, and dilute quantitatively, and stepwise if necessary, with absolute alcohol to obtain a solution having a known concentration of about 0.025 mg of isosorbide per mL.

Standard solution 3—Weigh accurately a quantity of USP Isosorbide RS, and dilute quantitatively, and stepwise if necessary, with absolute alcohol to obtain a solution having a known concentration of about 0.05 mg of isosorbide per mL.

Test solution—Transfer a portion of Diluted Isosorbide Mononitrate, equivalent to about 200 mg of isosorbide mononitrate, accurately weighed, to a suitable container, add 20.0 mL of absolute alcohol, sonicate for 10 minutes, and then centrifuge. Use the supernatant.

Application volume: 20 µL.

Developing solvent system: a mixture of absolute alcohol and toluene (8:2).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. After developing, dry the plate with warm air for about 10 minutes, dip the plate in a solution prepared by dissolving 1.25 g of potassium permanganate and 10.0 g of sodium hydroxide in 500 mL of water (prepared fresh for each plate), and heat at 105° for 5 minutes. Any spot in the chromatogram obtained from the *Test solution* and corresponding to the R_f value of the spots obtained from the *Standard solutions* is not more intense than the spot in the chromatogram obtained from *Standard solution 3:* not more than 0.5% of any individual impurity is found. If the spot in the chromatogram obtained from the *Test solution* is nearly as intense as the spot obtained from *Standard solution 3,* further dilute the *Test solution* with absolute alcohol (1:1), repeat the test, and compare the intensity of the isosorbide spot in the diluted *Test solution* with the intensity of the spots obtained from the *Standard solutions,* correcting the percentage level for the additional dilution of the *Test solution.*

TEST 2—

Mobile phase, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay.*

www.webofpharma.com

Isosorbide mononitrate related compound A standard solution—Prepare as directed for *Isosorbide mononitrate related compound A standard preparation* in the *Assay.*
Isosorbide dinitrate standard stock solution—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Dinitrate RS in methanol, sonicate and warm if necessary, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.125 mg of isosorbide dinitrate per mL.
Standard solution—Transfer a quantity of USP Diluted Isosorbide Mononitrate RS, accurately weighed, to a suitable volumetric flask. Dissolve in water, quantitatively add a volume of *Isosorbide mononitrate related compound A standard solution* and a volume of *Isosorbide dinitrate standard stock solution,* and dilute with water to volume to obtain a solution having a known concentration of about 2.0 mg of isosorbide mononitrate per mL, 0.005 mg of isosorbide mononitrate related compound A per mL, and 0.005 mg of isosorbide dinitrate per mL. Filter a portion of the solution, discarding the first few mL of the filtrate.
Test solution—Use the *Assay preparation,* prepared as directed in the *Assay.*
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of isosorbide mononitrate related compound A and isosorbide dinitrate relative to the amount of isosorbide mononitrate in the portion of Diluted Isosorbide Mononitrate taken by the formula:

$$100(CV/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of isosorbide mononitrate related compound A or isosorbide dinitrate, as appropriate, in the *Standard solution; V* is the volume, in mL, of the *Test solution; W* is the amount, in mg, of isosorbide mononitrate in the portion of Diluted Isosorbide Mononitrate used to prepare the *Test solution,* based on the label claim; and r_U and r_S are the peak areas of the corresponding components obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.25% of isosorbide mononitrate related compound A is found; and not more than 0.25% of isosorbide dinitrate is found. Calculate the percentage of each other impurity (other than isosorbide mononitrate related compound A or isosorbide dinitrate) in the portion of Diluted Isosorbide Mononitrate taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak area for each other impurity obtained from the *Test solution;* and r_s is the sum of the areas of all the peaks: not more than 0.5% of total impurities is found, including isosorbide mononitrate related compound A and isosorbide dinitrate; and not more than 0.5% of total impurities is found, the results for *Test 1* and *Test 2* being considered.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of water and methanol (95:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Transfer an accurately weighed quantity of USP Diluted Isosorbide Mononitrate RS to a suitable volumetric flask, dissolve in water, add a volume of methanol equivalent to 4% of the flask volume, and dilute with water to volume to obtain a solution having a known concentration of about 2.0 mg of isosorbide mononitrate per mL.
Isosorbide mononitrate related compound A standard preparation—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Mononitrate Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 1.0 mg of isosorbide mononitrate related compound A per mL. Quantitatively dilute a portion of this solution with water to obtain a solution having a known concentration of about 0.05 mg per mL.
Resolution solution—Transfer 10.0 mL of *Isosorbide mononitrate related compound A standard preparation,* 1.0 mL of the *Standard preparation,* and 4.0 mL of methanol to a 100-mL volumetric flask, and dilute with water to volume. Filter a portion of the solution, discarding the first few mL of the filtrate.
Assay preparation—Transfer an accurately weighed amount of Diluted Isosorbide Mononitrate, equivalent to 100 mg of isosorbide mononitrate, to a 50-mL volumetric flask, dissolve in about 25 mL of water, add 2 mL of methanol, dilute with water to volume, and mix. Filter a portion of the solution, discarding the first few mL of the filtrate.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4-mm × 12.5-cm column that contains packing L1. The flow rate is about 1.5 mL per minute, increasing to 3.0 mL per minute at about 8.5 minutes to ensure that the isosorbide mononitrate peak has completely eluted. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for isosorbide mononitrate related compound A, 1.0 for isosorbide mononitrate, and 4.1 for isosorbide dinitrate; and the resolution, *R,* between isosorbide mononitrate related compound A and isosorbide mononitrate is not less than 2.0. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the isosorbide mononitrate peaks. Calculate the quantity, in mg, of isosorbide mononitrate ($C_6H_9NO_6$) in the portion of Diluted Isosorbide Mononitrate taken by the formula:

$$CV(r_U/r_S)$$

in which C is the concentration, in mg per mL, of isosorbide mononitrate in the *Standard preparation; V* is the volume, in mL, of the *Assay preparation;* and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Isosorbide Mononitrate Tablets

» Isosorbide Mononitrate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$).

Packaging and storage—Preserve in tight containers. Store at a temperature between 20° and 30°.
USP Reference standards ⟨11⟩—
USP Isosorbide RS
[NOTE—The following Reference Standards are dry mixtures of an active component and suitable excipients to permit safe

www.webofpharma.com

handling. For quantitative applications, calculate the concentration of the active component based on the content stated on the label.]
USP Diluted Isosorbide Dinitrate RS
USP Diluted Isosorbide Mononitrate RS
USP Diluted Isosorbide Mononitrate Related Compound A RS
1,4:3,6-Dianhydro-D-glucitol 2-nitrate.
$C_6H_9NO_6$ 191.14

Identification—
A: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Test solution*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 120 mg of isosorbide mononitrate, to a suitable container, add 50.0 mL of absolute alcohol, sonicate for 10 minutes, and then centrifuge. Quantitatively dilute the supernatant (10 in 50) with absolute alcohol.
Standard solution: a solution of USP Diluted Isosorbide Mononitrate RS in absolute alcohol containing 0.5 mg of isosorbide mononitrate per mL.
Application volume: 20 µL.
Developing solvent system: a mixture of chloroform and methanol (95:5).
Spray reagent—Dissolve 1 g of soluble starch in 100 mL of boiling water. Cool, add 0.5 g of potassium iodide, and mix to dissolve.
Procedure—Examine the plate under short-wavelength UV light, marking any observed spots. Visualize nitrates on the plate by spraying with *Spray reagent* and illuminating with short-wavelength UV light for about 10 minutes. Isosorbide mononitrate and other nitrates appear as a violet spot on a white to light violet background.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: water; 900 mL, deaerated.
Apparatus 2: 50 rpm.
Time: 15 minutes.
Determine the amount of $C_6H_9NO_6$ dissolved by employing the following method.
Mobile phase—Proceed as directed in the *Assay.*
Standard solution—Transfer 20 mg, accurately weighed, of USP Diluted Isosorbide Mononitrate RS to a 200-mL volumetric flask, dilute with *Medium* to volume, and mix well. Transfer 20.0 mL of this solution to a 100-mL volumetric flask, dilute with *Medium* to volume, and mix well.
Test solution—Pass a portion of the solution under test through a suitable filter having a porosity of 0.45 µm, discarding the first few mL.
Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.* Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.5%.
Procedure—Proceed as directed in the *Assay.* Calculate the percentage of $C_6H_9NO_6$ dissolved by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times LC}$$

in which r_U and r_S are the peak responses for the *Test solution* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of the *Standard solution;* 900 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and *LC* is the Tablet label claim, in mg.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_6H_9NO_6$ is dissolved in 15 minutes.
Uniformity of dosage units ⟨905⟩: meet the requirements for *Content Uniformity.*

Related compounds—
TEST 1—
Adsorbent, Standard solution 1, Standard solution 2, Standard solution 3, Application volume, and *Developing solvent system*—Prepare as directed in *Related compounds, Test 1* under *Diluted Isosorbide Mononitrate.*
Test solution—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of isosorbide mononitrate, to a suitable container, add 20.0 mL of absolute alcohol, sonicate for 10 minutes, and then centrifuge. Use the supernatant.
Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. After developing, dry the plate with warm air for about 10 minutes, dip the plate in a solution prepared by dissolving 1.25 g of potassium permanganate and 10.0 g of sodium hydroxide in 500 mL of water (prepared fresh for each plate), and heat at 105° for 5 minutes. Any spot in the chromatogram obtained from the *Test solution* and corresponding to the R_F value of the spots obtained from the *Standard solutions* is not more intense than the spot in the chromatogram obtained from *Standard solution 3:* not more than 1.0% of any individual impurity is found. If the spot in the chromatogram obtained from the *Test solution* is nearly as intense as the spot obtained from *Standard solution 3,* further dilute the *Test solution* (1:1) with absolute alcohol, repeat the test, and compare the intensity of the isosorbide spot in the diluted *Test solution* with the intensity of the spots obtained from the *Standard solutions,* correcting the percentage level for the additional dilution of the *Test solution.*
TEST 2—
Mobile phase and *Resolution solution*—Proceed as directed in the *Assay.*
Isosorbide mononitrate related compound A standard stock solution—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Mononitrate Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.1 mg of isosorbide mononitrate related compound A per mL.
Isosorbide dinitrate standard stock solution—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Dinitrate RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.1 mg of isosorbide dinitrate per mL.
Standard solution—Transfer a quantity of USP Diluted Isosorbide Mononitrate RS, accurately weighed, to a suitable volumetric flask. Dissolve in water, quantitatively add a volume of *Isosorbide mononitrate related compound A standard stock solution* and a volume of *Isosorbide dinitrate standard stock solution,* and dilute with water to volume to obtain a solution having a known concentration of about 0.1 mg of isosorbide mononitrate per mL, 0.0005 mg of isosorbide mononitrate related compound A per mL, and 0.0005 mg of isosorbide dinitrate per mL. Filter a portion of the solution, discarding the first few mL of the filtrate.
Test solution—Use the *Assay preparation,* prepared as directed in the *Assay.*
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between isosorbide

USP Monographs

www.webofpharma.com

mononitrate related compound A and isosorbide mononitrate is not less than 1.5. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 10% for the isosorbide mononitrate related compound A and isosorbide dinitrate peaks.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Compare the peak areas of the corresponding impurity obtained from the *Test solution* and the *Standard solution,* respectively. The average peak area of the impurity in the *Test solution* is less than or equal to the average peak area of the corresponding peak in the *Standard solution:* not more than 0.5% of isosorbide mononitrate related compound A is found; and not more than 0.5% of isosorbide dinitrate is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and methanol (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Prepare a solution of USP Diluted Isosorbide Mononitrate RS and USP Diluted Isosorbide Mononitrate Related Compound A RS having a concentration of 0.0005 mg of each of isosorbide mononitrate and isosorbide mononitrate related compound A per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Diluted Isosorbide Mononitrate RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg of isosorbide mononitrate per mL. Pass a portion of this solution through a filter having a 0.45-µm or finer porosity, and use the filtrate.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of isosorbide mononitrate, to a 200-mL volumetric flask, add 100 mL of water, and sonicate for about 10 minutes. Dilute with water to volume, and mix. Pass a portion of this solution through a filter having a 0.45-µm or finer porosity, and use the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between isosorbide mononitrate related compound A and isosorbide mononitrate is not less than 1.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of isosorbide mononitrate ($C_6H_9NO_6$) in the portion of Tablets taken by the formula:

$$200C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of isosorbide mononitrate in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Isosorbide Mononitrate Extended-Release Tablets

DEFINITION

Isosorbide Mononitrate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$).

IDENTIFICATION

• **A. Thin-Layer Chromatographic Identification Test** ⟨201⟩

Standard solution: 0.5 mg/mL of isosorbide mononitrate from USP Diluted Isosorbide Mononitrate RS in absolute alcohol

Sample stock solution: To a portion of the powder from NLT 20 Tablets in a suitable container, nominally equivalent to 120 mg of isosorbide mononitrate, add 50.0 mL of absolute alcohol, sonicate for 10 min, and centrifuge.

Sample solution: Transfer 10 mL of supernatant from the *Sample stock solution* to a 50-mL volumetric flask, and dilute with absolute alcohol to volume.

Chromatographic system

Application volume: 20 µL

Developing solvent system: Chloroform and methanol (95:5)

Spray reagent: Dissolve 1 g of soluble starch in 100 mL of boiling water. Cool, and add 0.5 g of potassium iodide.

Analysis

Samples: *Standard solution* and *Sample solution*

Examine the plate under short-wavelength UV light, marking any observed spots. Visualize nitrates on the plate by spraying with *Spray reagent* and illuminating with short-wavelength UV light for 10 min.

Acceptance criteria: Isosorbide mononitrate and other nitrates appear as a violet spot on a white-to-light-violet background.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*

ASSAY

• **Procedure**

Mobile phase: Methanol and water (200:800)

Standard solution A: 0.15 mg/mL of isosorbide mononitrate related compound A from USP Diluted Isosorbide Mononitrate Related Compound A RS in water

Standard solution B: Equivalent to 0.12 mg/mL of isosorbide mononitrate from USP Diluted Isosorbide Mononitrate RS prepared as follows. Dissolve the sample in water, add methanol equivalent to 20% of the flask volume, then dilute with water to volume.

System suitability solution: Equivalent to 0.12 mg/mL of isosorbide mononitrate and 6 µg/mL of isosorbide mononitrate related compound A prepared as follows. Dissolve a suitable quantity of USP Diluted Isosorbide Mononitrate RS in water in a suitable volumetric flask, add a suitable amount of *Standard solution A* and methanol equivalent to 20% of the flask volume, and dilute with water to volume.

Sample solution: 0.12 mg/mL of isosorbide mononitrate from NLT 20 Tablets finely powdered, prepared as follows. Transfer a portion of the powder, nominally equivalent to 60 mg of isosorbide mononitrate, to a 100-mL volumetric flask. Add 50 mL of methanol, and sonicate for about 30 min with cooling. Warm to ambient temperature, dilute with methanol to volume, and mix. Centrifuge at about 3000 rpm for 10 min. Dilute the supernatant with water, and pass a portion of this solution through a suitable filter of 0.45-µm pore size.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4-mm × 12.5-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Samples: *Standard solution B* and *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between isosorbide mononitrate related compound A and isosorbide mononitrate, *System suitability solution*
Tailing factor: NMT 1.5, *Standard solution B*
Relative standard deviation: NMT 1.5%, *Standard solution B*
Analysis
Samples: *Standard solution B* and *Sample solution*
Calculate the percentage of the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of isosorbide mononitrate from the *Sample solution*
r_S = peak response of isosorbide mononitrate from *Standard solution B*
C_S = concentration of isosorbide mononitrate in *Standard solution B* (mg/mL)
C_U = nominal concentration of isosorbide mononitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩
Test 1
Medium: Water; 900 mL
Apparatus 2: 50 rpm; Tablets are placed in a metal helix prepared by winding 10 in of a 0.8-mm stainless steel wire around a 9/32-in shaft and pulling the coils to form a helix 1 in long.
Times: 1, 2, 4, 8, and 12 h
Mobile phase: Methanol and water (300:700)
Standard solution: (*L*/1000) of USP Diluted Isosorbide Mononitrate RS in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Use portions of the solution under test passed through a suitable nylon filter of 0.45-µm pore size, discarding the first 4–6 mL of the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 1.5%
Analysis
Samples: *Standard solution* and *Sample solution*
Determine the amount, in mg, of isosorbide mononitrate dissolved at each interval:

$$\text{Result} = (r_U/r_S) \times C_S \times V$$

r_U = peak response of isosorbide mononitrate from the *Sample solution*
r_S = peak response of isosorbide mononitrate from the *Standard solution*
C_S = concentration of isosorbide mononitrate in the *Standard solution* (mg/mL)
V = volume of *Medium* in the vessel at each time point (mL)

Calculate the amount, in mg, of isosorbide mononitrate removed by sampling at the previous time points:

$$\text{Result} = \Sigma AD \times (V_S/V)$$

AD = amount of isosorbide mononitrate dissolved at each time point (mg)
V_S = volume of the sample taken (mL)
V = volume of *Medium* in the vessel at each time point (mL)

Calculate the percentage of the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$) dissolved at each time point:

$$\text{Result} = (AD + AR) \times (100/L)$$

AD = amount of isosorbide mononitrate dissolved at each time point (mg)
AR = amount of isosorbide mononitrate removed at the previous time point (mg)
L = label claim (mg/Tablet)

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
1	15–35
2	28–48
4	43–68
8	65–90
12	NLT 80

The percentages of the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.
Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.
Medium: Simulated gastric fluid (without enzymes); 500 mL
Apparatus 2: 50 rpm
Times: 1, 2, 6, and 12 h
Mobile phase: Methanol and water (400:600)
Standard stock solution: 1.2 mg/mL of isosorbide mononitrate from USP Diluted Isosorbide Mononitrate RS diluted in *Medium*
Standard solution: 60 µg/mL of isosorbide mononitrate in *Medium* for Tablets labeled to contain 30 mg, and 120 µg/mL of isosorbide mononitrate in *Medium* for Tablets labeled to contain 60 mg, from the *Standard stock solution*
Sample solution: Pass portions of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC

USP Monographs

www.webofpharma.com

Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 10-µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i), in mg/mL, of isosorbide mononitrate removed at each time point (*i*):

$$\text{Result}_i = (r_{U(i)}/r_S) \times C_S$$

$r_{U(i)}$ = peak response of isosorbide mononitrate from the *Sample solution* at time point *i*
r_S = peak response of isosorbide mononitrate from the *Standard solution*
C_S = concentration of isosorbide mononitrate in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$) dissolved at each time point (*i*):

$$\text{Result} = \left\{C_i \times \left[V_0 - \left((i-1) \times V_t\right)\right] + \left(\sum_{j=1}^{i-1} C_j V_t\right)\right\} \times (100/L)$$

C_i = concentration of isosorbide mononitrate at time point *i* (mg/mL)
V_0 = initial volume of *Medium* (mL)
V_t = volume of sample removed at each sampling time (mL)
C_j = concentration of isosorbide mononitrate at time *j* (mg/mL)
L = label claim (mg/Tablet)

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
1	25–45
2	35–60
6	65–90
12	NLT 80

The percentages of the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.
Medium: Simulated gastric fluid (without enzymes); 500 mL
Apparatus 2: 50 rpm
Times: 1, 2, 6, and 12 h
Buffer: Transfer 15.4 g of ammonium acetate and 11.5 mL of acetic acid to a 1-L volumetric flask containing 500 mL of water. Adjust with acetic acid to a pH of 4.7, and dilute with water to volume.
Mobile phase: Methanol, *Buffer*, and water (300:100:600)
Standard stock solution: 0.12 mg/mL of isosorbide mononitrate from USP Diluted Isosorbide Mononitrate RS in *Medium*
Standard solution: For Tablets labeled to contain 60 mg, use the *Standard stock solution* with no further dilution (0.12 mg/mL). For Tablets labeled to contain 30 mg, prepare 0.06 mg/mL of isosorbide mononitrate in *Medium* from the *Standard stock solution*.
Sample solution: Pass portions of the solution under test through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i), in mg/mL, of isosorbide mononitrate at each time point (*i*):

$$\text{Result}_i = (r_{U(i)}/r_S) \times C_S$$

$r_{U(i)}$ = peak response of isosorbide mononitrate from the *Sample solution* at time point *i*
r_S = peak response of isosorbide mononitrate from the *Standard solution*
C_S = concentration of isosorbide mononitrate in the *Standard solution* (mg/mL)

Calculate the percentage of the label claim of isosorbide mononitrate ($C_6H_9NO_6$) dissolved at each time point (*i*):

$$\text{Result} = \left\{C_i \times \left[V_0 - \left((i-1) \times V_t\right)\right] + \left(\sum_{j=1}^{i-1} C_j V_t\right)\right\} \times (100/L)$$

C_i = concentration of isosorbide mononitrate at time point *i* (mg/mL)
V_0 = initial volume of *Medium* (mL)
V_t = volume of sample removed at each sampling time (mL)
C_j = concentration of isosorbide mononitrate at time *j* (mg/mL)
L = label claim (mg/Tablet)

Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved (%)
1	20–40
2	30–50
6	70–90
12	NLT 85

The percentages of the labeled amount of isosorbide mononitrate dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.
Medium: 0.2% sodium chloride in 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 50 rpm; sinker baskets (see *Dissolution* ⟨711⟩, *Figure 2a*)

www.webofpharma.com

Times: 1, 2, 6, and 12 h
Mobile phase: Methanol and water (180:820)
Standard solution: (*L*/500) mg/mL of isosorbide mononitrate from USP Diluted Isosorbide Mononitrate RS in *Medium*, where *L* is the label claim in mg/Tablet
Sample solution: Pass portions of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 30°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of isosorbide mononitrate ($C_6H_9NO_6$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S$$

r_i = peak response of isosorbide mononitrate from the *Sample solution* at time point *i*
r_S = peak response of isosorbide mononitrate from the *Standard solution*
C_S = concentration of isosorbide mononitrate in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amounts of isosorbide mononitrate ($C_6H_9NO_6$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

C_i = concentration of isosorbide mononitrate in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 500 mL
L = label claim (mg/Tablet)
V_S = volume of *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	20–40
2	2	30–55
3	6	60–90
4	12	NLT 85

The percentage of the labeled amount of isosorbide mononitrate dissolved at the times specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm; helix sinkers
Times: 1, 2, 4, 6, and 10 h
Mobile phase: Methanol and water (150:850)
System suitability solution: 0.033 mg/mL of isosorbide mononitrate from USP Diluted Isosorbide Mononitrate RS in *Medium*. Initially add *Medium* to fill 60% of total volume, shake for 30 min, sonicate for 5 min, then dilute with *Medium* to volume.
Standard solution: 0.067 mg/mL of isosorbide mononitrate from USP Diluted Isosorbide Mononitrate RS in *Medium*. Initially add *Medium* to fill 60% of total volume, shake for 30 min, sonicate for 5 min, then dilute with *Medium* to volume.
Sample solution: Pass portions of the solution under test through a suitable filter.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4-mm × 12.5-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Tailing factor: NMT 1.5
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of isosorbide mononitrate ($C_6H_9NO_6$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S$$

r_i = peak response of isosorbide mononitrate from the *Sample solution* at time point *i*
r_S = peak response of isosorbide mononitrate from the *Standard solution*
C_S = concentration of isosorbide mononitrate in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amounts of isosorbide mononitrate ($C_6H_9NO_6$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_5 = \{(C_5 \times V) + [(C_4 + C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of isosorbide mononitrate in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from *Medium* (mL)

Tolerances: See *Table 5*.

www.webofpharma.com

Table 5

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	20–40
2	2	30–50
3	4	50–70
4	6	65–85
5	10	NLT 80

The percentage of the labeled amount of isosorbide mononitrate dissolved at the times specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium, Apparatus 2, Times, Mobile phase, Standard solution, Sample solution, and **System suitability:** Proceed as directed in *Test 1*.

Chromatographic system: Proceed as directed in *Test 1* except for the *Injection volume*.
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Injection volume: 50 µL

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of isosorbide mononitrate ($C_6H_9NO_6$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = (r_i/r_S) \times C_S$$

r_i = peak response of isosorbide mononitrate from the *Sample solution* at time point *i*
r_S = peak response of isosorbide mononitrate from the *Standard solution*
C_S = concentration of isosorbide mononitrate in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amounts of isosorbide mononitrate ($C_6H_9NO_6$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$
$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$
$$\text{Result}_5 = \{(C_5 \times V) + [(C_4 + C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of isosorbide mononitrate in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from *Medium* (mL)

Tolerances: See *Table 6*.

Table 6

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	15–35
2	2	30–50
3	4	50–70
4	8	75–95
5	12	NLT 80

The percentage of the labeled amount of isosorbide mononitrate dissolved at the times specified conforms to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 7:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

Medium, Apparatus 2, Mobile phase, and **Chromatographic system:** Proceed as directed in *Test 5*.

Times: 1, 4, 8, and 12 h

Standard solution 1: 0.133 mg/mL of isosorbide mononitrate from USP Diluted Isosorbide Mononitrate RS in *Medium*. Initially add *Medium* to fill 60% of total volume, shake for 30 min, sonicate for 5 min, then dilute with *Medium* to volume.

Standard solution 2: 0.067 mg/mL of isosorbide mononitrate from USP Diluted Isosorbide Mononitrate RS in *Medium*. Initially add *Medium* to fill 60% of total volume, shake for 30 min, sonicate for 5 min, then dilute with *Medium* to volume.

Sample solution: Pass portions of the solution under test through a suitable filter.

System suitability
Sample: *Standard solution 1*
Suitability requirements
Relative standard deviation: NMT 2.0%
Tailing factor: NMT 1.5

Analysis
Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*
Calculate the response factor for *Standard solution 1* and *Standard solution 2*:

$$\text{Result} = C_S/r_S$$

C_S = concentration of isosorbide mononitrate in *Standard solution 1* or *Standard solution 2* (mg/mL)
r_S = peak response of isosorbide mononitrate from *Standard solution 1* or *Standard solution 2*

Calculate the concentration (C_i) of isosorbide mononitrate ($C_6H_9NO_6$) in the sample withdrawn from the vessel at time point *i*:

$$\text{Result}_i = r_i \times F_R$$

r_i = peak response of isosorbide mononitrate from the *Sample solution* at time point *i*
F_R = average response factor from *Standard solution 1* and *Standard solution 2*

Calculate the percentage of the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$
$$\text{Result}_2 = \{[C_2 \times (V - V_S)] + (C_1 \times V_S)\} \times (1/L) \times 100$$
$$\text{Result}_3 = (\{C_3 \times [V - (2 \times V_S)]\} + [(C_2 + C_1) \times V_S]) \times (1/L) \times 100$$
$$\text{Result}_4 = (\{C_4 \times [V - (3 \times V_S)]\} + [(C_3 + C_2 + C_1) \times V_S]) \times (1/L) \times 100$$

www.webofpharma.com

C_i = concentration of isosorbide mononitrate in the portion of sample withdrawn at time point *i* (mg/mL)
V = volume of *Medium*, 900 mL
L = label claim (mg/Tablet)
V_S = volume of the *Sample solution* withdrawn from *Medium* (mL)

Tolerances: See *Table 7*.

Table 7

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	15–35
2	4	40–60
3	8	60–80
4	12	NLT 80

The percentages of the labeled amount of isosorbide mononitrate dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 8-Oct-2018)

• **Uniformity of Dosage Units** ⟨905⟩
Procedure for content uniformity: Proceed as directed in the *Assay*, except use 1 Tablet instead of the portion of powdered Tablets used in the *Sample solution*.
Acceptance criteria: Meet the requirements

IMPURITIES

• **Organic Impurities, Procedure 1**
Standard solution A: 0.0125 mg/mL of USP Isosorbide RS in acetonitrile
Standard solution B: 0.025 mg/mL of USP Isosorbide RS in acetonitrile
Standard solution C: 0.05 mg/mL of USP Isosorbide RS in acetonitrile
Sample solution: Equivalent to 5 mg/mL of isosorbide mononitrate from a portion of powdered Tablets (NLT 20) in acetonitrile. Sonicate for 10 min, then centrifuge. Use the supernatant.
Chromatographic system
(See *Chromatography* ⟨621⟩, *General Procedures, Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 20 µL
Developing solvent system: Toluene, ethyl acetate, and isopropyl alcohol (53:32:15)
Detection solution: Dissolve 1.25 g of potassium permanganate and 10.0 g of sodium hydroxide in 500 mL of water (prepared fresh for each plate), and heat at 105° for 5 min.
Analysis
Samples: *Standard solutions* and *Sample solution*
Proceed as directed in the chapter. After developing, dry the plate with warm air for about 10 min, dip the plate in the *Detection solution*, and heat at 105° for 5 min.
Acceptance criteria: Any spot from the *Sample solution* and corresponding to the R_F value of the spots from the *Standard solutions* is not more intense than the spot from *Standard solution C*; NMT 1% of any individual impurity is found.
If the spot from the *Sample solution* is nearly as intense as the spot from *Standard solution C*, further dilute the *Sample solution* with acetonitrile (1:1), repeat the test, and compare the intensity of the isosorbide spot in the diluted *Sample solution* with the intensity of the spots from the *Standard solutions*, correcting the percentage level for the additional dilution of the *Sample solution*.
[Note—The R_F values of isosorbide and isosorbide mononitrate are about 0.2 and 0.6, respectively.]

• **Organic Impurities, Procedure 2**
Mobile phase: Methanol and water (250:750)
Isosorbide mononitrate related compound A standard stock solution: 0.3 mg/mL of isosorbide mononitrate related compound A from USP Diluted Isosorbide Mononitrate Related Compound A RS in water
Isosorbide dinitrate standard stock solution: 0.15 mg/mL of isosorbide dinitrate from USP Diluted Isosorbide Dinitrate RS in methanol
Standard stock solution: 6.0 µg/mL each of isosorbide mononitrate related compound A and isosorbide dinitrate from *Isosorbide mononitrate related compound A standard stock solution* and *Isosorbide dinitrate standard stock solution*, respectively, diluted with water
System suitability solution: Transfer a quantity of USP Diluted Isosorbide Mononitrate RS, equivalent to 24 mg of isosorbide mononitrate, to a 100-mL volumetric flask. Add 10.0 mL of the *Standard stock solution* and 20 mL of methanol, and dilute with water to volume.
Standard solution: Transfer 10.0 mL of the *Standard stock solution* and 20 mL of methanol to a 100-mL volumetric flask. Dilute with water to volume.
Sample solution: Transfer a portion of powder from NLT 20 Tablets, equivalent to 60 mg of isosorbide mononitrate, to a 50-mL volumetric flask. Add 40 mL of methanol, and sonicate for about 30 min with cooling. Warm to ambient temperature, dilute with methanol to volume, and mix. Centrifuge at about 3000 rpm for 10 min. Dilute 10 mL of the supernatant with water to 50 mL. Pass a portion of this solution through a suitable filter of 0.45-µm pore size, and use the filtrate.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—The relative retention times for isosorbide mononitrate related compound A, isosorbide mononitrate, and isosorbide dinitrate are about 0.9, 1.0, and 5.6, respectively.]
Suitability requirements
Resolution: NLT 1.0 between isosorbide mononitrate related compound A and isosorbide mononitrate, *System suitability solution*
Relative standard deviation: NMT 10% for the isosorbide mononitrate related compound A and isosorbide dinitrate peaks, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of isosorbide mononitrate related compound A and isosorbide dinitrate in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of isosorbide mononitrate related compound A or isosorbide dinitrate from the *Sample solution*
r_S = peak area of isosorbide mononitrate related compound A or isosorbide dinitrate from the *Standard solution*

USP Monographs

www.webofpharma.com

C_S = concentration of USP Diluted Isosorbide Mononitrate Related Compound A RS or USP Diluted Isosorbide Dinitrate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of isosorbide mononitrate in the *Sample solution* (mg/mL)

Calculate the percentage of each other impurity (other than isosorbide mononitrate related compound A or isosorbide dinitrate) in the portion of Tablets taken:

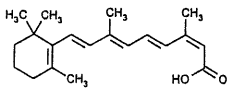
$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each impurity from the *Sample solution*
r_T = sum of all the peak areas from the *Sample solution*

Acceptance criteria
Individual impurities: NMT 0.25% each of isosorbide mononitrate related compound A and isosorbide dinitrate
Total other impurities: NMT 0.25%
Total impurities: NMT 0.5% including isosorbide mononitrate related compound A and isosorbide dinitrate

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at a temperature of 20°–30°.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Isosorbide RS
  The following Reference Standards are dry mixtures of an active component and suitable excipients to permit safe handling. For quantitative applications, calculate the concentration of the active component based on the content stated on the label.
  USP Diluted Isosorbide Dinitrate RS
  USP Diluted Isosorbide Mononitrate RS
  USP Diluted Isosorbide Mononitrate Related Compound A RS
  1,4:3,6-Dianhydro-D-glucitol 2-nitrate.
  $C_6H_9NO_6$ 191.14

Isotretinoin



$C_{20}H_{28}O_2$ 300.44
Retinoic acid, 13-*cis*-.
3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)2-*cis*-4-*trans*-6-*trans*-8-*trans*-nonatetraenoic acid [4759-48-2].

» Isotretinoin contains not less than 98.0 percent and not more than 102.0 percent of $C_{20}H_{28}O_2$, calculated on the dried basis.
[**CAUTION**—Isotretinoin is teratogenic. Avoid inhalation and skin contact.]

Packaging and storage—Preserve in tight containers, under an atmosphere of an inert gas, protected from light.
USP Reference standards ⟨11⟩—
USP Isotretinoin RS
USP Tretinoin RS

[NOTE—Avoid exposure to strong light, and use low-actinic glassware in the performance of the following procedures.]

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197M*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 4 µg per mL.
Medium: acidified isopropyl alcohol (prepared by diluting 1 mL of 0.01 N hydrochloric acid with isopropyl alcohol to 1000 mL). Absorptivities at 354 nm do not differ by more than 3.0%.
Loss on drying ⟨731⟩—Dry it in vacuum at room temperature for 16 hours: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.1%.

Limit of tretinoin—
Mobile phase—Prepare a suitable filtered and degassed mixture of isooctane, isopropyl alcohol, and glacial acetic acid (99.65: 0.25: 0.1).
Standard stock solution—Dissolve an accurately weighed quantity of USP Tretinoin RS in a minimum quantity of methylene chloride, add an amount of isooctane to obtain a solution having a known concentration of about 250 µg per mL, and mix.
Standard solution—Pipet 1 mL of *Standard stock solution* into a 100-mL volumetric flask, add isooctane to volume, and mix.
Test solution—Transfer about 25 mg of Isotretinoin, accurately weighed, to a 100-mL volumetric flask, dissolve in a minimum quantity of methylene chloride, add isooctane to volume, and mix.
System suitability stock solution—Dissolve a quantity of USP Isotretinoin RS in a minimum amount of methylene chloride, add an amount of isooctane to obtain an isotretinoin concentration of about 250 µg per mL, and mix.
System suitability solution—Pipet 1 mL of *Standard stock solution* into a 100-mL volumetric flask, add *System suitability stock solution* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 352-nm detector and a 4.0-mm × 25-cm column containing 5-µm packing L3. The flow rate is about 1 mL per minute. Chromatograph about 20 µL of the *System suitability solution*, and record the peak responses as directed for *Procedure*:the relative retention times for isotretinoin and tretinoin are about 0.84 and 1.00, respectively; the resolution, *R*, between isotretinoin and tretinoin is not less than 2.0; and the relative standard deviation determined from the tretinoin peak response in replicate injections is not more than 2.0%.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of tretinoin by the formula:

$$10(C/W)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Tretinoin RS in the *Standard solution*; W is the weight, in mg, of Isotretinoin taken; and r_U and r_S are the tretinoin peak responses obtained from the *Test solution* and the *Standard*

USP Monographs

www.webofpharma.com

solution, respectively. The content of tretinoin is not more than 1.0%.

Assay—Dissolve about 240 mg of Isotretinoin, accurately weighed, in 50 mL of dimethylformamide, add 3 drops of a solution of thymol blue in dimethylformamide (1 in 100), and titrate with 0.1 N sodium methoxide VS to a greenish endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 30.04 mg of $C_{20}H_{28}O_2$.

Isotretinoin Capsules

DEFINITION

Isotretinoin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$).

[**Caution**—Isotretinoin is teratogenic. Avoid inhalation and skin contact.]

Avoid exposure to strong light, and use low-actinic glassware in the performance of the following procedures.

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

Add the following:

▲• **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ 2S (USP41)

ASSAY

Change to read:

• **Procedure**

Protect the *System suitability solution*, *Standard solution*, *Sample stock solution*, and *Sample solution* from direct light.

Diluent: Heat 0.1 N sodium hydroxide to about 60°–70°. Cool to room temperature, purge with helium or nitrogen, and store in a plastic container.

Solution A: 0.5% acetic acid in methanol

Solution B: 0.5% acetic acid in water

Mobile phase: *Solution A* and *Solution B* (71:29)

System suitability solution: 0.04 mg/mL of USP Isotretinoin RS and 0.02 mg/mL of USP Tretinoin RS in *Diluent*

Standard solution: 0.04 mg/mL of USP Isotretinoin RS in *Diluent*

Sample stock solution: Nominally 0.4 mg/mL of isotretinoin in *Diluent*, prepared as follows. Transfer NLT 10 Capsules to a suitable volumetric flask. Add *Diluent* to the volumetric flask to fill about 50% of the volume, sonicate for 1 h with occasional shaking to disperse all of the contents, and dilute with *Diluent* to volume.

Sample solution: Nominally 0.04 mg/mL of isotretinoin in *Diluent* from the *Sample stock solution*. Pass the solution through a suitable membrane filter of 0.45-µm or finer pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 353 nm. ▲For *Identification B*, use a diode array detector in the range of 230–400 nm.▲ 2S (USP41)

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for isotretinoin and tretinoin are 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the isotretinoin and tretinoin peaks, *System suitability solution*

▲▲ 2S (USP41)

Tailing factor: NMT 2.0 for the isotretinoin peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Isotretinoin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of isotretinoin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩

▲Protect the solutions containing isotretinoin from light.▲ 2S (USP41)

Test 1

Medium

Stage 1: Simulated gastric fluid with pepsin, prepared fresh and purged with nitrogen

Stage 2: 0.13 N sodium hydroxide (5 g/L of sodium hydroxide in water). Prepare fresh, and purge with nitrogen.

Apparatus (see Disintegration ⟨701⟩)**:** No disks; the apparatus is adjusted so that the bottom of the basket-rack assembly descends to 1.0 ± 0.1 cm from the inside bottom surface of the vessel on the downward stroke; the 10-mesh stainless steel cloth in the basket-rack assembly is replaced with a 40-mesh stainless steel cloth; a 10-mesh stainless-steel cloth is fitted to the top of the basket-rack assembly.

Time: 60 min

Standard solution: Transfer 10 mg of USP Isotretinoin RS to a 200-mL volumetric flask. Add 25.0 mL of *Stage 1 Medium* and about 150 mL of *Stage 2 Medium*, sonicate until completely dissolved (about 20 min), and dilute with *Stage 2 Medium* to volume. Pass 20 mL of this solution through a suitable filter, discarding the first 5 mL. Dilute 5.0 mL of the filtrate with *Stage 2 Medium* to 50 mL.

Sample solutions: Perform a dissolution test on each of 6 Capsules. Place 1 Capsule in one of the tubes in each of six basket-rack assemblies. Place each basket in a 1-L beaker containing 100 mL of *Stage 1 Medium* in a bath having a temperature of 37.0 ± 0.5°. Allow to stand for 30 min. Carefully add 800 mL of *Stage 2 Medium* to each beaker. With the disintegration apparatus operating, connect each basket-rack assembly to the drive rod in a timed sequence. After 60 min, withdraw 20 mL of *Medium* (*Stage 1* and *Stage 2*), and immediately pass the solution through a suitable membrane filter of 0.45-µm pore size.

USP Monographs

www.webofpharma.com

Discard the first 5 mL, and collect the solution in argon-charged glassware. Dilute, if necessary, with *Stage 2 Medium* to obtain a theoretical concentration of 5.5 µg/mL of isotretinoin, assuming complete dissolution, based on the label claim.

Capsule shell correction solution: Empty the contents of 3 Capsules. Wash the Capsule shells in several 20-mL aliquots of chloroform. Allow the Capsule shells to air dry. Place the Capsule shells in a 1-L flask containing 100 mL of *Stage 1 Medium* and 800 mL of *Stage 2 Medium*. Allow the flask to stand for about 1 h in a bath with a temperature of 37.0 ± 0.5°, stirring occasionally. Filter, and dilute as described for the *Sample solutions*.

Analysis

Detector: UV 343 nm

Blank: *Medium* (*Stage 1* and *Stage 2*)

Samples: *Standard solution*, *Sample solutions*, and *Capsule shell correction solution*

Determine the amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved, correcting for the Capsule shell absorbance.

Calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved:

$$\text{Result} = [(A_U - A_C)/A_S] \times (C_S/L) \times D \times 100$$

A_U = absorbance of the *Sample solutions*
A_C = absorbance of the *Capsule shell correction solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Isotretinoin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
D = dilution factor for the *Sample solutions*

Tolerances: NLT 80% (*Q*) of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: 0.05 M phosphate buffer, pH 7.8, containing 0.5% (w/v) solid *N,N*-dimethyldodecylamine *N*-oxide; 900 mL

Apparatus 1: 20-mesh basket; 100 rpm

Time: 90 min

Buffer solution: 3.4 mg/mL of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.10 ± 0.05.

Mobile phase: Methanol and *Buffer solution* (81:19)

Standard solution: Transfer about 44 mg of USP Isotretinoin RS to a 100-mL volumetric flask. Add 15 mL of 1-propanol, and sonicate for about 15 min. Add 50 mL of *Medium*, and sonicate for 10 min. Dilute with *Medium* to volume. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, and dilute with *Medium* to volume. Dilute this solution with *Medium* to obtain a final concentration of about (*L*/1000) mg/mL, where *L* is the label claim, in mg/Capsule.

Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 358 nm

Column: 4.6-mm × 5-cm; 5-µm packing L1

Flow rate: 2.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

▲▲2S (USP41)

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

Medium: Borate buffer, pH 8.0, containing 0.5% cetrimide and 50 mg/L of pancreatin. Dissolve 12.37 g of boric acid and 14.91 g of potassium chloride in water, and dilute with water to 1 L. To 250 mL of this solution, add 19.5 mL of 0.2 M sodium hydroxide solution, and dilute with water to 1 L. Adjust with 0.2 M sodium hydroxide to a pH of 8.00 ± 0.05, if necessary. Add 5 g of cetrimide. Just before starting the test, dissolve a quantity of pancreatin to obtain a final concentration of 50 mg/L; 900 mL.

Apparatus 2: 75 rpm, with sinkers

Time: 90 min

Mobile phase: 0.5% acetic acid in methanol and 0.5% acetic acid in water (71:29)

Standard solution: 0.45 mg/mL of USP Isotretinoin RS in 0.1 N sodium hydroxide. Dilute this solution with *Medium* to obtain a final concentration of (*L*/1000) mg/mL, where *L* is the label claim, in mg/Capsule.

Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 353 nm

Column: 4.6-mm × 25-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

▲▲2S (USP41)

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 70% (*Q*) of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) is dissolved.

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.

www.webofpharma.com

Medium: 50 mM monobasic potassium phosphate at pH 7.4 containing 70 mg/L of pancreatin and 4.5% (v/v) of lauryl dimethyl amine oxide as a 30% solution, prepared as follows. Dissolve 6.8 g of monobasic potassium phosphate in 920 mL of water. Adjust by the addition of approximately 35 mL of 1 N sodium hydroxide to a pH of 7.4 ± 0.1. Add 70 mg of pancreatin and 45 mL of 30% solution of lauryl dimethyl amine oxide, and stir gently to mix. The pancreatin should be added on the day of use; 900 mL. [NOTE—Not all of the pancreatin visibly dissolves.]
Apparatus 2: 75 rpm, with spiral coated sinker. [NOTE—A suitable sinker is available as catalog number CAPWHT-02 from www.qla-llc.com.]
Time: 90 min
Mobile phase: Methanol, water, and glacial acetic acid (80: 20: 0.5)
Standard stock solution 1: 0.28 mg/mL of USP Isotretinoin RS, prepared as follows. Transfer USP Isotretinoin RS to a suitable volumetric flask, and add methanol equivalent to 10% of the final volume. Sonicate to dissolve, and dilute with *Medium* to volume.
Standard stock solution 2: 8.8 µg/mL of USP Tretinoin RS, prepared as follows. Transfer USP Tretinoin RS to a suitable volumetric flask, and add methanol equivalent to 20% of the final volume. Sonicate to dissolve, and dilute with *Medium* to volume to obtain a 0.22-mg/mL solution. Transfer 2 mL of this solution to a 50-mL volumetric flask, and dilute with *Medium* to volume.
Standard solution: 0.028 mg/mL of USP Isotretinoin RS in *Medium*, from *Standard stock solution 1*
System suitability solution: 45 µg/mL of USP Isotretinoin RS and 0.88 µg/mL of USP Tretinoin RS in *Medium* from *Standard stock solution 1* and *Standard stock solution 2*
Sample solution: Pass a portion of the solution under test through a suitable PVDF membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 353 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Temperatures
Autosampler: 4°
Column: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for isotretinoin and tretinoin are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the isotretinoin and tretinoin peaks, *System suitability solution*
Tailing factor: 0.8–1.3 for the isotretinoin peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Isotretinoin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (*Q*) of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) is dissolved.
Test 5: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 5*.
Medium: pH 7.7 phosphate buffer containing 50 mg/L of pancreatin and 1.5% *N*,*N*-dimethyldodecylamine-*N*-oxide, prepared as follows. Prepare a solution of 27.2 g/L of monobasic potassium phosphate in water (*Solution A*) and a solution of 8 g/L of sodium hydroxide pellets in water (*Solution B*). Transfer 250 mL of *Solution A* and 215 mL of *Solution B* to a 1000-mL volumetric flask, and dilute with water to volume. Adjust with *Solution B* to a pH of 7.7. Add 50 mg of pancreatin and 15 g of *N*,*N*-dimethyldodecylamine-*N*-oxide to this solution (the pancreatin should be added on the day of use); degassed; 900 mL.
Apparatus 2: 75 rpm, with sinkers
Time: 60 min
Mobile phase: Acetonitrile, water, and glacial acetic acid (85: 15: 0.5)
Standard solution: 0.45 mg/mL of USP Isotretinoin RS in methanol. Dilute this solution with *Medium* to obtain a final concentration of (*L*/900) mg/mL, where *L* is the label claim, in mg/Capsule.
Sample solution: Pass a portion of the solution under test through a suitable PVDF membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 353 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Isotretinoin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (*Q*) of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) is dissolved.
Test 6: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 6*.
▲▲25 (USP41)
Medium: 1% cetrimide (w/v) in pH 6.8 phosphate buffer containing an amount of pancreatin equivalent to 1750 USP Units of protease activity per liter; degassed; 900 mL
Apparatus 2: 100 rpm
Time: 120 min

www.webofpharma.com

Standard stock solutions: Prepare three solutions containing 0.08, 0.2, and 0.32 mg/mL of USP Isotretinoin RS in methanol. Sonicate if necessary.
Standard solutions: Prepare three solutions containing 0.8, 2.0, and 3.2 µg/mL of USP Isotretinoin RS in *Medium* from the *Standard stock solutions*. Pass a portion of the solutions through a suitable PVDF membrane filter of 0.45-µm pore size, and discard the first few milliliters of the filtrate.
Sample stock solution: Pass a portion of the solution under test through a suitable polyethylene full flow filter of 35-µm pore size, and then through a suitable PVDF membrane filter of 0.45-µm pore size. Discard the first few milliliters of the filtrate.
Sample solution: Transfer the *Sample stock solution* to a suitable volumetric flask and dilute with *Medium* to volume according to *Table 1*.

Table 1

Capsule Strength (mg)	Sample Stock Solution (mL)	Final Volume (mL)	Dilution Factor
10	6.0	25.0	4.2
20	3.0	25.0	8.3
30	4.0	50.0	12.5
40	3.0	50.0	16.7

Instrumental conditions
Mode: UV
Analytical wavelength: At maximum absorbance about 348 nm
Blank: *Medium*
Analysis
Samples: *Standard solutions* and *Sample solution*
Calibration curve: Construct a calibration curve of the concentration of USP Isotretinoin RS (µg/mL) versus absorbance from the *Standard solutions*. The linear regression coefficient is NLT 0.99.
Determine the concentration of isotretinoin in the *Sample solution* from the *Calibration curve*, and calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved:

$$\text{Result} = C_U \times V \times (D/L) \times (1/F) \times 100$$

C_U = nominal concentration of isotretinoin in the *Sample solution* (µg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution* (see *Table 1*)
L = label claim (mg/Capsule)
F = conversion factor, 1000 µg/mg

Tolerances: NLT 80% (Q) of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) is dissolved.
• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**
Methylene chloride reagent: Transfer 50 g of sodium bicarbonate to 1000 mL of methylene chloride, shake, and allow to stand overnight. At the time of use, filter suitable portions of this solution, and add 10 mg of butylated hydroxytoluene per mL of solution.
Mobile phase: Hexanes, ethyl acetate, and glacial acetic acid (970: 30: 0.1)
System suitability stock solution: 1 mg/mL each of USP Isotretinoin RS and USP Tretinoin RS in *Methylene chloride reagent*
System suitability solution: 0.01 mg/mL each of USP Isotretinoin RS and USP Tretinoin RS in hexanes from *System suitability stock solution*
Standard stock solution: 0.5 mg/mL of USP Tretinoin RS in *Methylene chloride reagent*
Standard solution: 1.0 µg/mL of USP Tretinoin RS from *Standard stock solution* in hexanes
Sample stock solution: Take a number of Capsules equivalent to about 200 mg of isotretinoin, and with a sharp blade carefully open the Capsules without loss of material. Transfer the contents by pipetting 5 mL of *Methylene chloride reagent* over each Capsule, and rinsing with hexanes. Collect the washings in a 500-mL volumetric flask, dilute with hexanes to volume, and mix.
Sample solution: Nominally 0.1 mg/mL of isotretinoin in hexanes. Transfer 50.0 mL of *Sample stock solution* to a 200-mL volumetric flask, and dilute with hexanes to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 365 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: 1 mL/min
Injection volume
System suitability: 20 µL
Analysis: 50 µL
Run time: NLT 2 times the retention time of isotretinoin
System suitability
Sample: *System suitability solution*
[Note—The relative retention times for isotretinoin and tretinoin are about 0.75 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between isotretinoin and tretinoin
Tailing factor: NMT 2.5 for the isotretinoin peak
Relative standard deviation: NMT 2.0% ▲for the tretinoin peak▲ 2S (USP41)
Analysis
Samples: *Standard solution* and *Sample solution*
▲Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_S = peak response of tretinoin from the *Standard solution*
C_S = concentration of USP Tretinoin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of isotretinoin in the *Sample solution* (mg/mL)▲ 2S (USP41)

Acceptance criteria
▲Any individual impurity: NMT 1.0%
Total impurities: NMT 1.5%▲ 2S (USP41)

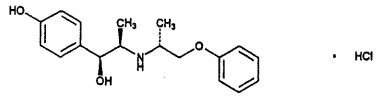
ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, protected from light. Store at controlled room temperature in a dry place.
• **Labeling:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

www.webofpharma.com

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Isotretinoin RS
USP Tretinoin RS

Isoxsuprine Hydrochloride



$C_{18}H_{23}NO_3 \cdot HCl$ 337.84

Benzenemethanol, 4-hydroxy-α-[1-[(1-methyl-2-phenoxyethyl)amino]ethyl]-, hydrochloride, stereoisomer.
p-Hydroxy-α-[1-[(1-methyl-2-phenoxyethyl)amino]ethyl] benzyl alcohol hydrochloride.
(±)-(α*R**)-*p*-Hydroxy-α-[(1*S**)-1-[[(1*S**)-1-methyl-2-phenoxyethyl]amino]ethyl]benzyl alcohol hydrochloride [579-56-6; 34331-89-0].

» Isoxsuprine Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of $C_{18}H_{23}NO_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.
USP Reference standards ⟨11⟩—
USP Isoxsuprine Hydrochloride RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K*▲ (CN 1-May-2020).

Change to read:

B: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)
Solution: 50 µg per mL.
Medium: water.

C: To 1 mL of a solution (1 in 100), obtained by heating as necessary, add 3 mL of a 1 in 15 solution of sodium nitrite in 2 N sulfuric acid. Add ammonium hydroxide dropwise: a yellow precipitate is formed and it dissolves upon the addition of sodium hydroxide solution (1 in 5).

D: To 1 mL of a solution (1 in 100) add 1 mL of phosphomolybdic acid solution (1 in 100): a pale yellow to white precipitate is formed.

pH ⟨791⟩: between 4.5 and 6.0, in a solution (1 in 100).
Loss on drying ⟨731⟩—Dry it at 105° for 1 hour: it loses not more than 0.5% of its weight.
Residue on ignition ⟨281⟩: not more than 0.2%.
Related compounds—To 10 mg, accurately weighed in a suitable vial, add 1 mL of N-trimethylsilylimidazole, and heat at 65° for 10 minutes. Add 5 mL of isooctane, wash with one 3-mL portion of water, and allow the layers to separate. Inject a 2-µL portion of the isooctane solution into a gas chromatograph equipped with a 0.3-cm × 2.0-m glass column packed with packing S1A containing 3% liquid phase G2 and a flame-ionization detector. The column temperature is maintained at 215°, and the injection port and detector are maintained at 250°. The carrier gas is nitrogen, flowing at the rate of 25 mL per minute. Adjust the instrument to provide full-scale response for the major component. Inject a second 2-µL portion of the isooctane solution with the attenuator adjusted to an 8-fold increase in sensitivity, and record the chromatogram from 0.5 to 1.5 relative to the retention time of the major peak. Measure the area of all minor peaks, and correct for differences in sensitivity settings. Calculate the percentage of related compounds present taken by the formula:

$$100A/B$$

in which *A* is the sum of the corrected area peaks for all minor peaks, and *B* is the sum of the corrected area peaks for the major and minor peaks. Not more than 2.0% is found.
Assay—Transfer about 50 mg of Isoxsuprine Hydrochloride, accurately weighed, to a 1000-mL volumetric flask, add water to volume, and mix. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Isoxsuprine Hydrochloride RS in the same medium having a known concentration of about 50 µg per mL in 1-cm cells at the wavelengths of maximum absorbance at about 269 and 300 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{18}H_{23}NO_3 \cdot HCl$ in the Isoxsuprine Hydrochloride taken by the formula:

$$C(A_{U269} - A_{U300})/(A_{S269} - A_{S300})$$

in which *C* is the concentration, in µg per mL, of USP Isoxsuprine Hydrochloride RS in the Standard solution; and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the assay solution (*U*) and the Standard solution (*S*), respectively.

Isoxsuprine Hydrochloride Injection

» Isoxsuprine Hydrochloride Injection is a sterile solution of Isoxsuprine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{18}H_{23}NO_3 \cdot HCl$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.
USP Reference standards ⟨11⟩—
USP Isoxsuprine Hydrochloride RS
Identification—To a 60-mL separator transfer 10 mL of pH 9.0 buffer (prepared by mixing equal volumes of 0.1 M monobasic potassium phosphate and 0.1 N sodium hydroxide and, using a pH meter, adjusting to a pH of 9.0 by adding, as necessary, more of either solution) add 1 mL of Injection, and mix. Add 2 mL of chloroform, shake vigorously for 1 minute, filter the chloroform extract through a pledget of cotton, and mix the filtrate with 500 mg of potassium bromide. Evaporate the chloroform, carefully removing the last trace of solvent in a small vacuum flask: the IR absorption spectrum of a potassium bromide dispersion of the isoxsuprine so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Isoxsuprine Hydrochloride RS that has been treated in the same manner.
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 35.70 USP Endotoxin Units per mg of isoxsuprine hydrochloride.
pH ⟨791⟩: between 4.9 and 6.0.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

USP Monographs

www.webofpharma.com

Assay—

pH 4.0 Citrate buffer—Mix equal volumes of 0.5 M citric acid and 0.5 M sodium citrate, and adjust, by the addition of either solution as necessary, the pH of the solution to 4.0 ± 0.2.

Mixed solvent—Shake 40 mL of ether, 160 mL of isooctane, and 10 mL of water in a separator, remove and discard the water phase, and pass the solvent phase through a large pledget of cotton to remove excess water.

Standard preparation—Transfer about 40 mg of USP Isoxsuprine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 2 N sulfuric acid to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with 2 N sulfuric acid to volume, and mix. The concentration of USP Isoxsuprine Hydrochloride RS in the *Standard preparation* is about 80 µg per mL.

Chromatographic column—Proceed as directed in *Chromatography* ⟨621⟩, *General Procedures, Column Chromatography*, packing a chromatographic tube with two segments of packing material. The lower segment is a mixture of 2 g of *Solid Support* and 1 mL of *pH 4.0 Citrate buffer*, and the upper segment is a mixture prepared as directed under *Assay preparation*.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 4 mg of isoxsuprine hydrochloride, to a 100-mL beaker, add 1 mL of dimethyl sulfoxide, and allow to stand for about 10 minutes, with occasional swirling. Add 1 mL of *pH 4.0 Citrate buffer* and 3 g of *Solid Support*, mix as directed under *Chromatographic column*, and transfer to the column. Pass 75 mL of *Mixed solvent* through the column, and discard the eluate. Elute the column with a solution prepared by mixing 0.2 mL of bis(2-ethylhexyl)phosphoric acid with 75 mL of *Mixed solvent*, and collect the eluate in a 125-mL separator. Extract the eluate with two 20-mL portions of 2 N sulfuric acid. Transfer the extracts to a 50-mL volumetric flask, dilute with 2 N sulfuric acid to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 275 nm, with a suitable spectrophotometer, using a column blank, prepared with *Mixed solvent*, to set the instrument. Calculate the quantity, in mg, of $C_{18}H_{23}NO_3 \cdot HCl$ in the portion of Injection taken by the formula:

$$0.05C(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Isoxsuprine Hydrochloride RS in the *Standard preparation;* and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Isoxsuprine Hydrochloride Tablets

» Isoxsuprine Hydrochloride Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{18}H_{23}NO_3 \cdot HCl$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Isoxsuprine Hydrochloride RS

Identification—Transfer a portion of finely powdered Tablets, equivalent to about 10 mg of isoxsuprine hydrochloride, to a 60-mL beaker, add about 20 mL of water, mix, and filter. Transfer the clear filtrate to a 60-mL separator, add 10 mL of pH 9.0 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), and shake vigorously to mix. Extract with 2 mL of chloroform, filter the extract through a pledget of cotton, and mix the filtrate with 500 mg of potassium bromide. Evaporate the chloroform, carefully removing the last trace of solvent in a small vacuum flask: the IR absorption spectrum of a potassium bromide dispersion of the isoxsuprine so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Isoxsuprine Hydrochloride RS that has been treated in the same manner.

Dissolution ⟨711⟩—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{18}H_{23}NO_3 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 269 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Isoxsuprine Hydrochloride RS in the same medium.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{18}H_{23}NO_3 \cdot HCl$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Buffer solution—Transfer about 1.32 g of anhydrous dibasic ammonium phosphate to a 1-liter volumetric flask, add about 950 mL of water, and mix. Adjust with phosphoric acid to a pH of 7.5, dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed mixture of methanol and *Buffer solution* (2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Isoxsuprine Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.4 mg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of isoxsuprine hydrochloride, to a 50-mL volumetric flask, and add about 25 mL of *Mobile phase*. Shake by mechanical means for 30 minutes, sonicate for ten minutes to dissolve, dilute with *Mobile phase* to volume, mix, and filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 274-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 1800 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

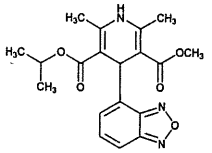
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{23}NO_3 \cdot HCl$ in the portion of Tablets taken by the formula:

$$50C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Isoxsuprine Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

www.webofpharma.com

Isradipine



$C_{19}H_{21}N_3O_5$ 371.39

3,5-Pyridinedicarboxylic acid, 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-, methyl 1-methylethyl ester, (±)-;
Isopropyl methyl (±)-4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate [75695-93-1].

DEFINITION

Isradipine contains NLT 98.0% and NMT 102.0% of isradipine ($C_{19}H_{21}N_3O_5$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)
- **B.** The retention time of the isradipine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Use low-actinic glassware throughout this procedure, and otherwise protect the test specimen, the Reference Standard, and all solutions containing them from unnecessary exposure to light.

Mobile phase: Methanol, tetrahydrofuran, and water (400:100:500)

Standard solution: 0.2 mg/mL of USP Isradipine RS and 0.01 mg/mL of USP Isradipine Related Compound A RS in *Mobile phase*. If necessary, use sonication and/or add 1 mL of methanol per 20 mL of *Mobile phase* to dissolve the Reference Standards.

Sample solution: 0.2 mg/mL of Isradipine prepared as follows. Transfer 20 mg of Isradipine to a 100-mL volumetric flask. Add sufficient methanol to dissolve, and sonicate if necessary. Dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 326 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 1.7 mL/min
Injection volume: 25 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between isradipine and isradipine related compound A
Relative standard deviation: NMT 1.5%

Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of isradipine ($C_{19}H_{21}N_3O_5$) in the portion of Isradipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of isradipine from the *Sample solution*
r_S = peak response of isradipine from the *Standard solution*
C_S = concentration of USP Isradipine RS in the *Standard solution* (mg/mL)
C_U = concentration of Isradipine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%
- **ORGANIC IMPURITIES**

Use low-actinic glassware throughout this procedure, and otherwise protect the test specimen, the Reference Standard, and all solutions containing them from unnecessary exposure to light.

Mobile phase: Prepare as directed in the *Assay*.

System suitability solution: 0.2 mg/mL of USP Isradipine RS and 0.01 mg/mL of USP Isradipine Related Compound A RS in *Mobile phase*. If necessary, use sonication and/or add 1 mL of methanol per 20 mL of *Mobile phase* to dissolve the Reference Standards.

Standard solution: 6 µg/mL of USP Isradipine RS in *Mobile phase*. If necessary, use sonication and/or add 1 mL of methanol per 20 mL of *Mobile phase* to dissolve the Reference Standards.

Sample solution: 2 mg/mL of Isradipine prepared as follows. Transfer 50 mg of Isradipine to a 25-mL volumetric flask, and add 5.0 mL of methanol to dissolve. Sonicate if necessary, and dilute with *Mobile phase* to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm × 10-cm; packing L1
Flow rate: 1.7 mL/min
Injection volume: 25 µL
Run time: NLT 3 times the retention time of isradipine for the *Sample solution*

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 1.5 between isradipine and isradipine related compound A
Relative standard deviation: NMT 1.5%

Analysis
Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The sum of the peak responses, other than that of isradipine, of the *Sample solution* is NMT 4 times the isradipine response from the *Standard solution* (NMT 1.2%); the response of the largest peak, other than that of isradipine, of the *Sample solution* is NMT 1.6 times greater than the isradipine response from the *Standard solution* (NMT 0.5%); no other peak response, other than that of isradipine, is greater than the isradipine response from the *Standard solution* (NMT 0.3%).

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨731⟩

Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Isradipine RS
USP Isradipine Related Compound A RS
Isopropyl methyl 4-(4-benzofurazanyl)-2,6-dimethyl-3,5-pyridinedicarboxylate.
$C_{19}H_{19}N_3O_5$ 369.38

USP Monographs

www.webofpharma.com

Isradipine Capsules

» Isradipine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isradipine ($C_{19}H_{21}N_3O_5$).

Packaging and storage—Store in a tight container at controlled room temperature. Protect from light.

USP Reference standards ⟨11⟩—
USP Isradipine RS
USP Isradipine Related Compound A RS
Isopropyl methyl 4-(4-benzofurazanyl)-2,6-dimethyl-3,5-pyridinedicarboxylate.
$C_{19}H_{19}N_3O_5$ 369.38

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Ultraviolet-Visible Spectroscopy: 197U*▲ (CN 1-May-2020)—
Medium: methanol.
Solution—Transfer the contents of 1 Capsule into a suitable volumetric flask, dissolve the contents in the *Medium* by mechanical shaking for 15 minutes, and dilute with *Medium* to obtain a solution containing 25 µg of isradipine per mL.
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: 0.1% aqueous solution of lauryl dimethyl amine oxide (prepared by transferring 500 mL of deaerated water into the dissolution vessel, adding 1.65 mL of 30% lauryl dimethyl amine oxide, and mixing); 500 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Procedure—Determine the amount of $C_{19}H_{21}N_3O_5$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 328 nm on filtered portions of the solution under test, suitably diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Isradipine RS in the same *Medium.*
Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{19}H_{21}N_3O_5$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.
[NOTE—Isradipine is light sensitive. Throughout the following procedures, protect test or assay specimens, the Reference Standards, and solutions containing them from unnecessary exposure to light. Use low-actinic glassware, unless otherwise directed.]

Chromatographic purity—
Mobile phase, Resolution solution, and *Chromatographic system*—Proceed as directed in the test for *Chromatographic purity* under *Isradipine.*
Standard solution—Dissolve an accurately weighed quantity of USP Isradipine RS in *Mobile phase,* with the aid of sonication if necessary, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1 µg per mL. [NOTE—If necessary, use 1 mL of methanol per 20 mL of *Mobile phase* to dissolve the Reference Standard prior to diluting with *Mobile phase.*]
Test solution—Use the *Assay preparation.*
Procedure—Separately inject equal volumes (about 25 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for all the peaks: the sum of all peak responses, other than that of isradipine, from the *Test solution* is not more than four times the isradipine response obtained from the *Standard solution* (2.0%); and no single peak response is greater than that of the isradipine peak response obtained from the *Standard solution* (0.5%).

Assay—
Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Isradipine.*
Assay preparation—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and mix the combined contents. Transfer an accurately weighed quantity, equivalent to about 25 mg of isradipine, to a 100-mL volumetric flask. Add 5.0 mL of methanol and 5.0 mL of *Mobile phase,* and sonicate at room temperature for 15 minutes. Shake for 15 minutes in a mechanical shaker. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 5 mL of the filtrate.
Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of isradipine ($C_{19}H_{21}N_3O_5$) in the portion of Capsules taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Isradipine RS in the *Standard preparation;* and r_U and r_S are the isradipine peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Isradipine Compounded Oral Suspension

DEFINITION
Isradipine Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of isradipine ($C_{19}H_{21}N_3O_5$).

Prepare Isradipine Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Isradipine capsules[a] or powder,[b] equivalent to	100 mg of isradipine
Glycerin, *USP*	3 mL
Syrup, *NF,* a sufficient quantity to make	100 mL

[a] DynaCirc 5-mg capsules, Sandoz Pharmaceuticals, East Hanover, NJ.
[b] Isradipine powder, Sandoz, East Hanover, NJ.

Calculate the required quantity of each ingredient for the total amount to be prepared. If using *Isradipine capsules,* empty the required number in a suitable mortar, or use *Isradipine powder.* Add sufficient *Glycerin* to wet the powder, and triturate to a fine paste. Add the *Syrup* in small portions. Add increasing volumes of the *Syrup* to make an isradipine liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Syrup* to bring to final volume, and mix well.

www.webofpharma.com

ASSAY

• **PROCEDURE**

Mobile phase: Methanol, tetrahydrofuran, and water (42:20:38). Filter, and degas.

Diluent: Prepare a solution of methanol and 95% ethanol (50:50).

Standard stock solution: 1.0 mg/mL of USP Isradipine RS in *Diluent*

Standard solution: Prepare 0.1 mg/mL of isradipine from *Standard stock solution* and *Diluent*, and pass through a filter of 0.22-µm pore size.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 0.1 mg/mL of isradipine from Oral Suspension and *Diluent*, and pass through a filter of 0.22-µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for isradipine is about 6.1 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of isradipine ($C_{19}H_{21}N_3O_5$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Isradipine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of isradipine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **PH** ⟨791⟩: 5.5–6.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator.

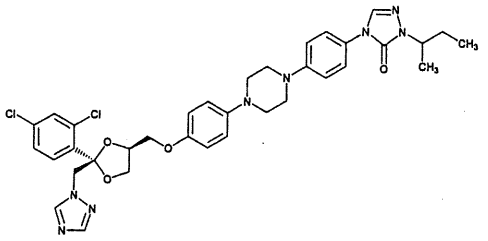
• **BEYOND-USE DATE:** NMT 30 days after the date on which it was compounded, when stored in a refrigerator

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Isradipine RS

Itraconazole



$C_{35}H_{38}Cl_2N_8O_4$ 705.63

3*H*-1,2,4-Triazol-3-one, 4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methylpropyl)-;

(±)-1-*sec*-Butyl-4-[*p*-[4-[*p*-[[(2*R**,4*S**)-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-Δ²-1,2,4-triazolin-5-one [84625-61-6].

DEFINITION

Itraconazole contains NLT 98.0% and NMT 102.0% of itraconazole ($C_{35}H_{38}Cl_2N_8O_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Prepare the solutions containing itraconazole immediately before use.

Solution A: 13.6 g/L of tetrabutylammonium hydrogen sulfate in water

Solution B: Acetonitrile

Diluent: Dilute 4.0 mL of hydrochloric acid with methanol to 1 L.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
12	50	50
16	80	20
20	80	20

Standard solution: 0.3 mg/mL of USP Itraconazole RS in *Diluent*

Sample solution: 0.3 mg/mL of Itraconazole in *Diluent*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

www.webofpharma.com

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of itraconazole ($C_{35}H_{38}Cl_2N_8O_4$) in the portion of Itraconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of itraconazole from the *Sample solution*
r_S = peak response of itraconazole from the *Standard solution*
C_S = concentration of USP Itraconazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Itraconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩
Sample: 1.0 g
Acceptance criteria: NMT 0.1%
• **Organic Impurities**
Solution B, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.
Solution A: 27.2 g/L of tetrabutylammonium hydrogen sulfate in water
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
2	80	20
22	50	50
27	50	50

System suitability solution: 10 mg/mL of USP Itraconazole System Suitability Mixture RS in *Diluent*
Standard solution: 10.0 µg/mL of USP Itraconazole RS in *Diluent*
Sample solution: 10 mg/mL of Itraconazole in *Diluent*
System suitability
Sample: *System suitability solution*
Suitability requirements
Peak-to-valley ratio: NLT 1.5
Calculate the peak-to-valley ratio (*p/v*), using the following:

$$p/v = H_p/H_v$$

H_p = height above the baseline of the peak due to the *n*-butyl isomer impurity
H_v = height above the baseline of the lowest point of the curve separating this peak from the itraconazole peak

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Itraconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of itraconazole from the *Standard solution*
C_S = concentration of USP Itraconazole RS in the *Standard solution* (mg/mL)
C_U = concentration of Itraconazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*. Disregard any peak less than 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
4-Methoxy derivative[a]	0.20	0.5
4-Triazolyl isomer[b]	0.74	0.5
Propyl analog[c]	0.86	0.5
Isopropyl analog[d]	0.86	0.5
Epimer[e]	0.93	0.5
Itraconazole	1.0	—
n-Butyl isomer[f]	1.05	0.5
Didioxolanyl analog[g]	1.3	0.5
Any unspecified impurity	—	0.10
Total impurities	—	1.25

[a] 2-*sec*-Butyl-4-{4-[4-(4-methoxyphenyl)piperazin-1-yl]phenyl}-2*H*-1,2,4-triazol-3(4*H*)-one.
[b] 4-(4-{4-[4-(((2*RS*,4*SR*)-2-[(4*H*-1,2,4-Triazol-4-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-*sec*-butyl-2*H*-1,2,4-triazol-3(4*H*)-one.
[c] 4-(4-{4-[4-(((2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-propyl-2*H*-1,2,4-triazol-3(4*H*)-one.
[d] 4-(4-{4-[4-(((2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-isopropyl-2*H*-1,2,4-triazol-3(4*H*)-one.
[e] 4-(4-{4-[4-(((2*RS*,4*RS*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-*sec*-butyl-2*H*-1,2,4-triazol-3(4*H*)-one.
[f] 4-(4-{4-[4-(((2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-butyl-2*H*-1,2,4-triazol-3(4*H*)-one.
[g] 4-(4-{4-[4-(((2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-({(2*RS*,4*SR*)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methyl)-2*H*-1,2,4-triazol-3(4*H*)-one.

SPECIFIC TESTS

• **Optical Rotation** ⟨781A⟩, *Procedures, Angular Rotation*
Sample: 100 mg/mL in methylene chloride. [Note—Use methylene chloride to zero the instrument.]
Acceptance criteria: −0.10° to +0.10° at 20°
• **Loss on Drying** ⟨731⟩
Sample: Dry 1 g at 105° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at room temperature.
• **USP Reference Standards** ⟨11⟩
USP Itraconazole RS
USP Itraconazole System Suitability Mixture RS
This mixture contains itraconazole and six other minor components (4-triazolyl isomer, propyl analog, isopropyl analog, epimer, *n*-butyl isomer, and didioxolanyl analog).

www.webofpharma.com

Add the following:

▲Itraconazole Capsules

DEFINITION

Itraconazole Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of itraconazole ($C_{35}H_{38}Cl_2N_8O_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 5.8 g/L of monobasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 2.0. Pass through a suitable filter of 0.45-µm pore size.
Solution B: Acetonitrile and tetrahydrofuran (90:10)
Mobile phase: *Solution A* and *Solution B* (45:55)
Diluent: Methanol and tetrahydrofuran (50:50)
Standard solution: 0.1 mg/mL of USP Itraconazole RS in *Diluent*. Sonicate, if necessary, to dissolve.
Sample stock solution: Nominally 1 mg/mL of itraconazole in *Diluent* prepared as follows. Transfer an amount nominally equivalent to 100 mg of itraconazole, from the contents of NLT 20 Capsules, to a 100-mL volumetric flask. Add 70 mL of *Diluent*, and sonicate for about 30 min. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size.
Sample solution: Nominally 0.1 mg/mL of itraconazole in *Diluent* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Run time: NLT 2 times the retention time of itraconazole
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of itraconazole ($C_{35}H_{38}Cl_2N_8O_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of itraconazole from the *Sample solution*
r_S = peak response of itraconazole from the *Standard solution*
C_S = concentration of USP Itraconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of itraconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION** ⟨711⟩

▲Test 1▲ (RB 1-Aug-2019)
Medium: 0.25% (w/v) sodium lauryl sulfate in 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 75 rpm; with a three-prong sinker
Time: 45 min
Standard stock solution: 0.55 mg/mL of USP Itraconazole RS in 40% glacial acetic acid. Sonicate, if necessary, to dissolve.
Standard solution: 0.02 mg/mL of USP Itraconazole RS in *Medium* from *Standard stock solution*
Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*, to obtain a concentration similar to that of the *Standard solution*.
Blank: *Medium*
Instrumental conditions
Mode: UV
Analytical wavelength: 260 nm
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0% for 5 replicate injections
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of itraconazole ($C_{35}H_{38}Cl_2N_8O_4$) released:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Itraconazole RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*, if applicable

Tolerances: NLT 80% (Q) of the labeled amount of itraconazole ($C_{35}H_{38}Cl_2N_8O_4$) is dissolved.

▲Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*. All solutions containing itraconazole should be stored in low-actinic or amber glassware and protected from light.
Medium: Simulated gastric fluid without enzymes, deaerated; 900 mL
Apparatus 2: 100 rpm; with a sinker
Time: 60 min
Standard stock solution: 0.55 mg/mL of USP Itraconazole RS in methanol prepared as follows. Transfer suitable amount of USP Itraconazole RS to a suitable volumetric flask and add about 80% of the flask volume of methanol. Heat the solution to 65° in a water bath, with intermittent stirring, until dissolved. Dilute with methanol to final volume.
Standard solution: 0.022 mg/mL of USP Itraconazole RS in *Medium* from *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter and dilute with *Medium*, if necessary.
Blank: *Medium*

www.webofpharma.com

Instrumental conditions
Mode: UV
Analytical wavelength: 255 nm
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of itraconazole ($C_{35}H_{38}Cl_2N_8O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Itraconazole RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution*, if applicable

Tolerances: NLT 80% (Q) of the labeled amount of itraconazole ($C_{35}H_{38}Cl_2N_8O_4$) is dissolved. ▲ (RB 1-Aug-2019)

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**
Solution A, Solution B, and **Diluent:** Prepare as directed in the *Assay*.
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
25	45	55
40	45	55
42	60	40
50	60	40

System suitability solution: 5 mg/mL of USP Itraconazole System Suitability Mixture RS in *Diluent*. Sonicate, if necessary, to dissolve.
Standard solution: 0.025 mg/mL of USP Itraconazole RS in *Diluent*. Sonicate, if necessary, to dissolve.
Sample solution: Nominally 5 mg/mL of itraconazole in *Diluent* prepared as follows. Combine the contents of NLT 20 Capsules and transfer a portion nominally equivalent to 500 mg of itraconazole to a 100-mL flask. Add about 70 mL of *Diluent* and sonicate for 30 min with intermittent shaking. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 30°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Resolution: NLT 1.5 between itraconazole and *n*-butyl isomer, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 10.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of any individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of itraconazole from the *Standard solution*
C_S = concentration of USP Itraconazole RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of itraconazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	▲ (RB 1-Aug-2019)	Acceptance Criteria, NMT (%)
4-Methoxy derivative[a, b]	0.28	▲ (RB 1-Aug-2019)	—
4-Triazolyl isomer[b, c]	0.64	▲ (RB 1-Aug-2019)	—
Propyl and isopropyl analog[b, d, e]	0.77	▲ (RB 1-Aug-2019)	—
Epimer[b, f]	0.84	▲ (RB 1-Aug-2019)	—
Itraconazole	1.0	▲ (RB 1-Aug-2019)	—
n-Butyl isomer[b, g]	1.1	▲ (RB 1-Aug-2019)	—
Didioxolanyl analog[b, h]	1.4	▲ (RB 1-Aug-2019)	—
Any individual unspecified impurity	—	▲ (RB 1-Aug-2019)	0.2

USP Monographs

www.webofpharma.com

Table 2 *(continued)*

Name	Relative Retention Time	▲▲ (RB 1-Aug-2019)	Acceptance Criteria, NMT (%)
Total impurities	—	▲▲ (RB 1-Aug-2019)	1.50

[a] 2-*sec*-Butyl-4-{4-[4-(4-methoxyphenyl)piperazin-1-yl]phenyl}-2*H*-1,2,4-triazol-3(4*H*)-one.

[b] Process-related impurity ▲included in the table for identification only. Process-related impurities are▲ (RB 1-Aug-2019) controlled in the drug substance▲ and are not to be reported or included in the total impurities of the drug product.▲ (RB 1-Aug-2019)

[c] 4-(4-{4-[4-(((2*RS*,4*SR*)-2-[(4*H*-1,2,4-Triazol-4-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-*sec*-butyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one.

[d] 4-(4-{4-[4-(((2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-propyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one.

[e] 4-(4-{4-[4-(((2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-isopropyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one.

[f] 4-(4-{4-[4-({(2*RS*,4*RS*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-*sec*-butyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one.

[g] 4-(4-{4-[4-(((2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-butyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one.

[h] Mixture of 4-(4-{4-[4-(((2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-({(2*RS*,4*SR*)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one and 4-(4-{4-[4-(((2*RS*,4*SR*)-2-[(1*H*-1,2,4-Triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-({(2*SR*,4*RS*)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl}methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one.

ADDITIONAL REQUIREMENTS

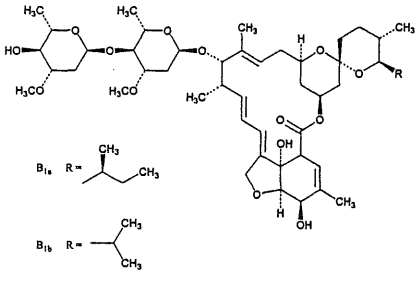
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

Add the following:

▲• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.▲ (RB 1-Aug-2019)

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Itraconazole RS
  USP Itraconazole System Suitability Mixture RS
  This is a mixture of itraconazole, 4-triazolyl isomer, propyl analog, epimer, *n*-butyl isomer, and didioxolanyl analog (other impurities may also be present).▲ (USP 1-Aug-2019)

Ivermectin



$C_{48}H_{74}O_{14}$ (Component H_2B_{1a}) 875.09

$C_{47}H_{72}O_{14}$ (Component H_2B_{1b}) 861.07

Component H_2B_{1a}:

Avermectin A_{1a}, 5-*O*-demethyl-22,23-dihydro-.

(2a*E*,4*E*,8*E*)-(5′*S*,6*S*,6′*R*,7*S*,11*R*,13*R*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-6′-(*S*)-*sec*-Butyl-3′,4′,5′,6,6′,7,10,11,14,15,17a,20,20a,20b-tetradecahydro-20,20b-dihydroxy[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-7-yl 2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-3-*O*-methyl-α-L-*arabino*-hexopyranoside [70161-11-4].

Component H_2B_{1b}:

Avermectin A_{1a}, 5-*O*-demethyl-25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl)-.

(2a*E*,4*E*,8*E*)-(5′*S*,6*S*,6′*R*,7*S*,11*R*,13*R*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-3′,4′,5′,6,6′,7,10,11,-oxospiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2′[2*H*]pyran]-7-yl 2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-3-*O*-methyl-α-L-*arabino*-hexopyranoside [70209-81-3].

» Ivermectin is a mixture of avermectin A_{1a}, 5-*O*-demethyl-22,23-dihydro-(component H_2B_{1a}), and avermectin A_{1a}, 5-*O*-demethyl-25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl)-(component H_2B_{1b}). It contains not less than 95.0 percent and not more than 102.0 percent for the sum of component H_2B_{1a} plus component H_2B_{1b}, calculated on the anhydrous and alcohol- and formamide-free basis; and the ratio (calculated by area percentage) of component $H_2B_{1a}/(H_2B_{1a} + H_2B_{1b})$ is not less than 90.0 percent. It may contain small amounts of suitable antioxidant and chelating agents.

Packaging and storage—Preserve in tight containers. Store between 2° and 8°. Where the use of an antioxidant is allowed, store at 25°, excursions permitted between 15° and 30°.

Labeling—If it is intended for veterinary use only, it is so labeled. Label it to state the name(s) and amount(s) of any added substance(s). Label it also to state that it is for manufacturing, processing, or repackaging.

USP Reference standards ⟨11⟩—
USP Ivermectin RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests* ⟨197⟩, *Infrared Spectroscopy: 197K.*▲ (CN 1-May-2020)

B: The retention times of the component H_2B_{1a} peak and the component H_2B_{1b} peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Specific rotation ⟨781S⟩**:** between −17° and −20° measured at 20°, calculated on the water-, alcohol-, and formamide-free basis.

Test solution: 25 mg per mL, in methanol.

Water Determination, *Method I* ⟨921⟩ **:** not more than 1.0%.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Limit of alcohol and formamide—

Internal standard solution—Dilute 0.5 mL of isopropyl alcohol with water to 100 mL, and mix.

Standard solution 1—Transfer 2.0 mL of dehydrated alcohol to a 100-mL volumetric flask, dilute with water to volume, and mix.

www.webofpharma.com

Standard solution 2—Transfer 1.0 mL of formamide to a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard solution 3—Transfer 5.0 mL of *Standard solution 1* and 5.0 mL of *Standard solution 2* to a 50-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having concentrations of formamide and alcohol of 0.001 and 0.002 mL per mL, respectively. Transfer 2.0 mL of this solution to a 15-mL centrifuge tube, add 2.0 mL of *m*-xylene, insert the stopper, mix, and centrifuge. Remove the upper *m*-xylene layer, and extract it with 2.0 mL of water. Discard the upper layer, combine the two retained lower aqueous layers, add 1.0 mL of *Internal standard solution,* and mix. Each mL of this solution contains about 0.0008 mL of alcohol and 0.0004 mL of formamide.

Standard solution 4—Transfer 10.0 mL of *Standard solution 1* and 10.0 mL of *Standard solution 2* to a 50-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having concentrations of alcohol and formamide of 0.004 and 0.002 mL per mL, respectively. Transfer 2.0 mL of this solution to a 15-mL centrifuge tube, add 2.0 mL of *m*-xylene, insert the stopper, mix, and centrifuge. Remove the upper *m*-xylene layer, and extract it with 2.0 mL of water. Discard the upper layer, combine the two retained lower aqueous layers, add 1.0 mL of *Internal standard solution,* and mix. Each mL of this solution contains about 0.0016 mL of alcohol and 0.0008 mL of formamide.

Test solution—Transfer 120 mg of Ivermectin, accurately weighed, to a 15-mL centrifuge tube, and dissolve in 2.0 mL of *m*-xylene, heating in a water bath at 45 ± 5°, if necessary. Add 2.0 mL of water, mix, and centrifuge. Transfer the *m*-xylene layer to a 15-mL centrifuge tube, and extract with 2.0 mL of water. Discard the upper *m*-xylene layer, combine the two retained lower aqueous layers, add 1.0 mL of *Internal standard solution,* and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m fused-silica analytical column coated with a 3-µm G43 stationary phase. The carrier gas is helium, with a 10:1 split ratio and a linear velocity of about 35 cm per second. The chromatograph is programmed as follows. The column temperature is maintained at about 40° for 5 minutes after injection, then increased at a rate of 20° per minute to 180° and maintained at 180° for 2 minutes. The injection port temperature is maintained at about 220°, and the detector temperature is maintained at about 280°.

Procedure—Separately inject equal volumes (about 2 µL) of *Standard solution 3, Standard solution 4,* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for alcohol, formamide, and isopropyl alcohol. Plot the ratios of the peak responses for alcohol and isopropyl alcohol and for formamide and isopropyl alcohol versus concentrations, in mL per mL, of alcohol and formamide, respectively, obtained from *Standard solution 3* and *Standard solution 4*. From the graphs so obtained, and using the ratios of the peak responses for alcohol and isopropyl alcohol and for formamide and isopropyl alcohol obtained from the chromatogram of the *Test solution,* determine the concentrations, *C,* of alcohol and formamide in the *Test solution.* [NOTE—In the event that the peak responses of the *Test solution* are significantly outside the ranges of peak responses obtained from *Standard solution 3* and *Standard solution 4,* prepare additional *Standard solutions,* and chromatograph them to obtain peak responses bracketing those obtained with the *Test solution.*] Calculate the percentages of alcohol and formamide in the portion of Ivermectin taken by the formula:

$$500{,}000CD/W$$

in which *C* is the concentration of alcohol or formamide, as appropriate, in mL per mL, in the *Test solution; D* is the density of alcohol (0.79) or formamide (1.13); and *W* is the weight, in mg, of Ivermectin taken: not more than 5.0% of alcohol and 3.0% of formamide are found.

Related compounds—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay.*

Standard solution—Proceed as directed for *Standard preparation* in the *Assay.*

Test solution—Use the *Assay preparation.*

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatogram of the *Test solution* for a period of time equivalent to twice the retention time of the main peak in the chromatogram obtained from the *Standard solution,* and measure the peak areas. Calculate the percentage of each impurity by the formula:

$$100r_i/(r_s - r_b)$$

in which r_i is the peak area for each individual impurity in the *Test solution* chromatogram; r_s is the sum of all peaks in the *Test solution* chromatogram; and r_b is the total area of all peaks in a blank chromatogram: not more than 2.5% is found for the sum of all peaks with a relative retention time of about 1.3 to 1.4 (corresponding to H_4B_{1a} isomers and $\Delta^{2,3}$ H_2B_{1a}); not more than 1% is found for the peak with a relative retention time of about 0.7 (corresponding to 8a-oxo-H_2B_{1a}); not more than 0.7% is found for the peak with a relative retention time of about 0.5 (corresponding to avermectin B_{1a}); not more than 0.5% is found for any other individual impurity peak; not more than 1% is found for the sum of all unidentified peaks; and not more than 4% is found for the sum of all the peaks, apart from the two main peaks (H_2B_{1a} and H_2B_{1b}). Disregard any peak that is calculated to be less than 0.05%.

Assay—

Mobile phase—Prepare a mixture of acetonitrile, methanol, and water (53: 27.5: 19.5), and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). Increasing the proportion of water increases the elution times and allows better separation of impurities.

Standard preparation—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol to obtain a solution having a known concentration of about 0.4 mg per mL.

Assay preparation—Transfer about 40 mg of Ivermectin, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Sonicate, if necessary.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.75 for component H_2B_{1b} and 1.0 for component H_2B_{1a}; the resolution, *R,* between component H_2B_{1b} and component H_2B_{1a} is not less than 3.0; the column efficiency determined from the component H_2B_{1a} peak is not less than 2000 theoretical plates; the tailing factor for component H_2B_{1a} peak is not more than 2.5; and the relative standard deviation for six replicate injections is not more than 1.0% determined from the component H_2B_{1a} peak.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for component H_2B_{1a} and component H_2B_{1b}.

www.webofpharma.com

Calculate the quantity, in mg, of component H_2B_{1a} ($C_{48}H_{74}O_{14}$) and component H_2B_{1b} ($C_{47}H_{72}O_{14}$) in the portion of Ivermectin taken by the formula:

$$DC(r_U/r_S)$$

in which D is the dilution factor, in mL, used to prepare the *Assay preparation;* C is the concentration, in mg per mL, of component H_2B_{1a} or component H_2B_{1b} in the *Standard preparation;* and r_U and r_S are the peak areas for component H_2B_{1a} or component H_2B_{1b} obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ivermectin Injection

» Ivermectin Injection is a sterile solution of Ivermectin in a suitable vehicle. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of ivermectin, calculated as the sum of component H_2B_{1a} ($C_{48}H_{74}O_{14}$) plus component H_2B_{1b} ($C_{47}H_{72}O_{14}$). The ratio of the contents, $H_2B_{1a}/(H_2B_{1a}+ H_2B_{1b})$, is not less than 90.0 percent.

Packaging and storage—Preserve in single-dose or in multi-dose containers, preferably of Type I glass or plastic. Store at a temperature of not more than 30°.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Ivermectin RS

Identification—
A: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Test solution*—Dissolve a volume of the Injection in methanol, dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution containing 0.5 mg per mL of ivermectin, and filter.
Injection volume: 2 µL.
Developing solvent system: unsaturated chamber, consisting of a freshly prepared and equilibrated mixture of methylene chloride, methanol, and ammonium hydroxide (90:9:1).
Procedure—Remove the plate, allow to air dry, and examine under short- and long-wavelength UV light: the retardation factor, R_F, of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution.*
B: The retention times of the two principal component peaks of ivermectin in the chromatogram of the *Assay preparation* corresponds to that of the two principal component peaks of ivermectin in the chromatogram of the *Standard preparation,* obtained as directed in the *Assay.*
Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.016 USP Endotoxin Unit per µg of ivermectin.
Sterility Tests ⟨71⟩ —It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

Chromatographic purity—
Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay.*
Standard solution—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol and dilute quantitatively, and stepwise if necessary, with methanol to obtain a 0.004 mg per mL solution.
Test solution—Dissolve a volume of the Injection in methanol and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution containing 0.4 mg of ivermectin per mL of solution, based on the label claim.
Procedure—Inject equal volumes (about 20 µL) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Injection taken by the formula:

$$100(C_S/C_T)(r_i/r_S)$$

in which C_S is the concentration, in mg per mL, of ivermectin in the *Standard solution;* C_T is the concentration, in mg per mL, of ivermectin in the *Test solution;* r_i is the peak response for each individual peak obtained from the *Test solution;* and r_S is the ivermectin peak response obtained from the *Standard solution.* Not more than 2.7% of the peak with a relative retention time of about 1.3 to 1.5 to that of the principal peak is found; not more than 1.0% of any other impurity is found; and not more than 6.0% of total impurities is found. Disregard any peak below 0.05%.
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—
Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, methanol, and water (106:55:39). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol and dilute quantitatively, and stepwise if necessary, with methanol to obtain a 0.4 mg per mL solution.
Assay preparation—Dilute a volume of Injection in methanol and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution containing 0.4 mg of ivermectin per mL of solution, based on the label claim.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with 245-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the first peak (component H_2B_{1b}) and the second peak (component H_2B_{1a}) is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%, determined from the component H_2B_{1a} peak.
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the component H_2B_{1a} plus component H_2B_{1b}. Calculate the percentage of label claim of ivermectin (H_2B_{1a} + H_2B_{1b}) in the portion of Injection taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Ivermectin RS in the *Standard preparation;* C_U is the concentration, in mg per mL, of ivermectin in the *Assay preparation;* r_U is the total peak response of H_2B_{1a} plus H_2B_{1b} peaks obtained from the *Assay preparation;* and r_S is the total peak response of H_2B_{1a} plus H_2B_{1b} peaks obtained from the *Standard preparation.* Calculate the ratio of the contents, in percent, of $H_2B_{1a}/(H_2B_{1a} + H_2B_{1b})$ in the portion of Injection taken by the formula:

www.webofpharma.com

$$100(r_1/r_U)$$

in which r_1 is the peak response of H_2B_{1a} obtained from the *Assay preparation;* and r_U is as defined above.

Add the following:

▲Ivermectin Compounded Oral Solution, Veterinary

DEFINITION

Ivermectin Compounded Oral Solution, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of ivermectin, calculated as the sum of components $H_2B_{1a}(C_{48}H_{74}O_{14})$ plus $H_2B_{1b}(C_{47}H_{72}O_{14})$.

Prepare Ivermectin Compounded Oral Solution, Veterinary 3 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Ivermectin Injection,[a] equivalent to	30 mg of ivermectin
Polyethylene Glycol 400, a sufficient quantity to make	10 mL

[a] Ivomec Injection 1%, Merial Ltd., Duluth, GA.

Accurately measure the *Ivermectin Injection* and place into a suitable container. Add sufficient *Polyethylene Glycol 400* to bring to final volume. Shake to mix well.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (45:40:15)

Standard solution: 0.12 mg/mL of USP Ivermectin RS in methanol

Sample solution: Transfer 1 mL of Oral Solution, Veterinary to a 25-mL volumetric flask, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability.*)

Mode: LC

Detector: UV 245 nm

Column: 4.6-mm × 25-cm; 3.6-µm packing L1

Temperatures

Autosampler: 15°

Column: 20°

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention times for component H_2B_{1b} and component H_2B_{1a} are about 23.8 and 31.6 min, respectively.]

Suitability requirements

Resolution: NLT 3.0 between component H_2B_{1b} and component H_2B_{1a}

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections determined from the component H_2B_{1a} peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ivermectin components $H_2B_{1a}(C_{48}H_{74}O_{14})$ plus $H_2B_{1b}(C_{47}H_{72}O_{14})$ in the portion of Oral Solution, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of H_2B_{1a} plus H_2B_{1b} from the *Sample solution*

r_S = peak response of H_2B_{1a} plus H_2B_{1b} from the *Standard solution*

C_S = concentration of USP Ivermectin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ivermectin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** ⟨791⟩: 5.6–6.6

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature.

• **LABELING:** Label to state that it is for veterinary use only and to state the *Beyond-Use Date.*

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Ivermectin RS▲ 25 (USP41)

Ivermectin Paste

DEFINITION

Ivermectin Paste contains NLT 90.0% and NMT 110.0% of the labeled amount of Ivermectin, calculated as the sum of component H_2B_{1a} ($C_{48}H_{74}O_{14}$) and component H_2B_{1b} ($C_{47}H_{72}O_{14}$). The ratio of the contents, $H_2B_{1a}/(H_2B_{1a} + H_2B_{1b})$, is NLT 90.0%.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨201⟩

Sample solution: 0.5 mg/mL of ivermectin dispersed in methanol from a quantity of Paste. Sonicate if necessary until completely dispersed.

Application volume: 2 µL

Developing solvent system: Methylene chloride, methanol, and ammonium hydroxide (90:9:1)

Analysis: Develop the chromatogram in an unsaturated chamber. Remove the plate, allow to air dry, and examine under short- and long-wavelength UV light.

Acceptance criteria: Meets the requirements

• **B.** The retention times of the two principal component peaks of ivermectin from the *Sample solution* correspond to those of the two principal component peaks of ivermectin from the *Standard solution*, as obtained in the *Assay.*

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (106:55:39)

Standard solution: 0.4 mg/mL of USP Ivermectin RS in methanol

Sample solution: Disperse a quantity of Paste in methanol, using sonication if necessary, to obtain a solution containing 0.4 mg/mL of ivermectin.

www.webofpharma.com

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability.*)
Mode: LC
Detector: UV 245 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between the first peak (component H_2B_{1b}) and the second peak (component H_2B_{1a})
Relative standard deviation: NMT 2.0%, determined from the component H_2B_{1a} peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ivermectin, component H_2B_{1a} ($C_{48}H_{74}O_{14}$) and component H_2B_{1b} ($C_{47}H_{72}O_{14}$), in the portion of Paste taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = sum of the peak responses for component H_2B_{1a} and component H_2B_{1b} from the *Sample solution*
r_S = sum of the peak responses for component H_2B_{1a} and component H_2B_{1b} from the *Standard solution*
C_S = concentration of USP Ivermectin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ivermectin in the *Sample solution* (mg/mL)

Calculate the ratio of the contents, in percentage, of the components, $H_2B_{1a}/(H_2B_{1a} + H_2B_{1b})$, in the portion of Paste taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of H_2B_{1a} from the *Sample solution*
r_T = sum of the peak responses for component H_2B_{1a} and component H_2B_{1b} from the *Sample solution*

Acceptance criteria: 90.0%–110.0% of the labeled amount of ivermectin, calculated as the sum of component H_2B_{1a} ($C_{48}H_{74}O_{14}$) and component H_2B_{1b} ($C_{47}H_{72}O_{14}$). The ratio of the contents, $H_2B_{1a}/(H_2B_{1a} + H_2B_{1b})$, is NLT 90.0%.

IMPURITIES
• **ORGANIC IMPURITIES**
Mobile phase, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*
Standard solution: 0.004 mg/mL of USP Ivermectin RS in methanol
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Paste taken, disregarding any peak below 0.05%:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response of the principal peak from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
C_U = nominal concentration of ivermectin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0% of any peak with a relative retention time of 1.3–1.5, relative to that of the principal peak
Any other impurity: NMT 1.0%
Total impurities: NMT 6.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at a temperature not higher than 30°.
• **LABELING:** Label it to indicate that it is for oral veterinary use only.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Ivermectin RS

Ivermectin Tablets

» Ivermectin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of Ivermectin components H_2B_{1a} ($C_{48}H_{74}O_{14}$) plus H_2B_{1b} ($C_{47}H_{72}O_{14}$). They may contain a suitable antioxidant.

Packaging and storage—Preserve in well-closed containers, and store at a temperature below 30°.
USP Reference standards ⟨11⟩—
USP Ivermectin RS
USP 3-*tert*-Butyl-4-hydroxyanisole RS
Identification—The retention times of the H_2B_{1a} and H_2B_{1b} peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩—
Medium: 0.01 M phosphate buffer, pH 7, with 0.5% of sodium dodecyl sulfate (prepared by dissolving 50 g of sodium dodecyl sulfate in approximately 9 L of water, adding 100 mL of 1 M monobasic sodium phosphate monohydrate, adjusting with sodium hydroxide to a pH of 7, and diluting with water to 10 L); 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.
Determine the amount of $C_{48}H_{74}O_{14}$ (component H_2B_{1a}) plus $C_{47}H_{72}O_{14}$ (component H_2B_{1b}) dissolved by employing the following method.
Mobile phase—Prepare a degassed solution of acetonitrile, methanol, and water (53:35:12).
Standard stock solution—Prepare a 0.13 mg per mL solution of USP Ivermectin RS in *Medium.*
Standard solution—Using the accompanying table, dilute the *Standard stock solution* with *Medium* to volume, and mix.

Tablet Strength (mg per Tablet)	Required Dilution Ratio	Volume of *Standard stock solution* (mL)	Volumetric Flask Size (mL)
3.0	1 in 40	5.0	200
6.0	1 in 20	5.0	100

Test solution—Pass a portion of the solution under test through a suitable filter, and use the filtrate.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 245-nm detector and a 4.6-mm × 10-cm column that contains 5-µm packing L1. The flow rate is about 1.2 mL per minute. The column

USP Monographs

www.webofpharma.com

temperature is maintained at 30°. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.81 for H_2B_{1b} and 1.0 for H_2B_{1a}; the resolution, *R,* between the H_2B_{1a} and H_2B_{1b} peaks is not less than 1.5; the capacity factor, *k′,* for the H_2B_{1a} peak is not less than 4; the column efficiency determined from both the H_2B_{1a} and H_2B_{1b} peaks is not less than 1500 theoretical plates; the tailing factor for the H_2B_{1a} peak is not more than 2; and the relative standard deviation for replicate injections for the H_2B_{1a} peak is not more than 2.0%.

Procedure—Separately inject equal volumes (about 100 µL) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the combined quantities, in percentage, of H_2B_{1a} plus H_2B_{1b} dissolved based on the peak responses obtained from the *Test solution* and the *Standard solution* by the formula:

$$[100(A_U)(W_S)(P)(D_U)]/[(A_S)(D_S)L]$$

in which A_U is the total peak area of H_2B_{1a} plus H_2B_{1b} obtained from the *Test solution;* W_S is the weight, in mg, of the USP Ivermectin RS taken to prepare the *Standard stock solution; P* is the purity of the USP Ivermectin RS (percent [w/w] H_2B_{1a} plus percent [w/w] H_2B_{1b}), expressed as a decimal; D_U is the *Test solution* dilution factor; A_S is the total peak area of H_2B_{1a} plus H_2B_{1b} obtained from the *Standard solution;* D_S is the *Standard solution* dilution factor; and *L* is the label claim of ivermectin, in mg per Tablet.

Tolerances—Not less than 80% (*Q*) of the labeled amount of $C_{48}H_{74}O_{14}$ (H_2B_{1a}) plus $C_{47}H_{72}O_{14}$ (H_2B_{1b}) is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements for *Content uniformity.*

PROCEDURE FOR CONTENT UNIFORMITY—

Mobile phase—Prepare as directed in the *Assay.*

Standard solution A—Use the *Standard preparation* from the *Assay.*

Standard solution B—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol to obtain a solution containing 0.125 mg per mL.

Stock sensitivity solution (1%)—Use the *Stock sensitivity solution (1%)* from the *Assay.*

Sensitivity solution (0.2%)—Use the *Sensitivity solution (0.2%)* from the *Assay.*

Test solution—Transfer 1 Tablet into each of ten 25-mL volumetric flasks. Add 5.0 mL of water, and sonicate for 10 minutes. Add approximately 15 mL of methanol, sonicate for 5 minutes, and mix. Allow the solution to cool to room temperature. Dilute with methanol to volume, and mix. Pass a portion of each solution through a 1.0- to 1.2-µm chemically resistant filter prior to analysis.

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the *Assay.*

Procedure—Separately inject equal volumes (about 10 µL) of *Standard solution A* (for the 6 mg per Tablet dose) or *Standard solution B* (for the 3 mg per Tablet dose), the *Sensitivity solution (0.2%),* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses of the ivermectin peaks. Calculate the quantity as a percentage of the label claim of ivermectin per Tablet taken by the formula:

$$[100(A_U)(W_S)(P)(D_U)]/[(A_S)(D_S)L]$$

in which A_U is the peak area of H_2B_{1a} plus the peak area of H_2B_{1b} obtained from the *Test solution;* W_S is the weight, in mg, of the USP Ivermectin RS taken to prepare *Standard solution A* or *Standard solution B; P* is the purity of USP Ivermectin RS (percent [w/w] H_2B_{1a} plus percent [w/w] H_2B_{1b}), expressed as a decimal; D_U is the *Test solution* dilution factor; A_S is the peak area of H_2B_{1a} plus the peak area of H_2B_{1b} obtained from *Standard solution A* or *Standard solution B;* D_S is the *Standard solution A* or *Standard solution B* dilution factor; and *L* is the label claim of ivermectin, in mg per Tablet.

Limit of 8a-oxo-H_2B_{1a}—

Mobile phase—Proceed as directed in the *Assay.*

BHA Working Standard solution—Dissolve an accurately weighed quantity of USP 3-*tert*-Butyl-4-hydroxyanisole RS in methanol, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.96 µg per mL.

Test solution—Use the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The column temperature is maintained at 30°. The flow rate is about 1.2 mL per minute. Chromatograph the *BHA Working Standard solution* and the *Test solution,* and record the peak responses as directed for *Procedure:* the relative retention times at 280 nm are about 0.24 for BHA, 0.77 for 8a-oxo-H_2B_{1a}, and 1.0 for H_2B_{1a}.

Procedure—Separately inject equal volumes (about 10 µL) of the *BHA Working Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of 8a-oxo-H_2B_{1a} as a percentage of the label claim of ivermectin in the portion of Tablets taken by the formula:

$$[100(A_D)(W_S)(P)(D_U)(C_F)]/[(A_S)(D_S)(N)(L)(F)]$$

in which A_D is the peak area of 8a-oxo-H_2B_{1a} obtained from the *Test solution;* W_S is the weight of USP 3-*tert*-Butyl-4-hydroxyanisole RS, in mg, taken to prepare the *BHA Working Standard solution; P* is the purity of USP 3-*tert*-Butyl-4-hydroxyanisole RS, expressed as a decimal; D_U is the *Test solution* dilution factor; C_F is the correction factor (equal to 0.98) used to convert mg of 8a-oxo-H_2B_{1a} to mg of ivermectin; A_S is the peak area of BHA obtained from the *BHA Working Standard solution;* D_S is the *BHA Working Standard* solution dilution factor; *N* is the number of Tablets taken to prepare the *Test solution; L* is the label claim of ivermectin, in mg per Tablet; and *F* is the relative response factor (equal to 1.0): not more than 2.0% of 8a-oxo-H_2B_{1a} is found. The correction factor, C_F, (equal to 0.98) is calculated by the following formula:

$$[0.90\ (\text{molecular weight of } H_2B_{1a}) + 0.10\ (\text{molecular weight of } H_2B_{1b})]/(\text{molecular weight of 8a-oxo-}H_2B_{1a}) = 873.10/889.10 = 0.98]$$

Assay—

Mobile phase—Prepare a mixture of acetonitrile, methanol, and water (53:35:12). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol to obtain a solution containing 0.25 mg per mL.

Stock sensitivity solution (1%)—Quantitatively prepare a 1 in 100 dilution of the *Standard solution* in methanol.

Sensitivity solution (0.2%)—Quantitatively prepare a 1 in 5 dilution of the *Stock sensitivity solution (1%)* in methanol.

www.webofpharma.com

Assay preparation—Transfer the appropriate number of Tablets into a 250-mL volumetric flask according to the accompanying table:

Tablet Strength (mg per Tablet)	Number of Tablets
3.0	20
6.0	10

Add approximately 25 mL of water, and sonicate for 10 minutes. Add methanol to fill the flask three-quarters full, sonicate for 5 minutes or until the Tablets are completely disintegrated, and shake until mixed well. Allow the solution to cool to room temperature. Dilute with methanol to volume, add a magnetic stirrer, and mix until no lumps are present in the solution. Pass a portion of this solution through a 1.0- to 1.2-µm chemically resistant filter prior to injection.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 245-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.2 mL per minute. The column temperature is maintained at 30°. Chromatograph the *Sensitivity solution (0.2%)* and the *Standard preparation* at 245-nm detection, and record the peak responses as directed for *Procedure:* the signal-to-noise ratio for the ivermectin peak obtained from the *Sensitivity solution (0.2%)* is not less than 10; obtained from the *Standard preparation,* the relative retention times are about 0.82 and 1.0 for components H_2B_{1b} and H_2B_{1a}, respectively; the capacity factor, *k'*, for the component H_2B_{1b} is not less than 3; the column efficiency for component H_2B_{1a} is not less than 1500 theoretical plates; the tailing factor for component H_2B_{1a} is not more than 2; and the relative standard deviation for the area response for total ivermectin (H_2B_{1a} plus H_2B_{1b}) for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for component H_2B_{1a} plus component H_2B_{1b}. Calculate the percentage of component H_2B_{1a} ($C_{48}H_{74}O_{14}$) plus component H_2B_{1b} ($C_{47}H_{72}O_{14}$) as a percentage of the label claim of ivermectin per Tablet taken by the formula:

$$[100(A_U)(W_S)(P)(D_U)]/[(A_S)(D_S)(N)(L)]$$

in which A_U is the total peak response of H_2B_{1a} plus H_2B_{1b} obtained from the *Assay preparation*; W_S is the weight of the USP Ivermectin RS, in mg, taken to prepare the *Standard preparation*; P is the purity of the USP Ivermectin RS (percent [w/w] H_2B_{1a} plus percent [w/w] H_2B_{1b}), expressed as a decimal; D_U is the sample dilution factor; A_S is the total peak area of H_2B_{1a} plus H_2B_{1b} obtained from the *Standard preparation*; D_S is the Standard dilution factor; N is the number of Tablets taken to prepare the *Assay preparation*; and L is the label claim of ivermectin, in mg per Tablet.

Ivermectin Topical Solution

» Ivermectin Topical Solution is a topical solution in a suitable vehicle. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of Ivermectin, calculated as the sum of component H_2B_{1a} ($C_{48}H_{74}O_{14}$) plus component H_2B_{1b} ($C_{47}H_{72}O_{14}$). The ratio of the contents, $H_2B_{1a}/(H_2B_{1a} + H_2B_{1b})$, is not less than 90.0 percent.

Packaging and storage—Preserve in well-closed containers. Store at a temperature not more than 30°.
Labeling—Label it to indicate that it is for veterinary use only.
USP Reference standards ⟨11⟩—
USP Ivermectin RS

Identification—

A: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Test solution*—Dissolve a volume of Topical Solution in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution containing 0.5 mg per mL of ivermectin, and filter.
Injection volume: 2 µL.
Developing solvent system: unsaturated chamber, freshly prepared and equilibrated mixture of methylene chloride, methanol, and ammonium hydroxide (90:9:1).
Procedure—Remove the plate, allow to air dry, and examine under short- and long-wavelength UV light: the retardation factor, R_F, of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution.*

B: The retention times of the two principal component peaks of ivermectin in the chromatogram of the *Assay preparation* corresponds to that of the two principal component peaks of ivermectin in the chromatogram of the *Standard preparation,* obtained as directed in the *Assay.*

Chromatographic purity—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay.*
Standard solution—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a 0.004 mg per mL solution.
Test solution—Dissolve a quantity of Topical Solution in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution containing 0.4 mg of ivermectin per mL of solution, based on the label claim.
Procedure—Inject equal volumes (about 20 µL) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Topical Solution taken by the formula:

$$100(C_S/C_T)(r_i/r_S)$$

in which C_S is the concentration, in mg per mL, of ivermectin in the *Standard solution*; C_T is the concentration, in mg per mL, of ivermectin in the *Test solution*; r_i is the peak response for each impurity from the *Test solution;* and r_S is the ivermectin peak response obtained from *Standard solution.* Not more than 2.7% of the peak with a relative retention time of about 1.3 to 1.5 to that of the principal peak is found; not more than 1.0% of any other impurity is found; and not more than 6.0% of total impurities is found. Disregard any peak below 0.05%.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, methanol, and water (106:55:39). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
Standard preparation—Dissolve an accurately weighed quantity of USP Ivermectin RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to

www.webofpharma.com

obtain a solution having a known concentration of about 0.4 mg per mL.

Assay preparation—Dilute a volume of Topical Solution, quantitatively, and stepwise if necessary, with methanol to obtain a solution containing 0.4 mg of ivermectin per mL of solution, based on the label claim.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 245-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the first peak (component H_2B_{1b}) and the second peak (component H_2B_{1a}) is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%, determined from the H_2B_{1a} peak.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the component H_2B_{1a} plus component H_2B_{1b}. Calculate the percentage of label claim of ivermectin (H_2B_{1a} + H_2B_{1b}) in the portion of Topical Solution taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Ivermectin RS in the *Standard preparation;* C_U is the concentration, in mg per mL, of ivermectin in the *Assay preparation;* r_U is the total peak response of H_2B_{1a} plus H_2B_{1b} peaks obtained from the *Assay preparation;* and r_S is the total peak response of H_2B_{1a} plus H_2B_{1b} peaks obtained from the *Standard preparation.* Calculate the ratio of the contents, in percent, of $H_2B_{1a}/(H_2B_{1a} + H_2B_{1b})$ in the portion of Topical Solution taken by the formula:

$$100(r_1/r_U)$$

in which r_1 is the peak response of H_2B_{1a} obtained from the *Assay preparation;* and r_U is as described above.

Ivermectin and Clorsulon Injection

» Ivermectin and Clorsulon Injection is a sterile solution of Ivermectin and Clorsulon in a suitable vehicle. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of ivermectin [component B_{1a} ($C_{48}H_{74}O_{14}$) plus component B_{1b} ($C_{47}H_{72}O_{14}$)] and not less than 95.0 percent and not more than 105.0 percent of the labeled amount of clorsulon ($C_8H_8Cl_3N_3O_4S_2$).

Packaging and storage—Preserve in single-dose or multi-dose containers, preferably of Type I glass or plastic. Store at a temperature not higher than 30°.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩—
USP Clorsulon RS
USP Ivermectin RS

Identification—

A: Thin-Layer Chromatographic Identification Test ⟨201⟩—*Test solution:* about 0.5 mg of ivermectin and 5 mg of clorsulon per mL in methanol.
Application volume: 2 µL.
Developing solvent system: a mixture of methylene chloride, methanol, and ammonium hydroxide (90:9:1).
B: The retention times of the two major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay for ivermectin.* The retention time of the major clorsulon peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay for clorsulon.*
[NOTE—The two major ivermectin components are not separated by this method.]

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 2.3 USP Endotoxin Units per mg of combined ivermectin and clorsulon.

Sterility Tests ⟨71⟩ **:** meets the requirements.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay for ivermectin—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, methanol, and water (530:350:70). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). Increasing the proportion of water increases the resolution.

Standard preparation—Prepare a solution of USP Ivermectin RS in methanol having a known concentration of about 0.3 mg per mL.

Assay preparation—Transfer an accurately measured portion of Injection, equivalent to about 30 mg of ivermectin, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure* [NOTE—The relative retention times are about 0.8 for component B_{1b} and 1.0 for component B_{1a}.] : the resolution, *R,* between component B_{1b} and component B_{1a} is not less than 2.0; the column efficiency determined from the component B_{1a} peak is not less than 2000 theoretical plates; and the relative standard deviation for replicate injections determined for the component B_{1a} peak is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of ivermectin component B_{1a} ($C_{48}H_{74}O_{14}$) plus ivermectin component B_{1b} ($C_{47}H_{72}O_{14}$) in the portion of Injection taken by the formula:

$$CP(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ivermectin RS in the *Standard preparation; P* is the designated percentage of the sum of component B_{1a} plus component B_{1b} in USP Ivermectin RS; and r_U and r_S are the sums of the peak area responses for ivermectin component B_{1a} plus ivermectin component B_{1b} obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for clorsulon—

Mobile phase—Prepare a filtered and degassed mixture of chloroform, methanol, and water (900:100:1). Make

www.webofpharma.com

adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Prepare a solution of USP Clorsulon RS in methanol having a known concentration of about 2.4 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with chloroform to volume, and mix.

Assay preparation—Transfer an accurately measured portion of Injection, equivalent to about 240 mg of clorsulon, to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with chloroform to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L3. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure* [NOTE—The relative retention times are about 0.6 for ivermectin (the major components of ivermectin co-elute) and 1.0 for clorsulon.] : the column efficiency determined from the clorsulon peak is not less than 4000 theoretical plates; the tailing factor for the clorsulon peak is not more than 2.0; and the relative standard deviation for replicate injections determined for the clorsulon peak is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of clorsulon ($C_8H_8Cl_3N_3O_4S_2$) in the portion of Injection taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Clorsulon RS in the *Standard preparation;* and r_U and r_S are the clorsulon peak area responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Ivermectin and Pyrantel Pamoate Tablets

» Ivermectin and Pyrantel Pamoate Tablets contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of ivermectin components H_2B_{1a} ($C_{48}H_{74}O_{14}$) plus H_2B_{1b} ($C_{47}H_{72}O_{14}$) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of pyrantel pamoate ($C_{34}H_{30}N_2O_6S$).

Packaging and storage—Preserve in tight containers, protected from light, at a temperature not exceeding 30°, and avoid freezing.

Labeling—Label Tablets to indicate that they are intended for veterinary use only. Tablets that can be chewed are so labeled.

USP Reference standards ⟨11⟩—
USP Ivermectin RS
USP Pyrantel Pamoate RS

Identification—The retention times of the two major ivermectin peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* obtained as directed in the *Assay for ivermectin.* The retention time of the pyrantel peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* obtained as directed in the *Assay for pyrantel pamoate.*

Microbial enumeration tests ⟨61⟩ and ***Tests for specified microorganisms*** ⟨62⟩—The total aerobic microbial count does not exceed 1000 cfu per g, and the total combined molds and yeasts count does not exceed 100 cfu per g. Tablets meet the requirements of the test for absence of *Escherichia coli.*

Uniformity of dosage units ⟨905⟩: The requirements for dosage uniformity are met if the amount of ivermectin and pyrantel pamoate in each of the 10 dosage units as determined from the Content Uniformity method lies within the range of 75.0% to 125.0% of the label claim, and the RSD is less than or equal to 7%.

If not more than 3 units are outside the range of 75.0% to 125.0% of label claim, and no unit is outside the range of 75.0% to 135.0% of label claim, or if the RSD is greater than 7%, or if both conditions prevail, test 20 additional units. The requirements are met if not more than 3 units of the 30 are outside the range of 75.0% to 125.0% of label claim, and no unit is outside the range of 75.0% to 135.0% of label claim and the RSD of the 30 dosage units does not exceed 9%.

pH ⟨791⟩: between 4 and 6, in a solution prepared as follows. Grind about 15 g of Tablets in a blender. Transfer 10 g of the coarse powder thus obtained to a blender jar, add 250 mL of water, previously adjusted to a pH of 7.0 with 0.01 N sodium hydroxide or 0.01 N hydrochloric acid, and blend for about 5 minutes. Allow to settle, and filter a portion of the supernatant. Determine the pH of the filtrate.

Assay for ivermectin—

Mobile phase—Prepare a mixture of acetonitrile, methanol, 0.05 M monobasic sodium phosphate, and water (1130:670:200:5), adjust with phosphoric acid to a pH of 3.0, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of methanol and water (95:5).

Alumina column—Add about 30 mL of water to about 500 g of neutral alumina, and shake for about 2 hours on a reciprocating shaker. Add about 4 g of the resulting suspension to a 10-mm × 10-cm chromatographic tube fitted with a stopcock, tapping the sides of the column to facilitate settling of the alumina to a height of between 5 and 6 cm. Prepare a separate column for each solution to be tested.

Standard stock solution—Prepare a solution of USP Ivermectin RS in methanol having a known concentration of about 0.28 mg of USP Ivermectin RS per mL. Transfer 4.0 mL of this solution and 5.0 mL of water to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.56 µg of USP Ivermectin RS per mL.

Standard preparation—Transfer 15.0 mL of the *Standard stock solution* to a 50-mL centrifuge tube, add 4.0 mL of water, and mix on a vortex mixer. Extract this solution with 10 mL of hexanes by shaking for about 10 minutes. Centrifuge, and discard the hexanes layer. Repeat the extraction with 5 mL of hexanes by shaking for 5 minutes. Centrifuge, and discard the hexanes layer. Add the aqueous layer to the *Alumina column,* and allow to elute. Discard the first 2 mL of eluant, and collect the next 5 mL of eluant in a stoppered tube.

Assay stock solution—Grind an accurately weighed Tablet until it is completely broken up and free from lumps and transfer to a screw-capped, wide-mouth, amber bottle of appropriate volume. Add an accurately measured volume of *Diluent* to the bottle to obtain the estimated concentration of ivermectin of about 0.55 µg per mL. Cap the bottle, and mix on a vortex mixer for about 1 minute. Ultrasonicate for 15 minutes then mechanically shake for an additional 30 minutes. Centrifuge a portion of the suspension thus obtained for about 5 minutes.

USP Monographs

www.webofpharma.com

Assay preparation—Transfer 15.0 mL of the *Assay stock solution* to a 50-mL centrifuge tube, add 4.0 mL of water, and mix on a vortex mixer. Proceed as directed under *Standard preparation,* beginning with "Extract this solution." The solution thus obtained is the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 245-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.2 mL per minute. The column temperature is maintained at about 30°. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the relative retention times are about 0.8 for component H_2B_{1b} and 1.0 for component H_2B_{1a}; the resolution, *R*, between the component H_2B_{1b} peak and the component H_2B_{1a} peak is not less than 2.5; the column efficiency determined from the component H_2B_{1a} peak is not less than 2000 theoretical plates; the tailing factor at 5.0% of peak height for the H_2B_{1a} peak is not more than 2.2; and the relative standard deviation for replicate injections determined from the sum of the component H_2B_{1b} peak and the component H_2B_{1a} peak is not more than 2.0%. [NOTE—After the column has been used, if the system suitability requirements are not met, regenerate the column as follows. Wash the column with 50 mL of water, slowly increasing the flow rate to 1 mL per minute. Make at least seven 100-µL injections of a dimethyl sulfoxide and water solution (1:1) at 5-minute intervals using water as a mobile phase. Purge the column with 100 mL of methanol, 200 mL of methylene chloride, and again with 100 mL of methanol, slowly increasing the flow rate to 1 mL per minute after each solvent changeover. Finally, purge the column with *Mobile phase,* increasing the flow rate to that used for the analysis.]

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of label claim of ivermectin [component H_2B_{1a} ($C_{48}H_{74}O_{14}$) plus component H_2B_{1b} ($C_{47}H_{72}O_{14}$)] in the Tablet taken by the formula:

$$100(P(C_S/C_U))(r_U/r_S)$$

in which C_S is the concentration, in µg per mL, of USP Ivermectin RS in the *Standard stock solution;* C_U is the nominal concentration, in µg per mL, of ivermectin in the *Assay stock solution;* *P* is the purity of the USP Ivermectin RS [percent (w/w) H_2B_{1a} plus percent (w/w) H_2B_{1b}], expressed as a decimal; and r_U and r_S are the sums of the peak areas for component H_2B_{1a} and component H_2B_{1b} obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Assay for pyrantel pamoate— [NOTE—Use amber glassware in preparing solutions of pyrantel pamoate, and otherwise protect the solutions from unnecessary exposure to bright light. Complete the *Assay for pyrantel pamoate* without prolonged interruption.]

Extraction solvent—Prepare a mixture of tetrahydrofuran and trifluoroacetic acid (94:6).

Mobile phase—Prepare a degassed mixture of acetonitrile, water, acetic acid, and triethylamine (940:25: 25:10). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). [NOTE—Increasing the amount of acetonitrile in the *Mobile phase* increases retention times.]

Standard preparation—Prepare a solution of USP Pyrantel Pamoate RS in *Extraction solvent* having a known concentration of about 1.7 mg per mL. Transfer 4.0 mL of this solution to a 25-mL volumetric flask, dilute with tetrahydrofuran to volume, and mix. This solution contains about 0.27 mg of USP Pyrantel Pamoate RS per mL. [NOTE—Stable for 72 hours if stored at room temperature in a dark area.]

Assay preparation—Grind an accurately weighed Tablet until it is completely broken up and free from lumps and transfer to a 300-mL stock bottle, add 50.0 mL of *Extraction solvent,* mix, sonicate for about 15 minutes, and shake by mechanical means for about 1 hour. Allow to settle, and decant the supernatant into a second 300-mL bottle. Add a second 50-mL portion of *Extraction solvent* to the stock bottle, and shake by mechanical means for about 1 hour. Allow to settle, and decant the supernatant to the second 300-mL bottle. Add a third 50-mL portion of *Extraction solvent* to the stock bottle, and shake by mechanical means for about 1 hour. Transfer the contents of the second 300-mL bottle to the stock bottle. Rinse the second bottle with 50.0 mL of *Extraction solvent,* and transfer this rinsing to the stock bottle. Sonicate the stock bottle for about 10 minutes, and centrifuge a portion of the liquid. Transfer an accurately measured volume of the clear supernatant, equivalent to about 6.5 mg of pyrantel pamoate, to a 25-mL volumetric flask, dilute with tetrahydrofuran to volume, and mix.

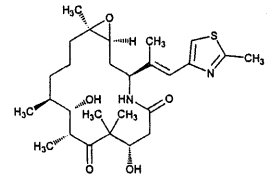
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 313-nm detector and a 4.6-mm × 25-cm column that contains packing L3. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the column efficiency determined from the pyrantel peak is not less than 8000 theoretical plates; the tailing factor is not more than 1.3; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of label claim of pyrantel pamoate ($C_{34}H_{30}N_2O_6S$) in the Tablet taken by the formula:

$$100\ (C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Pyrantel Pamoate RS in the *Standard preparation;* C_U is the nominal concentration of pyrantel pamoate in the *Assay preparation;* and r_U and r_S are the pyrantel peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively. [NOTE—Where the test for *Uniformity of dosage units* has been performed using the *Assay for pyrantel pamoate* procedure as a test for *Content uniformity,* use the average of these determinations as the *Assay for pyrantel pamoate* value.]

Ixabepilone



$C_{27}H_{42}N_2O_5S$ 506.70

17-Oxa-4-azabicyclo[14.1.0]heptadecane-5,9-dione, 7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[(1*E*)-1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-, (1*S*,3*S*,7*S*,10*R*,11*S*,12*S*,16*R*)-;

www.webofpharma.com

(1*S*,3*S*,7*S*,10*R*,11*S*,12*S*,16*R*)-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[(*E*)-1-(2-methylthiazol-4-yl)prop-1-en-2-yl]-17-oxa-4-azabicyclo[14.1.0]heptadecane-5,9-dione. [219989-84-1].

DEFINITION

Ixabepilone contains NLT 97.5% and NMT 102.0% of ixabepilone ($C_{27}H_{42}N_2O_5S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A. ▲Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer stock solution: 100 mM tris(hydroxymethyl) aminomethane in water prepared as follows. Dissolve 12.1 g of tris(hydroxymethyl)aminomethane in 1 L of water and adjust to a pH of 8.5.

Buffer: 5 mM tris(hydroxymethyl)aminomethane in water, from *Buffer stock solution*

Solution A: Acetonitrile and *Buffer* (10:90)

Solution B: Acetonitrile and *Buffer* (90:10)

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the system for 6 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
2.5	70	30
10	60.6	39.4
20	35	65
22	12.5	87.5
25	12.5	87.5

Standard solution: 1.0 mg/mL of USP Ixabepilone RS in acetonitrile. Sonicate to assist the dissolution.

Sample solution: 1.0 mg/mL of Ixabepilone in acetonitrile. Sonicate to assist the dissolution.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Autosampler temperature: 4°

Flow rate: 1.8 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 1.0% for six injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ixabepilone ($C_{27}H_{42}N_2O_5S$) in the portion of Ixabepilone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ixabepilone RS in the *Standard solution* (mg/mL)

C_U = concentration of Ixabepilone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.0% on the anhydrous basis

IMPURITIES

- **Organic Impurities**

Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 1.0 mg/mL of USP Ixabepilone System Suitability Mixture RS in acetonitrile. Sonicate to assist the dissolution.

Sensitivity solution: 0.3 µg/mL of USP Ixabepilone RS in acetonitrile from *Standard solution*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.0 between ixabepilone oxazine analog and desmethyl ixabepilone peaks, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Ixabepilone taken:

$$\text{Result} = r_U/\{\Sigma[r_U \times (1/F)] + r_T\} \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

F = relative response factor for each individual impurity (see *Table 2*)

r_T = peak response of ixabepilone from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.03%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ixabepilone diol	0.47	1.0	0.15
Ixabepilone oxazine analog	0.79	1.0	0.15
Desmethyl ixabepilone	0.85	1.0	0.4
Ixabepilone	1.0	—	—
(*Z*)-Ixabepilone	1.19	0.83	0.15
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.8

SPECIFIC TESTS

- **Water Determination** ⟨921⟩, *Method I, Method Ic*: NMT 0.2%
- **Bacterial Endotoxins Test** ⟨85⟩: It contains NMT 0.32 USP Endotoxin Units/mg of ixabepilone.

USP Monographs

www.webofpharma.com

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from light and store at 2°–8°.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Endotoxin RS

USP Ixabepilone RS

USP Ixabepilone System Suitability Mixture RS

It contains ixabepilone and small amounts of:

Ixabepilone diol;
(4*S*,7*R*,8*S*,9*S*,13*R*,14*R*,16*S*)-4,8,13,14-Tetrahydroxy-5,5,7,9,13-pentamethyl-16-[(*E*)-1-(2-methylthiazol-4-yl)prop-1-en-2-yl]azacyclohexadecane-2,6-dione.
$C_{27}H_{44}N_2O_6S$ 524.72

Ixabepilone oxazine analog;
(1*R*,2*R*,6*S*,7*S*,8*R*,11*S*,15*S*)-2,7,11-Trihydroxy-2,6,8,10,10-pentamethyl-15-[(*E*)-1-(2-methylthiazol-4-yl)prop-1-en-2-yl]-17-oxa-14-azabicyclo[11.3.1]heptadec-13-en-9-one.
$C_{27}H_{42}N_2O_5S$ 506.70

Desmethyl ixabepilone;
(1*S*,3*S*,7*S*,10*R*,11*S*,12*S*,16*R*)-7,11-Dihydroxy-8,8,10,12-tetramethyl-3-[(*E*)-1-(2-methylthiazol-4-yl)prop-1-en-2-yl]-17-oxa-4-azabicyclo[14.1.0]heptadecane-5,9-dione.
$C_{26}H_{40}N_2O_5S$ 492.68

(*Z*)-Ixabepilone;
(1*S*,3*S*,7*S*,10*R*,11*S*,12*S*,16*R*)-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[(*Z*)-1-(2-methylthiazol-4-yl)prop-1-en-2-yl]-17-oxa-4-azabicyclo[14.1.0]heptadecane-5,9-dione.
$C_{27}H_{42}N_2O_5S$ 506.70

[NOTE—It may contain other impurities.]

www.webofpharma.com

www.webofpharma.com

Combined Index to USP 43 and NF 38, Volumes 1–5

Page citations refer to the pages of Volumes 1, 2, 3, 4 and 5 of USP 43–NF 38. This index is repeated in its entirety in each volume.

1–2482 Volume 1
2483–4722 Volume 2
4723–6108 Volume 3
6109–7208 Volume 4
7209–8760 Volume 5

Numbers in angle brackets such as ⟨421⟩ refer to chapter numbers in the General Chapters section.

A

Abacavir
  oral solution, 19
  sulfate, 23
  tablets, 20
  and lamivudine tablets, 21
Abiraterone
  acetate, 25
  acetate tablets, 26
Absolute
  alcohol, 6133
  ether, 6131
Absorbable
  dusting powder, 1569
  gelatin film, 2092
  gelatin sponge, 2093
  surgical suture, 4189
Absorbent
  cotton, 6132
  gauze, 2091
Acacia, 5597
  syrup, 5597
Acamprosate calcium, 28
Acarbose, 30
  tablets, 31
Acebutolol hydrochloride, 33
  capsules, 35
Acepromazine maleate, 36
  injection, 37
  tablets, 38
Acesulfame potassium, 5598
Acetal, 6132
Acetaldehyde, 6132
  TS, 6225
Acetaldehyde ammonia trimer trihydrate, 6132
Acetaminophen, 38
  aspirin and caffeine tablets, 49
  and aspirin tablets, 48
  butalbital and caffeine capsules, 645
  butalbital and caffeine tablets, 646
  and caffeine tablets, 51
  capsules, 40
  and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, capsules containing at least three of the following, 52
  and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, oral powder containing at least three of the following, 54
  and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, oral solution containing at least three of the following, 56
  and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, tablets containing at least three of the following, 58
  chlorpheniramine maleate, and dextromethorphan hydrobromide tablets, 60
  and codeine phosphate capsules, 52
  and codeine phosphate oral solution, 54
  and codeine phosphate oral suspension, 56
  and codeine phosphate tablets, 66
  dextromethorphan hydrobromide, doxylamine succinate, and pseudoephedrine hydrochloride oral solution, 67
  and diphenhydramine citrate tablets, 69
  diphenhydramine hydrochloride, and pseudoephedrine hydrochloride tablets, 70
  and hydrocodone bitartrate tablets, 2224
  isometheptene mucate, and dichloralphenazone capsules, 2423
  and oxycodone capsules, 3341
  and oxycodone tablets, 3343
  and pentazocine tablets, 3460
  and pseudoephedrine hydrochloride tablets, 72
  oral solution, 41
  for effervescent oral solution, 42
  suppositories, 44
  oral suspension, 42
  tablets, 45
  extended-release tablets, 47
  and tramadol hydrochloride oral suspension, 4452
Acetanilide, 6132
Acetate
  methyl, 6178
Acetate buffer, 6145
  TS, 6225
Acetazolamide, 73
  for injection, 74
  oral suspension, 76
  tablets, 76
Acetic acid, 5599, 6132
  ammonium acetate buffer TS, 6225
  diluted, 5599, 6132, 6159
  double-normal (2 N), 6238
  glacial, 77, 6132, 6168
  glacial, TS, 6229
  and hydrocortisone otic solution, 2235
  irrigation, 78
  metaphosphoric, TS, 6231
  otic solution, 78
  strong, TS, 6225
  1 M TS, 6225
  2 M TS, 6225
Acetic acid in peptides, 6741
0.008 M Acetic acid TS, 6225
0.3 N Acetic acid TS, 6225
Acetic anhydride, 6132
Acetohydroxamic acid, 78
  tablets, 79
Acetone, 5600, 6132
  anhydrous, 6132
  neutralized, 6132, 6227
Acetonitrile, 6132
  spectrophotometric, 6132
Acetophenone, 6132
p-Acetotoluidide, 6132
Acetylacetone, 6132
Acetyl chloride, 6132
Acetylcholine chloride, 80, 6132
  for ophthalmic solution, 81
Acetylcysteine, 82
Acetylcysteine
  compounded solution, 83
  and isoproterenol hydrochloride inhalation solution, 84

www.webofpharma.com

Acetylcysteine *(continued)*
solution, 82
N-Acetylglucosamine, 4745
2-Acetylresorcinol, 6133
3-Acetylthio-2-methylpropanoic acid, 6133
Acetyltributyl citrate, 5601
Acetyltriethyl citrate, 5601
N-Acetyltyrosine, 4746
N-Acetyl-L-tyrosine ethyl ester, 6133
Acid
acrylic, 6133
alpha lipoic, 5124
dehydroacetic, 5741
ferric chloride TS, 6225
ferrous sulfate TS, 6225
Folic, compounded oral soultion, 2027
iminodiacetic, 6172
stannous chloride TS, 6225
stannous chloride TS, stronger, 6225
Acid-neutralizing capacity ⟨301⟩, 6661
Acidulated phosphate and sodium fluoride topical solution, 4074
Acitretin, 85
capsules, 86
Acoustic emission ⟨1005⟩, 7230, 7644, 8117
Acrylic acid, 6133
Activated
alumina, 6133
charcoal, 923, 6133
magnesium silicate, 6133
Acyclovir, 88
capsules, 89
for injection, 90
ointment, 91
oral suspension, 92
tablets, 92
Adamantane, 6133
Adapalene, 93
gel, 96
Ademetionine disulfate tosylate, 4747
Adenine, 97
sulfate, 6133
Adenosine, 98
injection, 99
Adipic acid, 5602, 6133
Admissions
to *NF 38*,
to *USP 43*, xxxi

Aerosol

Bacitracin and polymyxin B sulfate topical, 468
Benzocaine, butamben, and tetracaine hydrochloride topical, 513
Benzocaine and menthol topical, 518
Benzocaine topical, 503
Fluticasone propionate and salmeterol inhalation, 2005
Fluticasone propionate inhalation, 1988
Inhalation and nasal drug products: aerosols, sprays, and powders—performance quality tests ⟨601⟩, 6819
Isoproterenol sulfate inhalation, 2437
Lidocaine topical, 2622
Polymyxin B sulfate and bacitracin zinc topical, 3591
Povidone–iodine topical, 3635
Thimerosal topical, 4346
Tolnaftate topical, 4425
Triamcinolone acetonide topical, 4483

Agar, 5603, 6133
Agarose, 6133
Air, medical, 100
Air-helium certified standard, 6133
Alanine, 101
L-Alanyl-L-glutamine, 4748
Albendazole, 102
oral suspension, 102
tablets, 103
Albumen TS, 6225
Albumin
bovine serum, 6133
human, 104
rAlbumin human, 5604
Albuterol, 104
sulfate, 109
tablets, 105
extended-release tablets, 106
Alclometasone dipropionate, 110
cream, 111
ointment, 112
Alcohol, 113, 6133
70 percent, 80 percent, and 90 percent, 6133
absolute, 6133
aldehyde-free, 6134
alpha-(2-(methylamino)ethyl)benzyl, 6134
amyl, 6134
tert-amyl, 6134, 6137, 6173
butyl, 5648
dehydrated, 115, 6134, 6155
dehydrated isopropyl, 6134
denaturated, 6134
denaturated, TS, 6228
determination ⟨611⟩, 6848
in dextrose injection, 117
diluted, 5607, 6134
injection, dehydrated, 117
isobutyl, 6134
isopropyl, 6134
methyl, 6134
neutralized, 6134, 6182
phenol TS, 6225
n-propyl, 6134
rubbing, 118
secondary butyl, 6134
tertiary butyl, 6134
Alcoholic
ammonia TS, 6225
mercuric bromide TS, 6226
potassium hydroxide TS, 6226
potassium hydroxide TS 2, 6234
TS, 6225
Alcoholic potassium hydroxide 0.2 N VS, 6238
Alcoholometric table, 6346
Aldehyde dehydrogenase, 6134
Alendronate sodium, 119
tablets, 120
Alfadex, 5607
Alfentanil
hydrochloride, 122
injection, 123
Alfuzosin hydrochloride, 124
extended-release tablets, 125
Alginates assay ⟨311⟩, 6662
Alginic acid, 5608
Alizarin complexone, 6134
Alkaline
cupric citrate TS, 6226
cupric citrate TS 2, 6226
cupric iodide TS, 6226
mercuric-potassium iodide TS, 6226
phosphatase enzyme, 6134
picrate TS, 6226
pyrogallol TS, 6234
sodium hydrosulfite TS, 6226
Alkaline borate buffer, 6145
Alkaline cupric tartrate TS, 6226
Alkyl (C12-15) benzoate, 5609
Alkylphenoxypolyethoxyethanol, 6134
Allantoin, 130
Allopurinol, 131
oral suspension, 133
tablets, 134
Allyl isothiocyanate, 135
Almond oil, 5609
Almotriptan
tablets, 138
Almotriptan malate, 135
Aloe, 140
Alosetron
tablets, 142
Alosetron hydrochloride, 141
Alpha
lipoic acid, 5124
Alpha-chymotrypsin, 6134
Alpha cyclodextrin hydrate, 6134
Alpha-(2-(methylamino)ethyl)benzyl alcohol, 6134
Alphanaphthol, 6134
Alphazurine 2G, 6220
Alprazolam, 144
oral suspension, 145
tablets, 146
extended-release tablets, 148
orally disintegrating tablets, 152
Alprenolol hydrochloride, 6134
Alprostadil, 154
injection, 156
Alteplase, 157
for injection, 160
Alternative microbiological sampling methods for nonsterile inhaled and nasal products ⟨610⟩, 6846
Altretamine, 163
capsules, 164
Alum, 6134
ammonium, 165, 6134
potassium, 166, 6192
Alumina, 6134
activated, 6133, 6134, 6138
anhydrous, 6134
aspirin, codeine phosphate, and magnesia tablets, 404
aspirin, and magnesia tablets, 397
aspirin, and magnesium oxide tablets, 399
magnesia, and calcium carbonate chewable tablets, 169
magnesia, calcium carbonate, and simethicone chewable tablets, 170
magnesia, and calcium carbonate oral suspension, 168
magnesia, and simethicone oral suspension, 173
magnesia, and simethicone chewable tablets, 174
and magnesia oral suspension, 166
and magnesia tablets, 167
magnesium carbonate, and magnesium oxide tablets, 178
and magnesium carbonate oral suspension, 176
and magnesium carbonate tablets, 177
and magnesium trisilicate oral suspension, 179
and magnesium trisilicate tablets, 180
Aluminon, 6134
Aluminum, 6135
acetate topical solution, 181

www.webofpharma.com

Aluminum *(continued)*
chloride, 181
chlorohydrate, 182
chlorohydrate solution, 183
chlorohydrex polyethylene glycol, 184
chlorohydrex propylene glycol, 185
dichlorohydrate, 185
dichlorohydrate solution, 186
dichlorohydrex polyethylene glycol, 187
dichlorohydrex propylene glycol, 188
hydroxide gel, 188
hydroxide gel, dried, 189
hydroxide gel capsules, dried, 190
hydroxide gel tablets, dried, 190
monostearate, 5612
oxide, 5613
oxide, acid-washed, 6134, 6135
phosphate gel, 190
potassium sulfate, 6135
sesquichlorohydrate, 191
sesquichlorohydrate solution, 191
sesquichlorohydrex polyethylene glycol, 192
sesquichlorohydrex propylene glycol, 192
subacetate topical solution, 193
sulfate, 193
sulfate and calcium acetate tablets for topical solution, 195
zirconium octachlorohydrate, 195
zirconium octachlorohydrate solution, 196
zirconium octachlorohydrex gly, 197
zirconium octachlorohydrex gly solution, 198
zirconium pentachlorohydrate, 199
zirconium pentachlorohydrate solution, 199
zirconium pentachlorohydrex gly, 200
zirconium pentachlorohydrex gly solution, 201
zirconium tetrachlorohydrate, 202
zirconium tetrachlorohydrate solution, 203
zirconium tetrachlorohydrex gly, 203
zirconium tetrachlorohydrex gly solution, 204
zirconium trichlorohydrate, 205
zirconium trichlorohydrate solution, 206
zirconium trichlorohydrex gly, 206
zirconium trichlorohydrex gly solution, 207
Aluminum oxide strips, 6135
Aluminum tartrate, 6137
Aluminum ⟨206⟩, 6605
Aluminum chloride, 6135
Aluminum chloride hexahydrate, 6135
Aluminum sulfate
and calcium acetate for topical solution, 194
Amantadine hydrochloride, 208
capsules, 209
oral solution, 212
Amaranth, 6135
TS, 6226
Amcinonide, 214
cream, 214
ointment, 215
American ginseng, 4750
capsules, 4754
extract, powdered, 4753
powdered, 4752
tablets, 4757
Amifostine, 216
for injection, 217
Amikacin, 219
sulfate, 220
sulfate injection, 221
Amiloride hydrochloride, 222
and hydrochlorothiazide tablets, 224
tablets, 223
Amiloxate, 227
Aminoacetic acid, 6135
4-Aminoantipyrine, 6135
Aminobenzoate
potassium, 228
potassium capsules, 230
sodium, 231
Aminobenzoic acid, 233
gel, 234
p-Aminobenzoic acid, 6135
2-Aminobenzonitrile, 6135
Aminocaproic acid, 235
injection, 236
oral solution, 236
tablets, 237
4-Amino-6-chloro-1,3-benzenedisulfonamide, 6135
4-Amino-2-chlorobenzoic acid, 6135, 6151
2-Amino-5-chlorobenzophenone, 6151, 6151
7-Aminodesacetoxycephalosporanic acid, 6135
2-Aminoethyl diphenylborinate, 6135
1-(2-Aminoethyl)piperazine, 6135
Aminoguanidine bicarbonate, 6136
2-Aminoheptane, 6136
N-Aminohexamethyleneimine, 6136
Aminohippurate sodium injection, 237
Aminohippuric acid, 238
4-Amino-3-hydroxy-1-naphthalenesulfonic acid, 6136
Aminolevulinic acid
hydrochloride, 238
Amino methacrylate copolymer, 5614
1,2,4-Aminonaphtholsulfonic acid, 6136
Aminonaphtholsulfonic acid TS, 6226
Aminopentamide sulfate, 239
injection, 240
tablets, 240
2-Aminophenol, 6136
4-Aminophenol in acetaminophen-containing drug products ⟨227⟩, 6637
m-Aminophenol, 6136
p-Aminophenol, 6136
Aminophylline, 241
injection, 243
oral solution, 245
rectal solution, 246
suppositories, 247
tablets, 248
delayed-release tablets, 250
3-Amino-1-propanol, 6136
3-Aminopropionic acid, 6136
Aminosalicylic acid, 251
3-Aminosalicylic acid, 6136
Amiodarone
hydrochloride injection, 254
Amiodarone hydrochloride, 253
oral suspension, 257
Amiodarone hydrochloride
tablets, 257
Amitraz, 259
concentrate for dip, 260
Amitriptyline hydrochloride, 261
and chlordiazepoxide tablets, 938
and perphenazine tablets, 3487
tablets, 263
Amlodipine
oral suspension, 264
and olmesartan medoxomil tablets, 272
and benazepril hydrochloride capsules, 269
and valsartan tablets, 275
valsartan and hydrochlorothiazide tablets, 279
Amlodipine and atorvastatin
tablets, 265
Amlodipine besylate, 285
tablets, 286
Ammonia
alcoholic TS, 6225
detector tube, 6136
N 13 injection, 3183
nitrate TS, silver, 6234
solution, diluted, 6136
solution, strong, 5616
spirit, aromatic, 287
TS, 6136, 6226
TS 2, 6226
TS alcoholic, 6226
TS stronger, 6226
water, stronger, 6207, 6226
water, 25 percent, 6136
Ammonia-ammonium chloride buffer TS, 6226
Ammoniacal potassium ferricyanide TS, 6226
Ammonia-cyanide TS, 6226
Ammoniated cupric oxide TS, 6226
Ammonio methacrylate copolymer, 5616
dispersion, 5618
Ammonium
acetate, 6136
acetate TS, 6226
alum, 165
bicarbonate, 6136
bisulfate, 6136
bromide, 6136
carbonate, 5619, 6137
carbonate TS, 6226
carbonate TS 2, 6226
chloride, 288, 6137
chloride–ammonium hydroxide TS, 6226
chloride injection, 288
chloride, potassium gluconate, and potassium citrate oral solution, 3622
chloride delayed-release tablets, 288
chloride TS, 6226
citrate, dibasic, 6137, 6156
citrate, ferric, 289
citrate for oral solution, ferric, 290
dihydrogen phosphate, 6137
fluoride, 6137
formate, 6137
glycyrrhizate, 5619
hydroxide, 6137
hydroxide 6 N, 6137
molybdate, 290, 6137
molybdate injection, 291
molybdate TS, 6226
nitrate, 6137
nitrate, ceric TS, 6227
nitrate TS, silver, 6234
oxalate, 6137
oxalate TS, 6226
persulfate, 6137
phosphate, 5620
phosphate, dibasic, 6137, 6156
phosphate, dibasic, TS, 6226
phosphate, monobasic, 6137
polysulfide TS, 6226
pyrrolidinedithiocarbamate, 6137
pyrrolidinedithiocarbamate, saturated, TS, 6226
reineckate, 6137
reineckate TS, 6226
sulfamate, 6137
sulfate, 5621, 6137
sulfate, cupric TS, 6227

www.webofpharma.com

Ammonium *(continued)*
sulfate, ferric TS, 6229
sulfide TS, 6226
thiocyanate, 6137
thiocyanate, tenth-normal (0.1 N), 6238
thiocyanate TS, 6226
vanadate, 6137
vanadate TS, 6226
Ammonium hydrogen difluoride , 6137
Ammonium hydroxide
1 M TS, 6226
2 M TS, 6226
Amobarbital sodium, 291
for injection, 292
Amodiaquine, 292
hydrochloride, 293
hydrochloride tablets, 294
Amoxapine, 295
tablets, 297
Amoxicillin, 297
boluses, 299
capsules, 300
and clavulanate potassium for oral suspension, 308
and clavulanate potassium tablets, 308
and clavulanic acid extended-release tablets, 310
for injectable suspension, 302
intramammary infusion, 302
oral suspension, 303
for oral suspension, 303
tablets, 304
tablets for oral suspension, 306
Amphetamine
sulfate, 312
sulfate tablets, 313
Amphotericin B, 314
cream, 315
for injection, 315
lotion, 316
ointment, 316
Ampicillin, 316
boluses, 321
capsules, 321
for injectable suspension, 324
for injection, 323
and probenecid for oral suspension, 326
sodium, 327
soluble powder, 323
and sulbactam for injection, 328
for oral suspension, 324
tablets, 325
Amprolium, 329
soluble powder, 330
oral solution, 331
Amyl
acetate, 6134, 6137, 6173
alcohol, 6137
nitrite, 331
nitrite inhalant, 332
α-Amylase, 6137
Amylene hydrate, 5622
tert-Amyl alcohol, 6137
Anagrelide
capsules, 334
hydrochloride, 332
Analysis of biological assays ⟨1034⟩, 7351
Analytical data—interpretation and treatment ⟨1010⟩, 7233
Analytical instrument qualification ⟨1058⟩, 7522
Analytical methodologies based on scattering phenomena—electrophoretic light scattering (determination of zeta potential) ⟨1430.4⟩, 8389
Analytical methodologies based on scattering phenomena—general ⟨1430⟩, 8376
Analytical methodologies based on scattering phenomena—light diffraction measurements of particle size ⟨1430.2⟩, 8386
Analytical methodologies based on scattering phenomena—small angle x-ray scattering and small angle neutron scattering ⟨1430.5⟩, 8392
Analytical methodologies based on scattering phenomena—static light scattering ⟨1430.1⟩, 8379
Analytical procedures for recombinant therapeutic monoclonal antibodies ⟨129⟩, 6565
Anastrozole, 336
tablets, 337
Ancillary materials for cell, gene, and tissue-engineered products ⟨1043⟩, 7381
Andrographis, 4758
extract, powdered, 4762
powdered, 4760
Anethole, 5622
(*E*)-Anethole, 6138
Angustifolia
extract, powdered echinacea, 4941
powdered echinacea, 4938

Anhydrous

acetone, 6132
alumina, 6138
barium chloride, 6138
calcium chloride, 6138
calcium phosphate, dibasic, 710
citric acid, 1040
cupric sulfate, 6138
dibasic sodium phosphate, 6138
magnesium perchlorate, 6138
magnesium sulfate, 6138
methanol, 6138
potassium carbonate, 6138
sodium acetate, 6138
sodium carbonate, 6138
sodium phosphate, monobasic, 6205
sodium sulfate, 6138
sodium sulfite, 6138

Anileridine, 339
hydrochloride, 340
hydrochloride tablets, 341
injection, 339
Aniline, 6138
blue, 6138
sulfate, 6138
Animal drugs for use in animal feeds ⟨1152⟩, 7952
Anion-exchange resin
strong, lightly cross-linked, in the chloride form, 6138
50- to 100-mesh, styrene-divinylbenzene, 6138, 6208
styrene-divinylbenzene, 6138
p-Anisaldehyde, 6139
Anise oil, 5624
p-Anisidine, 6139
Anisole, 6139
Annotations
to *NF 38*,
to *USP 43*, xxxiii
Antazoline phosphate, 342
Anthracene, 6139
Anthralin, 343
cream, 344
ointment, 344
Anthrone, 6139
TS, 6226
Antibiotics—microbial assays ⟨81⟩, 6488
Anticoagulant
citrate dextrose solution, 345
citrate phosphate dextrose solution, 347
citrate phosphate dextrose adenine solution, 348
heparin solution, 2194
sodium citrate solution, 350
Anti-D reagent, 6139
Anti-D (Rh_o) reagent, 6139
Anti-factor Xa and anti-factor IIa assays for unfractionated and low molecular weight heparins ⟨208⟩, 6611
Antifoam reagent, 6139
Antihuman globulin reagent, 6139
Antimicrobial
agents—content ⟨341⟩, 6664
effectiveness testing ⟨51⟩, 6449
Antimony
pentachloride, 6139
potassium tartrate, 350
sodium tartrate, 351
trichloride, 6140
trichloride TS, 6226
Antipyrine, 351
and benzocaine otic solution, 352
benzocaine, and phenylephrine hydrochloride otic solution, 354
Antithrombin III, 6140
human, 355
Apomorphine hydrochloride, 357
tablets, 358
Apparent intrinsic dissolution—dissolution testing procedures for rotating disk and stationary disk ⟨1087⟩, 7681
Applications of mass spectrometry ⟨1736⟩, 8508
Applications of nuclear magnetic resonance spectroscopy ⟨1761⟩, 8529
Application of water activity determination to nonsterile pharmaceutical products ⟨1112⟩, 7820
Apraclonidine
hydrochloride, 359
ophthalmic solution, 359
Aprepitant, 360
capsules, 362
Aprobarbital, 6140
Aprotinin, 364
injection, 367
Aqua regia, 6140
Argatroban, 368
Arginine, 371
capsules, 4763
hydrochloride, 371
hydrochloride injection, 373
tablets, 4764
Aripiprazole, 373
orally disintegrating tablets, 377
tablets, 375
Aromatic
castor oil, 807
elixir, 5624
Arsanilic acid, 379
Arsenazo III acid, 6140
Arsenic
in reagents, 6128
trioxide, 6140

www.webofpharma.com

Arsenic ⟨211⟩, 6621
Articaine
  hydrochloride, 380
  hydrochloride and epinephrine injection, 381
Articles
  appearing in *USP 43* that were not included in *USP 40* including supplements, xxix
  of Incorporation, xxvii
Articles of botanical origin ⟨561⟩, 6774
Ascorbic acid, 383, 6140
  compounded oral solution, 385
  injection, 383
  oral solution, 384
  tablets, 385
  10 TS, 6227
Ascorbic acid chewable gels, 4765
Ascorbyl palmitate, 5624
Ashwagandha root, 4766
  extract, powdered, 4768
  powdered, 4771
Asian ginseng, 4774
  extract, powdered, 4776
  powdered, 4775
  tablets, 4778
Asparagine, 5625
L-Asparagine, 6140
Aspart
  insulin, 2339
Aspartame, 5626
  acesulfame, 5627
Aspartic acid, 386
L-Aspartic acid, 6140
Aspirin, 388
  acetaminophen and caffeine tablets, 49
  and acetaminophen tablets, 48
  alumina and magnesia tablets, 397
  alumina and magnesium oxide tablets, 399
  boluses, 388
  butalbital, and caffeine capsules, 648
  butalbital, caffeine, and codeine phosphate capsules, 651
  butalbital, and caffeine tablets, 649
  and butalbital tablets, 646
  caffeine, and dihydrocodeine bitartrate capsules, 402
  capsules, 389
  delayed-release capsules, 390
  carisoprodol, and codeine phosphate tablets, 780
  and carisoprodol tablets, 778
  codeine phosphate, alumina, and magnesia tablets, 404
  and codeine phosphate tablets, 403
  effervescent tablets for oral solution, 395
  orphenadrine citrate and caffeine tablets, 3288
  and oxycodone tablets, 3344
  and pentazocine tablets, 3461
  suppositories, 391
  tablets, 392
  tablets, buffered, 393
  delayed-release tablets, 394
  extended-release tablets, 396
Assay
  alginates ⟨311⟩, 6662
  antibiotics, iodometric ⟨425⟩, 6696
  for citric acid/citrate and phosphate ⟨345⟩, 6668
  dexpanthenol ⟨115⟩, 6547
  epinephrine ⟨391⟩, 6676
  folic acid ⟨411⟩, 6689
  niacin or niacinamide ⟨441⟩, 6704
  riboflavin ⟨481⟩, 6735
  single-steroid ⟨511⟩, 6750
  for steroids ⟨351⟩, 6669
  thiamine ⟨531⟩, 6756
  vitamin A ⟨571⟩, 6801
  vitamin B_{12} activity ⟨171⟩, 6584
  vitamin D ⟨581⟩, 6808
  vitamin E ⟨551⟩, 6767
Assays
  antibiotics—microbial ⟨81⟩, 6488
  design and analysis of biological ⟨111⟩, 6543
  insulin ⟨121⟩, 6547
Assessment of drug product performance—bioavailability, bioequivalence, and dissolution ⟨1090⟩, 7695
Assessment of drug product leachables associated with pharmaceutical packaging/delivery systems ⟨1664⟩, 8455
Assessment of extractables associated with pharmaceutical packaging/delivery systems ⟨1663⟩, 8442
Astaxanthin esters, 4779
Astragalus root, 4782
  dry extract, 4787
  powder, 4785
Atenolol, 405
  and chlorthalidone tablets, 409
  injection, 406
  oral solution, 407
  tablets, 407
Atenolol compounded
  oral suspension, 408
Atenolol compounded, veterinary
  oral suspension, 409
Atomic absorption spectroscopy ⟨852⟩, 7142
Atomic absorption spectroscopy—theory and practice ⟨1852⟩, 8632
Atomic masses, 6345
Atomic weights, 6344
Atomoxetine
  capsules, 412
Atomoxetine hydrochloride, 411
Atorvastatin calcium, 414
Atorvastatin calcium
  tablets, 418
Atovaquone, 422
  oral suspension, 423
Atracurium besylate, 424
  injection, 426
Atropine, 428
  sulfate, 428
  sulfate and diphenoxylate hydrochloride oral solution, 1438
  sulfate and diphenoxylate hydrochloride tablets, 1439
  sulfate injection, 430
  sulfate ophthalmic ointment, 431
  sulfate ophthalmic solution, 432
Attapulgite, activated, 433
  colloidal, 433
Aurothioglucose, 434
  injectable suspension, 434
Auxiliary packaging components ⟨670⟩, 6916
Avobenzone, 435
Azaperone, 435
  injection, 436
Azatadine maleate, 437
  tablets, 438
Azathioprine, 438
  oral suspension, 440
  sodium for injection, 442
  tablets, 441
Azelastine hydrochloride, 443
Azelastine hydrochloride ophthalmic solution, 445
Azithromycin, 446
  capsules, 450
  for injection, 451
  for oral suspension, 454
  tablets, 457
Azo violet, 6220
Aztec marigold zeaxanthin extract, 4789
Aztreonam, 460
  injection, 462
  for injection, 463
Azure A, 6140

B

Bacillus coagulans, 4791
Bacillus coagulans capsules, 4794
Bacillus subtilis subsp. *subtilis* menaquinone-7 extract, 5152
Bacitracin, 464
  for injection, 466
  methylene disalicylate, soluble, 467
  methylene disalicylate soluble powder, 467
  neomycin and polymyxin B sulfates and hydrocortisone acetate ointment, 3116
  neomycin and polymyxin B sulfates and hydrocortisone acetate ophthalmic ointment, 3116
  neomycin and polymyxin B sulfates and lidocaine ointment, 3117
  and neomycin and polymyxin B sulfates ointment, 3115
  and neomycin and polymyxin B sulfates ophthalmic ointment, 3115
  and neomycin sulfate ointment, 3104
  ointment, 466
  ophthalmic ointment, 467
  and polymyxin B sulfate topical aerosol, 468
  zinc, 468
  zinc, neomycin and polymyxin B sulfates, and hydrocortisone ointment, 3118
  zinc, neomycin and polymyxin B sulfates, and hydrocortisone ophthalmic ointment, 3119
  zinc, neomycin and polymyxin B sulfates, and hydrocortisone acetate ophthalmic ointment, 3120
  zinc, neomycin and polymyxin B sulfates, and lidocaine ointment, 3120
  zinc and neomycin and polymyxin B sulfates ointment, 3118
  zinc and neomycin and polymyxin B sulfates ophthalmic ointment, 3118
  zinc and neomycin sulfate ointment, 3104
  zinc ointment, 470
  zinc and polymyxin B sulfate topical aerosol, 3591
  zinc and polymyxin B sulfate ointment, 471
  zinc and polymyxin B sulfate ophthalmic ointment, 471
  zinc and polymyxin B sulfate topical powder, 3591
  zinc soluble powder, 470
Baclofen, 471
  oral suspension, 473
  tablets, 473

www.webofpharma.com

Bacopa, 4797
  extract, powdered, 4800
  powdered, 4798
Bacterial
  alkaline protease preparation, 6140
  endotoxins test ⟨85⟩, 6508
Bacteriostatic
  sodium chloride injection, 4067
  water for injection, 4652
Balances ⟨41⟩, 6449
Balsalazide disodium, 475
  capsules, 477
Banaba leaf, 4801
  extract, dry, 4805
  powder, 4803
Bandage
  adhesive, 478
  gauze, 478
Barbital sodium, 6140
Barbituric acid, 6140
Barium
  acetate, 6140
  chloride, 6140
  chloride, anhydrous, 6138, 6140
  chloride dihydrate, 6140
  chloride TS, 6227
  hydroxide, 6140
  hydroxide lime, 479
  hydroxide TS, 6227
  nitrate, 6141
  nitrate TS, 6227
  sulfate, 479
  sulfate for suspension, 482
  sulfate paste, 480
  sulfate suspension, 481
  sulfate tablets, 483
0.05 M Barium perchlorate VS, 6238
Basic fuchsin, 6141
Beclomethasone, 6141
Beclomethasone dipropionate, 483
Beclomethasone dipropionate compounded oral solution, 484
Beef extract, 6141
Behenoyl polyoxylglycerides, 5628
Belladonna
  leaf, 485
  extract, 486
  extract tablets, 487
  tincture, 488
Benazepril hydrochloride, 488
  and amlodipine hydrochloride capsules, 269
  tablets, 491
Benazepril hydrochloride compounded, veterinary
  oral suspension, 490
Bendamustine hydrochloride, 493
Bendamustine hydrochloride for injection, 495
Bendroflumethiazide, 496
  and nadolol tablets, 3062
  tablets, 497
Benoxinate hydrochloride, 498
  and fluorescein sodium ophthalmic solution, 1944
  ophthalmic solution, 499
Bentonite, 5629
  magma, 5632
  purified, 5630
Benzaldehyde, 5632, 6141
  elixir, compound, 5634
Benzalkonium chloride, 5634, 6141
  solution, 5636
Benzamidine hydrochloride hydrate, 6141
Benzanilide, 6141
Benzene, 6141
Benzenesulfonamide, 6141
Benzenesulfonyl chloride, 6141
Benzethonium chloride, 499
  concentrate, 500
  topical solution, 501
  tincture, 501
Benzhydrol, 6141
Benzil, 6141
Benzocaine, 502
  topical aerosol, 503
  and antipyrine otic solution, 352
  antipyrine, and phenylephrine hydrochloride otic solution, 354
  butamben, and tetracaine hydrochloride topical aerosol, 513
  butamben, and tetracaine hydrochloride gel, 514
  butamben, and tetracaine hydrochloride ointment, 516
  butamben, and tetracaine hydrochloride topical solution, 517
  cream, 505
  gel, 506
  lozenges, 507
  and menthol topical aerosol, 518
  ointment, 509
  otic solution, 510
  topical solution, 511
Benzoic
  acid, 520, 6141
  and salicylic acids ointment, 521
Benzoin, 522
  tincture, compound, 522
Benzonatate, 523
  capsules, 523
Benzophenone, 6141
p-Benzoquinone, 6197
Benzoyl
  chloride, 6141
  peroxide and erythromycin topical gel, 1692
  peroxide gel, 526
  peroxide, hydrous, 525
  peroxide lotion, 527
N-Benzoyl-L-arginine ethyl ester hydrochloride, 6141
3-Benzoylbenzoic acid, 6141
Benzoylformic acid, 6141
Benzphetamine hydrochloride, 6141
Benzphetamine hydrochloride, 528
  tablets, 530
Benztropine mesylate, 532
  injection, 532
  tablets, 533
Benzyl
  alcohol, 5639
  benzoate, 534
  benzoate lotion, 535
N-Benzylacetamide, 6142
2-Benzylaminopyridine, 6142
1-Benzylimidazole, 6142
Benzylpenicilloyl polylysine
  concentrate, 535
  injection, 536
Benzyltrimethylammonium chloride, 6142
Beta carotene, 536
  capsules, 538
  preparation, 4806
Betadex, 5641
  sulfobutyl ether sodium, 5643
Beta glucan, 4808
Betahistine hydrochloride, 540
Betaine hydrochloride, 540
Beta-lactoglobulin, 6174
Beta-lactoglobulin A, 6174
Betamethasone, 541
  acetate, 544
  acetate and betamethasone sodium phosphate injectable suspension, 551
  acetate and gentamicin sulfate ophthalmic solution, 2102
  benzoate, 544
  benzoate gel, 545
  cream, 541
  dipropionate, 546
  dipropionate and clotrimazole cream, 1124
  dipropionate cream, 547
  dipropionate lotion, 548
  dipropionate ointment, 548
  sodium phosphate, 549
  sodium phosphate and betamethasone acetate injectable suspension, 551
  sodium phosphate injection, 551
  oral solution, 542
  valerate, 552
  valerate cream, 553
  valerate and gentamicin sulfate ointment, 2103
  valerate and gentamicin sulfate otic solution, 2104
  valerate and gentamicin sulfate topical solution, 2105
  valerate lotion, 554
  valerate ointment, 556
Betanaphthol, 6142
  TS, 6227
Betaxolol
  hydrochloride, 557
  ophthalmic solution, 558
  tablets, 559
Bethanechol chloride, 561
  injection, 562
  oral solution, 563
  oral suspension, 564
  tablets, 564
Beta-lactamase, 6142, 6186
Bibenzyl, 6142, 6156
Bicalutamide, 566
  tablets, 567
Bifidobacterium animalis subsp. *lactis*, 4811
Bilberry
  extract, powdered, 4813
Bile salts, 6142, 6203
(S)-Binol, 6142
Bioburden control of nonsterile drug substances and products ⟨1115⟩, 7827
Biocompatibility of materials used in drug containers, medical devices, and implants, the ⟨1031⟩, 7313
Biological
  assay chapters—overview and glossary ⟨1030⟩, 7300
  assay validation ⟨1033⟩, 7337
  indicators—resistance performance tests ⟨55⟩, 6452
  indicators for sterilization ⟨1229.5⟩, 8216
  reactivity tests, in vitro ⟨87⟩, 6514
  reactivity tests, in vivo ⟨88⟩, 6516
Biologics ⟨1041⟩, 7379
Biotechnology products: stability testing of biotechnological/biological products, quality of ⟨1049⟩, 7454
Biotechnology-derived articles
  amino acid analysis ⟨1052⟩, 7481
  isoelectric focusing ⟨1054⟩, 7499
  peptide mapping ⟨1055⟩, 7501
  polyacrylamide gel electrophoresis ⟨1056⟩, 7508
  total protein assay ⟨1057⟩, 7515

www.webofpharma.com

Biotechnology products derived from cell lines of human or animal origin, viral safety evaluation of ⟨1050⟩, 7459
Biotin, 569
capsules, 570
tablets, 571
Biotin compounded oral suspension, 571
Biphenyl, 6142
2,2′-Bipyridine, 6162
Bis(4-sulfobutyl) ether disodium, 6143
Bisacodyl, 572
rectal suspension, 574
suppositories, 573
delayed-release tablets, 574
4,4′-Bis(4-amino-naphthylazo)-2,2′-stilbenedisulfonic acid, 6143
Bis(2-ethylhexyl)
maleate, 6143
(phosphoric acid), 6143
phthalate, 6143
sebacate, 6143
Bismuth, 6143
citrate, 576
iodide TS, potassium, 6233
milk of, 575
nitrate pentahydrate, 6143
nitrate, 0.01 mol/L, 6239
subcarbonate, 576
subgallate, 577
subnitrate, 578, 6143
subsalicylate, 579
subsalicylate magma, 580
subsalicylate oral suspension, 582
subsalicylate tablets, 583
sulfite, 6220
sulfite agar, 6143
Bisoctrizole, 583
Bisoprolol fumarate, 585
and hydrochlorothiazide tablets, 587
tablets, 586
Bis(trimethylsilyl)
acetamide, 6143
trifluoroacetamide, 6143
trifluoroacetamide with trimethylchlorosilane, 6143
Biuret reagent TS, 6227
Black cohosh, 4815
fluidextract, 4821
powdered, 4817
powdered extract, 4820
tablets, 4823
Black pepper, 4825
powdered, extract, 4829
powdered, 4827
Bleomycin
for injection, 588
sulfate, 589

Blood

Blood, 6143
Grouping reagent, anti-A, grouping reagent, anti-B, and grouping reagent, anti-AB, 6143
Technetium Tc 99m red blood cells injection, 4235

Blue
G TS, brilliant , 6227
tetrazolium, 6144
tetrazolium TS, 6227
Board of trustees
USP Convention (2015–2020), ix
Boiling or distilling range for reagents, 6128
Boldine, 6144
Boluses
amoxicillin, 299
ampicillin, 321
aspirin, 388
dihydrostreptomycin sulfate, 1391
neomycin, 3102
phenylbutazone, 3511
tetracycline, 4302
Borage seed oil, 4830
capsules, 4831
Boric acid, 5646, 6144
(–)-Bornyl acetate, 6144
Boron trifluoride, 6144
14% Boron trifluoride–methanol, 6144
Boswellia serrata, 4832
extract, 4834
Botanical
extracts ⟨565⟩, 6798
origin, identification of articles of ⟨563⟩, 6788
Bovine collagen, 6144
Bovine serum ⟨1024⟩, 7260
7 percent bovine serum albumin certified standard, 6144
Branched polymeric sucrose, 6144
Bretylium tosylate, 590
in dextrose injection, 591
injection, 590
Brilliant
blue G TS, 6227
green, 6220
yellow, 6220
Brimonidine tartrate, 591
Brinzolamide, 592
ophthalmic suspension, 594
Bromelain, 6144
Bromine, 6145
sodium acetate TS, 6227
tenth-normal (0.1 N), 6239
TS, 6227
α-Bromo-2′-acetonaphthone, 6145
p-Bromoaniline, 6145
TS, 6227
Bromocresol
blue, 6220
blue TS, 6220
green, 6220
green-methyl red TS, 6227
green sodium salt, 6220
green TS, 6220, 6227
purple, 6220
purple sodium salt, 6220
purple TS, 6227
Bromocriptine mesylate, 595
capsules, 596
tablets, 597
Bromodiphenhydramine hydrochloride, 599
and codeine phosphate oral solution, 600
oral solution, 599
Bromofluoromethane, 6145
Bromophenol blue, 6220
sodium, 6220
TS, 6227
N-Bromosuccinimide, 6145
Bromothymol blue, 6220
TS, 6227
Brompheniramine maleate, 601
injection, 602
and pseudoephedrine sulfate oral solution, 603
oral solution, 602
tablets, 603
Brucine sulfate, 6145
Budesonide, 604

Buffer

Acetate TS, 6225
Acetic acid–ammonium acetate TS, 6225
Acetone buffered, TS, 6227
Acid phthalate, 6145
Alkaline borate, 6145
Hydrochloric acid, 6145
Neutralized phthalate, 6145

Buffered acetone TS, 6227
Buffers, 6145
Buffer solutions, 6145, 6223
acetate buffer, 6145
alkaline borate buffer, 6145
hydrochloric acid buffer, 6145
neutralized phthalate buffer, 6145
phosphate buffer, 6145
Bulk density and tapped density of powders ⟨616⟩, 6850
Bulk pharmaceutical excipients—certificate of analysis ⟨1080⟩, 7647
Bulk powder sampling procedures ⟨1097⟩, 7732
Bumetanide, 606
injection, 607
tablets, 608
Bupivacaine hydrochloride, 610
in dextrose injection, 612
and epinephrine injection, 612
injection, 611
Bupivacaine Hydrochloride
fentanyl citrate compounded injection, 1850
Buprenorphine
hydrochloride, 614
Buprenorphine and naloxone
sublingual tablets, 616
Buprenorphine compounded, veterinary
buccal solution, 615
Bupropion hydrochloride, 618
tablets, 620
extended-release tablets, 621
Buspirone hydrochloride, 634
tablets, 637
Busulfan, 639
tablets, 640
Butabarbital, 640
sodium, 641
sodium oral solution, 642
sodium tablets, 643
Butalbital, 644
acetaminophen, and caffeine capsules, 645
acetaminophen, and caffeine tablets, 646
aspirin, and caffeine capsules, 648
aspirin, caffeine, and codeine phosphate capsules, 651
aspirin, and caffeine tablets, 649
and aspirin tablets, 646
Butamben, 653
benzocaine, and tetracaine hydrochloride topical aerosol, 513
benzocaine, and tetracaine hydrochloride gel, 514
benzocaine, and tetracaine hydrochloride ointment, 516

www.webofpharma.com

Butamben *(continued)*
benzocaine, and tetracaine hydrochloride topical solution, 517
Butane, 5647
Butane-1,2-diol, 6145
Butane-1,4-diol, 6145
Butane-2,3-diol, 6145
1,3-Butanediol, 6145
2,3-Butanedione, 6145, 6156
1-Butanesulfonic acid sodium salt, 6145
1,4-Butane sultone, 6145
Butanol, 6145
Butoconazole nitrate, 654
vaginal cream, 655
Butorphanol tartrate, 655
injection, 656
nasal spray, 657
Butyl
acetate, normal, 6145
alcohol, 5648, 6145, 6146, 6184
alcohol, normal, 6145
alcohol, secondary, 6134, 6146, 6198
alcohol, tertiary, 6134, 6209
benzoate, 6146
ether, 6146
methacrylate, 6146
palmitostearate, 5649
stearate, 5650
n-Butyl chloride, 6146, 6151
tert-Butyl methyl ether, 6146
n-Butylamine, 6146, 6184
tert-Butylamine, 6146, 6184
4-(Butylamino)benzoic acid, 6146
Butylated
hydroxyanisole, 5651
n-Butylboronic acid, 6146
tert-Butyldimethylchlorosilane in *N*-methyl-*N*-*tert*-butyldimethylsilyltrifluoroacetamide, (1 in 100), 6146
Butylene glycol, 5653
Butylparaben, 5655, 6146
4-*tert*-Butylphenol, 6146
t-Butylthiol, 6146
Butyraldehyde, 6146
Butyric acid, 6146
Butyrolactone, 6146
Butyrophenone, 6147

C

C 13
for oral solution, urea, 767
urea, 766
C 14
capsules, urea, 768
Cabergoline, 658
tablets, 659
Cadmium
acetate, 6147
nitrate, 6147
Caffeine, 661
acetaminophen and aspirin tablets, 49
and acetaminophen tablets, 51
aspirin and dihydrocodeine bitartrate capsules, 402
butalbital, and acetaminophen capsules, 645
butalbital, and acetaminophen tablets, 646
butalbital, and aspirin capsules, 648
butalbital, aspirin, and codeine phosphate capsules, 651
butalbital, and aspirin tablets, 649
citrate injection, 662
citrate oral solution, 663
and ergotamine tartrate suppositories, 1680
and ergotamine tartrate tablets, 1681
orphenadrine citrate and aspirin tablets, 3288
and sodium benzoate injection, 664
Calamine, 664
topical suspension, phenolated, 665
topical suspension, 665
Calcifediol, 666
capsules, 666
Calcipotriene, 667
ointment, 668
Calcitonin salmon, 670
injection, 674
nasal solution, 675
Calcitriol, 675
injection, 677
Calcium
acetate, 677, 6147
acetate and aluminum sulfate tablets for topical solution, 195
acetate tablets, 682
ascorbate, 682
carbonate, 683, 5656, 6147
carbonate, alumina, and magnesia chewable tablets, 169
carbonate, alumina, magnesia, and simethicone chewable tablets, 170
carbonate, alumina, and magnesia oral suspension, 168
carbonate, chelometric standard, 6147
carbonate lozenges, 685
carbonate, magnesia, and simethicone chewable tablets, 689
carbonate and magnesia chewable tablets, 688
carbonate oral suspension, 686
carbonate tablets, 687
caseinate, 6147
chloride, 692, 6147
chloride, anhydrous, 6138, 6147
chloride injection, 693
chloride TS, 6227
citrate, 693, 6147
citrate tablets, 4835
glubionate syrup, 695
gluceptate, 696
gluceptate injection, 697
gluconate, 697
gluconate injection, 700
gluconate tablets, 701
glycerophosphate, 4836
hydroxide, 701, 6147
hydroxide topical solution, 702
hydroxide TS, 6227
lactate, 702, 6147
lactate tablets, 703
lactobionate, 704
Leucovorin comounded oral solution, 2570
levulinate, 705
levulinate injection, 705
and magnesium carbonates oral suspension, 691
and magnesium carbonates tablets, 691
nitrate, 6148
pantothenate, 706
pantothenate assay ⟨91⟩, 6536
pantothenate, dextro, 6148
pantothenate, racemic, 708
pantothenate tablets, 707
phosphate, anhydrous dibasic, 710
phosphate tablets, dibasic, 712
phosphate, tribasic, 5657
phosphate dihydrate, dibasic, 708
polycarbophil, 713
propionate, 5658
saccharate, 713
silicate, 5659
stearate, 5661
succinate, 714
sulfate, 5662, 6148
sulfate TS, 6227
undecylenate, 714
and vitamin D with minerals tablets, 4845
with vitamin D tablets, 4843
Calcium acetate
and aluminum sulfate for topical solution, 194
Calcium acetate
capsules, 680
Calcium citrate malate, 694
Calcium glubionate
syrup, 695
Calcium L-5-methyltetrahydrofolate, 4838
capsules, 4841
tablets, 4842
Calconcarboxylic acid, 6148
triturate, 6148
Calf thymus DNA, 6148
dl-Camphene, 6148
Camphor, 715
spirit, 715
d-10-Camphorsulfonic acid, 6148
dl-10-Camphorsulfonic acid, 6148
Canada balsam, 6148
Candelilla wax, 5663
Candesartan cilexetil, 716
and hydrochlorothiazide tablets, 719
tablets, 717
Canola oil, 5664
Capecitabine, 722
tablets, 723
Capillary electrophoresis ⟨1053⟩, 7492
Capreomycin
for injection, 726
sulfate, 725
Capric acid, 6148
Caprylic acid, 5664
Caprylocaproyl polyoxylglycerides, 5665
Capsaicin, 727
Capsicum, 728
oleoresin, 730
tincture, 732

Capsules

Acebutolol hydrochloride, 35
Acetaminophen, 40
Containing at least three of the following—acetaminophen and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, 52
Acetaminophen and codeine phosphate, 52
Acitretin, 86
Acyclovir, 89
Altretamine, 164
Aluminum hydroxide gel, dried, 190
Amantadine hydrochloride, 209
Aminobenzoate potassium, 230
Amlodipine and benazepril hydrochloride, 269
Amoxicillin, 300
Ampicillin, 321

www.webofpharma.com

Capsules *(continued)*
Anagrelide, 334
Aprepitant, 362
Arginine, 4763
Aspirin, 389
Aspirin, caffeine, and dihydrocodeine bitartrate, 402
Aspirin delayed-release, 390
Atomoxetine, 412
Azithromycin, 450
Bacillus coagulans, 4794
Balsalazide disodium, 477
Benzonatate, 523
Beta carotene, 538
Biotin, 570
Borage seed oil, 4831
Bromocriptine mesylate, 596
Butalbital, acetaminophen, and caffeine, 645
Butalbital, aspirin, and caffeine, 648
Butalbital, aspirin, caffeine, and codeine phosphate, 651
Calcifediol, 666
Calcium acetate, 680
Calcium L-5-methyltetrahydrofolate, 4841
C 14, urea, 768
Castor oil, 805
Cat's claw, 4857
Cefaclor, 809
Cefadroxil, 815
Cefdinir, 827
Cephalexin, 897
Cephradine, 907
Chloral hydrate, 924
Chloramphenicol, 928
Chlordiazepoxide hydrochloride, 941
Chlordiazepoxide hydrochloride and clidinium bromide, 943
Chlorpheniramine maleate extended-release, 970
Chlorpheniramine maleate and pseudoephedrine hydrochloride extended-release, 972
Cholecalciferol, 986
Clindamycin hydrochloride, 1065
Clofazimine, 1085
Clofibrate, 1088
Clomipramine hydrochloride, 1093
Cloxacillin sodium, 1129
Cod liver oil, 4910
Crypthecodinium cohnii oil, 4925
Curcuminoids, 4929
Cyclobenzaprine hydrochloride extended-release capsules, 1197
Cycloserine, 1211
Cyclosporine, 1213
Danazol, 1234
Dantrolene sodium, 1236
Diazoxide, 1333
Dicloxacillin sodium, 1358
Dicyclomine hydrochloride, 1360
Didanosine delayed-release, 1364
Digitalis, 1380
Dihydrotachysterol, 1392
Diltiazem hydrochloride extended-release, 1399
Diphenhydramine hydrochloride, 1425
Diphenhydramine hydrochloride and ibuprofen, 1431
Diphenhydramine and pseudoephedrine, 1435
Disopyramide phosphate, 1450
Disopyramide phosphate extended-release, 1450
Divalproex sodium delayed-release, 1454
Docusate calcium, 1476
Docusate potassium, 1478
Docusate sodium, 1479
Doxepin hydrochloride, 1509
Doxycycline, 1519
Doxycycline extended-release, 1521
Doxycycline hyclate, 1534
Doxycycline hyclate delayed-release, 1536
Dronabinol, 1549
Duloxetine delayed-release, 1559
Echinacea species, 4966
Echinacea species dry extract, 4960
Efavirenz, 1588
Eleuthero root and rhizome dry extract, 4972
Eleuthero root and rhizome powder, 4976
Ephedrine sulfate, 1642
Ergocalciferol, 1666
Ergoloid mesylates, 1670
Erythromycin delayed-release, 1686
Erythromycin estolate, 1693
Esomeprazole magnesium delayed-release, 1716
Ethchlorvynol, 1766
Ethosuximide, 1774
Etodolac, 1786
Etoposide, 1794
Evening primrose oil, 4980
Fenofibrate, 1834
Fenoprofen calcium, 1845
Ferrous gluconate, 1859
Fexofenadine hydrochloride, 1870
Fish oil containing omega-3 acids, 4994
Fish oil containing omega-3 acids, delayed-release, 4997
Flax seed oil, 4999
Flucytosine, 1916
Fluoxetine, 1958
Fluoxetine delayed-release, 1959
Flurazepam hydrochloride, 1978
Flutamide, 1984
Fluvastatin, 2020
Gabapentin, 2058
Galantamine extended-release, 2074
Gemfibrozil, 2097
Ginger, 5032
Ginkgo, 5037
Ginseng, American, 4754
Griseofulvin, 2165
Guaifenesin, 2170
Guaifenesin and pseudoephedrine hydrochloride, 2174
Guaifenesin, pseudoephedrine hydrochloride, and dextromethorphan hydrobromide, 2175
Hydrochlorothiazide, 2219
Hydroxyurea, 2263
Hydroxyzine pamoate, 2271
Imipramine pamoate, 2304
Indomethacin, 2324
Indomethacin extended-release, 2325
Sodium iodide I 123, 2365
Sodium iodide I 131, 2368
Isometheptene mucate, dichloralphenazone, and acetaminophen, 2423
Isosorbide dinitrate extended-release, 2441
Isotretinoin, 2458
Isradipine, 2465
itraconazole, 2468
Kanamycin sulfate, 2503
Ketoprofen, 2510
Ketoprofen extended-release, 2511
Krill oil, 5104
Krill oil delayed-release, 5107
Lansoprazole delayed-release, 2553
Lincomycin hydrochloride, 2634
Alpha lipoic acid, 5125
Lithium carbonate, 2650
Lomustine, 2656
Loperamide hydrochloride, 2660
Loxapine, 2697
Lutein, 5128
Magnesium oxide, 2721
Meclofenamate sodium, 2754
Mefenamic acid, 2758
Menaquinone-7, 5147
Mesalamine extended-release, 2802
Methoxsalen, 2863
Methsuximide, 2870
Methyltestosterone, 2906
Metronidazole, 2934
Metyrosine, 2942
Mexiletine hydrochloride, 2944
Milk thistle, 5162
Minerals, 5164
Minocycline hydrochloride, 2965
Morphine sulfate extended-release, 3024
Mycophenolate mofetil, 3044
Nifedipine, 3161
Nitrofurantoin, 3174
Nizatidine, 3192
Nortriptyline hydrochloride, 3216
Oil- and water-soluble vitamins with minerals, 5435
Olanzapine and fluoxetine, 3237
Oleovitamin A and D, 3242
Omega-3 ethyl esters, 3253
Omeprazole delayed-release, 3258
Orlistat, 3280
Oseltamivir phosphate, 3293
Oxazepam, 3314
Oxycodone and acetaminophen, 3341
Oxytetracycline hydrochloride, 3362
Pancrelipase, 3384
Pancrelipase delayed-release, 3384
Paricalcitol, 3404
Paromomycin sulfate, 3408
Penicillamine, 3427
Phendimetrazine tartrate, 3493
Phenoxybenzamine hydrochloride, 3503
Phentermine hydrochloride, 3506
Phenytoin sodium, extended, 3531
Piroxicam, 3578
Potassium chloride extended-release, 3602
Prazosin hydrochloride, 3653
Procainamide hydrochloride, 3689
Procarbazine hydrochloride, 3696
Propranolol hydrochloride extended-release, 3747
Pseudoephedrine hydrochloride extended-release, 3762
Pygeum, 5200
Quinidine sulfate, 3813
Quinine sulfate, 3819
Ramipril, 3839
Rhodiola rosea, 5224
Ribavirin, 3863
Rifabutin, 3873
Rifampin, 3876
Rifampin and isoniazid, 3878
Ritonavir, 3914
Rivastigmine tartrate, 3925
Propafenone hydrochloride extended-release, 3729
St. John's wort flowering top dry extract, 5242
Salsalate, 3981
Saquinavir, 3984
Saw palmetto, 5259

www.webofpharma.com

Capsules *(continued)*
Schizochytrium oil, 5269
Secobarbital sodium, 4015
Selegiline hydrochloride, 4018
Simethicone, 4043
Soy isoflavones, 5276
Stavudine, 4120
Sulfinpyrazone, 4172
Tacrolimus, 4196
Tamsulosin hydrochloride, 4213
Temazepam, 4251
Temozolomide, 4253
Terazosin, 4261
Tetracycline hydrochloride, 4305
Tetracycline hydrochloride and nystatin, 4313
Thalidomide, 4318
Theophylline, 4321
Theophylline extended-release, 4322
Theophylline and guaifenesin, 4331
Thiothixene, 4359
Tienchi ginseng root and rhizome dry extract, 5309
Tienchi ginseng root and rhizome powder, 5304
Tolmetin sodium, 4423
Topiramate, 4432
Triamterene, 4495
Triamterene and hydrochlorothiazide, 4496
Trientine hydrochloride, 4510
Trihexyphenidyl hydrochloride extended-release, 4518
Trimethobenzamide hydrochloride, 4524
Ubidecarenone, 5320
Ursodiol, 4558
Valerian root dry extract , 5332
Valerian root powder, 5333
Valproic acid, 4573
Vancomycin hydrochloride, 4588
Venlafaxine hydrochloride extended-release, 4598
Verapamil hydrochloride extended-release, 4605
Vinpocetine, 5336
Vitamin A, 4633
Vitamin E, 4639
Vitamins with minerals, oil-soluble, 5361
Vitamins with minerals, oil- and water-soluble, 5435
Vitamins with minerals, water-soluble, 5523
Vitamins, oil-soluble, 5346
Vitamins, oil- and water-soluble, 5389
Vitamins, water-soluble, 5499
Zaleplon, 4669
Zidovudine, 4674
Ziprasidone, 4695
Zonisamide, 4719

Capsules—dissolution testing and related quality attributes ⟨1094⟩, 7725
Captopril, 733
and hydrochlorothiazide tablets, 737
oral solution, 734
oral suspension, 735
tablets, 735
Caramel, 5666
Caraway, 5666
oil, 5667
Carbachol, 738
intraocular solution, 739
ophthalmic solution, 739
Carbamazepine, 740
oral suspension, 742
tablets, 743
extended-release tablets, 746
Carbamide peroxide, 747
topical solution, 747
Carbazole sulfate, 6148
Carbenicillin
disodium, 748
indanyl sodium, 748
indanyl sodium tablets, 749
for injection, 748
Carbidopa, 750
and levodopa extended-release tablets, 753
and levodopa orally disintegrating tablets, 759
and levodopa tablets, 751
Carbinoxamine maleate, 761
pseudoephedrine hydrochloride, and dextromethorphan hydrobromide oral solution, 3767
tablets, 763
Carbol-fuchsin topical solution, 765
Carbomer
934, 5667
934P, 5668
940, 5670
941, 5671
1342, 5672
copolymer, 5673
homopolymer, 5676
interpolymer, 5679
Carbon
C 13 for oral solution, urea, 767
C 13, urea, 766
C 14 capsules, urea, 768
dioxide, 765
dioxide detector tube, 6148
disulfide, chromatographic, 6148
disulfide, CS, 6148
monoxide detector tube, 6148
tetrachloride, 6148
Carbonates
calcium and magnesium, oral suspension, 691
calcium and magnesium, tablets, 691
Carboplatin, 768
for injection, 769
Carboprost
tromethamine, 771
tromethamine injection, 772
Carboxylate (sodium form) cation-exchange resin (50- to 100-mesh), 6148
Carboxymethoxylamine hemihydrochloride, 6149
Carboxymethylcellulose
calcium, 5682
sodium, 773
sodium 12, 5684
sodium, low-substituted, 5682
sodium and microcrystalline cellulose, 5698
sodium paste, 774
sodium tablets, 774
Carboxymethylcellulose sodium
enzymatically-hydrolyzed, 5685
Cardamom
oil, 5688
seed, 5688
tincture, compound, 5689
Carisoprodol, 776
aspirin and codeine phosphate tablets, 780
and aspirin tablets, 778
tablets, 777
Carmellose, 5689
Carmine, 6149
Carmustine, 782
for injection, 784
Carotenes, 4850
Carprofen, 785
tablets, 786
Carrageenan, 5690
Carteolol hydrochloride, 788
ophthalmic solution, 789
tablets, 790
Carvedilol, 791
tablets, 793
(*R*)-(–)-Carvone, 6149
Casanthranol, 795
Cascara
fluidextract, aromatic, 801
sagrada, 797
sagrada extract, 798
sagrada fluidextract, 801
tablets, 800
Casein, 6149
hammersten, 6149
Castor oil, 802
aromatic, 807
capsules, 805
emulsion, 806
hydrogenated, 5691
polyoxyl 35, 5956
Catechol, 6149
Cation-exchange resin, 6149
carboxylate (sodium form) (50- to 100-mesh), 6148, 6149
polystyrene, 6149, 6192
styrene-divinylbenzene, 6149
styrene-divinylbenzene, strongly acidic, 6149
sulfonic acid, 6149, 6209
Cat's claw, 4852
capsules, 4857
extract, powdered, 4855
powdered, 4854
tablets, 4858
Cedar oil, 6149
Cefaclor, 807
capsules, 809
for oral suspension, 810
extended-release tablets, 811
Cefadroxil, 813
capsules, 815
for oral suspension, 816
tablets, 817
Cefamandole nafate, 818
for injection, 819
Cefazolin, 820
injection, 822
for injection, 823
sodium, 824
Cefdinir, 826
capsules, 827
for oral suspension, 830
Cefepime
hydrochloride, 834
for injection, 832
Cefixime, 836
for oral suspension, 837
tablets, 838
Cefmenoxime
hydrochloride, 839
for injection, 839
Cefmetazole, 840
injection, 841
for injection, 841
sodium, 842

www.webofpharma.com

Cefonicid
for injection, 842
sodium, 843
Cefoperazone
injection, 844
for injection, 844
sodium, 845
Ceforanide, 846
for injection, 846
Cefotaxime
injection, 847
for injection, 848
sodium, 850
Cefotetan, 853
disodium, 855
injection, 853
for injection, 854
Cefotiam
hydrochloride, 856
for injection, 857
Cefoxitin
injection, 859
for injection, 860
sodium, 858
Cefpiramide, 860
for injection, 862
Cefpodoxime proxetil, 863
for oral suspension, 864
tablets, 864
Cefprozil, 865
for oral suspension, 869
tablets, 870
Ceftazidime, 872
injection, 872
for injection, 873
Ceftiofur
hydrochloride, 875
sodium, 877
Ceftizoxime
injection, 880
for injection, 881
sodium, 880
Ceftriaxone
injection, 881
for injection, 882
sodium, 884
Cefuroxime
axetil, 887
axetil for oral suspension, 888
axetil tablets, 889
injection, 886
for injection, 886
sodium, 891
Celecoxib, 892
Cellaburate, 5694
Cellacefate, 5696
Cellular and tissue-based products ⟨1046⟩, 7400
Cellulose
acetate, 5701
chromatographic, 6149
microcrystalline, 5696, 6150
microcrystalline and carboxymethylcellulose sodium, 5698
mixture, chromatographic, 6150
oxidized, 893
oxidized regenerated, 894
powdered, 5700
silicified microcrystalline, 5699
sodium phosphate, 895
sodium phosphate for oral suspension, 896
Cellulose diethylaminoethyl, 6150
Centella asiatica, 4859
extract, powdered, 4863
powdered, 4861
triterpenes, 4864
Cephalexin, 896
capsules, 897
hydrochloride, 900
for oral suspension, 898
tablets, 899
tablets for oral suspension, 899
Cephalothin
injection, 901
for injection, 901
sodium, 902
Cephapirin
benzathine, 904
benzathine intramammary infusion, 905
for injection, 903
sodium, 905
sodium intramammary infusion, 906
Cephradine, 906
capsules, 907
for injection, 908
for oral suspension, 909
tablets, 909
Ceric
ammonium nitrate, 6150
ammonium nitrate TS, 6227
ammonium nitrate, twentieth-normal (0.05 N), 6239
ammonium sulfate, 6150
sulfate, 6150
sulfate, tenth-normal (0.1 N), 6239
Cesium chloride, 6150
Cetirizine hydrochloride, 910
and pseudoephedrine hydrochloride extended-release tablets, 917
oral solution, 912
tablets, 913
orally disintegrating tablets, 915
Cetostearyl alcohol, 5702
Cetrimide, 6150
Cetrimonium bromide, 5704
Cetyl
alcohol, 5705
esters wax, 5706
palmitate, 5708
Cetylpyridinium chloride, 921
lozenges, 922
topical solution, 923
Cetyltrimethylammonium bromide, 6150, 6169
Cetyltrimethylammonium chloride, 25 percent in water, 6150
Chamomile, 4866
Characterization of crystalline and partially crystalline solids by X-ray powder diffraction (XRPD) ⟨941⟩, 7201
Characterization of crystalline solids by microcalorimetry and solution calorimetry ⟨696⟩, 6931
Charcoal
activated, 923, 6133, 6150
Chaste tree, 4868
powdered, 4870
powdered, extract, 4872
Chelating ion exchange resin, 6150
Chemometrics ⟨1039⟩, 7363
Chenodeoxycholic acid, 6150
Cherry
juice, 5709
syrup, 5709
Chia seed
oil, 4877
Chinese salvia, 4878
powdered, 4880
Chinese skullcap root, 4883
powder, 4885
Chinese skullcap root dry extract, 4887
Chitosan, 5710
Chloral hydrate, 924
capsules, 924
oral solution, 924
TS, 6227
Chlorambucil, 925
tablets, 926
Chloramine T, 6150
Chloramphenicol, 927
capsules, 928
cream, 928
and hydrocortisone acetate for ophthalmic suspension, 932
injection, 928
ophthalmic ointment, 929
ophthalmic solution, 929
for ophthalmic solution, 930
otic solution, 931
palmitate, 933
palmitate oral suspension, 934
and polymyxin B sulfate ophthalmic ointment, 933
sodium succinate, 935
sodium succinate for injection, 936
oral solution, 930
tablets, 932
Chlordiazepoxide, 936
and amitriptyline hydrochloride tablets, 938
hydrochloride, 940
hydrochloride capsules, 941
hydrochloride and clidinium bromide capsules, 943
tablets, 937
Chlorhexidine
acetate, 945
acetate topical solution, 947
gluconate oral rinse, 949
gluconate solution, 948
gluconate topical solution, 951
hydrochloride, 952
Chlorhexidine gluconate
topical gel, 4741
Chloride
cobaltous, TS, 6227
ferric, TS, 6229
ferrous tetrahydrate, 6166
gold, 6169
gold, TS, 6229
platinic, 6191
platinic, TS, 6233
in reagents, 6129
stannous, 6041
and sulfate ⟨221⟩, 6636
Chlorine, 6150
detector tube, 6150
TS, 6227
m-Chloroacetanilide, 6150
p-Chloroacetanilide, 6150
1-Chloroadamantane, 6151
2-Chloro-4-aminobenzoic acid, 6151
5-Chloro-2-aminobenzophenone, 6151
3-Chloroaniline, 6151
p-Chloroaniline, 6151
Chlorobenzene, 6151
4-Chlorobenzoic acid, 6151
m-Chlorobenzoic acid, 6151
4-Chlorobenzophenone, 6151
2-Chlorobenzophenone, 6151
1-Chlorobutane, 6151
Chlorobutanol, 5714
Chlorocresol, 5715
2-Chloroethanol, 6151

www.webofpharma.com

2-Chloroethylamine monohydrochloride, 6151
Chloroform, 6151
alcohol-free, 6151
methyl, 6151
Chlorogenic acid, 6151
Chloromethylated polystyrene-divinylbenzene anion-exchange resin, 6151
1-Chloronaphthalene, 6151
4-Chloro-1-naphthol, 6151
2-Chloronicotinic acid, 6151
2-Chloro-4-nitroaniline, 99%, 6152
Chlorophyllin copper complex sodium, 954
Chloroplatinic acid, 6152
Chloroprocaine hydrochloride, 956
injection, 957
Chloroquine, 957
hydrochloride injection, 958
phosphate, 959
phosphate oral suspension, 960
phosphate tablets, 961
5-Chlorosalicylic acid, 6152
Chlorothiazide, 962
and reserpine tablets, 3859
sodium for injection, 966
oral suspension, 963
tablets, 964
Chlorothiazide compounded
oral suspension, 963
1-Chloro-2,2,2-trifluoroethylchlorodifluoromethyl ether, 6152
Chlorotrimethylsilane, 6152, 6215
Chloroxylenol, 966
Chlorpheniramine
dextromethorphan, pseudoephedrine, (salts of), and acetaminophen, capsules containing at least three of the following, 52
dextromethorphan, pseudoephedrine (salts of), and acetaminophen, oral powder containing at least three of the following, 54
dextromethorphan, pseudoephedrine (salts of), and acetaminophen, oral solution containing at least three of the following, 56
dextromethorphan, pseudoephedrine (salts of), and acetaminophen, tablets containing at least three of the following, 58
maleate, 968
maleate extended-release capsules, 970
maleate injection, 971
maleate, penicillin G procaine, dihydrostreptomycin sulfate, and dexamethasone injectable suspension, 3445
maleate and pseudoephedrine hydrochloride extended-release capsules, 972
maleate and pseudoephedrine hydrochloride oral solution, 973
maleate oral solution, 971
maleate tablets, 971
maleate, acetaminophen, and dextromethorphan hydrobromide tablets, 60
Chlorpromazine, 974
hydrochloride, 975
hydrochloride injection, 976
hydrochloride oral concentrate, 975
hydrochloride syrup, 976
hydrochloride tablets, 977
suppositories, 974
Chlorpropamide, 978
tablets, 978
Chlortetracycline
bisulfate, 979
hydrochloride, 980, 6152
hydrochloride ointment, 980
hydrochloride ophthalmic ointment, 987
hydrochloride soluble powder, 987
hydrochloride tablets, 987
and sulfamethazine bisulfates soluble powder, 980
Chlorthalidone, 982
and atenolol tablets, 409
and clonidine hydrochloride tablets, 1104
tablets, 983
Chlorzoxazone, 983
tablets, 984
Chocolate, 5715
syrup, 5716
Cholecalciferol, 985
capsules, 986
solution, 987
tablets, 988
Cholecalciferol chewable
gels, 4889
Cholestane, 6152
Cholestanol, 6152
Cholesterol, 5716, 6152
Cholesteryl
benzoate, 6152
n-heptylate, 6152
Cholestyramine
resin, 989
for oral suspension, 990
Choline
bitartrate, 4891
chloride, 4892, 6152
Chondroitin sulfate sodium, 4894
and glucosamine tablets, 5043
glucosamine, and methylsulfonylmethane tablets, 5051
shark, 4898
tablets, 4897
Chromate, sodium, Cr 51 injection, 992
Chromatographic
columns, 6252
fuller's earth, 6152
n-heptane, 6152
magnesium oxide, 6152
reagents, 6152
silica gel, 6152
silica gel mixture, 6152
siliceous earth, 6152
siliceous earth, silanized, 6152
solvent hexane, 6152
Chromatography ⟨621⟩, 6853
Chromatography, ion ⟨1065⟩, 7588
Chromic chloride, 990
injection, 991
Chromium
Cr 51 injection, sodium chromate, 992
picolinate, 4901
picolinate tablets, 4902
potassium sulfate dodecahydrate, 6152
trioxide, 6152
Chromogenic
substrate for amidolytic test, 6152
Chromotrope 2R, 6152
Chromotropic acid, 6152, 6202
disodium salt, 6153
TS, 6227
Chymotrypsin, 993
for ophthalmic solution, 994
Ciclopirox, 995
olamine, 997
olamine cream, 998
olamine topical suspension, 998
topical solution, 996
Cidofovir, 999
injection, 1000
Cilastatin
and imipenem for injectable suspension, 2298
and imipenem for injection, 2297
sodium, 1001
Cilostazol, 1003
tablets, 1004
Cimetidine, 1005
hydrochloride, 1008
injection, 1006
in sodium chloride injection, 1007
tablets, 1007
Cinchonidine, 6153
Cinchonine, 6153
Cinnamomum cassia
twig, 4902
twig powder, 4904
Ciprofloxacin, 1009
and dexamethasone otic suspension, 1021
extended-release tablets, 1017
hydrochloride, 1011
injection, 1012
ophthalmic ointment, 1014
ophthalmic solution, 1015
for oral suspension, 1019
tablets, 1015
Cisapride, 1024
Cisapride compounded, veterinary
injection, 1024
oral suspension, 1025
Cisatracurium besylate, 1026
injection, 1028
Cisplatin, 1030
for injection, 1032
Citalopram
hydrobromide, 1037
oral solution, 1034
tablets, 1035
Citicoline sodium, 4906
Citrate
cupric TS, alkaline, 6226, 6228
cupric TS 2, alkaline, 6226, 6228
Citric acid, 6153
anhydrous, 1040, 6153
and magnesium carbonate for oral solution, 2710
magnesium carbonate, and potassium citrate for oral solution, 2711
magnesium oxide, and sodium carbonate irrigation, 1044
monohydrate, 1042
and potassium citrate oral solution, 3617
and potassium and sodium bicarbonates effervescent tablets for oral solution, 3598
and sodium citrate oral solution, 4069
TS, 6227
Citrulline, 4908
Cladribine, 1045
injection, 1046
Clarithromycin, 1047
for oral suspension, 1049
tablets, 1049
extended-release tablets, 1051
Clavulanate
potassium, 1055
potassium and amoxicillin for oral suspension, 308
potassium and amoxicillin tablets, 308

www.webofpharma.com

Clavulanic acid
and amoxicillin extended-release tablets, 310
Clavulanic acid
and ticarcillin injection, 4371
and ticarcillin for injection, 4372
Cleaning glass apparatus ⟨1051⟩, 7481
Clemastine fumarate, 1057
tablets, 1059
Clenbuterol hydrochloride, 1060
Clidinium bromide, 1061
and chlordiazepoxide hydrochloride capsules, 943
Clindamycin
hydrochloride, 1063
hydrochloride capsules, 1065
hydrochloride oral solution, 1066
injection, 1062
for injection, 1063
palmitate hydrochloride, 1068
palmitate hydrochloride for oral solution, 1068
phosphate, 1069
phosphate gel, 1071
phosphate topical solution, 1072
phosphate topical suspension, 1072
phosphate vaginal cream, 1071
phosphate vaginal inserts, 1073
Clindamycin hydrochloride compounded oral solution, 1066
Clioquinol, 1074
cream, 1075
and hydrocortisone cream, 1077
and hydrocortisone ointment, 1078
ointment, 1076
topical powder, compound, 1076
Clobetasol propionate, 1079
cream, 1080
ointment, 1081
topical solution, 1082
Clocortolone pivalate, 1083
cream, 1084
Clofazimine, 1084
capsules, 1085
Clofibrate, 1087
capsules, 1088
Clomiphene citrate, 1088
tablets, 1090
Clomipramine compounded
oral suspension, veterinary, 1091
Clomipramine hydrochloride, 1092
capsules, 1093
Clonazepam, 1096
oral suspension, 1097
tablets, 1098
orally disintegrating tablets, 1099
Clonidine, 1100
hydrochloride, 1101
hydrochloride and chlorthalidone tablets, 1104
hydrochloride tablets, 1103
transdermal system, 1105
Clonidine hydrochloride compounded
oral suspension, 1103
Clopidogrel
bisulfate, 1109
tablets, 1111
Clopidogrel compounded
oral suspension, 1111
Cloprostenol
injection, 1113
sodium, 1113
Clorazepate dipotassium, 1114
tablets, 1115
Clorsulon, 1117
and ivermectin injection, 2477
Clotrimazole, 1118
and betamethasone dipropionate cream, 1124
cream, 1119
lotion, 1119
lozenges, 1120
topical solution, 1122
vaginal inserts, 1123
Clove oil, 5718
Clover, red, 5203
extract, powdered, 5210
powdered, 5207
tablets, 5212
Cloxacillin
benzathine, 1125
benzathine intramammary infusion, 1126
sodium, 1127
sodium capsules, 1129
sodium intramammary infusion, 1129
sodium for oral solution, 1130
Clozapine, 1130
tablets, 1132
Cloxapine N-oxide, 6153
Coal tar, 1133
ointment, 1133
topical solution, 1133
Cobalt
chloride, 6153
nitrate, 6153
platinum, TS, 6233
uranyl acetate TS, 6227
Cobaltous
acetate, 6153
chloride, 6153
chloride CS, 6224
chloride TS, 6227
Cocaine, 1133
hydrochloride, 1134
hydrochloride tablets for topical solution, 1134
Cocoa butter, 5718
Coconut
oil, 5719
oil, hydrogenated, 5720
Codeine, 1137
phosphate, 1137
phosphate and acetaminophen capsules, 52
phosphate and acetaminophen oral solution, 54
phosphate and acetaminophen oral suspension, 56
phosphate and acetaminophen tablets, 66
phosphate, aspirin, alumina, and magnesia tablets, 404
phosphate and aspirin tablets, 403
phosphate and bromodiphenhydramine hydrochloride oral solution, 600
phosphate, butalbital, aspirin, and caffeine capsules, 651
phosphate, carisoprodol, and aspirin tablets, 780
phosphate and guaifenesin oral solution, 2172
phosphate injection, 1138
phosphate tablets, 1139
phosphate and promethazine and phenylephrine hydrochloride oral solution, 3724
phosphate oral solution, 1138
sulfate, 1140
sulfate oral solution, 1141
sulfate tablets, 1142
and terpin hydrate oral solution, 4284
Cod liver oil, 1135
capsules, 4910
Coenzyme Q9, 6153
Coffee fruit dry
extract, 4912
Cohosh
black fluidextract, 4821
Coix seed, 4915
powder, 4917
Colchicine, 1143
injection, 1144
and probenecid tablets, 3687
tablets, 1145
Colestipol hydrochloride, 1145
for oral suspension, 1147
tablets, 1147
Colistimethate
for injection, 1149
sodium, 1148
Colistin
and neomycin sulfates and hydrocortisone acetate otic suspension, 1150
sulfate, 1149
sulfate for oral suspension, 1150
Collagen, 6153
rat tail, 6153
Collagenase, 6153
Collagenase I ⟨89.1⟩, 6524
Collagenase II ⟨89.2⟩, 6529
Collodion, 1151
flexible, 1151
Colloidal oatmeal, 1152
Color
and achromicity ⟨631⟩, 6865
instrumental measurement ⟨1061⟩, 7553
Colorimetric solutions (CS), 6224
Compactin, 6153
Completeness of solution ⟨641⟩, 6866
Compound cardamom tincture, 5689
Compounded topical gel
ondansetron, 3272
Compounding for phase I investigational studies, 7985
Conductivity of solutions ⟨644⟩, 6868
Congealing temperature ⟨651⟩, 6874
Congo red, 6153, 6220
TS, 6227
Constitution and bylaws, xxviii
Construct human fibroblasts in bilayer synthetic scaffold, 1153
Construct human fibroblasts in polyglactin scaffold, 1157
Container content for injections ⟨697⟩, 6934
Containers
glass ⟨660⟩, 6881
performance testing ⟨671⟩, 6922
Container specifications for capsules and tablets, 6259
Coomassie
blue G-250, 6153
brilliant blue R-250, 6153
Copovidone, 5721
Copper, 6153
gluconate, 1168
Copper sulfate pentahydrate, 6153
Coriander oil, 5723
Corn
oil, 5723
starch, 6044
syrup, 5724
high fructose syrup, 5727
syrup solids, 5731
Corticotropin
injection, 1170

www.webofpharma.com

Corticotropin *(continued)*
for injection, 1172
injection, repository, 1174
Cortisone, 6153
acetate, 1175
acetate tablets, 1176
Cosyntropin, 1178
Cotton
absorbent, 6153
purified, 1180
Cotton ⟨691⟩, 6929
Cottonseed oil, 5733
hydrogenated, 5734
Council of experts
(2015–2020), ix
Cr 51
injection, sodium chromate, 992
Cranberry
liquid preparation, 4919

Cream

Alclometasone dipropionate, 111
Amcinonide, 214
Amphotericin B, 315
Anthralin, 344
Benzocaine, 505
Betamethasone, 541
Betamethasone dipropionate, 547
Betamethasone valerate, 553
Butoconazole nitrate, vaginal, 655
Chloramphenicol, 928
Ciclopirox olamine, 998
Clindamycin phosphate, vaginal, 1071
Clioquinol, 1075
Clioquinol and hydrocortisone, 1077
Clobetasol propionate, 1080
Clocortolone pivalate, 1084
Clotrimazole, 1119
Clotrimazole and betamethasone dipropionate, 1124
Crotamiton, 1187
Desoximetasone, 1274
Dibucaine, 1336
Diflorasone diacetate, 1374
Dioxybenzone and oxybenzone, 1418
Estradiol, vaginal, 1725
estriol compounded vaginal, 1743
Estropipate, vaginal, 1754
Flumethasone pivalate, 1928
Fluocinolone acetonide, 1935
Fluocinonide, 1937
Fluorometholone, 1952
Fluorouracil, 1956
Flurandrenolide, 1975
Fluticasone propionate, 1987
Gentamicin sulfate, 2101
Gentian violet, 2108
Halcinonide, 2184
Hydrocortisone, 2229
Hydrocortisone acetate, 2237
Hydrocortisone butyrate, 2239
Hydrocortisone valerate, 2245
Hydroquinone, 2255
Lidocaine and prilocaine, 2630
Lindane, 2635
Mafenide acetate, 2703
Miconazole nitrate, 2949
Mometasone furoate, 3002
Mupirocin, 3040
Naftifine hydrochloride, 3065
Neomycin and polymyxin B sulfates, 3114
Neomycin and polymyxin B sulfates and gramicidin, 3122
Neomycin and polymyxin B sulfates, gramicidin, and hydrocortisone acetate, 3123
Neomycin and polymyxin B sulfates and hydrocortisone acetate, 3125
Neomycin and polymyxin B sulfates and lidocaine, 3126
Neomycin and polymyxin B sulfates and pramoxine hydrochloride, 3126
Neomycin sulfate, 3103
Neomycin sulfate and dexamethasone sodium phosphate, 3104
Neomycin sulfate and fluocinolone acetonide, 3107
Neomycin sulfate and flurandrenolide, 3107
Neomycin sulfate and hydrocortisone, 3109
Neomycin sulfate and hydrocortisone acetate, 3110
Neomycin sulfate and methylprednisolone acetate, 3113
Neomycin sulfate and triamcinolone acetonide, 3129
Nystatin, 3219
Nystatin, neomycin sulfate, gramicidin, and triamcinolone acetonide, 3222
Nystatin, neomycin sulfate, thiostrepton, and triamcinolone acetonide, 3223
Nystatin and triamcinolone acetonide, 3224
Piroxicam, 3579
Pramoxine hydrochloride, 3641
Prednicarbate, 3656
Prednisolone, 3660
Sulfadiazine, silver, 4150
Sulfa, vaginal, triple, 4137
Tetracaine hydrochloride, 4296
Tolnaftate, 4426
Tretinoin, 4478
Triamcinolone acetonide, 4484

Creatine, 4920
Creatinine, 5734
Cresol, 5735
red, 6220
red–thymol blue TS, 6227
red TS, 6227
m-Cresol purple, 6154
TS, 6227
Cromolyn sodium, 1180
inhalation powder, 1182
inhalation solution, 1182
nasal solution, 1184
ophthalmic solution, 1185
Croscarmellose sodium, 5736
Crospovidone, 5737
Crotamiton, 1186
cream, 1187
Cryopreservation of cells ⟨1044⟩, 7389
Crypthecodinium cohnii oil, 4922
capsules, 4925
Crystallinity ⟨695⟩, 6931
Crystal violet, 6220
TS, 6232
Cupric
acetate, 6154
acetate TS, 6227
acetate TS, stronger, 6236
ammonium sulfate TS, 6227
chloride, 1187, 6154
chloride injection, 1188
citrate, 6154
citrate TS, 6228
citrate TS, alkaline, 6226, 6228
citrate TS 2, alkaline, 6226, 6228
iodide TS, alkaline, 6226, 6228
nitrate hydrate, 6154
nitrate, tenth-normal (0.1 N), 6239
oxide, ammoniated, TS, 6226, 6228, 6234, 6234
sulfate, 1189, 6154
sulfate, anhydrous, 6138, 6154
sulfate CS, 6225
sulfate injection, 1190
sulfate test paper, 6222
sulfate TS, 6222, 6228
tartrate, alkaline, solution (Fehling's solution), 6239
tartrate TS, alkaline, 6226, 6228, 6229
Cupriethylenediamine hydroxide solution, 1.0 M, 6154
Curcuminoids, 4927
capsules, 4929
tablets, 4930
Cyanoacetic acid, 6154
Cyanocobalamin, 1190
injection, 1191
tablets, 1192
Cyanocobalamin chewable gels, 4931
Cyanogen bromide, 6154
4-Cyanophenol, 6154
4-Cyanopyridine, 6154
Cyclam, 6154
Cyclandelate, 1193
Cyclizine hydrochloride, 1194
tablets, 1195
Cyclobenzaprine hydrochloride, 1196
extended-release capsules, 1197
tablets, 1199
1,1-Cyclobutanedicarboxylic acid, 6154
α-Cyclodextrin, 6154
β-Cyclodextrin, 6154
Cyclohexane, 6154
Cyclohexanol, 6154
(1,2-Cyclohexylenedinitrilo)tetraacetic acid, 6154
Cyclohexylmethanol, 6154
3-Cyclohexylpropionic acid, 6154
Cyclomethicone, 5740
Cyclopentolate hydrochloride, 1200
ophthalmic solution, 1201
Cyclophosphamide, 1202
for injection, 1204
tablets, 1209
Cyclophosphamide compounded oral suspension, 1208
Cyclopropane, 1210
Cycloserine, 1211
capsules, 1211
Cyclosporine, 1212
capsules, 1213
injection, 1214
oral solution, 1216
Cyclosporine compounded, veterinary ophthalmic solution, 1217
Cyproheptadine hydrochloride, 1218
oral solution, 1219
tablets, 1220
Cyromazine, 1221
Cysteine hydrochloride, 1221
injection, 1222

www.webofpharma.com

Cystine, 4933
L-Cystine, 6154
Cytarabine, 1223
for injection, 1224

D

Dacarbazine, 1225
for injection, 1226
Dactinomycin, 1227
for injection, 1228
Dalfampridine, 1228
Dalteparin
sodium, 1230
Danazol, 1233
capsules, 1234
Dantrolene sodium, 1234
capsules, 1236
for injection, 1238
Dapsone, 1239
oral suspension, 1240
tablets, 1241
Daunorubicin hydrochloride, 1242
for injection, 1242
DEAE-Agarose, 6154
Decanol, 6154
Decoquinate, 1243
premix, 1243
Decyl sodium sulfate, 6154
Deferoxamine mesylate, 1244
for injection, 1245
Dehydrated alcohol, 6155
Dehydroacetic acid, 5741
Dehydrocholic acid, 1246
Delafield's hematoxylin TS, 6228
Deliverable volume ⟨698⟩, 6935
Delta-8-tetrahydrocannabinol, 6211
Demecarium bromide, 1246
ophthalmic solution, 1247
Demeclocycline, 1247
hydrochloride, 1248
hydrochloride tablets, 1249
Denatonium benzoate, 5741
Denaturated alcohol TS, 6228
Denigès' reagent, 6228
Density of solids ⟨699⟩, 6938
Dental paste
triamcinolone acetonide, 4485
Deoxyadenosine triphosphate, 6155
Deoxycytidine triphosphate, 6155
Deoxyguanosine triphosphate, 6155
Deoxyribonucleic acid polymerase, 6155
Deoxythymidine triphosphate, 6155
Depyrogenation ⟨1228⟩, 8176
Depyrogenation by filtration ⟨1228.3⟩, 8184
Depyrogenation by Rinsing, 8187
Deschloroclotrimazole, 6155
Description and relative solubility of USP and NF articles, 6273
Desflurane, 1253
Design, evaluation and characterization of viral clearance procedures ⟨1050.1⟩, 7472
Design and analysis of biological assays ⟨111⟩, 6543
Design and development of biological assays ⟨1032⟩, 7320
Desipramine hydrochloride, 1255
tablets, 1256
Deslanoside, 1258
injection, 1259
Desloratadine, 1259
tablets, 1261
orally disintegrating tablets, 1263
Desmopressin acetate, 1265
injection, 1266
nasal spray, 1267
Desogestrel, 1268
and ethinyl estradiol tablets, 1270
Desonide, 1271
Desoximetasone, 1272
cream, 1274
gel, 1275
ointment, 1275
Desoxycholic acid, 1276
Desoxycorticosterone
pivalate, 1278
pivalate injectable suspension, 1279
Desoxycorticosterone acetate, 6155
Desvenlafaxine, 1280
Desvenlafaxine fumarate, 1281
Desvenlafaxine succinate, 1283
Detection of irradiated dietary supplements ⟨2250⟩, 8722
Determination
methoxy ⟨431⟩, 6702
nitrogen ⟨461⟩, 6709
Deuterated ethanol, 6155
Deuterated methanol, 6155
Deuterated water, 6155
Deuterium
chloride, 6155
oxide, 6155
Deuterium chloride
solution, 6155
Deuterochloroform, 6155
Devarda's alloy, 6155
Dexamethasone, 1284
acetate, 1290
acetate injectable suspension, 1291
and ciprofloxacin otic suspension, 1021
elixir, 1286
injection, 1286
and neomycin and polymyxin B sulfates ophthalmic ointment, 3121
and neomycin and polymyxin B sulfates ophthalmic suspension, 3122
ophthalmic suspension, 1287
penicillin G procaine, dihydrostreptomycin sulfate, and chlorpheniramine maleate injectable suspension, 3445
sodium phosphate, 1292
sodium phosphate injection, 1294
sodium phosphate and neomycin sulfate cream, 3104
sodium phosphate and neomycin sulfate ophthalmic ointment, 3105
sodium phosphate and neomycin sulfate ophthalmic solution, 3106
sodium phosphate ophthalmic solution, 1296
oral solution, 1288
tablets, 1289
and tobramycin ophthalmic ointment, 4409
and tobramycin ophthalmic suspension, 4411
Dexamethasone sodium phosphate
compounded
injection, 1295
Dexbrompheniramine maleate, 1296
and pseudoephedrine sulfate oral solution, 1298
Dexchlorpheniramine maleate, 1299
oral solution, 1301
tablets, 1301
Dexmedetomidine
injection, 1304
Dexmedetomidine hydrochloride, 1302
Dexpanthenol, 1305
assay ⟨115⟩, 6547
preparation, 1306
Dextran
1, 1307
40, 1309
40 in dextrose injection, 1311
40 in sodium chloride injection, 1312
70, 1313
70 in dextrose injection, 1315
70 in sodium chloride injection, 1316
high molecular weight, 6155
Dextrates, 5742
Dextrin, 5743, 6155
Dextro calcium pantothenate, 6155
Dextroamphetamine sulfate, 1316
tablets, 1318
Dextromethorphan, 1319
chlorpheniramine, pseudoephedrine (salts of), and acetaminophen, capsules containing at least three of the following, 52
chlorpheniramine, pseudoephedrine (salts of), and acetaminophen, oral powder containing at least three of the following, 54
chlorpheniramine, pseudoephedrine (salts of), and acetaminophen, oral solution containing at least three of the following, 56
chlorpheniramine, pseudoephedrine (salts of), and acetaminophen, tablets containing at least three of the following, 58
hydrobromide, 1320
hydrobromide, acetaminophen, doxylamine succinate, and pseudoephedrine hydrochloride oral solution, 67
hydrobromide, guaifenesin, and pseudoephedrine hydrochloride capsules, 2175
hydrobromide, pseudoephedrine hydrochloride, and carbinoxamine maleate oral solution, 3767
hydrobromide oral solution, 1321
hydrobromide, acetaminophen, and chlorpheniramine maleate tablets, 60
Dextrose, 1321
adenine solution, anticoagulant citrate phosphate, 348
anhydrous, 6156
and dopamine hydrochloride injection, 1495
excipient, 5743
and half-strength lactated Ringer's injection, 3895
injection, 1323
injection, alcohol in, 117
injection, bretylium tosylate in, 591
injection, bupivacaine hydrochloride in, 612
injection, dobutamine in, 1470
injection, magnesium sulfate in, 2727
injection, potassium chloride in, 3607
injection and potassium chloride in lactated ringer's, 3610
injection and sodium chloride injection, potassium chloride in, 3608
injection, tetracaine hydrochloride in, 4300
injection, theophylline in, 4328

www.webofpharma.com

Dextrose *(continued)*
injection type 1 and multiple electrolytes, 1598
injection type 2 and multiple electrolytes, 1601
injection type 3 and multiple electrolytes, 1605
and lactated Ringer's injection, 3893
and lidocaine hydrochloride injection, 2628
and modified lactated Ringer's injection, 3898
and Ringer's injection, 3889
and sodium chloride injection, 1323
solution, anticoagulant citrate, 345
solution, anticoagulant citrate phosphate, 347
Diacetyl, 6156
Diacetylated monoglycerides, 5745
3,3′-Diaminobenzidine hydrochloride, 6156
2,3-Diaminonaphthalene, 6156
2,6-Diaminopyridine, 6156
Diatomaceous earth, 6156
flux-calcined, 6156
silanized, 6156
Diatomaceous silica
calcined, 6156, 6199
Diatrizoate
meglumine, 1324
meglumine and diatrizoate sodium injection, 1326
meglumine and diatrizoate sodium solution, 1327
meglumine injection, 1325
sodium, 1327
sodium and diatrizoate meglumine injection, 1326
sodium and diatrizoate meglumine solution, 1327
sodium injection, 1328
sodium solution, 1329
Diatrizoic acid, 1329
Diaveridine, 6156
Diazepam, 1330
injection, 1331
tablets, 1332
Diazobenzenesulfonic acid TS, 6228
Diazoxide, 1333
capsules, 1333
injection, 1334
oral suspension, 1335
Dibasic
ammonium citrate, 6156
ammonium phosphate, 6156
calcium phosphate, anhydrous, 710
calcium phosphate dihydrate, 708
calcium phosphate tablets, 712
potassium phosphate, 3628, 6156
sodium phosphate, 4087
Dibenzyl, 6156
2,6-Dibromoquinone-chlorimide, 6156
Dibucaine, 1336
cream, 1336
hydrochloride, 1337
hydrochloride injection, 1338
ointment, 1337
Dibutyl
phthalate, 5745, 6156
sebacate, 5746
Dibutylamine, 6156
Dibutylammonium phosphate, 6156
1,3-Dicaffeoylquinic acid, 6157
Dichloralphenazone, 1338
isometheptene mucate and acetaminophen capsules, 2423
Dichloroacetic acid, 6157
2,5-Dichloroaniline, 6157
2,6-Dichloroaniline, 6157
o-Dichlorobenzene, 6157
1,2-Dichloroethane, 6157
Dichlorofluorescein, 6157
TS, 6228
Dichlorofluoromethane, 6157
2,6-Dichloroindophenol sodium, 6157
Dichloromethane, 6157
2,4-Dichloro-1-naphthol, 6157
2,6-Dichlorophenol-indophenol sodium, 6157, 6157
Dichlorophenol-indophenol solution, standard, 6240
2,6-Dichlorophenylacetic acid, 6157
2,6-Dichloroquinone-chlorimide, 6157
Dichlorphenamide, 1339
tablets, 1340
Diclazuril, 1341
Diclofenac potassium, 1343
tablets, 1345
Diclofenac potassium for oral solution, 1344
Diclofenac sodium, 1347
and misoprostol delayed-release tablets, 1353
delayed-release tablets, 1348
extended-release tablets, 1350
Diclofenac sodium
topical solution, 1352
Dicloxacillin sodium, 1357
capsules, 1358
Dicyclohexyl, 6157
Dicyclohexylamine, 6158
Dicyclohexyl phthalate, 6157
Dicyclomine hydrochloride, 1359
capsules, 1360
injection, 1361
oral solution, 1361
tablets, 1362
Didanosine, 1363
delayed-release capsules, 1364
for oral solution, 1366
tablets for oral suspension, 1367

Dietary Supplements

Chinese skullcap root, 4883
Chinese skullcap root dry extract, 4887
Chinese skullcap root powder, 4885
L-alpha-glycerylphosphorylcholine, 5055
Omega-3 free fatty acids, 5188
N-acetylglucosamine, 4745
Ademetionine disulfate tosylate, 4747
L-Alanyl-L-glutamine, 4748
Andrographis, 4758
Andrographis, powdered, 4760
Andrographis extract, powdered, 4762
Arginine capsules, 4763
Arginine tablets, 4764
Ascorbic acid chewable gels, 4765
Ashwagandha root, 4766
Ashwagandha root extract, powdered, 4768
Ashwagandha root, powdered, 4771
Astaxanthin esters, 4779
Astragalus root, 4782
Astragalus root dry extract, 4787
Astragalus root powder, 4785
Aztec marigold zeaxanthin extract, 4789
Bacillus coagulans, 4791
Bacillus coagulans capsules, 4794
Bacillus subtilis subsp. *subtilis* menaquinone-7 extract, 5152
Bacopa, 4797
Bacopa, powdered, 4798
Bacopa extract, powdered, 4800
Banaba leaf, 4801
Banaba leaf dry extract, 4805
Banaba leaf powder, 4803
Beta carotene preparation, 4806
Beta glucan, 4808
Bifidobacterium animalis subsp. *lactis*, 4811
Bilberry extract, powdered, 4813
Black cohosh, 4815
Black cohosh, powdered, 4817
Black cohosh extract, powdered, 4820
Black cohosh tablets, 4823
Black pepper, 4825
Powdered black pepper extract, 4829
Powdered black pepper, 4827
Borage seed oil, 4830
Borage seed oil capsules, 4831
Boswellia serrata, 4832
Boswellia serrata extract, 4834
Calcium citrate tablets, 4835
Calcium L-5-methyltetrahydrofolate, 4838
Calcium L-5-methyltetrahydrofolate capsules, 4841
Calcium L-5-methyltetrahydrofolate tablets, 4842
Calcium and vitamin D with minerals tablets, 4845
Calcium with vitamin D tablets, 4843
Carotenes, 4850
Cat's claw, 4852
Cat's claw capsules, 4857
Cat's claw extract, powdered, 4855
Cat's claw, powdered, 4854
Cat's claw tablets, 4858
Centella asiatica, 4859
Centella asiatica, powdered, 4861
Centella asiatica extract, powdered, 4863
Centella asiatica triterpenes, 4864
Chamomile, 4866
Chaste tree, 4868
Chaste tree, powdered, 4870
Chaste tree extract, powdered, 4872
Chia seed oil, 4877
Chinese salvia, 4878
Chinese salvia, powdered, 4880
Cholecalciferol chewable gels, 4889
Choline bitartrate, 4891
Choline chloride, 4892
Chondroitin sulfate sodium, 4894
Chondroitin sulfate sodium, shark, 4898
Chondroitin sulfate sodium tablets, 4897
Chromium picolinate, 4901
Chromium picolinate tablets, 4902
Cinnamomum cassia twig, 4902
Cinnamomum cassia twig powder, 4904
Citrulline, 4908
Clover, red, 5203
Clover, powdered red, 5207
Clover extract, powdered red, 5210
Clover tablets, red, 5212
Cod liver oil capsules, 4910
Coffee fruit dry extract, 4912
Cohosh, black, fluidextract, 4821
Coix seed, 4915
Coixseedpowder, 4917
Cranberry liquid preparation, 4919
Crypthecodinium cohnii oil, 4922
Crypthecodinium cohnii oil capsules, 4925
Curcuminoids, 4927
Curcuminoids capsules, 4929
Curcuminoids tablets, 4930

www.webofpharma.com

Dietary Supplements *(continued)*
Cyanocobalamin chewable gels, 4931
Diosmin, 4934
Echinacea angustifolia, 4935
Echinacea angustifolia, powdered, 4938
Echinacea angustifolia extract, powdered, 4941
Echinacea pallida, 4944
Echinacea pallida, powdered, 4946
Echinacea pallida, powdered, extract, 4948
Echinacea purpurea aerial parts, 4950
Echinacea purpurea, powdered, 4955
Echinacea purpurea, powdered, extract, 4958
Echinacea purpurea root, 4953
Echinacea Species Dry Extract Capsules, 4960
Echinacea Species Dry Extract Tablets, 4963
Echinacea species powder capsules, 4966
Eleuthero, 4969
Eleuthero, powdered, 4975
Eleuthero extract, powdered, 4970
Eleuthero root and rhizome dry extract capsules, 4972
Eleuthero root and rhizome dry extract tablets, 4973
Eleuthero root and rhizome powder capsules, 4976
European elder berry dry extract, 4978
Evening primrose oil, 4980
Evening primrose oil capsules, 4980
Fenugreek seed, 4982
Fenugreek seed powder, 4984
Fenugreek seed powdered extract, 4987
Feverfew, 4989
Feverfew, powdered, 4990
Fish oil containing omega-3 acids, 4992
Fish oil containing omega-3 acids capsules, 4994
Fish oil containing omega-3 acids delayed-release capsules, 4997
Flax seed oil, 4998
Flax seed oil capsules, 4999
Forskohlii, 5000
Powdered forskohlii, 5002
Powdered forskohlii extract, 5003
Ganoderma lucidum fruiting body, 5004
Ganoderma lucidum fruiting body powder, 5008
Garcinia cambogia, 5011
Garcinia cambogia, powdered, 5012
Garcinia hydroxycitrate extract, powdered, 5014
Garcinia indica, 5015
Garcinia indica, powdered, 5016
Garlic, 5018
Garlic, powdered, 5020
Garlic extract, powdered, 5022
Garlic fluidextract, 5023
Garlic delayed-release tablets, 5024
Ginger, 5026
Ginger, powdered, 5028
Ginger capsules, 5032
Ginger tincture, 5030
Ginkgo, 5034
Ginkgo extract, powdered, 5038
Ginkgo capsules, 5037
Ginkgo tablets, 5041
Ginseng, American, 4750
Ginseng, American, capsules, 4754
Ginseng, American, powdered, 4752
Ginseng, American extract, powdered, 4753
Ginseng, American, tablets, 4757
Ginseng, Asian, 4774
Ginseng, Asian, powdered, 4775
Ginseng, Asian extract, powdered, 4776
Ginseng, Asian, tablets, 4778
Glucosamine and chondroitin sulfate sodium tablets, 5043
Glucosamine hydrochloride, 5045
Glucosamine tablets, 5046
Glucosamine sulfate potassium chloride, 5047
Glucosamine sulfate sodium chloride, 5048
Glucosamine and methylsulfonylmethane tablets, 5049
Glucosamine, chondroitin sulfate sodium, and methylsulfonylmethane tablets, 5051
Glutamic acid, 5053
Glutathione, 5054
Glycyl-L-glutamine, 5058
Glycyl-L-tyrosine, 5059
Goldenseal, 5061
Goldenseal, powdered extract, 5064
Goldenseal, powdered, 5063
Grape seeds oligomeric proanthocyanidins, 5065
Green tea extract, decaffeinated, powdered, 5067
Guggul, 5069
Guggul extract, native, 5070
Guggul extract, purified, 5071
Guggul tablets, 5072
Gymnema, 5074
Gymnema extract, native, 5077
Gymnema, powdered, 5075
Gymnema extract, purified, 5078
Hawthorn leaf with flower, 5080
Hawthorn leaf with flower, powdered, 5082
Hesperidin, 5084
Holy basil leaf, 5085
Holy basil leaf powdered, 5087
Holy basil leaf extract, powdered, 5089
Horse chestnut, 4873
Horse chestnut, powdered, 4875
Horse chestnut extract, powdered, 4876
5-Hydroxy-L-tryptophan, 5313
Japanese honeysuckle flower, 5091
Japanese honeysuckle flower dry extract, 5094
Japanese honeysuckle flower powder, 5097
Krill oil capsules, 5104
Krill oil delayed-release capsules, 5107
Lactobacillus acidophilus La-14, 5111
Lactobacillus acidophilus NCFM, 5113
Lactobacillus paracasei LPC-37, 5115
Lactobacillus rhamnosus HN001, 5117
Licorice, 5119
Licorice, powdered, 5120
Licorice extract, powdered, 5121
Ground limestone, 5121
Linoleic acids-free fatty acids, conjugated, 5123
Lipoic acid, alpha, 5124
Lipoic acid capsules, alpha, 5125
Lipoic acid tablets, alpha, 5126
Lutein, 5126
Lutein capsules, 5128
Lutein preparation, 5128
Lycopene, 5130
Lycopene preparation, 5131
Lysine hydrochloride tablets, 5135
Malabar-nut-tree, leaf, 5136
Malabar-nut-tree, leaf, powdered, 5137
Malabar-nut-tree, leaf extract, powdered, 5138
Maritime pine, 5139
Maritime pine extract, 5141
Melatonin, 5143
Melatonin tablets, 5144
Menaquinone-4, 5145
Menaquinone-7, 5146
Menaquinone-7 capsules, 5147
Menaquinone-7 preparation, 5149
Menaquinone-7 tablets, 5151
Methylcobalamin, 5153
Methylcobalamin tablets, 5154
Methylsulfonylmethane, 5155
Methylsulfonylmethane tablets, 5156
Milk thistle, 5157
Milk thistle, powdered, 5159
Milk thistle extract, powdered, 5160
Milk thistle capsules, 5162
Milk thistle tablets, 5163
Minerals capsules, 5164
Minerals tablets, 5172
Northern schisandra fruit, 5261
Northern schisandra fruit dry extract, 5263
Northern schisandra fruit powder, 5265
Oliveleaf, 5181
Olive leaf dry extract, 5182
Olive leaf powder, 5184
Omega-3 acids triglycerides, 5185
Phyllanthus amarus, 5191
Phyllanthus amarus, powdered, 5193
Plant stanol esters, 5194
Potassium citrate tablets, 5196
Powdered *Rhodiola rosea*, 5220
Powdered *Rhodiola rosea* extract, 5221
Powdered rosemary, 5232
Pygeum extract, 5198
Quercetin, 5202
Red clover aerial parts isoflavone aglycones dry extract, 5206
Rhodiola crenulata root and rhizome, 5213
Rhodiola crenulata root and rhizome dry extract, 5215
Rhodiola crenulata root and rhizome powder, 5216
Rhodiola rosea, 5218
Rhodiola rosea capsules, 5224
Rhodiola rosea tablets, 5227
Rhodiola rosea tincture, 5223
Ribose, 5229
Rosemary, 5230
Rosemary leaf dry aqueous extract, 5234
Rutin, 5235
St. John's wort flowering top, 5237
St. John's wort flowering top dry extract capsules, 5242
St. John's wort flowering top extract, dry, 5240
St. John's wort flowering top powder, 5238
St. John's wort flowering top dry extract tablets, 5244
Salix species bark, 5245
Salix species bark dry extract, 5247
Salix species bark powder, 5249
Saw palmetto, 5251
Saw palmetto, powdered, 5254
Saw palmetto capsules, 5259
Saw palmetto extract, 5256
Schizochytrium oil, 5266
Schizochytrium oil capsules, 5269
Selenomethionine, 5272
Sodium ferrous citrate, 5273
Soy isoflavones capsules, 5276
Soy isoflavones extract, powdered, 5274
Soy isoflavones tablets, 5278
Spirulina, 5279

www.webofpharma.com

Dietary Supplements *(continued)*
Spirulina tablets, 5283
Stinging nettle, 5286
Stinging nettle, powdered extract, 5290
Stinging nettle, powdered, 5288
Tangerine peel, 5292
Tangerine peel dry extract, 5294
Tangerine peel powder, 5296
Tienchi ginseng root and rhizome, 5299
Tienchi ginseng root and rhizome dry extract capsules, 5309
Tienchi ginseng root and rhizome dry extract, 5307
Tienchi ginseng root and rhizome powder, 5301
Tienchi ginseng root and rhizome powder capsules, 5304
Tienchi ginseng root and rhizome dry extract tablets, 5311
Tienchi ginseng root and rhizome powder tablets, 5306
Tomato extract containing lycopene, 5133
Turmeric, 5314
Turmeric, powdered, 5316
Turmeric extract, powdered, 5317
Ubidecarenone, 5319
Ubidecarenone capsules, 5320
Ubidecarenone tablets, 5321
Ubiquinol, 5322
Ubiquinol capsules, 5323
Valerian, 5324
Valerian, powdered, 5326
Valerian extract, powdered, 5327
Valerian root dry extract capsules, 5332
Valerian root powder capsules, 5333
Valerian tablets, 5329
Valerian tincture, 5330
Vinpocetine, 5335
Vinpocetine capsules, 5336
Vinpocetine tablets, 5337
Vitamin A oral liquid preparation, 4634
Vitamins capsules, oil- and water-soluble, 5389
Vitamins with minerals capsules, oil- and water-soluble, 5435
Vitamins with minerals capsules, water-soluble, 5523
Vitamins with minerals oral solution, water-soluble, 5543
Vitamins with minerals tablets, oil- and water-soluble, 5476
Vitamins with minerals tablets, water-soluble, 5552
Vitamins tablets, oil- and water-soluble, 5419
Vitamins capsules, oil-soluble, 5346
Vitamins capsules, water-soluble, 5499
Vitamins with minerals oral solution, oil- and water-soluble, 5462
Oil-soluble vitamins with minerals capsules, 5361
Oil-soluble vitamins with minerals oral solution, 5372
Oil-soluble vitamins with minerals tablets, 5378
Vitamins oral solution, oil- and water-soluble, 5409
Oil-soluble vitamins oral solution, 5353
Vitamins tablets, oil-soluble, 5356
Vitamins tablets, water-soluble, 5512
Vitamins with minerals oral powder, 5338
meso-Zeaxanthin, 5570
meso-Zeaxanthin preparation, 5572
Zinc citrate, 5574
Zinc citrate tablets, 5575
Zinc and vitamin C lozenges, 5576

Diethanolamine, 5747
Diethyl phthalate, 6158
Diethylamine, 6158
Diethylamine phosphate, 6158
N,N-Diethylaniline, 6158
Diethylcarbamazine citrate, 1368
tablets, 1369
Diethylene glycol, 6158
monoethyl ether, 5749
stearates, 5751
succinate polyester, 6158
Di(ethylene glycol) methyl ether, 6158
Diethylenetriamine, 6158
Di(2-ethylhexyl)phthalate, 6158
Diethyl phthalate, 5748
Diethylpropion hydrochloride, 1370
tablets, 1371
Diethylpyrocarbonate, 6158
Diethyl sebacate, 5748
Diethylstilbestrol, 1372
Diethyl sulfone, 6158
Diethyltoluamide, 1372
topical solution, 1373
Diflorasone diacetate, 1374
cream, 1374
ointment, 1375
Diflunisal, 1375
tablets, 1376
Digitalis, 1377
capsules, 1380
powdered, 1379
tablets, 1380
Digitonin, 6158
Digitoxin, 1381
injection, 1381
tablets, 1382
Digoxigenin, 6158
Digoxin, 1383
injection, 1384
oral solution, 1385
tablets, 1385
Dihydrocodeine bitartrate, 1386
aspirin and caffeine capsules, 402
Dihydroergotamine mesylate, 1388
injection, 1389
7,8-Dihydrofolic acid, 6158
24,25-Dihydrolanosterol, 6158
Dihydroquinidine hydrochloride, 6158
Dihydroquinine, 6158
Dihydrostreptomycin
injection, 1391
sulfate, 1390
sulfate boluses, 1391
sulfate, penicillin G procaine, chlorpheniramine maleate, and dexamethasone injectable suspension, 3445
sulfate and penicillin G procaine injectable suspension, 3444
sulfate and penicillin G procaine intramammary infusion, 3444
sulfate, penicillin G procaine, and prednisolone injectable suspension, 3447
Dihydrotachysterol, 1391
capsules, 1392
oral solution, 1392
tablets, 1393
Dihydroxyacetone, 1393
Dihydroxyaluminum
aminoacetate, 1394
aminoacetate magma, 1395
sodium carbonate, 1395
sodium carbonate chewable tablets, 1396
Dihydroxybenzaldehyde, 6159
2,5-Dihydroxybenzoic acid, 6159
2,7-Dihydroxynaphthalene, 6159
2,7-Dihydroxynaphthalene TS, 6228
Dihydroxyphenylacetone, 6159
4,5-Dihydroxy-3-(*p*-sulfophenylazo)-2,7-napthalenedisulfonic acid, trisodium salt, 6220
Diiodofluorescein, 6159
TS, 6228
Diisodecyl phthalate, 6159
Diisopropanolamine, 5752
Diisopropyl ether, 6159, 6163, 6174
Diisopropylamine, 6159
Diisopropylethylamine, 6159
n,n′-Diisopropylethylenediamine, 6159
1,2-Dilinoleoyl-3-oleoyl-rac-glycerol, 6159
1,2-Dilinoleoyl-3-palmitoyl-rac-glycerol, 6159
Diloxanide furoate, 1397
Diltiazem hydrochloride, 1398
extended-release capsules, 1399
oral solution, 1405
oral suspension, 1406
tablets, 1406
Diluted
acetic acid, 5599, 6159
alcohol, 6159
hydrochloric acid, 6159
lead subacetate TS, 6228
nitric acid, 6159
sulfuric acid, 6159
Dimenhydrinate, 1408
injection, 1409
oral solution, 1410
tablets, 1410
Dimercaprol, 1411
injection, 1412
Dimethicone, 5753
viscosity 500 centistokes, 6159
2,5-Dimethoxybenzaldehyde, 6159
1,2-Dimethoxyethane, 6160
Dimethoxymethane, 6160
(3,4-Dimethoxyphenyl)-acetonitrile, 6160
Dimethyl
phthalate, 6160
sulfone, 6160
sulfoxide, 1413, 6180
sulfoxide gel, 1413
sulfoxide irrigation, 1414
sulfoxide topical solution, 1414
sulfoxide spectrophotometric grade, 6160
N,N-Dimethylacetamide, 6160
p-Dimethylaminoazobenzene, 6160
p-Dimethylaminobenzaldehyde, 6160
TS, 6228
p-Dimethylaminocinnamaldehyde, 6160
2-Dimethylaminoethyl methacrylate, 6160
Dimethylaminophenol, 6160
Dimethylaniline ⟨223⟩, 6636
2,6-Dimethylaniline, 6160
N,N-Dimethylaniline, 6160
3,4-Dimethylbenzophenone, 6160
Dimethylcarbonate, 6161
5,5-Dimethyl-1,3-cyclohexanedione, 6161
N,N-Dimethyldecylamine, 6161
1,5-Dimethyl-1,5-diazaundecamethylene polymethobromide, 6161
N,N-Dimethyldodecylamine-*N*-oxide, 6161
Dimethylethyl(3-hydroxyphenyl)ammonium chloride, 6161
Dimethylformamide, 6161
N,N-Dimethylformamide diethyl acetal, 6161
1,3-Dimethyl-2-imidazolidinone, 6161

www.webofpharma.com

1,9-Dimethyl-methylene blue, 6161
N,N-Dimethyl-1-naphthylamine, 6161
N,N-Dimethyloctylamine, 6161
2,5-Dimethylphenol, 6161
2,6-Dimethylphenol, 6161
3,5-Dimethylphenol, 6161
3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, 6161
Dimethyltin dibromide, 6161
N,N-Dimethyl-*p*-phenylenediamine dihydrochloride, 6161
Dimidium
bromide, 6162
m-Dinitrobenzene, 6162
3,5-Dinitrobenzoyl chloride, 6162
2,4-Dinitrochlorobenzene, 6162
2,4-Dinitrofluorobenzene, 6162
2,4-Dinitrophenylhydrazine, 6162
Dinitrophenylhydrazine TS, 6228
Dinoprost tromethamine, 1415
injection, 1416
Dinoprostone, 1417
Dioctyl sodium sulfosuccinate, 6162
Diosmin, 4934
Dioxane, 6162
Dioxybenzone, 1418
and oxybenzone cream, 1418
Diphenhydramine
citrate, 1419
citrate and acetaminophen tablets, 69
citrate and ibuprofen tablets, 1421
hydrochloride, 1423
hydrochloride, acetaminophen, and pseudoephedrine hydrochloride tablets, 70
hydrochloride capsules, 1425
hydrochloride injection, 1426
hydrochloride oral solution, 1429
hydrochloride and ibuprofen capsules, 1431
and phenylephrine hydrochloride tablets, 1433
and pseudoephedrine capsules, 1435
Diphenoxylate hydrochloride, 1437
and atropine sulfate oral solution, 1438
and atropine sulfate tablets, 1439
Diphenyl ether, 6163, 6189
Diphenylamine, 6162
TS, 6228
Diphenylborinic acid, ethanolamine ester, 6135, 6162
Diphenylcarbazide, 6162
Diphenylcarbazone, 6162
TS, 6228
2,2-Diphenylglycine, 6162
Diphtheria antitoxin potency testing for human immune globulins ⟨162⟩, 6582
Dipicolinic acid, 6162
Dipicrylamine, 6162
Dipivefrin hydrochloride, 1440
ophthalmic solution, 1442
Dipropyl phthalate, 6162
Dipyridamole, 1443
injection, 1445
oral suspension, 1445
tablets, 1446
4,4′-Dipyridyl, 6162
α,α′-Dipyridyl, 6162
Direct red 80, 6197
Dirithromycin, 1447
delayed-release tablets, 1448
Disinfectants and antiseptics ⟨1072⟩, 7608
Disintegration ⟨701⟩, 6940
and dissolution of dietary supplements ⟨2040⟩, 8711
Disodium
chromotropate, 6162
ethylenediaminetetraacetate, 6162
Disopyramide phosphate, 1449
capsules, 1450
extended-release capsules, 1450
Dissolution ⟨711⟩, 6945
The dissolution procedure: development and validation ⟨1092⟩, 7707
Distilling range ⟨721⟩, 6955
Disulfiram, 1451
tablets, 1452
5,5′-Dithiobis (2-nitrobenzoic acid), 6162
Dithiothreitol, 6162
Dithizone, 6162
TS, 6228
Divalproex sodium, 1453
delayed-release capsules, 1454
delayed-release tablets, 1457
extended-release tablets, 1458
Dobutamine
in dextrose injection, 1470
hydrochloride, 1466
injection, 1468
for injection, 1469
Docetaxel, 1471
injection, 1474
Docusate
calcium, 1475
calcium capsules, 1476
potassium, 1477
potassium capsules, 1478
sodium, 1479
sodium capsules, 1479
sodium and ferrous fumarate extended-release tablets, 1856
sodium solution, 1480
sodium syrup, 1481
sodium tablets, 1481
1-Dodecanol, 6162
Dodecyl
alcohol, 6162
lithium sulfate, 6162
sodium sulfonate, 6163
3-(Dodecyldimethylammonio) propanesulfonate, 6162
Dodecyltriethylammonium phosphate, 0.5 M, 6163
Dodecyltrimethylammonium bromide, 6163
Dofetilide, 1482
Dolasetron mesylate, 1483
oral solution, 1485
oral suspension, 1485
Donepezil hydrochloride, 1486
tablets, 1488
orally disintegrating tablets, 1492
Dopamine hydrochloride, 1494
and dextrose injection, 1495
injection, 1495
Dorzolamide
Hydrochloride and timolol maleate ophthalmic solution, 1499
Dorzolamide hydrochloride
ophthalmic solution, 1497
Dorzolamide hydrochloride, 1496
Doxapram hydrochloride, 1502
injection, 1503
Doxazosin mesylate, 1504
tablets, 1506
Doxepin hydrochloride, 1507
capsules, 1509
oral solution, 1510
Doxercalciferol, 1510
Doxorubicin hydrochloride, 1512
injection, 1514
for injection, 1516
Doxycycline, 1518
calcium oral suspension, 1529
capsules, 1519
extended-release capsules, 1521
hyclate, 1532
hyclate capsules, 1534
hyclate delayed-release capsules, 1536
hyclate tablets, 1537
hyclate delayed-release tablets, 1539
for injection, 1523
for oral suspension, 1525
tablets, 1527
Doxycycline compounded, veterinary oral suspension, 1530
Doxylamine succinate, 1544
acetaminophen, dextromethorphan hydrobromide, and pseudoephedrine hydrochloride oral solution, 67
oral solution, 1546
tablets, 1546
Drabkin's reagent, 6163
Dragendorff's TS, 6228
Dried peptone, 6163
Dronabinol, 1547
capsules, 1549
Dronedarone
hydrochloride, 1549
tablets, 1551
Droperidol, 1552
injection, 1553
Drospirenone, 1554
and ethinyl estradiol tablets, 1556
Drug release ⟨724⟩, 6957
Dry heat depyrogenation ⟨1228.1⟩, 8181
Dry heat sterilization ⟨1229.8⟩, 8225
Duloxetine
delayed-release capsules, 1559
Duloxetine hydrochloride, 1565
Dusting powder, absorbable, 1569
Dutasteride, 1567
Dyclonine hydrochloride, 1570
gel, 1570
topical solution, 1571
Dydrogesterone, 1571
tablets, 1572
Dyphylline, 1572
and guaifenesin oral solution, 1575
and guaifenesin tablets, 1575
injection, 1573
oral solution, 1573
tablets, 1574

E

Earth, chromatographic, silanized, acid-base washed, 6163
Ecamsule
solution, 1576
Echinacea
angustifolia, 4935
angustifolia, powdered, 4938
angustifolia extract, powdered, 4941
pallida, 4944
pallida, powdered, 4946
pallida extract, powdered, 4948
purpurea aerial parts, 4950
purpurea, powdered, 4955
purpurea root, 4953

www.webofpharma.com

Echinacea *(continued)*
purpurea extract, powdered, 4958
Echinacea species
dry extract capsules, 4960
dry extract tablets, 4963
powder capsules, 4966
Echothiophate
iodide, 1578
iodide for ophthalmic solution, 1579
Econazole nitrate, 1580
Edetate
calcium disodium, 1582
calcium disodium injection, 1583
disodium, 1583, 6162, 6163
disodium injection, 1584
disodium TS, 6228
disodium, twentieth-molar (0.05 M), 6240
Edetate disodium
0.01 M TS, 6228
0.002 M VS, 6240
0.01 M VS, 6240
0.02 M VS, 6240
Edetic acid, 5754, 6163
Edrophonium
chloride, 1584
chloride injection, 1585
Efavirenz, 1585
capsules, 1588
Tablets, 1591
Egg phospholipids, 5755
n-Eicosane, 6163
Eicosanol, 6163
Elastomeric closures for injections ⟨381⟩, 6669
Electrolytes
and dextrose injection type 1, multiple, 1598
and dextrose injection type 2, multiple, 1601
and dextrose injection type 3, multiple, 1605
and polyethylene glycol 3350 for oral solution, 3587
injection type 1, multiple, 1593
injection type 2, multiple, 1595
Elemental contaminants in dietary supplements ⟨2232⟩, 8719
Elemental impurities—limits ⟨232⟩, 6641
Elemental impurities—procedures ⟨233⟩, 6645
Elements
injection, trace, 1608
Eleuthero, 4969
extract, powdered, 4970
powdered, 4975
Eleuthero
root and rhizome dry extract capsules, 4972
root and rhizome dry extract tablets, 4973
root and rhizome powder capsules, 4976

Elixir

Aromatic, 5624
Benzaldehyde, compound, 5634
Dexamethasone, 1286
Fluphenazine hydrochloride, 1971
Hyoscyamine sulfate, 2276

Elm, 1609
Emedastine
difumarate, 1610
ophthalmic solution, 1610
Enalapril maleate, 1611
and hydrochlorothiazide tablets, 1616
tablets, 1614
Enalaprilat, 1619
injection, 1619
Enalapril maleate
oral suspension, 1613
Enalapril maleate compounded, veterinary
oral suspension, 1613
Endotoxin indicator for depyrogenation, 1621
Endotoxin indicators for depyrogenation ⟨1228.5⟩, 8190
Enflurane, 1622
Enoxaparin sodium, 1623
injection, 1626
Enrofloxacin, 1628
Enrofloxacin compounded, veterinary
oral suspension, 1630
Ensulizole, 1631
Entacapone, 1631
tablets, 1632
Entecavir, 1634
oral solution, 1636
tablets, 1637
Enzacamene, 1639
Enzymatically-hydrolyzed carboxymethylcellulose sodium, 5685
Enzymes used as ancillary materials in pharmaceutical manufacturing ⟨89⟩, 6521
Eosin Y, 6163, 6220
TS, 6228
Ephedrine, 1640
hydrochloride, 1641
hydrochloride, theophylline, and phenobarbital tablets, 4330
sulfate, 1642
sulfate capsules, 1642
sulfate injection, 1643
sulfate nasal solution, 1644
sulfate oral solution, 1644
Epiandrosterone, 6163
4-Epianhydrotetracycline ⟨226⟩, 6636
15-Epicarboprost, 6163
Epinephrine, 1645
and articaine hydrochloride injection, 381
assay ⟨391⟩, 6676
bitartrate, 1650
bitartrate ophthalmic solution, 1651
bitartrate for ophthalmic solution, 1652
and bupivacaine hydrochloride injection, 612
inhalation solution, 1648
injection, 1648
and lidocaine hydrochloride injection, 2629
nasal solution, 1649
ophthalmic solution, 1649
and prilocaine injection, 3679
and procaine hydrochloride injection, 3694
Epinephryl borate ophthalmic solution, 1652
Epirubicin hydrochloride, 1653
injection, 1654
Epitetracycline hydrochloride, 1656
Epoetin, 1657
Eprinomectin, 1660
Eprosartan mesylate, 1662
Equilenin, 6163
Equilin, 1664
Ergocalciferol, 1665
capsules, 1666
oral solution, 1667
tablets, 1668
α-Ergocryptine, 6163
Ergoloid mesylates, 1669
capsules, 1670
oral solution, 1671
tablets, 1671
Ergonovine maleate, 1672
injection, 1673
tablets, 1674
Ergotamine tartrate, 1675
and caffeine suppositories, 1680
and caffeine tablets, 1681
injection, 1677
sublingual tablets, 1679
tablets, 1678
Eriochrome
black T, 6220
black TS, 6229
black T–sodium chloride indicator, 6222
black T trituration, 6221
cyanine R, 6163
cyanine TS, 6229
Erythorbic acid, 5756
Erythritol, 5757
Erythromycin, 1683
and benzoyl peroxide topical gel, 1692
delayed-release capsules, 1686
estolate, 1693
estolate capsules, 1693
estolate and sulfisoxazole acetyl oral suspension, 1695
estolate oral suspension, 1693
estolate for oral suspension, 1694
estolate tablets, 1694
ethylsuccinate, 1696
ethylsuccinate injection, 1698
ethylsuccinate, sterile, 1698
ethylsuccinate and sulfisoxazole acetyl for oral suspension, 1701
ethylsuccinate oral suspension, 1698
ethylsuccinate for oral suspension, 1699
ethylsuccinate tablets, 1699
topical gel, 1686
gluceptate, sterile, 1702
injection, 1687
intramammary infusion, 1687
lactobionate for injection, 1703
lactobionate, sterile, 1703
ointment, 1688
ophthalmic ointment, 1689
pledgets, 1690
topical solution, 1690
stearate, 1704
stearate tablets, 1706
tablets, 1691
delayed-release tablets, 1691
Erythropoietin bioassays ⟨124⟩, 6557
Escin, 6163
Escitalopram
oral solution, 1706
Escitalopram oxalate, 1711
Escitalopram
tablets, 1708
Esmolol hydrochloride, 1712
Esomeprazole magnesium, 1714
delayed-release capsules, 1716
Esomeprazole strontium, 1720
Estazolam, 1722
tablets, 1723
Estradiol, 1724
vaginal cream, 1725
vaginal inserts, 1726
transdermal system, 1728
tablets, 1732
benzoate, 1736

www.webofpharma.com

Estradiol *(continued)*
cypionate, 1737
cypionate injection, 1738
and norethindrone acetate tablets, 1733
valerate, 1739
valerate injection, 1741
Estriol, 1741
Estriol compounded vaginal cream, 1743
Estrogens
conjugated, 1744
esterified, 1749
tablets, conjugated, 1746
tablets, esterified, 1750
Estrone, 1751
injectable suspension, 1752
Estropipate, 1753
tablets, 1754
vaginal cream, 1754
Eszopiclone, 1755
tablets, 1757
Ethacrynate sodium for injection, 1759
Ethacrynic acid, 1760
tablets, 1761
Ethambutol hydrochloride, 1762
rifampin, isoniazid, and pyrazinamide tablets, 3881
Compounded oral suspension, 1763
tablets, 1764
Ethanesulfonic acid, 6163
Ethchlorvynol, 1765
capsules, 1766
Ether, 1767, 6163
absolute, 6131, 6163
diphenyl, 6163
isopropyl, 6163
nonyl phenyl polyethylene glycol, 6163
peroxide-free, 6164
Ethidium bromide, 6164
Ethinyl estradiol, 1768
and desogestrel tablets, 1270
and drospirenone tablets, 1556
and ethynodiol diacetate tablets, 1781
and levonorgestrel tablets, 2612
and norethindrone acetate tablets, 3208
and norethindrone tablets, 3201
and norgestimate tablets, 3211
and norgestrel tablets, 3214
tablets, 1768
Ethiodized oil injection, 1770
Ethionamide, 1771
tablets, 1771
Ethopabate, 1772
Ethosuximide, 1773
capsules, 1774
oral solution, 1776
Ethotoin, 1777
tablets, 1778
4′-Ethoxyacetophenone, 6164
2-Ethoxyethanol, 6164
Ethyl
acetate, 5759, 6164
acrylate, 6164
acrylate and methacrylic acid copolymer, 5878
acrylate and methacrylic acid copolymer, partially-neutralized, 5881
acrylate and methyl methacrylate copolymer dispersion, 5760
alcohol, 6164
arachidate, 6164
benzoate, 6164
chloride, 1779
cyanoacetate, 6164
ether, 6163, 6164
ether, anhydrous, 6131, 6163, 6164
maltol, 5761
oleate, 5762
salicylate, 6164
vanillin, 5763
Ethyl p-toluenesulfonate, 6164
2-Ethylaminopropiophenone hydrochloride, 6164
4-Ethylbenzaldehyde, 6164
Ethylbenzene, 6164
Ethylcellulose, 5763
aqueous dispersion, 5765
dispersion type b, 5766
Ethylene
dichloride, 6157, 6164
glycol, 6164
glycol, diethylene glycol, and triethylene glycol in ethoxylated substances ⟨469⟩, 6733
glycol monoethyl ether, 6164
glycol stearates, 5771
glycol and vinyl alcohol graft copolymer, 5769
oxide and dioxane ⟨228⟩, 6639
oxide in methylene chloride (50 mg/mL), 6165
Ethylenediamine, 1779, 6165
2-Ethylhexanoic acid, 6165
N-Ethylmaleimide, 6165
2-Ethyl-2-methylsuccinic acid, 6165
Ethylparaben, 5772, 6165
Ethylparaben sodium, 5773
1-Ethylquinaldinium iodide, 6165
Ethynodiol diacetate, 1780
and ethinyl estradiol tablets, 1781
and mestranol tablets, 1781
Etidronate disodium, 1782
tablets, 1784
Etodolac, 1785
capsules, 1786
tablets, 1787
extended-release tablets, 1788
Etomidate, 1789
injection, 1790
Etoposide, 1792
capsules, 1794
injection, 1795
Eucalyptol, 1797
Eucalyptus oil, 5774
Eugenol, 1797
European elder berry dry extract, 4978
Evaluation of plastic packaging systems and their materials of construction with respect to their user safety impact ⟨1661⟩, 8434
Evaluation of screening technologies for assessing medicine quality ⟨1850⟩, 8626
Evaluation of the inner surface durability of glass containers ⟨1660⟩, 8429
Evening primrose oil, 4980
capsules, 4980
Excipient biological safety evaluation guidelines ⟨1074⟩, 7613
Excipient performance ⟨1059⟩, 7528
Excipients
USP and NF, listed by category, 5584
Exemestane, 1798
Exenatide, 1800
injection, 1803
Expert committees (2015–2020), x
Food Chemicals Codex, xvi
National Formulary, xiv
United States Pharmacopeia, x
United States Pharmacopeia and the *Dietary Supplements Compendium*, xv
United States Pharmacopeia and *USP on Compounding*, xvi
Expert Panels for the Council of Experts Executive Committee, x
Extended release tablets
nevirapine, 3135

Extract

Andrographis, powdered, 4762
Ashwagandha root, powdered, 4768
Astragalus root, dry, 4787
Aztec Marigold Zeaxanthin Extract, 4789
Bacillus subtilis subsp. *subtilis* menaquinone-7, 5152
Bacopa, powdered, 4800
Banaba leaf, dry, 4805
Beef, 6141
Belladonna, 486
Belladonna tablets, 487
Bilberry, powdered, 4813
Black cohosh, powdered, 4820
Black pepper, powdered, 4829
Boswellia serrata, 4834
Cascara fluidextract, aromatic, 801
Cascara sagrada, 798
Cascara sagrada fluidextract, 801
Cat's claw, powdered, 4855
Centella asiatica, powdered, 4863
Chaste tree, powdered, 4872
Chinese skullcap root dry , 4887
Clover, red, powdered, 5210
coffee fruit dry, 4912
Echinacea angustifolia, powdered, 4941
Echinacea pallida, powdered, 4948
Echinacea purpurea, powdered, 4958
Eleuthero, powdered, 4970
European elder berry dry, 4978
Fenugreek seed, powdered, 4987
Garcinia hydroxycitrate, powdered, 5014
Garlic, powdered, 5022
Garlic fluidextract, 5023
Ginkgo, powdered, 5038
Ginseng, American, powdered, 4753
Ginseng, Asian, powdered, 4776
Goldenseal, powdered, 5064
Green tea, decaffeinated, powdered, 5067
Guggul, native, 5070
Guggul, purified, 5071
Gymnema, native, 5077
Gymnema, purified, 5078
Holy basil leaf powdered, 5089
Horse chestnut, powdered, 4876
Japanese honeysuckle flower, dry, 5094
Licorice, powdered, 5121
Licorice fluidextract, 5857
Malabar-nut-tree, leaf, powdered, 5138
Maritime pine, 5141
Milk thistle, powdered, 5160
Northern schisandra fruit, dry, 5263
Olive leaf dry, 5182
Powdered *Rhodiola rosea*, 5221
Pygeum, 5198
Pyrethrum, 3778
Red clover aerial parts isoflavone aglycones, dry, 5206
Rhodiola crenulata root and rhizome dry, 5215
Salix species bark dry, 5247
Saw palmetto, 5256
Senna fluidextract, 4025
Soy isoflavones, powdered, 5274
Stinging nettle, powdered, 5290

www.webofpharma.com

Extract *(continued)*
St. John's wort flowering top, dry, 5240
Tangerine peel, dry, 5294
Tienchi ginseng root and rhizome, dry, 5307
Tomato, containing lycopene, 5133
Turmeric, powdered, 5317
Valerian, powdered, 5327
Valerian root dry, capsules, 5332
Yeast, 6219

Ezetimibe, 1804
tablets, 1806

F

F 18
injection, fludeoxyglucose, 1946
injection, sodium fluoride, 1950
Factor IX complex, 1808
Factor X_a (activated factor X) for anti-factor X_a test, 6165
Famciclovir, 1808
tablets, 1812
Famciclovir compounded
oral suspension, 1812
Famotidine, 1815
injection, 1816
for oral suspension, 1818
tablets, 1819
Fast
blue B salt, 6165
blue BB salt, 6165
green FCF, 6165
Fat, hard, 5776
Fats and fixed oils ⟨401⟩, 6676
FD&C blue no. 1, 6165
Fehling's solution, 6229
Felbamate, 1821
oral suspension, 1822
tablets, 1824
Felodipine, 1826
extended-release tablets, 1827
Fenbendazole, 1831
Fennel oil, 5776
Fenofibrate, 1833
capsules, 1834
tablets, 1837
Fenoldopam mesylate, 1840
injection, 1842
Fenoprofen calcium, 1843
capsules, 1845
tablets, 1845
Fentanyl, 1846
Fentanyl citrate, 1848
injection, 1849
Fentanyl citrate
bupivacaine hydrochloride compounded injection, 1850
Fentanyl citrate compounded
injection, 1848
Fenugreek seed, 4982
powdered extract, 4987
powder, 4984
Ferric
ammonium citrate, 289, 6165
ammonium citrate for oral solution, 290
ammonium sulfate, 6166
ammonium sulfate, tenth-normal (0.1 N), 6241
ammonium sulfate TS, 6229
chloride, 6166
chloride CS, 6225
chloride TS, 6229
nitrate, 6166
oxide, 5776
subsulfate solution, 1852
sulfate, 1852, 6166
Ferrocyphen, 6166
Ferroin TS, 6229
Ferrosoferric oxide, 5777
Ferrous
ammonium sulfate, 6166
ammonium sulfate, tenth-normal (0.1 N), 6241
fumarate, 1853
fumarate and docusate sodium extended-release tablets, 1856
fumarate tablets, 1855
gluconate, 1857
gluconate capsules, 1859
gluconate oral solution, 1860
gluconate tablets, 1861
sulfate, 1862, 6166
sulfate, dried, 1864
sulfate oral solution, 1863
sulfate syrup, 1863
sulfate tablets, 1864
sulfate TS, 6229
sulfate, acid, TS, 6225, 6229
0.07 N Ferrous ammonium sulfate, 6241
Ferulic acid, 6166
Ferumoxides injection, 1866
Ferumoxsil oral suspension, 1868
Fetal bovine serum—quality attributes and functionality tests ⟨90⟩, 6533
Feverfew, 4989
powdered, 4990
Fexofenadine hydrochloride, 1869
capsules, 1870
and pseudoephedrine hydrochloride extended-release tablets, 1876
tablets, 1872
Fibroblast growth factor-2, 6166
Fibroblasts
bilayer synthetic scaffold, construct human, 1153
polyglactin scaffold, construct human, 1157
Filgrastim, 1885
Finasteride, 1889
tablets, 1890
Fingolimod
hydrochloride, 1891
Fish oil containing omega-3 acids, 4992
capsules, 4994
delayed-release capsules, 4997
Flame photometry for reagents, 6129
Flavoxate hydrochloride, 1892
tablets, 1894
Flax seed oil, 4998
capsules, 4999
Flecainide acetate, 1895
oral suspension, 1897
tablets, 1898
Flow cytometric enumeration of CD34+ cells ⟨127⟩, 6560
Flow cytometry ⟨1027⟩, 7281
Floxuridine, 1899
for injection, 1901
Fluconazole, 1901
in dextrose injection, 1907
for oral suspension, 1912
injection, 1903
in sodium chloride injection, 1909
tablets, 1914
Flucytosine, 1915
capsules, 1916
oral suspension, 1917
Fludarabine phosphate, 1917
injection, 1919
for injection, 1921
Fludeoxyglucose F18 injection, 1946
Fludrocortisone acetate, 1922
tablets, 1923
Flumazenil, 1925
injection, 1926
Flumethasone pivalate, 1927
cream, 1928
Flunisolide, 1929
nasal solution, 1929
Flunixin meglumine, 1930
granules, 1931
injection, 1932
paste, 1933
Fluocinolone acetonide, 1934
cream, 1935
and neomycin sulfate cream, 3107
ointment, 1935
topical solution, 1936
Fluocinonide, 1937
cream, 1937
gel, 1938
ointment, 1938
topical solution, 1939
Fluorene, 6166
(–)-1-(9-Fluorenyl) ethanol, 6166
(+)-1-(9-Fluorenyl) ethyl chloroformate solution, 6166
9-Fluorenylmethyl chloroformate, 6167
Fluorescamine, 6167
Fluorescein, 1940
injection, 1940
sodium, 1942
sodium and benoxinate hydrochloride ophthalmic solution, 1944
sodium ophthalmic strips, 1943
sodium and proparacaine hydrochloride ophthalmic solution, 1945
Fluorescence spectroscopy ⟨853⟩, 7146
Fluorescence spectroscopy—theory and practice ⟨1853⟩, 8641
Fluorine
F 18 injection, fludeoxyglucose, 1946
F 18 injection, sodium fluoride, 1950
4′-Fluoroacetophenone, 6167
Fluoroandrostadiene carboxylic acid, 6167
Fluorometholone, 1950
acetate, 1953
acetate and tobramycin ophthalmic suspension, 4412
cream, 1952
and neomycin sulfate ointment, 3107
ophthalmic suspension, 1952
Fluorouracil, 1954
cream, 1956
injection, 1957
topical solution, 1957
Fluoxetine
capsules, 1958
delayed-release capsules, 1959
hydrochloride, 1964
and olanzapine capsules, 3237
oral solution, 1961
tablets, 1962
Fluoxymesterone, 1965
tablets, 1966

www.webofpharma.com

Fluphenazine
decanoate, 1967
decanoate injection, 1968
enanthate, 1969
enanthate injection, 1970
hydrochloride, 1970
hydrochloride elixir, 1971
hydrochloride injection, 1972
hydrochloride oral solution, 1973
hydrochloride tablets, 1973
Flurandrenolide, 1974
cream, 1975
lotion, 1976
and neomycin sulfate cream, 3107
and neomycin sulfate lotion, 3108
and neomycin sulfate ointment, 3108
ointment, 1976
tape, 1976
Flurazepam hydrochloride, 1977
capsules, 1978
Flurbiprofen, 1979
sodium, 1981
sodium ophthalmic solution, 1982
tablets, 1980
Flutamide, 1983
capsules, 1984
Fluticasone
propionate and salmeterol inhalation aerosol, 2005
propionate and salmeterol inhalation powder, 2010
Fluticasone propionate, 1985
cream, 1987
inhalation aerosol, 1988
inhalation powder, 1993
lotion, 1998
nasal spray, 2000
ointment, 2003
Fluvastatin
capsules, 2020
sodium, 2018
Fluvoxamine maleate, 2022
tablets, 2023
Folic acid, 2026
assay ⟨411⟩, 6689
compounded oral solution, 2027
injection, 2027
tablets, 2028
Folin-ciocalteu phenol TS, 6229
Fondaparinux sodium, 2029
injection, 2033
Formaldehyde
solution, 2035, 6167, 6229
TS, 6229
Formamide, 6167
anhydrous, 6167
Formic acid, 6167
96 percent, 6167
anhydrous, 6168
Formoterol fumarate, 2036
Forskohlii, 5000
extract, powdered, 5003
powdered, 5002
Foscarnet sodium, 2037
Fosfomycin tromethamine, 2039
Fosinopril sodium, 2040
and hydrochlorothiazide tablets, 2043
tablets, 2042
Fosphenytoin sodium, 2045
injection, 2047
Fructose, 2048
injection, 2049
and sodium chloride injection, 2049
Fuchsin
basic, 2050, 6141, 6168
pyrogallol TS, 6229
sulfurous acid TS, 6229
Fuller's earth, chromatographic, 6152, 6168
Fulvestrant, 2050
Fumaric acid, 5780
Fuming
nitric acid, 6168
sulfuric acid, 6168
Furazolidone, 2052
oral suspension, 2052
tablets, 2053
Furfural, 6168
Furosemide, 2053
injection, 2054
oral solution, 2055
tablets, 2056

G

G designations, 6168
Ga 67 injection, gallium citrate, 2088
Gabapentin, 2057
capsules, 2058
tablets, 2060
Gadodiamide, 2061
injection, 2064
Gadolinium (Gd III) acetate hydrate, 6168
Gadolinium sulfate, 6168
Gadopentetate dimeglumine injection, 2065
Gadoteridol, 2067
injection, 2069
Gadoversetamide, 2070
injection, 2073
Galactose, 5781
Galageenan, 5782
Galantamine
extended-release capsules, 2074
hydrobromide, 2083
oral solution, 2079
tablets, 2081
Gallamine triethiodide, 2087
injection, 2087
Gallium citrate Ga 67 injection, 2088
Gamma cyclodextrin, 5739
Ganciclovir, 2088
for injection, 2089
oral suspension, 2090
Ganoderma lucidum fruiting body, 5004
Ganoderma lucidum fruiting body powder, 5008
Garcinia cambogia, 5011
powdered, 5012
Garcinia hydroxycitrate
extract, powdered, 5014
Garcinia indica, 5015
powdered, 5016
Garlic, 5018
delayed-release tablets, 5024
extract, powdered, 5022
fluidextract, 5023
powdered, 5020
Gaseous sterilization ⟨1229.7⟩, 8222
Gastric fluid, simulated, TS, 6234
Gauze
absorbent, 2091
petrolatum, 2092

Gel

Adapalene, 96
Aluminum hydroxide, 188
Aluminum hydroxide, dried, 189
Aluminum hydroxide capsules, dried, 190
Aluminum hydroxide tablets, dried, 190
Aluminum phosphate, 190
Aminobenzoic acid, 234
ascorbic acid chewable, 4765
Benzocaine, 506
Benzocaine, butamben, and tetracaine hydrochloride, 514
Benzoyl peroxide, 526
Betamethasone benzoate, 545
cholecalciferol chewable, 4889
Chromatographic silica, 6152
Chromatographic silica mixture, 6152
Clindamycin phosphate, 1071
Cyanocobalamin chewable, 4931
Desoximetasone, 1275
Dimethyl sulfoxide, 1413
Dyclonine hydrochloride, 1570
Erythromycin and benzoyl peroxide, topical, 1692
Erythromycin, topical, 1686
Fluocinonide, 1938
Gelatin, 5783
Gelatin film, absorbable, 2092
Gelatin sponge, absorbable, 2093
Gelatin TS, 6229
Hydrocortisone, 2230
Indomethacin, topical, 2330
Metronidazole, 2935
Naftifine hydrochloride, 3066
Phenol topical, camphorated, 3501
Phenytoin compounded topical, 3525
Salicylic acid, 3972
Selegiline compounded topical, 4021
Silica, 6199
Silica, binder-free, 6199
Silica, chromatographic, 6152, 6199
Silica, impregnated glass microfiber sheet, 6199
Silica mixture, chromatographic, 6152, 6199
Silica mixture, chromatographic, with chemically bound amino groups, 6199
Silica mixture, dimethylsilanized, chromatographic, 6199
Silica mixture, octadecylsilanized chromatographic, 6199
Silica mixture, octylsilanized, chromatographic, 6199
Silica, octadecylsilanized chromatographic, 6199
Silica, porous, 6199
Sodium fluoride and phosphoric acid, 4075
Sodium sulfide topical, 4096
Stannous fluoride, 4115
Tolnaftate, 4426
Tretinoin, 4479

Gelatin, 5783, 6168
film, absorbable, 2092
sponge, absorbable, 2093
TS, 6229
Gellan gum, 5785
Gemcitabine
for injection, 2094

www.webofpharma.com

Gemcitabine *(continued)*
hydrochloride, 2093
Gemfibrozil, 2096
capsules, 2097
tablets, 2098
Gene therapy products ⟨1047⟩, 7426

General chapters

⟨1⟩ Injections and implanted drug products (parenterals)—product quality tests, 6405
⟨2⟩ Oral drug products—product quality tests, 6410
⟨3⟩ Topical and transdermal drug products—product quality tests, 6415
⟨4⟩ Mucosal drug products—product quality tests, 6423
⟨5⟩ Inhalation and nasal drug products general information and product quality tests, 6427
⟨7⟩ Labeling, 6435
⟨11⟩ USP reference standards, 6442
⟨17⟩ Prescription container labeling, 6445
⟨31⟩ Volumetric apparatus, 6448
⟨41⟩ Balances, 6449
⟨51⟩ Antimicrobial effectiveness testing, 6449
⟨55⟩ Biological indicators—resistance performance tests, 6452
⟨60⟩ Microbiological examination of nonsterile products tests for Burkholderia Cepacia complex, 6454
⟨61⟩ Microbiological examination of nonsterile products: microbial enumeration tests, 6457
⟨62⟩ Microbiological examination of nonsterile products: tests for specified organisms, 6463
⟨63⟩ Mycoplasma tests, 6472
⟨71⟩ Sterility tests, 6481
⟨81⟩ Antibiotics—microbial assays, 6488
⟨85⟩ Bacterial endotoxins test, 6508
⟨87⟩ Biological reactivity tests, in vitro, 6514
⟨88⟩ Biological reactivity tests, in vivo, 6516
⟨89.1⟩ Collagenase I, 6524
⟨89.2⟩ Collagenase II, 6529
⟨89⟩ Enzymes used as ancillary materials in pharmaceutical manufacturing, 6521
⟨90⟩ Fetal bovine serum—quality attributes and functionality tests, 6533
⟨91⟩ Calcium pantothenate assay, 6536
⟨92⟩ Growth factors and cytokines used in cell therapy manufacturing, 6539
⟨111⟩ Design and analysis of biological assays, 6543
⟨115⟩ Dexpanthenol assay, 6547
⟨121⟩ Insulin assays, 6547
⟨121.1⟩ Physicochemical analytical procedures for insulins, 6550
⟨123⟩ Glucagon bioidentity tests, 6552
⟨124⟩ Erythropoietin bioassays, 6557
⟨126⟩ Somatropin bioidentity tests, 6558
⟨127⟩ Flow cytometric enumeration of CD34+ cells, 6560
⟨129⟩ Analytical procedures for recombinant therapeutic monoclonal antibodies, 6565
⟨130⟩ Protein A quality attributes, 6570
⟨151⟩ Pyrogen test, 6577
⟨161⟩ Medical devices—bacterial endotoxin and pyrogen tests, 6579
⟨162⟩ Diphtheria antitoxin potency testing for human immune globulins, 6582
⟨165⟩ Prekallikrein activator, 6583
⟨171⟩ Vitamin B_{12} activity assay, 6584
⟨181⟩ Identification—organic nitrogenous bases, 6587
⟨191⟩ Identification tests—general, 6587
⟨193⟩ Identification—tetracyclines, 6592
⟨197⟩ Spectrophotometric identification tests, 6593
⟨201⟩ Thin-layer chromatographic identification test, 6600
⟨202⟩ Identification of fixed oils by thin-layer chromatography, 6601
⟨203⟩ High-performance thin-layer chromatography procedure for identification of articles of botanical origin, 6602
⟨206⟩ Aluminum, 6605
⟨207⟩ Test for 1,6-anhydro derivative for enoxaparin sodium, 6605
⟨208⟩ Anti-factor Xa and anti-factor IIa assays for unfractionated and low molecular weight heparins, 6611
⟨209⟩ Low molecular weight heparin molecular weight determinations, 6614
⟨210⟩ Monosaccharide analysis, 6616
⟨211⟩ Arsenic, 6621
⟨212⟩ Oligosaccharide analysis, 6623
⟨221⟩ Chloride and sulfate, 6636
⟨223⟩ Dimethylaniline, 6636
⟨226⟩ 4-Epianhydrotetracycline, 6636
⟨227⟩ 4-Aminophenol in acetaminophen-containing drug products, 6637
⟨228⟩ Ethylene oxide and dioxane, 6639
⟨232⟩ Elemental impurities—limits, 6641
⟨233⟩ Elemental impurities—procedures, 6645
⟨241⟩ Iron, 6648
⟨251⟩ Lead, 6649
⟨261⟩ Mercury, 6650
⟨267⟩ Porosimetry by mercury intrusion, 6653
⟨268⟩ Porosity by nitrogen adsorption–desorption, 6655
⟨271⟩ Readily carbonizable substances test, 6660
⟨281⟩ Residue on ignition, 6660
⟨291⟩ Selenium, 6661
⟨301⟩ Acid-neutralizing capacity, 6661
⟨311⟩ Alginates assay, 6662
⟨341⟩ Antimicrobial agents—content, 6664
⟨345⟩ Assay for citric acid/citrate and phosphate, 6668
⟨351⟩ Assay for steroids, 6669
⟨381⟩ Elastomeric closures for injections, 6669
⟨391⟩ Epinephrine assay, 6676
⟨401⟩ Fats and fixed oils, 6676
⟨411⟩ Folic acid assay, 6689
⟨413⟩ Impurities testing in medical gases, 6692
⟨415⟩ Medical gases assay, 6693
⟨425⟩ Iodometric assay—antibiotics, 6696
⟨429⟩ Light diffraction measurement of particle size, 6697
⟨431⟩ Methoxy determination, 6702
⟨441⟩ Niacin or niacinamide assay, 6704
⟨451⟩ Nitrite titration, 6709
⟨461⟩ Nitrogen determination, 6709
⟨466⟩ Ordinary impurities, 6710
⟨467⟩ Residual solvents, 6712
⟨469⟩ Ethylene glycol, diethylene glycol, and triethylene glycol in ethoxylated substances, 6733
⟨471⟩ Oxygen flask combustion, 6735
⟨481⟩ Riboflavin assay, 6735
⟨501⟩ Salts of organic nitrogenous bases, 6741
⟨503⟩ Acetic acid in peptides, 6741
⟨503.1⟩ Trifluoroacetic acid (TFA) in peptides, 6743
⟨507⟩ Protein determination procedures, 6744
⟨509⟩ Residual DNA testing, 6748
⟨511⟩ Single-steroid assay, 6750
⟨525⟩ Sulfur dioxide, 6751
⟨531⟩ Thiamine assay, 6756
⟨541⟩ Titrimetry, 6764
⟨551⟩ Vitamin E assay, 6767
⟨561⟩ Articles of botanical origin, 6774
⟨563⟩ Identification of articles of botanical origin, 6788
⟨565⟩ Botanical extracts, 6798
⟨571⟩ Vitamin A assay, 6801
⟨580⟩ Vitamin C assay, 6805
⟨581⟩ Vitamin D assay, 6808
⟨591⟩ Zinc determination, 6817
⟨601⟩ Inhalation and nasal drug products: aerosols, sprays, and powders—performance quality tests, 6819
⟨602⟩ Propellants, 6844
⟨603⟩ Topical aerosols, 6845
⟨604⟩ Leak rate, 6846
⟨610⟩ Alternative microbiological sampling methods for nonsterile inhaled and nasal products, 6846
⟨611⟩ Alcohol determination, 6848
⟨616⟩ Bulk density and tapped density of powders, 6850
⟨621⟩ Chromatography, 6853
⟨630⟩ Visiual Comparison , 6865
⟨631⟩ Color and achromicity, 6865
⟨641⟩ Completeness of solution, 6866
⟨643⟩ Total organic carbon, 6867
⟨644⟩ Conductivity of solutions, 6868
⟨645⟩ Water conductivity, 6871
⟨651⟩ Congealing temperature, 6874
⟨659⟩ Packaging and storage requirements, 6876
⟨660⟩ Containers—glass, 6881
⟨661⟩ Plastic packaging systems and their materials of construction, 6887
⟨661.1⟩ Plastic materials of construction, 6893
⟨661.2⟩ Plastic packaging systems for pharmaceutical use, 6912
⟨670⟩ Auxiliary packaging components, 6916
⟨671⟩ Containers—performance testing, 6922
⟨691⟩ Cotton, 6929
⟨695⟩ Crystallinity, 6931
⟨696⟩ Characterization of crystalline solids by microcalorimetry and solution calorimetry, 6931
⟨697⟩ Container content for injections, 6934
⟨698⟩ Deliverable volume, 6935
⟨699⟩ Density of solids, 6938
⟨701⟩ Disintegration, 6940
⟨705⟩ Quality attributes of tablets labeled as having a functional score, 6944
⟨711⟩ Dissolution, 6945
⟨721⟩ Distilling range, 6955
⟨724⟩ Drug release, 6957

www.webofpharma.com

General chapters *(continued)*
⟨729⟩ Globule size distribution in lipid injectable emulsions, 6963
⟨730⟩ Plasma spectrochemistry, 6967
⟨731⟩ Loss on drying, 6970
⟨733⟩ Loss on ignition, 6970
⟨735⟩ X-ray fluorescence spectrometry, 6971
⟨736⟩ Mass spectrometry, 6975
⟨741⟩ Melting range or temperature, 6980
⟨755⟩ Minimum fill, 6982
⟨761⟩ Nuclear magnetic resonance spectroscopy, 6984
⟨771⟩ Ophthalmic products—quality tests, 6993
⟨776⟩ Optical microscopy, 6998
⟨781⟩ Optical rotation, 7001
⟨782⟩ Vibrational circular dichroism spectroscopy, 7002
⟨785⟩ Osmolality and osmolarity, 7008
⟨786⟩ Particle size distribution estimation by analytical sieving, 7010
⟨787⟩ Subvisible particulate matter in therapeutic protein injections, 7014
⟨788⟩ Particulate matter in injections, 7017
⟨789⟩ Particulate matter in ophthalmic solutions, 7020
⟨790⟩ Visible particulates in injections, 7021
⟨791⟩ pH, 7022
⟨795⟩ Pharmaceutical compounding—nonsterile preparations, 7025
⟨797⟩ Pharmaceutical compounding—sterile preparations, 7037
⟨800⟩ Hazardous drugs—handling in healthcare settings, 7071
⟨801⟩ Polarography, 7089
⟨811⟩ Powder fineness, 7093
⟨821⟩ Radioactivity, 7094
⟨823⟩ Positron emission tomography drugs for compounding, investigational, and research uses, 7100
⟨825⟩ Radiopharmaceuticals—preparation, compounding, dispensing, and repackaging, 7109
⟨831⟩ Refractive index, 7137
⟨841⟩ Specific gravity, 7137
⟨846⟩ Specific surface area, 7139
⟨852⟩ Atomic absorption spectroscopy, 7142
⟨853⟩ Fluorescence spectroscopy, 7146
⟨854⟩ Mid-infrared spectroscopy, 7151
⟨855⟩ Nephelometry, turbidimetry, and visual comparison, 7155
⟨856⟩ Near-infrared spectroscopy, 7161
⟨857⟩ Ultraviolet-visible spectroscopy, 7166
⟨861⟩ Sutures—diameter, 7175
⟨871⟩ Sutures—needle attachment, 7178
⟨881⟩ Tensile strength, 7179
⟨891⟩ Thermal analysis, 7180
⟨905⟩ Uniformity of dosage units, 7183
⟨911⟩ Viscosity—capillary methods, 7187
⟨912⟩ Viscosity—rotational methods, 7189
⟨913⟩ Viscosity—rolling ball method, 7193
⟨914⟩ Viscosity—pressure driven methods, 7195
⟨921⟩ Water determination, 7196
⟨941⟩ Characterization of crystalline and partially crystalline solids by X-ray powder diffraction (XRPD), 7201
⟨1004⟩ Mucosal drug products—performance tests, 7227
⟨1005⟩ Acoustic emission, 7230
⟨1010⟩ Analytical data—interpretation and treatment, 7233
⟨1024⟩ Bovine serum, 7260
⟨1025⟩ Pancreatin, 7272
⟨1027⟩ Flow cytometry, 7281
⟨1030⟩ Biological assay chapters—overview and glossary, 7300
⟨1031⟩ The biocompatibility of materials used in drug containers, medical devices, and implants, 7313
⟨1032⟩ Design and development of biological assays, 7320
⟨1033⟩ Biological assay validation, 7337
⟨1034⟩ Analysis of biological assays, 7351
⟨1039⟩ Chemometrics, 7363
⟨1041⟩ Biologics, 7379
⟨1043⟩ Ancillary materials for cell, gene, and tissue-engineered products, 7381
⟨1044⟩ Cryopreservation of cells, 7389
⟨1046⟩ Cellular and tissue-based products, 7400
⟨1047⟩ Gene therapy products, 7426
⟨1048⟩ Quality of biotechnological products: analysis of the expression construct in cells used for production of r-DNA derived protein products, 7452
⟨1049⟩ Quality of biotechnological products: stability testing of biotechnological/biological products, 7454
⟨1050⟩ Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin, 7459
⟨1050.1⟩ Design, evaluation and characterization of viral clearance procedures, 7472
⟨1051⟩ Cleaning glass apparatus, 7481
⟨1052⟩ Biotechnology-derived articles—amino acid analysis, 7481
⟨1053⟩ Capillary electrophoresis, 7492
⟨1054⟩ Biotechnology-derived articles—isoelectric focusing, 7499
⟨1055⟩ Biotechnology-derived articles—peptide mapping, 7501
⟨1056⟩ Biotechnology-derived articles—polyacrylamide gel electrophoresis, 7508
⟨1057⟩ Biotechnology-derived articles—total protein assay, 7515
⟨1058⟩ Analytical instrument qualification, 7522
⟨1059⟩ Excipient performance, 7528
⟨1061⟩ Color—instrumental measurement, 7553
⟨1062⟩ Tablet compression characterization, 7556
⟨1063⟩ Shear cell methodology for powder flow testing, 7566
⟨1064⟩ Identification of articles of botanical origin by high-performance thin-layer chromatography procedure, 7578
⟨1065⟩ Ion chromatography, 7588
⟨1066⟩ Physical environments that promote safe medication use, 7590
⟨1071⟩ Rapid sterility testing of short life products a risk based approach, 7602
⟨1072⟩ Disinfectants and antiseptics, 7608
⟨1074⟩ Excipient biological safety evaluation guidelines, 7613
⟨1078⟩ Good manufacturing practices for bulk pharmaceutical excipients, 7617
⟨1079.1⟩ Storage and transportation of investigational drug products, 7644
⟨1079⟩ Good storage and distribution practices for drug products, 7635
⟨1080⟩ Bulk pharmaceutical excipients—certificate of analysis, 7647
⟨1084⟩ Glycoprotein and glycan analysis—general considerations, 7655
⟨1085⟩ Guidelines on endotoxins test, 7665
⟨1086⟩ Impurities in drug substances and drug products, 7678
⟨1087⟩ Apparent intrinsic dissolution—dissolution testing procedures for rotating disk and stationary disk, 7681
⟨1088⟩ In vitro and in vivo evaluation of dosage forms, 7684
⟨1090⟩ Assessment of drug product performance—bioavailability, bioequivalence, and dissolution, 7695
⟨1091⟩ Labeling of inactive ingredients, 7706
⟨1092⟩ The dissolution procedure: development and validation, 7707
⟨1094⟩ Capsules—dissolution testing and related quality attributes, 7725
⟨1097⟩ Bulk powder sampling procedures, 7732
⟨1099⟩ Limit on number of large deviations when assessing content uniformity in large samples, 7744
⟨1102⟩ Immunological test methods—general considerations, 7746
⟨1103⟩ Immunological test methods—enzyme-linked immunosorbent assay (ELISA), 7753
⟨1104⟩ Immunological test methods—immunoblot analysis, 7763
⟨1105⟩ Immunological test methods—surface plasmon resonance, 7773
⟨1106⟩ Immunogenicity assays—design and validation of immunoassays to detect anti-drug antibodies, 7788
⟨1106.1⟩ Immunogenicity assays—design and validation of assays to detect anti-drug neutralizing antibody, 7802
⟨1111⟩ Microbiological examination of nonsterile products: acceptance criteria for pharmaceutical preparations and substances for pharmaceutical use, 7819
⟨1112⟩ Application of water activity determination to nonsterile pharmaceutical products, 7820
⟨1113⟩ Microbial characterization, identification, and strain typing, 7823
⟨1115⟩ Bioburden control of nonsterile drug substances and products, 7827
⟨1116⟩ Microbiological control and monitoring of aseptic processing environments, 7833
⟨1117⟩ Microbiological best laboratory practices, 7845
⟨1118⟩ Monitoring devices—time, temperature, and humidity, 7850
⟨1120⟩ Raman spectroscopy, 7856
⟨1121⟩ Nomenclature, 7862
⟨1125⟩ Nucleic acid-based techniques—general, 7865
⟨1126⟩ Nucleic acid-based techniques—extraction, detection, and sequencing, 7870
⟨1127⟩ Nucleic acid-based techniques—amplification, 7879
⟨1128⟩ Nucleic acid-based techniques—microarray, 7887
⟨1129⟩ Nucleic acid-based techniques—genotyping, 7893
⟨1130⟩ Nucleic acid-based techniques—approaches for detecting trace nucleic acids (residual DNA testing), 7897

www.webofpharma.com

General chapters *(continued)*
⟨1132⟩ Residual host cell protein measurement in biopharmaceuticals, 7900
⟨1136⟩ Packaging and repackaging—single unit containers, 7919
⟨1151⟩ Pharmaceutical dosage forms, 7929
⟨1152⟩ Animal drugs for use in animal feeds, 7952
⟨1160⟩ Pharmaceutical calculations in pharmacy practice, 7953
⟨1163⟩ Quality assurance in pharmaceutical compounding, 7979
⟨1168⟩ Compounding for phase I investigational studies, 7985
⟨1174⟩ Powder flow, 7993
⟨1176⟩ Prescription balances and volumetric apparatus, 7997
⟨1177⟩ Good packaging practices, 8003
⟨1178⟩ Good repackaging practices, 8006
⟨1180⟩ Human plasma, 8008
⟨1181⟩ Scanning electron microscopy, 8025
⟨1184⟩ Sensitization testing, 8035
⟨1191⟩ Stability considerations in dispensing practice, 8044
⟨1195⟩ Significant change guide for bulk pharmaceutical excipients, 8049
⟨1197⟩ Good distribution practices for bulk pharmaceutical excipients, 8060
⟨1207.1⟩ Package integrity and test method selection, 8086
⟨1207.2⟩ Package integrity leak test technologies, 8097
⟨1207.3⟩ Package seal quality test technologies, 8111
⟨1207⟩ Sterile product packaging—integrity evaluation, 8079
⟨1208⟩ Sterility testing—validation of isolator systems, 8113
⟨1210⟩ Statistical tools for procedure validation, 8117
⟨1211⟩ Sterility assurance, 8129
⟨1216⟩ Tablet friability, 8137
⟨1217⟩ Tablet breaking force, 8138
⟨1222⟩ Terminally sterilized pharmaceutical products—parametric release, 8142
⟨1223⟩ Validation of alternative microbiological methods, 8144
⟨1223.1⟩ Validation of alternative methods to antibiotic microbial assays, 8157
⟨1224⟩ Transfer of analytical procedures, 8164
⟨1225⟩ Validation of compendial procedures, 8166
⟨1226⟩ Verification of compendial procedures, 8171
⟨1227⟩ Validation of microbial recovery from pharmacopeial articles, 8172
⟨1228.4⟩ Depyrogenation by Rinsing, 8187
⟨1228⟩ Depyrogenation, 8176
⟨1228.1⟩ Dry heat depyrogenation, 8181
⟨1228.3⟩ Depyrogenation by filtration, 8184
⟨1228.5⟩ Endotoxin indicators for depyrogenation, 8190
⟨1229⟩ Sterilization of compendial articles, 8194
⟨1229.1⟩ Steam sterilization by direct contact, 8199
⟨1229.2⟩ Moist heat sterilization of aqueous liquids, 8202
⟨1229.3⟩ Monitoring of bioburden, 8206
⟨1229.4⟩ Sterilizing filtration of liquids, 8209
⟨1229.5⟩ Biological indicators for sterilization, 8216
⟨1229.6⟩ Liquid-phase sterilization, 8219
⟨1229.7⟩ Gaseous sterilization, 8222
⟨1229.8⟩ Dry heat sterilization, 8225
⟨1229.9⟩ Physicochemical integrators and indicators for sterilization, 8227
⟨1229.10⟩ Radiation sterilization, 8228
⟨1229.11⟩ Vapor phase sterilization, 8231
⟨1229.12⟩ New sterilization methods, 8233
⟨1229.13⟩ Sterilization-in-place, 8233
⟨1229.14⟩ Sterilization cycle development, 8235
⟨1229.15⟩ Sterilization filtration of gases, 8238
⟨1229.16⟩ Prion inactivation, 8239
⟨1230⟩ Water for hemodialysis applications, 8240
⟨1231⟩ Water for pharmaceutical purposes, 8242
⟨1234⟩ Vaccines for human use—polysaccharide and glycoconjugate vaccines, 8276
⟨1235⟩ Vaccines for human use—general considerations, 8291
⟨1237⟩ Virology test methods, 8319
⟨1238⟩ Vaccines for human use—bacterial vaccines, 8338
⟨1240⟩ Virus testing of human plasma for further manufacture, 8350
⟨1241⟩ Water–solid interactions in pharmaceutical systems, 8359
⟨1251⟩ Weighing on an analytical balance, 8363
⟨1265⟩ Written prescription drug information—guidelines, 8368
⟨1285⟩ Preparation of biological specimens for histologic and immunohistochemical analysis, 8370
⟨1285.1⟩ Hematoxylin and eosin staining of sectioned tissue for microscopic examination, 8374
⟨1430.1⟩ Analytical methodologies based on scattering phenomena—static light scattering, 8379
⟨1430.2⟩ Analytical methodologies based on scattering phenomena- light diffraction measurements of particle size, 8386
⟨1430.4⟩ Analytical methodologies based on scattering phenomena—electrophoretic light scattering (determination of zeta potential), 8389
⟨1430.5⟩ Analytical methodologies based on scattering phenomena—small angle x-ray scattering and small angle neutron scattering, 8392
⟨1430⟩ Analytical methodologies based on scattering phenomena—general, 8376
⟨1467⟩ Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures, 8404
⟨1601⟩ Products for nebulization—characterization tests, 8407
⟨1602⟩ Spacers and valved holding chambers used with inhalation aerosols—characterization tests, 8411
⟨1644⟩ Theory and practice of electrical conductivity measurements of solutions, 8423
⟨1660⟩ Evaluation of the inner surface durability of glass containers, 8429
⟨1661⟩ Evaluation of plastic packaging systems and their materials of construction with respect to their user safety impact, 8434
⟨1663⟩ Assessment of extractables associated with pharmaceutical packaging/delivery systems, 8442
⟨1664⟩ Assessment of drug product leachables associated with pharmaceutical packaging/delivery systems, 8455
⟨1664.1⟩ Orally inhaled and nasal drug products, 8467
⟨1724⟩ Semisolid drug products—performance tests, 8473
⟨1730⟩ Plasma spectrochemistry—theory and practice, 8484
⟨1735⟩ X-ray fluorescence spectrometry—theory and practice, 8490
⟨1736⟩ Applications of mass spectrometry, 8508
⟨1761⟩ Applications of nuclear magnetic resonance spectroscopy, 8529
⟨1771⟩ Ophthalmic products—performance tests, 8549
⟨1782⟩ Vibrational circular dichroism spectroscopytheory and practice, 8549
⟨1787⟩ Measurement of subvisible particulate matter in therapeutic protein injections, 8562
⟨1788⟩ Methods for the determination of particulate matter in injections and ophthalmic solutions, 8575
⟨1790⟩ Visual inspection of injections, 8587
⟨1821⟩ Radioactivity—theory and practice, 8604
⟨1823⟩ Positron emission tomography drugs—information, 8617
⟨1850⟩ Evaluation of screening technologies for assessing medicine quality, 8626
⟨1852⟩ Atomic absorption spectroscopy—theory and practice, 8632
⟨1853⟩ Fluorescence spectroscopy—theory and practice, 8641
⟨1854⟩ Mid-infrared spectroscopy—theory and practice, 8650
⟨1857⟩ Ultraviolet-visible spectroscopy—theory and practice, 8670
⟨1911⟩ Rheometry, 8679
⟨198⟩ Nuclear magnetic resonance spectroscopy identity testing of bacterial polysaccharides used in vaccine manufacture, 6596
⟨2021⟩ Microbial enumeration tests—nutritional and dietary supplements, 8687
⟨2022⟩ Microbiological procedures for absence of specified microorganisms—nutritional and dietary supplements, 8692
⟨2023⟩ Microbiological attributes of nonsterile nutritional and dietary supplements, 8698
⟨2030⟩ Supplemental information for articles of botanical origin, 8702
⟨2040⟩ Disintegration and dissolution of dietary supplements, 8711
⟨2091⟩ Weight variation of dietary supplements, 8718
⟨2232⟩ Elemental contaminants in dietary supplements, 8719
⟨2250⟩ Detection of irradiated dietary supplements, 8722

www.webofpharma.com

General chapters *(continued)*
⟨2251⟩ Screening for undeclared drugs and drug analogues, 8725
⟨2750⟩ Manufacturing practices for dietary supplements, 8742

General chapters

Applications of mass spectrometry ⟨1736⟩, 8508
Radiopharmaceuticals—preparation, compounding, dispensing, and repackaging ⟨825⟩, 7109
Acetic acid in peptides ⟨503⟩, 6741
Acid-neutralizing capacity ⟨301⟩, 6661
Acoustic emission ⟨1005⟩, 7230
Alcohol determination ⟨611⟩, 6848
Alginates assay ⟨311⟩, 6662
Alternative microbiological sampling methods for nonsterile inhaled and nasal products ⟨610⟩, 6846
Aluminum ⟨206⟩, 6605
4-Aminophenol in acetaminophen-containing drug products ⟨227⟩, 6637
Analysis of biological assays ⟨1034⟩, 7351
Analytical data—interpretation and treatment ⟨1010⟩, 7233
Analytical instrument qualification ⟨1058⟩, 7522
Analytical methodologies based on scattering phenomena—electrophoretic light scattering (determination of zeta potential) ⟨1430.4⟩, 8389
Analytical methodologies based on scattering phenomena—general ⟨1430⟩, 8376
Analytical methodologies based on scattering phenomena—light diffraction measurements of particle size ⟨1430.2⟩, 8386
Analytical methodologies based on scattering phenomena—small angle x-ray scattering and small angle neutron scattering ⟨1430.5⟩, 8392
Analytical methodologies based on scattering phenomena—static light scattering ⟨1430.1⟩, 8379
Analytical procedures for recombinant therapeutic monoclonal antibodies ⟨129⟩, 6565
Ancillary materials for cell, gene, and tissue-engineered products ⟨1043⟩, 7381
Animal drugs for use in animal feeds ⟨1152⟩, 7952
Antibiotics—microbial assays ⟨81⟩, 6488
Anti-factor Xa and anti-factor IIa assays for unfractionated and low molecular weight heparins ⟨208⟩, 6611
Antimicrobial agents—content ⟨341⟩, 6664
Antimicrobial effectiveness testing ⟨51⟩, 6449
Apparent intrinsic dissolution—dissolution testing procedures for rotating disk and stationary disk ⟨1087⟩, 7681
Applications of nuclear magnetic resonance spectroscopy ⟨1761⟩, 8529
Application of water activity determination to nonsterile pharmaceutical products ⟨1112⟩, 7820
Arsenic ⟨211⟩, 6621
Articles of botanical origin ⟨561⟩, 6774
Assay for citric acid/citrate and phosphate ⟨345⟩, 6668
Assay for steroids ⟨351⟩, 6669
Assessment of drug product performance—bioavailability, bioequivalence, and dissolution ⟨1090⟩, 7695
Assessment of drug product leachables associated with pharmaceutical packaging/delivery systems ⟨1664⟩, 8455
Assessment of extractables associated with pharmaceutical packaging/delivery systems ⟨1663⟩, 8442
Atomic absorption spectroscopy ⟨852⟩, 7142
Atomic absorption spectroscopy—theory and practice ⟨1852⟩, 8632
Auxiliary packaging components ⟨670⟩, 6916
Bacterial endotoxins test ⟨85⟩, 6508
Balances ⟨41⟩, 6449
Bioburden control of nonsterile drug substances and products ⟨1115⟩, 7827
The biocompatibility of materials used in drug containers, medical devices, and implants ⟨1031⟩, 7313
Biological assay chapters—overview and glossary ⟨1030⟩, 7300
Biological assay validation ⟨1033⟩, 7337
Biological indicators—resistance performance tests ⟨55⟩, 6452
Biological indicators for sterilization ⟨1229.5⟩, 8216
Biological reactivity tests, in vitro ⟨87⟩, 6514
Biological reactivity tests, in vivo ⟨88⟩, 6516
Biologics ⟨1041⟩, 7379
Biotechnology-derived articles—amino acid analysis ⟨1052⟩, 7481
Biotechnology-derived articles—isoelectric focusing ⟨1054⟩, 7499
Biotechnology-derived articles—peptide mapping ⟨1055⟩, 7501
Biotechnology-derived articles—polyacrylamide gel electrophoresis ⟨1056⟩, 7508
Biotechnology-derived articles—total protein assay ⟨1057⟩, 7515
Botanical extracts ⟨565⟩, 6798
Bovine serum ⟨1024⟩, 7260
Bulk density and tapped density of powders ⟨616⟩, 6850
Bulk pharmaceutical excipients—certificate of analysis ⟨1080⟩, 7647
Bulk powder sampling procedures ⟨1097⟩, 7732
Calcium pantothenate assay ⟨91⟩, 6536
Capillary electrophoresis ⟨1053⟩, 7492
Capsules—dissolution testing and related quality attributes ⟨1094⟩, 7725
Cellular and tissue-based products ⟨1046⟩, 7400
Characterization of crystalline and partially crystalline solids by X-ray powder diffraction (XRPD) ⟨941⟩, 7201
Characterization of crystalline solids by microcalorimetry and solution calorimetry ⟨696⟩, 6931
Chemometrics ⟨1039⟩, 7363
Chloride and sulfate ⟨221⟩, 6636
Chromatography ⟨621⟩, 6853
Cleaning glass apparatus ⟨1051⟩, 7481
Collagenase I ⟨89.1⟩, 6524
Collagenase II ⟨89.2⟩, 6529
Color and achromicity ⟨631⟩, 6865
Color—instrumental measurement ⟨1061⟩, 7553
Completeness of solution ⟨641⟩, 6866
Compounding for phase I investigational studies ⟨1168⟩, 7985
Conductivity of solutions ⟨644⟩, 6868
Congealing temperature ⟨651⟩, 6874
Container content for injections ⟨697⟩, 6934
Containers—glass ⟨660⟩, 6881
Containers—performance testing ⟨671⟩, 6922
Cotton ⟨691⟩, 6929
Cryopreservation of cells ⟨1044⟩, 7389
Crystallinity ⟨695⟩, 6931
Deliverable volume ⟨698⟩, 6935
Density of solids ⟨699⟩, 6938
Depyrogenation ⟨1228⟩, 8176
Depyrogenation by filtration ⟨1228.3⟩, 8184
Depyrogenation by Rinsing ⟨1228.4⟩, 8187
Design, evaluation and characterization of viral clearance procedures ⟨1050.1⟩, 7472
Design and analysis of biological assays ⟨111⟩, 6543
Design and development of biological assays ⟨1032⟩, 7320
Detection of irradiated dietary supplements ⟨2250⟩, 8722
Dexpanthenol assay ⟨115⟩, 6547
Dimethylaniline ⟨223⟩, 6636
Diphtheria antitoxin potency testing for human immune globulins ⟨162⟩, 6582
Disinfectants and antiseptics ⟨1072⟩, 7608
Disintegration ⟨701⟩, 6940
Disintegration and dissolution of dietary supplements ⟨2040⟩, 8711
Dissolution ⟨711⟩, 6945
The dissolution procedure: development and validation ⟨1092⟩, 7707
Distilling range ⟨721⟩, 6955
Drug release ⟨724⟩, 6957
Dry heat depyrogenation ⟨1228.1⟩, 8181
Dry heat sterilization ⟨1229.8⟩, 8225
Elastomeric closures for injections ⟨381⟩, 6669
Elemental contaminants in dietary supplements ⟨2232⟩, 8719
Elemental impurities—limits ⟨232⟩, 6641
Elemental impurities—procedures ⟨233⟩, 6645
Endotoxin indicators for depyrogenation ⟨1228.5⟩, 8190
Enzymes used as ancillary materials in pharmaceutical manufacturing ⟨89⟩, 6521
4-Epianhydrotetracycline ⟨226⟩, 6636
Epinephrine assay ⟨391⟩, 6676
Erythropoietin bioassays ⟨124⟩, 6557
Ethylene glycol, diethylene glycol, and triethylene glycol in ethoxylated substances ⟨469⟩, 6733
Ethylene oxide and dioxane ⟨228⟩, 6639
Evaluation of plastic packaging systems and their materials of construction with respect to their user safety impact ⟨1661⟩, 8434
Evaluation of screening technologies for assessing medicine quality ⟨1850⟩, 8626
Evaluation of the inner surface durability of glass containers ⟨1660⟩, 8429
Excipient biological safety evaluation guidelines ⟨1074⟩, 7613
Excipient performance ⟨1059⟩, 7528

www.webofpharma.com

General chapters *(continued)*
Fats and fixed oils ⟨401⟩, 6676
Fetal bovine serum—quality attributes and functionality tests ⟨90⟩, 6533
Flow cytometric enumeration of CD34+ cells ⟨127⟩, 6560
Flow cytometry ⟨1027⟩, 7281
Fluorescence spectroscopy ⟨853⟩, 7146
Fluorescence spectroscopy—theory and practice ⟨1853⟩, 8641
Folic acid assay ⟨411⟩, 6689
Gaseous sterilization ⟨1229.7⟩, 8222
Gene therapy products ⟨1047⟩, 7426
Globule size distribution in lipid injectable emulsions ⟨729⟩, 6963
Glucagon bioidentity tests ⟨123⟩, 6552
Glycoprotein and glycan analysis—general considerations ⟨1084⟩, 7655
Good distribution practices for bulk pharmaceutical excipients ⟨1197⟩, 8060
Good manufacturing practices for bulk pharmaceutical excipients ⟨1078⟩, 7617
Good packaging practices ⟨1177⟩, 8003
Good repackaging practices ⟨1178⟩, 8006
Good storage and distribution practices for drug products ⟨1079⟩, 7635
Growth factors and cytokines used in cell therapy manufacturing ⟨92⟩, 6539
Guidelines on endotoxins test ⟨1085⟩, 7665
Hazardous drugs—handling in healthcare settings ⟨800⟩, 7071
Hematoxylin and eosin staining of sectioned tissue for microscopic examination ⟨1285.1⟩, 8374
High-performance thin-layer chromatography procedure for identification of articles of botanical origin ⟨203⟩, 6602
Human plasma ⟨1180⟩, 8008
Identification of articles of botanical origin ⟨563⟩, 6788
Identification of articles of botanical origin by high-performance thin-layer chromatography procedure ⟨1064⟩, 7578
Identification of fixed oils by thin-layer chromatography ⟨202⟩, 6601
Identification—organic nitrogenous bases ⟨181⟩, 6587
Identification tests—general ⟨191⟩, 6587
Identification—tetracyclines ⟨193⟩, 6592
Immunogenicity assays—design and validation of assays to detect anti-drug neutralizing antibody ⟨1106.1⟩, 7802
Immunogenicity assays—design and validation of immunoassays to detect anti-drug antibodies ⟨1106⟩, 7788
Immunological test methods—surface plasmon resonance ⟨1105⟩, 7773
Immunological test methods—enzyme-linked immunosorbent assay (ELISA) ⟨1103⟩, 7753
Immunological test methods—general considerations ⟨1102⟩, 7746
Immunological test methods—immunoblot analysis ⟨1104⟩, 7763
Impurities in drug substances and drug products ⟨1086⟩, 7678
Impurities testing in medical gases ⟨413⟩, 6692
Inhalation and nasal drug products: aerosols, sprays, and powders—performance quality tests ⟨601⟩, 6819
Inhalation and nasal drug products—general information and product quality tests ⟨5⟩, 6427
Injections and implanted drug products (parenterals)—product quality tests ⟨1⟩, 6405
Insulin assays ⟨121⟩, 6547
In vitro and in vivo evaluation of dosage forms ⟨1088⟩, 7684
Iodometric assay—antibiotics ⟨425⟩, 6696
Ion chromatography ⟨1065⟩, 7588
Iron ⟨241⟩, 6648
Labeling ⟨7⟩, 6435
Labeling of inactive ingredients ⟨1091⟩, 7706
Lead ⟨251⟩, 6649
Leak rate ⟨604⟩, 6846
Light diffraction measurement of particle size ⟨429⟩, 6697
Limit on number of large deviations when assessing content uniformity in large samples ⟨1099⟩, 7744
Liquid-phase sterilization ⟨1229.6⟩, 8219
Loss on drying ⟨731⟩, 6970
Loss on ignition ⟨733⟩, 6970
Low molecular weight heparin molecular weight determinations ⟨209⟩, 6614
Manufacturing practices for dietary supplements ⟨2750⟩, 8742
Mass spectrometry ⟨736⟩, 6975
Measurement of subvisible particulate matter in therapeutic protein injections ⟨1787⟩, 8562
Medical devices—bacterial endotoxin and pyrogen tests ⟨161⟩, 6579
Medical gases assay ⟨415⟩, 6693
Melting range or temperature ⟨741⟩, 6980
Mercury ⟨261⟩, 6650
Methods for the determination of particulate matter in injections and ophthalmic solutions ⟨1788⟩, 8575
Methoxy determination ⟨431⟩, 6702
Microbial characterization, identification, and strain typing ⟨1113⟩, 7823
Microbial enumeration tests—nutritional and dietary supplements ⟨2021⟩, 8687
Microbiological attributes of nonsterile nutritional and dietary supplements ⟨2023⟩, 8698
Microbiological best laboratory practices ⟨1117⟩, 7845
Microbiological control and monitoring of aseptic processing environments ⟨1116⟩, 7833
Microbiological examination of nonsterile products: acceptance criteria for pharmaceutical preparations and substances for pharmaceutical use ⟨1111⟩, 7819
Microbiological examination of nonsterile products: microbial enumeration tests ⟨61⟩, 6457
Microbiological examination of nonsterile products: tests for specified organisms ⟨62⟩, 6463
Microbiological examination of nonsterile products tests for Burkholderia Cepacia complex ⟨60⟩, 6454
Microbiological procedures for absence of specified microorganisms—nutritional and dietary supplements ⟨2022⟩, 8692
Mid-infrared spectroscopy ⟨854⟩, 7151
Mid-infrared spectroscopy—theory and practice ⟨1854⟩, 8650
Minimum fill ⟨755⟩, 6982
Moist heat sterilization of aqueous liquids ⟨1229.2⟩, 8202
Monitoring devices—time, temperature, and humidity ⟨1118⟩, 7850
Monitoring of bioburden ⟨1229.3⟩, 8206
Monosaccharide analysis ⟨210⟩, 6616
Mucosal drug products—performance tests ⟨1004⟩, 7227
Mucosal drug products—product quality tests ⟨4⟩, 6423
Mycoplasma tests ⟨63⟩, 6472
Near-infrared spectroscopy ⟨856⟩, 7161
Nephelometry, turbidimetry, and visual comparison ⟨855⟩, 7155
New sterilization methods ⟨1229.12⟩, 8233
Niacin or niacinamide assay ⟨441⟩, 6704
Nitrite titration ⟨451⟩, 6709
Nitrogen determination ⟨461⟩, 6709
Nomenclature ⟨1121⟩, 7862
Nuclear magnetic resonance spectroscopy ⟨761⟩, 6984
Nuclear magnetic resonance spectroscopy identity testing of bacterial polysaccharides used in vaccine manufacture ⟨198⟩, 6596
Nucleic acid-based techniques—amplification ⟨1127⟩, 7879
Nucleic acid-based techniques—approaches for detecting trace nucleic acids (residual DNA testing) ⟨1130⟩, 7897
Nucleic acid-based techniques—extraction, detection, and sequencing ⟨1126⟩, 7870
Nucleic acid-based techniques—general ⟨1125⟩, 7865
Nucleic acid-based techniques—genotyping ⟨1129⟩, 7893
Nucleic acid-based techniques—microarray ⟨1128⟩, 7887
Oligosaccharide analysis ⟨212⟩, 6623
Ophthalmic products—performance tests ⟨1771⟩, 8549
Ophthalmic products—quality tests ⟨771⟩, 6993
Optical microscopy ⟨776⟩, 6998
Optical rotation ⟨781⟩, 7001
Oral drug products—product quality tests ⟨2⟩, 6410
Orally inhaled and nasal drug products ⟨1664.1⟩, 8467
Ordinary impurities ⟨466⟩, 6710
Osmolality and osmolarity ⟨785⟩, 7008
Oxygen flask combustion ⟨471⟩, 6735
Package integrity and test method selection ⟨1207.1⟩, 8086
Package integrity leak test technologies ⟨1207.2⟩, 8097
Package seal quality test technologies ⟨1207.3⟩, 8111
Packaging and repackaging—single unit containers ⟨1136⟩, 7919
Packaging and storage requirements ⟨659⟩, 6876
Pancreatin ⟨1025⟩, 7272
Particle size distribution estimation by analytical sieving ⟨786⟩, 7010
Particulate matter in injections ⟨788⟩, 7017
Particulate matter in ophthalmic solutions ⟨789⟩, 7020
pH ⟨791⟩, 7022
Pharmaceutical calculations in pharmacy practice ⟨1160⟩, 7953
Pharmaceutical compounding—nonsterile preparations ⟨795⟩, 7025

www.webofpharma.com

General chapters *(continued)*
Pharmaceutical compounding—sterile preparations ⟨797⟩, 7037
Pharmaceutical dosage forms ⟨1151⟩, 7929
Physical environments that promote safe medication use ⟨1066⟩, 7590
Physicochemical analytical procedures for insulins ⟨121.1⟩, 6550
Physicochemical integrators and indicators for sterilization ⟨1229.9⟩, 8227
Plasma spectrochemistry ⟨730⟩, 6967
Plasma spectrochemistry—theory and practice ⟨1730⟩, 8484
Plastic materials of construction ⟨661.1⟩, 6893
Plastic packaging systems and their materials of construction ⟨661⟩, 6887
Plastic packaging systems for pharmaceutical use ⟨661.2⟩, 6912
Polarography ⟨801⟩, 7089
Porosimetry by mercury intrusion ⟨267⟩, 6653
Porosity by nitrogen adsorption–desorption ⟨268⟩, 6655
Positron emission tomography drugs for compounding, investigational, and research uses ⟨823⟩, 7100
Positron emission tomography drugs—information ⟨1823⟩, 8617
Powder fineness ⟨811⟩, 7093
Powder flow ⟨1174⟩, 7993
Prekallikrein activator ⟨165⟩, 6583
Preparation of biological specimens for histologic and immunohistochemical analysis ⟨1285⟩, 8370
Prescription balances and volumetric apparatus ⟨1176⟩, 7997
Prescription container labeling ⟨17⟩, 6445
Prion inactivation ⟨1229.16⟩, 8239
Products for nebulization—characterization tests ⟨1601⟩, 8407
Propellants ⟨602⟩, 6844
Protein A quality attributes ⟨130⟩, 6570
Protein determination procedures ⟨507⟩, 6744
Pyrogen test ⟨151⟩, 6577
Quality assurance in pharmaceutical compounding ⟨1163⟩, 7979
Quality attributes of tablets labeled as having a functional score ⟨705⟩, 6944
Quality of biotechnological products: analysis of the expression construct in cells used for production of r-DNA derived protein products ⟨1048⟩, 7452
Quality of biotechnological products: stability testing of biotechnological/biological products ⟨1049⟩, 7454
Radiation sterilization ⟨1229.10⟩, 8228
Radioactivity ⟨821⟩, 7094
Radioactivity—theory and practice ⟨1821⟩, 8604
Raman spectroscopy ⟨1120⟩, 7856
Rapid sterility testing of short life products a risk based approach ⟨1071⟩, 7602
Readily carbonizable substances test ⟨271⟩, 6660
Refractive index ⟨831⟩, 7137
Residual DNA testing ⟨509⟩, 6748
Residual host cell protein measurement in biopharmaceuticals ⟨1132⟩, 7900
Residual solvents ⟨467⟩, 6712
Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures ⟨1467⟩, 8404
Residue on ignition ⟨281⟩, 6660
Rheometry ⟨1911⟩, 8679
Riboflavin assay ⟨481⟩, 6735
Salts of organic nitrogenous bases ⟨501⟩, 6741
Scanning electron microscopy ⟨1181⟩, 8025
Screening for undeclared drugs and drug analogues ⟨2251⟩, 8725
Selenium ⟨291⟩, 6661
Semisolid drug products—performance tests ⟨1724⟩, 8473
Sensitization testing ⟨1184⟩, 8035
Shear cell methodology for powder flow testing ⟨1063⟩, 7566
Significant change guide for bulk pharmaceutical excipients ⟨1195⟩, 8049
Single-steroid assay ⟨511⟩, 6750
Somatropin bioidentity tests ⟨126⟩, 6558
Spacers and valved holding chambers used with inhalation aerosols—characterization tests ⟨1602⟩, 8411
Specific gravity ⟨841⟩, 7137
Specific surface area ⟨846⟩, 7139
Spectrophotometric identification tests ⟨197⟩, 6593
Stability considerations in dispensing practice ⟨1191⟩, 8044
Statistical tools for procedure validation ⟨1210⟩, 8117
Steam sterilization by direct contact ⟨1229.1⟩, 8199
Sterile product packaging—integrity evaluation ⟨1207⟩, 8079
Sterility Assurance ⟨1211⟩, 8129
Sterility testing—validation of isolator systems ⟨1208⟩, 8113
Sterility tests ⟨71⟩, 6481
Sterilization cycle development ⟨1229.14⟩, 8235
Sterilization filtration of gases ⟨1229.15⟩, 8238
Sterilization-in-place ⟨1229.13⟩, 8233
Sterilization of compendial articles ⟨1229⟩, 8194
Sterilizing filtration of liquids ⟨1229.4⟩, 8209
Storage and transportation of investigational drug products ⟨1079.1⟩, 7644
Subvisible particulate matter in therapeutic protein injections ⟨787⟩, 7014
Sulfur dioxide ⟨525⟩, 6751
Supplemental information for articles of botanical origin ⟨2030⟩, 8702
Sutures—diameter ⟨861⟩, 7175
Sutures—needle attachment ⟨871⟩, 7178
Tablet breaking force ⟨1217⟩, 8138
Tablet compression characterization ⟨1062⟩, 7556
Tablet friability ⟨1216⟩, 8137
Tensile strength ⟨881⟩, 7179
Terminally sterilized pharmaceutical products—parametric release ⟨1222⟩, 8142
Test for 1,6-anhydro derivative for enoxaparin sodium ⟨207⟩, 6605
Theory and practice of electrical conductivity measurements of solutions ⟨1644⟩, 8423
Thermal analysis ⟨891⟩, 7180
Thiamine assay ⟨531⟩, 6756
Thin-layer chromatographic identification test ⟨201⟩, 6600
Titrimetry ⟨541⟩, 6764
Topical aerosols ⟨603⟩, 6845
Topical and transdermal drug products—product quality tests ⟨3⟩, 6415
Total organic carbon ⟨643⟩, 6867
Transfer of analytical procedures ⟨1224⟩, 8164
Trifluoroacetic acid (TFA) in peptides ⟨503.1⟩, 6743
Ultraviolet-visible spectroscopy ⟨857⟩, 7166
Ultraviolet-visible spectroscopy—theory and practice ⟨1857⟩, 8670
Uniformity of dosage units ⟨905⟩, 7183
USP reference standards ⟨11⟩, 6442
Vaccines for human use—bacterial vaccines ⟨1238⟩, 8338
Vaccines for human use—general considerations ⟨1235⟩, 8291
Vaccines for human use—polysaccharide and glycoconjugate vaccines ⟨1234⟩, 8276
Validation of alternative microbiological methods ⟨1223⟩, 8144
Validation of compendial procedures ⟨1225⟩, 8166
Validation of microbial recovery from pharmacopeial articles ⟨1227⟩, 8172
Validation of alternative methods to antibiotic microbial assays ⟨1223.1⟩, 8157
Vapor phase sterilization ⟨1229.11⟩, 8231
Verification of compendial procedures ⟨1226⟩, 8171
Vibrational circular dichroism spectroscopytheory and practice ⟨1782⟩, 8549
Vibrational circular dichroism spectroscopy ⟨782⟩, 7002
Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin ⟨1050⟩, 7459
Virology test methods ⟨1237⟩, 8319
Virus testing of human plasma for further manufacture ⟨1240⟩, 8350
Viscosity—capillary methods ⟨911⟩, 7187
Viscosity—pressure driven methods ⟨914⟩, 7195
Viscosity—rolling ball method ⟨913⟩, 7193
Viscosity—rotational methods ⟨912⟩, 7189
Visible particulates in injections ⟨790⟩, 7021
Visual Comparison ⟨630⟩, 6865
Visual inspection of injections ⟨1790⟩, 8587
Vitamin A assay ⟨571⟩, 6801
Vitamin B_{12} activity assay ⟨171⟩, 6584
Vitamin C assay ⟨580⟩, 6805
Vitamin D assay ⟨581⟩, 6808
Vitamin E assay ⟨551⟩, 6767
Volumetric apparatus ⟨31⟩, 6448
Water conductivity ⟨645⟩, 6871
Water determination ⟨921⟩, 7196
Water for hemodialysis applications ⟨1230⟩, 8240
Water for pharmaceutical purposes ⟨1231⟩, 8242
Water–solid interactions in pharmaceutical systems ⟨1241⟩, 8359
Weighing on an analytical balance ⟨1251⟩, 8363
Weight variation of dietary supplements ⟨2091⟩, 8718
Written prescription drug information—guidelines ⟨1265⟩, 8368
X-ray fluorescence spectrometry ⟨735⟩, 6971
X-ray fluorescence spectrometry—theory and practice ⟨1735⟩, 8490

www.webofpharma.com

General chapters *(continued)*
Zinc determination ⟨591⟩, 6817

General notices and requirements, 1
Conformance to standards, 4
Monograph components, 6
Monographs and general chapters, 5
Official status and legal recognition, 3
Prescribing and dispensing, 12
Preservation, packaging, storage, and labeling, 12
Terms and definitions, 9
Test results, 9
Testing practices and procedures, 8
Title and revision, 3
General tests for reagents, 6128
Geneticin, 6168
Gentamicin
injection, 2099
and prednisolone acetate ophthalmic ointment, 2106
and prednisolone acetate ophthalmic suspension, 2107
sulfate, 2100
sulfate and betamethasone acetate ophthalmic solution, 2102
sulfate and betamethasone valerate ointment, 2103
sulfate and betamethasone valerate otic solution, 2104
sulfate and betamethasone valerate topical solution, 2105
sulfate cream, 2101
sulfate ointment, 2101
sulfate ophthalmic ointment, 2102
sulfate ophthalmic solution, 2102
uterine infusion, 2099
Gentian violet, 2107
cream, 2108
topical solution, 2109
Ginger, 5026
capsules, 5032
powdered, 5028
tincture, 5030
Ginkgo, 5034
capsules, 5037
extract, powdered, 5038
tablets, 5041
Ginseng
American, 4750
Asian, 4774
capsules, American, 4754
extract, powdered American, 4753
extract, powdered Asian, 4776
powdered, American, 4752
powdered, Asian, 4775
tablets, American, 4757
tablets, Asian, 4778
Tienchi, root and rhizome, 5299
Tienchi, root and rhizome dry extract, 5307
Tienchi, root and rhizome powder, 5301
Girard reagent T, 6168
Gitoxin, 6168
Glacial acetic acid, 77, 6168
TS, 6229
Glass wool, 6168
Glaze, pharmaceutical, 5786
Glimepiride, 2109
and pioglitazone tablets, 3554
tablets, 2111
Glipizide, 2113
and metformin hydrochloride tablets, 2116
tablets, 2115
Globule size distribution in lipid injectable emulsions ⟨729⟩, 6963
Globulin
immune, 2119
reagent, anti-human, 6139
RH_o (D) immune, 2120
Glucagon, 2120
for injection, 2122
Glucagon bioidentity tests ⟨123⟩, 6552
Glucoamylase, 6168
D-Gluconic acid, 50 percent in water, 6168
Gluconolactone, 2123
Glucosamine
and chondroitin sulfate sodium tablets, 5043
chondroitin sulfate sodium, and methylsulfonylmethane tablets, 5051
hydrochloride, 5045
and methylsulfonylmethane tablets, 5049
sulfate potassium chloride, 5047
sulfate sodium chloride, 5048
tablets, 5046
Glucose, 6168
enzymatic test strip, 2123
liquid, 5788
oxidase-chromogen TS, 6229
Glucose oxidase, 6168
D-Glucuronolactone, 6168
Glutamic acid, 5053, 6168
L-Glutamic acid, 6168
hydrochloride, 5789
Glutamine, 2123
L-Glutamine, 6168
Glutaral
concentrate, 2124
disinfectant solution, 5789
Glutathione, 5054
Glyburide, 2125
and metformin hydrochloride tablets, 2128
tablets, 2126
Glycerin, 2132, 6168
base TS, 6229
ophthalmic solution, 2133
oral solution, 2134
suppositories, 2134
Glyceryl
behenate, 5790
dibehenate, 5791
distearate, 5792
monocaprylate, 5799
monocaprylocaprate, 5802
monolinoleate, 5804
monooleate, 5805
monostearate, 5807
tristearate, 5810
Glyceryl mono and dicaprylate, 5794
Glyceryl mono and dicaprylocaprate, 5796
Glyceryl monostearate, 6168
Glyceryl tricaprylate, 5808
Glycine, 2134
irrigation, 2136
Glycolic acid, 6168
Glycoprotein and glycan analysis—general considerations ⟨1084⟩, 7655
Glycopyrrolate, 2136
injection, 2139
tablets, 2140
Glycyl-L-glutamine, 5058
Glycyl-L-tyrosine, 5059
Glyoxal solution, 6169
Gold
chloride, 6169
chloride TS, 6229
sodium thiomalate, 2142
sodium thiomalate injection, 2143
Goldenseal, 5061
extract, powdered, 5064
powdered, 5063
Gonadorelin
acetate, 2144
hydrochloride, 2146
for injection, 2143
Gonadotropin
chorionic, 2148
chorionic, for injection, 2149
Good distribution practices for bulk pharmaceutical excipients ⟨1197⟩, 8060
Good manufacturing practices for bulk pharmaceutical excipients ⟨1078⟩, 7617
Good packaging practices ⟨1177⟩, 8003
Good repackaging practices ⟨1178⟩, 8006
Good storage and distribution practices for drug products ⟨1079⟩, 7635
Goserelin acetate, 2150
Goserelin Implants, 2152
Government liaisons to expert committees and expert panels, xvi
Graftskin, 1162
Gramicidin, 2156
and neomycin and polymyxin B sulfates cream, 3122
and neomycin and polymyxin B sulfates and hydrocortisone acetate cream, 3123
and neomycin and polymyxin B sulfates ophthalmic solution, 3123
and neomycin sulfate ointment, 3108
nystatin, neomycin sulfate, and triamcinolone acetonide cream, 3222
nystatin, neomycin sulfate, and triamcinolone acetonide ointment, 3222
Granisetron, 2156
Granisetron hydrochloride, 2158
injection, 2159
oral suspension, 2161
tablets, 2161
Granules
Flunixin meglumine, 1931
Montelukast sodium, oral, 3012
Grape seeds oligomeric proanthocyanidins, 5065
Gravity, specific ⟨841⟩, 7137
Green
brilliant, 6144, 6176, 6220
FCF, fast, 6165
soap, 2163
soap tincture, 2164
Green tea
extract, decaffeinated, powdered, 5067
Griseofulvin, 2164
capsules, 2165
oral suspension, 2166
tablets, 2166
tablets, ultramicrosize, 2168
Growth factors and cytokines used in cell therapy manufacturing ⟨92⟩, 6539
Guaiacol, 6169
Guaifenesin, 2169, 6169
capsules, 2170
and codeine phosphate oral solution, 2172
compounded injection, veterinary, 2170
and dyphylline oral solution, 1575
and dyphylline tablets, 1575
and pseudoephedrine hydrochloride capsules, 2174
pseudoephedrine hydrochloride, and dextromethorphan hydrobromide capsules, 2175
and theophylline capsules, 4331
and theophylline oral solution, 4332

www.webofpharma.com

Guaifenesin *(continued)*
for injection, 2171
oral solution, 2171
tablets, 2172
Guanabenz acetate, 2176
tablets, 2177
Guanethidine monosulfate, 2178
tablets, 2179
Guanfacine
hydrochloride, 2180
tablets, 2180
Guanidine hydrochloride, 6169
Guanidine isothiocyanate, 6169
Guanine hydrochloride, 6169
Guar gum, 5813
Guggul, 5069
extract, native, 5070
extract, purified, 5071
tablets, 5072
Guide to general chapters
charts, 6353
table of contents, 13
Guidelines on endotoxins test ⟨1085⟩, 7665
Gutta percha, 2182
Gymnema, 5074
extract, native, 5077
extract, purified, 5078
powdered, 5075

H

Halazone, 2182
tablets for solution, 2182
Halcinonide, 2183
cream, 2184
ointment, 2185
topical solution, 2186
Halobetasol propionate, 2187
Haloperidol, 2188
decanoate, 2191
injection, 2189
oral solution, 2189
tablets, 2190
Halothane, 2192
Hawthorn leaf
with flower, 5080
with flower, powdered, 5082
Hazardous drugs—handling in healthcare settings ⟨800⟩, 7071
Heavy metals in reagents, 6130
Helium, 2193
oxygen certified standard, 6185
Hematein, 6169
Hematoxylin, 6169
TS, Delafield's, 6228
Hematoxylin and eosin staining of sectioned tissue for microscopic examination ⟨1285.1⟩, 8374
Hemoglobin, bovine, 6169
Heparin
lock flush solution, 2194
sodium, 2195
sodium injection, 2200
Hepatitis B
immune globulin, 2201
1-Heptadecanol, 6169
Heptafluorobutyric acid, 6169
Heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin, 6169
n-Heptane, 6169
chromatographic, 6152, 6169
Heptyl *p*-hydroxybenzoate, 6169
Hesperidin, 5084
Hexachlorophene, 2201
cleansing emulsion, 2202
liquid soap, 2202
Hexadecyl hexadecanoate, 6169
Hexadecyltrimethylammonium bromide, 6169
Hexadimethrine bromide, 6170
Hexamethyldisilazane, 6170
Hexamethyleneimine, 6170
Hexamethylenetetramine, 6170
n-Hexane, 6170
Hexane, solvent, 6170, 6189, 6207
chromatographic, 6152, 6170
Hexanes, 6170
Hexanitrodiphenylamine, 6162, 6170
Hexanophenone, 6170
Hexylamine, 6170
Hexylene glycol, 5814
Hexylresorcinol, 2203
lozenges, 2204
High-performance thin-layer chromatography procedure for identification of articles of botanical origin ⟨203⟩, 6602
Histamine
dihydrochloride, 6170
phosphate, 2205
phosphate injection, 2205
Histidine, 2206
L-Histidine hydrochloride monohydrate, 6170
Holy basil leaf, 5085
extract, powdered, 5089
powdered, 5087
Homatropine
hydrobromide, 2207
hydrobromide ophthalmic solution, 2208
methylbromide, 2208
methylbromide and hydrocodone bitartrate tablets, 2225
methylbromide tablets, 2210
Homosalate, 2210
Honey, purified, 5816
Horse chestnut, 4873
extract, powdered, 4876
powdered, 4875
Horseradish peroxidase conjugated to goat anti-mouse IgG, 6170
Human plasma ⟨1180⟩, 8008
Hyaluronidase
injection, 2211
for injection, 2211
Hydralazine hydrochloride, 2213
injection, 2214
oral solution, 2215
tablets, 2215
Hydrazine
dihydrochloride, 6170
hydrate, 85% in water, 6170
sulfate, 6170
Hydrindantin, 6170
Hydriodic acid, 6170
Hydrobromic acid, 6170
Hydrochloric acid, 5816, 6170
alcoholic, tenth-molar (0.1 M), 6242
buffer, 6145
diluted, 5817, 6159, 6170
half-normal (0.5 N), 6242
half-normal (0.5 N) in methanol, 6242
injection, 2217
normal (1 N), 6242
0.001 N TS, 6229
0.01 M TS, 6229
0.025 N TS, 6229
0.36 N TS, 6229
0.05 N TS, 6229
2 N TS, 6229
3 N TS, 6229
5 N TS, 6229
6 N TS, 6230
0.01 N VS, 6241
0.1 N VS, 6242
0.02 N VS, 6241
Hydrochloric acid
25 percent, 6171
4 N Hydrochloric acid
TS, 6229
0.08 N hydrochloric acid TS, 6230
0.125 N hydrochloric acid TS, 6230
Hydrochloride
fingolimod, 1891
nile blue, 6221
Hydrochlorothiazide, 2217
and amiloride hydrochloride tablets, 224
amlodipine, valsartan, tablets, 279
and bisoprolol fumarate tablets, 587
candesartan cilexetil, tablets, 719
capsules, 2219
and captopril tablets, 737
and enalapril maleate tablets, 1616
and fosinopril tablets, 2043
and irbesartan tablets, 2404
and lisinopril tablets, 2646
and losartan potassium tablets, 2690
and methyldopa tablets, 2878
and metoprolol tartrate tablets, 2928
and moexipril hydrochloride and tablets, 2996
and propranolol hydrochloride tablets, 3751
and quinapril tablets, 3803
and spironolactone oral suspension, 4113
and spironolactone tablets, 4113
tablets, 2222
and telmisartan tablets, 4246
and timolol maleate tablets, 4391
and triamterene capsules, 4496
and triamterene tablets, 4499
and valsartan tablets, 4580
Hydrocodone bitartrate, 2223
and acetaminophen tablets, 2224
and homatropine methylbromide tablets, 2225
tablets, 2224
Hydrocodone diol, 6171
Hydrocortisone, 2228
acetate, 2236
acetate and chloramphenicol for ophthalmic suspension, 932
acetate and colistin and neomycin sulfates otic suspension, 1150
acetate cream, 2237
acetate lotion, 2237
acetate, neomycin and polymyxin B sulfates, and bacitracin ointment, 3116
acetate, neomycin and polymyxin B sulfates, and bacitracin ophthalmic ointment, 3116
acetate, neomycin and polymyxin B sulfates, and bacitracin zinc ophthalmic ointment, 3120
acetate and neomycin and polymyxin B sulfates cream, 3125
acetate, neomycin and polymyxin B sulfates, and gramidicin cream, 3123
acetate and neomycin and polymyxin B sulfates ophthalmic suspension, 3125
acetate and neomycin sulfate cream, 3110
acetate and neomycin sulfate lotion, 3110

www.webofpharma.com

Hydrocortisone *(continued)*
acetate and neomycin sulfate ointment, 3110
acetate and neomycin sulfate ophthalmic suspension, 3110
acetate ointment, 2238
acetate and oxytetracycline hydrochloride ophthalmic suspension, 3363
acetate, penicillin G, neomycin, polymyxin B, and hydrocortisone sodium succinate topical suspension, 3430
acetate, penicillin G procaine, and neomycin and polymyxin B sulfates topical suspension, 3448
and acetic acid otic solution, 2235
and clioquinol cream, 1077
and clioquinol ointment, 1078
and neomycin and polymyxin B sulfates ophthalmic suspension, 3123
and neomycin and polymyxin B sulfates otic solution, 3124
and neomycin and polymyxin B sulfates otic suspension, 3124
and neomycin sulfate cream, 3109
and neomycin sulfate ointment, 3109
and neomycin sulfate otic suspension, 3109
and oxytetracycline hydrochloride ointment, 3364
and polymyxin B sulfate otic solution, 3592
butyrate, 2238
butyrate cream, 2239
cream, 2229
gel, 2230
hemisuccinate, 2240
lotion, 2232
neomycin and polymyxin B sulfates and bacitracin zinc ointment, 3118
neomycin and polymyxin B sulfates and bacitracin zinc ophthalmic ointment, 3119
ointment, 2232
rectal suspension, 2233
sodium phosphate, 2241
sodium phosphate injection, 2242
sodium succinate, 2243
sodium succinate for injection, 2243
sodium succinate, penicillin G, neomycin, polymyxin B, and hydrocortisone acetate topical suspension, 3430
tablets, 2234
valerate, 2245
valerate cream, 2245
valerate ointment, 2246
Hydroflumethiazide, 2247
tablets, 2247
Hydrofluoric acid, 6171
Hydrogen
peroxide, 10 percent, 6171
peroxide, 30 percent, 6171
peroxide, 30 percent, unstabilized, 6171
peroxide, 50 percent in water, 6171
peroxide concentrate, 2248
peroxide solution, 6171
peroxide topical solution, 2249
peroxide TS, 6230
sulfide, 6171
sulfide detector tube, 6171
sulfide TS, 6230
Hydrogenated lanolin, 5850
Hydrogenated polydextrose, 5936
Hydrogenated vegetable oil, 6099
Hydromorphone hydrochloride, 2249
injection, 2252
oral solution, 2252
tablets, 2254
Hydroquinone, 2255, 6171
cream, 2255
topical solution, 2255
Hydroxocobalamin, 2256
injection, 2257
Hydroxy naphthol blue, 6171
3′-Hydroxyacetophenone, 6171
4′-Hydroxyacetophenone, 6171
Hydroxyamphetamine hydrobromide, 2258
ophthalmic solution, 2258
Hydroxyanisole, butylated, 5651
p-Hydroxybenzoic acid, 6171
4-Hydroxybenzoic acid isopropyl ester, 6171
2-Hydroxybenzyl alcohol, 6171
4-Hydroxybutane-1-sulfonic acid, 6171
4-Hydroxy-2-butanone, 6171
Hydroxychloroquine sulfate, 2259
tablets, 2259
Hydroxyethyl cellulose, 5817
N-(2-Hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid), 6171
Hydroxylamine hydrochloride, 6171
TS, 6230
5-Hydroxymethylfurfural, 6172
10β-Hydroxynorandrostenedione, 6172
2′-(4-Hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1*H*-benzimidazole trihydrochloride pentahydrate, 6172
4-(4-Hydroxyphenyl)-2-butanone, 6172
3-Hydroxyphenyldimethylethyl ammonium chloride, 6172
D-α-4-Hydroxyphenylglycine, 6172
4-Hydroxy-4-phenylpiperidine, 6171
Hydroxyprogesterone caproate, 2260
injection, 2261
Hydroxypropyl
betadex, 5818
cellulose, 5821
cellulose, low-substituted, 5822
cellulose ocular system, 2262
corn starch, 6045
pea starch, 6054
potato starch, 6060
Hydroxypropyl-β-cyclodextrin, 6172
Hydroxypropyl cellulose, 6172
8-Hydroxyquinoline, 6172
TS, 6230
Hydroxytoluene
butylated, 5652
butylated, reagent, 6146
Hydroxyurea, 2262
capsules, 2263
Hydroxyzine
hydrochloride, 2264
hydrochloride injection, 2265
hydrochloride oral solution, 2266
hydrochloride tablets, 2267
pamoate, 2269
pamoate capsules, 2271
Hymetellose, 5824
Hyoscyamine, 2273
hydrobromide, 2274
sulfate, 2275
sulfate elixir, 2276
sulfate injection, 2277
sulfate oral solution, 2277
sulfate tablets, 2278
tablets, 2273
Hypophosphorous acid, 5825
50 percent, 6172
Hypoxanthine, 6172
Hypromellose, 2279
acetate succinate, 5825
ophthalmic solution, 2281
phthalate, 5828

I

I 123
capsules, sodium iodide, 2365
injection, iobenguane, 2365
solution, sodium iodide, 2366
I 125
albumin injection, iodinated, 2367
injection, iothalamate sodium, 2367
I 131
albumin injection, iodinated, 2368
capsules, sodium iodide, 2368
solution, sodium iodide, 2368
Ibuprofen, 2281
and diphenhydramine citrate tablets, 1421
diphenhydramine hydrochloride capsules, 1431
and pseudoephedrine hydrochloride tablets, 2286
oral suspension, 2283
tablets, 2285
Ibutilide fumarate, 2287
Ichthammol, 2289
ointment, 2289
Idarubicin hydrochloride, 2290
injection, 2291
for injection, 2291
Identification
of articles of botanical origin ⟨563⟩, 6788
of articles of botanical origin by high-performance thin-layer chromatography procedure ⟨1064⟩, 7578
of fixed oils by thin-layer chromatography ⟨202⟩, 6601
organic nitrogenous bases ⟨181⟩, 6587
test, thin-layer chromatographic ⟨201⟩, 6600
tests—general ⟨191⟩, 6587
tests, spectrophotometric ⟨197⟩, 6593
tetracyclines ⟨193⟩, 6592
Idoxuridine, 2292
ophthalmic ointment, 2293
ophthalmic solution, 2293
Ifosfamide, 2294
for injection, 2296
IgG-coated red cells, 6172
Imidazole, 6172
Imidurea, 5829
Iminodiacetic acid, 6172
Imipenem, 2296
and cilastatin for injectable suspension, 2298
and cilastatin for injection, 2297
Imipramine pamoate, 2303
Imipramine hydrochloride, 2300
injection, 2301
tablets, 2301
Imipramine pamoate
capsules, 2304
Imiquimod, 2307
cream, 2309
Immunogenicity assays—design and validation of assays to detect anti-drug neutralizing antibody ⟨1106.1⟩, 7802
Immunogenicity assays—design and validation of immunoassays to detect anti-drug antibodies ⟨1106⟩, 7788

www.webofpharma.com

Immunological test methods—surface plasmon resonance ⟨1105⟩, 7773
Immunological test methods
enzyme-linked immunosorbent assay (ELISA) ⟨1103⟩, 7753
general considerations ⟨1102⟩, 7746
immunoblot analysis ⟨1104⟩, 7763
Impurities
ordinary ⟨466⟩, 6710
testing in medical gases ⟨413⟩, 6692
Impurities in drug substances and drug products ⟨1086⟩, 7678
Inamrinone, 2310
injection, 2311
Indapamide, 2313
tablets, 2314
Indene, 6172
Indicator and test papers, 6219
Indicators, 6220
indicator papers, 6219
reagents, and solutions, 6127
test papers, 6219
Indigo carmine, 6172
TS, 6230
Indigotindisulfonate sodium, 2315
injection, 2316
Indinavir sulfate, 2316
Indium In 111
capromab pendetide injection, 2318
chloride solution, 2318
oxyquinoline solution, 2320
pentetate injection, 2320
pentetreotide injection, 2321
Indocyanine green, 2322
for injection, 2322
Indole, 6172
Indole-3-carboxylic acid, 6172
Indomethacin, 2323
capsules, 2324
extended-release capsules, 2325
for injection, 2330
topical gel, 2330
oral suspension, 2332
sodium, 2334
suppositories, 2331
Indophenol-acetate TS, 6230
Inhalant
amyl nitrite, 332
propylhexedrine, 3754

Inhalation

Acetylcysteine and isoproterenol hydrochloride solution, 84
Cromolyn sodium powder, 1182
Cromolyn sodium solution, 1182
Epinephrine solution, 1648
Fluticasone propionate aerosol, 1988
Fluticasone propionate powder, 1993
Isoetharine solution, 2416
Isoproterenol solution, 2431
Isoproterenol sulfate aerosol, 2437
Isoproterenol sulfate solution, 2438
Levalbuterol solution, 2576
Racepinephrine solution, 3825
Ribavirin for solution, 3865
Salmeterol powder, 3973
Sodium chloride, solution, 4068
Sterile water for, 4652
Tobramycin solution, 4406

Inhalation and nasal drug products: aerosols, sprays, and powders—performance quality tests ⟨601⟩, 6819
Inhalation and nasal drug products general information and product quality tests ⟨5⟩, 6427

Injection

Acepromazine maleate, 37
Acetazolamide for, 74
Acyclovir for, 90
Adenosine, 99
Alcohol, dehydrated, 117
Alcohol in dextrose, 117
Alfentanil, 123
Alprostadil, 156
Alteplase for, 160
Amifostine for, 217
Amikacin sulfate, 221
Aminocaproic acid, 236
Aminohippurate sodium, 237
Aminopentamide sulfate, 240
Aminophylline, 243
Amiodarone hydrochloride, 254
Ammonium chloride, 288
Ammonium molybdate, 291
Amobarbital sodium for, 292
Amphotericin B for, 315
Ampicillin for, 323
Ampicillin and sulbactam for, 328
Anileridine, 339
Aprotinin, 367
Arginine hydrochloride, 373
Articaine hydrochloride and epinephrine, 381
Ascorbic acid, 383
Atenolol, 406
Atracurium besylate, 426
Atropine sulfate, 430
Azaperone, 436
Azathioprine sodium for, 442
Azithromycin for, 451
Aztreonam, 462
Aztreonam for, 463
Bacitracin for, 466
Bacteriostatic sodium chloride, 4067
Bacteriostatic water for, 4652
Bendamustine hydrochloride, 495
Benztropine mesylate, 532
Benzylpenicilloyl polylysine, 536
Betamethasone sodium phosphate, 551
Bethanechol chloride, 562
Bleomycin for, 588
Bretylium tosylate, 590
Bretylium tosylate in dextrose, 591
Brompheniramine maleate, 602
Bumetanide, 607
Bupivacaine hydrochloride, 611
Bupivacaine hydrochloride in dextrose, 612
Bupivacaine hydrochloride and epinephrine, 612
Butorphanol tartrate, 656
Caffeine citrate, 662
Caffeine and sodium benzoate, 664
Calcitonin salmon, 674
Calcitriol, 677
Calcium chloride, 693
Calcium gluceptate, 697
Calcium gluconate, 700
Calcium levulinate, 705
Capreomycin for, 726
Carbenicillin for, 748
Carboplatin for, 769
Carboprost tromethamine, 772
Carmustine for, 784
Cefamandole nafate for, 819
Cefazolin, 822
Cefazolin for, 823
Cefepime for, 832
Cefmenoxime for, 839
Cefmetazole, 841
Cefmetazole for, 841
Cefonicid for, 842
Cefoperazone, 844
Cefoperazone for, 844
Ceforanide for, 846
Cefotaxime, 847
Cefotaxime for, 848
Cefotetan, 853
Cefotetan for, 854
Cefotiam for, 857
Cefoxitin, 859
Cefoxitin for, 860
Cefpiramide for, 862
Ceftazidime, 872
Ceftazidime for, 873
Ceftizoxime, 880
Ceftizoxime for, 881
Ceftriaxone, 881
Ceftriaxone for, 882
Cefuroxime, 886
Cefuroxime for, 886
Cephalothin, 901
Cephalothin for, 901
Cephapirin for, 903
Cephradine for, 908
Chloramphenicol, 928
Chloramphenicol sodium succinate for, 936
Chloroprocaine hydrochloride, 957
Chloroquine hydrochloride, 958
Chlorothiazide sodium for, 966
Chlorpheniramine maleate, 971
Chlorpromazine hydrochloride, 976
Chorionic gonadotropin for, 2149
Chromic chloride, 991
Cimetidine, 1006
Cimetidine in sodium chloride, 1007
Ciprofloxacin, 1012
Cisapride compounded, veterinary, 1024
Cisatracurium besylate, 1028
Cisplatin for, 1032
Cladribine, 1046
Clavulanic acid and ticarcillin, 4371
Clindamycin, 1062
Clindamycin for, 1063
Cloprostenol, 1113
Codeine phosphate, 1138
Colchicine, 1144
Colistimethate for, 1149
Corticotropin, 1170
Corticotropin for, 1172
Corticotropin, repository, 1174
Cr 51, sodium chromate, 992
Cupric chloride, 1188
Cupric sulfate, 1190
Cyanocobalamin, 1191
Cyclophosphamide for, 1204
Cyclosporine, 1214
Cysteine hydrochloride, 1222
Cytarabine for, 1224
Dacarbazine for, 1226
Dactinomycin for, 1228
Dantrolene sodium for, 1238
Daunorubicin hydrochloride for, 1242

www.webofpharma.com

Injection *(continued)*
Deferoxamine mesylate for, 1245
Dehydrated alcohol, 117
Deslanoside, 1259
Desmopressin acetate, 1266
Dexamethasone, 1286
Dexamethasone sodium phosphate, 1294
Dexamethasone sodium phosphate compounded, 1295
Dexmedetomidine, 1304
Dextran 40 in dextrose, 1311
Dextran 40 in sodium chloride, 1312
Dextran 70 in dextrose, 1315
Dextran 70 in sodium chloride, 1316
Dextrose, 1323
Dextrose and sodium chloride, 1323
Diatrizoate meglumine, 1325
Diatrizoate meglumine and diatrizoate sodium, 1326
Diatrizoate sodium, 1328
Diazepam, 1331
Diazoxide, 1334
Dibucaine hydrochloride, 1338
Dicyclomine hydrochloride, 1361
Digitoxin, 1381
Digoxin, 1384
Dihydroergotamine mesylate, 1389
Dihydrostreptomycin, 1391
Dimenhydrinate, 1409
Dimercaprol, 1412
Dinoprost tromethamine, 1416
Diphenhydramine hydrochloride, 1426
Dipyridamole, 1445
Dobutamine, 1468
Dobutamine for, 1469
Dobutamine in dextrose, 1470
Docetaxel, 1474
Dopamine hydrochloride, 1495
Dopamine hydrochloride and dextrose, 1495
Doxapram hydrochloride, 1503
Doxorubicin hydrochloride, 1514
Doxorubicin hydrochloride for, 1516
Doxycycline for, 1523
Droperidol, 1553
Dyphylline, 1573
Edetate calcium disodium, 1583
Edetate disodium, 1584
Edrophonium chloride, 1585
Electrolytes and dextrose type 1, multiple, 1598
Electrolytes and dextrose type 2, multiple, 1601
Electrolytes and dextrose type 3, multiple, 1605
Electrolytes type 1, multiple, 1593
Electrolytes type 2, multiple, 1595
Elements, trace, 1608
Enalaprilat, 1619
Enoxaparin sodium, 1626
Ephedrine sulfate, 1643
Epinephrine, 1648
Epirubicin hydrochloride, 1654
Ergonovine maleate, 1673
Ergotamine tartrate, 1677
Erythromycin, 1687
Erythromycin ethylsuccinate, 1698
Erythromycin lactobionate for, 1703
Estradiol cypionate, 1738
Estradiol valerate, 1741
Ethacrynate sodium for, 1759
Ethiodized oil, 1770
Etomidate, 1790
Etoposide, 1795
Exenatide, 1803
Famotidine, 1816
Fenoldopam mesylate, 1842
Fentanyl citrate, 1849
Fentanyl citrate and bupivacaine hydrochloride compounded injection, 1850
Fentanyl citrate compounded, 1848
Ferumoxides, 1866
Floxuridine for, 1901
Fluconazole, 1901
Fluconazole in dextrose, 1907
Fluconazole in sodium chloride, 1909
Fludarabine phosphate, 1919
Fludarabine phosphate for, 1921
Fludeoxyglucose F18, 1946
Flumazenil, 1926
Flunixin meglumine, 1932
Fluorescein, 1940
F 18, sodium fluoride, 1950
Fluorouracil, 1957
Fluphenazine decanoate, 1968
Fluphenazine enanthate, 1970
Fluphenazine hydrochloride, 1972
Folic acid, 2027
Fondaparinux sodium, 2033
Fosphenytoin sodium, 2047
Fructose, 2049
Fructose and sodium chloride, 2049
Furosemide, 2054
Gadodiamide, 2064
Gadopentetate dimeglumine, 2065
Gadoteridol, 2069
Gadoversetamide, 2073
Gallamine triethiodide, 2087
Gallium citrate Ga 67, 2088
Ganciclovir for, 2089
Gemcitabine for, 2094
Gentamicin, 2099
Glucagon for, 2122
Glycopyrrolate, 2139
Gold sodium thiomalate, 2143
Gonadorelin for, 2143
Gonadotropin, chorionic for, 2149
Granisetron hydrochloride, 2159
Guaifenesin compounded, veterinary, 2170
Guaifenesin for, 2171
Haloperidol, 2189
Heparin sodium, 2200
Histamine phosphate, 2205
Hyaluronidase, 2211
Hyaluronidase for, 2211
Hydralazine hydrochloride, 2214
Hydrochloric acid, 2217
Hydrocortisone sodium phosphate, 2242
Hydrocortisone sodium succinate for, 2243
Hydromorphone hydrochloride, 2252
Hydroxocobalamin, 2257
Hydroxyprogesterone caproate, 2261
Hydroxyzine hydrochloride, 2265
Hyoscyamine sulfate, 2277
I 123, iobenguane, 2365
I 125, iothalamate sodium, 2367
I 125, albumin, iodinated, 2367
I 131, albumin, iodinated, 2368
Idarubicin hydrochloride, 2291
Idarubicin hydrochloride for, 2291
Ifosfamide for, 2296
Imipenem and cilastatin for, 2297
Imipramine hydrochloride, 2301
Inamrinone, 2311
Indigotindisulfonate sodium, 2316
Indium In 111 capromab pendetide, 2318
Indium In 111 pentetate, 2320
Indium In 111 pentetreotide, 2321
Indocyanine green for, 2322
Indomethacin for, 2330
injection, 1000
Insulin, 2338
Insulin aspart, 2341
Insulin glargine, 2344
Insulin human, 2348
Insulin, human, and human insulin isophane suspension, 2349
Insulin lispro, 2355
Inulin in sodium chloride, 2361
Invert sugar, 4133
Iodipamide meglumine, 2370
Iodixanol, 2373
Iohexol, 2381
Iopamidol, 2383
Iopromide, 2386
Iothalamate meglumine, 2387
Iothalamate meglumine and iothalamate sodium, 2388
Ioversol, 2390
Ioxaglate meglumine and ioxaglate sodium, 2391
Ioxilan, 2394
Irinotecan hydrochloride, 2409
Iron dextran, 2411
Iron sorbitex, 2412
Iron sucrose, 2413
Isoniazid, 2425
Isoproterenol hydrochloride, 2433
Isoxsuprine hydrochloride, 2462
Ivermectin, 2472
Ivermectin and clorsulon, 2477
Kanamycin, 2501
Ketamine hydrochloride, 2506
Ketorolac tromethamine, 2514
Labetalol hydrochloride, 2518
Leucovorin calcium, 2568
leucovorin calcium for, 2569
Levetiracetam, 2601
Levocarnitine, 2599
Levorphanol tartrate, 2614
Lidocaine hydrochloride, 2625
Lidocaine hydrochloride and dextrose, 2628
Lidocaine hydrochloride and epinephrine, 2629
Lincomycin, 2632
Lorazepam, 2682
Magnesium sulfate, 2727
Magnesium sulfate in dextrose, 2727
Manganese chloride, 2731
Mannitol, 2736
Mannitol in sodium chloride, 2737
Mechlorethamine hydrochloride for, 2749
Meperidine hydrochloride, 2783
Mepivacaine hydrochloride, 2788
Mepivacaine hydrochloride and levonordefrin, 2789
Meropenem for, 2798
Metaraminol bitartrate, 2817
Methadone hydrochloride, 2837
Methocarbamol, 2851
Methohexital sodium for, 2853
Methotrexate, 2857
Methotrexate for, 2858
Methotrimeprazine, 2861
Methyldopate hydrochloride, 2880
Methylene blue, 2882
Methylene blue, veterinary, 2884
Methylergonovine maleate, 2886
Methylprednisolone sodium succinate for, 2903
Metoclopramide, 2909
Metoprolol tartrate, 2923
Metronidazole, 2936

www.webofpharma.com

Injection *(continued)*
Midazolam, 2953
Milrinone lactate, 2959
Minocycline for, 2963
Mitomycin for, 2986
Mitoxantrone, 2988
Morphine sulfate, 3027
Morphine sulfate compounded, 3028
Mycophenolate mofetil for, 3046
N 13, ammonia, 3183
Nafcillin, 3063
Nafcillin for, 3063
Nalorphine hydrochloride, 3067
Naloxone hydrochloride, 3068
Nandrolone decanoate, 3073
Neomycin for, 3102
Neostigmine methylsulfate, 3130
Niacin, 3140
Niacinamide, 3147
Nicardipine hydrochloride, 3149
Nitroglycerin, 3187
Norepinephrine bitartrate, 3198
Ondansetron, 3265
Orphenadrine citrate, 3284
Oxacillin, 3296
Oxacillin for, 3297
Oxaliplatin, 3301
Oxaliplatin for, 3303
Oxymorphone hydrochloride, 3353
Oxytetracycline, 3360
Oxytetracycline for, 3362
Oxytocin, 3367
Paclitaxel, 3370
Pamidronate disodium for, 3379
Pancuronium bromide, 3386
Papaverine hydrochloride, 3398
Paricalcitol, 3406
Particulate matter in injections ⟨788⟩, 7017
Pemetrexed, 3422
Penicillin G potassium, 3437
Penicillin G potassium for, 3437
Penicillin G sodium for, 3450
Pentazocine, 3465
Pentobarbital sodium, 3469
Phenobarbital sodium, 3500
Phentolamine mesylate for, 3509
Phenylbutazone, 3511
Phenylephrine hydrochloride, 3517
Phenytoin sodium, 3534
Physostigmine salicylate, 3535
Phytonadione injectable emulsion, 3538
Piperacillin for, 3565
Piperacillin and tazobactam for, 3567
Polymyxin B for, 3590
Potassium acetate, 3595
Potassium chloride concentrate for, 3604
Potassium chloride in dextrose, 3607
Potassium chloride in dextrose and sodium chloride, 3608
Potassium chloride in lactated ringer's and dextrose, 3610
Potassium chloride in sodium chloride, 3612
Potassium phosphates, 3629
Potassium phosphates compounded, 3629
Pralidoxime chloride for, 3637
Prednisolone sodium phosphate, 3667
Prednisolone sodium succinate for, 3668
Prilocaine and epinephrine, 3679
Prilocaine hydrochloride, 3678
Procainamide hydrochloride, 3690
Procaine hydrochloride, 3694
Procaine hydrochloride and epinephrine, 3694
Procaine and tetracaine hydrochlorides and levonordefrin, 3695
Prochlorperazine edisylate, 3699
Progesterone, 3705
Promazine hydrochloride, 3712
Promethazine hydrochloride, 3716
Propofol injectable emulsion, 3742
Propoxycaine and procaine hydrochlorides and levonordefrin, 3744
Propoxycaine and procaine hydrochlorides and norepinephrine bitartrate, 3745
Propranolol hydrochloride, 3749
Protamine sulfate, 3758
Pyridostigmine bromide, 3780
Pyridoxine hydrochloride, 3783
Quinidine gluconate, 3809
Ranitidine, 3845
Ranitidine in sodium chloride, 3848
Repository corticotropin, 1174
Riboflavin, 3869
Rifampin for, 3877
Ringer's, 3887
Ringer's and dextrose, 3889
Ringer's and dextrose, half-strength lactated, 3895
Ringer's and dextrose, lactated, 3893
Ringer's and dextrose, modified, lactated, 3898
Ringer's, lactated, 3891
Ritodrine hydrochloride, 3911
Ropivacaine hydrochloride, 3945
Rubidium chloride Rb 82, 3958
Sargramostim for, 3988
Scopolamine hydrobromide, 4012
Selenious acid, 4022
Sm 153 lexidronam, samarium, 3983
Sodium acetate, 4054
Sodium bicarbonate, 4060
Sodium bicarbonate compounded, 4061
Sodium bromide, veterinary, 4063
Sodium chloride, 4066
Sodium chloride, bacteriostatic, 4067
Sodium chromate Cr 51, 992
Sodium lactate, 4078
Sodium nitrite, 4082
Sodium nitroprusside for, 4084
Sodium phosphates, 4088
Sodium phosphates compounded, 4089
Sodium sulfate, 4096
Sodium thiosulfate, 4097
Somatropin for, 4099
Strontium chloride Sr 89, 4126
Streptomycin, 4125
Streptomycin for, 4125
Succinylcholine chloride, 4129
Sufentanil citrate, 4133
Sugar, invert, 4133
Sulfadiazine sodium, 4152
Sulfamethoxazole and trimethoprim, 4162
Sumatriptan, 4182
Technetium Tc 99m albumin aggregated, 4226
Technetium Tc 99m bicisate, 4227
Technetium Tc 99m disofenin, 4227
Technetium Tc 99m exametazime, 4228
Technetium Tc 99m mebrofenin, 4229
Technetium Tc 99m medronate, 4230
Technetium Tc 99m mertiatide, 4231
Technetium Tc 99m oxiseonate, 4232
Technetium Tc 99m pentetate, 4232
Technetium Tc 99m pertechnetate, sodium, 4233
Technetium Tc 99m pyrophosphate, 4234
Technetium Tc 99m (pyro- and trimeta-) phosphates, 4235
Technetium Tc 99m red blood cells, 4235
Technetium Tc 99m sestamibi, 4236
Technetium Tc 99m succimer, 4237
Technetium Tc 99m sulfur colloid, 4238
Technetium Tc 99m tetrofosmin, 4238
Temozolomide for injection, 4256
Teniposide, 4259
Terbutaline sulfate, 4272
Teriparatide, 4280
Testosterone cypionate, 4289
Testosterone enanthate, 4290
Testosterone propionate, 4291
Tetracaine hydrochloride, 4297
Tetracaine hydrochloride for, 4298
Tetracaine hydrochloride in dextrose, 4300
Tetracycline hydrochloride for, 4307
Thallous chloride Tl 201, 4318
Theophylline in dextrose, 4328
Thiamine hydrochloride, 4338
Thiopental sodium for, 4352
Thiotepa for, 4357
Thiothixene hydrochloride, 4360
Thiothixene hydrochloride for, 4361
Ticarcillin and clavulanic acid, 4371
Ticarcillin and clavulanic acid for, 4372
Ticarcillin for, 4370
Tigecycline for, 4381
Tiletamine and zolazepam for, 4383
Tilmicosin, 4385
Tobramycin, 4402
Tobramycin for, 4403
Tolazoline hydrochloride, 4418
Tolbutamide for, 4419
Tranexamic acid, 4463
Trifluoperazine hydrochloride, 4512
Triflupromazine hydrochloride, 4515
Trimethobenzamide hydrochloride, 4525
Tripelennamine hydrochloride, 4531
Tromethamine for, 4538
Tubocurarine chloride, 4548
Tylosin, 4550
Urea for, 4557
Valproate sodium, 4572
Vancomycin, 4585
Vancomycin hydrochloride for, 4588
Vasopressin, 4593
Verapamil hydrochloride, 4607
Verteporfin for, 4617
Vinblastine sulfate for, 4624
Vincristine sulfate, 4626
Vincristine sulfate for, 4628
Vinorelbine, 4631
Warfarin sodium for, 4648
Water for, bacteriostatic, 4652
Water for, sterile, 4653
Water for, 4651
Xylazine, 4659
Yohimbine, 4664
Yttrium Y 90 ibritumomab tiuxetan, 4665
Zidovudine, 4675
Zinc chloride, 4684
Zinc sulfate, 4692
Zolazepam and tiletamine for injection, 4383

Injections and implanted drug products (parenterals)—product quality tests ⟨1⟩, 6405
Inosine, 6172
Inositol, 5829, 6172
Insoluble matter in reagents, 6130
Insulin, 2335
aspart, 2339

www.webofpharma.com

Insulin *(continued)*
assays ⟨121⟩, 6547
glargine, 2342
glargine injection, 2344
human, 2346
human injection, 2348
human isophane suspension and human insulin injection, 2349
human suspension, isophane, 2352
injection, 2338
lispro, 2353
lispro injection, 2355
suspension, isophane, 2351
zinc suspension, 2356
zinc suspension, extended, 2357
zinc suspension, prompt, 2359
Insulin aspart
injection, 2341
Intestinal fluid, simulated, TS, 6230, 6234
Intramammary infusion
amoxicillin, 302
cloxacillin benzathine, 1126
Intrauterine contraceptive system
progesterone, 3705
Intrinsic viscosity table, 6349
Inulin, 2360
in sodium chloride injection, 2361
Invert sugar, 5832
In vitro
and in vivo evaluation of dosage forms ⟨1088⟩, 7684
biological reactivity tests ⟨87⟩, 6514
In vivo
biological reactivity tests ⟨88⟩, 6516
and in vitro evaluation of dosage forms ⟨1088⟩, 7684
Iobenguane
I 123 injection, 2365
sulfate, 6173
Iodic acid, 6173
Iodinated
I 125 albumin injection, 2367
I 131 albumin injection, 2368
Iodine, 2362, 6172
diluted TS, 6230
hundredth-normal (0.01 N), 6242
I 123 capsules, sodium iodide, 2365
I 123 injection, iobenguane, 2365
I 123 solution, sodium iodide, 2366
I 125 albumin injection, iodinated, 2367
I 125 injection, iothalamate sodium, 2367
I 131 albumin injection, iodinated, 2368
I 131 capsules, sodium iodide, 2368
I 131 solution, sodium iodide, 2368
monobromide, 6173
monochloride, 6173
monochloride TS, 6230
and potassium iodide TS 1, 6230
and potassium iodide TS 2, 6230
and potassium iodide TS 3, 6230
solution, strong, 2363
topical solution, 2362
tenth-normal (0.1 N), 6230, 6243
tincture, 2363
tincture, strong, 2364
TS, 6230
twentieth-normal (0.05 N), 6242
Iodipamide, 2369
meglumine injection, 2370
Iodixanol, 2370
injection, 2373
Iodobromide TS, 6230
Iodochloride TS, 6230
Iodoethane, 6173
Iodoform, 2376
Iodometric assay—antibiotics ⟨425⟩, 6696
p-Iodonitrotetrazolium violet, 6173
Iodoplatinate TS, 6230
Iodoquinol, 2377
tablets, 2378
Iohexol, 2378
injection, 2381
Ion chromatography ⟨1065⟩, 7588
Ion-exchange resin, 6173
Iopamidol, 2382
injection, 2383
Iopromide, 2384
injection, 2386
Iothalamate
meglumine injection, 2387
meglumine and iothalamate sodium injection, 2388
sodium I 125 injection, 2367
sodium and iothalamate meglumine injection, 2388
Iothalamic acid, 2389
Ioversol, 2390
injection, 2390
Ioxaglate
meglumine and ioxaglate sodium injection, 2391
sodium and ioxaglate meglumine injection, 2391
Ioxaglic acid, 2391
Ioxilan, 2392
injection, 2394
Ipecac, 2395
powdered, 2396
oral solution, 2397
Ipratropium bromide, 2398
Ipratropium bromide and albuterol sulfate
inhalation solution, 2399
Irbesartan, 2402
and hydrochlorothiazide tablets, 2404
tablets, 2403
Irinotecan hydrochloride, 2406
injection, 2409
Iron
carbonyl, 2410
dextran injection, 2411
phenol TS, 6230
powder, 6173
salicylate TS, 6230
sorbitex injection, 2412
sucrose injection, 2413
wire, 6173
Iron ⟨241⟩, 6648
Isoamyl
alcohol, 6173
Isobutane, 5833
Isobutyl
acetate, 6173
alcohol, 5833, 6134, 6173
4-Isobutylacetophenone, 6173
Isobutyl chloroformate, 6173
N-Isobutylpiperidone, 6173
Isoetharine
hydrochloride, 2415
inhalation solution, 2416
mesylate, 2416
Isoflupredone acetate, 2418, 6173
injectable suspension, 2419
neomycin sulfate and tetracaine hydrochloride ointment, 3111
neomycin sulfate and tetracaine hydrochloride topical powder, 3112
Isoflurane, 2420
Isoleucine, 2422
L-Isoleucine, 6173
Isomalt, 5836
Isomaltotriose, 6173
Isometheptene mucate, 2423
dichloralphenazone, and acetaminophen capsules, 2423
2-Isoniazid, 2424, 6173
injection, 2425
and rifampin capsules, 3878
rifampin, pyrazinamide, and ethambutol hydrochloride tablets, 3881
rifampin and pyrazinamide tablets, 3879
oral solution, 2426
tablets, 2426
Isonicotinamide, 6173
Isonicotinic acid, 6173
hydrazide, 6173
Isooctane, 6174
Isopropamide iodide, 2427
tablets, 2428
Isopropyl
acetate, 6174
alcohol, 2428, 6134, 6174, 6194
alcohol, azeotropic, 2430
alcohol, dehydrated, 6134, 6174
alcohol, rubbing, 2430
ether, 6174
iodide, 6174
myristate, 5839, 6174
palmitate, 5840
salicylate, 6174
Isopropylamine, 6174
Isopropyl isostearate, 5838
Isopropyl stearate, 6174
Isoproterenol
hydrochloride, 2431
hydrochloride and acetylcysteine inhalation solution, 84
hydrochloride injection, 2433
hydrochloride tablets, 2434
inhalation solution, 2431
sulfate, 2436
sulfate inhalation aerosol, 2437
sulfate inhalation solution, 2438
Isorhamnetin, 6174
Isosorbide
concentrate, 2438
dinitrate extended-release capsules, 2441
dinitrate chewable tablets, 2444
dinitrate, diluted, 2440
dinitrate sublingual tablets, 2447
dinitrate extended-release tablets, 2445
mononitrate, diluted, 2448
mononitrate tablets, 2449
mononitrate extended-release tablets, 2451
oral solution, 2440
Isostearic acid, 6174
Isostearyl isostearate, 5840
Isotretinoin, 2457
capsules, 2458
Isovaleric acid, 6174
Isoxsuprine hydrochloride, 2462
injection, 2462
tablets, 2463
Isradipine, 2464
capsules, 2465
oral suspension, 2465
Itraconazole, 2466
capsules, 2468
Ivermectin, 2470
and clorsulon injection, 2477
injection, 2472
paste, 2473
and pyrantel pamoate tablets, 2478
topical solution, 2476
tablets, 2474

www.webofpharma.com

Ivermectin compounded
oral solution, veterinary, 2473
Ixabepilone, 2479

J

Japanese honeysuckle flower, 5091
dry extract, 5094
powder, 5097
Juniper tar, 2501

K

Kaempferol, 6174
Kanamycin
injection, 2501
sulfate, 2502
sulfate capsules, 2503
Kaolin, 2504
Kerosene, 6174
Ketamine hydrochloride, 2505
injection, 2506
Ketoconazole, 2506
oral suspension, 2507
tablets, 2508
Ketoprofen, 2509
capsules, 2510
extended-release capsules, 2511
Ketorolac tromethamine, 2513
injection, 2514
tablets, 2515
Krill oil
capsules, 5104
delayed-release capsules, 5107

L

L designations, 6174
Labeling ⟨7⟩, 6435
Labeling of inactive ingredients ⟨1091⟩, 7706
Labetalol hydrochloride, 2517
injection, 2518
oral suspension, 2519
tablets, 2519
alpha-Lactalbumin, 5841
Lactase, 2520
Lactic acid, 2520, 6174
Lactitol, 5845
Lactobacillus acidophilus
La-14, 5111
NCFM, 5113
Lactobacillus paracasei LPC-37, 5115
Lactobacillus rhamnosus HN001, 5117
Lactobionic acid, 5846
Lactose, 6174
anhydrous, 5847
beta, 6174
monohydrate, 5849
monohydrate, alpha, 6174
Lactulose
concentrate, 2521
solution, 2522
Lamivudine, 2523
oral solution, 2526
tablets, 2527
and abacavir tablets, 21
and zidovudine tablets, 2528
Lamotrigine, 2530
tablets, 2532
Lamotrigine
extended-release tablets, 2534
tablets for oral suspension, 2542
Lamotrigine compounded
oral suspension, 2545
Lanolin, 2545
alcohols, 5850
modified, 2548
Lansoprazole, 2551
delayed-release capsules, 2553
Lansoprazole compounded
oral suspension, 2556
Lanthanum
alizarin complexan mixture, 6175
chloride, 6175
nitrate hexahydrate, 6175
nitrate TS, 6230
oxide, 6175
Latanoprost, 2556
Lauric acid, 5852
Lauroyl polyoxylglycerides, 5852
Lauryl dimethyl amine oxide, 6175
Lead
acetate, 6175
acetate paper, 6175
acetate test paper, 6222
acetate TS, 6230
acetate TS, alcoholic, 6231
monoxide, 6175
nitrate, 6175
nitrate, hundredth-molar (0.01 M), 6243
nitrate stock solution TS, 6231
perchlorate, 6175
perchlorate, hundredth-molar (0.01 M), 6243
perchlorate, tenth-molar (0.1 M), 6243
solution, standard, 6236
subacetate TS, 6231
subacetate TS, diluted, 6228, 6231
tetraacetate, 6175
Lead ⟨251⟩, 6649
Leak rate ⟨604⟩, 6846
Lecithin, 5853, 6175
Leflunomide, 2558
tablets, 2560
Leflunomide compounded
oral suspension, 2560
Lemon
oil, 5856
tincture, 5857
Letrozole, 2562
tablets, 2563
Leucine, 2565
Leucovorin calcium, 2566
compounded oral suspension, 2570
injection, 2568
tablets, 2571
Leucovorin calcium for injection, 2569
Leuprolide acetate, 2572
Levalbuterol
inhalation solution, 2576
Levalbuterol hydrochloride, 2574
Levamisole hydrochloride, 2578
tablets, 2579
Levetiracetam, 2580
extended-release tablets, 2587
injection, 2582
oral solution, 2583
tablets, 2584
Levmetamfetamine, 2593
Levobunolol hydrochloride, 2594
ophthalmic solution, 2595
Levocabastine hydrochloride, 2596
Levocarnitine, 2597
injection, 2599
oral solution, 2600
tablets, 2601
Levocetirizine dihydrochloride
tablets, 2603
Levodopa, 2604
Levodopa
and carbidopa extended-release tablets, 753
and carbidopa orally disintegrating tablets, 759
and carbidopa tablets, 751
Levofloxacin, 2606
oral solution, 2608
tablets, 2609
Levonordefrin, 2611
and mepivacaine hydrochloride injection, 2789
and procaine and tetracaine hydrochlorides injection, 3695
and propoxycaine and procaine hydrochlorides injection, 3744
Levonorgestrel, 2612
and ethinyl estradiol tablets, 2612
Levorphanol tartrate, 2613
injection, 2614
tablets, 2614
Levothyroxine sodium, 2615
oral powder, 2618
tablets, 2618
L-alpha-glycerylphosphorylcholine, 5055
Licorice, 5119
extract, powdered, 5121
fluidextract, 5857
powdered, 5120
Lidocaine, 2620
topical aerosol, 2622
hydrochloride, 2623
hydrochloride and dextrose injection, 2628
hydrochloride and epinephrine injection, 2629
hydrochloride injection, 2625
hydrochloride jelly, 2625
hydrochloride oral topical solution, 2626
hydrochloride topical solution, 2627
neomycin and polymyxin B sulfates and bacitracin ointment, 3117
neomycin and polymyxin B sulfates and bacitracin zinc ointment, 3120
and neomycin and polymyxin B sulfates cream, 3126
ointment, 2622
and prilocaine cream, 2630
oral topical solution, 2623
Light diffraction measurement of particle size ⟨429⟩, 6697
Lime, 2632
Limestone
ground, 5121
Limit on number of large deviations when assessing content uniformity in large samples, 7744
(*R*)-(+)-Limonene, 6175
Linalool, 6175
Lincomycin
hydrochloride, 2633
hydrochloride capsules, 2634
hydrochloride soluble powder, 2634
injection, 2632

www.webofpharma.com

Lincomycin *(continued)*
oral solution, 2632
Lindane, 2634
cream, 2635
lotion, 2636
shampoo, 2636
Linezolid, 2637
Linoleic acid, 6175
Linoleic acids-free fatty acids
conjugated, 5123
Linolenic acid, 6175
Linoleoyl polyoxylglycerides, 5857
Liothyronine sodium, 2638
tablets, 2640
Liotrix tablets, 2640
Lipid injectable emulsion, 2641
Lipoic acid
alpha, 5124
capsules, alpha, 5125
tablets, alpha, 5126
α-Lipoic acid, 6175
Liquid petrolatum, 6175
Liquid-phase sterilization ⟨1229.6⟩, 8219
Lisinopril, 2642
and hydrochlorothiazide tablets, 2646
oral suspension, 2644
tablets, 2644
Lithium, 6175
carbonate, 2649
carbonate capsules, 2650
carbonate tablets, 2650
carbonate extended-release tablets, 2651
chloride, 6175
citrate, 2653
hydroxide, 2654, 6175
metaborate, 6175
methoxide, fiftieth-normal (0.02 N) in methanol, 6243
methoxide, tenth-normal (0.1 N) in chlorobenzene, 6243
methoxide, tenth-normal (0.1 N) in methanol, 6244
methoxide, tenth-normal (0.1 N) in toluene, 6244
nitrate, 6176
perchlorate, 6176
oral solution, 2648
sulfate, 6176
Lithocholic acid, 6176
Litmus, 6176, 6221
paper, blue, 6222
paper, red, 6222
TS, 6231
Litmus paper, neutral, 6222
Littorine hydrochloride (*R*)-(–)-, 6176
Locke-Ringer's
solution, 6231
TS, 6231
Locust bean gum, 6176
Lomustine, 2655
capsules, 2656
Loperamide hydrochloride, 2658
capsules, 2660
oral solution, 2661
tablets, 2661
Lopinavir, 2663
Lopinavir
and ritonavir oral solution, 2666
and ritonavir tablets, 2670
Loratadine, 2672
chewable tablets, 2676
oral solution, 2674
tablets, 2675
orally disintegrating tablets, 2678
Lorazepam, 2680
injection, 2682
oral concentrate, 2683
tablets, 2684
Losartan potassium, 2686
and hydrochlorothiazide tablets, 2690
tablets, 2687
Loss on drying ⟨731⟩, 6970
Loss on drying for reagents, 6131
Loss on ignition ⟨733⟩, 6970

Lotion

Amphotericin B, 316
Benzoyl peroxide, 527
Benzyl benzoate, 535
Betamethasone dipropionate, 548
Betamethasone valerate, 554
Clotrimazole, 1119
Flurandrenolide, 1976
Fluticasone propionate, 1998
Hydrocortisone, 2232
Hydrocortisone acetate, 2237
Lindane, 2636
Malathion, 2730
Methylbenzethonium chloride, 2873
Neomycin sulfate and flurandrenolide, 3108
Neomycin sulfate and hydrocortisone acetate, 3110
Nystatin, 3220
Padimate O, 3372
Triamcinolone acetonide, 4485

Lovastatin, 2694
Low molecular weight heparin molecular weight determinations ⟨209⟩, 6614
Loxapine
capsules, 2697
succinate, 2696
Lufenuron, 2697
Lumefantrine, 2699
Lutein, 5126
capsules, 5128
preparation, 5128
Lycopene, 5130
preparation, 5131
tomato extract containing, 5133
Lysine
acetate, 2700
hydrochloride, 2701
hydrochloride tablets, 5135
L-Lysine, 6176
Lysolecithin, 6176

M

Mafenide acetate, 2702
cream, 2703
for topical solution, 2704
Magaldrate, 2705
and simethicone chewable tablets, 2707
and simethicone oral suspension, 2706
Magnesia
alumina and calcium carbonate chewable tablets, 169
alumina, calcium carbonate, and simethicone chewable tablets, 170
alumina and calcium carbonate oral suspension, 168
alumina and simethicone chewable tablets, 174
alumina and simethicone oral suspension, 173
and alumina oral suspension, 166
and alumina tablets, 167
aspirin and alumina tablets, 397
aspirin, codeine phosphate, and alumina tablets, 404
calcium carbonate and simethicone chewable tablets, 689
and calcium carbonate chewable tablets, 688
milk of, 2708
mixture TS, 6231
tablets, 2709
Magnesium, 6176
acetate, 6176
aluminometasilicate, 5858
aluminosilicate, 5859
aluminum silicate, 5861
and calcium carbonates oral suspension, 691
and calcium carbonates tablets, 691
carbonate, 2709
carbonate and citric acid for oral solution, 2710
carbonate, citric acid, and potassium citrate for oral solution, 2711
carbonate and sodium bicarbonate for oral suspension, 2712
carbonate, alumina, and magnesium oxide tablets, 178
carbonate and alumina oral suspension, 176
carbonate and alumina tablets, 177
chloride, 2712, 6176
chloride, 0.01 M, 6244
citrate, 2713
citrate oral solution, 2714
citrate for oral solution, 2715
gluconate, 2716
gluconate tablets, 2717
hydroxide, 2718
hydroxide paste, 2719
nitrate, 6176
oxide, 2719, 6176
oxide, alumina, and magnesium carbonate tablets, 178
oxide, aspirin, and alumina tablets, 399
oxide capsules, 2721
oxide, chromatographic, 6152, 6176
oxide, citric acid, and sodium carbonate irrigation, 1044
oxide tablets, 2721
perchlorate, anhydrous, 6138, 6176
phosphate, 2722
salicylate, 2723
salicylate tablets, 2724
silicate, 5863
silicate, activated, 6133, 6176
silicate, chromatographic, 6176
stearate, 5864
sulfate, 2726, 6176
sulfate, anhydrous, 6138, 6176
sulfate in dextrose injection, 2727
sulfate injection, 2727
sulfate TS, 6231
trisilicate, 2728
trisilicate and alumina oral suspension, 179
trisilicate and alumina tablets, 180

www.webofpharma.com

Magnesium *(continued)*
trisilicate tablets, 2729
Magnesium carbonate, 6176
Malabar-nut-tree, leaf, 5136
powdered, 5137
extract, powdered, 5138
Malachite green
G, 6176
oxalate, 6221
TS, 6231
Malathion, 2729
lotion, 2730
Maleic acid, 5867, 6176
Malic acid, 5869
Mallory's stain, 6231
Malonic acid, 6176
Maltitol, 5869
solution, 5871
Maltodextrin, 5872
Maltol, 5875
Maltose, 5875
Maltotriose, 6176
Mandelic acid, 5876
Manganese, 6176
chloride, 2731
chloride injection, 2731
chloride for oral solution, 2732
dioxide, 6176
dioxide, activated, 6176, 6177
gluconate, 2732
sulfate, 2734
Manganese chloride tetrahydrate, 6177
Manganese sulfate, 6176
Mannitol, 2734
injection, 2736
in sodium chloride injection, 2737
D-Mannitol, 6177
Manufacturing practices for dietary supplements ⟨2750⟩, 8742
Maprotiline hydrochloride, 2737
tablets, 2739
Marbofloxacin compounded, veterinary
oral suspension, 2741
Marfey's reagent, 6177
Maritime pine, 5139
extract, 5141
Mass spectrometry ⟨736⟩, 6975
Mayer's reagent, 6231
Mazindol, 2742
Measurement of subvisible particulate matter in therapeutic protein injections ⟨1787⟩, 8562
Mebendazole, 2742
oral suspension, 2744
tablets, 2744
Mebrofenin, 2746
Mecamylamine hydrochloride, 2747
tablets, 2748
Mechlorethamine hydrochloride, 2749
for injection, 2749
Meclizine hydrochloride, 2750
tablets, 2751
Meclofenamate sodium, 2753
capsules, 2754
Medical air, 100
Medical devices—bacterial endotoxin and pyrogen tests ⟨161⟩, 6579
Medical gases assay ⟨415⟩, 6693
Medium-chain triglycerides, 6095, 6177
Medroxyprogesterone acetate, 2754
injectable suspension, 2755
tablets, 2756
Mefenamic acid, 2757
capsules, 2758
Mefloquine hydrochloride, 2760
tablets, 2761
Megestrol acetate, 2762
oral suspension, 2763
tablets, 2764
Meglumine, 2765
Melamine, 6177
Melatonin, 5143
tablets, 5144
Melengestrol acetate, 2766
Meloxicam, 2767
oral suspension, 2770
tablets, 2771
Melphalan, 2773
tablets, 2774
Melting range or temperature ⟨741⟩, 6980
Memantine hydrochloride, 2774
tablets, 2776
Members of the United States Pharmacopeial Convention, xviii
Menadiol sodium diphosphate, 2779
Menadione, 2780
Menaquinone-4, 5145
Menaquinone-7, 5146
capsules, 5147
extract, *Bacillus subtilis* subsp. *subtilis*, 5152
preparation, 5149
tablets, 5151
Menthol, 2781
and benzocaine topical aerosol, 518
lozenges, 2782
and tetracaine ointment, 4294
Meperidine hydrochloride, 2782
injection, 2783
oral solution, 2784
tablets, 2784
Mephobarbital, 2785
tablets, 2785
Mepivacaine hydrochloride, 2786
injection, 2788
and levonordefrin injection, 2789
Meprobamate, 2790
tablets, 2791
Meradimate, 2792
2-Mercaptoethanol, 6177
3-Mercaptopropionic acid, 6177
Mercaptopurine, 2793
tablets, 2794
Mercuric
acetate, 6177
acetate TS, 6231
ammonium thiocyanate TS, 6231
bromide, 6177
bromide test paper, 6222
bromide TS, alcoholic, 6226, 6231
chloride, 6177
chloride TS, 6231
iodide, red, 6177
iodide, TS, 6231
nitrate, 6177
nitrate, tenth-molar (0.1 M), 6244
nitrate TS, 6231
oxide, yellow, 6177, 6219
potassium iodide TS, 6231
potassium iodide TS, alkaline, 6226, 6231, 6232
sulfate, 6177
sulfate TS, 6228, 6231
thiocyanate, 6177
Mercurous nitrate
dihydrate, 6177
TS, 6231
Mercury, 6177
ammoniated, 2796
Mercury ⟨261⟩, 6650
Meropenem, 2796
for injection, 2798
Mesalamine, 2800
extended-release capsules, 2802
rectal suspension, 2803
delayed-release tablets, 2805
Mesityl oxide, 6177
Mesna, 2807
tablets, 2809
Mestranol, 2810
and ethynodiol diacetate tablets, 1781
and norethindrone tablets, 3203
Metacresol, 2811
Metanil
yellow, 6177
Metaphenylenediamine hydrochloride, 6177
TS, 6231
Metaphosphoric-acetic acid TS, 6231
Metaphosphoric acid, 6177
Metaproterenol sulfate, 2812
oral solution, 2813
tablets, 2814
Metaraminol bitartrate, 2815
injection, 2817
Metaxalone, 2817
tablets, 2819
Metformin hydrochloride, 2821
extended-release tablets, 2824
and glipizide tablets, 2116
and glyburide tablets, 2128
and pioglitazone tablets, 3558
tablets, 2822
Methacholine chloride, 2833
Methacrylic acid, 6177
and ethyl acrylate copolymer, 5878
and ethyl acrylate copolymer, partially-neutralized, 5881
and methyl methacrylate copolymer, 5880
Methacycline hydrochloride, 2835
oral suspension, 2835
Methadone hydrochloride, 2836
injection, 2837
oral concentrate, 2836
oral solution, 2838
tablets, 2838
tablets for oral suspension, 2839
Methamphetamine hydrochloride, 2840
tablets, 2840
Methanesulfonic acid, 6177
Methanol, 6134, 6138, 6177
aldehyde-free, 6177
anhydrous, 6177
deuterated, 6155
spectrophotometric, 6177
Methazolamide, 2841
tablets, 2842
Methenamine, 2843, 6170, 6177
hippurate, 2844
hippurate tablets, 2845
mandelate, 2845
mandelate tablets, 2846
tablets, 2843
Methimazole, 2847
tablets, 2848
Methionine, 2848
L-Methionine sulfoxide, 6177
Methocarbamol, 2850
injection, 2851
tablets, 2851
Methods for the determination of particulate matter in injections and ophthalmic solutions ⟨1788⟩, 8575
Methohexital, 2853
sodium for injection, 2853

www.webofpharma.com

Methotrexate, 2854
injection, 2857
for injection, 2858
tablets, 2860
Methotrimeprazine, 2860
injection, 2861
Methoxsalen, 2862
capsules, 2863
topical solution, 2865
5-Methoxy-1*H*-benzimidazole-2-thiol, 6178
Methoxyacetophenone *p*-, 6177
7-Methoxycoumarin, 6178
Methoxy determination ⟨431⟩, 6702
Methoxyethanol, 6178
2-Methoxyethanol, 6178
Methoxyflurane, 2866
5-Methoxy-2-methyl-3-indoleacetic acid, 6178
Methoxyphenylacetic acid, 6178
Methoxyphenylacetic TS, 6231
Methscopolamine bromide, 2866
tablets, 2868
Methsuximide, 2870
capsules, 2870
Methyclothiazide, 2871
tablets, 2872
Methyl
acetate, 6178
alcohol, 5882
4-aminobenzoate, 6178
arachidate, 6178
behenate, 6178
benzenesulfonate, 6178
caprate, 6178
caprylate, 6178
carbamate, 6178
chloroform, 6151, 6178, 6214
erucate, 6178
ethyl ketone, 6178
green, 6178
green–iodomercurate paper, 6222
heptadecanoate, 6178
iodide, 6179
isobutyl ketone, 5883, 6179
laurate, 6179
lignocerate, 6179
linoleate, 6179
linolenate, 6179
methacrylate, 6179
methacrylate and ethyl acrylate copolymer dispersion, 5760
myristate, 6179
oleate, 6179
orange, 6221
orange TS, 6231
palmitate, 6179
purple TS, 6231
red, 6179, 6221
red–methylene blue TS, 6231, 6232
red sodium, 6221
red TS, 6231
red TS 2, 6232
red TS, methanolic, 6232
salicylate, 5884
stearate, 6180
sulfoxide, 6180
violet TS, 6232
yellow, 6180, 6221
yellow–methylene blue TS, 6232
yellow paper, 6222
yellow TS, 6232
3-Methyl-2-benzothiazolinone hydrazone hydrochloride TS, 6232
Methylamine, 40 percent in water, 6180
Methylamine hydrochloride, 6180
p-Methylaminophenol sulfate, 6180
Methylbenzethonium chloride, 2873
lotion, 2873
4-Methylbenzophenone, 6180
Methylbenzothiazolone hydrazone hydrochloride, 6180
(*R*)-(+)-alpha-Methylbenzyl isocyanate, 6180
(*S*)-(–)-α-Methylbenzyl isocyanate, 6180
Methylcellulose, 2874
ophthalmic solution, 2876
oral solution, 2876
tablets, 2876
Methylcobalamin, 5153
tablets, 5154
Methyldopa, 2877
and hydrochlorothiazide tablets, 2878
tablets, 2878
Methyldopate hydrochloride, 2880
injection, 2880
Methyl cis-11-eicosenoate, 6178
Methylene
blue, 2881, 6180
blue injection, 2882
blue injection, veterinary, 2884
blue TS, 6232
chloride, 5885, 6157, 6180
5,5′-Methylenedisalicylic acid, 6180
Methylergonovine maleate, 2885
injection, 2886
tablets, 2887
3-*O*-Methylestrone, 6180
1-Methylimidazole, 6180
2-Methylimidazole, 6180
Methyl isobutyrate, 6179
Methyl 12-Ketostearate, 6179
Methyl methacrylate
and methacrylic acid copolymer, 5880
Methylnaltrexone bromide, 2888
2-Methyl-5-nitroimidazole, 6180
N-Methyl-*N*-nitroso-*p*-toluenesulfonamide, 6180
Methylparaben, 5886
sodium, 5887
4-Methylpentan-2-ol, 6180
2-Methylpentane, 6180
4-Methyl-2-pentanone, 6179, 6180
3-Methyl-2-pentanone, 6180
Methylphenidate hydrochloride, 2890
tablets, 2892
extended-release tablets, 2892
Methylprednisolone, 2898
acetate, 2899
acetate injectable suspension, 2901
acetate and neomycin sulfate cream, 3113
hemisuccinate, 2901
sodium succinate, 2902
sodium succinate for injection, 2903
tablets, 2899
2-Methyl-2-propyl-1,3-propanediol, 6180
Methyl *p*-toluenesulfonate, 6180
N-Methylpyrrolidine, 6181
Methylpyrrolidone, 5888
Methylsulfonylmethane, 5049
and glucosamine tablets, 5049
glucosamine, and chondroitin sulfate sodium tablets, 5051
tablets, 5156
Methyltestosterone, 2904
capsules, 2906
tablets, 2906
Methylthionine perchlorate TS, 6232
Methysergide maleate, 2907
Metoclopramide
hydrochloride, 2908
injection, 2909
oral solution, 2910
tablets, 2911
Metolazone, 2912
oral suspension, 2913
tablets, 2914
Metoprolol
fumarate, 2915
succinate, 2917
succinate extended-release tablets, 2918
tartrate, 2922
tartrate and hydrochlorothiazide tablets, 2928
tartrate injection, 2923
tartrate oral solution, 2925
tartrate oral suspension, 2926
tartrate tablets, 2926
Metrifonate, 2930
Metronidazole, 2931
benzoate, 2932
capsules, 2934
gel, 2935
injection, 2936
tablets, 2937
extended-release tablets, 2939
Metronidazole benzoate compounded oral suspension, 2933
Metyrapone, 2941
Metyrosine, 2942
capsules, 2942
Mexiletine hydrochloride, 2943
capsules, 2944
Mibolerone, 2944
oral solution, 2945
Miconazole, 2946
compounded ophthalmic solution, 2946
nitrate, 2947
nitrate cream, 2949
nitrate topical powder, 2949
nitrate vaginal suppositories, 2951
Microbial characterization, identification, and strain typing ⟨1113⟩, 7823
Microbial enumeration tests—nutritional and dietary supplements ⟨2021⟩, 8687
Microbiological attributes of nonsterile nutritional and dietary supplements ⟨2023⟩, 8698
Microbiological best laboratory practices ⟨1117⟩, 7845
Microbiological control and monitoring of aseptic processing environments ⟨1116⟩, 7833
Microbiological examination of nonsterile products: acceptance criteria for pharmaceutical preparations and substances for pharmaceutical use ⟨1111⟩, 7819
Microbiological examination of nonsterile products: microbial enumeration tests ⟨61⟩, 6457
Microbiological examination of nonsterile products: tests for specified microorganisms ⟨62⟩, 6463
Microbiological examination of nonsterile products tests for Burkholderia Cepacia complex ⟨60⟩, 6454
Microbiological procedures for absence of specified microorganisms—nutritional and dietary supplements ⟨2022⟩, 8692
Microscopy, optical ⟨776⟩, 6998
Midazolam, 2952
injection, 2953
Mid-infrared spectroscopy ⟨854⟩, 7151
Mid-infrared spectroscopy—theory and practice ⟨1854⟩, 8650

www.webofpharma.com

Midodrine hydrochloride, 2954
  tablets, 2955
Milbemycin oxime, 2957
Milk thistle, 5157
  capsules, 5162
  extract, powdered, 5160
  powdered, 5159
  tablets, 5163
Millon's reagent, 6232
Milrinone, 2958
Milrinone lactate
  injection, 2959
Mineral
  acid, 6181
  oil, 2961
  oil emulsion, 2962
  oil, light, 5889
  oil, rectal, 2962
  oil, topical light, 2962
Minerals
  with calcium and vitamin D tablets, 4845
  capsules, 5164
  oil- and water-soluble vitamins with, capsules, 5435
  oil- and water-soluble vitamins with, oral solution, 5462
  oil- and water-soluble vitamins with, tablets, 5476
  tablets, 5172
  water-soluble vitamins with, capsules, 5523
  water-soluble vitamins with, oral solution, 5543
  water-soluble vitamins with, tablets, 5552
Minimum fill ⟨755⟩, 6982
Minocycline
  hydrochloride, 2964
  hydrochloride capsules, 2965
  periodontal system, 2970
  hydrochloride oral suspension, 2966
  hydrochloride tablets, 2966
  hydrochloride extended-release tablets, 2967
  for injection, 2963
Minoxidil, 2972
  topical solution, 2975
  tablets, 2974
Mirtazapine, 2976
  tablets, 2978
  orally disintegrating tablets, 2980
Mirtazapine compounded, veterinary
  oral suspension, 2977
Misoprostol, 2981
  dispersion, 2983
Mission
  and preface, v
  statement, v
Mitomycin, 2984
  for injection, 2986
Mitotane, 2986
  tablets, 2987
Mitoxantrone
  hydrochloride, 2987
  injection, 2988
Modafinil, 2990
  tablets, 2991
Moexipril hydrochloride, 2992
Moexipril hydrochloride
  and hydrochlorothiazide tablets, 2996
  tablets, 2994
Moist heat sterilization of aqueous liquids ⟨1229.2⟩, 8202
Molindone hydrochloride, 2999
  tablets, 3000
Molybdenum, 6181
Molybdic acid, 6181
Molybdo-phosphotungstate TS, 6232
Mometasone furoate, 3001
  cream, 3002
  ointment, 3003
  topical solution, 3005
Monensin, 3006
  granulated, 3007
  premix, 3008
  sodium, 3009
Monitoring devices—time, temperature, and humidity ⟨1118⟩, 7850
Monitoring of bioburden ⟨1229.3⟩, 8206
Monobasic
  potassium phosphate, 5979, 6181
  sodium phosphate, 4088, 6181
0.1 M Monobasic sodium phosphate TS, 6232
0.05 M Monobasic sodium phosphate TS, 6232
Monobenzone, 3009
Monochloroacetic acid, 6181
Mono- and di-glycerides, 5890
Monoethanolamine, 5891, 6181
Monoglyceride citrate, 5891
Monograph and reference material donors 2014 recognition, xxv
Monosaccharide Analysis, 6616
Monosodium glutamate, 5892, 6181
Monothioglycerol, 5892
Montelukast
  sodium oral granules, 3012
  sodium tablets, 3015
  sodium chewable tablets, 3018
Montelukast sodium, 3010
Montelukast sodium hydrate, 6181
Morantel tartrate, 3021
Moricizine hydrochloride, 3021
Morin, 6181
Morphine sulfate, 3023
  extended-release capsules, 3024
  injection, 3027
  suppositories, 3028
Morphine sulfate
  compounded injection, 3028
Morpholine, 6181
4-Morpholine propane sulfonic acid, 6181
Moxidectin, 3029
Moxifloxacin
  hydrochloride, 3032
  ophthalmic solution, 3034
  tablets, 3036
Mucosal drug products—performance tests ⟨1004⟩, 7227
Mucosal drug products—product quality tests ⟨4⟩, 6423
Mupirocin, 3038
  calcium, 3038
  cream, 3040
  ointment, 3041
  nasal ointment, 3042
Mycophenolate
  sodium, 3052
Mycophenolate mofetil, 3043
  capsules, 3044
  for injection, 3046
  for oral suspension, 3047
  tablets, 3049
Mycophenolic acid
  delayed-release tablets, 3054
Mycoplasma tests ⟨63⟩, 6472
Myristic acid, 5893
Myristyl alcohol, 5895
Myristyltrimethylammonium bromide, 6181
Myrrh, 3056
  topical solution, 3057

N

N 13 injection, ammonia, 3183
Nabumetone, 3058
  tablets, 3059
Nadolol, 3059
  and bendroflumethiazide tablets, 3062
  tablets, 3061
Nafcillin
  injection, 3063
  for injection, 3063
  sodium, 3064
Naftifine hydrochloride, 3064
  cream, 3065
  gel, 3066
Nalorphine hydrochloride, 3066
  injection, 3067
Naloxone
  hydrochloride, 3067
  hydrochloride injection, 3068
  and pentazocine tablets, 3463
Naltrexone hydrochloride, 3069
  tablets, 3071
Nandrolone
  decanoate, 3072
  decanoate injection, 3073
Naphazoline hydrochloride, 3074
  nasal solution, 3075
  ophthalmic solution, 3076
  and pheniramine maleate ophthalmic solution, 3076
Naphthalene, 6181
1,3-Naphthalenediol, 6181
2,7-Naphthalenediol, 6159, 6181
2-Naphthalenesulfonic acid, 6181
Naphthol
  dipotassium disulfonate, 6181
  disodium disulfonate, 6181
1-Naphthol, 6134, 6181
  reagent, 6232
  TS, 6232
2-Naphthol, 6142, 6181
  TS, 6227, 6232
p-Naphtholbenzein, 6182, 6221
  TS, 6232
β-Naphthoquinone-4-sodium sulfonate, 6182
Naphthoresorcinol, 6182
1-Naphthylamine, 6182
1-Naphthylamine hydrochloride, 6182
2-Naphthyl chloroformate, 6182
N-(1-Naphthyl)ethylenediamine
  dihydrochloride, 6182
  TS, 6232
Naproxen, 3077
  sodium, 3082
  sodium tablets, 3082
  oral suspension, 3079
  tablets, 3079
  delayed-release tablets, 3081
Naproxen compounded
  oral suspension, 3078
Naproxen sodium and pseudoephedrine hydrochloride extended-release tablets, 3084
Narasin
  granular, 3088
  premix, 3090
Naratriptan
  hydrochloride, 3093
  hydrochloride oral suspension, 3094
  tablets, 3091

www.webofpharma.com

Nasal solution

Calcitonin salmon, 675
Cromolyn sodium, 1184
Ephedrine sulfate, 1644
Epinephrine, 1649
Flunisolide, 1929
Naphazoline hydrochloride, 3075
Oxymetazoline hydrochloride, 3349
Phenylephrine hydrochloride, 3518
Tetrahydrozoline hydrochloride, 4315
Xylometazoline hydrochloride, 4661

Nasal spray

Butorphanol tartrate, 657
Desmopressin acetate, 1267
Fluticasone propionate, 2000
Natamycin, 3095
  ophthalmic suspension, 3096
Nateglinide, 3096
  tablets, 3098
Near-infrared spectroscopy ⟨856, 7161
Nefazodone hydrochloride, 3099
  tablets, 3100
Neohesperidin dihydrochalcone, 5896
Neomycin
  boluses, 3102
  and colistin sulfates and hydrocortisone acetate otic suspension, 1150
  for injection, 3102
  penicillin G, polymyxin B, hydrocortisone acetate, and hydrocortisone sodium succinate topical suspension, 3430
  and polymyxin B sulfates, bacitracin, and hydrocortisone acetate ointment, 3116
  and polymyxin B sulfates, bacitracin, and hydrocortisone acetate ophthalmic ointment, 3116
  and polymyxin B sulfates, bacitracin, and lidocaine ointment, 3117
  and polymyxin B sulfates and bacitracin ointment, 3115
  and polymyxin B sulfates and bacitracin ophthalmic ointment, 3115
  and polymyxin B sulfates, bacitracin zinc, and hydrocortisone ointment, 3118
  and polymyxin B sulfates, bacitracin zinc, and hydrocortisone ophthalmic ointment, 3119
  and polymyxin B sulfates, bacitracin zinc, and hydrocortisone acetate ophthalmic ointment, 3120
  and polymyxin B sulfates, bacitracin zinc, and lidocaine ointment, 3120
  and polymyxin B sulfates and bacitracin zinc ointment, 3118
  and polymyxin B sulfates and bacitracin zinc ophthalmic ointment, 3118
  and polymyxin B sulfates cream, 3114
  and polymyxin B sulfates and dexamethasone ophthalmic ointment, 3121
  and polymyxin B sulfates and dexamethasone ophthalmic suspension, 3122
  and polymyxin B sulfates and gramicidin cream, 3122
  and polymyxin B sulfates, gramicidin, and hydrocortisone acetate cream, 3123
  and polymyxin B sulfates and gramicidin ophthalmic solution, 3123
  and polymyxin B sulfates and hydrocortisone ophthalmic suspension, 3123
  and polymyxin B sulfates and hydrocortisone otic solution, 3124
  and polymyxin B sulfates and hydrocortisone otic suspension, 3124
  and polymyxin B sulfates and hydrocortisone acetate cream, 3125
  and polymyxin B sulfates and hydrocortisone acetate ophthalmic suspension, 3125
  and polymyxin B sulfates and lidocaine cream, 3126
  and polymyxin B sulfates ophthalmic ointment, 3114
  and polymyxin B sulfates ophthalmic solution, 3115
  and polymyxin B sulfates, penicillin G procaine, and hydrocortisone acetate topical suspension, 3448
  and polymyxin B sulfates and pramoxine hydrochloride cream, 3126
  and polymyxin B sulfates and prednisolone acetate ophthalmic suspension, 3127
  and polymyxin B sulfates solution for irrigation, 3114
  sulfate, 3102
  sulfate and bacitracin ointment, 3104
  sulfate and bacitracin zinc ointment, 3104
  sulfate cream, 3103
  sulfate and dexamethasone sodium phosphate cream, 3104
  sulfate and dexamethasone sodium phosphate ophthalmic ointment, 3105
  sulfate and dexamethasone sodium phosphate ophthalmic solution, 3106
  sulfate and fluocinolone acetonide cream, 3107
  sulfate and fluorometholone ointment, 3107
  sulfate and flurandrenolide cream, 3107
  sulfate and flurandrenolide lotion, 3108
  sulfate and flurandrenolide ointment, 3108
  sulfate and gramicidin ointment, 3108
  sulfate and hydrocortisone cream, 3109
  sulfate and hydrocortisone ointment, 3109
  sulfate and hydrocortisone otic suspension, 3109
  sulfate and hydrocortisone acetate cream, 3110
  sulfate and hydrocortisone acetate lotion, 3110
  sulfate and hydrocortisone acetate ointment, 3110
  sulfate and hydrocortisone acetate ophthalmic suspension, 3110
  sulfate, isoflupredone acetate, and tetracaine hydrochloride ointment, 3111
  sulfate, isoflupredone acetate, and tetracaine hydrochloride topical powder, 3112
  sulfate and methylprednisolone acetate cream, 3113
  sulfate, nystatin, gramicidin, and triamcinolone acetonide cream, 3222
  sulfate, nystatin, gramicidin, and triamcinolone acetonide ointment, 3222
  sulfate, nystatin, thiostrepton, and triamcinolone acetonide cream, 3223
  sulfate, nystatin, thiostrepton, and triamcinolone acetonide ointment, 3223
  sulfate ointment, 3103
  sulfate ophthalmic ointment, 3103
  sulfate and prednisolone acetate ophthalmic suspension, 3128
  sulfate oral solution, 3103
  sulfate tablets, 3104
  sulfate and triamcinolone acetonide cream, 3129
Neostigmine
  bromide, 3129
  bromide tablets, 3129
  methylsulfate, 3130
  methylsulfate injection, 3130
Neotame, 5898
Nephelometry, turbidimetry, and visual comparison ⟨855⟩, 7155
Nessler's reagent, 6232
Neutralized
  alcohol, 6182
  phthalate buffer, 6145
Neutral red, 6221
  TS, 6232
Nevirapine, 3131
Nevirapine
  extended release tablets, 3135
  oral suspension, 3132
  tablets, 3134
New sterilization methods ⟨1229.12⟩, 8233
Niacin, 3138
  extended-release tablets, 3141
  injection, 3140
  or niacinamide assay ⟨441⟩, 6704
  tablets, 3140
Niacinamide, 3146
  injection, 3147
  or niacin assay ⟨441⟩, 6704
  tablets, 3147
Nicardipine hydrochloride, 3148
  injection, 3149
Nickel-aluminum catalyst, 6182
Nickel, 6182
  standard solution TS, 6232
  sulfate, 6182
  (II) sulfate heptahydrate, 6182
Nickel nitrate hexahydrate, 6182
β-Nicotinamide adenine dinucleotide, 6182
Nicotinamide adenine dinucleotide phosphate-adenosine-5′-triphosphate mixture, 6182
Nicotine, 3152
  polacrilex, 3156
  polacrilex gum, 3159
  transdermal system, 3153
Nicotinic acid, 6182
Nifedipine, 3160
  capsules, 3161
  extended-release tablets, 3163
Nile blue hydrochloride, 6221
Nilutamide, 3170
Nimodipine, 3171
Ninhydrin, 6182
  TS, 6232
Nitrate
  mercurous, dihydrate, 6177
  mercurous, TS, 6231
  ophthalmic solution, silver, 4041
  in reagents, 6131
  silver, 4041, 6200
  silver, TS, 6234
  tenth-normal (0.1 N), silver, 6234, 6247
  toughened silver, 4042
Nitric
  acid, 5900, 6182
  acid, diluted, 6159, 6182
  acid, fuming, 6168, 6182
  acid, lead-free, 6182

www.webofpharma.com

Nitric *(continued)*
oxide–nitrogen dioxide detector tube, 6182
Nitric acid
0.01 N TS, 6232
0.2 N TS, 6232
1 N TS, 6232
2 N TS, 6232
ultratrace, 6182
Nitrilotriacetic acid, 6182
Nitrite titration ⟨451⟩, 6709
4′-Nitroacetophenone, 6182
o-Nitroaniline, 6183
p-Nitroaniline, 6183
TS, 6232
Nitrobenzene, 6183
p-Nitrobenzenediazonium tetrafluoroborate, 6183
4-Nitrobenzoic acid, 6183
p-Nitrobenzyl bromide, 6183
4-(*p*-Nitrobenzyl) pyridine, 6183
Nitrofurantoin, 3172
capsules, 3174
oral suspension, 3179
tablets, 3180
Nitrofurazone, 3181
ointment, 3182
topical solution, 3183
Nitrogen, 5900
97 percent, 5901
certified standard, 6183
compounds in reagents, 6131
determination ⟨461⟩, 6709
N 13 injection, ammonia, 3183
Nitroglycerin
diluted, 3186
injection, 3187
ointment, 3187
sublingual tablets, 3188
Nitromersol, 3189
topical solution, 3190
Nitromethane, 6183
5-Nitro-1,10-phenanthroline, 6182
Nitrophenanthroline TS, 6232
1-Nitroso-2-naphthol, 6183
Nitroso R salt, 6183
Nitrous
oxide, 3190
oxide certified standard, 6183
Nizatidine, 3191
capsules, 3192
Nomenclature ⟨1121⟩, 7862
Nonadecane, 6183
Nonanoic acid, 6183
Nonionic wetting agent, 6183, 6184
Nonoxynol 9, 3193, 6184
1-Nonyl alcohol, 6184
n-Nonylamine, 6184
Nonylphenol polyoxyethylene ether, 6184
Nonylphenoxypoly(ethyleneoxy)ethanol, 6184
Norelgestromin, 3196
Norepinephrine bitartrate, 3197
injection, 3198
and propoxycaine and procaine hydrochlorides injection, 3745
Norethindrone, 3198
acetate, 3204
acetate and estradiol tablets, 1733
acetate and ethinyl estradiol tablets, 3208
acetate tablets, 3205
and ethinyl estradiol tablets, 3201
and mestranol tablets, 3203
tablets, 3199
Norgestimate, 3209
and ethinyl estradiol tablets, 3211
Norgestrel, 3212
and ethinyl estradiol tablets, 3214
tablets, 3213
Normal
butyl acetate, 6145
butyl alcohol, 6145
butylamine, 6184
Northern schisandra fruit, 5261
dry extract, 5263
powder, 5265
Nortriptyline hydrochloride, 3214
capsules, 3216
oral solution, 3216
Noscapine, 3217
Novobiocin
sodium, 3218
sodium intramammary infusion, 3218
sodium and penicillin G procaine intramammary infusion, 3448
sodium, tetracycline hydrochloride, and prednisolone tablets, 4312
sodium and tetracycline hydrochloride tablets, 4312
Nuclear magnetic resonance spectroscopy ⟨761⟩, 6984
Nuclear magnetic resonance spectroscopy identity testing of bacterial polysaccharides used in vaccine manufacture ⟨198⟩, 6596
Nucleic acid-based techniques
amplification ⟨1127⟩, 7879
approaches for detecting trace nucleic acids (residual DNA testing) ⟨1130⟩, 7897
extraction, detection, and sequencing ⟨1126⟩, 7870
general ⟨1125⟩, 7865
genotyping ⟨1129⟩, 7893
microarray ⟨1128⟩, 7887
Nystatin, 3218
cream, 3219
lotion, 3220
lozenges, 3220
neomycin sulfate, gramicidin, and triamcinolone acetonide cream, 3222
neomycin sulfate, gramicidin, and triamcinolone acetonide ointment, 3222
neomycin sulfate, thiostrepton, and triamcinolone acetonide cream, 3223
neomycin sulfate, thiostrepton, and triamcinolone acetonide ointment, 3223
ointment, 3220
topical powder, 3220
oral suspension, 3221
for oral suspension, 3221
tablets, 3221
and tetracycline hydrochloride capsules, 4313
and triamcinolone acetonide cream, 3224
and triamcinolone acetonide ointment, 3225
vaginal inserts, 3222
vaginal suppositories, 3220

O

n-Octadecane, 6184
Octadecyl silane, 6184
Octane-*n*, 6184
Octanesulfonic acid sodium salt, 6204
Octanesulfonic acid sodium salt monohydrate, 6184
1-Octanol, 6184
Octanophenone, 6184
Octinoxate, 3225
Octisalate, 3226
Octocrylene, 3227
Octoxynol 9, 5901, 6184
Octreotide acetate, 3228
Octylamine, 6184
Octyldodecanol, 5904
(*p-tert*-Octylphenoxy)nonaethoxyethanol, 6163, 6184
(*p-tert*-Octylphenoxy)polyethoxyethanol, 6184
Octyl sulfate, sodium salt, 6184
Officers (2015–2020), ix
Ofloxacin, 3230
ophthalmic solution, 3231
tablets, 3232

Oil

Almond, 5609
Anise, 5624
Borage seed, 4830
Borage seed, capsules, 4831
Canola, 5664
Caraway, 5667
Cardamom, 5688
Castor, 802
Castor, aromatic, 807
Castor, capsules, 805
Castor, emulsion, 806
Castor, hydrogenated, 5691
Cedar, 6149
Chia seed, 4877
Clove, 5718
Coconut, 5719
Coconut, hydrogenated, 5720
Cod liver, 1135
Cod liver, capsules, 4910
Coriander, 5723
Corn, 5723
Cottonseed, 5733
Cottonseed, hydrogenated, 5734
Crypthecodinium cohnii, 4922
Crypthecodinium cohnii, capsules, 4925
Ethiodized injection, 1770
Evening primrose, 4980
Evening primrose, capsules, 4980
Fats and fixed oils ⟨401⟩, 6676
Fennel, 5776
Flax seed, 4998
Flax seed, capsules, 4999
Krill, capsules, 5104
Krill delayed-release capsules, 5107
Lemon, 5856
Mineral, 2961
Mineral emulsion, 2962
Mineral, light, 5889
Mineral, rectal, 2962
Mineral, topical light, 2962
Olive, 5910
Orange, 5914
Palm, 5916
Palm, hydrogenated, 5916
Palm kernel, 5917
Peanut, 5921
Peppermint, 5923
Polyoxyl 35 castor, 5956
Polyoxyl 40 hydrogenated castor, 5958
Fully hydrogenated rapeseed, 5998

www.webofpharma.com

Oil *(continued)*
Superglycerinated fully hydrogenated rapeseed, 5999
Rose, 6001
Safflower, 3968
Schizochytrium, 5266
Schizochytrium, capsules, 5269
Sesame, 6003
Soybean, 4105
Soybean, hydrogenated, 6040
Star anise, 6043
Sunflower, 6085
Vegetable, hydrogenated, 6099
Vitamins capsules, oil- and water-soluble, 5389
Vitamins capsules, oil-soluble, 5346
Vitamins with minerals capsules, oil- and water-soluble, 5435
Vitamins with minerals oral solution, oil- and water-soluble, 5462
Vitamins with minerals tablets, oil- and water-soluble, 5476
Vitamins oral solution, oil- and water-soluble, 5409
Vitamins tablets, oil- and water-soluble, 5419
Vitamins tablets, oil-soluble, 5356

Oil-soluble vitamins
capsules, 5346
tablets, 5356
Oil- and water-soluble vitamins
capsules, 5389
with minerals capsules, 5435
with minerals oral solution, 5462
with minerals tablets, 5476
oral solution, 5409
tablets, 5419

Ointment

Acyclovir, 91
Alclometasone dipropionate, 112
Amcinonide, 215
Amphotericin B, 316
Anthralin, 344
Atropine sulfate ophthalmic, 431
Bacitracin ophthalmic, 467
Bacitracin zinc, 470
Bacitracin zinc and polymyxin B sulfate, 471
Bacitracin zinc and polymyxin B sulfate ophthalmic, 471
Benzocaine, 509
Benzocaine, butamben, and tetracaine hydrochloride, 516
Benzoic and salicylic acids, 521
Betamethasone dipropionate, 548
Betamethasone valerate, 556
Bland lubricating ophthalmic, 3273
Calcipotriene, 668
Chloramphenicol and polymyxin B sulfate ophthalmic, 933
Chloramphenicol ophthalmic, 929
Chlortetracycline hydrochloride, 980
Chlortetracycline hydrochloride ophthalmic, 987
Ciprofloxacin ophthalmic, 1014
Clioquinol, 1076
Clioquinol and hydrocortisone, 1078
Clobetasol propionate, 1081
Coal tar, 1133
Desoximetasone, 1275
Dibucaine, 1337
Diflorasone diacetate, 1375
Erythromycin, 1688
Erythromycin ophthalmic, 1689
Fluocinolone acetonide, 1935
Fluocinonide, 1938
Flurandrenolide, 1976
Fluticasone propionate, 2003
Gentamicin and prednisolone acetate ophthalmic, 2106
Gentamicin sulfate, 2101
Gentamicin sulfate and betamethasone valerate, 2103
Gentamicin sulfate ophthalmic, 2102
Halcinonide, 2185
Hydrocortisone, 2232
Hydrocortisone acetate, 2238
Hydrocortisone valerate, 2246
Hydrophilic, 3233
Ichthammol, 2289
Idoxuridine ophthalmic, 2293
Lidocaine, 2622
Mometasone furoate, 3003
Mupirocin, 3041
Mupirocin nasal, 3042
Neomycin and polymyxin B sulfates and bacitracin, 3115
Neomycin and polymyxin B sulfates, bacitracin, and hydrocortisone acetate, 3116
Neomycin and polymyxin B sulfates, bacitracin, and hydrocortisone acetate ophthalmic, 3116
Neomycin and polymyxin B sulfates, bacitracin, and lidocaine, 3117
Neomycin and polymyxin B sulfates and bacitracin ophthalmic, 3115
Neomycin and polymyxin B sulfates and bacitracin zinc, 3118
Neomycin and polymyxin B sulfates, bacitracin zinc, and hydrocortisone, 3118
Neomycin and polymyxin B sulfates, bacitracin zinc, and hydrocortisone acetate ophthalmic, 3120
Neomycin and polymyxin B sulfates, bacitracin zinc, and hydrocortisone ophthalmic, 3119
Neomycin and polymyxin B sulfates, bacitracin zinc, and lidocaine, 3120
Neomycin and polymyxin B sulfates and bacitracin zinc ophthalmic, 3118
Neomycin and polymyxin B sulfates and dexamethasone ophthalmic, 3121
Neomycin and polymyxin B sulfates ophthalmic, 3114
Neomycin sulfate, 3103
Neomycin sulfate and bacitracin, 3104
Neomycin sulfate and bacitracin zinc, 3104
Neomycin sulfate and dexamethasone sodium phosphate ophthalmic, 3105
Neomycin sulfate and fluorometholone, 3107
Neomycin sulfate and flurandrenolide, 3108
Neomycin sulfate and gramicidin, 3108
Neomycin sulfate and hydrocortisone, 3109
Neomycin sulfate and hydrocortisone acetate, 3110
Neomycin sulfate, isoflupredone acetate, and tetracaine hydrochloride, 3111
Neomycin sulfate ophthalmic, 3103
Nitrofurazone, 3182
Nitroglycerin, 3187
Nystatin, 3220
Nystatin, neomycin sulfate, gramicidin, and triamcinolone acetonide, 3222
Nystatin, neomycin sulfate, thiostrepton, and triamcinolone acetonide, 3223
Nystatin and triamcinolone acetonide, 3225
Oxytetracycline hydrochloride and hydrocortisone, 3364
Oxytetracycline hydrochloride and polymyxin B sulfate, 3364
Oxytetracycline hydrochloride and polymyxin B sulfate ophthalmic, 3365
Polyethylene glycol, 5941
Povidone–iodine, 3635
Prednicarbate, 3658
Resorcinol ointment, compound, 3861
Rose water, 3946
Sodium chloride ophthalmic, 4068
Sulfacetamide sodium ophthalmic, 4142
Sulfacetamide sodium and prednisolone acetate ophthalmic, 4144
Sulfur, 4176
Tetracaine, 4294
Tetracaine and menthol, 4294
Tetracycline hydrochloride, 4307
Tetracycline hydrochloride ophthalmic, 4308
Tobramycin and dexamethasone ophthalmic, 4409
Tobramycin ophthalmic, 4405
Triamcinolone acetonide, 4485
Undecylenic acid, compound, 4554
White, 3233
Yellow, 3233
Zinc oxide, 4689

Olanzapine, 3234
and fluoxetine capsules, 3237
tablets, 3235
Olanzapine orally disintegrating tablets, 3240
Olefin detector tube, 6184
Oleic acid, 5906
Oleoresin, capsicum, 730
Oleovitamin A and D, 3242
capsules, 3242
Oleoyl polyoxylglycerides, 5908
Oleyl
alcohol, 5909
oleate, 5910
Oligo-deoxythymidine, 6184
Oligosaccharide analysis ⟨212⟩, 6623
Olive leaf, 5181
dry extract, 5182
powder, 5184
Olive oil, 5910
Olmesartan medoxomil, 3242
and amlodipine tablets, 272
tablets, 3244
Olopatadine hydrochloride ophthalmic solution, 3249
Omega-3
acids triglycerides, 5185
ethyl esters capsules, 3253
ethyl esters, 3250
Omega-3 free fatty acids, 5188
Omeprazole, 3256
delayed-release capsules, 3258
magnesium, 3260

www.webofpharma.com

Ondansetron, 3262
compounded topical gel, 3272
hydrochloride, 3263
hydrochloride oral suspension, 3268
injection, 3265
oral solution, 3266
tablets, 3268
orally disintegrating tablets, 3271

Ophthalmic ointment

Atropine sulfate, 431
Bacitracin, 467
Bacitracin zinc and polymyxin B sulfate, 471
Bland lubricating, 3273
Chloramphenicol, 929
Chloramphenicol and polymyxin B sulfate, 933
Chlortetracycline hydrochloride, 987
Ciprofloxacin, 1014
Erythromycin, 1689
Gentamicin and prednisolone acetate, 2106
Gentamicin sulfate, 2102
Idoxuridine, 2293
Neomycin and polymyxin B sulfates, 3114
Neomycin and polymyxin B sulfates and bacitracin, 3115
Neomycin and polymyxin B sulfates, bacitracin, and hydrocortisone acetate, 3116
Neomycin and polymyxin B sulfates and bacitracin zinc, 3118
Neomycin and polymyxin B sulfates, bacitracin zinc, and hydrocortisone, 3119
Neomycin and polymyxin B sulfates, bacitracin zinc, and hydrocortisone acetate, 3120
Neomycin and polymyxin B sulfates and dexamethasone, 3121
Neomycin sulfate, 3103
Neomycin sulfate and dexamethasone sodium phosphate, 3105
Oxytetracycline hydrochloride and polymyxin B sulfate, 3365
Sodium chloride, 4068
Sulfacetamide sodium, 4142
Sulfacetamide sodium and prednisolone acetate, 4144
Tetracycline hydrochloride, 4308
Tobramycin, 4405
Tobramycin and dexamethasone, 4409

Ophthalmic products—performance tests ⟨1771⟩, 8549
Ophthalmic products—quality tests ⟨771⟩, 6993

Ophthalmic solution

Acetylcholine chloride for, 81
Apraclonidine, 359
Atropine sulfate, 432
Azelastine hydrochloride, 445
Benoxinate hydrochloride, 499
Betaxolol, 558
Carbachol, 739
Carteolol hydrochloride, 789
Chloramphenicol, 929
Chloramphenicol for, 930
Chymotrypsin for, 994
Ciprofloxacin, 1015
Cromolyn sodium, 1185
Cyclopentolate hydrochloride, 1201
Cyclosporine compounded, veterinary, 1217
Demecarium bromide, 1247
Dexamethasone sodium phosphate, 1296
Dipivefrin hydrochloride, 1442
Dorzolamide hydrochloride, 1497
Dorzolamide hydrochloride and timolol maleate, 1499
Echothiophate iodide for, 1579
Emedastine, 1610
Epinephrine, 1649
Epinephrine bitartrate, 1651
Epinephrine bitartrate for, 1652
Epinephryl borate, 1652
Fluorescein sodium and benoxinate hydrochloride, 1944
Fluorescein sodium and proparacaine hydrochloride, 1945
Flurbiprofen sodium, 1982
Gentamicin sulfate, 2102
Gentamicin sulfate and betamethasone acetate, 2102
Glycerin, 2133
Homatropine hydrobromide, 2208
Hydroxyamphetamine hydrobromide, 2258
Hypromellose, 2281
Idoxuridine, 2293
Levobunolol hydrochloride, 2595
Methylcellulose, 2876
Miconazole compounded, 2946
Moxifloxacin, 3034
Naphazoline hydrochloride, 3076
Naphazoline hydrochloride and pheniramine maleate, 3076
Neomycin and polymyxin B sulfates, 3115
Neomycin and polymyxin B sulfates and gramicidin, 3123
Neomycin sulfate and dexamethasone sodium phosphate, 3106
Ofloxacin, 3231
Olopatadine hydrochloride, 3249
Oxymetazoline hydrochloride, 3350
Phenylephrine hydrochloride, 3519
Physostigmine salicylate, 3536
Pilocarpine hydrochloride, 3543
Pilocarpine nitrate, 3545
Polymyxin B sulfate and trimethoprim, 3592
Prednisolone sodium phosphate, 3668
Proparacaine hydrochloride, 3739
Scopolamine hydrobromide, 4013
Silver nitrate, 4041
Sodium chloride, 4068
Sulfacetamide sodium, 4143
Tetracaine hydrochloride, 4299
Tetrahydrozoline hydrochloride, 4315
Timolol maleate, 4389
Tobramycin, 4408
Travoprost, 4471
Tropicamide, 4541
Voriconazole compounded, veterinary, 4645
Zinc sulfate, 4693

Ophthalmic suspension

Brinzolamide, 594
Chloramphenicol and hydrocortisone acetate for, 932
Dexamethasone, 1287
Fluorometholone, 1952
Gentamicin and prednisolone acetate, 2107
Natamycin, 3096
Neomycin and polymyxin B sulfates and dexamethasone, 3122
Neomycin and polymyxin B sulfates and hydrocortisone, 3123
Neomycin and polymyxin B sulfates and hydrocortisone acetate, 3125
Neomycin and polymyxin B sulfates and prednisolone acetate, 3127
Neomycin sulfate and hydrocortisone acetate, 3110
Neomycin sulfate and prednisolone acetate, 3128
Oxytetracycline hydrochloride and hydrocortisone acetate, 3363
Prednisolone acetate, 3663
Rimexolone, 3887
Sulfacetamide sodium and prednisolone acetate, 4145
Tetracycline hydrochloride, 4310
Tobramycin and dexamethasone, 4411
Tobramycin and fluorometholone acetate, 4412

Opium, 3273
powdered, 3274
tincture, 3274
Optical
microscopy ⟨776⟩, 6998
rotation ⟨781⟩, 7001
Oral drug products—product quality tests ⟨2⟩, 6410
Orally inhaled and nasal drug products ⟨1664.1⟩, 8467

Oral powder

Containing at least three of the following—acetaminophen and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, 54
Levothyroxine sodium, 2618
Sodium bicarbonate, 4061

Oral solution

Abacavir, 19
Acacia syrup, 5597
Acetaminophen, 41
Containing at least three of the following—acetaminophen and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, 56
Acetaminophen and codeine phosphate, 54

www.webofpharma.com

Oral solution *(continued)*
Acetaminophen, dextromethorphan hydrobromide, doxylamine succinate, and pseudoephedrine hydrochloride, 67
Acetaminophen for effervescent, 42
Amantadine hydrochloride, 212
Aminocaproic acid, 236
Aminophylline, 245
Amprolium, 331
Aromatic elixir, 5624
Ascorbic acid, 384
Ascorbic acid compounded, 385
Aspirin effervescent tablets for, 395
Atenolol, 407
Beclomethasone dipropionate compounded, 484
Benzaldehyde elixir, compound, 5634
Betamethasone, 542
Bethanechol chloride, 563
Bromodiphenhydramine hydrochloride, 599
Bromodiphenhydramine hydrochloride and codeine phosphate, 600
Brompheniramine maleate, 602
Brompheniramine maleate and pseudoephedrine sulfate, 603
Butabarbital sodium, 642
Caffeine citrate, 663
Calcium glubionate syrup, 695
Captopril, 734
C 13 for, urea, 767
Cetirizine hydrochloride, 912
Cherry syrup, 5709
Chloral hydrate, 924
Chloramphenicol, 930
Chlorpheniramine maleate, 971
Chlorpheniramine maleate and pseudoephedrine hydrochloride, 973
Chlorpromazine hydrochloride syrup, 976
Chocolate syrup, 5716
Citalopram, 1034
Clindamycin hydrochloride, 1066
clindamycin hydrochloride compounded, 1066
Clindamycin palmitate hydrochloride for, 1068
Cloxacillin sodium for, 1130
Codeine phosphate, 1138
Codeine sulfate, 1141
Cyclosporine, 1216
Cyproheptadine hydrochloride, 1219
Dexamethasone, 1288
Dexamethasone elixir, 1286
Dexbrompheniramine maleate and pseudoephedrine sulfate, 1298
Dexchlorpheniramine maleate, 1301
Dextromethorphan hydrobromide, 1321
Diclofenac potassium for, 1344
Dicyclomine hydrochloride, 1361
Didanosine for, 1366
Digoxin, 1385
Dihydrotachysterol, 1392
Diltiazem hydrochloride, 1405
Dimenhydrinate, 1410
Diphenhydramine hydrochloride, 1429
Diphenoxylate hydrochloride and atropine sulfate, 1438
Docusate sodium syrup, 1481
Dolasetron mesylate, 1485
Doxepin hydrochloride, 1510
Doxylamine succinate, 1546
Dyphylline, 1573
Dyphylline and guaifenesin, 1575
Entecavir, 1636
Ephedrine sulfate, 1644
Ergocalciferol, 1667
Ergoloid mesylates, 1671
Escitalopram, 1706
Ethosuximide, 1776
Ferric ammonium citrate for, 290
Ferrous gluconate, 1860
Ferrous sulfate, 1863
Ferrous sulfate syrup, 1863
Fluoxetine, 1961
Fluphenazine hydrochloride, 1973
Fluphenazine hydrochloride elixir, 1971
Folic acid, compounded, 2027
Furosemide, 2055
Galantamine, 2079
Glycerin, 2134
Guaifenesin, 2171
Guaifenesin and codeine phosphate, 2172
Haloperidol, 2189
Hydralazine hydrochloride, 2215
Hydromorphone hydrochloride, 2252
Hydroxyzine hydrochloride, 2266
Hyoscyamine sulfate, 2277
Hyoscyamine sulfate elixir, 2276
Ipecac, 2397
Isoniazid, 2426
Isosorbide, 2440
Ivermectin compounded, veterinary, 2473
Lamivudine, 2526
Leucovorin calcium compounded, 2570
Levetiracetam, 2583
Levocarnitine, 2600
Levofloxacin, 2608
Lincomycin, 2632
Lithium, 2648
Loperamide hydrochloride, 2661
Lopinavir and ritonavir, 2666
Loratadine, 2674
Magnesium carbonate, citric acid, and potassium citrate for, 2711
Magnesium carbonate and citric acid for, 2710
Manganese chloride for, 2732
Magnesium citrate, 2714
Magnesium citrate for, 2715
Meperidine hydrochloride, 2784
Metaproterenol sulfate, 2813
Methadone hydrochloride, 2838
Methylcellulose, 2876
Metoclopramide, 2910
Metoprolol tartrate, 2925
Mibolerone, 2945
Neomycin sulfate, 3103
Nortriptyline hydrochloride, 3216
Ondansetron, 3266
Orange syrup, 5915
Oxybutynin chloride, 3328
Oxycodone hydrochloride, 3337
Paromomycin, 3409
Penicillin G potassium for, 3439
Penicillin V potassium for, 3456
Phenobarbital, 3498
Piperazine citrate syrup, 3575
Polyethylene glycol 3350 and electrolytes for, 3587
Potassium bicarbonate effervescent tablets for, 3596
Potassium bicarbonate and potassium chloride for effervescent, 3597
Potassium bicarbonate and potassium chloride effervescent tablets for, 3597
Potassium bicarbonate, potassium chloride, and potassium citrate effervescent tablets for, 3609
Potassium bromide, veterinary, 3600
Potassium chloride, 3604
Potassium chloride for, 3605
Potassium citrate and citric acid, 3617
Potassium gluconate, 3619
Potassium gluconate and potassium chloride, 3621
Potassium gluconate and potassium chloride for, 3621
Potassium gluconate and potassium citrate, 3622
Potassium gluconate, potassium citrate, and ammonium chloride, 3622
Potassium iodide, 3624
Potassium and sodium bicarbonates and citric acid effervescent tablets for, 3598
Prednisolone, 3661
Prednisolone sodium phosphate compounded, 3667
Prednisone, 3671
Prochlorperazine, 3697
Promazine hydrochloride, 3713
Promazine hydrochloride syrup, 3713
Promethazine and phenylephrine hydrochloride, 3721
Promethazine and phenylephrine hydrochloride and codeine phosphate, 3724
Promethazine hydrochloride, 3717
Pseudoephedrine hydrochloride, 3762
Pseudoephedrine hydrochloride, carbinoxamine maleate, and dextromethorphan hydrobromide, 3767
Pyridostigmine bromide, 3780
Ranitidine, 3846
Risperidone, 3905
Ritonavir, 3917
Saccharin sodium, 3967
Senna, 4026
Sertraline hydrochloride, 4032
Sodium benzoate compounded, 4056
Sodium bromide, veterinary, 4063
Sodium citrate and citric acid, 4069
Sodium fluoride, 4072
Sodium phosphates, 4090
Stavudine for, 4122
Sulfaquinoxaline, 4169
Syrup, 6087
Terpin hydrate, 4284
Terpin hydrate and codeine, 4284
Theophylline, 4325
Theophylline and guaifenesin, 4332
Theophylline sodium glycinate, 4334
Thiamine hydrochloride, 4338
Thiamine mononitrate, 4341
Thioridazine hydrochloride, 4354
Thiothixene hydrochloride, 4361
Tolu balsam syrup, 6090
Triamcinolone diacetate, 4491
Tricitrates, 4504
Trifluoperazine, 4511
Trihexyphenidyl hydrochloride, 4519
Trikates, 4521
Trimeprazine, 4522
Triprolidine hydrochloride, 4533
Triprolidine and pseudoephedrine hydrochlorides, 4534
Valproic acid, 4574
Vancomycin hydrochloride for, 4590
Vancomycin hydrochloride compounded, 4589
Vehicle for, 5913
Vehicle for, sugar free, 5913
Verapamil hydrochloride, 4609
Vigabatrin for, 4619
Vitamins with minerals, oil-soluble, 5372

www.webofpharma.com

Oral solution *(continued)*
Vitamins with minerals, oil- and water-soluble, 5462
Vitamins with minerals, water-soluble, 5543
Vitamins, oil-soluble, 5353
Vitamins, oil- and water-soluble, 5409
Zidovudine, 4676
Zinc acetate, 4682
Zinc sulfate, 4693

Oral suspension

Acetaminophen, 42
Acetaminophen and codeine phosphate, 56
Acetazolamide, 76
Acyclovir, 92
Albendazole, 102
Allopurinol, 133
Alprazolam, 145
Alumina and magnesia, 166
Alumina, magnesia, and calcium carbonate, 168
Alumina, magnesia, and simethicone, 173
Alumina and magnesium carbonate, 176
Alumina and magnesium trisilicate, 179
Amiodarone hydrochloride, 257
Amlodipine, 264
Amoxicillin, 303
Amoxicillin and clavulanate potassium for, 308
Amoxicillin for, 303
Amoxicillin tablets for, 306
Ampicillin for, 324
Ampicillin and probenecid for, 326
Atenolol compounded, 408
Atenolol compounded, veterinary, 409
Atovaquone, 423
Azathioprine, 440
Azithromycin for, 454
Baclofen, 473
Benazepril hydrochloride compounded, veterinary, 490
Bethanechol chloride, 564
Biotin compounded, 571
Bismuth subsalicylate, 582
Calcium carbonate, 686
Calcium and magnesium carbonates, 691
Captopril, 735
Carbamazepine, 742
Cefaclor for, 810
Cefadroxil for, 816
Cefdinir for, 830
Cefixime for, 837
Cefpodoxime proxetil for, 864
Cefprozil for, 869
Cefuroxime axetil for, 888
Cellulose sodium phosphate for, 896
Cephalexin for, 898
Cephalexin tablets for, 899
Cephradine for, 909
Chloramphenicol palmitate, 934
Chloroquine phosphate, 960
Chlorothiazide, 963
Chlorothiazide compounded, 963
Cholestyramine for, 990
Ciprofloxacin for, 1019
Cisapride compounded, veterinary, 1025
Clarithromycin for, 1049
Clavulanate potassium and amoxicillin for, 308
Clomipramine compounded, veterinary, 1091
Clonazepam, 1097
Clonidine hydrochloride compounded, 1103
Clopidogrel compounded, 1111
Colestipol hydrochloride for, 1147
Colistin sulfate for, 1150
Cyclophosphamide compounded, 1208
Dapsone, 1240
Diazoxide, 1335
Didanosine tablets for, 1367
Diltiazem hydrochloride, 1406
Dipyridamole, 1445
Dolasetron mesylate, 1485
Doxycycline for, 1525
Doxycycline calcium, 1529
Doxycycline compounded, veterinary, 1530
Enalapril maleate, 1613
Enalapril maleate compounded, veterinary, 1613
Enrofloxacin compounded, veterinary, 1630
Erythromycin estolate, 1693
Erythromycin estolate for, 1694
Erythromycin estolate and sulfisoxazole acetyl, 1695
Erythromycin ethylsuccinate, 1698
Erythromycin ethylsuccinate for, 1699
Erythromycin ethylsuccinate and sulfisoxazole acetyl for, 1701
Ethambutol hydrochloride compounded, 1763
Famciclovir compounded, 1812
Famotidine for, 1818
Felbamate, 1822
Ferumoxsil, 1868
Flecainide acetate, 1897
Fluconazole for, 1912
Flucytosine, 1917
Furazolidone, 2052
Ganciclovir, 2090
Granisetron hydrochloride, 2161
Griseofulvin, 2166
Ibuprofen, 2283
Indomethacin, 2332
Isradipine, 2465
Ketoconazole, 2507
Labetalol hydrochloride, 2519
Lamotrigine compounded, 2545
Lamotrigine tablets, 2542
Lansoprazole compounded, 2556
Leflunomide compounded, 2560
Lisinopril, 2644
Magaldrate and simethicone, 2706
Magnesium carbonate and sodium bicarbonate for, 2712
Marbofloxacin compounded, veterinary, 2741
Mebendazole, 2744
Megestrol acetate, 2763
Meloxicam, 2770
Methacycline hydrochloride, 2835
Methadone hydrochloride tablets for, 2839
Metolazone, 2913
Metoprolol tartrate, 2926
Metronidazole benzoate compounded, 2933
Minocycline hydrochloride, 2966
Mirtazapine compounded, veterinary, 2977
Mycophenolate mofetil for, 3047
Naproxen, 3079
Naproxen compounded, 3078
Naratriptan hydrochloride, 3094
Nevirapine, 3132
Nitrofurantoin, 3179
Nystatin, 3221
Nystatin for, 3221
Ondansetron hydrochloride, 3268
Oxcarbazepine, 3317
Oxfendazole, 3322
Penicillin G benzathine, 3433
Penicillin V for, 3452
Penicillin V benzathine, 3454
Pentoxifylline, 3470
Pergolide, veterinary, 3478
Phenobarbital, 3498
Phenoxybenzamine hydrochloride compounded, 3504
Phenytoin, 3526
Phytonadione compounded, 3538
Piroxicam compounded, 3579
Prazosin hydrochloride compounded, 3655
Prednisolone compounded, veterinary, 3664
Primidone, 3684
Propylthiouracil, 3755
Psyllium hydrophilic mucilloid for, 3771
Pyrantel pamoate, 3774
Pyrazinamide, 3776
Pyrimethamine, 3787
Pyrvinium pamoate, 3789
Quinidine sulfate, 3814
Rifabutin, 3874
Rifampin, 3877
Sildenafil citrate, 4040
Simethicone, 4045
Sodium phenylbutyrate, 4086
Sotalol hydrochloride, 4104
Spironolactone, 4111
Spironolactone and hydrochlorothiazide, 4113
Spironolactone compounded, 4111
Sulfadimethoxine, 4153
Sulfamethizole, 4158
Sulfamethoxazole, 4161
Sulfamethoxazole and trimethoprim, 4163
Sulfisoxazole acetyl, 4175
Sumatriptan succinate, 4188
Tacrolimus, 4202
Tadalafil compounded, 4207
Temozolomide, 4257
Terbinafine, 4267
Terbutaline, 4273
Tetracycline, 4302
Tetracycline hydrochloride, 4310
Theophylline, 4327
Thiabendazole, 4335
Tiagabine hydrochloride, 4367
Topiramate compounded, 4436
Tramadol hydrochloride, 4443
Tramadol hydrochloride and acetaminophen, 4452
Tramadol hydrochloride compounded, veterinary, 4443
Trifluprom azine, 4514
Trisulfapyrimidines, 4536
Ursodiol, 4559
Valacyclovir, 4561
Vehicle for, 5913
Verapamil hydrochloride, 4609
Zonisamide compounded, 4721

Orange
G, 6184
oil, 5914
peel tincture, sweet, 5915

www.webofpharma.com

Orange *(continued)*
spirit, compound, 5914
syrup, 5915
Orbifloxacin, 3275
tablets, 3276
Orcinol, 6185
Ordinary impurities ⟨466⟩, 6710
Organic
nitrogenous bases—identification ⟨181⟩, 6587
nitrogenous bases, salts of ⟨501⟩, 6741
Orlistat, 3277
capsules, 3280
Orphenadrine citrate, 3281
aspirin and caffeine tablets, 3288
injection, 3284
extended-release tablets, 3285
Orthophenanthroline, 6185
TS, 6232
Oseltamivir phosphate, 3291
capsules, 3293
Osmium tetroxide, 6185
Osmolality and osmolarity ⟨785⟩, 7008
Otic solution
acetic acid, 78
antipyrine and benzocaine, 352
antipyrine, benzocaine, and phenylephrine hydrochloride, 354
benzocaine, 510
chloramphenicol, 931
gentamicin sulfate and betamethasone valerate, 2104
hydrocortisone and acetic acid, 2235
neomycin and polymyxin B sulfates and hydrocortisone, 3124
polymyxin B sulfate and hydrocortisone, 3592
Otic suspension
Ciprofloxacin and dexamethasone, 1021
Oxacillin
injection, 3296
for injection, 3297
sodium, 3294
Oxalic acid, 6185
tenth-normal (0.1 N), 6244
TS, 6232
Oxaliplatin, 3297
injection, 3301
for injection, 3303
Oxandrolone, 3306
tablets, 3308
Oxaprozin, 3310
tablets, 3311
Oxazepam, 3312
capsules, 3314
Oxcarbazepine, 3315
oral suspension, 3317
tablets, 3319
Oxfendazole, 3322
oral suspension, 3322
Oxidized cellulose, 893
regenerated, 894
Oxprenolol hydrochloride, 3323
extended-release tablets, 3324
Oxtriphylline, 3324
extended-release tablets, 3325
Oxybenzone, 3326
and dioxybenzone cream, 1418
Oxybutynin chloride, 3326
oral solution, 3328
tablets, 3328
tablets, extended-release, 3329
Oxycodone
and acetaminophen capsules, 3341
and acetaminophen tablets, 3343
and aspirin tablets, 3344
terephthalate, 3345
Oxycodone hydrochloride, 3335
oral solution, 3337
tablets, 3338
extended-release tablets, 3339
3,3′-Oxydipropionitrile, 6185
Oxygen, 3347
21 percent certified standard, 6185
93 percent, 3347
93 percent certified standard, 6185
certified standard, 6185
flask combustion ⟨471⟩, 6735
helium certified standard, 6185
Oxymetazoline hydrochloride, 3348
nasal solution, 3349
ophthalmic solution, 3350
Oxymetholone, 3350
tablets, 3351
Oxymorphone hydrochloride, 3352
injection, 3353
tablets, 3354
extended-release tablets, 3356
Oxyquinoline sulfate, 5915
Oxytetracycline, 3359
for injection, 3362
hydrochloride, 3361
hydrochloride capsules, 3362
hydrochloride and hydrocortisone acetate ophthalmic suspension, 3363
hydrochloride and hydrocortisone ointment, 3364
hydrochloride and polymyxin B sulfate ointment, 3364
hydrochloride and polymyxin B sulfate ophthalmic ointment, 3365
hydrochloride and polymyxin B sulfate topical powder, 3365
hydrochloride and polymyxin B sulfate vaginal inserts, 3366
hydrochloride soluble powder, 3363
injection, 3360
tablets, 3361
Oxytocin, 3366
injection, 3367

P

Package integrity and test method selection ⟨1207.1⟩, 8086
Package integrity leak test technologies ⟨1207.2⟩, 8097
Package seal quality test technologies ⟨1207.3⟩, 8111
Packaging and repackaging—single unit containers ⟨1136⟩, 7919
Packaging and storage requirements ⟨659⟩, 6876
Paclitaxel, 3367
injection, 3370
Padimate O, 3371
lotion, 3372
Paired ion chromatography reagent, 6185
Paliperidone, 3373
Palladium
catalyst, 6185
chloride, 6185
chloride TS, buffered, 6232
Palladous chloride, 6185
Pallida
echinacea, 4944
extract, powdered echinacea, 4948
powdered echinacea, 4946
Palm
oil, 5916
oil, hydrogenated, 5916
kernel oil, 5917
Palmitic acid, 5918
Palonosetron
hydrochloride, 3374
Pamabrom, 3376
Pamidronate disodium, 3377
for injection, 3379
Pancreatic digest of casein, 6185, 6216
Pancreatin, 3379, 6185
tablets, 3381
Pancreatin ⟨1025⟩, 7272
Pancrelipase, 3382
capsules, 3384
delayed-release capsules, 3384
tablets, 3385
Pancuronium bromide, 3385
injection, 3386
Panthenol, 3388
Pantoprazole sodium, 3388
delayed-release tablets, 3391
Papaic digest of soybean meal, 6185
Papain, 3396
tablets for topical solution, 3396
Papaverine hydrochloride, 3397
injection, 3398
tablets, 3398
Paper
lead acetate, 6175
Para-aminobenzoic acid, 6135, 6185
Parachlorophenol, 3399
camphorated, 3399
Paraffin, 5920
synthetic, 5921
Paraformaldehyde, 6186
Paraldehyde, 3400
Paregoric, 3401
Paricalcitol, 3402
capsules, 3404
injection, 3406
Paromomycin
oral solution, 3409
sulfate, 3408
sulfate capsules, 3408
Paroxetine
hydrochloride, 3409
tablets, 3412
extended-release tablets, 3413
Partially-neutralized methacrylic acid and ethyl acrylate copolymer, 5881
Particle size distribution estimation by analytical sieving ⟨786⟩, 7010
Particulate matter in injections ⟨788⟩, 7017
Particulate matter in ophthalmic solutions ⟨789⟩, 7020
Peanut oil, 5921
Pea starch, 6053
Pectate lyase, 6186
Pectin, 3418
Pemetrexed
disodium, 3420
for injection, 3422
Penbutolol sulfate, 3424
tablets, 3425
Penicillamine, 3426
capsules, 3427
tablets, 3429
Penicillin
G benzathine, 3432
G benzathine injectable suspension, 3433

www.webofpharma.com

Penicillin *(continued)*
G benzathine and penicillin G procaine injectable suspension, 3434
G benzathine oral suspension, 3433
G benzathine tablets, 3434
G, neomycin, polymyxin B, hydrocortisone acetate, and hydrocortisone sodium succinate topical suspension, 3430
G potassium, 3436
G potassium injection, 3437
G potassium for injection, 3437
G potassium for oral solution, 3439
G potassium tablets, 3439
G procaine, 3441
G procaine, dihydrostreptomycin sulfate, chlorpheniramine maleate, and dexamethasone injectable suspension, 3445
G procaine and dihydrostreptomycin sulfate injectable suspension, 3444
G procaine and dihydrostreptomycin sulfate intramammary infusion, 3444
G procaine, dihydrostreptomycin sulfate, and prednisolone injectable suspension, 3447
G procaine injectable suspension, 3443
G procaine for injectable suspension, 3443
G procaine intramammary infusion, 3442
G procaine, neomycin and polymyxin B sulfates, and hydrocortisone acetate topical suspension, 3448
G procaine and novobiocin sodium intramammary infusion, 3448
G procaine and penicillin G benzathine injectable suspension, 3434
G sodium, 3449
G sodium for injection, 3450
V, 3451
V benzathine, 3453
V benzathine oral suspension, 3454
V potassium, 3454
V potassium for oral solution, 3456
V potassium tablets, 3456
V for oral suspension, 3452
V tablets, 3453
Penicillinase, 6186
Pentadecane, 6186
1-Pentadecanol, 6186
Pentafluoropropionic acid, 6186
Pentamidine isethionate, 3457
Pentane, 6186
1-Pentanesulfonic acid sodium salt, 6186
2-Pentanone, 6186
Pentazocine, 3458
and acetaminophen tablets, 3460
and aspirin tablets, 3461
hydrochloride, 3459
injection, 3465
and naloxone tablets, 3463
Pentetic acid, 3466
Pentobarbital, 3467
sodium, 3468
sodium injection, 3469
Pentoxifylline, 3469
oral suspension, 3470
extended-release tablets, 3471
People, ix
Peppermint, 5922
oil, 5923
spirit, 3474
water, 5923
Pepsin, 6186
purified, 6188
Peptic digest of animal tissue, 6188
Peptone, dried, 6163, 6188
Perchloric acid, 6189
tenth-normal (0.1 N) in dioxane, 6244
tenth-normal (0.1 N) in glacial acetic acid, 6244
TS, 6233
Perflubron, 3474
Perflutren protein-type A microspheres injectable suspension, 3475
Pergolide
mesylate, 3477
oral suspension veterinary, 3478
tablets, 3478
Perindopril
erbumine, 3480
erbumine tablets, 3483
Periodic acid, 6189
Periodontal system
minocycline, 2970
Peroxidase, 6189
Perphenazine, 3485
and amitriptyline hydrochloride tablets, 3487
tablets, 3486
Pertussis
immune globulin, 3488
Petrolatum, 3488
hydrophilic, 3489
white, 3489
Petroleum benzin, 6189
Petroleum ether, 6189
pH ⟨791⟩, 7022
Pharmaceutical calculations in pharmacy practice ⟨1160⟩, 7953
Pharmaceutical compounding
nonsterile preparations ⟨795⟩, 7025
sterile preparations ⟨797⟩, 7037
Pharmaceutical dosage forms ⟨1151⟩, 7929
Phellandrene
(R)-(–)-alpha, 6189
Phenacetin, 6189
1,10-Phenanthroline, 6185, 6189
o-Phenanthroline monohydrochloride monohydrate, 6189
Phenazopyridine hydrochloride, 3490
tablets, 3491
Phendimetrazine tartrate, 3492
capsules, 3493
tablets, 3494
Phenelzine sulfate, 3495
tablets, 3496
Pheniramine maleate, 3497
and naphazoline hydrochloride ophthalmic solution, 3076
Phenobarbital, 3497
sodium, 3499
sodium injection, 3500
sodium for injection, 3500
oral solution, 3498
oral suspension, 3498
tablets, 3499
theophylline and ephedrine hydrochloride tablets, 4330
Phenol, 3501, 6189
alcohol TS, 6225
topical gel, camphorated, 3501
iron, TS, 6230
liquefied, 3502
red, 6189, 6221
red, sodium, 6189
red TS, 6233
red TS, pH 4.7, 6233
camphorated, topical solution, 3502
TS, 6233
Phenolated
calamine topical suspension, 665
Phenoldisulfonic acid TS, 6233
Phenolphthalein, 6221
paper, 6222
Phenolphthalein TS, 6233
Phenolsulfonphthalein, 5924, 6189
Phenoxybenzamine hydrochloride, 3503, 6189
capsules, 3503
Phenoxybenzamine hydrochloride compounded
oral suspension, 3504
3-Phenoxybenzoic acid, 6189
2-Phenoxyethanol, 6189
Phenoxyethanol, 5925
Phentermine hydrochloride, 3505
capsules, 3506
tablets, 3507
Phentolamine mesylate, 3508
for injection, 3509
Phenyl
ether, 6189
isocyanate, 6189
2-Phenylacetamide, 6189
Phenylacetic acid, 6189
Phenylalanine, 3509, 6189
dl-Phenylalanine, 6189
Phenylbutazone, 3510
boluses, 3511
injection, 3511
tablets, 3512
p-Phenylenediamine
dihydrochloride, 6189
hydrochloride, 6189
o-Phenylenediamine dihydrochloride, 6189
Phenylephrine
bitartrate, 3513
diphenhydramine, hydrochloride tablets, 1433
hydrochloride, 3515
hydrochloride, antipyrine, and benzocaine otic solution, 354
hydrochloride and promethazine and codeine phosphate oral solution, 3724
hydrochloride and promethazine oral solution, 3721
hydrochloride injection, 3517
hydrochloride nasal jelly, 3518
hydrochloride nasal solution, 3518
hydrochloride ophthalmic solution, 3519
hydrochloride tablets, 3520
Phenylethyl alcohol, 3522
Phenylglycine, 6190
Phenylhydrazine, 6190
acetate TS, 6233
hydrochloride, 6190
sulfuric acid TS, 6233
Phenylmercuric
acetate, 5926
nitrate, 5926
Phenylmethylsulfonyl fluoride, 6190
3-Phenylphenol, 6190
Phenylpropanolamine
hydrochloride, 3522
Phenyltoloxamine citrate, 3523
Phenytoin, 3524
chewable tablets, 3528
sodium, 3529
sodium capsules, extended, 3531
sodium injection, 3534
oral suspension, 3526
Phenytoin compounded topical gel, 3525
pH indicator paper, short-range, 6222
Phloroglucinol, 6190
TS, 6233

www.webofpharma.com

Phloxine B, 6190
Phosphatase enzyme, alkaline, 6134, 6190
Phosphate
  acidulated, and sodium fluoride topical solution, 4074
  buffer, 6145
  diethylamine, 6158
  in reagents, 6131
Phosphatic enzyme, 6190
  TS, 6233
Phosphomolybdic acid, 6190
  TS, 6233
Phosphoric acid, 5927, 6190
  diluted, 5927
  and sodium fluoride gel, 4075
  0.01% TS, 6233
  0.05 M TS, 6233
  0.06 M TS, 6233
  10 TS, 6233
  1 N TS, 6233
  20% TS, 6233
  0.01 M TS, 6233
18 N Phosphoric acid
  TS, 6233
1 M Phosphoric acid
  TS, 6233
0.75 M Phosphoric acid TS, 6233
0.02 M Phosphoric acid TS, 6233
1.5 M Phosphoric acid TS, 6233
Phosphorous acid, 6190
Phosphorus
  pentoxide, 6190
  red, 6190, 6197
Phosphotungstic acid, 6190
  TS, 6233
o-Phthalaldehyde, 6190
Phthalazine, 6191
Phthalic
  acid, 6191
  anhydride, 6191
Phthalimide, 6191
Phyllanthus amarus, 5191
  powdered, 5193
Physical environments that promote safe medication use ⟨1066⟩, 7590
Physicochemical analytical procedures for insulins ⟨121.1⟩, 6550
Physicochemical integrators and indicators for sterilization ⟨1229.9⟩, 8227
Physostigmine
  salicylate, 3535
  salicylate injection, 3535
  salicylate ophthalmic solution, 3536
Phytonadione, 3537
  injectable emulsion, 3538
  tablets, 3539
Phytonadione compounded
  oral suspension, 3538
2-Picoline, 6191
Picrate TS, alkaline, 6226, 6233
Picric acid, 6191, 6215
  TS, 6233
Picrolonic acid, 6191
Pilocarpine, 3539
  hydrochloride, 3542
  hydrochloride ophthalmic solution, 3543
  hydrochloride tablets, 3543
  nitrate, 3545
  nitrate ophthalmic solution, 3545
  ocular system, 3541
Pimobendan, 3546
Pimozide, 3547
  tablets, 3548
Pindolol, 3549
  tablets, 3550
Pinene
  (+)-alpha, 6191
  beta, 6191
Pioglitazone
  and glimepiride tablets, 3554
  hydrochloride, 3551
  and metformin hydrochloride tablets, 3558
  tablets, 3553
Pipemidic acid, 6191
Piperacillin, 3562
  for injection, 3565
  sodium, 3564
  and tazobactam for injection, 3567
Piperazine, 3573, 6191
  adipate, 3573
  citrate, 3574
  citrate syrup, 3575
  citrate tablets, 3575
  dihydrochloride, 3575
  phosphate, 3576
Piperidine, 6191
Piroxicam, 3577
  capsules, 3578
  cream, 3579
Piroxicam compounded
  oral suspension, 3579
Plantago seed, 3580
Plant Stanol Esters, 5194
Plasma protein fraction, 3581
Plasma spectrochemistry ⟨730⟩, 6967
Plasma spectrochemistry—theory and practice ⟨1730⟩, 8484
Plastic materials of construction ⟨661.1⟩, 6893
Plastic packaging systems and their materials of construction ⟨661⟩, 6887
Plastic packaging systems for pharmaceutical use ⟨661.2⟩, 6912
Platinic
  chloride, 6191
  chloride TS, 6233
Platinum
  cobalt TS, 6233
Podophyllum, 3581
  resin, 3582
  resin topical solution, 3582
Polacrilin potassium, 5928
Polarography ⟨801⟩, 7089
Policies, USP, xxviii
Poloxalene, 3582
Poloxamer, 5929
Poly(dimethylsiloxane-co-methylphenylsiloxane), 6191
Polycarbophil, 3583
  calcium, 713
Polydecene
  hydrogenated, 5932
Polydextrose, 5933
  hydrogenated, 5936
Polydimethylsiloxane, viscosity 0.65 centistokes, 6191
Polyethylene
  glycol, 5939
  glycol 200, 6191
  glycol 600, 6191
  glycol 20,000, 6191
  glycol 3350 and electrolytes for oral solution, 3587
  glycol monomethyl ether, 5942
  glycol ointment, 5941
  oxide, 5944
Polyethylene glycol 3350, 3584
Polyethylene glycol 400, 6191
Polyethylene glycol 4000, 6191
Polyethylene glycol standards with molecular weights of 1000, 2000, 3000, 4000, and 6000 daltons (g/mol), 6192
Polyglyceryl
  3 diisostearate, 5948
  dioleate, 5946
Polyisobutylene, 5949
Polymyxin B
  for injection, 3590
  and neomycin sulfates, bacitracin, and hydrocortisone acetate ointment, 3116
  and neomycin sulfates, bacitracin, and hydrocortisone acetate ophthalmic ointment, 3116
  and neomycin sulfates, bacitracin, and lidocaine ointment, 3117
  and neomycin sulfates and bacitracin ointment, 3115
  and neomycin sulfates and bacitracin ophthalmic ointment, 3115
  and neomycin sulfates, bacitracin zinc, and hydrocortisone acetate ophthalmic ointment, 3120
  and neomycin sulfates, bacitracin zinc, and hydrocortisone ointment, 3118
  and neomycin sulfates, bacitracin zinc, and hydrocortisone ophthalmic ointment, 3119
  and neomycin sulfates, bacitracin zinc, and lidocaine ointment, 3120
  and neomycin sulfates and bacitracin zinc ointment, 3118
  and neomycin sulfates and bacitracin zinc ophthalmic ointment, 3118
  and neomycin sulfates cream, 3114
  and neomycin sulfates and dexamethasone ophthalmic ointment, 3121
  and neomycin sulfates and dexamethasone ophthalmic suspension, 3122
  and neomycin sulfates and gramidicin cream, 3122
  and neomycin sulfates, gramidicin, and hydrocortisone acetate cream, 3123
  and neomycin sulfates and gramidicin ophthalmic solution, 3123
  and neomycin sulfates and hydrocortisone acetate cream, 3125
  and neomycin sulfates and hydrocortisone acetate ophthalmic suspension, 3125
  and neomycin sulfates and hydrocortisone ophthalmic suspension, 3123
  and neomycin sulfates and hydrocortisone otic solution, 3124
  and neomycin sulfates and hydrocortisone otic suspension, 3124
  and neomycin sulfates and lidocaine cream, 3126
  and neomycin sulfates ophthalmic ointment, 3114
  and neomycin sulfates ophthalmic solution, 3115
  and neomycin sulfates, penicillin G procaine, and hydrocortisone acetate topical suspension, 3448
  and neomycin sulfates and pramoxine hydrochloride cream, 3126
  and neomycin sulfates and prednisolone acetate ophthalmic suspension, 3127
  and neomycin sulfates solution for irrigation, 3114
  penicillin G, neomycin, hydrocortisone acetate, and hydrocortisone sodium succinate topical suspension, 3430
  sulfate, 3588
  sulfate and bacitracin topical aerosol, 468

www.webofpharma.com

Polymyxin B *(continued)*
sulfate and bacitracin zinc topical aerosol, 3591
sulfate and bacitracin zinc ointment, 471
sulfate and bacitracin zinc ophthalmic ointment, 471
sulfate and bacitracin zinc topical powder, 3591
sulfate and chloramphenicol ophthalmic ointment, 933
sulfate and hydrocortisone otic solution, 3592
sulfate and oxytetracycline hydrochloride ointment, 3364
sulfate and oxytetracycline hydrochloride ophthalmic ointment, 3365
sulfate and oxytetracycline hydrochloride topical powder, 3365
sulfate and oxytetracycline hydrochloride vaginal inserts, 3366
sulfate and trimethoprim ophthalmic solution, 3592
Polyoxyethylene 10 lauryl ether, 6192
Polyoxyethylene (20) sorbitan monolaurate, 6192
Polyoxyethylene (23) lauryl ether, 6192
Polyoxyl
10 oleyl ether, 5950
15 hydroxystearate, 5951
20 cetostearyl ether, 5955
35 castor oil, 5956
40 hydrogenated castor oil, 5958
lauryl ether, 5960
oleate, 5961
stearate, 5961
stearyl ether, 5962
Polypeptone, 6192
Polypropylene glycol 11 stearyl ether, 5963
Polysaccharide molecular weight standards, 6192
Polysorbate
20, 5965
40, 5966
60, 5966
80, 5967
Polysorbate 20, 6192
Polysorbate 80, 6192
Polystyrene
cation-exchange resin, 6192
Polytef, 6192
Polyvinyl
acetate, 5969
acetate dispersion, 5972
acetate phthalate, 5974
alcohol, 3593, 6192
alcohol and ethylene glycol graft copolymer, 5769
Porosimetry by mercury intrusion ⟨267⟩, 6653
Porosity by nitrogen adsorption–desorption ⟨268⟩, 6655
Positron emission tomography drugs for compounding, investigational, and research uses ⟨823⟩, 7100
Positron emission tomography drugs—information ⟨1823⟩, 8617
Potash, sulfurated, 3594
Potassium
acetate, 3595, 6192
acetate injection, 3595
acetate TS, 6233
alginate, 5975
alum, 166, 6192
arsenate monobasic, 6192
arsenite, tenth-normal (0.1 N), 6245
benzoate, 5976
bicarbonate, 3596, 6192
bicarbonate effervescent tablets for oral solution, 3596
bicarbonate and potassium chloride for effervescent oral solution, 3597
bicarbonate and potassium chloride effervescent tablets for oral solution, 3597
bicarbonate, potassium chloride, and potassium citrate effervescent tablets for oral solution, 3609
biphosphate, 6192
biphthalate, 6192
bismuth iodide TS, 6233
bisulfate, 6192
bitartrate, 3599
bromate, 6192
bromate, tenth-normal (0.1 N), 6245
bromide, 3600, 6192
bromide-bromate, tenth-normal (0.1 N), 6245
bromide oral solution, veterinary, 3600
carbonate, 3601, 6193
carbonate, anhydrous, 6138, 6193
carbonate TS, 6233
chlorate, 6193
chloride, 3601, 6193
chloride extended-release capsules, 3602
chloride in dextrose injection, 3607
chloride in dextrose and sodium chloride injection, 3608
chloride for injection concentrate, 3604
chloride in lactated ringer's and dextrose injection, 3610
chloride, potassium bicarbonate, and potassium citrate effervescent tablets for oral solution, 3609
chloride and potassium bicarbonate for effervescent oral solution, 3597
chloride and potassium bicarbonate effervescent tablets for oral solution, 3597
chloride and potassium gluconate oral solution, 3621
chloride and potassium gluconate for oral solution, 3621
chloride in sodium chloride injection, 3612
chloride oral solution, 3604
chloride for oral solution, 3605
chloride extended-release tablets, 3605
chloroplatinate, 6193
chromate, 6193
chromate TS, 6234
citrate, 3613
citrate and citric acid oral solution, 3617
citrate, magnesium carbonate, and citric acid for oral solution, 2711
citrate, potassium chloride, and potassium bicarbonate effervescent tablets for oral solution, 3609
citrate, potassium gluconate, and ammonium chloride oral solution, 3622
citrate and potassium gluconate oral solution, 3622
citrate tablets, 5196
citrate extended-release tablets, 3614
cyanide, 6193
dichromate, 6193
dichromate, tenth-normal (0.1 N), 6245
dichromate TS, 6234
ferricyanide, 6193
ferricyanide TS, 6234
ferricyanide, twentieth-molar (0.05 M), 6245
ferrocyanide, 6193
ferrocyanide TS, 6234
gluconate, 3618
gluconate and potassium chloride oral solution, 3621
gluconate and potassium chloride for oral solution, 3621
gluconate, potassium citrate, and ammonium chloride oral solution, 3622
gluconate and potassium citrate oral solution, 3622
gluconate oral solution, 3619
gluconate tablets, 3620
guaiacolsulfonate, 3623
hyaluronate, 6193
hydrogen sulfate, 6193
hydroxide, 5977, 6193
hydroxide, alcoholic, half-normal (0.5 N), 6234, 6246
hydroxide, alcoholic, tenth-molar (0.1 M), 6246
hydroxide, methanolic, tenth-normal (0.1 N), 6246
hydroxide, normal (1 N), 6246
hydroxide TS, 6234
hydroxide 2 N TS, 6234
hydroxide TS, alcoholic, 6234
hydroxide TS 2, alcoholic, 6234
iodate, 6193
iodate, twentieth-molar (0.05 M), 6246
iodide, 3624, 6193
iodide and iodine TS 1, 6230
iodide and iodine TS 2, 6230
iodide and iodine TS 3, 6230
iodide oral solution, 3624
iodide and starch TS, 6234
iodide tablets, 3624
iodide delayed-release tablets, 3625
iodide TS, 6234
iodide 20% TS, 6234
iodoplatinate TS, 6234
metabisulfite, 5978, 6193
metaphosphate, 5978
nitrate, 3625, 6193
nitrate solution, 3626
nitrite, 6193
perchlorate, 3627, 6193
periodate, 6193
permanganate, 3627, 6193
permanganate, tenth-normal (0.1 N), 6234, 6246
permanganate TS, 6234
persulfate, 6193
phosphate, dibasic, 3628, 6156, 6193
phosphate, dibasic, trihydrate, 6193
phosphate, monobasic, 5979, 6181, 6192, 6193
phosphate, tribasic, 6194
phosphates injection, 3629
pyroantimonate, 6194
pyroantimonate TS, 6234
pyrophosphate, 6194
pyrosulfate, 6194
and sodium bicarbonates and citric acid effervescent tablets for oral solution, 3598
sodium tartrate, 3630, 6194
sorbate, 5980
sulfate, 6194
sulfate TS, 6234
tellurite, 6194
thiocyanate, 6194
thiocyanate, tenth-normal (0.1 N), 6247
thiocyanate TS, 6234
0.025 N Potassium dichromate VS, 6245

www.webofpharma.com

Potassium hydroxide
1.8 N TS, 6234
45% TS, 6234
10 M TS, 6234
0.1 N VS, 6246
Potassium phosphate
0.02 M TS, 6234
0.2 M TS, 6234
Potassium phosphates
compounded injection, 3629
Potato starch, 6058, 6194
Povidone, 3631
Povidone–iodine, 3634
topical aerosol, 3635
cleansing solution, 3635
ointment, 3635
topical solution, 3636

Powder

Absorbable dusting, 1569
Ampicillin soluble, 323
Amprolium soluble, 330
Astragalus root, 4785
Bacitracin methylene disalicylate soluble, 467
Bacitracin zinc soluble, 470
Banaba leaf, 4803
Chinese skullcap root, 4885
Chlortetracycline and sulfamethazine bisulfates soluble, 980
Chlortetracycline hydrochloride soluble, 987
Cinnamomum cassia twig, 4904
Compound clioquinol topical, 1076
Coix seed, 4917
Cromolyn sodium inhalation, 1182
Echinacea species, 4966
Eleuthero root and rhizome, capsules, 4976
Fenugreek seed, 4984
Fluticasone propionate and salmeterol, inhalation, 2010
Fluticasone propionate inhalation, 1993
Ganoderma lucidum fruiting body, 5008
Iron, 6173
Japanese honeysuckle flower, 5097
Levothyroxine sodium oral, 2618
Lincomycin hydrochloride soluble, 2634
Miconazole nitrate topical, 2949
Neomycin sulfate, isoflupredone acetate, and tetracaine hydrochloride topical, 3112
Northern schisandra fruit, 5265
Nystatin topical, 3220
Olive leaf, 5184
Oral, containing at least three of the following—acetaminophen and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, 54
Oxytetracycline hydrochloride and polymyxin B sulfate topical, 3365
Oxytetracycline hydrochloride soluble, 3363
Polymyxin B sulfate and bacitracin zinc topical, 3591
Rhodiola crenulata root and rhizome, 5216
St. John's wort flowering top, 5238
Salix species bark, 5249
Salmeterol inhalation, 3973
Sodium bicarbonate oral, 4061
Soy isoflavones, powdered extract, 5274
Sulfadimethoxine soluble, 4153
Tangerine peel, 5296
Tetracycline hydrochloride soluble, 4309
Tienchi ginseng root and rhizome, 5301
Tolnaftate topical, 4426
Valerian root, capsules, 5333
Vitamins with minerals oral, 5338
Zinc oxide, 4690

Powdered

American ginseng, 4752
American ginseng extract, 4753
andrographis, 4760
andrographis extract, 4762
ashwagandha root, 4771
ashwagandha root extract, 4768
Asian ginseng, 4775
Asian ginseng extract, 4776
bilberry extract, 4813
black cohosh, 4817
black cohosh extract, 4820
black pepper, 4827
black pepper extract, 4829
cat's claw, 4854
cat's claw extract, 4855
cellulose, 5700
Chinese salvia, 4880
digitalis, 1379
Echinacea angustifolia, 4938
Echinacea angustifolia extract, 4941
Echinacea pallida, 4946
Echinacea pallida extract, 4948
Echinacea purpurea, 4955
Echinacea purpurea extract, 4958
eleuthero, 4975
eleuthero extract, 4970
fenugreek seed, extract, 4987
feverfew, 4990
garlic, 5020
garlic extract, 5022
ginger, 5028
ginkgo extract, 5038
goldenseal, 5063
goldenseal extract, 5064
green tea extract, decaffeinated, 5067
gymnema, 5075
hawthorn leaf with flower, 5082
holy basil leaf, 5087
holy basil leaf extract, 5089
horse chestnut, 4875
horse chestnut extract, 4876
ipecac, 2396
licorice, 5120
licorice extract, 5121
malabar-nut-tree, leaf, 5137
milk thistle, 5159
milk thistle extract, 5160
opium, 3274
Phyllanthus amarus, 5193
rauwolfia serpentina, 3850
Rhodiola rosea, 5220
Rhodiola rosea extract, 5221
rosemary, 5232
saw palmetto, 5254
stinging nettle, 5288
stinging nettle extract, 5290
turmeric, 5316
turmeric extract, 5317
valerian, 5326
valerian extract, 5327
zinc chloride, anhydrous, 6219
Powder fineness ⟨811⟩, 7093
Powder flow ⟨1174⟩, 7993
Pralidoxime
chloride, 3636
chloride for injection, 3637
Pramipexole dihydrochloride, 3639
Pramoxine
hydrochloride, 3640
hydrochloride cream, 3641
hydrochloride jelly, 3642
hydrochloride and neomycin and polymyxin B sulfates cream, 3126
Prasugrel hydrochloride, 3642
Pravastatin sodium, 3645
tablets, 3647
Praziquantel, 3649
tablets, 3650
Prazosin hydrochloride, 3651
capsules, 3653
Prazosin hydrochloride compounded oral suspension, 3655
Prednicarbate, 3655
cream, 3656
ointment, 3658
Prednisolone, 3659
acetate, 3662
acetate and gentamicin ophthalmic ointment, 2106
acetate and gentamicin ophthalmic suspension, 2107
acetate injectable suspension, 3663
acetate and neomycin and polymyxin B sulfates ophthalmic suspension, 3127
acetate and neomycin sulfate ophthalmic suspension, 3128
acetate ophthalmic suspension, 3663
acetate and sulfacetamide sodium ophthalmic ointment, 4144
acetate and sulfacetamide sodium ophthalmic suspension, 4145
cream, 3660
hemisuccinate, 3665
penicillin G procaine, and dihydrostreptomycin sulfate injectable suspension, 3447
sodium phosphate, 3665
sodium phosphate injection, 3667
sodium phosphate ophthalmic solution, 3668
sodium succinate for injection, 3668
oral solution, 3661
tablets, 3661
tebutate, 3669
tebutate injectable suspension, 3670
tetracycline hydrochloride and novobiocin sodium tablets, 4312
Prednisolone compounded oral suspension, veterinary, 3664
Prednisolone sodium phosphate compounded
oral solution, 3667
Prednisone, 3670
injectable suspension, 3672
oral solution, 3671
tablets, 3672
Preface
and mission, v
Pregabalin, 3674
Pregnenolone acetate, 6194
Prekallikrein activator ⟨165⟩, 6583
Preparation of biological specimens for histologic and immunohistochemical analysis ⟨1285⟩, 8370

www.webofpharma.com

Prescription balances and volumetric apparatus ⟨1176⟩, 7997
Prescription container labeling ⟨17⟩, 6445
Prilocaine, 3675
and epinephrine injection, 3679
hydrochloride, 3676
hydrochloride injection, 3678
and lidocaine cream, 2630
Primaquine phosphate, 3680
tablets, 3681
Primidone, 3683
oral suspension, 3684
tablets, 3685
Prion inactivation ⟨1229.16⟩, 8239
Probenecid, 3686
and ampicillin for oral suspension, 326
and colchicine tablets, 3687
tablets, 3687
Procainamide hydrochloride, 3688
capsules, 3689
injection, 3690
tablets, 3690
extended-release tablets, 3691
Procaine
hydrochloride, 3693
hydrochloride and epinephrine injection, 3694
hydrochloride injection, 3694
and propoxycaine hydrochlorides and levonordefrin injection, 3744
and propoxycaine hydrochlorides and norepinephrine bitartrate injection, 3745
and tetracaine hydrochlorides and levonordefrin injection, 3695
Procarbazine hydrochloride, 3696
capsules, 3696
Prochlorperazine, 3697
edisylate, 3699
edisylate injection, 3699
maleate, 3700
maleate tablets, 3701
oral solution, 3697
suppositories, 3698
Procyclidine hydrochloride, 3703
tablets, 3704
Products for nebulization—characterization tests ⟨1601⟩, 8407
Progesterone, 3704
injectable suspension, 3706
injection, 3705
intrauterine contraceptive system, 3705
vaginal suppositories, 3707
Proguanil hydrochloride, 3708
Proline, 3711
Promazine hydrochloride, 3712
injection, 3712
oral solution, 3713
syrup, 3713
tablets, 3714
Promethazine
and phenylephrine hydrochloride and codeine phosphate oral solution, 3724
and phenylephrine hydrochloride oral solution, 3721
Promethazine hydrochloride, 3714
injection, 3716
oral solution, 3717
suppositories, 3718
tablets, 3719
Propafenone hydrochloride, 3727
extended-release capsules, 3729
tablets, 3734
Propane, 5982
Propanediol, 5983
Propantheline bromide, 3736
tablets, 3737
Proparacaine hydrochloride, 3738
and fluorescein sodium ophthalmic solution, 1945
ophthalmic solution, 3739
Propellants ⟨602⟩, 6844
Propionaldehyde, 6194
Propionic
acid, 5985
anhydride, 6194
Propionic acid, 6194
Propiophenone, 6194
Propofol, 3739
injectable emulsion, 3742
Propoxycaine
hydrochloride, 3743
and procaine hydrochlorides and levonordefrin injection, 3744
and procaine hydrochlorides and norepinephrine bitartrate injection, 3745
Propranolol hydrochloride, 3746
extended-release capsules, 3747
and hydrochlorothiazide tablets, 3751
injection, 3749
tablets, 3750
iso-Propyl alcohol, 6194
n-Propyl alcohol, 6134, 6194
Propyl gallate, 5985
Propylamine hydrochloride, 6194
Propylene
carbonate, 5986
glycol, 3753
glycol alginate, 5986
glycol dicaprylate/dicaprate, 5988
glycol dilaurate, 5989
glycol monocaprylate, 5990
glycol monolaurate, 5991
glycol monostearate, 5993
Propylene glycol, 6194
Propylene Glycol Diacetate, 5987
Propylhexedrine, 3754
inhalant, 3754
Propyliodone, 3754
Propylparaben, 5994, 6194
sodium, 5995
Propylthiouracil, 3755
oral suspension, 3755
tablets, 3756
Protamine sulfate, 3757
injection, 3758
Protein
molecular weight standard, 6194
standard solution (8 g/dL), 6194
Protein A quality attributes ⟨130⟩, 6570
Protein determination procedures ⟨507⟩, 6744
Protocatechuic acid, 6194
Protriptyline hydrochloride, 3759
tablets, 3760
Pseudoephedrine
chlorpheniramine, dextromethorphan (salts of), and acetaminophen, capsules containing at least three of the following, 52
chlorpheniramine, dextromethorphan (salts of), and acetaminophen, oral powder containing at least three of the following, 54
chlorpheniramine, dextromethorphan (salts of), and acetaminophen, oral solution containing at least three of the following, 56
chlorpheniramine, dextromethorphan (salts of) and acetaminophen, tablets containing at least three of the following, 58
and diphenhydramine capsules, 1435
hydrochloride, 3761
hydrochloride, acetaminophen, dextromethorphan hydrobromide, and doxylamine succinate oral solution, 67
hydrochloride, acetaminophen, and diphenhydramine hydrochloride tablets, 70
hydrochloride and acetaminophen tablets, 72
hydrochloride extended-release capsules, 3762
hydrochloride, carbinoxamine maleate, and dextromethorphan hydrobromide oral solution, 3767
hydrochloride and chlorpheniramine maleate extended-release capsules, 972
hydrochloride and chlorpheniramine maleate oral solution, 973
hydrochloride and guaifenesin capsules, 2174
hydrochloride, guaifenesin, and dextromethorphan hydrobromide capsules, 2175
hydrochloride and ibuprofen tablets, 2286
hydrochloride oral solution, 3762
hydrochloride tablets, 3763
hydrochloride extended-release tablets, 3764
hydrochloride and cetrizine hydrochloride extended-release tablets, 917
hydrochloride and fexofenadine hydrochloride extended-release tablets, 1876
sulfate, 3768
sulfate and brompheniramine maleate oral solution, 603
sulfate and dexbrompheniramine maleate oral solution, 1298
and triprolidine hydrochlorides oral solution, 4534
and triprolidine hydrochlorides tablets, 4535
Psyllium
hemicellulose, 3768
husk, 3770
hydrophilic mucilloid for oral suspension, 3771
Pullulan, 5996
Pullulanase, 6194
5,800, 23,700, and 100,000 molecular weight (MW) pullulan standards, 6181
Pumice, 3772, 6195
Pure steam, 4654
Purine, 6196
Purpurea
extract, powdered *Echinacea*, 4958
powdered *Echinacea*, 4955
root, *Echinacea*, 4953
Putrescine dihydrochloride, 6196
Pygeum, 5198
capsules, 5200
extract, 5198
Pyrantel pamoate, 3772
and ivermectin tablets, 2478
oral suspension, 3774
Pyrantel tartrate, 3775
Pyrazinamide, 3776
rifampin, isoniazid, and ethambutol hydrochloride tablets, 3881
rifampin and isoniazid tablets, 3879

www.webofpharma.com

Pyrazinamide *(continued)*
oral suspension, 3776
tablets, 3777
Pyrazole, 6196
Pyrene, 6196
Pyrethrum extract, 3778
4-(2-Pyridylazo)resorcinol, 6196
Pyridine, 6196
dried, 6196
Pyridine, anhydrous, 6196
Pyridine–pyrazolone TS, 6234
Pyridostigmine bromide, 3778
injection, 3780
oral solution, 3780
tablets, 3781
Pyridoxal
hydrochloride, 6196
5-phosphate, 6196
Pyridoxamine dihydrochloride, 6196
Pyridoxine hydrochloride, 3782
injection, 3783
tablets, 3784
1-(2-Pyridylazo)-2-naphthol, 6196
3-(2-Pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′, 5″-disulfonic acid, disodium salt, 6196
Pyrilamine maleate, 3785
tablets, 3786
Pyrimethamine, 3786
and sulfadoxine tablets, 4155
oral suspension, 3787
tablets, 3787
Pyrogallol, 6196
TS, alkaline, 6234
Pyrogen test ⟨151⟩, 6577
Pyroxylin, 3788
Pyrrole, 6196
Pyruvic acid, 6196
Pyrvinium pamoate, 3789
oral suspension, 3789
tablets, 3790

Q

Quality assurance in pharmaceutical compounding ⟨1163⟩, 7979
Quality attributes of tablets labeled as having a functional score ⟨705⟩, 6944
Quality of biotechnological products
analysis of the expression construct in cells used for production of r-DNA derived protein products ⟨1048⟩, 7452
stability testing of biotechnological/biological products ⟨1049⟩, 7454
Quazepam, 3790
tablets, 3791
Quercetin, 5202
Quetiapine
tablets, 3792
extended-release tablets, 3795
Quetiapine fumarate, 3800
Quinaldine red, 6221
TS, 6234
Quinapril
hydrochloride, 3802
and hydrochlorothiazide tablets, 3803
tablets, 3806
Quinhydrone, 6197
Quinidine gluconate, 3807
injection, 3809
extended-release tablets, 3810
Quinidine sulfate, 3812
capsules, 3813
oral suspension, 3814
tablets, 3814
extended-release tablets, 3816
Quinine sulfate, 3818
capsules, 3819
tablets, 3821
Quinone, 6197
TS, 6234

R

Rabeprazole
sodium, 3822
Rabies
immune globulin, 3824
Racemethionine, 5997
Racemic
calcium pantothenate, 708
Racepinephrine, 3824
hydrochloride, 3826
inhalation solution, 3825
Ractopamine hydrochloride
suspension, 3826
Radiation sterilization ⟨1229.10⟩, 8228
Radioactivity ⟨821⟩, 7094
Radioactivity—theory and practice ⟨1821⟩, 8604

Radiopharmaceuticals

C 13, urea, 766
C 13, urea for oral solution, 767
C 14, urea capsules, 768
Cr 51, sodium chromate injection, 992
F 18, fludeoxyglucose injection, 1946
F 18, sodium fluoride injection, 1950
Ga 67 injection, gallium citrate, 2088
Indium In 111 capromab pendetide injection, 2318
Indium In 111 chloride solution, 2318
Indium In 111 oxyquinoline solution, 2320
Indium In 111 pentetate injection, 2320
Indium In 111 pentetreotide injection, 2321
I 123, iobenguane injection, 2365
I 123, sodium iodide capsules, 2365
I 123, sodium iodide solution, 2366
I 125, iodinated albumin injection, 2367
I 125, iothalamate sodium injection, 2367
I 131, iodinated albumin injection, 2368
I 131, sodium iodide capsules, 2368
I 131, sodium iodide solution, 2368
N 13, ammonia injection, 3183
Rubidium chloride Rb 82 injection, 3958
Samarium Sm 153 lexidronam injection, 3983
Sr 89 injection, strontium chloride, 4126
Technetium Tc 99m albumin aggregated injection, 4226
Technetium Tc 99m bicisate injection, 4227
Technetium Tc 99m disofenin injection, 4227
Technetium Tc 99m exametazime injection, 4228
Technetium Tc 99m mebrofenin injection, 4229
Technetium Tc 99m medronate injection, 4230
Technetium Tc 99m mertiatide injection, 4231
Technetium Tc 99m oxidronate injection, 4232
Technetium Tc 99m pentetate injection, 4232
Technetium Tc 99m pertechnetate injection, sodium, 4233
Technetium Tc 99m pyrophosphate injection, 4234
Technetium Tc 99m (pyro- and trimeta-) phosphates injection, 4235
Technetium Tc 99m red blood cells injection, 4235
Technetium Tc 99m sestamibi injection, 4236
Technetium Tc 99m succimer injection, 4237
Technetium Tc 99m sulfur colloid injection, 4238
Technetium Tc 99m tetrofosmin injection, 4238
Thallous chloride Tl 201 injection, 4318
Xenon Xe 133, 4656
Yttrium Y 90 ibritumomab tiuxetan injection, 4665

Radiopharmaceuticals—preparation, compounding, dispensing, and repackaging ⟨825⟩, 7109
Raloxifene hydrochloride, 3828
tablets, 3830
Raltegravir
chewable tablets, 3835
tablets, 3834
Raltegravir potassium, 3832
Raman spectroscopy ⟨1120⟩, 7856
Ramipril, 3837
capsules, 3839
tablets, 3841
Ranitidine
hydrochloride, 3843
injection, 3845
in sodium chloride injection, 3848
oral solution, 3846
tablets, 3847
Rapeseed oil
fully hydrogenated, 5998
superglycerinated fully hydrogenated, 5999
Rapid sterility testing of short life products a risk based approach ⟨1071⟩, 7602
Rat tail collagen, 6153
Rauwolfia serpentina, 3849
powdered, 3850
tablets, 3851
Rayon, 6197
purified, 3851
Rb 82
injection, rubidium chloride, 3958
Readily carbonizable substances test ⟨271⟩, 6660
Reagents, 6128
arsenic in, 6128
boiling or distilling range for, 6128
chloride in, 6129
flame photometry for, 6129
general tests for, 6128
heavy metals in, 6130
indicators and solutions, 6127
insoluble matter in, 6130

www.webofpharma.com

Reagents *(continued)*
loss on drying for, 6131
nitrate in, 6131
nitrogen compounds in, 6131
phosphate in, 6131
residue on ignition in, 6131
sulfate in, 6131
Rectal solution
aminophylline, 246
sodium phosphates, 4090
Red
80, direct, 6197
phosphorus, 6197
Red-cell lysing agent, 6197
Red clover
aerial parts isoflavone aglycones dry extract, 5206
Reference standards
USP ⟨11⟩, 6442
Reference tables, 6259
Alcoholometric, 6346
Atomic weights, 6344
Container specifications for capsules and tablets, 6259
Description and relative solubility of USP and NF articles, 6273
Intrinsic viscosity table, 6349
Relative atomic masses and half-lives of selected radionuclides, 6345
Solubilities, 6334
Refractive index ⟨831⟩, 7137
Rehydration salts, oral, 3851
Relative atomic masses and half-lives of selected radionuclides, 6345
Repaglinide, 3854
tablets, 3856
Resazurin (sodium), 6197
Reserpine, 3857
and chlorothiazide tablets, 3859
tablets, 3858
Residual DNA testing ⟨509⟩, 6748
Residual host cell protein measurement in biopharmaceuticals ⟨1132⟩, 7900
Residual solvents ⟨467⟩, 6712
Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures, 8404
Residue on ignition ⟨281⟩, 6660
Residue on ignition in reagents, 6131

Resin

Anion-exchange, 50- to 100-mesh, styrene-divinylbenzene, 6138, 6208
Anion-exchange, chloromethylated polystyrene-divinylbenzene, 6138, 6151
Anion-exchange, strong, lightly cross-linked, in the chloride form, 6138
Anion-exchange, styrene-divinylbenzene, 6138
Capsicum oleoresin, 730
Carboxylate (sodium form) cation-exchange (50- to 100-mesh), 6148
Cation-exchange, 6149
Cation-exchange, carboxylate (sodium form) 50- to 100-mesh, 6148, 6149
Cation-exchange, polystyrene, 6149, 6192
Cation-exchange, styrene-divinylbenzene, 6149
Cation-exchange, styrene-divinylbenzene, strongly acidic, 6149, 6208
Cation-exchange, sulfonic acid, 6149, 6209
Chloromethylated polystyrene-divinylbenzene anion-exchange, 6151
Cholestyramine, 989
Ion-exchange, 6173
Podophyllum, 3582
Podophyllum topical solution, 3582
Polystyrene cation-exchange, 6192
Styrene-divinylbenzene anion-exchange, 50- to 100-mesh, 6208
Styrene-divinylbenzene cation-exchange, strongly acidic, 6208
Sulfonic acid cation-exchange, 6209

Resorcinol, 3861, 6197
monoacetate, 3862
ointment, compound, 3861
and sulfur topical suspension, 3862
TS, 6234
6*Z*-retinoic acid, 6197
Retinyl palmitate, 6197
Reverse transcriptase, 6197
Rheometry ⟨1911⟩, 8679
Rhodamine 6G, 6197
Rhodamine B, 6197
Rhodiola crenulata
root and rhizome dry extract, 5215
root and rhizome powder, 5216
Rhodiola crenulata
root and rhizome, 5213
Rhodiola rosea, 5218
capsules, 5224
extract, 5221
powdered, 5220
tablets, 5227
tincture, 5223
Ribavirin, 3863
capsules, 3863
for inhalation solution, 3865
tablets, 3866
Riboflavin, 3869
assay ⟨481⟩, 6735
injection, 3869
5′-phosphate sodium, 3870
tablets, 3870
Ribonuclease inhibitor, 6197
Ribose, 5229
Rifabutin, 3872
capsules, 3873
oral suspension, 3874
Rifampin, 3875
capsules, 3876
for injection, 3877
and isoniazid capsules, 3878
isoniazid, pyrazinamide, and ethambutol hydrochloride tablets, 3881
isoniazid, and pyrazinamide tablets, 3879
oral suspension, 3877
Riluzole, 3882
tablets, 3883
Rimantadine hydrochloride, 3884
tablets, 3885
Rimexolone, 3886
ophthalmic suspension, 3887
Ringer's
and dextrose injection, 3889
and dextrose injection, half-strength lactated, 3895
and dextrose injection, lactated, 3893
and dextrose injection, modified lactated, 3898
injection, 3887
injection, lactated, 3891
irrigation, 3900
lactated, and dextrose injection, potassium chloride in, 3610
Risedronate sodium, 3900
tablets, 3902
Risperidone, 3904
oral solution, 3905
tablets, 3907
orally disintegrating tablets, 3908
Ritodrine hydrochloride, 3910
injection, 3911
tablets, 3911
Ritonavir, 3912
capsules, 3914
and lopinavir oral solution, 2666
and lopinavir tablets, 2670
oral solution, 3917
tablets, 3920
Rivastigmine, 3922
Rivastigmine tartrate, 3924
capsules, 3925
Rizatriptan benzoate, 3927
tablets, 3928
orally disintegrating tablets, 3930
Rocuronium bromide, 3932
Root and rhizome
dry extract capsules and eleuthero, 4972
dry extract and *Rhodiola crenulata*, 5215
dry extract tablets and eleuthero root, 4973
powder capsules and eleuthero, 4976
powder and *Rhodiola crenulata*, 5216
and *Rhodiola crenulata*, 5213
Ropinirole
tablets, 3934
extended-release tablets, 3936
Ropinirole hydrochloride, 3940
Ropivacaine hydrochloride, 3943
injection, 3945
Rose
bengal sodium, 6197
oil, 6001
water ointment, 3946
water, stronger, 6001
Rosiglitazone maleate, 3946
Rosuvastatin
tablets, 3950
Rosuvastatin calcium, 3948
Rotigotine, 3953
Rotigotine transdermal system, 3955
Roxarsone, 3957
Rubidium chloride Rb 82 injection, 3958
Rufinamide, 3959
tablets, 3960
Rules and procedures, xxviii
Ruthenium red, 6198
TS, 6234
Rutin, 5235

S

Sabinene, 6198
Saccharin, 6001
calcium, 3963
sodium, 3965
sodium oral solution, 3967
sodium tablets, 3968
Saccharose, 6198
Safflower oil, 3968
Safranin O, 6198
St. John's wort flowering top, 5237
dry extract capsules, 5242

www.webofpharma.com

St. John's wort flowering top *(continued)*
extract, dry, 5240
powder, 5238
dry extract tablets, 5244
Salicylaldazine, 6198
Salicylaldehyde, 6198
Salicylamide, 3969
Salicylic acid, 3970, 6198
and benzoic acids ointment, 521
collodion, 3972
gel, 3972
plaster, 3973
topical foam, 3972
and zinc paste, 4690
Saline TS, 6202, 6234
pyrogen-free, 6234
Salix species bark, 5245
dry extract, 5247
powder, 5249
Salmeterol
fluticasone propionate, inhalation aerosol, 2005
fluticasone propionate, inhalation powder, 2010
inhalation powder, 3973
Salmeterol xinafoate, 3978
Salsalate, 3980
capsules, 3981
tablets, 3982
Salt
octanesulfonic acid sodium, 6204
Salts of organic nitrogenous bases ⟨501⟩, 6741
Samarium Sm 153 lexidronam injection, 3983
Sand
standard 20- to 30-mesh, 6198, 6207
washed, 6198, 6218
Saquinavir mesylate, 3983
capsules, 3984
Sargramostim, 3985
for injection, 3988
Sawdust, purified, 6198
Saw palmetto, 5251
capsules, 5259
extract, 5256
powdered, 5254
Scaffold
bovine dermis, 3990
human dermis, 3998
porcine bladder, 4001
silk fibroin, 4006
Scaffold human amniotic membrane allograft, 3993
Scandium oxide, 6198
Scanning electron microscopy ⟨1181⟩, 8025
Schizochytrium oil, 5266
capsules, 5269
Schweitzer's reagent, 6234
Scopolamine hydrobromide, 4010
injection, 4012
ophthalmic solution, 4013
tablets, 4013
Screening for undeclared drugs and drug analogues ⟨2251⟩, 8725
S designations, 6198
Secobarbital, 4013
sodium, 4014
sodium capsules, 4015
Secondary butyl alcohol, 6198
Selamectin, 4016
Selegiline hydrochloride, 4017
capsules, 4018
tablets, 4020
Selegiline hydrochloride compounded topical gel, 4021
Selenious acid, 4022, 6198
injection, 4022
Selenium, 6199
sulfide, 4023
sulfide topical suspension, 4023
Selenium ⟨291⟩, 6661
Selenomethionine, 5272, 6199
Semisolid drug products—performance tests ⟨1724⟩, 8473
Senna
fluidextract, 4025
leaf, 4023
pods, 4025
oral solution, 4026
Sennosides, 4026
tablets, 4028
Sensitization testing ⟨1184⟩, 8035
Serine, 4029
Sertraline
hydrochloride, 4030
hydrochloride oral solution, 4032
hydrochloride tablets, 4033
Sesame oil, 6003
Sevoflurane, 4036
Shear cell methodology for powder flow testing ⟨1063⟩, 7566
Shellac, 6005
Significant change guide for bulk pharmaceutical excipients ⟨1195⟩, 8049
Sildenafil
tablets, 4038
Sildenafil citrate, 4039
oral suspension, 4040
Silica
calcined diatomaceous, 6199
chromatographic, silanized, flux-calcined, acid-washed, 6199
colloidal, hydrophobic, 6007
dental-type, 6006
gel, 6199
gel, binder-free, 6152, 6199
gel, chromatographic, 6152, 6199
gel-impregnated glass microfiber sheet, 6199
gel mixture, chromatographic, 6152, 6199
gel mixture, chromatographic, with chemically bound amino groups, 6199
gel mixture, dimethylsilanized, chromatographic, 6199
gel mixture, octadecylsilanized chromatographic, 6199
gel mixture, octylsilanized, chromatographic, 6199
gel, octadecylsilanized chromatographic, 6199
gel, porous, 6199
microspheres, 6199
Silica gel, pre-coated plates, with fluorescence indicator F254, 6199
Siliceous earth
chromatographic, 6152, 6199
chromatographic, silanized, 6152, 6200
purified, 6008
Silicic
acid, 6200
acid—impregnated glass microfilament sheets with fluorescent indicator, 6200
Silicon
carbide, 6200
dioxide, 6009
dioxide colloidal, 6009
Silicone
75 percent phenyl, methyl, 6200
Silicotungstic acid, *n*-hydrate, 6200
Silicified
microcrystalline cellulose, 5699
Silver
diethyldithiocarbamate, 6200
diethyldithiocarbamate TS, 6234
nitrate, 4041, 6200
nitrate ophthalmic solution, 4041
nitrate, tenth-normal (0.1 N), 6234, 6247
nitrate, toughened, 4042
nitrate TS, 6234
oxide, 6200
Silver–ammonia–nitrate TS, 6234
Silver–ammonium nitrate TS, 6234
Silver nitrate
0.002 N VS, 6247
0.05 N VS, 6247
Silver sulfate, 6200
Simethicone, 4042
alumina, magnesia, and calcium carbonate chewable tablets, 170
alumina and magnesia oral suspension, 173
alumina and magnesia chewable tablets, 174
calcium carbonate and magnesia chewable tablets, 689
capsules, 4043
emulsion, 4044
and magaldrate chewable tablets, 2707
and magaldrate oral suspension, 2706
oral suspension, 4045
tablets, 4045
Simulated gastric fluid TS, 6234
Simulated intestinal fluid TS, 6234
Simvastatin, 4046
tablets, 4047
Single-steroid assay ⟨511⟩, 6750
Sipuleucel-T, 4049
Sitagliptin
phosphate, 4052
tablets, 4051
β-Sitosterol, 6200
Six-month implementation guideline, ii
Sm 153 lexidronam injection, samarium, 3983
Soda lime, 6010, 6201
Sodium phosphate
tribasic, anhydrous, 6205
Sodium, 6201
acetate, 4053, 6201
acetate, anhydrous, 6138, 6201
acetate injection, 4054
acetate solution, 4055
acetate TS, 6234
alendronate, tablets, 120
alginate, 6011
alizarinsulfonate, 6201
alizarinsulfonate TS, 6235
aminoacetate TS, 6235
ammonium phosphate, 6201
arsenate, 6201
arsenite, 6201
arsenite, twentieth-molar (0.05 M), 6247
ascorbate, 4055
azide, 6201
benzoate, 6011
benzoate and caffeine injection, 664
bicarbonate, 4056, 6201
bicarbonate injection, 4060
bicarbonate and magnesium carbonate for oral suspension, 2712
bicarbonate oral powder, 4061
bicarbonate tablets, 4062
biphenyl, 6201

www.webofpharma.com

Sodium *(continued)*
biphosphate, 6202
bisulfite, 6202
bisulfite TS, 6235
bitartrate, 6202
bitartrate TS, 6235
borate, 6012, 6202
borohydride, 6202
bromide, 4062, 6202
bromide injection, veterinary, 4063
bromide oral solution, veterinary, 4063
butyrate, 4064
caprylate, 6013
carbonate, 6014, 6202
carbonate, anhydrous, 6138, 6202
carbonate, citric acid, and magnesium oxide irrigation, 1044
carbonate, monohydrate, 6202
carbonate TS, 6235
carboxymethylcellulose, 773
carboxymethylcellulose, and microcrystalline cellulose, 5698
carboxymethylcellulose, paste, 774
carboxymethylcellulose, tablets, 774
carboxymethylcellulose, 12, 5684
cefazolin, 824
cefmetazole, 842
cefoperazone, 845
cefotaxime, 850
cetostearyl sulfate, 6014
chloride, 4064, 6202
chloride and dextrose injection, 1323
chloride and fructose injection, 2049
chloride inhalation solution, 4068
chloride injection, 4066
chloride injection, bacteriostatic, 4067
chloride injection, dextran 40 in, 1312
chloride injection, dextran 70 in, 1316
chloride injection, mannitol in, 2737
chloride injection, potassium chloride in, 3612
chloride injection, potassium chloride in dextrose injection and, 3608
chloride injection, ranitidine in, 3848
chloride irrigation, 4067
chloride ophthalmic ointment, 4068
chloride ophthalmic solution, 4068
chloride solution, isotonic, 6202
chloride tablets, 4068
chloride tablets for solution, 4069
chloride TS, alkaline, 6235
cholate hydrate, 6202
chromate, 6202
chromate, Cr 51 injection, 992
chromotropate, 6202
cilastatin, 1001
citrate, 4069
citrate and citric acid oral solution, 4069
citrate dihydrate, 6203
citrate TS, 6235
citrate TS, alkaline, 6235
cobaltinitrite, 6203
cobaltinitrite TS, 6235
cyanide, 6203
dalteparin, 1230
1-decanesulfonate, 6203
dehydroacetate, 6016
desoxycholate, 6203
dichromate, 6203
diethyldithiocarbamate, 6203
2,2-dimethyl-2-silapentane-5-sulfonate, 6203
dithionite, 6203
dodecyl sulfate, 6203, 6204
ethylparaben, 5773
ferrocyanide, 6203
fluconazole, chloride injection, 1909
fluorescein, 6203
fluoride, 4071, 6203
fluoride and acidulated phosphate topical solution, 4074
fluoride F18 injection, 1950
fluoride and phosphoric acid gel, 4075
fluoride oral solution, 4072
fluoride tablets, 4073
fluoride TS, 6235
formaldehyde sulfoxylate, 6017
gluconate, 4076
glycocholate, 6203
1-heptanesulfonate, 6203
1-heptanesulfonate, monohydrate, 6203
1-hexanesulfonate, 6203
1-hexanesulfonate, monohydrate, 6203
hydrogen sulfate, 6203
hydrosulfite, 6203
hydrosulfite TS, alkaline, 6226, 6235
hydroxide, 6018, 6204
hydroxide, alcoholic, tenth-normal (0.1 N), 6248
hydroxide, normal (1 N), 6248
hydroxide TS, 6235
hydroxide TS 2, 6235
hydroxide TS 3, 6235
hypobromite TS, 6235
hypochlorite solution, 4076, 6235
hypochlorite topical solution, 4077
hypochlorite TS, 6235
iodate, 6204
iodide, 4077
iodide I 123 capsules, 2365
iodide I 123 solution, 2366
iodide I 131 capsules, 2368
iodide I 131 solution, 2368
iodohydroxyquinolinesulfonate TS, 6235
lactate injection, 4078
lactate solution, 4078
lauryl sulfate, 6019, 6204
low-substituted carboxymethylcellulose, 5682
metabisulfite, 6020, 6204
metaperiodate, 6204
methoxide, 6204
methoxide, half-normal (0.5 N) in methanol, 6248
methoxide, tenth-normal (0.1 N) in toluene, 6248
molybdate, 6204
monofluorophosphate, 4079
montelukast, oral granules, 3012
montelukast, tablets, 3015
montelukast, chewable tablets, 3018
mycophenolate, 3052
nitrate, 6204
nitrite, 4080, 6204
nitrite injection, 4082
nitrite, tenth-molar (0.1 M), 6249
nitroferricyanide, 6204
nitroferricyanide TS, 6235
nitroprusside, 4083
nitroprusside for injection, 4084
1-octanesulfonate, 6204
oxalate, 6204
(tri) pentacyanoamino ferrate, 6204
1-pentanesulfonate, 6186, 6205
1-pentanesulfonate, anhydrous, 6205
perchlorate, 6205
peroxide, 6205
pertechnetate Tc 99m injection, 4233
phenylbutyrate, 4085
phenylbutyrate oral suspension, 4086
phosphate, dibasic, 4087, 6205
phosphate, dibasic, anhydrous, 6138, 6205
phosphate, dibasic, dihydrate, 6205
phosphate, dibasic, dodecahydrate, 6205
phosphate, dibasic, heptahydrate, 6205
phosphate, dibasic, TS, 6235
phosphate, monobasic, 4088, 6181, 6205
phosphate, monobasic, anhydrous, 6205
phosphate, monobasic, dihydrate, 6205
phosphates injection, 4088
phosphates oral solution, 4090
phosphates rectal solution, 4090
phosphate, tribasic, 6021, 6205
phosphite pentahydrate, 6205
phosphotungstate TS, 6235
picosulfate, 4090
polystyrene sulfonate, 4092
polystyrene sulfonate suspension, 4092
and potassium bicarbonates and citric acid effervescent tablets for oral solution, 3598
propionate, 6022
pyrophosphate, 6205
pyruvate, 6205
rabeprazole, 3822
salicylate, 4093, 6205
salicylate tablets, 4094
selenite, 6206
starch glycolate, 6022
stearate, 6024
stearyl fumarate, 6025
sulfate, 4095, 6206
sulfate, anhydrous, 6138, 6206
sulfate decahydrate, 6206
sulfate injection, 4096
sulfide, 4096, 6206
sulfide topical gel, 4096
sulfide TS, 6235
sulfite, 6027, 6206
sulfite, anhydrous, 6138, 6206
p-sulfophenylazochromotropate, 6206
tartrate, 6028, 6206
tartrate TS, 6235
tetraphenylborate, 6206
tetraphenylboron, 6206
tetraphenylboron, fiftieth-molar (0.02 M), 6249
tetraphenylboron TS, 6236
thioglycolate, 6206
thioglycolate TS, 6236
thiosulfate, 4097, 6207
thiosulfate injection, 4097
thiosulfate, tenth-normal (0.1 N), 6236, 6249
thiosulfate TS, 6236
L-thyroxine, 6207
3-(trimethylsilyl)-1-propane sulfonate, 6203, 6207
tungstate, 6207
Sodium benzoate compounded oral solution, 4056
Sodium bicarbonate compounded injection, 4061
Sodium butyrate, 6202
Sodium chloride
TS, 6235
0.5 M TS, 6235
0.1 N Sodium chloride TS, 6235
Sodium 1-dodecanesulfonate, 6203
Sodium ferrous citrate, 5273
Sodium hydroxide
0.0025 N TS, 6235
0.2 N, TS, 6235
0.02 N TS, 6235

www.webofpharma.com

Sodium hydroxide *(continued)*
10 N TS, 6235
2.5 N TS, 6235
2 N TS, 6235
5 N, TS, 6235
0.025 N VS, 6247
0.1 N VS, 6248
0.01 N VS, 6247
0.5 N VS, 6247
50 percent sodium hydroxide TS, 6235
Sodium oleate, 6204
Sodium phenylbutyrate, 4085
Sodium phosphate, 6205
Sodium phosphates compounded injection, 4089
Sodium propionate, 6205
Sodium succinate, 6026
Sodium taurocholate, 6206
Sodium taurodeoxycholate, 6206
Sodium thiosulfate
0.002 N VS, 6249
0.01 M VS, 6249
Solubilities, 6334
Soluble starch, 6207

Solution

Acetaminophen and codeine phosphate oral, 54
Acetaminophen, dextromethorphan hydrobromide, doxylamine succinate, and pseudoephedrine hydrochloride oral, 67
Acetaminophen for effervescent oral, 42
Acetaminophen oral, 41
Acetic acid otic, 78
Acetylcholine chloride for ophthalmic, 81
Acetylcysteine, 82
Acidulated phosphate and sodium fluoride topical, 4074
Aluminum acetate topical, 181
Aluminum chlorohydrate, 183
Aluminum dichlorohydrate, 186
Aluminum sesquichlorohydrate, 191
Aluminum subacetate topical, 193
Aluminum sulfate and calcium acetate for topical, 194
Aluminum sulfate and calcium acetate tablets for topical, 195
Aluminum zirconium octachlorohydrate, 196
Aluminum zirconium octachlorohydrex gly, 198
Aluminum zirconium pentachlorohydrate, 199
Aluminum zirconium pentachlorohydrex gly, 201
Aluminum zirconium tetrachlorohydrate, 203
Aluminum zirconium tetrachlorohydrex gly, 204
Aluminum zirconium trichlorohydrate, 206
Aluminum zirconium trichlorohydrex gly, 207
Amantadine hydrochloride oral, 212
Aminocaproic acid oral, 236
Aminophylline oral, 245
Aminophylline rectal, 246
Ammonia, diluted, 6136
Ammonia, strong, 5616
Amprolium oral, 331
Anticoagulant citrate dextrose, 345
Anticoagulant citrate phosphate dextrose, 347
Anticoagulant citrate phosphate dextrose adenine, 348
Anticoagulant heparin, 2194
Anticoagulant sodium citrate, 350
Antipyrine and benzocaine otic, 352
Antipyrine, benzocaine, and phenylephrine hydrochloride otic, 354
Apraclonidine ophthalmic, 359
Aromatic elixir, 5624
Ascorbic acid oral, 384
Aspirin effervescent tablets for oral, 395
Atenolol oral, 407
Atropine sulfate ophthalmic, 432
Benoxinate hydrochloride ophthalmic, 499
Benzaldehyde elixir, compound, 5634
Benzalkonium chloride, 5636
Benzethonium chloride topical, 501
Benzocaine, butamben, and tetracaine hydrochloride topical, 517
Benzocaine otic, 510
Benzocaine topical, 511
Betamethasone oral, 542
Betaxolol ophthalmic, 558
Bethanechol chloride oral, 563
Bromodiphenhydramine hydrochloride and codeine phosphate oral, 600
Bromodiphenhydramine hydrochloride oral, 599
Brompheniramine maleate and pseudoephedrine sulfate oral, 603
Brompheniramine maleate oral, 602
Buprenorphine compounded buccal, veterinary, 615
Butabarbital sodium oral, 642
Caffeine citrate oral, 663
Calcitonin salmon nasal, 675
Calcium hydroxide topical, 702
Captopril oral, 734
Carbachol intraocular, 739
Carbachol ophthalmic, 739
Carbamide peroxide topical, 747
Carbol-fuchsin topical, 765
C 13 for oral, urea, 767
Carteolol hydrochloride ophthalmic, 789
Cetylpyridinium chloride topical, 923
Chloral hydrate oral, 924
Chloramphenicol for ophthalmic, 930
Chloramphenicol ophthalmic, 929
Chloramphenicol oral, 930
Chloramphenicol otic, 931
Chlorhexidine gluconate, 948
Chlorpheniramine maleate and pseudoephedrine hydrochloride oral, 973
Chlorpheniramine maleate oral, 971
Cholecalciferol, 987
Chymotrypsin for ophthalmic, 994
Ciprofloxacin ophthalmic, 1015
Clindamycin hydrochloride oral, 1066
Clindamycin palmitate hydrochloride for oral, 1068
Clindamycin phosphate topical, 1072
Clobetasol propionate topical, 1082
Clotrimazole topical, 1122
Cloxacillin sodium for oral, 1130
Coal tar topical, 1133
Cocaine hydrochloride tablets for topical, 1134
Codeine sulfate oral, 1141
Cromolyn sodium ophthalmic, 1185
Cupriethylenediamine hydroxide, 1.0 M, 6154
Cyclopentolate hydrochloride ophthalmic, 1201
Cyclosporine oral, 1216
Cyproheptadine hydrochloride oral, 1219
Demecarium bromide ophthalmic, 1247
Dexamethasone elixir, 1286
Dexamethasone oral, 1288
Dexamethasone sodium phosphate ophthalmic, 1296
Dexbrompheniramine maleate and pseudoephedrine sulfate oral, 1298
Dexchlorpheniramine maleate oral, 1301
Dextromethorphan hydrobromide oral, 1321
Diatrizoate meglumine and diatrizoate sodium, 1327
Diatrizoate sodium, 1329
Dichlorophenol–indophenol, standard, 6240
Dicyclomine hydrochloride oral, 1361
Diethyltoluamide topical, 1373
Digoxin oral, 1385
Dihydrotachysterol oral, 1392
Diltiazem hydrochloride oral, 1405
Dimenhydrinate oral, 1410
Dimethyl sulfoxide topical, 1414
Diphenhydramine hydrochloride oral, 1429
Diphenoxylate hydrochloride and atropine sulfate oral, 1438
Dipivefrin hydrochloride ophthalmic, 1442
Docusate sodium, 1480
Dolasetron mesylate oral, 1485
Dorzolamide hydrochloride and timolol maleate ophthalmic, 1499
Doxepin hydrochloride oral, 1510
Doxylamine succinate oral, 1546
Dyclonine hydrochloride topical, 1571
Dyphylline and guaifenesin oral, 1575
Dyphylline oral, 1573
Ecamsule, 1576
Echothiophate iodide for ophthalmic, 1579
Emedastine ophthalmic, 1610
Ephedrine sulfate oral, 1644
Epinephrine bitartrate for ophthalmic, 1652
Epinephrine bitartrate ophthalmic, 1651
Epinephrine ophthalmic, 1649
Epinephryl borate ophthalmic, 1652
Ergocalciferol oral, 1667
Ergoloid mesylates oral, 1671
Erythromycin topical, 1690
Escitalopram oral, 1706
Ethosuximide oral, 1776
Fehling's, 6229
Ferric ammonium citrate for oral, 290
Ferric subsulfate, 1852
Ferrous gluconate oral, 1860
Ferrous sulfate oral, 1863
Fluocinolone acetonide topical, 1936
Fluocinonide topical, 1939
(+)-1-(9-Fluorenyl) ethyl chloroformate, 6166
Fluorescein sodium and benoxinate hydrochloride ophthalmic, 1944
Fluorescein sodium and proparacaine hydrochloride ophthalmic, 1945
Fluorouracil topical, 1957
Fluoxetine oral, 1961
Fluphenazine hydrochloride elixir, 1971
Fluphenazine hydrochloride oral, 1973
Flurbiprofen sodium ophthalmic, 1982
Formaldehyde, 2035, 6167, 6229
Furosemide oral, 2055
Gentamicin sulfate and betamethasone acetate ophthalmic, 2102

www.webofpharma.com

Solution *(continued)*
Gentamicin sulfate and betamethasone valerate otic, 2104
Gentamicin topical, 2105
Gentamicin sulfate ophthalmic, 2102
Gentian violet topical, 2109
Glutaral disinfectant, 5789
Glycerin ophthalmic, 2133
Glycerin oral, 2134
Guaifenesin and codeine phosphate oral, 2172
Guaifenesin oral, 2171
Halazone tablets for, 2182
Halcinonide topical, 2186
Haloperidol oral, 2189
Heparin lock flush, 2194
Homatropine hydrobromide ophthalmic, 2208
Hydralazine hydrochloride oral, 2215
Hydrocortisone and acetic acid otic, 2235
Hydrogen peroxide, 6171
Hydrogen peroxide topical, 2249
Hydroquinone topical, 2255
Hydroxyamphetamine hydrobromide ophthalmic, 2258
Hydroxyzine hydrochloride oral, 2266
Hyoscyamine sulfate elixir, 2276
Hyoscyamine sulfate oral, 2277
Hypromellose ophthalmic, 2281
Idoxuridine ophthalmic, 2293
Indium In 111 chloride, 2318
Indium In 111 oxyquinoline, 2320
Iodine, strong, 2363
Sodium iodide I 123, 2366
Sodium iodide I 131, 2368
Iodine topical, 2362
Ipecac oral, 2397
Ipratropium bromide and albuterol sulfate inhalation, 2399
Isoniazid oral, 2426
Isosorbide oral, 2440
Ivermectin topical, 2476
Lactulose, 2522
Lead, standard, 6236
Levalbuterol inhalation, 2576
Levobunolol hydrochloride ophthalmic, 2595
Levocarnitine oral, 2600
Levofloxacin oral, 2608
Lidocaine hydrochloride topical, 2627
Lincomycin oral, 2632
Lithium oral, 2648
Locke-Ringer's, 6231
Loperamide hydrochloride oral, 2661
Loratadine oral, 2674
Mafenide acetate for topical, 2704
Magnesium carbonate and citric acid for oral, 2710
Magnesium carbonate, citric acid, and potassium citrate for oral, 2711
Manganese chloride for oral, 2732
Magnesium citrate for oral, 2715
Magnesium citrate oral, 2714
Maltitol, 5871
Meperidine hydrochloride oral, 2784
Metaproterenol sulfate oral, 2813
Methadone hydrochloride oral, 2838
Methoxsalen topical, 2865
Methylcellulose ophthalmic, 2876
Methylcellulose oral, 2876
Metoclopramide oral, 2910
Metoprolol tartrate oral, 2925
Mibolerone oral, 2945
Minoxidil topical, 2975
Mometasone furoate topical, 3005
Moxifloxacin ophthalmic, 3034
Myrrh topical, 3057
Naphazoline hydrochloride ophthalmic, 3076
Naphazoline hydrochloride and pheniramine maleate ophthalmic, 3076
Neomycin and polymyxin B sulfates and gramicidin ophthalmic, 3123
Neomycin and polymyxin B sulfates and hydrocortisone otic, 3124
Neomycin and polymyxin B sulfates for irrigation, 3114
Neomycin and polymyxin B sulfates ophthalmic, 3115
Neomycin sulfate and dexamethasone sodium phosphate ophthalmic, 3106
Neomycin sulfate oral, 3103
Nickel standard TS, 6232
Nitrofurazone topical, 3183
Nitromersol topical, 3190
Nortriptyline hydrochloride oral, 3216
Ofloxacin ophthalmic, 3231
Olopatadine hydrochloride ophthalmic, 3249
Ondansetron, oral, 3266
Oral, containing at least three of the following—acetaminophen and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, 56
Oxybutynin chloride oral, 3328
Oxycodone hydrochloride oral, 3337
Oxymetazoline hydrochloride ophthalmic, 3350
Papain tablets for topical, 3396
Paromomycin oral, 3409
Penicillin G potassium for oral, 3439
Penicillin V potassium for oral, 3456
Phenobarbital oral, 3498
Phenol, topical, camphorated, 3502
Phenylephrine hydrochloride ophthalmic, 3519
Physostigmine salicylate ophthalmic, 3536
Pilocarpine hydrochloride ophthalmic, 3543
Pilocarpine nitrate ophthalmic, 3545
Podophyllum resin topical, 3582
Polyethylene glycol 3350 and electrolytes for oral, 3587
Polymyxin B sulfate and hydrocortisone otic, 3592
Polymyxin B sulfate and trimethoprim ophthalmic, 3592
Potassium bicarbonate effervescent tablets for oral, 3596
Potassium bicarbonate and potassium chloride for effervescent oral, 3597
Potassium bicarbonate and potassium chloride effervescent tablets for oral, 3597
Potassium bicarbonate, potassium chloride, and potassium citrate effervescent tablets for oral, 3609
Potassium bromide oral, veterinary, 3600
Potassium chloride for oral, 3605
Potassium chloride oral, 3604
Potassium citrate and citric acid oral, 3617
Potassium gluconate and potassium chloride for oral, 3621
Potassium gluconate and potassium chloride oral, 3621
Potassium gluconate, potassium citrate, and ammonium chloride oral, 3622
Potassium gluconate and potassium citrate oral, 3622
Potassium gluconate oral, 3619
Potassium iodide oral, 3624
Potassium nitrate, 3626
Potassium and sodium bicarbonates and citric acid effervescent tablets for oral, 3598
Povidone–iodine cleansing, 3635
Povidone–iodine topical, 3636
Prednisolone oral, 3661
Prednisolone sodium phosphate compounded oral, 3667
Prednisolone sodium phosphate ophthalmic, 3668
Prednisone oral, 3671
Prochlorperazine oral, 3697
Promazine hydrochloride oral, 3713
Promethazine and phenylephrine hydrochloride and codeine phosphate oral, 3724
Promethazine and phenylephrine hydrochloride oral, 3721
Promethazine hydrochloride oral, 3717
Proparacaine hydrochloride ophthalmic, 3739
Protein standard (8 g/dL), 6194
Pseudoephedrine hydrochloride, carbinoxamine maleate, and dextromethorphan hydrobromide oral, 3767
Pseudoephedrine hydrochloride oral, 3762
Pyridostigmine bromide oral, 3780
Ranitidine oral, 3846
Risperidone oral, 3905
Saccharin sodium oral, 3967
Scopolamine hydrobromide ophthalmic, 4013
Senna oral, 4026
Silver nitrate ophthalmic, 4041
Sodium acetate, 4055
Sodium bromide oral, veterinary, 4063
Sodium chloride, isotonic, 6202
Sodium chloride ophthalmic, 4068
Sodium chloride tablets for, 4069
Sodium citrate and citric acid oral, 4069
Sodium fluoride and acidulated phosphate topical, 4074
Sodium fluoride oral, 4072
Sodium hypochlorite, 4076, 6235, 6235
Sodium hypochlorite topical, 4077
Sodium lactate, 4078
Sodium phosphates oral, 4090
Sodium phosphates rectal, 4090
Sorbitol, 4101
Sorbitol noncrystallizing, 6036
Sorbitol sorbitan, 6038
Stavudine for oral, 4122
Sulfacetamide sodium ophthalmic, 4143
Sulfaquinoxaline oral, 4169
Terpin hydrate and codeine oral, 4284
Terpin hydrate oral, 4284
Tetracaine hydrochloride ophthalmic, 4299
Tetracaine hydrochloride topical, 4299
Tetracycline hydrochloride for topical, 4309
Tetrahydrozoline hydrochloride ophthalmic, 4315
Tetramethylammonium hydroxide, in methanol, 6211
Theophylline and guaifenesin oral, 4332
Theophylline oral, 4325
Theophylline sodium glycinate oral, 4334
Thiamine hydrochloride oral, 4338
Thiamine mononitrate oral, 4341
Thimerosal topical, 4347
Thioridazine hydrochloride oral, 4354
Thiothixene hydrochloride oral, 4361

www.webofpharma.com

Solution *(continued)*
Timolol maleate ophthalmic, 4389
Tobramycin ophthalmic, 4408
Tolnaftate topical, 4427
Travoprost ophthalmic, 4471
Tretinoin topical, 4479
Triamcinolone diacetate oral, 4491
Tricitrates oral, 4504
Trifluoperazine oral, 4511
Trihexyphenidyl hydrochloride oral, 4519
Trikates oral, 4521
Trimeprazine oral, 4522
Triprolidine hydrochloride oral, 4533
Triprolidine and pseudoephedrine hydrochlorides oral, 4534
Tropicamide ophthalmic, 4541
Valproic acid oral, 4574
Valrubicin intravesical, 4577
Vancomycin hydrochloride for oral, 4590
Vehicle for oral, 5913
Vehicle for oral, sugar free, 5913
Verapamil hydrochloride oral, 4609
Vitamins with minerals, water-soluble oral, 5543
Vitamins with minerals, oil- and water-soluble oral, 5462
Vitamins, oil- and water-soluble oral, 5409
Xanthan gum, 6104
Zidovudine oral, 4676
Zinc sulfate ophthalmic, 4693
Zinc sulfate oral, 4693

Solutions,
reagents, and indicators, 6127
Solvent hexane, 6207
Somatropin, 4098
for injection, 4099
Somatropin bioidentity tests ⟨126⟩, 6558
Sorbic acid, 6029
Sorbitan
monolaurate, 6030
monooleate, 6031
monopalmitate, 6032
monostearate, 6033
sesquioleate, 6033
sorbitol, solution, 6038
trioleate, 6034
Sorbitol, 6035, 6207
solution, 4101
solution noncrystallizing, 6036
sorbitan solution, 6038
Sotalol hydrochloride, 4102
oral suspension, 4104
tablets, 4105
Soybean oil, 4105
hydrogenated, 6040
Soy isoflavones
capsules, 5276
powdered extract, 5274
tablets, 5278
Spacers and valved holding chambers used with inhalation aerosols—characterization tests ⟨1602⟩ , 8411
Specific gravity ⟨841⟩, 7137
Specific surface area ⟨846⟩, 7139
Spectinomycin
hydrochloride, 4108
for injectable suspension, 4108
Spectrophotometric identification tests ⟨197⟩, 6593
Spironolactone, 4109
and hydrochlorothiazide oral suspension, 4113
and hydrochlorothiazide tablets, 4113
tablets, 4112
Spironolactone compounded
oral suspension, 4111
Spironolactone compounded, veterinary
oral suspension, 4111
Spirulina, 5279
tablets, 5283
Squalane, 6041
Sr 89 injection, strontium chloride, 4126
Stability considerations in dispensing practice ⟨1191⟩, 8044
Stachyose hydrate, 6207
Standard sand, 20- to 30-mesh, 6207
Stannous
chloride, 6041, 6207
chloride acid, stronger, TS, 6225, 6236
chloride acid TS, 6225, 6236
fluoride, 4114
fluoride gel, 4115
Stanozolol, 4117
tablets, 4118
Star anise
oil, 6043
Starch
corn, 6044
corn, pregelatinized hydroxypropyl, 6047
hydrolysate, hydrogenated, 6049
hydroxypropyl corn, 6045
iodate paper, 6222
iodide-free TS, 6236
iodide paper, 6222
iodide paste TS, 6236
modified, 6052
pea, 6053
pea, pregelatinized hydroxypropyl, 6056
potassium iodide TS, 6236
potassium iodide and, TS, 6234
potato, 6058, 6194, 6207
potato, pregelatinized hydroxypropyl, 6061
pregelatinized, 6063
pregelatinized modified, 6064
sodium, glycolate, 6022
soluble, 6207
soluble, purified, 6207
tapioca, 6066
topical, 4119
TS, 6236
wheat, 6066
Stavudine, 4119
capsules, 4120
for oral solution, 4122
Steam, pure, 4654
Steam sterilization by direct contact ⟨1229.1⟩, 8199
Stearic acid, 6068, 6207
purified, 6070
Stearoyl polyoxylglycerides, 6071
Stearyl alcohol, 6072, 6207

Sterile

Erythromycin ethylsuccinate, 1698
Erythromycin gluceptate, 1702
Erythromycin lactobionate, 1703
Pharmaceutical compounding—sterile preparations ⟨797⟩, 7037
Sterile product packaging—integrity evaluation ⟨1207⟩, 8079
Sterility assurance ⟨1211⟩, 8129
Sterility testing—validation of isolator systems ⟨1208⟩, 8113
Water, purified, 4654
Water for inhalation, 4652
Water for injection, 4653
Water for irrigation, 4653

Sterile product packaging—integrity evaluation ⟨1207⟩, 8079
Sterility
testing—validation of isolator systems ⟨1208⟩, 8113
tests ⟨71⟩, 6481
Sterility assurance ⟨1211⟩, 8129
Sterilization cycle development, 8235
Sterilization filtration of gases, 8238
Sterilization-in-place ⟨1229.13⟩, 8233
Sterilization of compendial articles ⟨1229⟩, 8194
Sterilizing filtration of liquids ⟨1229.4⟩, 8209
Stinging nettle, 5286
extract, powdered, 5290
powdered, 5288
Storax, 4123
Streptomycin
injection, 4125
for injection, 4125
sulfate, 4124
Stronger
ammonia water, 6207
cupric acetate TS, 6236
Strontium
acetate, 6207
chloride Sr 89 injection, 4126
hydroxide, 6208
Strychnine sulfate, 6208
Styrene-divinylbenzene
anion-exchange resin, 50- to 100-mesh, 6208
cation-exchange resin, strongly acidic, 6208
copolymer beads, 6208
Subvisible particulate matter in therapeutic protein injections ⟨787⟩, 7014
Succinic acid, 6074, 6208
Succinylcholine chloride, 4127
injection, 4129
Sucralfate, 4130
tablets, 4131
Sucralose, 6074
Sucrose, 6076
octaacetate, 6077
palmitate, 6078
stearate, 6079
Sudan
III, 6208
III TS, 6236
IV, 6208
IV TS, 6236
Sufentanil citrate, 4132
injection, 4133
Sugar
compressible, 6081
confectioner's, 6082
free suspension structured vehicle, 6087
injection, invert, 4133
spheres, 6083
Sulbactam
and ampicillin for injection, 328
sodium, 4134
Sulconazole nitrate, 4135
Sulfa
vaginal cream, triple, 4137
vaginal inserts, triple, 4137
Sulfabenzamide, 4138

www.webofpharma.com

Sulfacetamide, 4139
sodium, 4140
sodium ophthalmic ointment, 4142
sodium ophthalmic solution, 4143
sodium and prednisolone acetate ophthalmic ointment, 4144
sodium and prednisolone acetate ophthalmic suspension, 4145
sodium topical suspension, 4143
Sulfachlorpyridazine, 4146
Sulfadiazine, 4147
cream, silver, 4150
silver, 4149
sodium, 4151
sodium injection, 4152
tablets, 4148
Sulfadimethoxine, 4152
sodium, 4154
soluble powder, 4153
oral suspension, 4153
tablets, 4153
Sulfadoxine, 4154
and pyrimethamine tablets, 4155
Sulfaguanidine, 6209
Sulfamerazine, 6209
Sulfamethazine, 4156
and chlortetracycline bisulfates soluble powder, 980
granulated, 4156
Sulfamethizole, 4157
oral suspension, 4158
tablets, 4158
Sulfamethoxazole, 4159
oral suspension, 4161
tablets, 4161
and trimethoprim injection, 4162
and trimethoprim oral suspension, 4163
and trimethoprim tablets, 4166
Sulfamic acid, 6209
Sulfan blue, 6209
Sulfanilamide, 6209
Sulfanilic
acid, 6209
acid, diazotized TS, 6236
acid TS, 6236
1-naphthylamine TS, 6236
α-naphthylamine TS, 6236
Sulfapyridine, 4167
tablets, 4167
Sulfaquinoxaline, 4168
oral solution, 4169
Sulfasalazine, 4169
tablets, 4170
delayed-release tablets, 4170
Sulfatase enzyme preparation, 6209
Sulfate
acid, ferrous, TS, 6225, 6229
and chloride ⟨221⟩, 6636
ferrous, TS, 6229
magnesium, TS, 6231
mercuric, TS, 6228, 6231
potassium, 6194
potassium, TS, 6234
in reagents, 6131
strychnine, 6208
Sulfathiazole, 4171
sodium, 6209
Sulfinpyrazone, 4172
capsules, 4172
tablets, 4173
Sulfiram, 6209
Sulfisoxazole, 4174
acetyl, 4175
acetyl and erythromycin estolate oral suspension, 1695
acetyl and erythromycin ethylsuccinate for oral suspension, 1701
acetyl oral suspension, 4175
tablets, 4175
Sulfomolybdic acid TS, 6236
Sulfonic acid cation-exchange resin, 6209
2-(4-Sulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonic acid, trisodium salt, 6220, 6221
Sulfosalicylic acid, 6209
Sulfur, 6209
dioxide, 6084
dioxide detector tube, 6209
ointment, 4176
precipitated, 4176
and resorcinol topical suspension, 3862
sublimed, 4176
Sulfur dioxide ⟨525⟩, 6751
Sulfuric acid, 6084, 6209
diluted, 6159, 6209
fluorometric, 6209
fuming, 6168, 6209
half-normal (0.5 N) in alcohol, 6250
nitrogen free, 6209
normal (1 N), 6250
phenylhydrazine, TS, 6233
TS, 6236
0.02 N TS, 6236
0.2 N TS, 6236
0.5 N TS, 6236
1 M TS, 6236
2 N TS, 6236
6 N TS, 6236
7 N TS, 6236
0.01 N VS, 6250
0.02 N Sulfuric acid VS, 6250
10 N Sulfuric acid TS, 6236
Sulfuric acid–formaldehyde TS, 6236
0.05 N Sulfuric acid VS, 6250
Sulfurous acid, 6209
Sulindac, 4177
tablets, 4178
Sulisobenzone, 4180
Sumatriptan, 4180
nasal spray, 4183
injection, 4182
succinate, 4187
succinate oral suspension, 4188
tablets, 4185
Sunflower oil, 6085, 6209
Supplemental information for articles of botanical origin ⟨2030⟩, 8702

Suppositories

Acetaminophen, 44
Aminophylline, 247
Aspirin, 391
Bisacodyl, 573
Chlorpromazine, 974
Ergotamine tartrate and caffeine, 1680
Glycerin, 2134
Indomethacin, 2331
Miconazole nitrate vaginal, 2951
Morphine sulfate, 3028
Nystatin vaginal, 3220
Prochlorperazine, 3698
Progesterone vaginal, 3707
Promethazine hydrochloride, 3718
Thiethylperazine maleate, 4342

Suspension

Acetaminophen and codeine phosphate oral, 56
Acetaminophen oral, 42
Acetazolamide oral, 76
Acyclovir oral, 92
Albendazole oral, 102
Allopurinol oral, 133
Alprazolam oral, 145
Alumina, magnesia, and calcium carbonate oral, 168
Alumina and magnesia oral, 166
Alumina, magnesia, and simethicone oral, 173
Alumina and magnesium carbonate oral, 176
Alumina and magnesium trisilicate oral, 179
Amoxicillin and clavulanate potassium for oral, 308
Amoxicillin for oral, 303
Amoxicillin for injectable, 302
Amoxicillin oral, 303
Amoxicillin tablets for oral, 306
Ampicillin for injectable, 324
Ampicillin for oral, 324
Ampicillin and probenecid for oral, 326
Atenolol compounded oral, 408
Atenolol compounded oral, veterinary, 409
Atovaquone oral, 423
Aurothioglucose injectable, 434
Azathioprine oral, 440
Azithromycin for oral, 454
Baclofen oral, 473
Barium sulfate, 481
Barium sulfate for, 482
Benazepril hydrochloride compounded oral, veterinary, 490
Betamethasone sodium phosphate and betamethasone acetate injectable, 551
Bethanechol chloride oral, 564
Bisacodyl rectal, 574
Bismuth subsalicylate oral, 582
Brinzolamide ophthalmic, 594
Calamine topical, 665
Calamine topical, phenolated, 665
Calcium carbonate oral, 686
Calcium and magnesium carbonates oral, 691
Captopril oral, 735
Carbamazepine oral, 742
Cefaclor for oral, 810
Cefadroxil for oral, 816
Cefdinir for oral, 830
Cefixime for oral, 837
Cefpodoxime proxetil for oral, 864
Cefprozil for oral, 869
Cefuroxime axetil for oral, 888
Cellulose sodium phosphate for oral, 896
Cephalexin for oral, 898
Cephradine for oral, 909
Chloramphenicol and hydrocortisone acetate for ophthalmic, 932
Chloramphenicol palmitate oral, 934
Chlorothiazide oral, 963
Cholestyramine for oral, 990
Ciclopirox olamine topical, 998
Ciprofloxacin and dexamethasone otic, 1021
Clarithromycin for oral, 1049
Clavulanate potassium and amoxicillin for oral, 308

www.webofpharma.com

Suspension *(continued)*
Clindamycin phosphate topical, 1072
Clonazepam oral, 1097
Clopidogrel compounded oral, 1111
Colestipol hydrochloride for oral, 1147
Colistin and neomycin sulfates and hydrocortisone acetate otic, 1150
Colistin sulfate for oral, 1150
Desoxycorticosterone pivalate injectable, 1279
Dexamethasone acetate injectable, 1291
Dexamethasone ophthalmic, 1287
Diazoxide oral, 1335
Didanosine tablets for oral, 1367
Diltiazem hydrochloride oral, 1406
Dipyridamole oral, 1445
Dolasetron mesylate oral, 1485
Doxycycline calcium oral, 1529
Doxycycline compounded oral, veterinary, 1530
Doxycycline for oral, 1525
Enalapril maleate compounded oral, veterinary, 1613
Erythromycin estolate for oral, 1694
Erythromycin estolate oral, 1693
Erythromycin estolate and sulfisoxazole acetyl oral, 1695
Erythromycin ethylsuccinate for oral, 1699
Erythromycin ethylsuccinate oral, 1698
Erythromycin ethylsuccinate and sulfisoxazole acetyl for oral, 1701
Estrone injectable, 1752
Famotidine for oral, 1818
Ferumoxsil oral, 1868
Flucytosine oral, 1917
Fluorometholone ophthalmic, 1952
Furazolidone oral, 2052
Ganciclovir oral, 2090
Gentamicin and prednisolone acetate ophthalmic, 2107
Griseofulvin oral, 2166
Hydrocortisone rectal, 2233
Ibuprofen oral, 2283
Imipenem and cilastatin for injectable, 2298
Indomethacin oral, 2332
Isophane insulin human, 2352
Human insulin isophane and human insulin injection, 2349
Isophane insulin, 2351
Insulin zinc, 2356
Insulin zinc, extended, 2357
Insulin zinc, prompt, 2359
Isoflupredone acetate injectable, 2419
Ketoconazole oral, 2507
Labetalol hydrochloride oral, 2519
Lamotrigine compounded oral, 2545
Lansoprazole compounded oral, 2556
Magaldrate and simethicone oral, 2706
Magnesium carbonate and sodium bicarbonate for oral, 2712
Mebendazole oral, 2744
Medroxyprogesterone acetate injectable, 2755
Megestrol acetate oral, 2763
Meloxicam oral, 2770
Mesalamine rectal, 2803
Methacycline hydrochloride oral, 2835
Methadone hydrochloride tablets for oral, 2839
Methylprednisolone acetate injectable, 2901
Metolazone oral, 2913
Metoprolol tartrate oral, 2926
Metronidazole benzoate compounded oral, 2933
Minocycline hydrochloride oral, 2966
Naproxen oral, 3079
Natamycin ophthalmic, 3096
Neomycin and polymyxin B sulfates and dexamethasone ophthalmic, 3122
Neomycin and polymyxin B sulfates and hydrocortisone otic, 3124
Neomycin and polymyxin B sulfates and hydrocortisone acetate ophthalmic, 3125
Neomycin and polymyxin B sulfates and hydrocortisone ophthalmic, 3123
Neomycin and polymyxin B sulfates and prednisolone acetate ophthalmic, 3127
Neomycin sulfate and hydrocortisone otic, 3109
Neomycin sulfate and hydrocortisone acetate ophthalmic, 3110
Neomycin sulfate and prednisolone acetate ophthalmic, 3128
Nevirapine oral, 3132
Nitrofurantoin oral, 3179
Nystatin for oral, 3221
Nystatin oral, 3221
Ondansetron hydrochloride oral, 3268
Oxfendazole oral, 3322
Oxytetracycline hydrochloride and hydrocortisone acetate ophthalmic, 3363
Penicillin G benzathine injectable, 3433
Penicillin G benzathine and penicillin G procaine injectable, 3434
Penicillin G benzathine oral, 3433
Penicillin G, neomycin, polymyxin B, hydrocortisone acetate, and hydrocortisone sodium succinate topical, 3430
Penicillin G procaine, dihydrostreptomycin sulfate, chlorpheniramine maleate, and dexamethasone injectable, 3445
Penicillin G procaine and dihydrostreptomycin sulfate injectable, 3444
Penicillin G procaine, dihydrostreptomycin sulfate, and prednisolone injectable, 3447
Penicillin G procaine, neomycin and polymyxin B sulfates, and hydrocortisone acetate topical, 3448
Penicillin G procaine injectable, 3443
Penicillin G procaine for injectable, 3443
Penicillin V benzathine oral, 3454
Penicillin V for oral, 3452
Perflutren protein-type A microspheres injectable, 3475
Pergolide, oral, veterinary, 3478
Phenoxybenzamine hydrochloride compounded oral, 3504
Phenytoin oral, 3526
Piroxicam compounded oral, 3579
Prednisolone acetate injectable, 3663
Prednisolone acetate ophthalmic, 3663
Prednisolone compounded oral, veterinary, 3664
Prednisone injectable, 3672
Prednisolone tebutate injectable, 3670
Primidone oral, 3684
Progesterone injectable, 3706
Psyllium hydrophilic mucilloid for oral, 3771
Pyrantel pamoate oral, 3774
Pyrvinium pamoate oral, 3789
Quinidine sulfate oral, 3814
Ractopamine hydrochloride, 3826
Resorcinol and sulfur topical, 3862
Rifampin oral, 3877
Rimexolone ophthalmic, 3887
Selenium sulfide topical, 4023
Simethicone oral, 4045
Sodium polystyrene sulfonate, 4092
Spectinomycin for injectable, 4108
Spironolactone compounded oral, 4111
Structured vehicle, 6086
Structured vehicle, sugar-free, 6087
Sulfacetamide sodium and prednisolone acetate ophthalmic, 4145
Sulfacetamide sodium topical, 4143
Sulfadimethoxine oral, 4153
Sulfamethizole oral, 4158
Sulfamethoxazole oral, 4161
Sulfamethoxazole and trimethoprim oral, 4163
Sulfisoxazole acetyl oral, 4175
Sumatriptan succinate oral, 4188
Temozolomide oral, 4257
Testosterone injectable, 4287
Tetracycline hydrochloride ophthalmic, 4310
Tetracycline hydrochloride oral, 4310
Tetracycline oral, 4302
Thiabendazole oral, 4335
Tobramycin and dexamethasone ophthalmic, 4411
Tobramycin and fluorometholone acetate ophthalmic, 4412
Topiramate compounded oral, 4436
Triamcinolone acetonide injectable, 4490
Triamcinolone diacetate injectable, 4491
Triamcinolone hexacetonide injectable, 4493
Trifluprom azine oral, 4514
Trisulfapyrimidines oral, 4536
Vehicle for oral, 5913
Verapamil hydrochloride oral, 4609
Zinc sulfide topical, 4695

Suspension structured vehicle, 6086
sugar-free, 6087
Suture
absorbable surgical, 4189
nonabsorbable surgical, 4191
Sutures
diameter ⟨861⟩, 7175
needle attachment ⟨871⟩, 7178

Syrup

Acacia, 5597
Calcium glubionate, 695
Cherry, 5709
Chlorpromazine hydrochloride, 976
Chocolate, 5716
Corn, 5724
Corn, solids, 5731
High fructose corn, 5727
Docusate sodium, 1481
Ferrous sulfate, 1863
Orange, 5915
Piperazine citrate, 3575
Promazine hydrochloride, 3713
Syrup, 6087
Tolu balsam, 6090

www.webofpharma.com

T

Tablet breaking force ⟨1217⟩, 8138
Tablet compression characterization, 7556
Tablet friability ⟨1216⟩, 8137

Tablets

Abacavir, 6066
Abiraterone acetate, 26
Acarbose, 31
Acepromazine maleate, 38
Acetaminophen, 45
Containing at least three of the following—acetaminophen and (salts of) chlorpheniramine, dextromethorphan, and pseudoephedrine, 58
Acetaminophen and aspirin, 48
Acetaminophen, aspirin, and caffeine, 49
Acetaminophen and caffeine, 51
Acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide, 60
Acetaminophen and codeine phosphate, 66
Acetaminophen and diphenhydramine citrate, 69
Acetaminophen, diphenhydramine hydrochloride, and pseudoephedrine hydrochloride, 70
Acetaminophen extended-release, 47
Acetaminophen and hydrocodone bitartrate, 2224
Acetaminophen and pseudoephedrine hydrochloride, 72
Acetazolamide, 76
Acetohydroxamic acid, 79
Acyclovir, 92
Albendazole, 103
Albuterol, 105
Albuterol extended-release, 106
Alendronate sodium, 120
Alfuzosin hydrochloride extended-release, 125
Allopurinol, 134
Almotriptan, 138
Alosetron, 142
Alprazolam, 146
Alprazolam extended-release, 148
Alprazolam orally disintegrating, 152
Alumina and magnesia, 167
Alumina, magnesia, and calcium carbonate chewable, 169
Alumina, magnesia, calcium carbonate, and simethicone chewable, 170
Alumina, magnesia, and simethicone chewable, 174
Alumina and magnesium carbonate, 177
Alumina, magnesium carbonate, and magnesium oxide, 178
Alumina and magnesium trisilicate, 180
Aluminum hydroxide gel, dried, 190
Aluminum sulfate and calcium acetate for topical solution, 195
Amiloride hydrochloride, 223
Amiloride hydrochloride and hydrochlorothiazide, 224
Aminocaproic acid, 237
Aminopentamide sulfate, 240
Aminophylline, 248
Aminophylline delayed-release, 250
Amiodarone hydrochloride, 257
Amitriptyline hydrochloride, 263
amlodipine and atorvastatin, 265
Amlodipine and olmesartan medoxomil, 272
Amlodipine and valsartan, 275
Amlodipine besylate, 286
Amlodipine, valsartan and hydrochlorothiazide, 279
Ammonium chloride delayed-release, 288
Amodiaquine hydrochloride, 294
Amoxapine, 297
Amoxicillin, 304
Amoxicillin and clavulanic acid extended-release, 310
Amoxicillin and clavulanate potassium, 308
Amphetamine sulfate, 313
Ampicillin, 325
Anastrozole, 337
Anileridine hydrochloride, 341
Apomorphine hydrochloride, 358
Arginine, 4764
Aripiprazole, 375
Aripiprazole orally disintegrating, 377
Ascorbic acid, 385
Aspirin, 392
Aspirin, alumina, and magnesia, 397
Aspirin, alumina, and magnesium oxide, 399
Aspirin, buffered, 393
Aspirin and codeine phosphate, 403
Aspirin, codeine phosphate, alumina, and magnesia, 404
Aspirin delayed-release, 394
Aspirin effervescent, for oral solution, 395
Aspirin extended-release, 396
Atenolol, 407
Atenolol and chlorthalidone, 409
Atorvastatin calcium, 418
Azatadine maleate, 438
Azathioprine, 441
Azithromycin, 457
Baclofen, 473
Barium sulfate, 483
Belladonna extract, 487
Benazepril hydrochloride, 491
Bendroflumethiazide, 497
benzphetamine hydrochloride, 530
Benztropine mesylate, 533
Betaxolol, 559
Bethanechol chloride, 564
Bicalutamide, 567
Biotin, 571
Bisacodyl delayed-release, 574
Bismuth subsalicylate, 583
Bisoprolol fumarate, 586
Bisoprolol fumarate and hydrochlorothiazide, 587
Black cohosh, 4823
Bromocriptine mesylate, 597
Brompheniramine maleate, 603
Bumetanide, 608
Buprenorphine and naloxone sublingual, 616
Bupropion hydrochloride, 620
Bupropion hydrochloride extended-release, 621
Buspirone hydrochloride, 637
Busulfan, 640
Butabarbital sodium, 643
Butalbital, acetaminophen, and caffeine, 646
Butalbital and aspirin, 646
Butalbital, aspirin, and caffeine, 649
Cabergoline, 659
Calcium acetate, 682
Calcium carbonate, 687
Calcium carbonate, magnesia, and simethicone chewable, 689
Calcium citrate, 4835
Calcium gluconate, 701
Calcium L-5-methyltetrahydrofolate, 4842
Calcium lactate, 703
Calcium and magnesium carbonates, 691
Calcium pantothenate, 707
Calcium phosphate, dibasic, 712
Calcium with vitamin D, 4843
Calcium and vitamin D with minerals, 4845
Candesartan cilexetil, 717
Candesartan cilexetil and hydrochlorothiazide, 719
Capecitabine, 723
Captopril, 735
Captopril and hydrochlorothiazide, 737
Carbamazepine, 743
Carbamazepine extended-release, 746
Carbenicillin indanyl sodium, 749
Carbidopa and levodopa, 751
Levodopa and carbidopa extended-release, 753
Carbidopa and levodopa orally disintegrating, 759
Carbinoxamine maleate, 763
Calcium carbonate and magnesia chewable, 688
Carboxymethylcellulose sodium, 774
Carisoprodol, 777
Carisoprodol, aspirin, and codeine phosphate, 780
Carisoprodol and aspirin, 778
Carprofen, 786
Carteolol hydrochloride, 790
Carvedilol, 793
Cascara, 800
Cat's claw, 4858
Cefaclor extended-release, 811
Cefadroxil, 817
Cefixime, 838
Cefpodoxime proxetil, 864
Cefprozil, 870
Cefuroxime axetil, 889
Cephalexin, 899
Cephalexin, for oral suspension, 899
Cephradine, 909
Cetirizine hydrochloride, 913
Cetirizine hydrochloride orally disintegrating, 915
Cetrizine hydrochloride and pseudoephedrine hydrochloride extended-release, 917
Chlorambucil, 926
Chloramphenicol, 932
Chlordiazepoxide, 937
Chlordiazepoxide and amitriptyline hydrochloride, 938
Chloroquine phosphate, 961
Chlorothiazide, 964
Chlorpheniramine maleate, 971
Chlorpromazine hydrochloride, 977
Chlorpropamide, 978
Chlortetracycline hydrochloride, 987
Chlorthalidone, 983
Chlorzoxazone, 984
Cholecalciferol, 988
Chondroitin sulfate sodium, 4897
Chromium picolinate, 4902
Cilostazol, 1004
Cimetidine, 1007
Ciprofloxacin, 1015
Ciprofloxacin extended-release, 1017

www.webofpharma.com

Tablets *(continued)*
Citalopram, 1035
Clarithromycin, 1049
Clarithromycin extended-release, 1051
Clemastine fumarate, 1059
Clomiphene citrate, 1090
Clonazepam, 1098
Clonazepam orally disintegrating, 1099
Clonidine hydrochloride, 1103
Clonidine hydrochloride and chlorthalidone, 1104
Clopidogrel, 1111
Clorazepate dipotassium, 1115
Clover, red, 5212
Clozapine, 1132
Cocaine hydrochloride, for topical solution, 1134
Codeine phosphate, 1139
Codeine sulfate, 1142
Colchicine, 1145
Colestipol hydrochloride, 1147
Cortisone acetate, 1176
Curcuminoids, 4930
Cyanocobalamin, 1192
Cyclizine hydrochloride, 1195
Cyclobenzaprine hydrochloride, 1199
Cyclophosphamide, 1209
Cyproheptadine hydrochloride, 1220
Dapsone, 1241
Demeclocycline hydrochloride, 1249
Desipramine hydrochloride, 1256
Desloratadine, 1261
Desloratadine orally disintegrating, 1263
Desogestrel and ethinyl estradiol, 1270
Dexamethasone, 1289
Dexchlorpheniramine maleate, 1301
Dextroamphetamine sulfate, 1318
Diazepam, 1332
Dichlorphenamide, 1340
Diclofenac potassium, 1345
Diclofenac sodium and misoprostol delayed-release, 1353
Diclofenac sodium delayed-release, 1348
Diclofenac sodium extended-release, 1350
Dicyclomine hydrochloride, 1362
Didanosine, for oral suspension, 1367
Diethylcarbamazine citrate, 1369
Diethylpropion hydrochloride, 1371
Diflunisal, 1376
Digitalis, 1380
Digitoxin, 1382
Digoxin, 1385
Dihydrotachysterol, 1393
Dihydroxyaluminum sodium carbonate chewable, 1396
Diltiazem hydrochloride, 1406
Dimenhydrinate, 1410
Diphenhydramine citrate and ibuprofen, 1421
Diphenhydramine and phenylephrine hydrochloride, 1433
Diphenoxylate hydrochloride and atropine sulfate, 1439
Dipyridamole, 1446
Dirithromycin delayed-release, 1448
Disulfiram, 1452
Divalproex sodium delayed-release, 1457
Divalproex sodium extended-release, 1458
Docusate sodium, 1481
Donepezil hydrochloride, 1488
Donepezil hydrochloride orally disintegrating, 1492
Doxazosin, 1506
Doxycycline, 1527
Doxycycline hyclate, 1537
Doxycycline hyclate delayed-release, 1539
Doxylamine succinate, 1546
Dronedarone, 1551
Drospirenone and ethinyl estradiol, 1556
Dydrogesterone, 1572
Dyphylline, 1574
Dyphylline and guaifenesin, 1575
Echinacea Species dry extract tablets, 4963
Efavirenz, 1591
Eleuthero root and rhizome dry extract, 4973
Enalapril maleate, 1614
Enalapril maleate and hydrochlorothiazide, 1616
Entacapone, 1632
Entecavir, 1637
Ergocalciferol, 1668
Ergoloid mesylates, 1671
Ergonovine maleate, 1674
Ergotamine tartrate, 1678
Ergotamine tartrate and caffeine, 1681
Ergotamine tartrate sublingual, 1679
Erythromycin, 1691
Erythromycin delayed-release, 1691
Erythromycin estolate, 1694
Erythromycin ethylsuccinate, 1699
Erythromycin stearate, 1706
Escitalopram, 1708
Estazolam, 1723
Estradiol, 1732
Estradiol and norethindrone acetate, 1733
Estrogens, conjugated, 1746
Estrogens, esterified, 1750
Estropipate, 1754
Eszopiclone, 1757
Ethacrynic acid, 1761
Ethambutol hydrochloride, 1764
Ethinyl estradiol, 1768
Ethionamide, 1771
Ethotoin, 1778
Ethynodiol diacetate and ethinyl estradiol, 1781
Ethynodiol diacetate and mestranol, 1781
Etidronate disodium, 1784
Etodolac, 1787
Etodolac extended-release, 1788
Ezetimibe, 1806
famciclovir, 1812
Famotidine, 1819
Felbamate, 1824
Felodipine extended-release, 1827
Fenofibrate, 1837
Fenoprofen calcium, 1845
Ferrous fumarate, 1855
Ferrous fumarate and docusate sodium extended-release, 1856
Ferrous gluconate, 1861
Ferrous sulfate, 1864
Fexofenadine hydrochloride, 1872
Fexofenadine hydrochloride and pseudoephedrine hydrochloride extended-release, 1876
Finasteride, 1890
Flavoxate hydrochloride, 1894
Flecainide acetate, 1898
Fluconazole, 1914
Fludrocortisone acetate, 1923
Fluoxetine, 1962
Fluoxymesterone, 1966
Flurbiprofen, 1980
Fluvoxamine maleate, 2023
Folic acid, 2028
Fosinopril sodium, 2042
Fosinopril sodium and hydrochlorothiazide, 2043
Furazolidone, 2053
Furosemide, 2056
Gabapentin, 2060
Galantamine, 2081
Garlic delayed-release, 5024
Gemfibrozil, 2098
Ginkgo, 5041
Ginseng, American, 4757
Ginseng, Asian, 4778
Glimepiride, 2111
Glipizide, 2115
Glipizide and metformin hydrochloride, 2116
Glucosamine, 5046
Glucosamine and chondroitin sodium sulfate, 5043
Glucosamine, chondroitin sulfate sodium, and methylsulfonylmethane, 5051
Glucosamine and methylsulfonylmethane, 5049
Glyburide, 2126
Glyburide and metformin hydrochloride, 2128
Glycopyrrolate, 2140
Granisetron hydrochloride, 2161
Griseofulvin, 2166
Griseofulvin, ultramicrosize, 2168
Guaifenesin, 2172
Guanabenz acetate, 2177
Guanethidine monosulfate, 2179
Guanfacine, 2180
Guggul, 5072
Halazone for solution, 2182
Haloperidol, 2190
Homatropine methylbromide, 2210
Hydralazine hydrochloride, 2215
Hydrochlorothiazide, 2222
Hydrochlorothiazide and amiloride hydrochloride, 224
Hydrocodone bitartrate, 2224
Hydrocodone bitartrate and acetaminophen, 2224
Hydrocodone bitartrate and homatropine methylbromide, 2225
Hydrocortisone, 2234
Hydroflumethiazide, 2247
Hydromorphone hydrochloride, 2254
Hydroxychloroquine sulfate, 2259
Hydroxyzine hydrochloride, 2267
Hyoscyamine, 2273
Hyoscyamine sulfate, 2278
Ibuprofen, 2285
Ibuprofen and pseudoephedrine hydrochloride, 2286
Imipramine hydrochloride, 2301
Indapamide, 2314
Iodoquinol, 2378
Irbesartan, 2403
Irbesartan and hydrochlorothiazide, 2404
Isoniazid, 2426
Isopropamide iodide, 2428
Isoproterenol hydrochloride, 2434
Isosorbide dinitrate chewable, 2444
Isosorbide dinitrate extended-release, 2445
Isosorbide dinitrate sublingual, 2447
Isosorbide mononitrate, 2449
Isosorbide mononitrate extended-release, 2451
Isoxsuprine hydrochloride, 2463
Ivermectin, 2474
Ivermectin and pyrantel pamoate, 2478
Ketoconazole, 2508
Ketorolac tromethamine, 2515
Labetalol hydrochloride, 2519
Lamivudine, 2527

www.webofpharma.com

Tablets *(continued)*
Lamivudine and zidovudine, 2528
Lamotrigine, 2532
Lamotrigine extended-release, 2534
Lamotrigine, for oral suspension, 2542
Leflunomide, 2560
Letrozole, 2563
Leucovorin calcium, 2571
Levamisole hydrochloride, 2579
Levetiracetam, 2584
Levetiracetam extended-release, 2587
Levocarnitine, 2601
Levocetirizine dihydrochloride, 2603
Levofloxacin, 2609
Levonorgestrel and ethinyl estradiol, 2612
Levorphanol tartrate, 2614
Levothyroxine sodium, 2618
Liothyronine sodium, 2640
Liotrix, 2640
Lipoic acid, alpha, 5126
Lisinopril, 2644
Lisinopril and hydrochlorothiazide, 2646
Lithium carbonate, 2650
Lithium carbonate extended-release, 2651
Loperamide hydrochloride, 2661
Lopinavir and ritonavir, 2670
Loratadine, 2675
Loratadine chewable, 2676
Loratadine orally disintegrating, 2678
Lorazepam, 2684
Losartan potassium, 2687
Losartan potassium and hydrochlorothiazide, 2690
Lysine hydrochloride, 5135
Magaldrate and simethicone chewable, 2707
Magnesia, 2709
Magnesium gluconate, 2717
Magnesium oxide, 2721
Magnesium salicylate, 2724
Magnesium trisilicate, 2729
Maprotiline hydrochloride, 2739
Mebendazole, 2744
Mecamylamine hydrochloride, 2748
Meclizine hydrochloride, 2751
Medroxyprogesterone acetate, 2756
Mefloquine hydrochloride, 2761
Megestrol acetate, 2764
Melatonin, 5144
Meloxicam, 2771
Melphalan, 2774
Memantine hydrochloride, 2776
Menaquinone-7, 5151
Meperidine hydrochloride, 2784
Mephobarbital, 2785
Meprobamate, 2791
Mercaptopurine, 2794
Mesalamine delayed-release, 2805
Mesna, 2809
Metaproterenol sulfate, 2814
Metaxalone, 2819
Metformin hydrochloride, 2822
Metformin hydrochloride extended-release, 2824
Methadone hydrochloride, 2838
Methamphetamine hydrochloride, 2840
Methazolamide, 2842
Methenamine, 2843
Methenamine hippurate, 2845
Methenamine mandelate, 2846
Methimazole, 2848
Methocarbamol, 2851
Methotrexate, 2860
Methscopolamine bromide, 2868
Methyclothiazide, 2872
Methylcellulose, 2876
Methylcobalamin, 5154
Methyldopa, 2878
Methyldopa and hydrochlorothiazide, 2878
Methylergonovine maleate, 2887
Methylphenidate hydrochloride, 2892
Methylphenidate hydrochloride extended-release, 2892
Methylprednisolone, 2899
Methylsulfonylmethane, 5156
Methyltestosterone, 2906
Metoclopramide, 2911
Metolazone, 2914
Metoprolol succinate extended-release, 2918
Metoprolol tartrate, 2926
Metoprolol tartrate and hydrochlorothiazide, 2928
Metronidazole, 2937
Metronidazole extended-release, 2939
Midodrine hydrochloride, 2955
Milk thistle, 5163
Minerals, 5172
Minocycline hydrochloride, 2966
Minocycline hydrochloride extended-release, 2967
Minoxidil, 2974
Mirtazapine, 2978
Mirtazapine orally disintegrating, 2980
Mitotane, 2987
Modafinil, 2991
Memantine hydrochloride, 2994
Moexipril hydrochloride and hydrochlorothiazide, 2996
Molindone hydrochloride, 3000
Montelukast sodium, 3015
Montelukast sodium chewable, 3018
Moxifloxacin, 3036
Mycophenolate mofetil, 3049
Mycophenolic acid delayed-release, 3054
Nabumetone, 3059
Nadolol, 3061
Nadolol and bendroflumethiazide, 3062
Naltrexone hydrochloride, 3071
Naproxen, 3079
Naproxen delayed-release, 3081
Naproxen sodium, 3082
Naproxen sodium and pseudoephedrine hydrochloride extended-release, 3084
Naratriptan, 3091
Nateglinide, 3098
Nefazodone hydrochloride, 3100
Neomycin sulfate, 3104
Neostigmine bromide, 3129
Nevirapine, 3134
Niacin, 3140
Niacinamide, 3147
Niacin extended-release, 3141
Nifedipine extended-release, 3163
Nitrofurantoin, 3180
Nitroglycerin sublingual, 3188
Norethindrone, 3199
Norethindrone acetate, 3205
Norethindrone acetate and ethinyl estradiol, 3208
Norethindrone and ethinyl estradiol, 3201
Norethindrone and mestranol, 3203
Norgestimate and ethinyl estradiol, 3211
Norgestrel, 3213
Norgestrel and ethinyl estradiol, 3214
Nystatin, 3221
Ofloxacin, 3232
Olanzapine, 3235
Olanzapine orally disintegrating, 3240
Olmesartan medoxomil, 3244
Ondansetron, 3268
Ondansetron orally disintegrating, 3271
Orbifloxacin, 3276
Orphenadrine citrate, aspirin, and caffeine, 3288
Orphenadrine citrate extended-release, 3285
Oxandrolone, 3308
Oxaprozin, 3311
Oxcarbazepine, 3319
Oxprenolol hydrochloride extended-release, 3324
Oxtriphylline extended-release, 3325
Oxybutynin chloride, 3328
Oxybutynin chloride extended-release, 3329
Oxycodone and acetaminophen, 3343
Oxycodone and aspirin, 3344
Oxycodone hydrochloride, 3338
Oxycodone hydrochloride extended-release, 3339
Oxymetholone, 3351
Oxymorphone hydrochloride, 3354
Oxymorphone hydrochloride extended-release, 3356
Oxytetracycline, 3361
Pancreatin, 3381
Pancrelipase, 3385
Pantoprazole sodium delayed-release, 3391
Papain, for topical solution, 3396
Papaverine hydrochloride, 3398
Paroxetine, 3412
Paroxetine extended-release, 3413
Penbutolol sulfate, 3425
Penicillamine, 3429
Penicillin G benzathine, 3434
Penicillin G potassium, 3439
Penicillin V, 3453
Penicillin V potassium, 3456
Pentazocine and acetaminophen, 3460
Pentazocine and aspirin, 3461
Pentazocine and naloxone, 3463
Pentoxifylline extended-release, 3471
Pergolide, 3478
Perindopril erbumine, 3483
Perphenazine, 3486
Perphenazine and amitriptyline hydrochloride, 3487
Phenazopyridine hydrochloride, 3491
Phendimetrazine tartrate, 3494
Phenelzine sulfate, 3496
Phenobarbital, 3499
Phentermine hydrochloride, 3507
Phenylbutazone, 3512
Phenylephrine hydrochloride, 3520
Phenytoin chewable, 3528
Phytonadione, 3539
Pilocarpine hydrochloride, 3543
Pimozide, 3548
Pindolol, 3550
Pioglitazone, 3553
Pioglitazone and glimepiride, 3554
Pioglitazone and metformin hydrochloride, 3558
Piperazine citrate, 3575
Potassium and sodium bicarbonates and citric acid effervescent, for oral solution, 3598
Potassium bicarbonate effervescent, for oral solution, 3596
Potassium bicarbonate and potassium chloride effervescent, for oral solution, 3597
Potassium chloride extended-release, 3605

www.webofpharma.com

Tablets *(continued)*
Potassium chloride, potassium bicarbonate, and potassium citrate effervescent, for oral solution, 3609
Potassium citrate, 5196
Potassium citrate extended-release, 3614
Potassium gluconate, 3620
Potassium iodide, 3624
Potassium iodide delayed-release, 3625
Pravastatin sodium, 3647
Praziquantel, 3650
Prednisolone, 3661
Prednisone, 3672
Primaquine phosphate, 3681
Primidone, 3685
Probenecid, 3687
Probenecid and colchicine, 3687
Procainamide hydrochloride, 3690
Procainamide hydrochloride extended-release, 3691
Prochlorperazine maleate, 3701
Procyclidine hydrochloride, 3704
Promazine hydrochloride, 3714
Promethazine hydrochloride, 3719
Propafenone hydrochloride, 3734
Propantheline bromide, 3737
Propranolol hydrochloride, 3750
Propranolol hydrochloride and hydrochlorothiazide, 3751
Propylthiouracil, 3756
Protriptyline hydrochloride, 3760
Pseudoephedrine hydrochloride, 3763
Pseudoephedrine hydrochloride extended-release, 3764
Pyrazinamide, 3777
Pyridostigmine bromide, 3781
Pyridoxine hydrochloride, 3784
Pyrilamine maleate, 3786
Pyrimethamine, 3787
Pyrvinium pamoate, 3790
Quazepam, 3791
Quetiapine, 3792
Quetiapine, extended-release, 3795
Quinapril, 3806
Quinapril and hydrochlorothiazide, 3803
Quinidine gluconate extended-release, 3810
Quinidine sulfate, 3814
Quinidine sulfate extended-release, 3816
Quinine sulfate, 3821
Raloxifene hydrochloride, 3830
Raltegravir, 3834
Raltegravir chewable, 3835
Ramipril, 3841
Ranitidine, 3847
Rauwolfia serpentina, 3851
Repaglinide, 3856
Reserpine, 3858
Reserpine and chlorothiazide, 3859
Rhodiola rosea, 5227
Ribavirin, 3866
Riboflavin, 3870
Rifampin, isoniazid, and pyrazinamide, 3879
Rifampin, isoniazid, pyrazinamide, and ethambutol hydrochloride, 3881
Riluzole, 3883
Rimantadine hydrochloride, 3885
Risedronate sodium, 3902
Risperidone, 3907
Risperidone orally disintegrating, 3908
Ritodrine hydrochloride, 3911
Ritonavir, 3920
Rizatriptan benzoate, 3928
Rizatriptan benzoate orally disintegrating, 3930
Ropinirole, 3934
Ropinirole extended-release, 3936
Rosuvastatin, 3950
Rufinamide, 3960
Saccharin sodium, 3968
St. John's wort flowering top dry extract, 5244
Salsalate, 3982
Scopolamine hydrobromide, 4013
Selegiline hydrochloride, 4020
Sennosides, 4028
Sertraline hydrochloride, 4033
Sildenafil, 4038
Simethicone, 4045
Simvastatin, 4047
Sitagliptin, 4051
Sodium bicarbonate, 4062
Sodium chloride, 4068
Sodium chloride, for solution, 4069
Sodium fluoride, 4073
Sodium salicylate, 4094
Sotalol hydrochloride, 4105
Soy isoflavones, 5278
Spironolactone, 4112
Spironolactone and hydrochlorothiazide, 4113
Spirulina, 5283
Stanozolol, 4118
Sucralfate, 4131
Sulfadiazine, 4148
Sulfadimethoxine, 4153
Sulfadoxine and pyrimethamine, 4155
Sulfamethizole, 4158
Sulfamethoxazole, 4161
Sulfamethoxazole and trimethoprim, 4166
Sulfapyridine, 4167
Sulfasalazine, 4170
Sulfasalazine delayed-release, 4170
Sulfinpyrazone, 4173
Sulfisoxazole, 4175
Sulindac, 4178
Sumatriptan, 4185
Tadalafil, 4205
Tamoxifen citrate, 4210
Telmisartan, 4240
Telmisartan and amlodipine, 4242
Telmisartan and hydrochlorothiazide, 4246
Terazosin, 4263
Terbinafine, 4268
Terbutaline sulfate, 4273
Testolactone, 4286
Tetracycline hydrochloride, 4311
Tetracycline hydrochloride and novobiocin sodium, 4312
Tetracycline hydrochloride, novobiocin sodium, and prednisolone, 4312
Theophylline, 4328
Theophylline, ephedrine hydrochloride, and phenobarbital, 4330
Theophylline sodium glycinate, 4334
Thiabendazole chewable, 4336
Thiamine hydrochloride, 4339
Thiethylperazine maleate, 4343
Thioguanine, 4350
Thioridazine hydrochloride, 4355
Thyroid, 4364
Ticlopidine hydrochloride, 4378
Tienchi ginseng root and rhizome dry extract, 5311
Tienchi ginseng root and rhizome powder, 5306
Timolol maleate, 4389
Timolol maleate and hydrochlorothiazide, 4391
Tizanidine, 4399
Tolazamide, 4417
Tolbutamide, 4419
Tolcapone, 4421
Tolmetin sodium, 4424
Topiramate, 4434
Torsemide, 4439
Tramadol hydrochloride, 4444
Tramadol hydrochloride and acetaminophen, 4450
Tramadol hydrochloride extended-release, 4446
Trandolapril, 4454
Trandolapril and verapamil hydrochloride extended-release, 4456
Tranexamic acid, 4464
Tranylcypromine, 4466
Trazodone hydrochloride, 4474
Triamcinolone, 4482
Triamterene and hydrochlorothiazide, 4499
Triazolam, 4502
Trifluoperazine hydrochloride, 4512
Triflupromazine hydrochloride, 4515
Trihexyphenidyl hydrochloride, 4519
Trimeprazine tartrate, 4523
Trimethoprim, 4526
Trioxsalen, 4529
Tripelennamine hydrochloride, 4531
Triprolidine hydrochloride, 4534
Triprolidine and pseudoephedrine hydrochlorides, 4535
Trisulfapyrimidines, 4537
Trospium chloride, 4543
Ubidecarenone, 5321
Ursodiol, 4559
Valacyclovir, 4562
Valerian, 5329
Valganciclovir, 4566
Valsartan, 4579
Valsartan and hydrochlorothiazide, 4580
Venlafaxine, 4595
Verapamil hydrochloride, 4610
Verapamil hydrochloride extended-release, 4611
Vigabatrin, 4621
Vinpocetine, 5337
Vitamin A, 4635
Vitamins with minerals, oil-soluble, 5378
Vitamins with minerals, oil- and water-soluble, 5476
Vitamins with minerals, water-soluble, 5552
Vitamins, oil-soluble, 5356
Vitamins, oil- and water-soluble, 5419
Vitamins, water-soluble, 5512
Warfarin sodium, 4650
Zalcitabine, 4667
Zidovudine, 4678
Zinc citrate, 5575
Zinc gluconate, 4686
Zinc sulfate, 4694
Zolmitriptan, 4706
Zolmitriptan orally disintegrating, 4709
Zolpidem tartrate, 4712
Zolpidem tartrate extended-release, 4714

Tacrolimus, 4193
capsules, 4196
oral suspension, 4202
Tadalafil, 4203
tablets, 4205

www.webofpharma.com

Tadalafil compounded
oral suspension, 4207
Tagatose, 6087
Talc, 4207
Tamoxifen citrate, 4209
tablets, 4210
Tamsulosin hydrochloride, 4211
capsules, 4213
Tangerine peel, 5292
dry extract, 5294
powder , 5296
Tannic acid, 4223, 6209
TS, 6236
Tape, adhesive, 4223
Tapioca starch, 6066
Tartaric acid, 6088, 6209
TS, 6237
Taurine, 4223
Tazobactam, 4224
and piperacillin for injection, 3567
Tc 99m
albumin aggregated injection, technetium, 4226
bicisate injection, technetium, 4227
disofenin injection, technetium, 4227
exametazime injection, technetium, 4228
mebrofenin injection, technetium, 4229
medronate injection, technetium, 4230
mertiatide injection, technetium, 4231
oxidronate injection, technetium, 4232
pentetate injection, technetium, 4232
pertechnetate injection, sodium, 4233
(pyro- and trimeta-) phosphates injection, technetium, 4235
pyrophosphate injection, technetium, 4234
red blood cells injection, technetium, 4235
sestamibi injection, technetium, 4236
succimer injection, technetium, 4237
sulfur colloid injection, technetium, 4238
tetrofosmin injection, technetium, 4238
T-dodecyl mercaptan ethoxylate, 6163
Technetium
Tc 99m albumin aggregated injection, 4226
Tc 99m bicisate injection, 4227
Tc 99m disofenin injection, 4227
Tc 99m exametazime injection, 4228
Tc 99m mebrofenin injection, 4229
Tc 99m medronate injection, 4230
Tc 99m mertiatide injection, 4231
Tc 99m oxidronate injection, 4232
Tc 99m pentetate injection, 4232
Tc 99m pertechnetate injection, sodium, 4233
Tc 99m pyrophosphate injection, 4234
Tc 99m (pyro- and trimeta-) phosphates injection, 4235
Tc 99m red blood cells injection, 4235
Tc 99m sestamibi injection, 4236
Tc 99m succimer injection, 4237
Tc 99m sulfur colloid injection, 4238
Tc 99m tetrofosmin injection, 4238
Telmisartan, 4239
and hydrochlorothiazide tablets, 4246
tablets, 4240
Telmisartan and amlodipine
tablets, 4242
Temazepam, 4250
capsules, 4251
Temozolomide, 4252
capsules, 4253
for injection, 4256
oral suspension, 4257
Temperature
congealing ⟨651⟩, 6874
Teniposide, 4258
injection, 4259
Tensile strength ⟨881⟩, 7179
Terazosin
capsules, 4261
hydrochloride, 4264
tablets, 4263
Terbinafine
hydrochloride, 4269
oral suspension, 4267
tablets, 4268
Terbutaline
sulfate, 4271
sulfate injection, 4272
sulfate tablets, 4273
oral suspension, 4273
Terconazole, 4274
Teriparatide, 4275
injection, 4280
Terminally sterilized pharmaceutical products—parametric release ⟨1222⟩, 8142
Terpin hydrate, 4283
and codeine oral solution, 4284
oral solution, 4284
tert-Butyl hydroperoxide solution, 6209
Tertiary butyl alcohol, 6134, 6209
Test for 1,6-anhydro derivative for enoxaparin sodium ⟨207⟩, 6605
Testolactone, 4285
tablets, 4286
Testosterone, 4287
benzoate, 6209
cypionate, 4288
cypionate injection, 4289
enanthate, 4289
enanthate injection, 4290
injectable suspension, 4287
propionate, 4290
propionate injection, 4291
topical solution, 4291
Test papers
and indicator, 6219
indicators and indicator, 6220
Test solutions, 6225
Tetanus
immune globulin, 4292
2′,4′,5′,7′-Tetrabromofluorescein, 6209
Tetrabromophenolphthalein ethyl ester, 6209
TS, 6237
Tetrabutylammonium
bromide, 6209
hydrogen sulfate, 6209
hydrogen sulfate ion pairing reagent, 6209
hydroxide, 1.0 M in methanol, 6210
hydroxide, 0.4 M aqueous, 6210
hydroxide 30-hydrate, 6210
hydroxide in methanol/isopropyl alcohol (0.1 N), 6251
hydroxide, tenth-normal (0.1 N), 6250
iodide, 6210
phosphate, 6210
Tetrabutylammonium hydrogen sulfate
0.02 M TS, 6237
Tetrabutylammonium hydroxide
25% TS, 6237
Tetrabutylammonium hydroxide, 40 percent in water, 6210
Tetracaine, 4292
hydrochloride, 4295
hydrochloride, benzocaine, and butamben topical aerosol, 513
hydrochloride, benzocaine, and butamben gel, 514
hydrochloride, benzocaine, and butamben ointment, 516
hydrochloride, benzocaine, and butamben topical solution, 517
hydrochloride cream, 4296
hydrochloride in dextrose injection, 4300
hydrochloride injection, 4297
hydrochloride for injection, 4298
hydrochloride, neomycin sulfate, and isoflupredone acetate ointment, 3111
hydrochloride, neomycin sulfate, and isoflupredone acetate topical powder, 3112
hydrochloride ophthalmic solution, 4299
hydrochloride topical solution, 4299
and menthol ointment, 4294
ointment, 4294
and procaine hydrochlorides and levonordefrin injection, 3695
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, ^{13}C-labeled, 6210
2,3,7,8-Tetrachlorodibenzofuran, ^{13}C-labeled, 6210
1,1,2,2-Tetrachloroethane, 6210
Tetracosane, 6210
Tetracycline, 4301
boluses, 4302
hydrochloride, 4303
hydrochloride capsules, 4305
hydrochloride for injection, 4307
hydrochloride, novobiocin sodium, and prednisolone tablets, 4312
hydrochloride and novobiocin sodium tablets, 4312
hydrochloride and nystatin capsules, 4313
hydrochloride ointment, 4307
hydrochloride ophthalmic ointment, 4308
hydrochloride ophthalmic suspension, 4310
hydrochloride soluble powder, 4309
hydrochloride for topical solution, 4309
hydrochloride oral suspension, 4310
hydrochloride tablets, 4311
oral suspension, 4302
Tetradecane, 6210
Tetradecylammonium bromide, 6210
Tetraethylammonium chloride, 6210
Tetraethylammonium perchlorate, 6210
Tetraethylene glycol, 6210
Tetraethylenepentamine, 6210
Tetraheptylammonium bromide, 6210
Tetrahexylammonium hydrogen sulfate, 6210
Tetrahydrofuran, 6211
peroxide-free, 6211
stabilizer-free, 6211
Tetrahydro-2-furancarboxylic acid, 6211
N-(2-Tetrahydrofuroyl)piperazine, 6211
1,2,3,4-Tetrahydronaphthalene, 6211
Tetrahydrozoline hydrochloride, 4314
nasal solution, 4315
ophthalmic solution, 4315
Tetramethylammonium
bromide, 6211
bromide, tenth-molar (0.1 M), 6251
chloride, 6211
chloride, tenth-molar (0.1 M), 6251
hydroxide, 6211
hydroxide, pentahydrate, 6211
hydroxide solution in methanol, 6211
hydroxide TS, 6237
nitrate, 6211
Tetramethylbenzidine, 6211
1,1,3,3-Tetramethylbutylamine, 6211
4,4′-Tetramethyldiaminodiphenylmethane, 6211
Tetramethylethylenediamine, 6211
Tetramethylsilane, 6211

www.webofpharma.com

Tetrapropylammonium
chloride, 6211
Tetrasodium ethylenediaminetetraacetate, 6212
Thalidomide, 4316
capsules, 4318
Thallous chloride, 6212
TI 201 injection, 4318
Theobromine, 6212
Theophylline, 4319
capsules, 4321
extended-release capsules, 4322
in dextrose injection, 4328
ephedrine hydrochloride, and phenobarbital tablets, 4330
and guaifenesin capsules, 4331
and guaifenesin oral solution, 4332
sodium glycinate, 4333
sodium glycinate oral solution, 4334
sodium glycinate tablets, 4334
oral solution, 4325
oral suspension, 4327
tablets, 4328
Theory and practice of electrical conductivity measurements of solutions ⟨1644⟩, 8423
Thermal analysis ⟨891⟩, 7180
Thiabendazole, 4335
chewable tablets, 4336
oral suspension, 4335
Thiamine
hydrochloride, 4337
hydrochloride injection, 4338
hydrochloride oral solution, 4338
hydrochloride tablets, 4339
mononitrate, 4340
mononitrate oral solution, 4341
Thiamine assay ⟨531⟩, 6756
Thiazole yellow, 6212
paper, 6222
Thiethylperazine maleate, 4342
suppositories, 4342
tablets, 4343
Thimerosal, 4344
topical aerosol, 4346
topical solution, 4347
tincture, 4348
Thin-layer chromatographic identification test ⟨201⟩, 6600
Thioacetamide, 6212
TS, 6237
Thioacetamide-glycerin base TS, 6237
2-Thiobarbituric acid, 6212
2,2′-Thiodiethanol, 6212
Thioglycolic acid, 6212
Thioguanine, 4349
tablets, 4350
Thionine acetate, 6212
Thiopental sodium, 4351
for injection, 4352
Thioridazine, 4353
hydrochloride, 4353
hydrochloride oral solution, 4354
hydrochloride tablets, 4355
Thiostrepton, 4355
nystatin, neomycin sulfate, and triamcinolone acetonide cream, 3223
nystatin, neomycin sulfate, and triamcinolone acetonide ointment, 3223
Thiotepa, 4356
for injection, 4357
Thiothixene, 4358
capsules, 4359
hydrochloride, 4360
hydrochloride injection, 4360
hydrochloride for injection, 4361
hydrochloride oral solution, 4361
Thiourea, 6212
Thorium nitrate, 6212
TS, 6237
Threonine, 4362
Thrombin human, 6212
Thromboplastin, 6212
Thymidine, 6212
Thymol, 6088, 6212
blue, 6221
blue TS, 6237
Thymolphthalein, 6221
TS, 6237
Thyroglobulin, 6213
Thyroid, 4362
tablets, 4364
Tiagabine hydrochloride, 4365
oral suspension, 4367
Tiamulin, 4368
fumarate, 4369
Ticarcillin
and clavulanic acid injection, 4371
and clavulanic acid for injection, 4372
disodium, 4373
for injection, 4370
monosodium, 4375
Ticlopidine hydrochloride, 4376
tablets, 4378
Tienchi ginseng root and rhizome, 5299
dry extract capsules, 5309
powder capsules, 5304
dry extract, 5307
powder, 5301
dry extract tablets, 5311
powder tablets, 5306
Tigecycline, 4379
for injection, 4381
Tiletamine
hydrochloride, 4382
and zolazepam for injection, 4383
Tilmicosin, 4384
injection, 4385
Timolol
maleate, 4387
maleate and hydrochlorothiazide tablets, 4391
maleate ophthalmic solution, 4389
maleate tablets, 4389
Timolol maleate
and dorzolamide hydrochloride ophthalmic solution, 1499
Tin, 6213

Tincture

Belladonna, 488
Benzethonium chloride, 501
Benzoin, compound, 522
Capsicum, 732
Cardamom, compound, 5689
Ginger, 5030
Green soap, 2164
Iodine, 2363
Iodine, strong, 2364
Lemon, 5857
Opium, 3274
Orange peel, sweet, 5915
Rhodiola rosea, 5223
Thimerosal, 4348
Tolu balsam, 6090
Valerian, 5330
Vanilla, 6098

Tinidazole, 4392
Tioconazole, 4393
Tissue human amnion chorion membrane dehydrated, 4395
Titanium
dioxide, 4396
tetrachloride, 6213
trichloride, 6213
trichloride–sulfuric acid TS, 6237
trichloride, tenth-normal (0.1 N), 6251
trichloride TS, 6237
Titration, nitrite ⟨451⟩, 6709
Titrimetry ⟨541⟩, 6764
Tizanidine
hydrochloride, 4397
tablets, 4399
TI 201
injection, thallous chloride, 4318
Tobramycin, 4401
and dexamethasone ophthalmic ointment, 4409
and dexamethasone ophthalmic suspension, 4411
and fluorometholone acetate ophthalmic suspension, 4412
inhalation solution, 4406
injection, 4402
for injection, 4403
ophthalmic ointment, 4405
ophthalmic solution, 4408
sulfate, 4414
Tocopherols excipient, 6089
Tolazamide, 4415
tablets, 4417
Tolazoline hydrochloride, 4417
injection, 4418
Tolbutamide, 4418
for injection, 4419
tablets, 4419
Tolcapone, 4420
tablets, 4421
o-Tolidine, 6213
Tolmetin sodium, 4422
capsules, 4423
tablets, 4424
Tolnaftate, 4425
topical aerosol, 4425
cream, 4426
gel, 4426
topical powder, 4426
topical solution, 4427
Tolterodine tartrate, 4427
Tolualdehyde, 6213
p-Tolualdehyde, 6213
Tolu balsam, 4429
syrup, 6090
tincture, 6090
Toluene, 6213
p-Toluenesulfonic acid, 6213
TS, 6237
p-Toluenesulfonyl-L-arginine methyl ester hydrochloride, 6213
p-Toluic acid, 6213
Toluidine
blue, 6213
blue O, 6213
o-Toluidine, 6213
p-Toluidine, 6213
Tomato extract containing lycopene, 5133
Topical aerosols ⟨603⟩, 6845

www.webofpharma.com

Topical and transdermal drug products—product quality tests ⟨3⟩, 6415

Topical solution

Aluminum acetate, 181
Aluminum subacetate, 193
Aluminum sulfate and calcium acetate for, 194
Aluminum sulfate and calcium acetate tablets for, 195
Benzethonium chloride, 501
Benzocaine, 511
Benzocaine, butamben, and tetracaine hydrochloride, 517
Calcium hydroxide, 702
Carbamide peroxide, 747
Carbol-fuchsin, 765
Cetylpyridinium chloride, 923
Chlorhexidine acetate, 947
Chlorhexidine gluconate, 951
Ciclopirox, 996
Clindamycin phosphate, 1072
Clobetasol propionate, 1082
Clotrimazole, 1122
Coal tar, 1133
Cocaine hydrochloride tablets for, 1134
Diclofenac sodium , 1352
Diethyltoluamide, 1373
Dimethyl sulfoxide, 1414
Dyclonine hydrochloride, 1571
Erythromycin, 1690
Fluocinolone acetonide, 1936
Fluocinonide, 1939
Fluorouracil, 1957
Gentamicin sulfate and betamethasone valerate, 2105
Gentian violet, 2109
Halcinonide, 2186
Hydrogen peroxide, 2249
Hydroquinone, 2255
Iodine, 2362
Ivermectin, 2476
Lidocaine hydrochloride, 2627
Mafenide acetate for, 2704
Methoxsalen, 2865
Minoxidil, 2975
Mometasone furoate, 3005
Myrrh, 3057
Nitrofurazone, 3183
Nitromersol, 3190
Papain tablets for, 3396
Phenol, camphorated, 3502
Podophyllum resin, 3582
Povidone–iodine, 3636
Sodium fluoride and acidulated phosphate, 4074
Sodium hypochlorite, 4077
Testosterone, 4291
Tetracaine hydrochloride, 4299
Tetracycline hydrochloride for, 4309
Thimerosal, 4347
Tolnaftate, 4427
Tretinoin, 4479

Topical suspension

Calamine, 665
Calamine, phenolated, 665
Ciclopirox olamine, 998
Clindamycin phosphate, 1072
Penicillin G, neomycin, polymyxin B, hydrocortisone acetate, and hydrocortisone sodium succinate, 3430
Penicillin G procaine, neomycin and polymyxin B sulfates, and hydrocortisone acetate, 3448
Resorcinol and sulfur, 3862
Selenium sulfide, 4023
Sulfacetamide sodium, 4143
Zinc sulfide, 4695

Topiramate, 4429
  capsules, 4432
  tablets, 4434
Topiramate compounded
  oral suspension, 4436
Torsemide, 4437
  tablets, 4439
Tosylchloramide sodium, 6213
Total organic carbon ⟨643⟩, 6867
Tragacanth, 6091
Tramadol hydrochloride, 4441
  and acetaminophen oral suspension, 4452
  and acetaminophen tablets, 4450
  oral suspension, 4443
  tablets, 4444
  extended-release tablets, 4446
Tramadol hydrochloride compounded, veterinary
  oral suspension, 4443
Trandolapril, 4453
  tablets, 4454
Trandolapril and verapamil hydrochloride extended-release tablets, 4456
Tranexamic acid, 4462
  injection, 4463
Transdermal system
  clonidine, 1105
  nicotine, 3153
Transfer of analytical procedures ⟨1224⟩, 8164
Tranylcypromine
  sulfate, 4468
  tablets, 4466
Travoprost, 4469
  ophthalmic solution, 4471
Trazodone hydrochloride, 4472
  tablets, 4474
Trehalose, 6091
Trenbolone acetate, 4476
Tretinoin, 4478
  cream, 4478
  gel, 4479
  topical solution, 4479
Triacetin, 4480, 6213
n-Triacontane, 6213
Triamcinolone, 4481
  acetonide, 4482
  acetonide cream, 4484
  acetonide dental paste, 4485
  acetonide injectable suspension, 4490
  acetonide topical aerosol, 4483
  acetonide lotion, 4485
  acetonide and neomycin sulfate cream, 3129
  acetonide and nystatin cream, 3224
  acetonide, nystatin, neomycin sulfate, and gramicidin cream, 3222
  acetonide, nystatin, neomycin sulfate, and gramicidin ointment, 3222
  acetonide, nystatin, neomycin sulfate and thiostrepton cream, 3223
  acetonide, nystatin, neomycin sulfate, and thiostrepton ointment, 3223
  acetonide and nystatin ointment, 3225
  acetonide ointment, 4485
  acetonide nasal spray, 4485
  diacetate, 4490
  diacetate injectable suspension, 4491
  diacetate oral solution, 4491
  hexacetonide, 4492
  hexacetonide injectable suspension, 4493
  tablets, 4482
2,4,6-Triamino-5-nitrosopyrimidine, 6213
Triamterene, 4494
  capsules, 4495
  and hydrochlorothiazide capsules, 4496
  and hydrochlorothiazide tablets, 4499
Triazolam, 4501, 6213
  tablets, 4502
Tribasic calcium phosphate, 5657
Tribasic sodium phosphate, 6021
Tributyl
  citrate, 6093
  phosphate, 6213
Tributylethylammonium hydroxide, 6214
Tributyrin, 6214
Trichlormethiazide, 4502
Trichloroacetic acid, 6214
Trichloroethane, 6214
Trichloroethanol2,2,2-, 6214
Trichlorofluoromethane, 6214
Trichloromonofluoromethane, 6093
Trichlorotrifluoroethane, 6214
Tricitrates oral solution, 4504
Triclocarban, 4505
Triclosan, 4507
Trientine hydrochloride, 4509
  capsules, 4510
Triethanolamine, 6214
Triethylamine, 6214
  hydrochloride, 6214
  phosphate, 6214
Triethylammonium Acetate
  1M, 6214
Triethyl citrate, 6094
Triethylenediamine, 6214
Triethylene glycol, 6214
Trifluoperazine
  hydrochloride, 4511
  hydrochloride injection, 4512
  hydrochloride tablets, 4512
  oral solution, 4511
Trifluoroacetic
  acid, 6214
  anhydride, 6214
Trifluoroacetic acid ethyl ester, 6214
Trifluoroacetic acid (TFA) in peptides ⟨503.1⟩, 6743
0.1% Trifluoroacetic acid TS, 6237
2,2,2-Trifluoroethanol, 6215
2,2,2-Trifluoroethyldifluoromethyl ether, 6215
(*m*-Trifluoromethylphenyl) trimethylammonium hydroxide in methanol, 6215
5-(Trifluoromethyl)uracil, 6215
α,α,α-Trifluoro-*p*-cresol, 6215
Trifluorovinyl chloride polymer, 6215
Triflupromazine, 4513
  hydrochloride, 4514
  hydrochloride injection, 4515
  hydrochloride tablets, 4515
  oral suspension, 4514
Trifluridine, 4516
Triglycerides medium-chain, 6095

www.webofpharma.com

Trihexyphenidyl hydrochloride, 4517
extended-release capsules, 4518
oral solution, 4519
tablets, 4519
Trikates oral solution, 4521
Triketohydrindene hydrate
TS, 6232, 6237
Trimeprazine
oral solution, 4522
tartrate, 4521
tartrate tablets, 4523
Trimethobenzamide hydrochloride, 4524
capsules, 4524
injection, 4525
Trimethoprim, 4525
and polymyxin B sulfate ophthalmic solution, 3592
and sulfamethoxazole injection, 4162
and sulfamethoxazole oral suspension, 4163
and sulfamethoxazole tablets, 4166
sulfate, 4527
tablets, 4526
Trimethylacethydrazide ammonium chloride, 6168, 6215
Trimethylamine hydrochloride, 6215
Trimethylchlorosilane, 6215
2,2,4-Trimethylpentane, 6174, 6215
2,4,6-Trimethylpyridine, 6215
N-(Trimethylsilyl)-imidazole, 6215
Trimethyltin bromide, 6215
Trimipramine maleate, 4527
2,4,6-Trinitrobenzenesulfonic acid, 6215
Trinitrophenol, 6215
TS, 6233, 6237
Trioctylphosphine oxide, 6215
Trioxsalen, 4529
tablets, 4529
Tripelennamine hydrochloride, 4530
injection, 4531
tablets, 4531
1,3,5-Triphenylbenzene, 6215
Triphenylene, 6215
Triphenylmethane, 6215
Triphenylmethanol, 6216
Triphenylphosphine oxide, 6216
Triphenyltetrazolium
chloride, 6216
chloride TS, 6237
Triprolidine
hydrochloride, 4532
hydrochloride oral solution, 4533
hydrochloride tablets, 4534
and pseudoephedrine hydrochlorides oral solution, 4534
and pseudoephedrine hydrochlorides tablets, 4535
Tris(2-aminoethyl)amine, 6216
Tris(hydroxymethyl)aminomethane, 6216
acetate, 6216
hydrochloride, 6216
N-Tris(hydroxymethyl)methylglycine, 6216
Trisulfapyrimidines
oral suspension, 4536
tablets, 4537
Tritirachium album proteinase K, 6216
Trolamine, 6097
salicylate, 4537
Tromethamine, 4538, 6216
carboprost, 771
carboprost, injection, 772
for injection, 4538
Tropaeolin OO, 6216
Tropic acid, 6216
Tropicamide, 4539
ophthalmic solution, 4541
Tropine, 6216
Trospium chloride, 4541
tablets, 4543
Trypan blue, 6216
Trypsin, crystallized, 4545
Tryptamine hydrochloride, 6216
Tryptone, 6216
Tryptophan, 4546
5-Hydroxy-L-, 5313
L-Tryptophane, 6216
Tuberculin purified protein derivative *(Tuberculin PPD)*, 6216
Tubocurarine chloride, 4547, 6217
injection, 4548
Tungstic acid, 6217
Turmeric, 5314
powdered, 5316
extract, powdered, 5317
Turmeric paper, 6222
Tylosin, 4548
granulated, 4549
injection, 4550
tartrate, 4550
Tyloxapol, 4551
Tyrosine, 4553
L-Tyrosine disodium, 6217
Tyrosol, 6217
Tyrothricin, 4554

U

Ubidecarenone, 5319
capsules, 5320
tablets, 5321
Ubiquinol, 5322
capsules, 5323
Ultraviolet-visible spectroscopy ⟨857⟩, 7166
Ultraviolet-visible spectroscopy—theory and practice ⟨1857⟩, 8670
Undecylenic acid, 4554
ointment, compound, 4554
Uniformity of dosage units ⟨905⟩, 7183
Uracil, 6217
Uranyl acetate, 6217
cobalt, TS, 6227
zinc, TS, 6237
Urea, 4555, 6217
C 13, 766
C 13 for oral solution, 767
C 14 capsules, 768
for injection, 4557
Urea compounded irrigation, 4556
Urethane, 6217
Uridine, 6217
Ursodiol, 4557
capsules, 4558
oral suspension, 4559
tablets, 4559
USP and NF excipients listed by category, 5584
USP policies, xxviii
USP reference standards ⟨11⟩, 6442

V

Vaccines for human use
bacterial vaccines ⟨1238⟩, 8338
general considerations ⟨1235⟩, 8291
polysaccharide and glycoconjugate vaccines ⟨1234⟩, 8276
Vaccinia immune globulin, 4561
Valacyclovir
oral suspension, 4561
tablets, 4562
Valacyclovir hydrochloride, 4563
Valerian, 5324
extract, powdered, 5327
powdered, 5326
tablets, 5329
tincture, 5330
Valerian root
powder capsules, 5333
Valerian root dry
extract capsules, 5332
Valeric acid, 6217
Valerophenone, 6217
Valganciclovir
hydrochloride, 4568
tablets, 4566
Validation
of alternative microbiological methods ⟨1223⟩, 8144
of compendial procedures ⟨1225⟩, 8166
of microbial recovery from pharmacopeial articles ⟨1227⟩, 8172
Validation of alternative methods to antibiotic microbial assays ⟨1223.1⟩, 8157
Valine, 4571
Valproate sodium
injection, 4572
Valproic acid, 4572
capsules, 4573
oral solution, 4574
Valrubicin, 4575
intravesical solution, 4577
Valsartan, 4577
tablets, 4579
amlodipine, and hydrochlorothiazide tablets, 279
and amlodipine tablets, 275
and hydrochlorothiazide tablets, 4580
Vanadium pentoxide, 6217
Vanadyl sulfate, 6217
Vancomycin, 4583
hydrochloride, 4586
hydrochloride capsules, 4588
hydrochloride for injection, 4588
hydrochloride for oral solution, 4590
injection, 4585
Vancomycin hydrochloride compounded
oral solution, 4589
Vanilla, 6097
tincture, 6098
Vanillin, 6098
Vapor phase sterilization ⟨1229.11⟩, 8231
Varicella-zoster immune globulin, 4592
Vasopressin, 4592
injection, 4593
Vecuronium bromide, 4593
Vegetable oil, hydrogenated, 6099
Vehicle
for oral solution, 5913
for oral solution, sugar free, 5913
for oral suspension, 5913
suspension structured, 6086
suspension structured, sugar-free, 6087

www.webofpharma.com

Venlafaxine
hydrochloride, 4597
hydrochloride extended-release capsules, 4598
tablets, 4595
Verapamil hydrochloride, 4604
extended-release capsules, 4605
injection, 4607
oral solution, 4609
oral suspension, 4609
tablets, 4610
extended-release tablets, 4611
Vardenafil
hydrochloride, 4590
Verification of compendial procedures ⟨1226⟩, 8171
Verteporfin, 4615
for injection, 4617

Veterinary

Atenolol compounded oral suspension, 409
Benazepril hydrochloride compounded oral suspension, 490
Buprenorphine compounded buccal solution, 615
Doxycycline compounded oral suspension, veterinary, 1530
Enalapril maleate compounded oral suspension, 1613
Methylene blue injection, 2884
Pergolide oral suspension, 3478
Potassium bromide oral solution, 3600
Prednisolone compounded oral suspension, 3664
Sodium bromide injection, 4063
Sodium bromide oral solution, 4063
Spironolactone compounded oral suspension, 4111
Tramadol hydrochloride compounded oral suspension, 4443
Voriconazole compounded ophthalmic solution, 4645

Vibrational circular dichroism spectroscopy ⟨782⟩, 7002
Vibrational circular dichroism spectroscopytheory and practice ⟨1782⟩, 8549
Vigabatrin, 4618
for oral solution, 4619
tablets, 4621
Vinblastine sulfate, 4623
for injection, 4624
Vincristine sulfate, 4625
injection, 4626
for injection, 4628
Vinorelbine
injection, 4631
tartrate, 4630
Vinpocetine, 5335
capsules, 5336
tablets, 5337
Vinyl acetate, 6217
2-Vinylpyridine, 6218
Vinylpyrrolidinone, 6218
Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin ⟨1050⟩, 7459
Virology test methods ⟨1237⟩, 8319
Virus testing of human plasma for further manufacture ⟨1240⟩, 8350
Viscosity—capillary methods ⟨911⟩, 7187
Viscosity—pressure driven methods ⟨914⟩, 7195
Viscosity—rolling ball method ⟨913⟩, 7193
Viscosity—rotational methods ⟨912⟩, 7189
Visible particulates in injections ⟨790⟩, 7021
Visual Comparison ⟨630⟩, 6865
Visual inspection of injections, 8587
Vitamin
A, 4632
A assay ⟨571⟩, 6801
A capsules, 4633
A oral liquid preparation, 4634
A tablets, 4635
B_{12} activity assay ⟨171⟩, 6584
C assay ⟨580⟩, 6805
C and zinc lozenges, 5576
D assay ⟨581⟩, 6808
D and calcium with minerals tablets, 4845
D with calcium tablets, 4843
E, 4637
E assay ⟨551⟩, 6767
E capsules, 4639
E polyethylene glycol succinate, 6099
E preparation, 4641
Vitamins
capsules, oil-soluble, 5346
capsules, oil- and water-soluble, 5389
capsules, water-soluble, 5499
with minerals capsules, oil- and water-soluble, 5435
with minerals capsules, water-soluble, 5523
with minerals oral solution, oil- and water-soluble, 5462
with minerals oral solution, water-soluble, 5543
with minerals tablets, oil- and water-soluble, 5476
with minerals tablets, water-soluble, 5552
with minerals capsules, oil-soluble, 5361
with minerals oral solution, oil-soluble, 5372
with minerals tablets, oil-soluble, 5378
oral solution, oil-soluble, 5353
oral solution, oil- and water-soluble, 5409
tablets, oil-soluble, 5356
tablets, oil- and water-soluble, 5419
tablets, water-soluble, 5512
Vitamins with minerals oral powder, 5338
Volumetric
apparatus ⟨31⟩, 6448
solutions, 6237
Voriconazole, 4643
Voriconazole compounded, veterinary ophthalmic solution, 4645

W

Warfarin sodium, 4646
for injection, 4648
tablets, 4650
Washed sand, 6218

Water

ammonia, stronger, 6207, 6207, 6226, 6226
ammonia, 25 percent, 6136
ammonia-free, 6218
carbon dioxide-free, 6218
cetyltrimethylammonium chloride, 25 percent in, 6150
conductivity ⟨645⟩, 6871
deaerated, 6218
determination ⟨921⟩, 7196
deuterated, 6155
D-Gluconic acid, 50 percent in, 6168
for hemodialysis, 4651
for hemodialysis applications ⟨1230⟩, 8240
hydrazine hydrate, 85% in, 6170
for inhalation, sterile, 4652
for injection, 4651
for injection, bacteriostatic, 4652
for injection, sterile, 4653
for irrigation, sterile, 4653
methylamine, 40 percent in, 6180
organic-free, 6218
particle-free, 6218
peppermint, 5923
for pharmaceutical purposes ⟨1231⟩, 8242
pure steam, 4654
purified, 4653
purified, sterile, 4654
rose, ointment, 3946
rose, stronger, 6001
solid interactions in pharmaceutical systems ⟨1241⟩, 8359
soluble vitamins capsules, 5499
soluble vitamins with minerals capsules, 5523
soluble vitamins with minerals oral solution, 5543
soluble vitamins with minerals tablets, 5552
soluble vitamins tablets, 5512
Stronger ammonia, 6207
vapor detector tube, 6218
vitamins capsules, and oil-soluble, 5389
vitamins with minerals capsules, and oil-soluble, 5435
vitamins with minerals oral solution, and oil-soluble, 5462
vitamins with minerals tablets, and oil-soluble, 5476
vitamins oral solution, and oil-soluble, 5409
vitamins tablets, and oil-soluble, 5419

Wax
carnauba, 6101
emulsifying, 6101
microcrystalline, 6101
white, 6102
yellow, 6103
Weighing on an analytical balance ⟨1251⟩, 8363
Weight variation of dietary supplements ⟨2091⟩, 8718
Wheat
bran, 4654
starch, 6066
Witch hazel, 4656
Wound matrix small intestinal submucosa, 4002
Wright's stain, 6218

www.webofpharma.com

Written prescription drug information—guidelines ⟨1265⟩, 8368

X

Xanthan gum, 6103
solution, 6104
Xanthine, 6218
Xanthydrol, 6218
Xenon Xe 133, 4656
X-ray fluorescence spectrometry ⟨735⟩, 6971
X-ray fluorescence spectrometry—theory and practice ⟨1735⟩, 8490
Xylazine, 4656
hydrochloride, 4658
injection, 4659
Xylene, 6218
m-Xylene, 6218
o-Xylene, 6218
p-Xylene, 6218
Xylene cyanole FF, 6219
Xylenol orange, 6221
TS, 6237
Xylitol, 6105
Xylometazoline hydrochloride, 4659, 6219
nasal solution, 4661
Xylose, 4662, 6219

Y

Yeast extract, 6219
Yellow mercuric oxide, 6219
Yohimbine
hydrochloride, 4663
injection, 4664
Yttrium Y 90 ibritumomab tiuxetan
injection, 4665

Z

Zalcitabine, 4666
tablets, 4667
Zaleplon, 4668
capsules, 4669
Zanamivir, 4671
meso-Zeaxanthin, 5570
preparation, 5572
Zein, 6106
Zidovudine, 4673
capsules, 4674
injection, 4675
and lamivudine tablets, 2528
oral solution, 4676
tablets, 4678
Zileuton, 4679
Zinc, 6219
acetate, 4681, 6219
acetate oral solution, 4682
activated, 6219
amalgam, 6219
carbonate, 4682
chloride, 4683
chloride, anhydrous, powdered, 6219
chloride injection, 4684
citrate, 5574
citrate tablets, 5575
determination ⟨591⟩, 6817
gluconate, 4684
gluconate tablets, 4686
oxide, 4687
oxide neutral, 4688
oxide ointment, 4689
oxide paste, 4690
oxide and salicylic acid paste, 4690
stearate, 4691
sulfate, 4692
sulfate heptahydrate, 6219
sulfate injection, 4692
sulfate ophthalmic solution, 4693
sulfate oral solution, 4693
sulfate tablets, 4694
sulfate, twentieth-molar (0.05 M), 6252
sulfide topical suspension, 4695
undecylenate, 4695
uranyl acetate TS, 6237
and vitamin C lozenges, 5576
Zinc oxide
powder, 4690
Zinc sulfate
0.02 M VS, 6252
0.1 M VS, 6252
Ziprasidone
capsules, 4695
Ziprasidone hydrochloride, 4699
Zirconyl
nitrate, 6219
Zolazepam
hydrochloride, 4701
and tiletamine for injection, 4383
Zolmitriptan, 4702
nasal spray, 4704
tablets, 4706
orally disintegrating tablets, 4709
Zolpidem tartrate, 4711
tablets, 4712
extended-release tablets, 4714
Zonisamide, 4718
capsules, 4719
Zonisamide compounded
oral suspension, 4721

www.webofpharma.com